Assessing human exposure to acrylamide

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Abstract

Acrylamide is considered as probably carcinogenic to humans. The International Agency for Research on Cancer (IARC) rates it as a Group 2A reagent. Therefore, human exposure to acrylamide in occupational settings has been of concern for a long time. Until recently, tobacco smoke was assumed to be the only major source of acrylamide exposure in the general population. Now, it is well established that acrylamide is formed in food during processing or cooking at high temperatures. Surveys showed that acrylamide can be found in most types of food, with highest amounts measured in French fries and potato chips. The concentrations found in some foods exceed the concentrations found for other environmental contaminants, such as pesticides.

The Division of Laboratory Sciences at CDC’s National Center for Environmental Health, through a biomonitoring program project, is assessing people’s exposure to this chemical. The goal is to better assess the magnitude and distribution of human exposure to acrylamide and risks associated with this exposure in the general population. Project researchers are measuring biomarkers of exposure such as hemoglobin adducts of acrylamide and its primary metabolite glycidamide. First assessments show that biomarker levels mainly range between 27 pmol/g globin and 148 pmol/g globin for acrylamide and glycidamide adducts, respectively. Biomarker values in smokers are about 3 to 4 times higher than in non-smokers. An initial study looked at the effects of acrylamide in food on biomarkers of acrylamide exposure. Results indicate that acrylamide consumed through food has a more profound effect on glycidamide adduct levels than on acrylamide adduct levels. The acrylamide biomarker concentrations determined so far are within the range reported by other investigators. Smoking appears to be an important contributor to the background exposure found in people.

Keywords: acrylamide, glycidamide, exposure assessment, hemoglobin adducts.
1 Introduction

Acrylamide is an industrial and environmental chemical known to be neurotoxic to animals and humans, mutagenic to male germ cells and carcinogenic in animals [1-4]. Consequently, the International Agency for Research on Cancer (IARC) classifies acrylamide as probably carcinogenic to humans and the Occupational Safety and Health Agency rates it as a potential occupational carcinogen [4, 5]. Because acrylamide can be a health hazard to humans, the assessment of human exposure to acrylamide is important to protect people’s health and to evaluate potential health effects caused by this chemical.

Early exposure assessments in people focused on occupational activities, mainly in the production and use of acrylamide as cement binder, in the production of polymers and gels, and in scientific research. For occupational exposure, the main exposure paths are considered inhalation and skin, and occupational exposure assessments were made by measuring the acrylamide concentration in the air of the workplace (findings are summarized in [6]). The development of methods to determine the amounts of acrylamide occurring in blood allows more accurate information about the actual acrylamide exposure in humans to be obtained. Using these methods, acrylamide exposure can be quantitatively described and different degrees of exposure in workers during regular occupational activities or occupational accidents can be distinguished [2, 7, 8]. Further investigations on the occurrence of acrylamide in the blood of people not occupationally exposed to the chemical found that smoking is another source of acrylamide exposure [8-10].

The search for sources of exposure seen in non-occupationally exposed, non-smoking people finally led to the discovery of food being a source of exposure to acrylamide in the general population [11, 12]. Acrylamide is not a food contaminant, such as pesticides, but is formed in food from the reaction of endogenous non-protein-bound amino acids, mainly asparagine, and sugars during heating at temperatures of 120°C or higher [13, 14]. Because most foods contain this amino acid, acrylamide is found in a wide variety of food (Table 1). For comparison, pesticide residues found in the total diet study foods in 2002 ranged between 0.1–166 µg/kg for the most frequently occurring pesticides (DDT, chlorpyrifos-methyl, malathion, endosulfan and dieldrin) [16]. Thus acrylamide occurs in food in concentrations higher than found for other food contaminants such as pesticides.

The fact that the general population is exposed to acrylamide, which is suspected to be carcinogenic in humans, initiated further health risk assessments [17, 18]. These assessments revealed the need for more information about acrylamide exposure in the general population. To address this need, the Division of Laboratory Sciences at CDC’s National Center for Environmental Health initiated a project to determine the acrylamide exposure in the general U.S. population as part of its human biomonitoring program.

This includes the development of methods suitable to measure low background exposure levels in people in large population studies that can be used with different types of specimen, depending on specimen availability.
These methods are measuring biomarkers of acrylamide exposure, which are reaction products of acrylamide and its primary metabolite glycidamide with hemoglobin, specifically the N-terminal valine of the hemoglobin protein chains. These biomarkers already have been applied successfully by other researchers and shown to be suitable for assessing human exposure to acrylamide.

Table 1: Acrylamide concentrations in food compiled from the 2005 Joint FAO/WHO Expert Committee on Food Additives report [15].

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Mean Concentration (µg/kg)</th>
<th>Coefficient of Variation (%)</th>
<th>Reported maximum concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals and cereal-based products</td>
<td>343</td>
<td>156</td>
<td>7,834</td>
</tr>
<tr>
<td>Meat and offals</td>
<td>19</td>
<td>174</td>
<td>313</td>
</tr>
<tr>
<td>Milk and milk products</td>
<td>5.8</td>
<td>119</td>
<td>36</td>
</tr>
<tr>
<td>Nuts and oil seeds</td>
<td>84</td>
<td>233</td>
<td>1,925</td>
</tr>
<tr>
<td>Roots and tubers</td>
<td>477</td>
<td>108</td>
<td>5,312</td>
</tr>
<tr>
<td>Coffee, cocoa and tea</td>
<td>509</td>
<td>120</td>
<td>7,300</td>
</tr>
</tbody>
</table>

This report describes a method that uses established procedures to analyze acrylamide exposure using hemoglobin adducts. The study compares that method to a new method that allows measurements of acrylamide exposure in large population studies and in different types of specimen.

2 Materials and methods

Initial biomarker measurements were based on methods and procedures described in literature. In brief, globin was isolated from erythrocytes by precipitation as described by Mowrer et al. [19]. The N-terminal valine adducts (N-(2-carbamoyl-ethyl)valine for acrylamide adducts and N-(2-carbamoyl-2-hydroxyethyl)-valine for glycidamide adducts) were removed from globin by modified Edman reaction using pentafluorophenylisothiocyanate. They were then isolated using liquid-liquid extraction with isopropylether as described by Tornqvist et al. [20]. The extracted analytes were analyzed by HPLC/MS/MS (ThermoFinnigan TSQ Quantum MS with ThermoFinnigan Surveyor HPLC) as described by Ospina et al. [21]. We used octapeptides (VHLTPEEK) with acrylamide or glycidamide added at the N-terminal valine as calibrators. The same peptides containing $^{15}$C$_5$$^{15}$N-labeled valine instead of non-labeled valine were used as internal standards (Bachem, Prince of Prussia, PA).

To be able to automate this method we modified it by introducing liquid-liquid extraction on diatomaceous earth. We also assessed the applicability of this modified method for direct use with EDTA-whole blood and red blood cells. Therefore, 50 mg globin or 200 µL of EDTA-whole blood or red blood cells
were used for modified Edman reaction. The reaction mixture was then applied on diatomaceous earth (Chem Elute, Varian, Palo Alto, CA) and the analytes were extracted with a mixture of toluene/ethyl acetate/isopropyl ether (10/40/50 v/v/v). The eluate was concentrated and applied to HPLC/MS/MS as described previously [21]. Using this method, all sample handling steps could be performed in 48 deep wellplates using a Tecan Genesis (Tecan, Durham, NC) and Gilson 215 (Gilson, Middleton, WI) liquid handling system. The total hemoglobin content in the whole blood samples and erythrocytes was determined spectrophotometrically after oxidation by ferricyanide using a conventional clinical assay (Stanbio Hemoglobin, Stanbio Laboratory, Boerne, TX).

3 Results and discussion

The initial method setup in our laboratory allows the reliable measurement of hemoglobin adducts of acrylamide and glycidamide in smokers and non-smoking people. A coefficient of variability is determined at 2 different concentration levels of an average 16% for acrylamide and glycidamide adducts. The detection limit of this method was 0.5 pmol of adduct on column.

The hemoglobin adduct concentrations measured with this method were, in smokers, 148 pmol/g globin (SD: ±8 pmol/g globin) and 69 pmol/g globin (SD: ±23 pmol/g globin) for acrylamide and glycidamide adducts, respectively. For non-smokers, the adduct concentrations were 41 pmol/g globin (SD: ±6 pmol/g globin) and 27 pmol/g globin (±6 pmol/g globin) for acrylamide and glycidamide adducts, respectively. The concentrations in smokers analyzed so far are 3 to 4 times higher than in non-smokers. These findings are similar to those reported by other researchers [22-25]. Judging from these results, smoking seems to cause the highest exposure values in non-occupationally exposed people and thus seems to be the major contributor to the background exposure in the general population.

CDC researchers have also used this method to obtain preliminary information about the effects of eating potato chips with high amounts of endogenous acrylamide on biomarkers of acrylamide exposure [26]. The findings that potato chips consumption seem to have a more pronounced affect on glycidamide adducts than acrylamide adducts appears to be in agreement with findings in animal studies where acrylamide exposure in food resulted in higher concentrations of free glycidamide than acrylamide in blood [27]. The findings from these studies show the importance of measuring acrylamide adducts and glycidamide adducts to obtain more comprehensive information about the acrylamide exposure in a person.

While results obtained with this initial (conventional) method agreed well with findings by other researchers, the procedures are highly labor-intensive, allowing a throughput of only 30 samples per day. To enable the measurement of large numbers of samples within a reasonable period of time, this method was modified by replacing a manual liquid-liquid extraction with extraction on diatomaceous earth. While this change allowed the automation of the extraction
procedure, it appeared to be less efficient compared to the original extraction procedure. The use of a new extraction solvent helped to overcome this problem. Preliminary comparison between the initial (conventional) method and our new (biomonitoring) method shows no significant difference between both methods (Figure 1) with the mean bias being 0.5 pmol/g globin (95% CI: -0.5–1.5 pmol/g globin) for glycidamide adducts and 1.41 pmol/g globin (95% CI: -0.1–2.9 pmol/g globin) for acrylamide adducts.

Figure 1: Bias plot assessing the differences in results between our initial (conventional) method and new (biomonitoring) method for measurement of hemoglobin adducts of acrylamide and glycidamide (n=18). Dotted line: mean bias, dashed-dotted line: ±2 SD.

To further simplify this method, the use of whole blood and erythrocytes in place of isolated globin was assessed. To express results relative to the amount of hemoglobin used, the hemoglobin content of the whole blood and erythrocyte samples was assessed using a conventional spectrophotometric assay performed in microtiter plates as compared to manually weighing the necessary amount of
globin for each sample in the conventional method. First assessments show that the method is able to reproducibly measure acrylamide and glycidamide adducts in whole blood and erythrocytes with an average coefficient of variation of 10% for both types of specimen. Deming regression of results obtained with whole blood and erythrocytes showed a close correlation to results obtained with isolated globin from the same individuals (Figure 2). The coefficients of correlation are 0.995 for erythrocytes and 0.979 for whole blood.

![Graph showing correlation between acrylamide and glycidamide adducts measured in globin and in whole blood or erythrocytes.](image)

Figure 2: Regression analysis to assess correlation between acrylamide adducts and glycidamide adducts measured in globin and in whole blood (circles with full line) or erythrocytes (squares with dashed line).

No systematic biases to results obtained with globin samples were detected with whole blood and erythrocytes for acrylamide or glycidamide adducts. Only results obtained with acrylamide adducts of whole blood showed a proportional bias to globin samples (Table 2). The detection limits and coefficients of
variation are similar to those of the initial manual method. Using the modified method, 196 samples can be processed per day using automated systems.

Table 2: Results of Deming regression assessing the correlation of acrylamide and glycidamide adducts values obtained with globin to results obtained with whole blood and erythrocytes.

<table>
<thead>
<tr>
<th>Adduct</th>
<th>Specimen</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>Whole Blood</td>
<td>1.128 (0.944 – 1.312)</td>
<td>5.75 (-11.9 – 23.4)</td>
</tr>
<tr>
<td></td>
<td>Erythrocytes</td>
<td>1.319 (1.206 – 1.432)</td>
<td>-2.28 (-13.2 – 8.61)</td>
</tr>
<tr>
<td>Glycidamide</td>
<td>Whole Blood</td>
<td>1.075 (0.884 – 1.267)</td>
<td>5.52 (-7.67 – 18.7)</td>
</tr>
<tr>
<td></td>
<td>Erythrocytes</td>
<td>1.092 (0.988 – 1.195)</td>
<td>3.65 (-3.46 – 10.8)</td>
</tr>
</tbody>
</table>

4 Conclusion

Initial assessments on human exposure to acrylamide using conventional methods and procedures to measure acrylamide and glycidamide adducts to hemoglobin gave results that agree with findings by other researchers. Modifications made to this conventional method allowed the automation of the sample preparation procedure. Initial assessments show a good agreement between the new automated method and the traditional method initially used. This method can be used with isolated globin, erythrocytes or whole blood. Results obtained with erythrocytes and whole blood correlate closely with results obtained with globin. Studies with larger sample sizes to confirm these initial findings are in progress. Once confirmed, this new method will be used to measure acrylamide exposure in large population studies.

References


[20] Tornqvist, M., Mowrer, J., Jensen, S., & Ehrenberg, L., Monitoring of environmental cancer initiators through hemoglobin adducts by a


