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Towards a biological monitoring guidance value for acrylamide

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HIGHLIGHTS

- We report a study of workers at a UK acrylamide production plant.
- Biological monitoring data (urine metabolites and haemoglobin adducts) are given.
- Their relationship with environmental (air and hand wash) levels were investigated.
- We propose urinary acrylamide-mercapturic acid as the preferred biomarker.
- A guidance value is proposed.

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ABSTRACT

Acrylamide is classified as a potential human carcinogen and neurotoxicant. Biological monitoring is a useful tool for monitoring worker exposure. However, other sources of exposure to acrylamide (including cigarette smoke and diet) also need to be considered. This study has performed repeat measurements of the urinary mercapturic acids of acrylamide (AAMA) and its metabolite glycidamide (GAMA) and determined globin adducts in 20 production-plant workers at a UK acrylamide production facility. The relationship between biomarker levels and environmental monitoring data (air levels and hand washes) was investigated.

Good correlations were found between all of the biomarkers (r^2 = 0.86–0.91) and moderate correlations were found between the biomarkers and air levels (r^2 = 0.56–0.65). Our data show that urinary AAMA is a reliable biomarker of acrylamide exposure.

Occupational hygiene data showed that acrylamide exposure at the company was well within the current UK Workplace Exposure Limit. The 90th percentile of urinary AAMA in non-smoking production-plant workers (537 μmol/mol creatinine (n = 59 samples)) is proposed as a possible biological monitoring guidance value. This 90th percentile increased to 798 μmol/mol if smokers were included (n = 72 samples). These values would be expected following an airborne exposure of less than 0.07 mg/m^3, well below the current UK workplace exposure limit of 0.3 mg/m^3. Comparison of biomarker levels in non-occupationally exposed individuals suggests regional variations (between UK and Germany), possibly due to differences in diet.

1. Introduction

Acrylamide is an important industrial chemical with a range of applications, mainly in the manufacture of a variety of polymers. It is synthesised by the hydration of acrylonitrile with water in the presence of Raney copper catalyst to produce a 50% aqueous solution in a continuous "closed process", although there is potential for exposure during in-process sampling and testing and plant maintenance. It is also formed in food during cooking at high temperatures, particularly during baking or frying starchy foods (Tareke et al., 2002) and is present in cigarette smoke (Urban et al., 2006). Acrylamide is a solid at room temperature. Exposure to acrylamide has been linked with adverse health effects including cancer and neurotoxicity, although evidence from human studies remains unclear (Bachmann et al., 1992; Lipworth et al., 2012, 2013; Mulloy 1996). Acrylamide can be absorbed through

Abbreviations: AA, globin-acrylamide adduct; AAMA, acrylamide-mercapturic acid urinary metabolite; GAMA, glycidamide-mercapturic acid urinary metabolite.

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inhalation, dermally and ingestion. Biological monitoring offers a straightforward means of assessing total uptake by all routes (including dermal and ingestion) and all sources, including environmental exposure (from smoking and dietary sources of acrylamide) (Manno et al., 2010).

Acrylamide is metabolised in vivo to glycidamide, a more chemically reactive epoxide, which may be more carcinogenic (Besaratinia and Pfeifer 2005). Acrylamide and glycidamide subsequently undergo phase II metabolism to form their respective mercapturic acids (AAMA and GAMA), prior to excretion via urine. Methods for quantifying acrylamide and glycidamide in blood and urine are well established (Bjellaas et al., 2005; Boettcher and Angerer 2005; Fennell et al., 2006; Urban et al., 2006). Analytical methods using blood have determined haemoglobin adduct levels, the excretion of which depends on the life span of the red cells. Consequently, haemoglobin adducts in blood samples reflect an average of the exposure over approximately 12 weeks prior to sampling. The half-lives of excretion for the urinary metabolites are 17 and 25 h for AAMA and GAMA respectively (Fuhr et al., 2006). This means that urine sampling will mostly reflect that day and the previous day's exposure to acrylamide. There may be some accumulation of GAMA over the working week. The different biological fluids can therefore be used to look at acrylamide exposures over different timescales.

This study describes the results of a biological monitoring programme at an acrylamide production-plant in the UK. Biological monitoring data are compared with environmental analysis (air levels and hand washes) with the aim of improving the understanding of the relationships between work-related dermal and inhalation exposures and biomarker levels.

2. Methods

2.1. Sampled workers

All workers with potentially significant exposure to acrylamide (as judged by the occupational hygienist) were eligible to participate. Participants gave their informed consent. Different job categories on site have been previously described (Bull et al., 2005).

Samples were collected in two stages, separated by about a year. In stage one, samples were collected from 20 shift managers and operators in the acrylamide production-plant (subsequently referred to as 'production workers') and a further 13 individuals working on the wider site, including tanker loading operators ('other jobs'). The production workers provided four end-of-shift urine samples each covering four different shifts—one 'clean' sample (provided after several days off work), one 'indoor log' shift (working in the control room), one 'outdoor log' shift (working on site) and one 'outdoor sampling' shift (breaking into production lines to take quality control samples). Each urine sample (except 'clean' samples) was accompanied by a personal air sample (8 h time weighted average) and a hand rinse sample (cumulative sample during the shift). Two blood samples (collected approximately 3 months apart to reflect the red cell life span) were collected from all 20 production workers and nine other workers. The four monitored shifts took place during this 3 month period between the blood samples.

In stage two, 18 of the same production workers (two did not participate in stage 2) provided three urine samples (clean, outdoor log and outdoor sampling). In addition, seven powder workers (producing polyacrylamide powders from the acrylamide produced on site) and 12 office staff (unexposed controls) provided one urine sample each. Again, a personal air sample and a hand rinse sample accompanied each urine sample (except 'clean' samples). No blood samples were taken during stage 2. A previous study at this site (Bull et al., 2005) showed that production workers had the highest potential exposure to acrylamide; powder workers had potential inhalation exposure but very low dermal exposure.

2.2. Air sampling, extraction and analysis

The personal air sampling method used pumped sampling into glass tubes filled with silica gel (225 mg, SKC 226-10). Where solid acrylamide was in use, a pre-filter was attached to collect any particulate, and the total mass determined was the sum of the vapour and dust collected. Each personal monitoring pump was calibrated to approximately 100 ml/min with a sampling tube in place and the actual flow rate was recorded. The collected acrylamide was desorbed from the tube packing with methanol: water (1 ml, 50:50, v/v) by agitation for 30 min. The supernatant was then analysed. The detection limit was 0.005 mg/m^3 for a 48 l air sample. Analysis was by high performance liquid chromatography ultraviolet detection (210 nm) using a reverse phase C18 column (250 × 4.6 mm, 4 μm particles). The mobile phase consisted of 0.1% phosphoric acid (solvent A) and acetonitrile (solvent B). A gradient elution of 0–10% solvent B over 12 min was used at a flow rate of 0.5–2.0 ml/min. A standard calibration stock solution of acrylamide was prepared in methanol:water 50:50 (v/v), from which serial dilutions were made over the range of 4–200 μg/ml. Quality control samples of known concentration were run after every 10 samples. Acceptance limits were set as the mean ± 2 standard deviations with a coefficient of variation of 10%.

2.3. Hand-wash sampling, extraction and analysis

A simple method for estimating the amount of acrylamide present on worker's hands was developed. Workers rinsed both hands in one litre of water in a container every time they would normally wash their hands throughout a shift. The same container was used for the entire shift therefore reflecting aggregated skin contamination during that shift. Samples were analysed using the same method as for air samples (limit of detection was 0.2 μg/l).

Operators wore gloves for tasks involving contact with plant or equipment (e.g. sampling and breaking into lines). Hand wash samples were collected after removing the gloves.

2.4. Urine analysis

Mercapturic acid derivatives of acrylamide (AAMA) and glycidamide (GAMA—an un-resolved mixture of GAMA and iso-GAMA) were custom synthesised along with d_3-AAMA as internal standard (Sheffield Hallam University, UK). Stock solutions were prepared in methanol and stored at −20 °C.

All urine samples were stored frozen until analysis. Samples were analysed in duplicate. Urine (1 ml) was dispensed into glass tubes then internal standard, 1 ml deionised water and 100 μl 1% formic acid were added to all tubes. Quality control samples, prepared separately and stored frozen in aliquots, were included in every analytical batch. A pair of quality control samples was run after the standard curve then after each group of 5 duplicate samples. A standard curve was prepared by adding the required volume of working solution containing AAMA and GAMA to 1 ml urine to achieve a concentration range of 20–1000 μg/l. Samples were extracted using TELOS ENV (100 mg, 3 ml) solid-phase extraction cartridges (Kinesis). Cartridges were conditioned with 2 ml methanol, followed by 2 ml 0.1% formic acid. The urine sample (2 ml) was then loaded onto the SPE cartridge, followed by washing with 2 ml 0.1% formic acid and 0.8 ml 90:10 0.1% formic acid: methanol. Cartridges were eluted with 2 ml methanol containing 1% formic acid. The eluate was evaporated to dryness under a stream of nitrogen, reconstituted with 100 μl 0.1% formic acid and transferred to sample vials ready for analysis.