Biological Monitoring of Exposure to 3-Chloro-4-fluoroaniline by Determination of a Urinary Metabolite and a Hemoglobin Adduct

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In two studies, involving 75 and 72 workers, potential exposure to 3-chloro-4-fluoroaniline (CFA) was biologically monitored by determination of its main urinary metabolite 2-amino-4-chloro-5-fluorophenol sulfate (CFA-S). As this method only allows the detection of recent exposure, analysis of CFA adducts bound to hemoglobin (Hb) was investigated as a method that allows biological monitoring of exposure to CFA over longer periods. The median CFA-S concentration in 67 samples from the first study was 0.14 μmole/g creatinine (range <0.05 – 2.82) and in 201 samples from the second study 0.21 μmole/g creatinine (range <0.05 – 6.05). In addition, urine samples, collected after shifts with supposed incidental exposure, slightly higher concentrations were measured: 0.27 μmole/g creatinine (range <0.05 – 122; 18 samples) and 0.76 μmole/g creatinine (range <0.05 – 18.5; 46 samples), respectively. The median Hb adduct concentration in 75 samples from the first study was 9 pmoles CFA/g Hb (range <5 – 640) and in 46 samples from the second study 12 pmoles/g Hb (range 3 – 240). In 24 blood samples collected after incidents, a median concentration of 13 pmoles CFA/g Hb (range <5 – 52) was found. Urinary CFA-S and Hb adducts correlated well in samples collected shortly after incidental exposures. However, in 25% of the operators, no CFA-S was detected during routine biological monitoring while Hb adduct analysis showed clear evidence of exposure. This indicates that because of the stability of Hb adducts of CFA in blood, intermittent exposure to CFA is more reliably biologically monitored by determination of Hb adducts of CFA than by assessment of urinary CFA-S. — Environ Health Perspect 102(Suppl 6):23–25 (1994)

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Introduction

3-Chloro-4-fluoroaniline (CFA) is an intermediate in herbicide manufacture. A major pathway in the metabolism of CFA in man is ring-oxidation to 2-amino-4-chloro-5-fluorophenol, which is rapidly excreted in the urine as its sulfate conjugate (CFA-S) (1). It was estimated in workers exposed to low concentrations of CFA (<0.3 mg/m³, 8-hr time-weighted average) that more than 25% of the inhaled CFA is excreted as CFA-S in urine voided during the shift (NJ van Sittert, unpublished observations). Despite the high sensitivity of the determination of CFA-S in urine, the method can only be used to detect exposure during the past 24 hr because of this rapid elimination. A minor pathway in the metabolism of arylamines is N-oxidation to the corresponding hydroxylamine, which may be further oxidized in an autocatalytic co-oxidation process with hemoglobin (Hb) yielding the nitrosoureine (2). The nitrosoureine can react with the β-93 cysteine residue of Hb to a sulfinic acid amide (Figure 1). Because Hb adducts are stable in vivo (3), measurement of CFA bound to Hb should, theoretically, allow the detection of exposure over a much longer period (4). In this study, workers potentially exposed to CFA were biologically monitored by determination of both urinary CFA-S and CFA adducts bound to Hb.

Materials and Methods

Chemicals

CFA-S was obtained from Sintingbourne Research Center (Shell Research Ltd., UK) and was >99% pure. All other reagents were purchased commercially and of the highest purity.

Biological Monitoring of Industrial Workers

All workers potentially exposed to CFA in the Shell herbicide plant in Pernis are routinely monitored by determination of urinary CFA-S. The men are employed on a five-shift system and postshift urine samples are collected at the end of every third night shift. During herbicide production in 1991 and 1992, the workers also were monitored by determination of CFA adducts bound to Hb. For that purpose blood samples were collected at the end of each production period. The operators also were asked to provide additional urine samples at the end of shifts in which incidental exposure to CFA might have occurred. In 1992, these samples were analyzed within 24 hr and if the concentration of CFA-S was higher than 0.5 μmole/g creatinine, an extra blood sample was collected the next day for Hb adduct analysis.

Determination of Urinary CFA-S

The concentration of CFA-S in urine was determined by HPLC with fluorescence detection by a method previously developed in our laboratory (1). To allow correction for differences in urinary output, the creatinine (CR) content of the samples was assessed by the Jaffe method. All results are expressed as μmole/g CR.

Determination of Hb Adducts of CFA

Blood samples were collected by venapuncture from the antecubital vein in 10-ml heparinized vacuum tubes and centrifuged.

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The plasma and buffy coat were removed, and the erythrocytes were washed three times with 10 ml physiologic saline. The erythrocytes were then resuspended with purified water and lysed by subsequent freezing and thawing. After sedimentation of membranes, the solution was dialyzed during 48 hr against purified water, and the amount of Hb was assessed spectrophotometrically using a Systex K-1000 blood analyzer. After addition of 4-chloroaniline (pCA) as internal standard, aliquots of the dialysate were hydrolyzed by incubation with 0.1 M NaOH (final concentration) during 2 hr at room temperature and protected from light. The hydrolysates were prepared for analysis according to Stillwell et al. (5) with some minor modifications. Briefly, the CFA was extracted into dichloromethane (DCM), reacted with pentafluoroproponic acid anhydride but without the addition of trimethylamine. The DCM was evaporated with a gentle stream of N2 at room temperature. The residue was dissolved in ethyl acetate by sonification and after centrifugation the clear supernatant was analyzed on a Hewlett Packard 5890 series II gas chromatograph equipped with a 5971A mass selective detector in the electron impact mode (electron energy fixed at 70 eV, ion source at 180°C). The GC column was a 60 m x 0.25 mm fused silica capillary, coated with a 0.1-μm film of Durabond-1. Helium was used as carrier gas at a flow of 1 ml/min. One microliter injections were made using an HP7673 autosampler in the splitless mode with an injector temperature of 250°C. The initial oven temperature was 60°C for 1 min, followed by a temperature ramp (20°C/min) to 170°C, another ramp (10°C/min) to 300°C and an isothermal phase at 300°C for 5 min. The following ions were monitored with dwell times of 50 ms each: m/z 293 (CFA) and m/z 273 (pCA). Calibration graphs were constructed by plotting the ratio of the integrated peak areas of the ions of CFA and pCA against the ratio of the amounts CFA and pCA in a series of Hb samples prepared from control blood spiked with varying amounts of CFA and prepared in the same way as the unknown samples. The concentration CFA in the unknown sample was calculated from this graph and divided by the concentration of Hb in the sample.

**Results and Discussion**

Recently, a method was developed in our laboratory that allows quantification of CFA-S in urine (1). Due to its rapid urinary elimination, determination of CFA-S only allows detection of exposure during the last 24 hr after cessation of exposure. As Hb adducts of aryamines are thought to be rather stable in vivo (3), the assessment of Hb adducts is a promising tool for the biological monitoring of aryamines in the case of intermittent exposure and chronic exposures to low concentrations. We adapted the method developed by Stillwell and co-workers (5) and applied it to workers potentially exposed to low concentrations of CFA. The method was found to be both specific and sensitive as in persons not occupationally exposed to CFA, Hb adduct levels were below the limit of quantification, which was less than 5 pmole/g Hb.

Both methods, determination of CFA-S in urine and CFA Hb adducts in blood, were applied during the production of CFA-based herbicides in 1991 and 1992. In 1991, 56 operators provided 85 urinary samples: 67 routine samples and 18 samples that were collected after accidents that, in their opinion, might have led to exposure. The median concentration in the routine samples was 0.14 μmole/g cr (range <0.05–2.82) and in the incident samples 0.27 μmole/g cr (range <0.05–12.2). A blood sample was obtained from 75 operators. The concentration of CFA adducts to Hb ranged from <5 to 640 pmole/g Hb (median 9 pmole CFA/g Hb) in 1992, 201 routine and 46 incident urine samples were collected from 69 operators. The median concentration of CFA-S in the routine samples was 0.21 μmole/g cr (range <0.05–6.05). In the incident samples, the median CFA-S concentration was 0.76 μmole/g cr (range <0.05–18.5). Forty-six blood samples were collected at the end of the production period. The concentration of CFA adducts to Hb ranged from <5 to 24 pmole CFA/g Hb (medium 12 pmole CFA/g Hb). Twenty-two blood samples were collected after supposed incidental exposure. The median concentration of adducts in these samples was only 13 pmole CFA/g Hb (range <5–52).

Twice in 1991 and on 19 occasions in 1992, mostly after a supposed incidental exposure, a blood sample was collected within 1 day after the collection of a urine sample. It appeared that the concentrations of CFA bound to Hb correlated very well with the concentration of CFA-S in the corresponding urine samples (Figure 2).

![Figure 1. Oxidative metabolism of 3-chloro-4-fluoroaniline (CFA). (A) CFA is ring oxidized and subsequently sulfated to 2-amino-4-chloro-5-fluorophenyl (CFA-S) (B), which is excreted into the urine. A minor oxidative pathway leads, via the hydroxylamine, to the nitrosoarene, which forms hemoglobin adducts through the β3 cysteine residue of hemoglobin (C). The sulfamidate is hydrolyzed under mild alkaline conditions yielding the original CFA (A).](image)

![Figure 2. Correlation between excretion of 2-amino-4-chloro-5-fluorophenol in urine and formation of hemoglobin adducts of 3-chloro-4-fluoroaniline in workers occupationally exposed to low concentrations 3-chloro-4-fluoroaniline CFA (n = 21, r = 0.987).](image)
In the case of an accidental exposure in which an operator spilled some CFA-containing fluid on unprotected skin, an adduct level of 640 pmoles CFA/g Hb was found. The man provided another blood sample 20 days later. Assuming first-order kinetics and that no exposure had occurred during those 20 days, a half-life for the adduct of 17 days was calculated for the disappearance of the Hb adduct of CFA in blood. Although this is considerably shorter than expected from the life-span of the erythrocyte (i.e., 126 days), it is long enough to allow detection several weeks after exposure. In 1991, 51 of the 56 operators who provided one or more urine samples during the production period also provided a blood sample at the end of that period. In 13 of these 51 operators, no detectable amount of CFA-S (i.e., less than 0.05 µmole/g cr) was found in urine while in all blood samples Hb adducts of CFA were readily detectable (up to 185 pmoles CFA/g Hb). This confirms that exposure that has occurred more than one day before the collection of the urine sample will be missed by measurement of urinary CFA-S but may very well be detected by a much less frequent assessment of Hb adducts of CFA because of the much longer half-life of the latter. From this study, it may therefore be concluded that the determination of Hb adducts provides a useful method for the biologic monitoring of exposure to low concentrations of CFA over prolonged periods and also for incidental higher exposures.

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