Hemoglobin Adducts of Acrylamide and Acrylonitrile in Laboratory Workers, Smokers and Nonsmokers

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Acrylamide is a chemical which is extensively used in research laboratories for the preparation of polyacrylamide gels for electrophoresis (PAGE). Blood samples were collected from laboratory personnel who were working with PAGE, from smokers, and from nonsmokers. Hemoglobin adducts of acrylamide, acrylonitrile, and ethylene oxide were determined using the modified Edman degradation procedure. Acrylamide adducts were detected in all persons. The PAGE workers (mean 54 pmol/g) had a significantly increased adduct level compared to nonsmoking controls (mean 31 pmol/g). The acrylamide adducts in smokers (mean 116 pmol/g) correlated with the number of cigarettes smoked per day. This confirms the presence of acrylamide in tobacco smoke and shows that it is an important source of acrylamide exposure. The increased level of acrylamide adducts in the PAGE workers corresponds to an uptake of acrylamide from about 3 cigarettes per day. It is not possible from this study to draw any conclusion as to which step in the working procedure is most critical for exposure. The PAGE workers are probably not at risk for neurotoxic damage to the peripheral nervous system. However, it needs to be investigated whether the exposure to acrylamide in PAGE workers represents a risk for genotoxic and reproductive effects. The high background of acrylamide adducts in nonsmoking controls was unexpected. The origin of this background is not known. Acrylonitrile adducts were below the detection limit (<2 pmol/g) in nonsmoking controls. In the smokers (mean 106 pmol/g) this adduct correlated with cigarettes/day and with ethylene oxide adducts. Acrylonitrile adducts could be a better indicator of tobacco smoking than ethylene oxide adducts since the latter are showing a background of endogenous origin.

Introduction

Acrylamide is an important industrial chemical used mainly for the preparation of polyacrylamides (*1*). These are used for, *e.g.*, purification of waste water and drinking water and in the petroleum and paper industry. Acrylamide is also extensively used in research laboratories for the preparation of polyacrylamide gels for electrophoresis (PAGE).[1] The personnel in such laboratories is the largest group occupationally exposed to acrylamide. The United States Environmental Protection Agency (*2*) estimated the number of laboratory workers exposed to acrylamide to be 100 000−200 000 in the United States alone. There are several recent reports of higher cancer rates in workers in laboratories than in other comparable occupations (*3*). It is, therefore, of interest to study the exposure to chemicals commonly used in laboratories. Moreover, it has not been of common knowledge that acrylamide is a component of tobacco smoke. However, there is a study by Schumacher *et al.* (*4*) in which acrylamide was identified in the water soluble portion of cigarette smoke.

Acrylamide has well-known neurotoxic effects (*1*) and is also carcinogenic in experimental animals (*5*, *6*). Occupational exposure, which probably occurs by a combination of inhalation and skin absorption, has been monitored by hemoglobin adduct measurements (*7*). Hemoglobin adducts give a measure of the blood dose of a chemical over the past 4 months and are a biomarker which takes into account the exposure via multiple routes and the effect of individual metabolic differences (*8*). Measurements of hemoglobin adducts in blood samples have been used to monitor exposure to several reactive chemicals, from both occupational and environmental exposure (*9*), and hemoglobin adduct studies have also been used for quantitative cancer risk assessments (*10*).

Human exposure to acrylamide was determined by hemoglobin adduct measurements in a factory in the People's Republic of China (*7*). These workers were exposed to high levels of acrylamide during synthesis of the compound, and all had elevated levels of hemoglobin adducts (mean 9500 pmol/g of globin). Neurological examinations of the workers showed that several had signs and symptoms of peripheral neuropathy (*11*). The neurotoxicity was found to correlate with the levels of acrylamide adducts. An estimation of the risk to develop peripheral neuropathy during chronic exposure to acrylamide was made on the basis of this study and gave values for NOAEL (no observed adverse effect level) = 2000 pmol/g of globin and LOAEL (lowest observed adverse effect level) = 6000 pmol/g of globin.[2] These figures correspond to steady-state levels of adducts of acrylamide to N-terminal valine in hemoglobin.

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[1] Abbreviations: AAVal, *N*-(2-carbamoylethyl)-L-valine; AAVal-PFPTH, *N*-(2-carbamoylethyl)-L-valine-PFPTH; d_8-AAVal, *N*-(2-carbamoylethyl)-[$^2H_8$]-L-valine; d_7-AAVal-PFPTH, *N*-(2-carbamoylethyl)-[$^2H_7$]-L-valine-PFPTH; ANVal, *N*-(2-cyanoethyl)-L-valine; ANVal-PFPTH, *N*-(2-cyanoethyl)-L-valine-PFPTH; d_8-ANVal, *N*-(2-cyanoethyl)-[$^2H_8$]-L-valine; d_7-ANVal-PFPTH, *N*-(2-cyanoethyl)-[$^2H_7$]-L-valine-PFPTH; EOVal, *N*-(2-hydroxyethyl)valine; EOVal-PFPTH, *N*-(2-hydroxyethyl)-valine-PFPTH; d_4-EOVal, *N*-(2-hydroxy[$^2H_4$]ethyl)valine; d_4-EOVal-PFPTH, *N*-(2-hydroxy[$^2H_4$]ethyl)valine-PFPTH; GC/MS-MS, gas chromatography−tandem mass spectrometry; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; PAGE, polyacrylamide gel electrophoresis; PFPITC, pentafluorophenyl isothiocyanate; PFPTH, (pentafluorophenyl)thiohydantoin; TLC, thin layer chromatography.

[2] Calleman *et al.*, unpublished results.

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Acrylonitrile is another industrial chemical and has been shown to be carcinogenic in experimental animals. Acrylonitrile is also a component of tobacco smoke (10 μg/cigarette, mainstream). Hemoglobin adducts of acrylonitrile have been measured in smokers (*12*) and in the factory workers in China (mean 18 700 pmol/g; *7*); these workers were exposed also to high levels of acrylonitrile because the acrylamide was synthesized from acrylonitrile.

In the present study, hemoglobin adducts of acrylamide and acrylonitrile have been analyzed in laboratory workers and controls with smokers represented in both groups. The purpose of the study was to investigate whether PAGE work leads to exposure and uptake of acrylamide and to study the exposure of acrylonitrile due to tobacco smoking. In order to accomplish this, the method used by Bergmark *et al.* (*7*) has been refined toward higher sensitivity. Adducts of ethylene oxide were analyzed in the same samples and were tested for a correlation with acrylonitrile adducts. Ethylene oxide is a metabolite of ethene which is present in tobacco smoke, and a correlation of ethylene oxide adducts with cigarette smoking has been shown by Törnqvist *et al.* (*13*). Ethene is also produced endogenously, as discussed in the Discussion.

Materials and Methods

Caution: *The following chemicals are hazardous and should be handled carefully: acrylamide, acrylonitrile, ethylene oxide, and PFPITC.*

Chemicals. Acrylamide, acrylonitrile, and L-valine were obtained from Aldrich-Chemie (Steinheim, Germany). [$^2H_8$]-L-Valine was purchased from MSD isotopes (Montreal, Canada). Myoglobin from horse skeletal muscle was from Sigma Chemical Co. (St. Louis, MO). Methanol/HCl, 1.25 M, was prepared by dripping concentrated H_2SO_4 on NaCl and dissolving the HCl gas formed in dry methanol. Pentafluorophenyl isothiocyanate (PFPITC), purum, was obtained from Fluka (Buchs, Switzerland) and was purified on a SEP-PAK silica cartridge (Millipore, Waters Assoc., Milford, MA) prior to use. Formamide (Merck, Darmstadt, Germany) was extracted with pentane before use. Other organic solvents and salts were of analytical grade and were used without further purification.

N-(2-Carbamoylethyl)-L-valine (AAVal) and *N*-(2-carbamoylethyl)-[$^2H_8$]-L-valine (d_8-AAVal) were prepared as described by Bergmark *et al.* (*7*). Ethylene oxide and [$^2H_4$]ethylene oxide alkylated globins (18 and 1.5 μmol/g of globin of *N*-(2-hydroxyethyl)valine (EOVal) and *N*-(2-hydroxy[$^2H_4$]ethyl)valine (d_4-EOVal), respectively) were prepared as described by Farmer *et al.* (*14*).

Synthesis of Acrylonitrile Alkylated Valines. *N*-(2-Cyanoethyl)-L-valine (ANVal) was prepared by dissolving 58.5 mg (0.5 mmol) of L-valine in 1 mL of H_2O and 87.5 μL (0.625 mmol) of triethylamine and adding 65.8 μL (1 mmol) of acrylonitrile. After 5 h at room temperature, the product was precipitated with acetone and recrystallized in H_2O/ethanol. The product (yield 70%) was judged to be pure by thin layer chromatography (TLC) and GC/MS analysis of the (pentafluorophenyl)thiohydantoin (PFPTH). The R_f value on TLC silica plate, eluted with methanol/$CHCl_3$/NH_3 (2:2:1), was 0.72.

ANVal was derivatized with PFPITC according to Mowrer *et al.* (*15*), and the resulting PFPTH was analyzed by GC/MS in the chemical ionization mode. The negative ion spectrum was identical to that published by Osterman-Golkar *et al.* (*12*).

N-(2-Cyanoethyl)-[$^2H_8$]-L-valine (d_8-ANVal) was synthetized as the nondeuterated analogue, as described above, with a yield of 52%. The compound was pure as jugded by TLC and GC/MS analysis of the PFPTH.

Preparation and Characterization of *in Vitro* Alkylated Globins. Human blood was treated *in vitro* with acrylamide or acrylonitrile, and globin was precipitated. Samples of 30 mg of globin were dissolved in 6 M HCl to a concentration of 10 mg/mL. The deuterated reference compounds, d_8-AAVal and d_8-ANVal, serving as internal standards, were added to give 1 μmol/g of globin. The samples were hydrolyzed for 15 h at 120 °C under vacuum. The hydrolysates were evaporated to dryness, dissolved in 1.5 mL of H_2O, and incubated for 1 h at 37 °C. The hydrolysates (750 μL) were evaporated to dryness, 800 μL of 1.25 M methanol/HCl was added, and the samples were incubated in 80 °C for 2 h, and were evaporated again. The samples were derivatized with PFPITC by the modified Edman method for free amino acids (*15, 16*). The samples were dissolved in 200−400 μL of toluene, of which 1 μL was injected for quantitative analysis by GC/MS. The GC/MS analyses were carried out as described for PFPTH derivatives in Bergmark *et al.* (*7*). The methyl esters of AAVal-PFPTH and ANVal-PFPTH had both the same chemical structure, the 2-carbamoylethyl and the 2-cyanoethyl groups being converted to a 2-carboxyethyl group during acid hydrolysis, and were monitored at $m/z = 409$ and 416 $[M - H]^-$ for the nondeuterated and the deuterated compounds, respectively. The quantification was based on the ratio between peak areas of the analytes and the internal standard. Calibration curves for the quantification of adduct levels in the *in vitro* alkylated globins were prepared as follows: samples of control globin (from a nonexposed person) were dissolved in 6 M HCl, and nondeuterated AAVal was added in different amounts to give 0−4 μmol/g of globin. The calibration samples were then analyzed after acid hydrolysis and derivatization as described above. The adduct levels in the globins were 1.4 and 6.5 μmol/g for the acrylamide- and acrylonitrile-treated globins, respectively.

Human Samples. Blood samples of 10−20 mL were collected in 1993 from 40 persons working at the Stockholm University. The blood was drawn in heparinized vacutainer tubes. Twenty-two persons were working with PAGE. The 18 controls were personnel and students in other laboratories or office workers. Smokers were represented in both groups (total of 17). The subjects were asked to fill out a questionnaire form about their work and smoking habits. The ages varied between 21 and 55 years, and 58% were women. The PAGE workers had used PAGE for a period of time between 3 months and 9 years.

Analysis of Hemoglobin Adducts with the Modified Edman Degradation Procedure. Globin was isolated from whole blood as described previously (*17*). Samples of 50 mg of globin were derivatized according to Törnqvist *et al.* (*18*). The amount of PFPITC reagent was 10 μL. For ethylene oxide adducts, [$^2H_4$]ethylene oxide alkylated globin was used as an internal standard and added to give a concentration of 150 pmol/g of globin. Toluene solutions of d_7-AAVal-PFPTH (40 pmol/mL) and d_7-ANVal-PFPTH (80 pmol/mL) were added (50 μL of each) just before the ether extractions to serve as internal standards for acrylamide and acrylonitrile adducts. The concentration of these solutions was determined spectroscopically assuming an ϵ value of 18 000 M^{-1} cm^{-1} (the ϵ value of similar PFPTH's varies between 16 000 and 20 000 M^{-1} cm^{-1}; *19*). The derivatized samples were dissolved in 50 μL of toluene, and 2 μL was injected for the quantitative analysis by GC/MS-MS.

Gas Chromatography−Tandem Mass Spectrometry (GC/MS-MS) Analysis. The analyses were carried out using a Varian 3400 gas chromatograph linked to a Finnigan TSQ700 tandem mass spectrometer. The operating conditions for the gas chromatograph were as follows: helium carrier gas at constant pressure of 8 psi; the samples were injected with an septum equipped programmable on-column injector (Varian) programmed from 60 °C (injection temperature) to 320 °C, 186 °C/min; the GC oven was programmed from 1 min at 100 °C, 20 °C/min to 240 °C, 10 °C/min to 320 °C for 8 min. A 30 m DB-5MS (0.32 mm i.d., 1 μm phase thickness) fused silica capillary column (J&W Scientific, Rancho Cordova, CA) coupled to a precolumn (2.5 m, 0.53 mm i.d.) was used. The mass spectrometer was operated in the negative ion chemical ioniza-

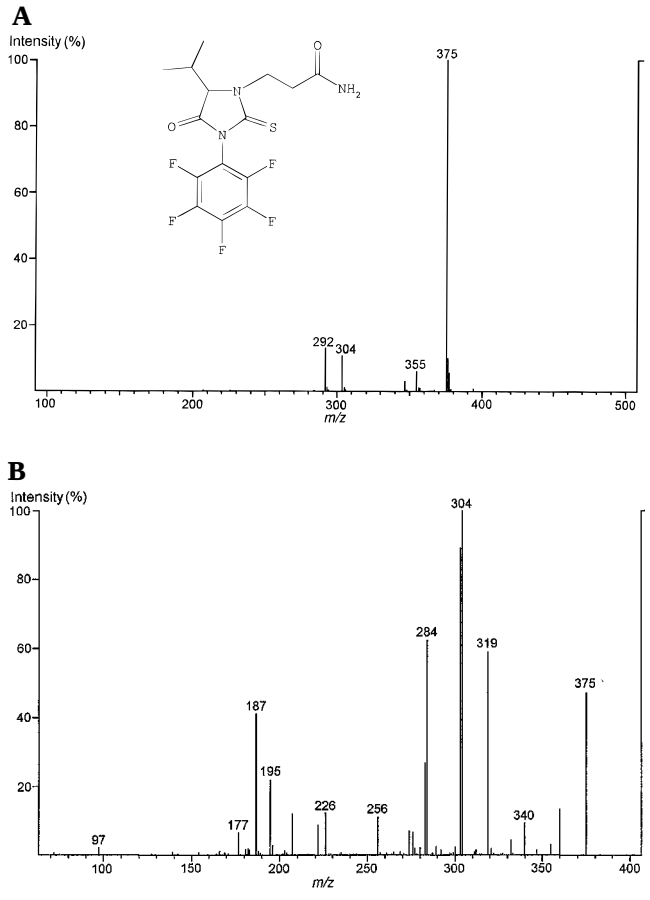

Figure 1. (A) Negative ion chemical ionization mass spectrum of the PFPTH derivative of AAVal. (B) Collision induced daughter ion spectrum of m/z 375 $[M - HF]^-$ from AAVal-PFPTH.

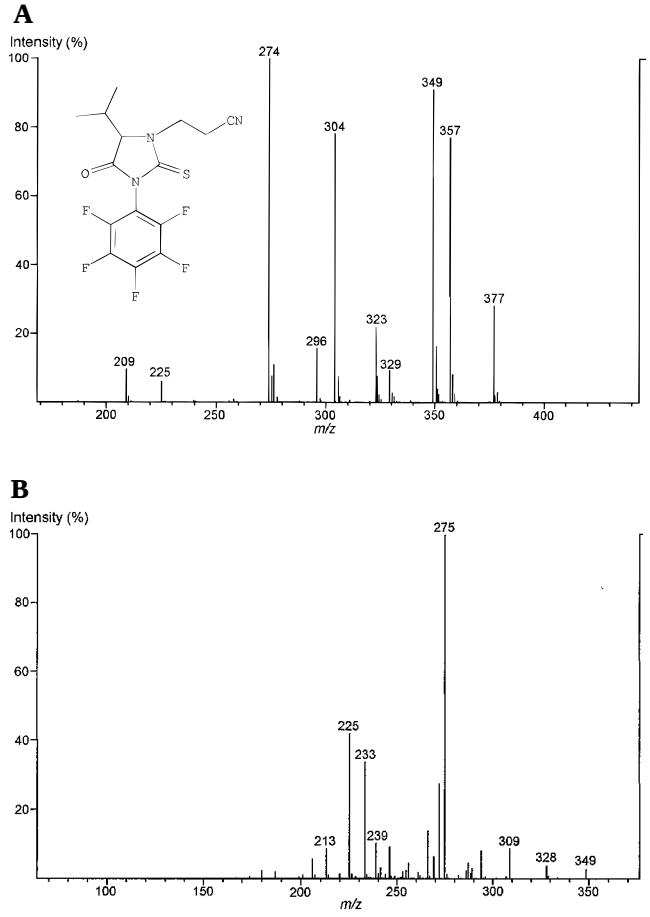

Figure 2. (A) Negative ion chemical ionization mass spectrum of the PFPTH derivative of ANVal. (B) Collision induced daughter ion spectrum of m/z 349 $[M - CO]^-$ from ANVal-PFPTH.

tion mode with methane as reagent gas. Collision induced daughter ions were produced by using argon as collision gas (pressure 1 mTorr). Chemical ionization full spectra and daughter ion spectra of AAVal-PFPTH and ANVal-PFPTH are shown in Figures 1 and 2, respectively. Selected ion monitoring for specific daughter ions of each analyte following fragmentation of specific parent ions was used. The daughter ions monitored were as follows (parent ion given in parenthesis): for EOVal-PFPTH m/z 318 (m/z 348 $[M - HF]^-$); for d_4-EOVal-PFPTH m/z 320 (m/z 352 $[M - HF]^-$); for AAVal-PFPTH m/z 303, 304, and 319 (m/z 375 $[M - HF]^-$); for d_7-AAVal-PFPTH m/z 310 and 326 (m/z 382 $[M - HF]^-$); for ANVal-PFPTH m/z 275 (m/z 349 $[M - CO]^-$); and for d_7-ANVal-PFPTH m/z 282 (m/z 356 $[M - CO]^-$). The collision energy was 10 eV for the parent ions of EOVal-PFPTH and ANVal-PFPTH's and 15 eV for the parent ions of AAVal-PFPTH's. The retention times were 11:15, 11:20, and 13:30 min for ANVal-PFPTH, EOVal-PFPTH, and AAVal-PFPTH, respectively. The quantification was based on the ratio of peak areas of the analyte and their respective deuterated internal standard. A chromatogram from a GC/MS-MS analysis of a sample from a PAGE worker is shown in Figure 3.

Calibration. Calibration curves for the human samples were prepared by addition of different amounts of *in vitro* alkylated (with acrylamide, acrylonitrile, or ethylene oxide) globins to 50 mg myoglobin samples. The adduct levels in the calibration samples corresponded to 50−300 pmol/g of globin. Internal standards were added as described above for the human globin samples. The calibration samples were derivatized with PFPITC and analyzed with GC/MS-MS as described above. The calibration curves were linear over the range of adduct levels studied (Figure 4).

Results

Adducts to N-terminal valine in hemoglobin of acrylamide, acrylonitrile, and ethylene oxide were determined simultaneously by means of the modified Edman degradation procedure. In a previous study (*7*) the internal standard used was [$^2H_4$]-ethylene oxide alkylated globin and the samples were quantified with GC/MS. The detection limit and precision were not sufficient for analysis of globin samples with low or background adduct levels. In this study, the sensitivity was increased by using deuterated internal standards for each analyte, and by using tandem mass spectrometry for detection.

The internal standards d_8-AAVal and d_8-ANVal were synthesized and derivatized with PFPITC. GC/MS analysis showed that the PFPTH's formed had only 7 deuteriums. One deuterium, at the α-carbon in valine, was lost in the derivatization procedure. The standards d_7-AAVal-PFPTH and d_7-ANVal-PFPTH, dissolved in toluene, were added to the globin samples. A drawback of adding the internal standard as a PFPTH is that these compounds do not correct for possible variations in yield during derivatization. However, the calibrations curves were linear (Figure 4 for AAVal), and experience in our laboratory from several studies based on the modified Edman method shows that the method is very reproducible. To confirm the quantification, it was possible to calculate the acrylamide and acrylonitrile adduct levels also by using d_4-EOVal as an internal standard, since every sample contained all three standards. This gave similar values (not shown) as when using d_7-AAVal-PFPTH and d_7-ANVal-PFPTH, respectively.

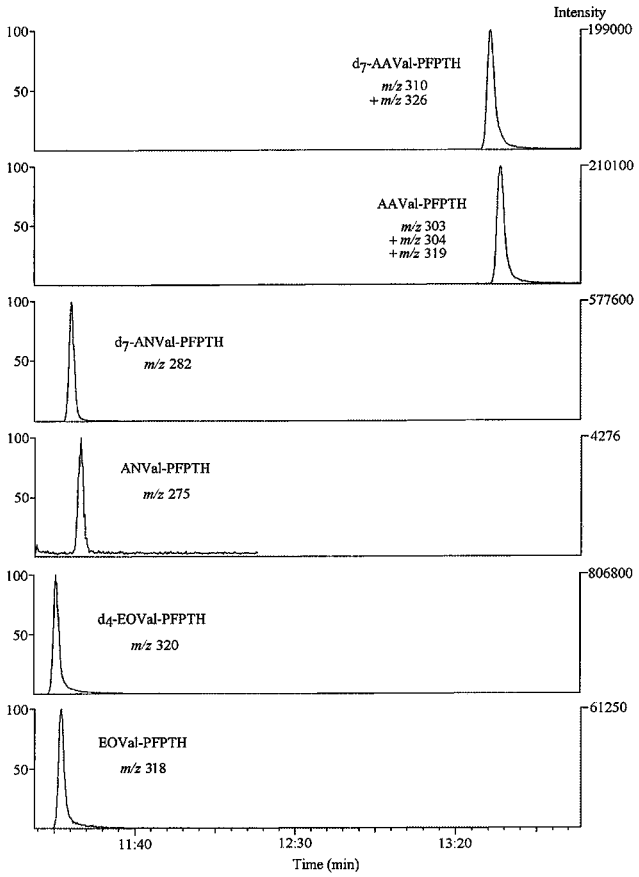

Figure 3. Typical ion chromatogram from a GC/MS-MS analysis of a globin from a PAGE worker. Selected daughter ions of each analyte, following fragmentation of specific parent ions, were monitored. The ion chromatograms represent the sum of the signals from the daughter ions monitored for each analyte.

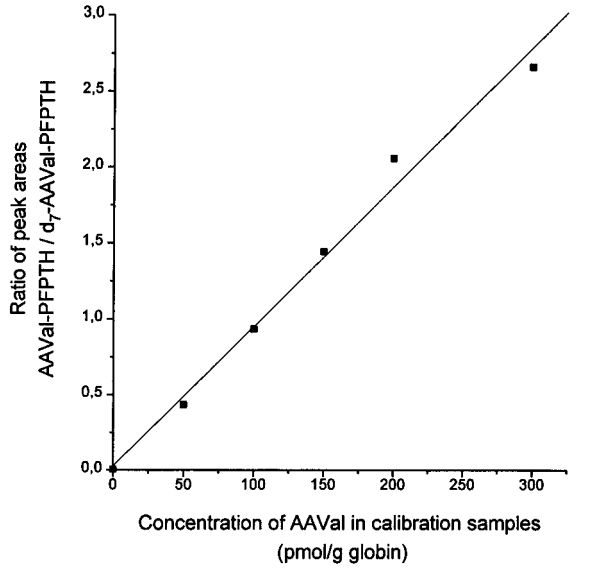

Figure 4. Calibration curve for acrylamide adducts. A globin alkylated *in vitro* with acrylamide, with a known adduct level, was added to calibration samples to give different concentrations of AAVal. The samples were analyzed by GC/MS-MS, and the ratios between peak areas of the daughter ions for AAVal-PFPTH and for d_7-AAVal-PFPTH were measured.

The monitoring of daughter ions in the GC/MS-MS gave a better sensitivity than GC/MS, mainly due to reduction of disturbing peaks (Figure 3). The detection limits for acrylamide adducts and acrylonitrile adducts were estimated as 4 and 2 pmol/g of globin, respectively.

In vitro-alkylated globins were used for the preparation of calibration curves. Determination of adduct levels of these globins was made on hydrolysates. In the acid hydrolysis, the adduct of acrylamide, 2-carbamoylethyl, and the adduct of acrylonitrile, 2-cyanoethyl, were converted to 2-carboxyethyl adducts. Prior to derivatization with PFPITC, the hydrolysates were converted to methyl esters in order for the derivatives to be extractable and amenable to analysis by GC/MS.

Data on hemoglobin adduct levels in the human samples are presented in Table 1 together with data on smoking habits and PAGE work. In this group of PAGE workers, all but one (AS7) ordered the acrylamide as a solid and prepared the water solutions themselves. Most wore gloves, lab coats, and masks during weighing which was performed either on the bench or in the hood. During the preparation of gels, all wore gloves and most wore lab coats. Regarding spills of acrylamide powder, the answers varied between never, sometimes, and every time, whereas almost everybody said that they spilled often during preparation of the gels.

Linear regression was made on different variables, and the correlation coefficients are shown in Table 2. There was no correlation between adduct levels of acrylamide and any of the indicators of use of acrylamide in the PAGE workers. Adduct levels of acrylamide (Figure 5A), acrylonitrile (Figure 5B), and ethylene oxide showed a high statistical significance for the correlation with cigarette smoking. There was a statistically significant increase (T-test, one tail, $p = 0.01$) in the acrylamide adduct level in the nonsmoking PAGE workers (mean 54 pmol/g) as compared to nonsmoking controls (mean 31 pmol/g) (Figure 6).

There was a background of acrylamide adducts in nonsmoking controls (mean 31 pmol/g). There was no, or very low, background of acrylonitrile (<2 pmol/g). However, as seen in Figure 3, the acrylonitrile adduct in nonsmokers is detectable, but the peaks are too small to be correctly quantified. The adduct levels of acrylonitrile in smokers were comparable to previous data (*12*). The adduct levels of ethylene oxide were also comparable to published data and have been shown to correlate with cigarette smoking (*13*).

The *in vivo* doses[3] corresponding to the adduct levels of acrylamide were calculated (cf. ref 7) and are presented in Table 3 together with an estimation of the uptake of acrylamide in $\mu g \cdot kg^{-1} \cdot day^{-1}$. The elimination rate of acrylamide in humans has been estimated by Calleman *et al.* (unpublished results and ref 21) to be about a fifth of the rate in the rat (0.5 h^{-1}; *11*). Therefore, the value 0.1 h^{-1} was used for calculation of the uptake.

Discussion and Conclusion

Acrylamide. PAGE workers are potentially exposed to the acrylamide monomer during weighing of the compound, preparation of solutions, and the preparation of polyacrylamide gels. Air concentrations of acrylamide during PAGE work have been measured (*22*), and levels up to 1.5 mg/m^3 were monitored during weighing of acrylamide powder and preparation of monomer solution. Uptake of the monomer, probably through the skin and by inhalation, is actually taking place, which has been

[3] *In vivo* dose (D) is defined as the time integral of the concentration of an electrophilic compound (RX): $D = \int[RX]\,dt$ (*20*). *In vivo* dose is equal to the area under the curve, AUC.

Table 1. Hemoglobin Adducts of Acrylamide, Acrylonitrile, and Ethylene Oxide in Human Samples

		PAGE work		hemoglobin adducts (pmol/g)			
person no.[a]	smoking (cig/day)	gels/week	weighing of AA (times/year)	acrylamide	acrylonitrile	ethylene oxide	mean
C1				27	<2	22	AAVal = 31
C2				49	<2	14	ANVal = <2
C3				35	<2	11	EOVal = 17
C4				30	<2	13	
C5				29	<2	20	
C6				27	<2	28	
C7				30	<2	11	
C8				24	<2	15	
CS1	5.5			67	36	29	AAVal = 116
CS2	6			27	25	26	ANVal = 106
CS3	8			130	63	78	EOVal = 126
CS4	10			136	178	176	
CS5	10			124	107	194	
CS6	10			97	37	53	
CS7	15			148	113	182	
CS8	20			137	170	152	
CS9	20			148	163	172	
CS10	20			146	172	196	
A1		0.25	6	82	<2	16	AAVal = 54
A2		0.375	4	47	<2	21	ANVal = <2
A3		0.75	5	39	<2	13	EOVal = 18
A4		1	24	29	<2	16	
A5		1	4	69	<2	17	
A6[b]		1	4	24	<2	18	
A7		1	0	54	<2	15	
A8		1.5	3.5	52	<2	19	
A9		2	2	40	<2	18	
A10		2.5	1.5	34	<2	20	
A11		2.5	1	71	<2	17	
A12		3	2	55	<2	17	
A13		5	24	116	<2	17	
A14		5	12	43	<2	28	
A15		6	24	58	<2	16	
AS1	party[c]	5	2	43	9	24	AAVal = 71
AS2	party	1.5	18	65	15	30	ANVal = 24
AS3	party	2.5	6	83	2	15	EOVal = 34
AS4	1	2	24	52	8	22	
AS5	1	1	0	85	23	45	
AS6	5	1	0	85	41	42	
AS7	12.5	1	0	80	72	59	

[a] Group labels: C = control, nonsmoker; CS = control, smoker, A = PAGE worker, nonsmoker; AS = PAGE worker, smoker. [b] This person uses a mask during gel-making and claims to never spill. [c] "Party smoker", smoking occasionally.

Table 2. Correlation Coefficients (Simple Linear Regression) and Levels of Statistical Significance for the Relationships between Different Variables

x-variable	*y*-variable	groups	*n*	corr coeff	*p* value
AAVal	gels/week	A	15	0.33	0.22
AAVal	weighings/year	A	15	0.27	0.32
AAVal	time working with PAGE (months)	A	15	−0.18	0.53
AAVal	cig/day	C + CS	18	0.91	<0.001
AAVal	ANVal[a]	C + CS	18	0.92	<0.001
AAVal	EOVal	C + CS	18	0.92	<0.001
ANVal[a]	cig/day	all	37[b]	0.94	<0.001
ANVal[a]	EOVal	all	40	0.95	<0.001
EOVal	cig/day	all	37[b]	0.89	<0.001

[a] For the linear regression, values of <2 pmol/g have been set to 1 pmol/g. [b] "Party smokers" exluded.

shown in this study by quantification of the adduct of acrylamide to the N-terminal valine in hemoglobin. The increased adduct level in the PAGE workers corresponds to an uptake of acrylamide from about 3 cigarettes/day. This was calculated from the linear regression shown in Figure 5A. The average adduct levels of the PAGE workers (54 pmol/g) were well below the NOAEL (2000 pmol/g) for neurotoxic damage to the peripheral nervous system, and it is therefore reasonable to believe that no risk is present for this type of effect. However, it needs to be investigated whether the exposure to acrylamide in PAGE workers represents a non-neglible risk for genotoxic and reproductive effects. From hemoglobin adduct levels, it is possible to calculate *in vivo* doses, which can be used to estimate the uptake of acrylamide, as shown in Table 3. Such data may be useful for risk assessments.

Acrylamide is metabolized to an epoxide, glycidamide (*23*). This metabolite has been suggested to be responsible for the carcinogenic and genotoxic effects of acrylamide. A DNA adduct, *N*-7-(2-carbamoyl-2-hydroxyethyl)-guanine, has been identified in mice and rats administred acrylamide (*24*). Hemoglobin adducts of glycidamide have been measured in workers heavily exposed to acrylamide (*7*). The *in vivo* dose of glycidamide was estimated to be 30% of the *in vivo* dose of acrylamide. The method used for analysis of glycidamide adducts (determination after total hydrolysis) was not sensitive enough to determine a possible background level. For further studies of acrylamide exposure and for cancer risk estimation, a method should be developed for the determination of glycidamide adducts in humans with low acrylamide exposure.

From this study, it is not possible to draw any conclusion as to which step in the working procedure is most

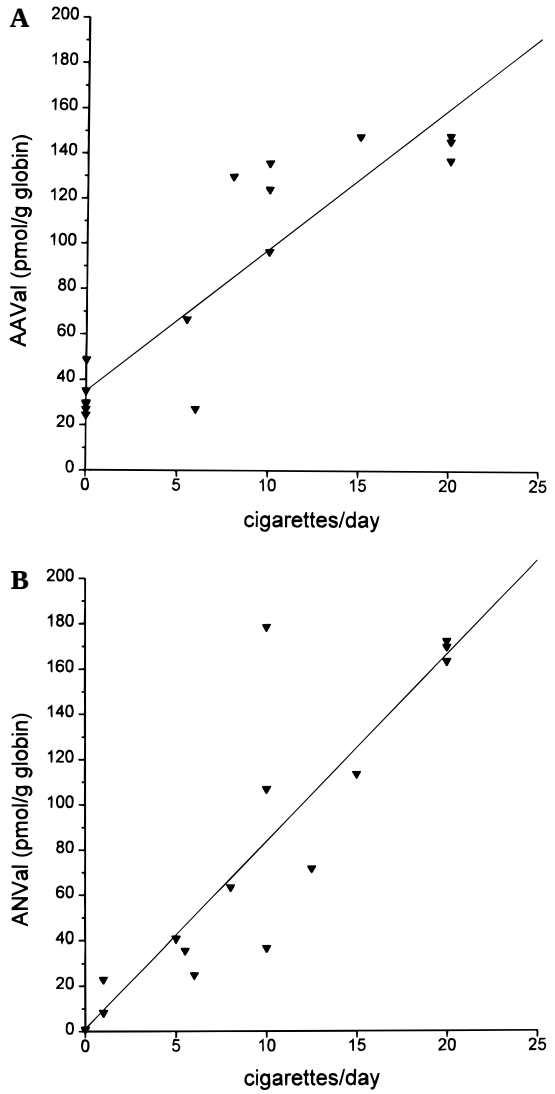

Figure 5. (A) Correlation between acrylamide adducts (AAVal; pmol/g of globin) and cigarettes/day in smokers and controls (n = 18, PAGE workers excluded). Linear regression, r = 0.91. (B) Correlation between acrylonitrile adducts (ANVal; pmol/g of globin) and cigarettes/day in smokers and controls (n = 37). Linear regression, r = 0.94.

critical for exposure. It is possible that the exposure to acrylamide would be diminished by ordering acrylamide as a water solution in order to reduce the exposure during weighing of the chemical and preparation of solutions. Commercially prepared polyacrylamide gel plates are also available for some types of applications, the use of which would further reduce the exposure.

The finding of acrylamide adducts in smokers (mean 116 pmol/g) suggests that acrylamide is a component of tobacco smoke. This is not an unexpected finding due to the large number of chemicals present in tobacco smoke; however, acrylamide was only mentioned in one article (*4*). In addition, hemoglobin adducts of several other compounds in tobacco smoke have been measured in previous studies (*9*).

It was unexpected to find such high background of acrylamide adducts in the nonsmoking controls (mean 31 pmol/g). The origin of this background is not known. The estimated uptake from drinking water and environmental tobacco smoke is too low to account for these levels. In addition, acrylamide is not known as an air pollutant. Other possible origins have to be investigated, *e.g.*, food, beverages, or endogenous metabolites.

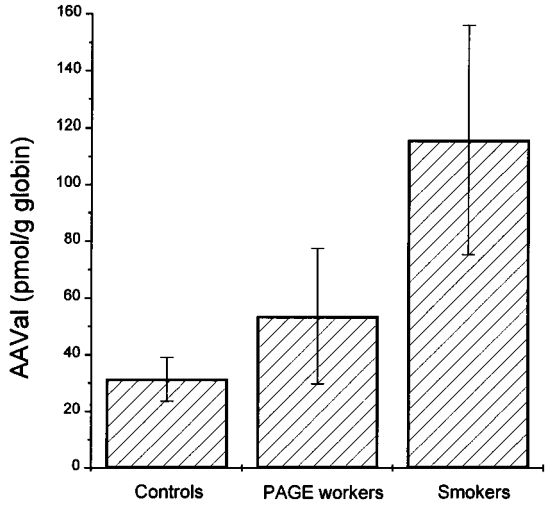

Figure 6. Means (±SD) for acrylamide adducts (AAVal) in controls (group C), PAGE workers (group A), and smokers (group CS).

Table 3. Daily *in Vivo* Dose and Daily Uptake of Acrylamide

group	AAVal (pmol/g of globin)	*in vivo* dose[a] of acrylamide [(μM·h)/day]	estimated uptake[b] (μg·kg^{-1}·day^{-1})
controls (C)	31	0.1174	0.8
PAGE (A)	54	0.2045	1.4
smokers (CS)	116	0.4394	3.1

[a] The *in vivo* dose (D; see also footnote 3) is calculated from the adduct level (*7*), as shown below, where k is the rate constant for reaction between valine in hemoglobin and acrylamide:

$$D\ (\mu\text{M·h}) = \frac{[\text{steady-state adduct level (pmol/g of globin)}]}{(60\ \text{days}) \times [(k)(4.4 \times 10^{-6}\ \text{L·(g of globin)}^{-1}\text{·h}^{-1})]}$$

[b] Uptake calculated from the *in vivo* dose:

$$\text{uptake}\ (\mu\text{g·kg}^{-1}\text{·day}^{-1}) = [D\ (\mu\text{M·h})] \times [\text{elimination rate}\ (0.1\ \text{h}^{-1})] \times (71\ \text{g/mol}) \times (1\ \text{L/kg})$$

Acrylonitrile. In nonsmoking controls, there was none or a very low background level, <2 pmol/g of globin, which was the detection limit of the method. The acrylonitrile adduct seems to be very specific for tobacco smoking and correlates with cigarettes/day, ethylene oxide adducts, and acrylamide adducts (in smokers and controls). Acrylonitrile adducts could be a better indicator of tobacco smoking than ethylene oxide adducts since the latter show a background level of endogenous origin (*25*). It might also be possible to study exposure to passive smoking by measuring acrylonitrile adducts to hemoglobin.

In summary, the monitoring of adducts of acrylamide to hemoglobin in humans has shown the following: PAGE work does lead to an uptake of acrylamide in this group of workers; cigarette smoke contains acrylamide and is an important source to acrylamide exposure; persons without known exposure of acrylamide have a background level of adducts. Acrylamide may be a carcinogenic risk to humans; therefore, it is important to reduce exposure and to investigate the sources of the background.

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