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Determination of acrylamide in rat serum and sciatic nerve by gas chromatography–electron-capture detection

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ABSTRACT

A modified method for the derivatization and determination of acrylamide as 2-bromopropenamide by gas chromatography–electron-capture detection was developed and applied to serum and sciatic nerve from rats. The method was accurate and precise over the calibration range 2.24–7.47 μg/ml in serum diluted 1:125 and 4–122 μg/g in sciatic nerve homogenate (5 mg/ml). Limits of detection were estimated to be 1200 ng/ml in undiluted serum and 3 μg/g in intact sciatic nerve. The use of less dilute samples to allow for lower limits of detection appears feasible. The time-course of acrylamide in serum and sciatic nerve was studied after acute dosing and indicated elimination half-lives of 1.8 and 2.0 h for serum and sciatic nerve, respectively. A dose–effect relationship was established for each matrix after acute dosing and the measured acrylamide concentrations in serum (μg/ml) were approximately the same as in sciatic nerve (μg/g).

INTRODUCTION

Acrylamide is a commercially important monomer with a wide variety of industrial applications [1]. Polyacrylamides, produced from the acrylamide monomer, are used as flocculent agents in the treatment of waste water and drinking water, stabilizers in paper products, and until recently in pipe grouting materials. Annual production volume for acrylamide in 1985 was estimated at $6.35 \cdot 10^7$ kg, with projected growth at 4% per year through 1989 [2]. There is also a large human population with potential for inhalation and dermal exposure; over 10 000 people are exposed in occupational settings [3]. Additionally, estimates indicate that up to 100 000 workers may be exposed (inhalation, dermal) to acrylamide via laboratory chromatography gels, and between 4 and 30 million people may be exposed via drinking water [2]. The neurotoxicity of acrylamide has been widely established, although questions remain concerning the mechanism. Case reports in humans [4,5] as well as extensive animal research [1,6,7] indicate a degenerative neuropathy of large sensory and motor fibers.

The current exposure guideline for acrylamide, the Reference Dose (RfD), is based on pathological changes in the peripheral nervous system of the rat following a 90-day exposure [8,9]. The RfD approach to non-cancer risk assessment utilizes a number of uncertainty factors [10]. One

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uncertainty factor used in RfD calculation for acrylamide was for the extrapolation from less than lifetime exposures. An ongoing multidisciplinary research program has been investigating the role of dose rate in the neurotoxic effects of acrylamide [11]. One part of this research was to define the relationship between the administered and/or target dose of acrylamide and the neurotoxic outcome. Establishing the relationship was necessary to address the question of whether the slow onset of effect in repeated dosing studies was due to an accumulation of acrylamide in target tissues or to an accumulation of damage or both. Although recent work with hemoglobin adducts has provided insight into the cumulative dose that may be useful in exposure assessment [12], these methods measure a "bound" hemoglobin–acrylamide adduct and may not estimate the available, neurotoxic concentrations in peripheral nerves.

Acrylamide has been analyzed in aqueous media using both high-performance liquid chromatography (HPLC) [13] and gas chromatography (GC) [14,15]. A method for the analysis of acrylamide in water was described by Hashimoto [14] in which a brominated acrylamide derivative (2,3-dibromopropionamide) was determined by GC with electron-capture detection (ECD). This method was later adapted by Poole *et al.* [15] for the analysis of acrylamide in sciatic nerve; a packed GC column was used in conjunction with ECD. It was also observed that the 2,3-dibromopropionamide could undergo substantial decomposition at injector/column/detector temperatures exceeding 180°C and that this would increase quantitative uncertainties. Andrawes *et al.* [16] confirmed this decomposition and solved the problem by conversion of the 2,3-dibromopropionamide to 2-bromopropenamide using triethylamine prior to GC analysis. However, the method as modified by Andrawes was not used or validated for acrylamide in tissue. It seemed that the method of Poole modified to include Andrawes' conversion to 2-bromopropenamide would be a good approach to the analysis of acrylamide in sciatic nerve or serum. The chemical reaction sequence of the derivatization is shown in Fig. 1.

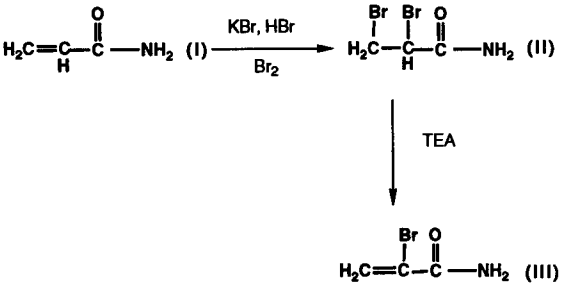

Fig. 1. Reaction sequence showing conversion of acrylamide (I) to 2,3-dibromopropionamide (II) in the presence of KBr, HBr, and Br_2 and finally to 2-bromopropenamide (III) after reaction with triethylamine (TEA).

A number of questions remained to be answered prior to the routine use in biological matrices of Poole's method modified to include Andrawes' conversion. An important step in the validation of the method is to verify the identity of the apparent derivative, 2-bromopropenamide, in the derivatized extract. Another issue to be addressed is the stability of the samples after removal from the animal and before derivatization in addition to the stability of the extract once the acrylamide derivative has been formed. It is also important to understand the potential impact of tissue concentration on the derivatization/analysis scheme. Increased concentrations of the biological matrix might impact both the yield of the derivatization step and the limit of detection (LOD). For example, trace levels of interferents might become significant with more concentrated samples. This paper describes the validation of this modified method and the application of the validated method to the study of acrylamide kinetics in serum and sciatic nerve from rats following acute dosing.

EXPERIMENTAL

Animals

Male Long Evans hooded rats (Charles River, Kingston, NJ, USA) were obtained at approximately 60 days of age, and were housed singly in standard plastic hanging cages (0.24 × 0.20 × 0.45 m). All animals were given a ten-day acclimation period and were maintained on a 12–12 h

photoperiod (light, 06:00; dark, 18:00). Food (Purina Lab Chow, Barnes Supply, Durham, NC, USA) and water were provided *ad libitum*. Temperature was maintained at 21.0 ± 2°C and relative humidity at 50 ± 10%. All experimental protocols were approved by the Animal Care Committee of the Health Effects Research Laboratory, US EPA.

Dosing and tissue preparation

Acrylamide (electrophoresis grade, 99.9%, Bio-Rad, Richmond, CA, USA) was administered i.p. in saline (1.0 ml/kg). Animals were killed by decapitation. Trunk blood was collected, allowed to clot, and serum was separated by centrifugation at 12 500 *g* for 5 min. The serum was then diluted 1:125 (v/v) with deionized water. The mid-thigh region of the sciatic nerve was dissected out, weighed, and diluted to 5 mg wet weight per ml deionized water. The samples were then homogenized on ice using a Polytron (setting 6, two 1-min bursts; Brinkmann Instruments, Westbury, NY, USA). Samples were frozen overnight at −80°C.

Derivatization and analysis of samples

The method of Poole *et al.* [15] was modified and used as described below. To a 250-ml glass stoppered flask, 50 ml of Milli-Q (Millipore, Bedford, MA, USA) water, 7.5 g of ACS reagent grade KBr (Aldrich, Milwaukee, WI, USA), and 1.0 ml of HBr (47–49%, Aldrich) were added. Prior to use, the KBr was heated to 450°C in a tube furnace and purged with cryogenically cleaned nitrogen for 2 h. The flask was wrapped in aluminum foil to exclude light and 0.5 ml of the sample (sciatic nerve 5 mg/ml in water, or serum diluted 1:125 with water) was added along with 2.5 ml of saturated bromine water. The latter was prepared by mixing 60 ml of Milli-Q water with excess molecular bromine (Aldrich, 99.99 + %) and letting the mixture equilibrate, protected from light, at 4°C. The reaction was then allowed to proceed in darkness at 0°C for 2 h. After this time, excess bromine was decomposed by dropwise addition of 1.0 *M* $Na_2S_2O_3$ (Fisher Scientific, Pittsburgh, PA, USA) solution until the solution was colorless. To facilitate extraction of 2,3-dibromopropionamide (Fig. 1, structure II) from the aqueous medium, 15 g of Na_2SO_4 (Baker-analyzed, J. T. Baker, Phillipsburg, NJ, USA) were added to the reaction mixture with vigorous stirring and the resulting solution was transferred to a 125-ml separatory funnel. The solution was then extracted with 15 ml of purified ethyl acetate (Fisher HPLC grade) which had been passed through activated alumina (Woelm N, Super I, Universal Scientific, Atlanta, GA, USA). The ethyl acetate layer was removed and the aqueous solution was extracted again with 10 ml of ethyl acetate. The organic extracts were combined. The organic phase was dried using approximately 1 g of anhydrous Na_2SO_4. The dried extract was transferred to a small vial and the Na_2SO_4 was rinsed twice with 1 ml of ethyl acetate. The ethyl acetate rinses were added to the extract, 1.00 ml of an ethyl acetate solution of 530 ng/ml *p*-dibromobenzene (the internal standard) was added, and the volume was reduced to 1 ml by warming on a 50°C water bath and a gentle stream of cryogenically cleaned nitrogen. The concentrated extract was transferred to a 1-dram amber vial, and 50 μl of triethylamine (Baker-analyzed, J. T. Baker) were added to convert the dibrominated compound to 2-bromopropenamide (Fig. 1, structure III).

Separation was accomplished on a DB-23, 30 m × 0.25 mm I.D. fused-silica capillary column with a 0.25-μm film (J&W Scientific, Folsom, CA, USA), installed in a Varian 3700 gas chromatograph (Varian Instruments, Walnut Creek, CA, USA) fitted with an electron-capture detector. A 1 m × 0.25 mm I.D. deactivated fused-silica precolumn (retention gap) was installed before the analytical column. The temperature was programmed from 75°C (hold 1 min) to 155°C at 2°C/min with a 5-min hold at the final temperature. Splitless–split injection (30 s) was used for the introduction of 1.0 μl of the extract. The injector temperature was 200°C and the electron-capture detector temperature was 250°C. An ethyl acetate blank was also prepared each day and analyzed to be certain that there were no interferences from the solvent. This blank was pre-

pared by reducing a 25-ml volume of solvent to 1 ml as for the samples. Retention times and areas of the chromatographic peaks were collected using a Nelson Analytical chromatographic data system (Model 4400, Version 7.2, PE-Nelson, Cupertino, CA, USA). A solution containing known amounts of the internal standard and 2-bromopropenamide was analyzed daily to verify retention times and ECD response for 2-bromopropenamide relative to the internal standard. For each GC analysis, the peak area measured for 2-bromopropenamide was divided by the peak area measured for *p*-dibromobenzene to normalize the samples for slight differences in final volume.

Structural verification

Verification of the structure of the acrylamide derivative was accomplished using both ^{1}H nuclear magnetic resonance (NMR) and GC in conjunction with ion trap mass spectrometry (GC–ITMS). Based on the work of Andrawes *et al.* [16], 2-bromopropenamide was synthesized for these structural analyses as follows. A 150-μl volume of triethylamine (TEA, 99%, Baker) was added to 53.5 mg of 2,3-dibromopropionamide (CTC Organics, Atlanta, GA, USA) dissolved in 2 ml of ethyl acetate. The resulting white precipitate, trietylammonium bromide, was removed by filtration of the solution through a 0.45-μm filter (Acro LC-13, Gelman Sciences, Ann Arbor, MI, USA). The solvent was then evaporated at 50°C with the aid of a gentle stream of cryogenically cleaned nitrogen. The vial containing the residue was placed into a vacuum oven at room temperature for a total of 9 min after which it was weighed to determine the mass of product formed. An aliquot of the product (18.5 mg) was dissolved in 0.5 ml of deuterochloroform for analysis by ^{1}H NMR (Bruker WM-250, 250 MHz, Bruker Instruments, Billerica, MA, USA) to verify conversion to 2-bromopropenamide.

The chemical identity and chromatographic retention time of the acrylamide derivative isolated from tissue samples containing acrylamide were verified as follows. Aliquots of sciatic nerve homogenate (5 mg/ml) and serum (diluted 1:125) from rats were spiked with acrylamide to provide a concentration of approximately 680 ng/ml in each matrix. Each of the samples was derivatized as described above. Analysis of each extract and the standard solution of 2-bromopropenamide was performed via GC–ITMS. The ITMS system (Finnigan-MAT, San Jose, CA, USA) was operated in the electron-impact (EI) mode using a 6-ms ionization time at 70 eV followed by a scan from mass 45 to 290 in 1 s. The pressure in the instrument during analysis was $2.7 \cdot 10^{-4}$ kg/m^2 ($2 \cdot 10^{-5}$ Torr), and the analyzer and transfer line temperatures were 110 and 150°C, respectively.

Calibration curve

Calibration curves for acrylamide were prepared by spiking 5-ml aliquots of each matrix (serum or sciatic nerve) with 0.100 ml of aqueous acrylamide standards to provide for five mixtures with concentrations ranging from 1.8 to 600 ng/ml in the dilute samples. The aqueous acrylamide standards were freshly prepared and used within 24 h. After addition of the standard to the matrix, the samples were allowed to equilibrate for 0.5 h before 0.5-ml aliquots were taken through the analytical procedure in duplicate. The response relative to the internal standard was determined at each level and these values were plotted as a function of concentration in intact sciatic nerve and undiluted serum using SigmaPlot (Version 4.1, Jandel Scientific, Corte Madera, CA, USA). The data were also fit to both a first- and second-order polynomial in SigmaPlot to obtain calibration equations.

Storage of samples prior to derivatization

Rats ($n = 3$ for each dose level) were dosed with 0, 5, 20, or 80 mg/kg acrylamide, and serum and sciatic nerve samples were collected. The samples were divided into three aliquots. The first aliquot was subjected to derivatization and analysis the following day. The other two aliquots were stored at −80°C for 7 and 21 days, respectively, before derivatization and analysis. Changes in the relative response as a function of storage time were studied.

Stability of derivatized extract

This set of experiments was conducted to study derivatized samples for changes in the relative response (2-bromopropenamide/internal standard) as a function of time of storage at room temperature. Rats ($n = 3$) were dosed with acrylamide at a level of 20 mg/kg. Aliquots (0.5 ml) of serum (diluted with water 1:125) and sciatic nerve (5 mg/ml) samples were derivatized in triplicate using the method described above. Each extract was then analyzed by GC–ECD the next day (day 1) and then reanalyzed on days 2, 3, 6, and 8. The average relative response measured for each sample was then plotted as a function of storage time to assess changes in the ECD response.

Effect of tissue or serum concentration

The impact of tissue or serum concentration on the measured acrylamide concentration was studied by adding the same mass of acrylamide to serum and sciatic nerve homogenates prepared at different concentrations. That is, all matrices contained the same concentration of acrylamide with different concentrations of tissue or serum. Serum from non-dosed rats was diluted (v/v) with water at 1:60, 1:125, 1:250, and 1:1000, and sciatic nerve tissue homogenates were prepared at concentrations of 1, 5, 10, and 20 mg/ml. Aliquots of 0.5 ml were derivatized and analyzed in triplicate using the method described above. Each sample was then spiked with acrylamide to give a concentration of 120 ng/ml in each sample matrix and dilution. Relative response was studied as a function of tissue concentration.

Time-course study

To determine the kinetics of acrylamide in the rat, 21 animals were dosed (saline *versus* 75 mg/kg) and sacrificed at 0 (saline), 15, 30, and 60 min, and 2, 4, 8, and 24 h post-administration. Three animals were used for each time point; samples were collected and analyzed as described above. From the time course of the acrylamide concentrations, the time of maximum concentration in each matrix was determined. This collection time would then be used in the dose–response study (see below). The concentrations measured in each of the three samples at each time point were averaged and the elimination half-life was determined in each matrix using Rstrip exponential stripping and parameter estimation software (Version 5.0, MicroMath Scientific Software, Salt Lake City, UT, USA) assuming a bolus dose.

Dose–effect study

To determine the acute dose–effect, acrylamide was administered intraperitoneally ($n = 3$ per dose) at levels of 0 (saline), 0.93, 1.87, 3.75, 7.5, 15, 20, 37.5, 75, or 150 mg/kg, and samples were obtained 15 min post-administration. Here, "effect" refers to extractable acrylamide in serum and sciatic nerve after dosing. Samples from each dose group were derivatized the day following dosing and analyzed by GC–ECD on the following day whenever possible. The concentrations in the samples were determined from the calibration curves and the results plotted as a function of dose.

RESULTS

Derivatization and analysis of samples

Fig. 2 shows a comparison of gas chromatograms obtained after analysis of blank (control) or acrylamide-spiked serum and sciatic nerve homogenate. There were many peaks in the control samples but none of these interfere with either the internal standard or the acrylamide derivative (indicated in chromatogram). A component is sometimes observed in the retention window for 2-bromopropenamide in sciatic nerve, but this small, somewhat variable peak does not present a problem except at concentrations near the LOD. It should be noted that the majority of the peaks observed in the chromatograms are derived from the inorganic reagents. The magnitude of the impurities was reduced after heat treatment of the KBr as indicated in the methods section. Thermal treatment of the Na_2SO_4 did not improve the background (data not presented).

Structural verification

A total of 29.2 mg of product was collected from the synthesis; this corresponds to an 84%

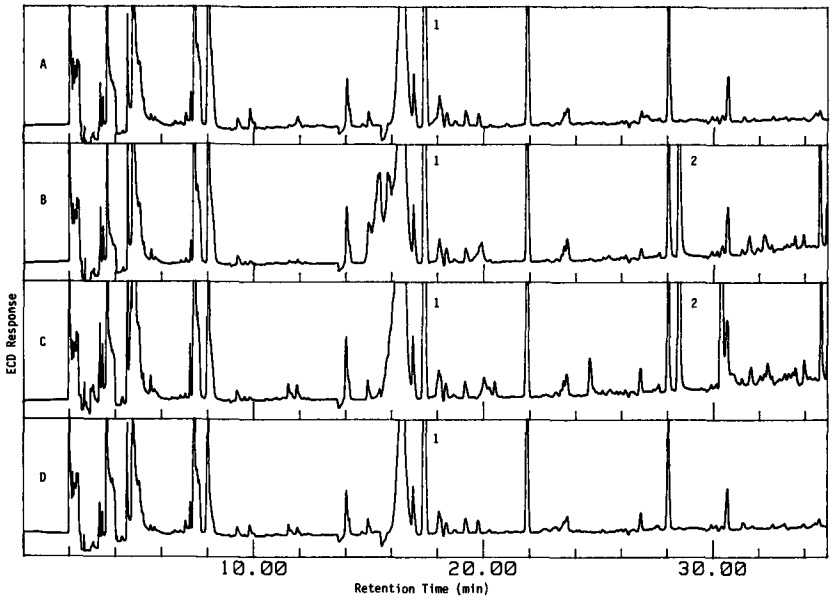

Fig. 2. Chromatograms obtained after derivatization and analysis of extracts from blank (control) serum (A), serum spiked with acrylamide at approximately 12 000 ng/ml (B), sciatic nerve spiked with acrylamide at approximately 18 ng/ml (C), and blank (control) sciatic nerve (D). Peaks: 1 = the internal standard, *p*-dibromobenzene; 2 = the acrylamide derivative 2-bromopropenamide. All chromatograms are 250 mV full scale.

yield relative to theoretical. Results of the ^{1}H NMR analysis did not show the spectral features in the region 3.6–4.8 ppm associated with the 2,3-dibromopropionamide reported previously [16], but did show two strong resonances at 6.0 and 6.9 ppm as compared to the two strong resonances at approximately 6.1 and 6.8 ppm reported by Andrawes *et al.* [16] for 2-bromopropenamide. These results indicate that the conversion to 2-bromopropenamide was successful. Small shifts in the peaks might be expected given that the published spectra were obtained in deuterated acetone as compared to the deuterated chloroform used in the current work.

The GC–ITMS results indicated the same retention times for the synthetic 2-bromopropenamide and the presumed acrylamide derivative in both serum and sciatic nerve tissue homogenate. Fig. 3 compares the mass spectra obtained from the standard and from the derivatized serum; the same spectrum was recorded from the sciatic nerve tissue homogenate. The expected parent ion and key fragment ions were found in the sample and the identity of the acrylamide derivative was confirmed. There was no evidence of 2,3-dibromopropionamide in the chromatogram nor other brominated material in the retention windows of either the internal standard or the acrylamide derivative.

Calibration curve

The calibration curves obtained for serum and sciatic nerve homogenate are shown in Figs. 4 and 5, respectively. In each case, the second-order polynomial provided a better fit to the data providing r^2 values of 0.99996 and 0.99993 for serum and sciatic nerve, respectively, than did a linear function. The ECD responses associated with the lowest concentrations were only slightly higher than the corresponding background. Based on an approximate signal-to-noise ratio of 4, the lower level of detection of the method was estimated to be 3 μg/g in intact sciatic nerve and 1200 ng/ml in undiluted serum.

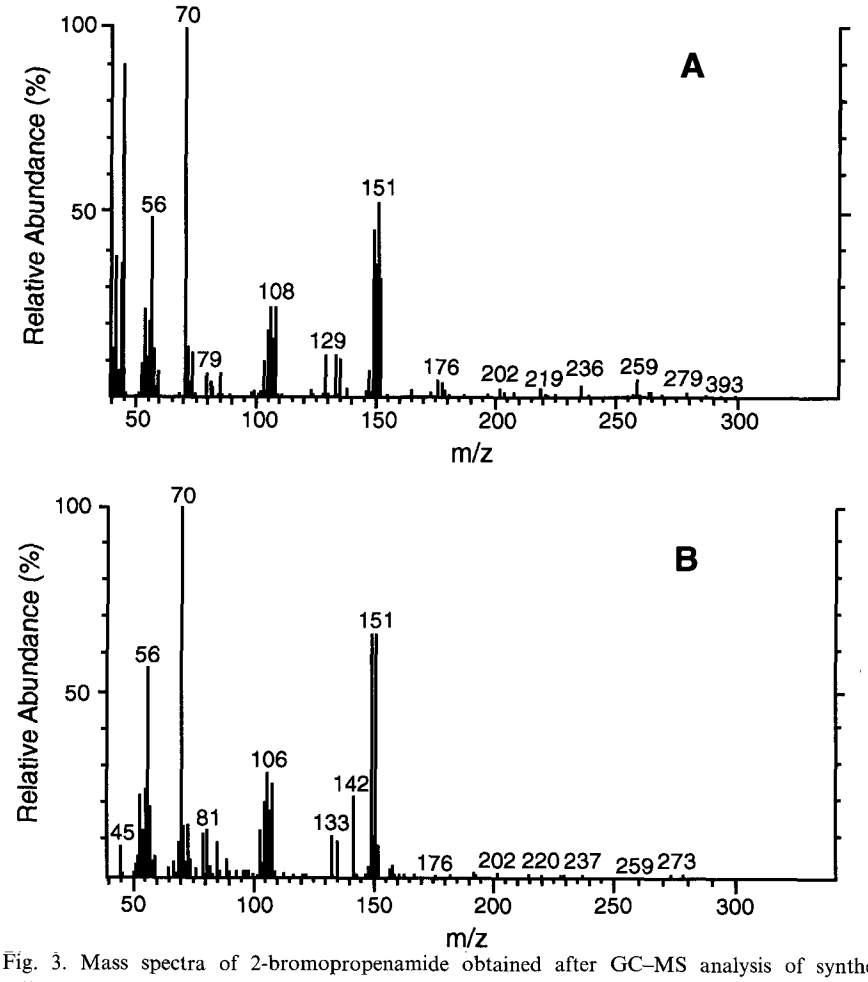

Fig. 3. Mass spectra of 2-bromopropenamide obtained after GC–MS analysis of synthetic compound (A) and acrylamide-spiked and derivatized rat serum (B).

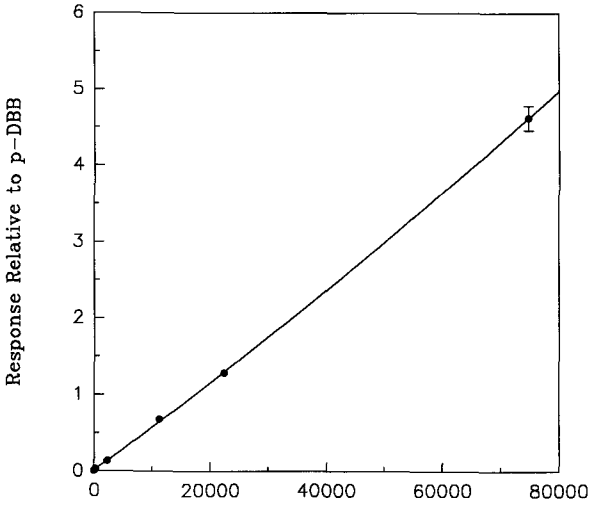

Fig. 4. Calibration curve for acrylamide in rat serum ($n = 2$ per concentration). Response of acrylamide derivative is relative to the internal standard, *p*-dibromobenzene (*p*-DBB). Error bar indicates standard deviation.

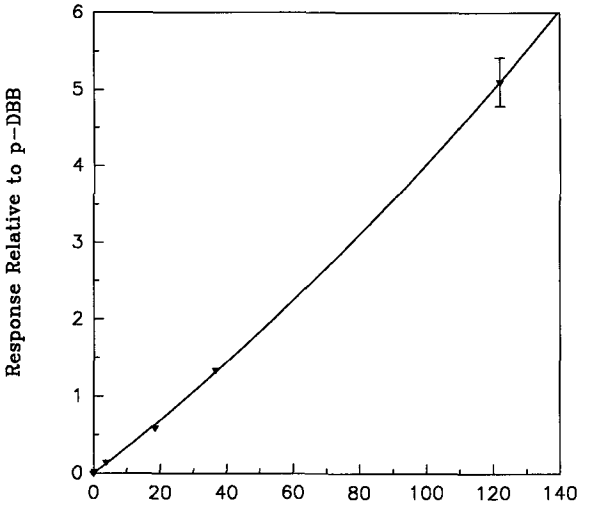

Fig. 5. Calibration curve for acrylamide in sciatic nerve ($n = 2$ per concentration). Response of acrylamide derivative is relative to the internal standard, *p*-dibromobenzene (*p*-DBB). Error bar indicates standard deviation.

Storage of samples prior to derivatization

The results of this experiment are presented in Table I. Note that relative response is presented. The mass of internal standard in each sample was not the same as for the calibration curve presented above so any predictions of concentration are inappropriate. Superimposed on these data are inter-day method variability. Note that sciatic nerve and serum data follow the same trends. The relative responses for the samples from the highest dosing level show trends different from the lower levels and suggest changes with storage. Although not definitive, these results suggest that samples should be derivatized and analyzed as soon as possible after collection. All samples in further studies were derivatized within two days following sample collection.

Stability of derivatized extract

The relative response ratios measured for the replicate serum and sciatic nerve derivatized extracts as a function of storage are shown graphically in Fig. 6. The response for the acrylamide derivative did not appear to be altered with storage time for the serum samples while a slight decrease, that might or might not be significant, was noted with time for the sciatic nerve samples. For sciatic nerve samples, storage of the prepared extract for up to three or four days at room temperature should not present a problem. The chromatographic profiles showed changes in the extract as a function of time but these changes did not induce major interferences in the elution windows for either the internal standard or the acrylamide derivative.

TABLE I

RELATIVE RESPONSES MEASURED FOR SAMPLES STORED PRIOR TO DERIVATIZATON AND ANALYSIS

Matrix[a]	Dosing level (mg/kg)	Response relative to internal standard after indicated storage time		
		1 day	7 days	21 days
Sciatic nerve	0 (control)	ND[b]	ND	ND
	5	0.78	0.62	0.54
	20	2.44	2.83	2.48
	80	7.32	9.81	11.5
Serum	0	ND	ND	ND
	5	1.13	1.59	1.50
	20	4.2	6.57	6.31
	80	6.3	14.4	22.4

[a] Sciatic nerve homogenate and diluted serum stored for indicated times at −80°C.

[b] Not detected.

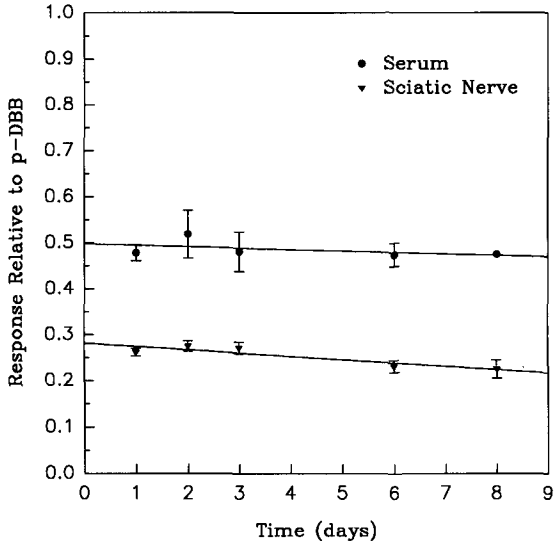

Fig. 6. Relative response data as a function of extract storage time. Error bars indicate the standard deviations for the replicates. The lines shown are from linear regression of the data. MBPA = 2-bromopropenamide; pDBB = *p*-dibromobenzene.

Effect of tissue or serum concentration

The responses from 2-bromopropenamide relative to *p*-dibromobenzene for each sample dilution are shown in Figs. 7 and 8 for serum and sciatic nerve, respectively. The relative standard deviations (R.S.D.) for the replicate samples ranged from 2 to 4% in each matrix. The non-spiked serum samples in this set showed a variable chromatographic peak in the elution window for 2-bromopropenamide. For sciatic nerve, no impurities coeluted with the 2-bromopropenamide, thus no response was measured in ten non-spiked samples. The relative response did not depend on the mass of tissue or serum present during derivatization.

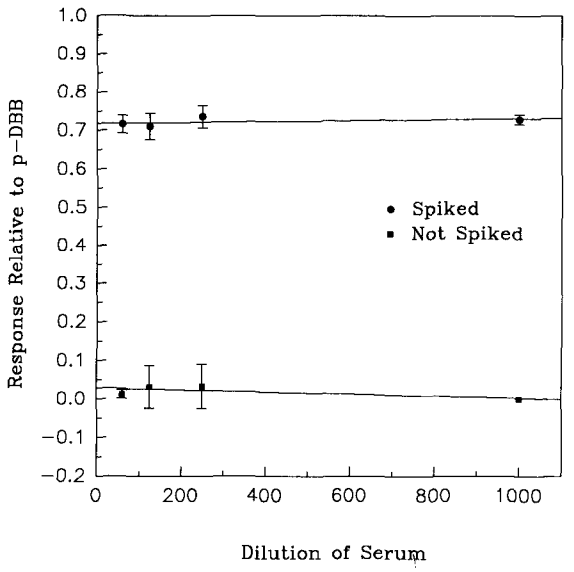

Fig. 7. Response of 2-bromopropenamide (MBPA) relative to *p*-dibromobenzene (*p*-DBB) as a function of serum dilution ($n = 3$ per dilution). The lines shown are from linear regression of the data. Error bars indicate standard deviation.

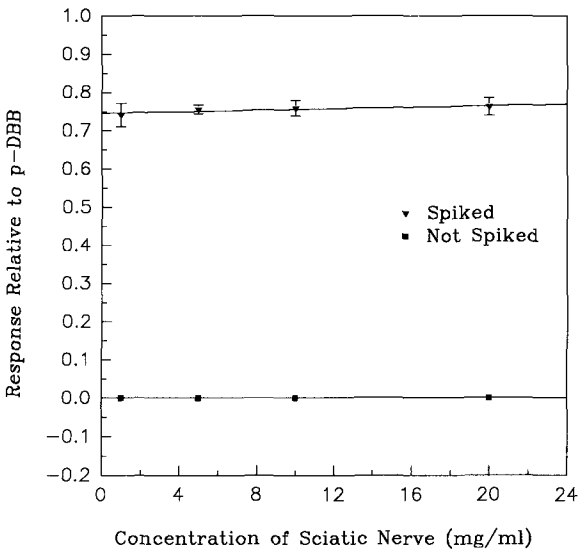

Fig. 8. Response of 2-bromopropenamide (MBPA) relative to *p*-dibromobenzene (*p*-DBB) as a function of sciatic nerve homogenate concentration ($n = 3$ per dilution). The lines shown are from linear regression of the data. Error bars indicate standard deviation.

Time-course study

The concentrations of acrylamide measured in serum and sciatic nerve as a function of time after dosing are shown in Fig. 9. Acrylamide was not detected in any of the 24-h post-dose samples. The elimination half-lives were estimated to be 1.8 h in serum and 2.0 h in sciatic nerve. The associated 95% confidence intervals from the curve fitting result in half-life ranges of 1.1–4.5 h in serum and 1.3–5.0 h in sciatic nerve.

Dose–effect study

Concentrations measured in serum and sciatic nerve homogenates (the "effect") as a function of dose are depicted graphically in Figs. 10 and 11. No acrylamide was detected in any of the sciatic nerve samples after the 0.93 and 1.87 mg/kg dosings. In each case, a second-order polynomial provided a better fit to the data than did a linear function. The equation of best fit for the serum was

$$\text{acrylamide concentration (ng/ml)} \times 10^{-3} = -0.25 + 1.10(\text{dose, mg/kg}) - 0.001(\text{dose, mg/kg})^2$$

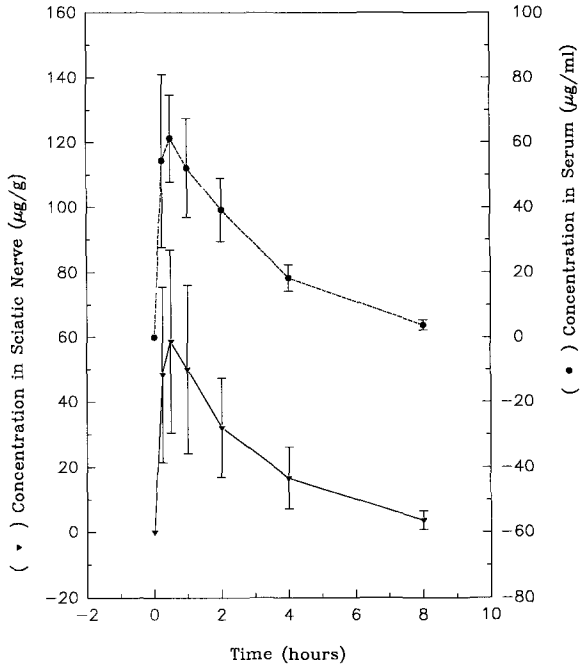

Fig. 9. Concentration of acrylamide in serum and sciatic nerve as a function of time after an acute dose ($n = 3$ at each time point). Acrylamide was not detected in the 24-h post-dose samples. Error bars indicate the standard deviation about the mean at each point.

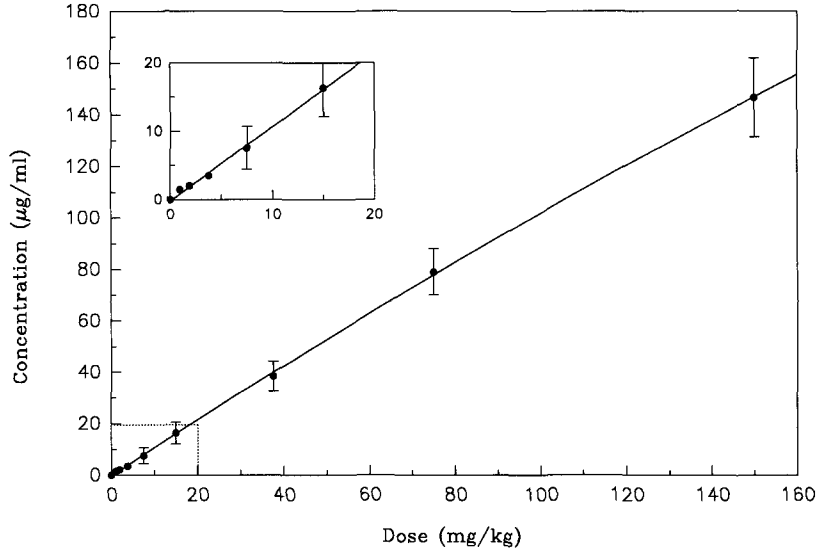

Fig. 10. Concentration of acrylamide measured in serum as a function of dose ($n = 3$ per dose). Inset shows the relationship for doses of 0–20 mg/kg. The limit of detection in undiluted serum was determined to be 1200 ng/ml. Error bars indicate standard deviation.

and the equation of best fit for sciatic nerve was

$$\text{acrylamide concentration } (\mu g/g) = -0.96 + 1.18 \text{ (dose, mg/kg)} - 0.001 \text{ (dose, mg/kg)}^2$$

The value of r^2 was 0.9999 for each curve. For the six samples where acrylamide was measured both in serum and sciatic nerve, the concentration in serum was approximately the same as in sciatic nerve.

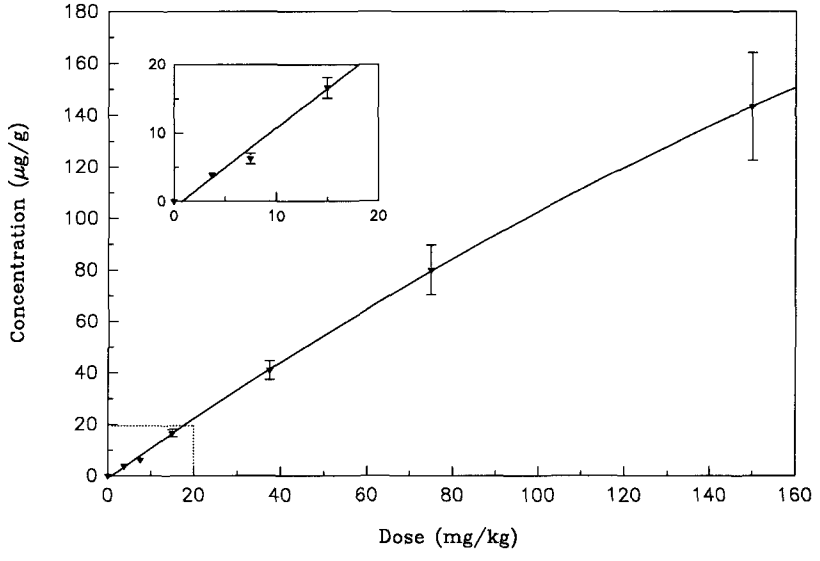

Fig. 11. Concentration of acrylamide measured in sciatic nerve as a function of dose ($n = 3$ per dose). Inset shows the relationship for doses of 0–20 mg/kg; concentrations of acrylamide were below the limit of detection for the method (3 μg/g) after doses of 0.93 and 1.87 mg/kg. Error bars indicate standard deviation.

DISCUSSION

During this study, a method was developed for the analysis of acrylamide based on chemical derivatization to 2,3-dibromopropionamide, as described by Poole *et al.* [15], followed by conversion to 2-bromopropenamide using triethylamine, a modification described by Andrawes *et al.* [16]. The integrated method was then applied to samples of rat serum and sciatic nerve. The method was evaluated in terms of storage for underivatized samples and for derivatized extracts, and the impact on the method for increased amounts of tissue or serum used in the derivatization procedure. The method was applied to a study of the uptake and elimination of acrylamide in rats after a single dose. The validation of the method and its application to some initial biological studies provide the basis for designing dosing experiments that are optimal from both an analytical and biological sense.

Validation of the analytical method

The general absence of co-eluting compounds in the chromatographic analyses of the extracts indicates that the derivatization/analysis scheme can be applied to serum and sciatic nerve homogenate without any pre-fractionation of the sample before derivatization and analysis. The ^{1}H NMR study demonstrated that the conversion of 2,3-dibromopropionamide to 2-bromopropeneamide was successful. The GC–ITMS work revealed that the chromatographic peak assumed to be 2-bromopropenamide, based on the association of increased peak area with increased spike level, is indeed 2-bromopropenamide; no evidence of unconverted 2,3-dibromopropionamide was found.

From the calibration studies, the LOD was estimated to be 1200 ng/ml in undiluted serum (or approximately 9.6 ng/ml in serum diluted 1:125 with water) and 3 μg/g in sciatic nerve homogenate containing 5 mg tissue per ml of homogenate. This compares favorably with the acrylamide LOD of 3.6 μg/g reported by Poole *et al.* [15] for derivatization and analysis (as 2,3-dibromopropionamide) of a 0.5-ml volume of 5 mg/ml sciatic nerve homogenate. This value also compares relatively well to the LOD of 3.2 ng/ml for a sample size of 0.5 ml reported by Hashimoto [14] for the derivatization and analysis of acrylamide, as 2,3-dibromopropionamide, in water.

The precision and accuracy of the calibration data indicated that the method is reproducible and accurate in each matrix from the estimated LODs to the highest concentrations studied (74 700 ng/ml in serum and 122 μg/g in sciatic nerve). The studies in which the serum dilution and sciatic nerve concentration were changed suggest that lower concentrations of acrylamide may be detectable by using larger amounts of tissue. Changes in the apparent concentration of acrylamide with storage time for dilute samples indicate that samples should be derivatized soon after collection. Once derivatized, there appears to be some flexibility in how much time can pass before GC analysis; storage for up to at least eight days for serum and three to four days for sciatic nerve should not adversely impact the results. This finding is important in the event of instrument failure after sample preparation or if there is a desire to derivatize several groups of samples and to do the analyses as a batch using an autosampler. It should also be noted that storage of the extracts was at room temperature. Storage at reduced temperatures (refrigerator or freezer) would likely improve the stabilities.

Biological aspects

The time-course study indicated that acrylamide concentrations in serum and sciatic nerve parallel each other. The highest concentration in each matrix after an acute dosing was measured at the 30-min post-dose sample. The elimination half-life for acrylamide was estimated to be 1.8 h in serum and 2.0 h in sciatic nerve. The value for serum agrees very well with the value of 1.7 h reported by Miller *et al.* [17] for [2,3-^{14}C]acrylamide in the plasma of Fisher-344 rats and with the value of 1.9 h reported by Edwards [18] for [1-^{14}C]acrylamide in the blood of Porton rats after intravenous dosing. The half-life of 1.8 h estimated in this work also agrees fairly well with the 1.4-h half-life for acrylamide in blood after

intravenous dosing of rats, as calculated from the data of Calleman *et al.* [19]. However, the 2.0-h half-life for sciatic nerve tissue is lower than the 5.0-h elimination half-life estimated by Miller and Spencer [7].

The dose–effect data (measurable acrylamide after an acute dose) indicated that acrylamide could be measured in serum after the lowest dose of 0.94 mg/kg, although this concentration was very near the LOD of the method. Concentrations of acrylamide in sciatic nerve did not exceed the LOD of the method until after a dose of 3.75 mg/kg. In both cases, no acrylamide was detected in pre-dose samples and the concentrations of acrylamide in each matrix increased with increasing dose. As for the time-course study above, the acrylamide concentrations measured in serum paralleled those measured in sciatic nerve. This is clearly shown by the similarity of the equations derived from the data. A comparison of the detectable concentrations in sciatic nerve to those in the corresponding serum samples after six different dosing levels showed acrylamide to be approximately the same in serum (μg/ml) and in sciatic nerve (μg/g). Thus, acrylamide concentrations in sciatic nerve can be estimated from serum measurements. These data demonstrate the adequacy of the procedure to detect acrylamide across the wide range of dosages employed in neurotoxicology studies.

CONCLUSIONS

A method for the determination of acrylamide in serum and sciatic nerve from rats was validated. The method was accurate and precise over the concentration range 2240–74 700 ng/ml in serum after a dilution of 1:125 and 4–122 μg/g in sciatic nerve present at 5 mg/ml. The use of less dilute samples to allow for lower limits of detection appears feasible. The time-course of acrylamide in serum and sciatic nerve was studied after acute dosing and indicated elimination half-lives of 1.8 and 2.0 h for serum and sciatic nerve, respectively. A dose–extractable acrylamide relationship was established for each matrix after acute dosing and the acrylamide concentrations in serum (μg/ml) were approximately the same in sciatic nerve (μg/g). Experiments in which the concentrations of acrylamide are measured in serum and sciatic nerve after repeated dosing are underway. Such repetitive or chronic dosings mimic the types of intake from exposures that are most commonly experienced by segments of the human population.

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REFERENCES

1 J. O'Donoghue (Editor), *Neurotoxicity of Industrial and Commercial Chemicals*, Vol. II, CRC Press, Boca Raton, FL, 1985, pp. 169–178.
2 *US EPA Assessment of Health Risks from Exposure to Acrylamide*, Office of Toxic Substances, US Environmental Protection Agency, Washington, DC, 1990.
3 National Institute for Occupational Safety and Health (NIOSH), *Monitoring at Manufacturing/Processing Sites*, US Department of Health and Human Services (USDHHS), Report to EPA, 1985.
4 T. Garland and M. Patterson, *Br. Med. J.*, 4 (1967) 134.
5 H. Igisu, I. Goto, Y. Kawamura, M. Kato, K. Izumi and Y. Kuroiwa, *J. Neurol. Neurosurg. Psychiat.*, 38 (1975) 581.
6 H. A. Tilson, *Neurobehav. Toxicol. Teratol.*, 3 (1981) 445.
7 M. S. Miller and P. Spencer, *Ann. Rev. Pharmacol. Toxicol.*, 25 (1985) 643.
8 J. Burrek, R. Albee, J. Beyer, T. Bell, R. Carreion, D. Morden, C. Wade, E. Hermann and S. Gorzinski, *J. Environ. Pathol. Toxicol.*, 4 (980) 157.
9 *US EPA RfD for Acrylamide*, IRIS Database, US Enviornmental Protection Agency, Washington, DC, 1992.
10 G. Barnes and M. Dourson, *Regul. Toxicol. Pharmacol.*, 8 (1988) 471.
11 K. M. Crofton, L. J. Somerholter, J. Farmer, S. Padilla and R. C. MacPhail, *Toxicologist*, 11 (1991) 307.
12 C. J. Calleman, E. Bergmark and L. G. Costa, *Chem. Res. Toxicol.*, 3 (1990) 406.
13 N. L. Freshour, P. W. Langvardt, S. W. Frantz and M. D. Dryzga, *J. Chromatogr.*, 346 (1985) 376.
14 A. Hashimoto, *Analyst*, 101 (1976) 932.
15 C. F. Poole, W. F. Sye, A. Zlatkis and P. S. Spencer, *J. Chromatogr.*, 217 (1981) 239.
16 F. Andrawes, S. Greenhouse and D. Draney, *J. Chromatogr.*, 399 (1987) 269.
17 M. J. Miller, D. E. Carter and I. G. Sipes, *Toxicol. Appl. Pharmacol.*, 63 (1982) 36.
18 P. M. Edwards, *Biochem. Pharmacol.*, 24 (1975) 1277.
19 C. J. Calleman, L. G. Stern, E. Bergmark and L. G. Costa, *Cancer Epidemiology, Biomarkers Prevention*, 1 (1992) 361.