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The study of human nails as an intake monitor for arsenic using neutron activation analysis

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Arsenic is toxic to humans with the lethal dose being approximately 1 mg/kg/day. At much lower long-term exposures, arsenic is hypothesized to increase the risk of certain cancers. We have developed an irradiation position for the neutron activation analysis (NAA) of nail specimens for arsenic, in support of a case-control study involving New Hampshire residents consuming well water above the EPA Safe Drinking Water Standard of 0.050 ppm. Arsenic is bound to nail keratin through sulfhydryl groups proportional to intake providing a convenient means of integrating arsenic intake in population-based studies. Our objective was to develop the necessary facilities and procedures by which relatively small samples (i.e. 20 to 100 mg) could be accurately analyzed for arsenic, so that affordable nutritional epidemiology investigations, requiring large numbers of samples (>1000 in this case), could be undertaken. A high-flux reflector position, with minimal axial variation throughout the fuel cycle, suitable for pneumatic-tube irradiations, was characterized by measurement of the neutron flux distribution (thermal and epithermal) within the irradiation capsule over time. Results from application of the method to a case-control study of basal and squamous cell skin cancer will be presented.

Introduction+

All humans are exposed to low levels of arsenic (As), which is widely distributed in the environment. Food is the largest source of As intake for most people, with lower amounts from drinking water and air. Most ingested As is absorbed through the stomach and intestines and then enters the bloodstream. Inhaled As is well-absorbed through the lungs and goes to the bloodstream.[1] Absorbed As is converted by the liver to a less-toxic form that is efficiently excreted in urine.[1] Therefore, blood and urine As concentrations reflect only recent exposure.[2]

Increased As exposure is due to a variety of situations. Natural mineral deposits in some geographic areas contain large amounts of As, which may result in elevated levels of inorganic As in water.[1] Some waste-chemical disposal sites contain large amounts of As, which may escape into the water.[1] Elevated levels of As in soil may lead to exposure from ingesting the soil.[1] Manufacturing of copper and other metals releases inorganic As into the air.[1,3] Burning of fossil fuels results in emissions of inorganic As into the air.[1] Workers involved in the manufacture or use of arsenic-containing pesticides may be exposed to As, and widespread application of the pesticides may lead to soil or water contamination.[1,3] Manufacturing of glass, semiconductors, and various pharmaceutical substances may release air-borne As.[3] Ingestion of As has been reported to increase the risk of skin, liver, bladder, kidney and lung cancer.[1]

There is an interest in the level of As in drinking water due to the epidemiological evidence of a association between As intake and cancer in the southwest coast of Taiwan. The residents of the area were exposed to arsenic-containing artesian well water for more than 45 years. The water from artesian wells was used due to the high salinity of the water from the shallow wells.[3,4] The prevalence of blackfoot disease, a peripheral vascular disorder, was found to increase proportionally with the As content of drinking water from these wells.[3–6] There was a dose-response relationship between the occurrence of non-melanoma skin cancer and the concentration of As in drinking water from the artesian wells.[4,6–8] There was also a significant dose-response relationship between the prevalence of skin cancer and cumulative As exposure index.[6] A positive dose-response relationship was observed between artesian well water exposure and bladder, lung, and liver cancer.[3,8] A significant dose-response relationship was observed between mortality from lung, bladder, and liver cancer and the duration of drinking artesian well water.[3,8] The standardized mortality ratios (SMRs) were significantly higher in the blackfoot-endemic area than in the general population of Taiwan for cancers of bladder, kidney, skin, lung, liver and colon.[3,4,8] The SMRs for bladder, kidney, skin, lung and liver cancers were highest in villages where only artesian wells were used and lowest in villages where only shallow wells were used.[4,8] The increase in mortality rates for lung and skin cancers in the blackfoot disease-endemic area is related to As exposure.[4]

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There have been reports of elevated levels of As in water supplies in several states of the USA including California,[9] Oregon,[10] Alaska[11] and New Hampshire.[12] Elevated levels of As were detected in several water supplies in New Hampshire, especially from artesian wells, following the implementation of the Safe Drinking Water Act of 1974. Private wells, which serve fewer than 15 households or 25 individuals, are not regulated by the Safe Drinking Water Standard, but are frequently used in rural areas. Private wells serve approximately 25% of the households in New Hampshire, and approximately 5% of these wells are estimated to have As levels above the present EPA standard of 0.05 ppm. Arsenic, at detectable levels, appears to be present in the water supplies in every town in New Hampshire.[12]

Previous epidemiological studies of arsenic and cancer have associated water concentrations in geographical areas with cancer occurrence in those areas. The studies have not evaluated water As concentrations and disease status of individuals and have not used any biologic monitors of exposure. Arsenic is rapidly cleared from the blood, so blood levels of As do not reflect long term exposure. Arsenic has an affinity for sulfhydryl groups of keratin and accumulates in the hair and nails, which can be used to track arsenic poisoning.[13,14] Toenail clippings are easy to collect and store, and can be analyzed for As along with other trace elements. They also reflect long-term exposure over a period of several months,[15] and should be a good biologic monitor for As exposure.

KARAGAS et al.[2] conducted a study to look at toenail As concentrations as an indicator of ingestion of arsenic-containing water. Water samples and toenail clippings were collected from households in New Hampshire, where elevated As levels had been detected in private water supplies.[2] Arsenic concentrations in toenail samples were highest in subjects whose well water samples contained higher As concentrations.[2] A 10-fold increase in well water As concentrations was reflected by about a 2-fold increase in toenail As concentrations.[2] It was concluded that toenail As concentrations provide a useful biologic monitor of As exposure on an individual level, especially among people whose drinking water contains As.[2]

Assessment of long-term reproducibility of a biologic monitor will determine how well a single measurement reflects long-term exposure. GARLAND et al.[16] assessed the reproducibility of As in toenails by comparing concentrations in toenails from 127 women collected in 1982–1983 with toenails from the same women collected in 1988. The As concentrations were significantly positively correlated over time.[16] The high degree of reproducibility over a 6-year period for toenail As concentrations implies that a single measurement of the toenail monitor reflects long-term exposure.[16]

The current study is a case-control study of skin cancer in a geographically defined US population with a high prevalence of arsenic-containing drinking water. Toenail clippings are being analyzed as a biologic monitor for As. The subjects in the non-melanoma skin cancer study are New Hampshire residents, between 25 and 74 years of age, newly diagnosed with primary basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) for the three-year period (July 1, 1993 through June 30, 1996). There are approximately 600 patients with BCC and 300 patients with SCC age and sex matched with approximately 600 control subjects. The development of the irradiation position and procedure to analyze relatively small samples for As via instrumental neutron activation analysis (INAA) will be discussed.

Experimental

Vertical flux profile

The MURR has an eight-element core of highly-enriched ^{235}U that is configured to produce a right-cylinder with a flux-trap annulus. The active fuel height is approximately 61 cm and is completely surrounded by a beryllium annulus having a thickness of 5 cm. Control is accomplished by a bank of 4 boron carbide control blades and a single stainless steel regulating blade, for fine adjustment, operating in a water gap between the core's pressure vessel and the beryllium reflector. The control blades have an active height of 86.4 cm and are indexed to the near-bottom of the reflector tank plate spanning the fuel assembly and overlapping the active fuel approximately 10 cm on the top and 15 cm on the bottom, with the reactor in the shut-down mode. From this position, it is possible, with eight new fuel elements (~0 MW days) to achieve criticality when the four control blades, moving in unison, reach a height of approximately 30 cm. However, the MURR fuel cycle introduces new fuel elements to the cycle as a two-element pair interspersed with six older elements such that the eight-element core has a cumulative history of approximately 550 MW days. For this fuel configuration, criticality is not reached until the control blades have reached approximately 41 cm placing the bottom of the blades ~5 cm below vertical core centerline. Ten MW operation is sustained for the 150 hour fuel cycle by additional control-blade travel of approximately 15 cm, most of that occurring in the first 24 hours of operation. This 15 cm span, approximately twice the active height of the shuttle rabbit, by design occurs above the Row II rabbit position to minimize the impact on the vertical flux profile in the rabbit. For most NAA experiments conducted at the MURR utilizing the pneumatic-tube facility, a single sample, occupying a volume of 1.5 cm^3 or less, is irradiated for short periods,

typically less than 60 seconds. For this study, we wanted to use the full height allowed by the shuttle rabbit (~8 cm). The Row II irradiation position, used in this study, is relatively new and had not been characterized over the height of the rabbit for the approximate 15 cm of control-blade travel that typically occurs at the MURR over 150 continuous hours operating at 10 MW. To characterize this position, thin gold wires (diameter = 0.25 mm) approximately 9 mm in length were threaded into capillary tubes and centered radially in the shuttle rabbit to measure the vertical flux profile. The gold flux monitors were irradiated for 20 seconds at control blade heights of 41.0, 42.3, 45.5, 48.3, 52.6, 54.7 and 56.9 cm, allowed to decay for 15 to 75 hours, attached to a plexiglass cylinder having a circumference of 8 cm and counted in segments through a 5 mm pin-hole lead collimator having a thickness of 5 cm. This technique produced 12 equal segments of approximately 6.7 mm. Each segment was counted for 60 seconds with a sample-to-detector distance of 36 cm using the high-resolution gamma-ray spectrometer described later. Then the cylinder was rotated 30 degrees to expose the detector to a new segment; repeating this process until the complete 12-segment cycle was counted in triplicate.

Arsenic standard preparation

Standards containing nominally 0.1 μg arsenic were prepared from a 1000±10 ppm arsenic standard solution (Fisher Scientific AA Standard #SO-A-449, lot # 862797-24). This 1000 ppm As stock solution was diluted by 100 to produce a 10 ppm As standard. Aliquots (10 μl) of this solution were pipetted, using a calibrated pipet, onto five 1 cm filter paper disks (Whatman Int. Ltd.) contained in 0.2 ml high-density polyethylene (HDPE) vials. As a confirmation of the pipet calibration, the mass of each aliquot was measured and recorded. The vials were allowed to air dry and were then capped with a friction-fit lid.

Keratin powder

Approximately 50 mg of ICN Keratin Powder (ICN Nutritional Biochemicals, Cat # 902111, lot # 20945) with a dry weight correction of 0.95 were placed in each tared 0.2 ml HDPE vial and weighed using a Mettler AT261 balance. After lyophilization (Hetovac VR-1), Whatman filter paper disks were placed on top of the keratin powder in the vial. The vials were capped with a friction-fit lid.

Sample preparation

Samples were collected for a case-control study of basal and squamous cell skin cancers. This case-control study involves New Hampshire residents consuming well water above the EPA Safe Drinking Water Standard of 0.050 ppm arsenic. The study involves about 600 BCC participants, 300 SCC participants and 600 control subjects. The subjects ranged in age from 25 to 74 years of age. Nail samples were collected after showering or bathing and were placed in coin envelopes. The subjects were instructed to towel dry and scrape the nails just prior to clipping. The samples are coded and no details regarding the participants (e.g., case-control status, age, gender, etc.) are known to the NAA laboratory personnel.

The nail samples ranged in mass from 23 mg to 124 mg with a median sample being approximately 60 mg. Any nails that contained nail polish were rinsed with acetone. The samples were placed in scintillation vials. The scintillation vials were filled 3/4 full with DI water and were sonicated (Branson Ultrasonic Cleaner) for 10 minutes to remove external surface contamination. The water was suction filtered off of the nail samples which were collected on a filter paper. The samples were thoroughly rinsed with approximately 15 ml of DI water, twice, to remove all traces of external sodium contamination on the nail. The samples were then freeze-dried. The dried samples were weighed into tared 0.2 ml HDPE vials. The vials were closed with a friction-fit cap and then heat sealed. To ensure that there was no As present in the sample preparation materials, blanks were prepared in the same manner as the samples.

Instrumental neutron activation analysis

Eight samples, one arsenic standard and one keratin sample are loaded into a polyethylene (PE) sleeve that is 7.5 cm long and 1.0 cm wide. The PE sleeve is double-sealed on top, bottom and one side (see Fig. 1).

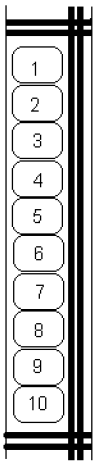

Fig. 1. Polyethylene sleeve

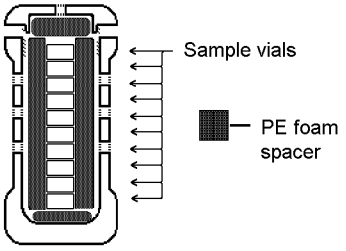

Fig. 2. Ventilated polyethylene rabbit with spacers and a sleeve containing samples

Table 1. Experimental parameters for arsenic analysis in toenails

Thermal flux	$4.6 \cdot 10^{13}$ cm$^{-2} \cdot$s^{-1}
Epithermal flux	$2 \cdot 10^{12}$ cm$^{-2} \cdot$s^{-1}
Irradiation time	10 min
Cycles	6
Time between cycles	10 min
Decay after last cycle	~24 hours
^{76}As half-life	26.32 hours
Gamma-ray energy	559.1 keV
Data reduction	Standard comparison

The samples are loaded such that the keratin and standard are incrementally translated up and down the length of the rabbit. This ensures, over the course of several sequential irradiations, that the standard and keratin vials are irradiated in all rabbit positions, and with the same relative proximities to each other as found for the samples and arsenic standards. The sleeve is then loaded inside a PE insert that is 8 cm long and 2.5 cm wide. A PE spacer is placed in the bottom of a HDPE rabbit (Fig. 2) that had been drilled with ventilation holes on its sides and with one in its screw-cap lid. Then, the PE insert and sleeve containing the samples and standards is placed inside the rabbit and another spacer is placed on top and the rabbit is closed with its screw cap. Generally, there are two rabbits prepared and these are irradiated for a total of 60 minutes in 10 minute cycles alternating between the two rabbits. During the cooling period, the rabbit is briefly submerged in liquid nitrogen allowing the expanded PE spacer material to absorb coolant and lower the temperature of the rabbit and its contents to ambient or below. Experimental parameters are given in Table 1. After undergoing a total of 60 minutes of irradiation, the samples were decayed for approximately 24 hours, and then live-time counted for 2 hours using HPGe detectors (EG&G Ortec) having an efficiency of 30% and automatic sample changers. The gamma-ray ($E_\gamma = 559.1$ keV) from the decay of ^{76}As ($T_{1/2} = 26.32$ hours) is used for analysis, and concentrations are determined by standard comparison. In addition to the HPGe detector, the spectrometer

included an Ortec 572 spectroscopy amplifier and a Nuclear Data 570 ADC. Data acquisition and peak extraction were done using a Microvax 3100, model 38, using version 2.1 Canberra/ND applications software.

Results and discussion

Vertical flux profile

The data from the 7 gold flux monitors irradiated beginning at start-up and continuing well past the fission-product-poison equilibrium point are summarized in Fig. 3. As desired, the neutron flux in the Row II rabbit position remained stable throughout the entire fuel cycle having a mean of $4.60\pm0.14\cdot10^{13}$ n·cm^{-2}·s^{-1}. Also as desired, the vertical flux profiles, measured at start-up and continuing past equilibrium, were consistent throughout having a mean relative standard deviation of 3.2% for the 7 measurements with a range of 2.3 to 5.6%. Among these Row II profiles, the mean flux is 5.0% higher in the top of the rabbit relative to the bottom and this difference is observed at all control-blade positions (at 10 MW). This small difference is statistically significant ($p=0.024$). A correction could be made for this small flux gradient. However, as discussed in the following section, dispersing standards and quality control materials throughout the vertical irradiation space in the shuttle rabbit produces acceptable results for the measurement of arsenic in these relatively small samples.

Keratin powder

Keratin Powder, used as a quality assurance standard for all analyses of toenails in this study, does not have a certified concentration for As. However, the previously reported value for As in this keratin powder is 0.28± 0.02 ppm using the k_0 methodology[17] compared to 0.31 ±0.02 ppm for the 150 samples analyzed in this study. This small variation between the two techniques is acceptable for the study undertaken.

The keratin sample was incrementally translated in sequential irradiations up and down the sleeve (see Fig. 1) starting at position 1 with an As comparison standard in position 8, until the standard is in position 1 and keratin is in position 8. This allows for the evaluation of any systematic error introduced by an axial flux profile over the height of the rabbit. As previously discussed the flux distribution from top-to-bottom of the rabbit is relatively constant for the entire fuel cycle. Consequently, all positions (1–8), without applying a flux gradient correction, gave As concentrations in individual keratin samples that were not significantly different from the mean (Table 2).

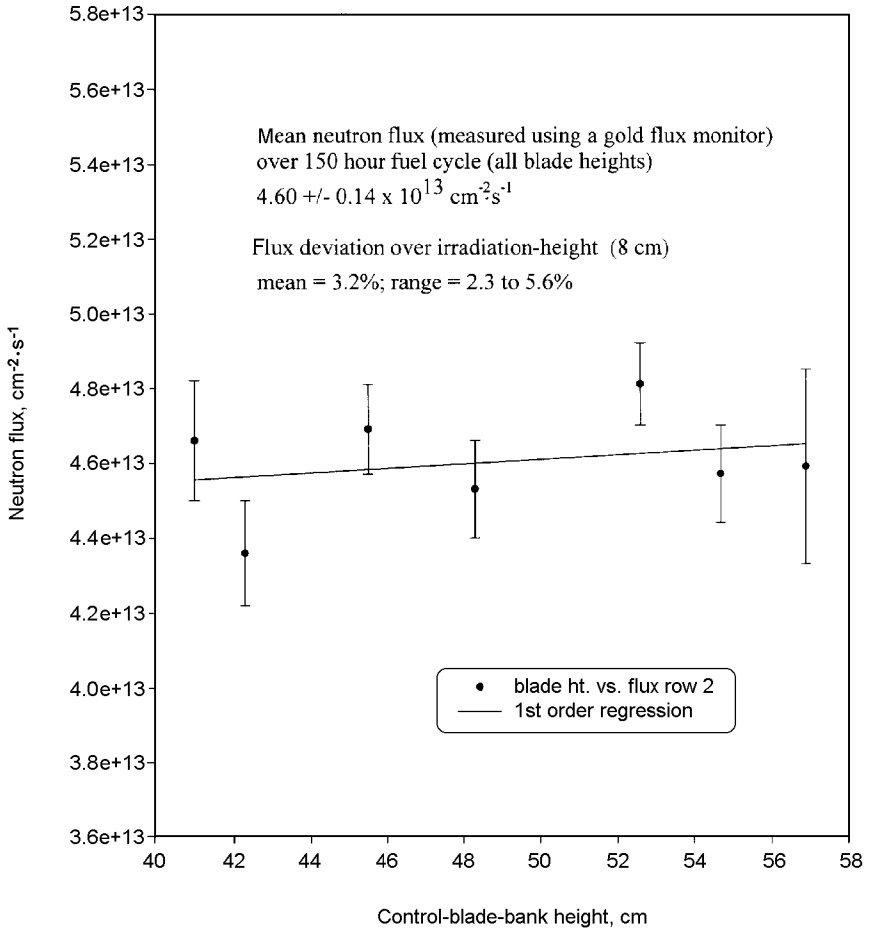

Fig. 3. Neutron flux in Row II as a function of control blade height

Table 2. Concentration (in ppm) of arsenic in the keratin quality control material as a function of rabbit irradiation position

Position	As, ppm	Mean, ppm	*t*-test
1	0.311 ± 0.029	0.309 ± 0.022	$p = 0.685$
2	0.318 ± 0.025	0.309 ± 0.022	$p = 0.186$
3	0.310 ± 0.019	0.309 ± 0.022	$p = 0.741$
4	0.319 ± 0.018	0.309 ± 0.022	$p = 0.078$
5	0.312 ± 0.015	0.309 ± 0.022	$p = 0.553$
6	0.300 ± 0.021	0.309 ± 0.022	$p = 0.068$
7	0.304 ± 0.015	0.309 ± 0.022	$p = 0.380$
8	0.301 ± 0.026	0.309 ± 0.022	$p = 0.192$

Case-control study

Previous studies have found an apparent systematic bias, which is inversely proportional to the mass of the sample.[18] However, this bias is not observed in samples having a mass greater than 23 mg. For this reason, samples containing less than 23 mg were screened out of the initial study and will not be used unless required to enhance the statistical power of the epidemiological conclusions.

One thousand thirty-three samples have been analyzed to date in this case-control study. The mean concentration observed is 0.118 ppm As. The median value is 0.088 ppm with a high of 2.573 ppm As and a low of 0.009 ppm As. These As concentrations are similar to those reported in two other nested population-based studies in which our laboratory has collaborated and different from a third study. These studies, and the one reported here, are summarized in Table 3. The As concentrations measured in the previous studies were done by k_0 NAA and the concentrations measured in this study were done via standard comparison. Subjects participating in the Nurses' Health Study (NHS)[16] and the Occupational Exposure Health Study (OEHS)[19] had similar mean As concentrations in their nails compared to those found in this work. Participants in the South Dakota Regional Health Study (SDRHS)[20] had significantly higher As concentrations.

Table 3. Arsenic concentration (in ppm) in human nail specimens

	SDRHS[a]	NHS-82[b]	NHS-88[c]	OEHS[d]	This work
n	169	120	113	128	1033
Mean, ppm	0.174	0.11	0.12	0.12	0.118
Std. dev.	0.195	0.17	0.27	0.13	0.13
Median, ppm	—	0.083	0.074	—	0.088
Minimum, ppm	0.024	—	—	0.022	0.009
Maximum, ppm	1.44	—	—	0.89	2.57

[a] South Dakota Regional Health Study (SDRHS), Harvard Medical School. Subjects (males and females) enrolled in this nested study at the time of sample collection lived (for at least one year) in a nine-county area comprising 7 counties in Western South Dakota and 2 counties in Eastern Wyoming.
[b] Nurses' Health Study (NHS), Harvard Medical School. Subjects (all females) enrolled in this nested study at the time of sample collection lived in one of 11 U.S. States and contributed the first sample set in 1982.
[c] Same study and subjects as described in Note b except samples were collected in 1988.
[d] Occupational Exposure Health Study (OEHS), Harvard Medical School. Subjects (all males) enrolled in this nested study at the time of sample collection were either currently active as carpenters, or had once been active in that occupation. Occupational exposure to heavy metals routinely occurred in this cohort as a result of the diverse nature of their activities.

Conclusions

Instrumental Neutron Activation Analysis is an accurate means to measure As in nails. The method reported here is relatively fast, cost effective and has a sample throughput capability adequate and affordable for case-control studies of the type to which it is being applied. The flux profile in the Row II irradiation position is not greatly affected by the control-rod movement during the approximate 150 hour fuel cycle used at the MURR. The axial flux profile in the rabbit has a mean standard deviation of 3.2% and a range of 2.3 to 5.6% for all rod heights. Consequently, flux corrections for the samples and standards that span the usable height of the rabbit (~8 cm) are not required. Arsenic concentrations determined for quality control samples and the nail specimens, using this pneumatic-tube based procedure, compare well with other NAA work done in our laboratory using the k_0 NAA approach.

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