ELSEVIER

Mutation Research 431 (1999) 247–257

Fundamental and Molecular Mechanisms of Mutagenesis

www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Effects of arsenic exposure on the frequency of *HPRT*-mutant lymphocytes in a population of copper roasters in Antofagasta, Chile: a pilot study

Karen Harrington-Brock [a], Marta Cabrera [b], Deborah D. Collard [a], Carolyn L. Doerr [a], Rob McConnell [c], Martha M. Moore [a], Hernan Sandoval [d], James C. Fuscoe [a,*]

[a] *Environmental Carcinogenesis Division, Mail Drop 68, National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA*
[b] *Department of Community Medicine, Mount Sinai School of Medicine, New York, NY 10029, USA*
[c] *Department of Preventive Medicine, University of Southern California, Los Angeles, CA 90033, USA*
[d] *Asociacion Chilena de Seguridad, Subgerencia de Medicina Preventiva, Santiago, Chile*

Received 8 April 1999; received in revised form 16 July 1999; accepted 3 August 1999

Abstract

A pilot biomarker study was conducted to investigate the feasibility of using the hypoxanthine guanine phosphoribosyltransferase (*HPRT*) gene in peripheral blood lymphocytes as a biomarker for detecting genetic effects of arsenic exposure. Blood and urine samples were obtained from workers highly exposed to arsenic in a copper roasting plant in Antofagasta, Chile. Individuals were classified according to their job titles into three potential exposure groups: high, medium, and low. To confirm exposure, arsenic concentration was determined in urine samples. The *HPRT* mutant frequencies were measured in lymphocytes from 15 individuals ranging in age from 24 to 66 years. The mean mutant frequencies for the three exposure groups were: low (9×10^{-6}), medium (11×10^{-6}), and high (24×10^{-6}). An increased mutant frequency was observed in the highly exposed group, but the response was so slight that it is not likely that this assay will be capable of providing dose–response information across a range of lower, more typical environmental arsenic levels.

Keywords: *HPRT*; Mutagenesis; Arsenic; Genotoxicity; Human exposure

1. Introduction

Inorganic arsenic (As) is a human carcinogen for the skin and lung [1,2]. In addition, there is suggestive evidence that internal cancers of the bladder and kidney may also be caused by environmental As ingestion [3]. Despite a large body of evidence indicating that As is a human carcinogen, animal models have failed to detect its direct carcinogenic effect [1,2]. In attempts to understand the mechanism of As carcinogenicity, many studies have examined the genotoxicity of As, both in in vitro systems and in

* Corresponding author. Tel.: +1-919-541-3918; fax: +1-919-541-0694.
E-mail address: fuscoe.jim@epa.gov (J.C. Fuscoe)

0027-5107/99/$ - see front matter
PII: S0027-5107(99)00167-0

exposed human populations. Arsenic is not mutagenic in bacteria [4,5], and does not appear to directly induce point mutations in mammalian cells [6]. Recently, the mutagenicity of As has been demonstrated in the human–hamster A_L cell assay [7] and the LY5178 $TK^{+/-}$ mouse lymphoma assay [8]. Unlike other mammalian cell assays, these assays are capable of detecting mutations caused by large chromosomal alterations, including deletions. Arsenic has also been shown to induce chromosome translocations and sister chromatid exchanges in vitro [9–11]. Studies on occupationally or environmentally exposed human populations have yielded evidence for cytogenetic effects [4,12–15], although interpretation of early studies requires caution because of methodological problems [4]. It has also been shown that As can potentiate the mutagenic effects of carcinogens, including ultraviolet light [16], ethyl methanesulfonate [17], and diepoxybutane [10]. Little is known about the factors influencing the metabolism of As in humans and its relationship to toxic effects. Recently, Vahter et al. [18] have shown a unique metabolism of As in native Andean women in a geographically isolated region of north–western Argentina and suggest that individual variation in methylation should be taken into consideration in the risk assessment process.

The US Environmental Protection Agency (EPA) is considering lowering the maximum contaminant level (MCL) for arsenic in drinking water from the present value of 50 μg/l. This current value was established in the 1940s by the US Public Health Service by applying a safety factor to human toxicity data and did not consider the potential carcinogenicity of arsenic. More recently, the EPA conducted a quantitative risk assessment for arsenic in drinking water [19] based on data from Taiwan [20]. The study of Tseng et al. [20] used subjects from an agrarian population exposed to high levels (>170 μg/ml) of arsenic in drinking water from artesian wells, and evaluated the risk for cancer. If one uses an excess lifetime cancer risk of 1 in 10,000, and applies a linearized multistage model to the Taiwan data, the acceptable MCL would be 2 μg/l. If this level is established as the MCL for arsenic in finished drinking water, the cost to bring all existing US water supplies into compliance runs into the billion dollar range [21]. This potential cost in combination with uncertainties in the Taiwanese study have led the Agency to consider carefully whether a linearized multistage model is appropriate in predicting the low-dose region of the arsenic dose–response curve.

Our long-term goal is to identify a biomarker that would provide information on the shape of the dose–effect relationship at low exposure levels (equivalent to 2–50 μg/l in drinking water) for use in As risk assessment. The purpose of this study was to investigate the feasibility of using mutagenesis of the hypoxanthine guanine phosphoribosyltransferase (*HPRT*) gene in T-lymphocytes as a quantitative biomarker for detection of biological damage caused by As. Because of the potentiating effects of As (as described above), the possible biological effects of As may be much more significant when exposure occurs in a complex mixture of other potentially mutagenic compounds (as occurs in humans) than would be expected based upon in vitro mutagenesis studies with pure As. The population that we chose to examine was exposed to much higher levels of As than is typically found, but would provide a good test of the responsiveness of *HPRT* to environmental As exposure. If effects were seen at these relatively high levels, then it may be fruitful to examine populations with lower, more relevant exposures. Alternatively, if no or little effect on *HPRT* mutagenesis was found, then it might be concluded that the *HPRT* biomarker would not be useful in providing insight into the shape of the human dose–response curve for low exposures to As.

2. Materials and methods

2.1. Study population

This study was carried out on workers employed at a copper roasting plant located a few miles northeast of Antofagasta, Chile. The plant had been operating since August 1988 and employed approximately 350 workers, of whom 100 were directly exposed to As in the oven areas. Other workers were involved in administration, construction, maintenance, and transport/loading. The plant operates 24 h/day, 7 days/week, and processes 100 tons of

copper concentrate each day. The copper concentrate roasted in this facility contained 30% copper and 0.1%–8% As. The purpose of the roasting operation was to further concentrate the copper by reducing the As concentration of the ore. Sulphur was added to the copper concentrate and the resulting mixture roasted under anaerobic conditions. Insoluble As_2S_3 was precipitated and recovered.

In January 1993, when this study was initiated, the plant had been in operation for 63 months. The working population at the plant was very stable, with the mean duration of employment at that time being 43 months (range: 4–60 months). There was an on-going medical surveillance program for all workers. Urinary total As levels were determined every 3 months, and for production workers (high exposure occupations) the average was 0.443 μg/ml with a range of 0.09–1200 μg/ml.

2.2. Sample collection and shipment

All participants volunteered and informed consent was obtained as approved by the Mt. Sinai Institutional Review Board. Thirty-milliliter blood samples were obtained by venipuncture into two heparinized tubes. These samples were shipped daily in insulated containers without refrigeration to the EPA Laboratory in Research Triangle Park, NC. All samples reported here arrived within 48 h of being drawn. Upon arrival of the samples, lymphocytes were immediately isolated using lymphocyte separation medium (Organon Teknica-Cappel, Durham, NC). Cells were cryopreserved using a programmable Cryomed freezer and stored in liquid nitrogen.

2.3. Urinary as measurement

Data on urinary total As was obtained from the plant's management for all workers as of June 1993. Urine samples were also obtained at the time blood samples were drawn, and urinary total As concentrations were determined as described [22].

2.4. Mutant frequency

Quantitative determination of *HPRT* mutant T-cells was as described by Moore et al. [23]. Briefly, the lymphocytes were thawed and cultured for 21–24 h at 37°C in 5% CO_2 at 1×10^6 lymphocytes per ml in RPMI 1640 medium containing 20% HL-1 medium, 5% fetal bovine serum, and 1 μg/ml phytohemagglutinin. The cells were then pelleted, counted, and plated at 1, 2, 5, and 10 cells per well in 96-well plates for determination of cloning efficiencies. The remainder of the cells were plated with 10 μM 6-thioguanine at 5×10^3 cells per well for mutant selection. Colonies were counted after 10–12 days, and the mutant frequency was calculated using the Poisson relationship.

2.5. Mutant clone isolation and analysis

HPRT mutant clones were isolated directly from the culture well and analyzed for deletion mutations by PCR. Crude cellular extracts were prepared as described by Moore et. al. [23] and the samples were stored at −80°C until use. Five to ten microliters, representing DNA from $1–4 \times 10^3$ cells, were used in a 50-μl multiplex *HPRT* PCR assay for amplification of all nine exons [24]. The primer concentrations and reaction conditions were optimized for this assay of crude cell extracts as described by Fuscoe et al. [26]. One-half of the reactions were analyzed by 1.4% agarose, 0.5× Tris–borate gel electrophoresis [25]. Mutants that displayed a wild-type pattern contained alterations such as point mutations below our detection limit. Missing exon fragments or shifts in the sizes of the *HPRT* exons indicated insertions or deletions. Mutants that produced weak or no *HPRT* exon fragments were candidates for total *HPRT* gene deletions. These extracts were further examined by co-amplification of the *K-RAS* gene plus *HPRT* exon 6 as described [26]. The presence of *K-RAS* plus the absence of *HPRT* exon 6 verified a total *HPRT* gene deletion.

2.6. Cytogenetics

Purified lymphocytes were placed into culture tubes at $0.5–1 \times 10^6$/ml of RPMI 1640 medium containing 15% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 1% phytohemagglutinin, and incubated at 37°C in 5% CO_2. Colcemid (0.1 μg/ml) was added for the 2 h preceding harvest at 48 h of culture. Centrifuged cells were treated with hypotonic KCl (0.075 M) for 12 min,

fixed with methanol:acetic acid (3:1), and dropped onto slides. Slides were stained for 6 min in 4% Gurr Giemsa. Fifty to one hundred metaphase spreads were analyzed from each individual for gross chromosome aberrations, including chromatid and chromosome breaks and rearrangements as described [8]. Chromatid and chromosome gaps were not included as aberrations.

3. Results

3.1. As exposure

Fifteen male workers participating in this study were categorized as being exposed to low, medium, or high levels of arsenic based on their job titles at the copper roasting operation in Antofagasta, Chile (Table 1). The workers ranged in age from 24 to 66 years with a mean age of 38 years. Two independent measures of exposure were assessed: (1) determination of total arsenic in a urine sample obtained at the time of blood collection and (2) analysis of urinary As levels from the copper roaster company's occupational surveillance records. These measurements generally supported the exposure categories based on job title, with the low exposure group having a mean urinary total As at the time of the blood draw of 0.12 μg/ml, the medium group 0.19 μg/ml, and the high group 0.26 μg/ml. The average of the last two quarterly measurements paralleled these values, indicating a relatively constant exposure. Fig. 1 shows the urinary total As measurement from the company records for these fifteen workers. When large exposures were indicated, workers were often reassigned to lower exposure jobs. For example, subject 9 worked in the smelter operation (high exposure group) but was moved to an office job (low exposure group) after a high urinary As determination (see Fig. 1). Subsequent measures were low.

Table 1
Relationship between exposure category and urinary arsenic levels

Exposure category[a]	Subject number[b]	Age (year)	Job title	Study As level (μg/ml)[c]	Prior two quarter As level (μg/ml)[d]	Range (μg/ml)[e]
Low	3	46	Guard	0.14	0.21	0.02–0.36
	9	38	Office worker[f]	0.11	0.11	0.10–0.88
	10	28	Office worker	0.10	0.17	0.17–0.47
	13	42	Office worker	NA[g]	0.19	0.04–0.36
				Mean = 0.12	Mean = 0.17	
Medium	1	35	Welder	0.19	0.27	0.11–0.35
	2	35	Mechanic	0.19	0.30	0.06–0.50
	6	25	Driver	0.14	0.33	0.08–0.57
	7	36	Electrician	NA	0.26	0.02–0.99
	11	66	Carpenter	0.09	0.33	0.14–0.54
	12	24	Safety officer	0.09	0.26	0.13–0.38
	14	43	Welder	0.42	0.30	0.15–0.81
	15	31	Painter	0.18	0.25	0.02–0.55
				Mean = 0.19	Mean = 0.29	
High	4	45	Roaster operator	0.19	0.25	0.09–0.72
	5	38	Roaster operator	0.21	0.44	0.18–0.60
	8	35	Roaster operator	0.38	0.47	0.18–0.57
				Mean = 0.26	Mean = 0.39	

[a] Exposure category was based on job title.
[b] Identification code.
[c] Urinary As measurements obtained at the time of the blood collection.
[d] The mean of last two quarterly measurements obtained from company surveillance records.
[e] The range of As levels for the 15 quarterly measurements.
[f] This worker was recently reassigned to an office position from a smelter position due to high urinary As level.
[g] Not available.

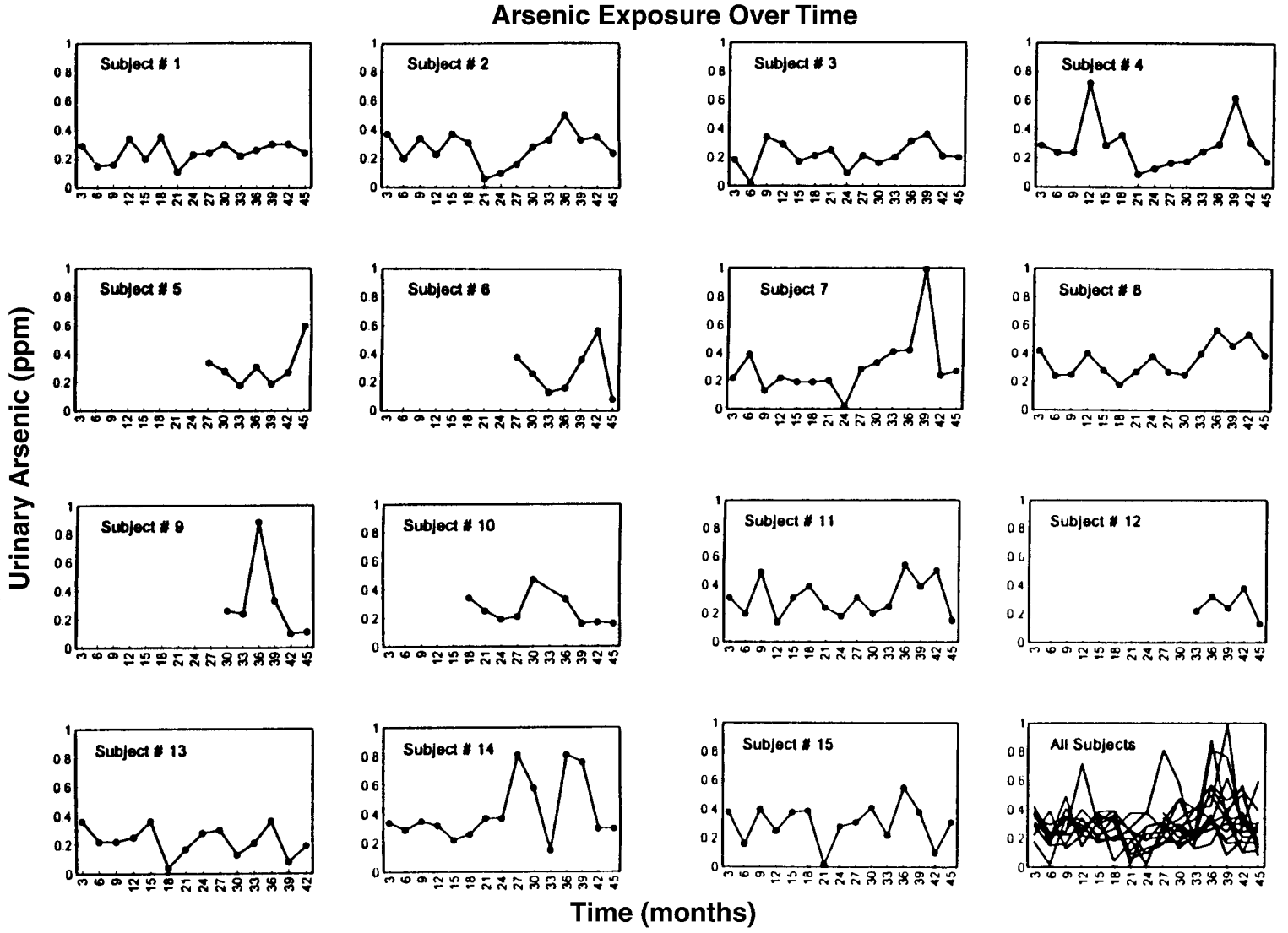

Fig. 1. Workers were monitored by a quarterly surveillance program for urinary As levels. Shown are urinary total As values in ppm (μg/ml) obtained from company records covering 45 months prior to the start of this study (after the 45-month time point). Values are from the 15 workers analyzed for *HPRT* mutations. The last panel shows the plots from all 15 individuals.

3.2. HPRT mutant frequency

The frequency of *HPRT*-deficient T-lymphocytes in the peripheral blood of the 15 subjects was determined (Table 2). The mean *HPRT* mutant frequencies for the three As exposure groups were: low exposure occupations, 9×10^{-6}, range $6–13 \times 10^{-6}$; medium exposure occupations, 11×10^{-6}, range $4–18 \times 10^{-6}$; high exposure occupations, 24×10^{-6}, range $9–42 \times 10^{-6}$. Although there was a dose-dependent increase in the mean mutant frequency, there was significant overlap among the mutant frequency values of the three groups. Also, all values except for one (42×10^{-6} in the high exposure group) were within the range we have previously found in unexposed populations [23], as well as within the range for healthy adult populations throughout the world [27]. Because of the small number of subjects examined, no statistical analysis was appropriate. The cloning efficiency of the lymphocytes ranged from 0.05 to 0.47 (mean = 0.23, standard deviation = 0.13).

3.3. Mutation analysis

One hundred fifty-six *HPRT* mutant lymphocyte clones isolated from nine individuals were analyzed for large genetic changes in the *HPRT* gene by multiplex PCR. In this assay, primers flanking each exon were used to amplify the region, and the resultant PCR products were displayed on an agarose gel. Small deletions were detected as the absence, or shift in size, of the exon-specific PCR products (see Fig. 2). Absence of all exons, indicating a deletion of the entire gene, was verified by co-amplifying a nonselected gene segment (*K-RAS* on chromosome 12) with *HPRT* exon 6 as described previously [26]. Successful amplification of *K-RAS* but not *HPRT*

Table 2
HPRT mutant frequencies from 15 individuals working in a Chilean copper roasting operation

Exposure category[a]	Subject number[b]	Plating efficiency (%)[c]	Selection plates (+TG)[d]		*HPRT* mutant frequency ($\times 10^{-6}$)[e]
			Negative wells	Total wells	
Low	3	10	670	672	6
	9	37	1322	1344	9
	10	5	1244	1248	13
	13	47	1507	1536	8
					Mean = 9
Medium	1	33	2148	2184	10
	2	17	1336	1344	7
	6	20	1513	1535	14
	7	10	382	384	10
	11	12	2282	2298	12
	12	20	1322	1344	16
	14	37	835	864	18
	15	29	1431	1440	4
					Mean = 11
High	4	43	788	864	42
	5	30	2412	2496	22
	8	43	894	912	9
					Mean = 24

[a] Exposure category was based on job title.
[b] Identification code.
[c] 1, 2, 5, and 10 lymphocytes were plated per well in 96-well microtiter plates in non-selective medium.
[d] 5×10^3 lymphocytes were plated per well in 96-well microtiter plates in thioguanine (TG)-containing medium.
[e] Mutant frequency was calculated as the ratio of the cellular cloning efficiency in the presence and the absence of TG.

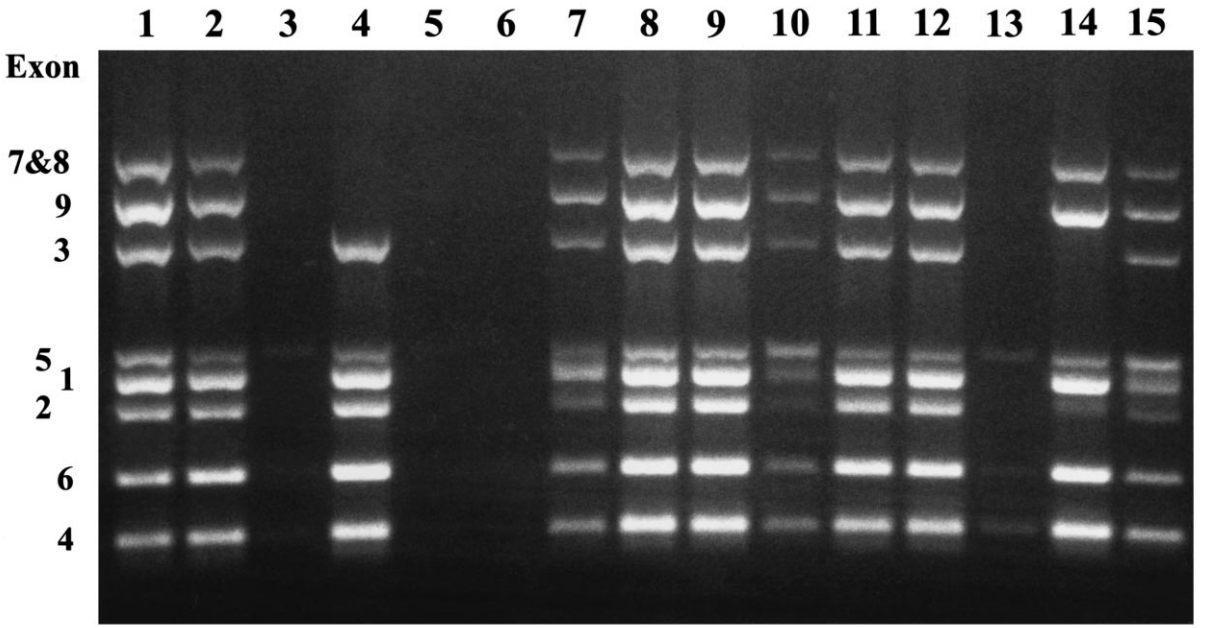

Fig. 2. Multiplex PCR analysis of *HPRT*-deficient lymphocyte clones from subject 8 in the high exposure group. The wild-type pattern is shown in lane 1 (human placenta DNA). Lanes 2–15 are from individual clones from subject 8. Lane 4 shows deletion of exons 7–9, and lane 14 shows deletion of exons 2 + 3. Samples shown in lanes 3, 5, 6, and 13 were reanalyzed using more DNA in the PCR, and the sample from lane 13 then showed a wild-type pattern. Weak or no fragments were seen in the others. The samples from lanes 3, 5, and 6 failed to support amplification of the unlinked K-RAS gene segment and thus the DNA was too dilute or inhibited the PCRs; they were not included in the mutant summary in Table 3.

Table 3
HPRT mutation characteristics from 15 individuals working in a Chilean copper roasting operation

Exposure category[a]	Subject number[b]	*HPRT* mutant frequency ($\times 10^{-6}$)	Number of mutants analyzed	Partial *HPRT* deletions[c]		Total *HPRT* deletions (%)[d]
				Number (%)	Exon(s) deleted	
Low	3	6	–			
	9	9	11	2 (18)	2 + 3, 2 + 3	0
	10	13	–			
	13	8	22	1 (5)	9	0
		Mean = 9				
Medium	1	10	19	2 (11)	2 + 3, partial 2	0
	2	7	–			
	6	14	12	0		1 (8)
	7	10	–			
	11	12	–			
	12	16	12	1 (8)	partial 1	0
	14	18	13	1 (8)	2 + 3	2 (15)
	15	4	–			
		Mean = 11				
High	4	42	18	2 (11)	1 + 2, partial 3	0
	5	22	37	5 (14)	partial 1, partial 4, 4–9, 4–9, 2–9	1(3)
	8	9	12	3 (25)	2 + 3, 1 + 2, 7–9	0
		Mean = 24				

[a] Exposure category was based on job title.
[b] Identification code.
[c] The nine *HPRT* exons were examine by PCR as described in the Materials and Methods section. One or more (but not all) exons were altered in size or absent.
[d] All nine *HPRT* exons were deleted as determined by PCR as described in the Materials and Methods section.

exon 6 confirmed the total deletion. The results of this molecular analysis of the mutants are shown in Table 3. A total of 33 mutants from two individuals in the low exposure group, 56 mutants from four individuals in the medium exposure group, and 67 mutants from three individuals in the high exposure group were analyzed. The percentages of mutants with partial deletion of the *HPRT* gene were 9.1, 7.1 and 14.9 in the low, medium, and high exposure groups, respectively. Although there is an apparent increase in the proportion of partial deletion mutations at the highest exposure, the average for the low, medium, and high exposure groups (10.9%) does not differ markedly from the previously reported value of 9.9% for a group of 223 mutants isolated from healthy adults [28]. The number of mutants with total *HPRT* gene deletions was small with 0 (0%), 3 (4.5%), and 1 (3%) found in the low, medium, and high exposure groups, respectively. Thus, there was no dose-dependent increase, and these values are not elevated above the previously reported 7.2% among the 223 mutants previously analyzed from healthy adults [28]. The types of deletions are summarized in Table 3.

Table 4
Chromosome aberrations in peripheral blood cells of five individuals working in a Chilean copper roaster operation

Exposure category[a]	Subject number[b]	Aberrant cells (%)[c]
Low	16	2
	17	2
	18	2
High	19	3
	20	0

[a] Low exposure group included office worker, guard, and laboratory technician. High exposure group included smelter and roaster operators.
[b] Identification code.
[c] Percentage of cells with chromosome aberrations; 50–100 metaphases were examined.

3.4. Chromosome aberrations

Peripheral blood lymphocytes from five subjects (three from low exposure occupations and two from high exposure occupations) were examined for chromosome aberrations (Table 4). These individuals were not part of the mutant frequency analysis. No evidence of increased chromosome aberrations was found.

4. Discussion

Here, we describe the results of a small pilot study to assess mutation at the *HPRT* locus in peripheral blood lymphocytes as a biomarker for genetic effects caused by As. This biomarker has been used extensively in examining the potential genetic effects produced by environmental and occupational exposures [27,29]. Advantages of this marker are the ready access of tissue, the quantitative nature of the assay, and the ability to propagate the mutant T-cell clones for subsequent detailed molecular analysis. Limitations of this biomarker include the relatively small target size and its functional or physical hemizygosity which limit the ability to recover large chromosomal alterations. This marker appears to be useful mainly for detection of point mutations, although it has been shown that deletions of more than a megabase can be recovered [30,31]. Whereas in vitro mutagenesis studies with As have shown As to be clastogenic, such studies have failed to provide strong evidence that As can cause point mutations (see references in Section 1) which may suggest that the *HPRT* biomarker may not be responsive to As. Despite these in vitro findings, additional studies have shown that As can synergistically enhance the mutagenicity of the point mutagen ultraviolet light [16]. It, therefore, seemed plausible that the *HPRT* biomarker may respond to As exposure coupled with possible environmental exposures to other mutagens/carcinogens, and this study was initiated to evaluate this possibility.

The study population we chose consisted of workers in a copper roasting plant in Antofagasta, Chile. Company surveillance records showed a sustained high exposure over a period of years as reflected by quarterly urinary As measurements. These high exposure values were confirmed by urinary As measurements performed at the time of blood collection for the *HPRT* assay. In addition to the As exposure from the copper roasting plant, other sources of As were drinking water and diet (seafood), although these probably accounted for a relatively small proportion of the total exposure. Other exposures included cigarette smoking, with all workers in the study population being smokers (60%) or ex-smokers (40%). Cigarette consumption per day, however, was moderate at 3–20.

From this pilot study, we conclude that the *HPRT* biomarker may not be useful for detecting potential genetic effects of low-level As exposure. Even though there was a slight increase in the *HPRT* mutant frequency at the highest exposure, this exposure level was exceedingly high. Our ultimate goal is to identify a biomarker for detecting potential genetic effects of As that could readily be applied to examining populations exposed to much lower levels. In particular, effects occurring at exposure levels less than the current US drinking water standard of 50 μg/l are a high priority. Recently, Maki-Paakkanen et al. [14] found urinary total As concentrations to be approximately 0.18 μg/ml when a population is exposed to approximately 400 μg/l in drinking water. This urinary As value is comparable to the values found in the Chilean copper roaster workers (see Table 1). Thus, the urinary As levels reported in the copper roasting plant population are comparable to exposures of perhaps 10 times the 50 μg/l drinking water level. Since marginal effects on the frequency of *HPRT*-deficient T-cells were seen in this study, it is unlikely that effects will be seen at the lower exposure levels.

Because As appears to cause chromosome aberrations both in vitro and in vivo, suggesting DNA breakage, we also examined the *HPRT*-deficient T-cells for large scale alterations (insertions and deletions) in the *HPRT* gene by multiplex PCR amplification of the nine exons. Even if the overall mutant frequency was not notably increased, the spectrum of mutations may be altered. Again, we did not find major effects on the frequency of partial *HPRT* gene deletions or on total *HPRT* gene deletions. A subclass of the partial *HPRT* gene deletions is due to the illegitimate activity of V(D)J recombinase which results in deletion of exons 2 + 3 [32]. In prior

studies, we have found that this type of mutation accounts for 2%–3% of all the *HPRT* mutations in adults [28,33], not different from the present study (3.2%). No evidence of chromosomal aberration induction was observed, although only a few individuals were examined. Other studies have required large sample size to show significant cytogenetic effects (compare Refs. [34,12]).

We would like to emphasize that the main question we are addressing in this pilot study is whether the *HPRT* biomarker would be useful for future studies aimed at detecting genetic changes caused by low-level environmental As exposures. The finding of little, if any, effect at the extremely high As exposures evaluated in this study suggests that *HPRT* may not be an optimal biomarker for low-level As exposures. This may not be too surprising in light of the lack of point mutagenic properties of As, and does not disparage the usefulness of the *HPRT* biomarker for detecting the genetic effects of other environmental pollutants. Indeed, genetic effects of potent mutagens have been readily detected with the *HPRT* system [27,29]. The question of whether As exposure is capable of inducing a statistically elevated *HPRT* mutant frequency was not addressed in this small pilot study. Although we found an apparent increase in *HPRT* mutant frequency at the highest exposure level, only one value was higher than historical controls. It is therefore possible that increasing the sample size may allow detection of a statistically significant mutagenic effect on the *HPRT* gene at these very high exposure levels. In light of the recent suggestion of possible polymorphism in the methyltransferases involved in As metabolism [18,35], it may be fruitful for these studies to focus on human populations with altered As metabolism. However, our goal is to identify biomarkers for future investigations that will provide useful information on the shape of the dose–response relationship at exposure levels of 10–100 fold below even the lowest exposure level in this study population; it appears unlikely that effects will be seen on *HPRT* mutant frequencies at such low As exposures when such a small (if any) effect was seen at the high exposures.

In conclusion, the *HPRT* T-cell assay does not appear to have sufficient sensitivity to be useful as a biomarker of genetic effects caused by low-level As exposure. This is in agreement with a study by Ostrosky-Wegman et al. [34], who used the short-term *HPRT* assay utilizing BrdU incorporation to find no significant induction of mutations in a population exposed to 390 μg As/l in drinking water. Other biomarkers will be needed in order to evaluate possible biological effects of low-level environmental As exposure.

Acknowledgements

This article has been reviewed by the US EPA and approved for publication. Approval does not signify that the contents necessarily reflect the views or policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

[1] IARC, Some metals and metallic compounds, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 23 IARC, Lyon, 1980.
[2] IARC, Arsenic and arsenic compounds, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 7 IARC, Lyon, 1987, Suppl.
[3] A.H. Smith, M. Goycolea, M.L. Biggs, Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water, Am. J. Epidemiol. 147 (1998) 660–669.
[4] IARC, Genetic and related effects, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 6 IARC, Lyon, 1987, Suppl.
[5] E.T. Snow, Metal carcinogenesis: mechanistic implications, Pharmacol. Ther. 53 (1992) 31–65.
[6] T.G. Rossman, D. Stone, M. Molina, W. Troll, Absence of arsenite mutagenicity in *E. coli* and Chinese hamster cells, Environ. Mutagen. 2 (1980) 371–379.
[7] T.K. Hei, S.X. Liu, C. Waldren, Mutagenicity of arsenic in mammalian cells: role of reactive oxygen species, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 8103–8107.
[8] M.M. Moore, K. Harrington-Brock, C.L. Doerr, Relative genotoxic potency of arsenic and its methylated metabolites, Mutat. Res. 386 (1997) 279–290.
[9] D. Jacobson-Kram, D. Montalbano, The reproductive effects assessment group's report on the mutagenicity of inorganic arsenic, Environ. Mutagen. 7 (1985) 787–804.
[10] J.K. Wiencke, J.W. Yager, Specificity of arsenite in potentiating cytogenetic damage induced by the DNA crosslinking agent diepoxybutane, Environ. Mol. Mutagen. 19 (1992) 195–200.

[11] R.E. Rasmussen, D.B. Menzel, Variation in arsenic-induced sister chromatid exchange in human lymphocytes and lymphoblastoid cell lines, Mutat. Res. 386 (1997) 299–306.
[12] M.E. Gonsebatt, L. Vega, A.M. Salazar, R. Montero, P. Guzman, J. Blas, L.M. Del Razo, G. Garcia-Vargas, A. Albores, M.E. Cebrian, M. Kelsh, P. Ostrosky-Wegman, Cytogenetic effects in human exposure to arsenic, Mutat. Res. 386 (1997) 219–228.
[13] L.E. Moore, A.H. Smith, C. Hopenhayn-Rich, M.L. Biggs, D.A. Kalman, M.T. Smith, Micronuclei in exfoliated bladder cells among individuals chronically exposed to arsenic in drinking water, Cancer Epidemiol., Biomarkers Prev. 6 (1997) 31–36.
[14] J. Maki-Paakkanen, P. Kurttio, A. Paldy, J. Pekkanen, Association between the clastogenic effect in peripheral lymphocytes and human exposure to arsenic through drinking water, Environ. Mol. Mutagen. 32 (1998) 301–313.
[15] F.N. Dulout, C.A. Grillo, A.I. Seoane, C.R. Maderna, R. Nilsson, M. Vahter, F. Darroudi, A.T. Natarajan, Chromosomal aberrations in peripheral blood lymphocytes from native Andean women and children from northwestern Argentina exposed to arsenic in drinking water, Mutat. Res. 370 (1996) 151–158.
[16] J.L. Yang, M.F. Chen, C.W. Wu, T.C. Lee, Posttreatment with sodium arsenite alters the mutational spectrum induced by ultraviolet light irradiation in Chinese hamster ovary cells, Environ. Mol. Mutagen. 20 (1992) 156–164.
[17] K.Y. Jan, R.Y. Huang, T.C. Lee, Different modes of action of sodium arsenite, 3-aminobenzamide, and caffeine on the enhancement of ethyl methanesulfonate clastogenicity, Cytogenet. Cell Genet. 41 (1986) 202–208.
[18] M. Vahter, G. Concha, B. Nermell, R. Nilsson, F. Dulout, A.T. Natarajan, A unique metabolism of inorganic arsenic in native Andean women, Eur. J. Pharmacol. 293 (1995) 455–462.
[19] US EPA, Special report on ingested arsenic, EPA/625/3-87/013, Risk Assessment Forum, US Environmental Protection Agency, Washington, DC, 1988.
[20] W.P. Tseng, H.M. Chu, S.W. How, J.M. Fong, C.S. Lin, S. Yeh, Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan, J. Natl. Cancer Inst. 40 (1968) 453–463.
[21] J.B. Kempic, Arsenic removal technologies: an evaluation of cost and performance, in: C.O. Abernathy, R.L. Calderon, W.R. Chappell (Eds.), Arsenic, Chapman and Hall, London, 1997, pp. 393–405.
[22] M. Buratti, G. Calzaferri, G. Caravelli, A. Colombi, M. Maroni, V. Foa, Significance of arsenic metabolic forms in urine: Part I. Chemical speciation, Int. J. Environ. Anal. Chem. 17 (1984) 25–34.
[23] M.M. Moore, K. Harrington-Brock, L.J. Zimmerman, L.P. Burnette, T.W. Smith, R.B. Everson, J.P. O'Neill, J.C. Fuscoe, Quantification and molecular characterization of *HPRT* mutants of human T-lymphocytes, Environ. Health Perspect. 101 (Suppl. 3) (1993) 219–224.
[24] R.A. Gibbs, P.N. Nguyen, A. Edwards, A.B. Civitello, C.T. Caskey, Multiplex DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch–Nyhan families, Genomics 7 (1990) 235–244.
[25] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989.
[26] J.C. Fuscoe, L.J. Zimmerman, K. Harrington-Brock, M.M. Moore, Large deletions are tolerated at the *HPRT* locus of in vivo derived human T-lymphocytes, Mutat. Res. 283 (1992) 255–262.
[27] J. Cole, T.R. Skopek, Somatic mutant frequency, mutation rates and mutational spectra in the human population in vivo, Mutat. Res. 304 (1994) 33–105.
[28] J.C. Fuscoe, L.J. Zimmerman, K. Harrington-Brock, M.M. Moore, Multiplex PCR analysis of in vivo-arising deletion mutations in the *HPRT* gene of human T-lymphocytes, Environ. Mol. Mutagen. 23 (1994) 89–95.
[29] R.J. Albertini, J.A. Nicklas, J.P. O'Neill, Somatic cell gene mutations in humans: biomarkers for genotoxicity, Environ. Health Perspect. 101 (Suppl. 3) (1993) 193–201.
[30] J.C. Fuscoe, A.J. Nelsen, G. Pilia, Detection of deletion mutations extending beyond the *HPRT* gene by multiplex PCR analysis, Somatic Cell Mol. Genet. 20 (1994) 39–46.
[31] M.J. Lippert, J.A. Nicklas, T.C. Hunter, R.J. Albertini, Pulsed field analysis of *HPRT* T-cell large deletions: telomeric region breakpoint spectrum, Mutat. Res. 326 (1995) 51–64.
[32] J.C. Fuscoe, L.J. Zimmerman, M.J. Lippert, J.A. Nicklas, J.P. O'Neill, R.J. Albertini, V(D)J recombinase-like activity mediates *HPRT* gene deletion in human fetal T-lymphocytes, Cancer Res. 51 (1991) 6001–6005.
[33] J.C. Fuscoe, L.J. Zimmerman, K. Harrington-Brock, L. Burnette, M.M. Moore, J.A. Nicklas, J.P. O'Neill, R.J. Albertini, V(D)J recombinase-mediated deletion of the *HPRT* gene in T-lymphocytes from adult humans, Mutat. Res. 283 (1992) 13–20.
[34] P. Ostrosky-Wegman, M.E. Gonsebatt, R. Montero, L. Vega, H. Barba, J. Espinosa, A. Palao, C. Cortinas, G. Garcia-Vargas, L.M. del Razo *et al.*, Lymphocyte proliferation kinetics and genotoxic findings in a pilot study on individuals chronically exposed to arsenic in Mexico, Mutat. Res. 250 (1991) 477–482.
[35] G. Concha, B. Nermell, M.V. Vahter, Metabolism of inorganic arsenic in children with chronic high arsenic exposure in northern Argentina, Environ. Health Perspect. 106 (1998) 355–359.