Environmental Health Perspectives
Vol. 95, pp. 205–210, 1991

Arsenite as the Probable Active Species in the Human Carcinogenicity of Arsenic: Mouse Micronucleus Assays on Na and K Arsenite, Orpiment, and Fowler's Solution

by H. Tinwell,* S. C. Stephens,† and J. Ashby*

Sodium arsenite, potassium arsenite, and Fowler's solution (arsenic trioxide dissolved in potassium bicarbonate) are equally active in the mouse bone marrow micronucleus assay (~ 10 mg/kg by IP injection). The natural ore orpiment (principally As_2S_3) was inactive despite blood levels of arsenic of 300 to 900 ng/mL in treated mice at 24 hr. Sodium arsenite was active in three strains of mice. It is suggested that the human lung cancer observed among arsenic ore smelters and the skin cancer among people exposed therapeutically to Fowler's solution, have, as their common origin, the genotoxic arsenite ion AsO_2^-. The difficulty experienced when attempting to demonstrate rodent carcinogenicity for derivatives of arsenic suggests that the bone marrow micronucleus assay may act as a useful assay for potentially carcinogenic arsenic derivatives.

Introduction

Attention has recently been focused on the clastogenic activity of most of the human carcinogens (including arsenic) to the rodent bone marrow (*1,2*). The International Agency for Research on Cancer (IARC) (*3,4*) has classified arsenic and its derivatives as having sufficient evidence for carcinogenicity in man and limited evidence in animals, but the active species is not clear. Shelby (*1*) quoted the positive mouse bone marrow micronucleus assay data reported for sodium arsenite by Deknudt et al. (*5*), but an earlier negative bone marrow cytogenetic study on arsenic trioxide also exists (*6*). Given the range of arsenic derivatives to which man has been exposed and the uncertainty surrounding the active carcinogenic species (*7*), we decided to conduct mouse bone marrow micronucleus assays on a range of derivatives of arsenic.

There are two major associations between human cancer and exposure to derivatives of arsenic: lung cancer among arsenic ore smelters and skin cancer among people exposed therapeutically to Fowler's solution (potassium arsenite). Arsenic occurs in nature mainly in combination with sulfur, the main ores being arsenic trisulfide (orpiment, As_2S_3), arsenic disulfide (red orpiment or realgar, As_4S_4), and arsenopyrite (FeAsS). It also occurs in these forms and as arsenides (*e.g.*, *niccolite*, NiAs) as an impurity (up to 3%) in many nonferrous ores such as in those of zinc and copper.

Natural weathering of sulfide ores can lead to natural deposits of oxides of arsenic. Orpiment (As_2S_3) was mentioned by Aristotle and was being roasted to yield arsenic trioxide (white arsenic; As_2O_3) as early as the first century A.D. (*8*). However, apart from the roasting of FeAsS in Japan, the specific roasting of arsenic ores is not now commercially practiced. Rather, the incidental roasting of arsenic ores during the smelting of other nonferrous ores provides the major source of arsenic as arsenic trioxide trapped in flues. The main incidences of human cancer appear to be associated with the culling and further purification of these flue deposits.

Fowler's solution is prepared by dissolving As_2O_3 in aqueous potassium bicarbonate and is formally aqueous potassium arsenite. Its medicinal use was mainly for the treatment of a range of skin conditions, but it is no longer used therapeutically. There is therefore a possible link between the human lung cancer induced by the refining of As_2O_3 and the skin cancer induced by the application of potassium arsenite, which is prepared from As_2O_3. We therefore decided to repeat the positive mouse bone marrow micronucleus assay of Deknudt et al. (*5*) on sodium arsenite

*ICI Central Toxicology Laboratory, Alderly Park, Cheshire, U.K.
†ICI Colours and Fine Chemicals, Blackley, Lancashire, U.K.
Address reprint requests to J. Ashby, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK104TJ U.K.

along with assays on potassium arsenite, Fowler's solution, and orpiment. selected determinations were made of blood levels of arsenic in treated animals in order to relate genetic activity to bioavailability of arsenic.

Materials and Methods

Nomenclature of Arsenic and Its Derivatives

Arsenic was known to the ancient Greeks in the form of the poisonous gold/red-colored sulfide ore (*Zarnig*, Iranian for gold; *arrhen*, Greek for virile, thus, *arsenikon* for the gold-hued poison, As_2S_3, the Latin *auri pigmentum* led to the name orpiment). There is the potential for confusion with the terminology applied to arsenites. In chemistry books written at the turn of the century (*9*), arsenious acid is given as $As(OH)_3$, thus, sodium arsenite is Na_2AsO_3. A dehydrated acid is also described as *meta*-arsenious acid As(O)OH, whose sodium salt would be sodium *meta*-arsenite, $NaAsO_2$. These separate terminologies are still used, but the separate existence of arsenites and *meta*-arsenites is no longer considered valid. Thus, the 11th edition of the *Merck Index* (*10*) lists sodium *meta*-arsenite as an alternative name to sodium arsenite, providing an approximate structure of $NaAsO_2$. Potassium arsenite is listed as being $KAsO_2$:$HAsO_2$ and the *meta*-arsenite is not mentioned. Likewise, the *Merck Index* names $KAsO_2$ as the product formed from As_2O_3 in Fowler's solution, i.e., potassium arsenite. Confirming this confusion of terms, Scheele's green, copper hydrogen arsenite, is given by Merck in both of the above forms: $CuHAsO_3$ and $Cu(AsO_2)$. In this paper the names and molecular species used are sodium arsenite ($NaAsO_2$), potassium arsenite ($KAsO_2$:$HAsO_2$), arsenious trioxide (As_2O_3), Fowler's solution (regarded as potassium arsenite), and orpiment (As_2S_3). In the final experiment (Experiment 8 in Table 1) all dosed animals received equimolar arsenic, independent of the chemical species under test.

Chemicals

Cyclophosphamide (CP) was purchased from the Sigma Chemical Company (Poole, Dorset, U.K.), sodium and potassium arsenite were obtained from BDH Chemicals Ltd.(Poole, Dorset, U.K.). Fowler's solution was prepared by dissolving As_2O_3 (Ventron, Alfa, Coventry, U.K.) (1 g) in water (50 mL) and a solution of potassium bicarbonate (0.76 g) in water (50 mL). Ethanol was added to produce a 3% solution. A sample of this solution (760 μL) was added to water (10 mL) for the dosing solution (10 mL/kg gave equimolar arsenic to $NaAsO_2$ [10 mg/kg]). Orpiment was purchased from a mineral shop in Brussels, but the address was not noted and its location cannot be found. The sample used was bright yellow with red streaks and metallic veins. The yellow regions are As_2S_3, and the orange-colored ones are realgar (As_2S_4). Leaves of arsenic metal could be seen by their ability to reflect light. Inductively coupled plasma emission spectrometry revealed a composition of approximately 61.6% arsenic, 38.5% sulfur, and 0.5% antimony (theoretical values for As_2S_3 are 61.0% As and 39.0% S). The mineral was friable and was ground to a fine powder using a pestle and mortar. The fragments of metallic arsenic were removed. The yellow powder was added to corn oil (experiment 1). In experiments 2, 6, and 7, the corn oil suspension was ball-milled for either 24 hr (experiment 2) or 72 hr (experiments 6 and 7; Table 1). Finally, the suspensions were homogenized using an ultra-Turrax homogenizer (*11*) for 0.5 hr immediately before dosing. In the arsenic determinations, the following reagents were used: nickel nitrate hexahydrate, analar grade (BDH Chemicals, Ltd.) Triton X-100 (BDH Chemicals Ltd.), arsenic stock solution, 1000 μg/mL, spectrosol grade (BDH Chemicals Ltd.).

Animals and Dosing

Male CBA/CafAP/Alpk mice (experiments 1, 2, 5, 6, and 7), male BALB/cfBL10/Alpk mice (experiments 3 and 4) and male C57BL/6JfBL10/Alpk mice (experiment 8) age 8 to 12 weeks were used. Animals were housed and maintained as described previously (*12*) and were allowed Porton diet and water *ad libitum*. With the exception of experiment 1, each animal received a single oral or IP dose (10 mL/kg or as stated below) of the test chemical 24 hr before sacrifice. Animals used in experiment 1 received three daily oral gavages of the test compounds, and the bone marrow was sampled 24 hr after the final dose. The dose-level of orpiment (experiments 2, 6, 7) was determined by estimating the amount of material remaining from the original amount ball-milled, i.e., the suspension was removed and its volume measured and any remaining solid determined gravimetrically after washing with acetone and drying. Adjustments to the dosing volume were then made to give a maximum dose of 500 mg/kg orpiment. To ease dosing, orpiment was administered using a dosing volume of 20 mL/kg body weight in experiments 2, 6, and 7, as was corn oil in experiment 7. The dose level of 500 mg/kg suspension used in experiment 7 (Table 1) was analyzed for arsenic (see below) and found to be within 95% of theory (i.e., 1.2% arsenic). Initial dose levels of sodium arsenite (used as a positive control) were based on literature values (*5*). However, its initial inactivity in the mouse bone marrow (experiment 2) led to a more in-depth evaluation of its maximum lethal dose (MLD) and optimum route and vehicle (experiments 3, 4, and 5). A combination of a single IP injection of 10 mg/kg (80% 4-day MLD) sodium arsenite dissolved in distilled water gave optimum results (experiments 4 and 5). CP was used at a dose level previously used in this laboratory (*13*).

Micronucleus Assays

Bone marrow smears were prepared as described previously (*14*) with a minor alteration. Briefly, the femoral cells were flushed out with fetal calf serum (FCS; Northumbria Biologicals, Northumberland, U. K.) containing 25 mM EDTA, resuspended and filtered through bolting cloth (150 μm; Henry Simon Ltd., Stockport, U.K.). After spinning (2000 rpm for 5 min), the resultant marrow button was resuspended in a small volume of supernatant, and smears were prepared by placing a drop of this suspension onto the center of a methanol-cleaned slide. A second slide placed on top of the first spread this drop by capilliary action. The two resulting smears were then slid apart, air dried, and fixed in methanol for 5 min. These were stained with acridine orange as described previously (*14*). A total of 2000 polychromatic erythrocytes (PE) per animal were scored for micronucleated PE (MPE). The erythropoietic ratio (poly-

Table 1. Results of eight experiments to evaluate and compare the micronucleus-inducing ability of derivatives of arsenic.[a]

Experiment no.	Compound	Dose, mg/kg/route	No. of animals	MPE/1000 PE		PE/NE ± SD	As/mL of combined blood from test group, ng/mL
				Individual animal data	Group mean ± SD		
Experiment 1	Corn oil	10 mL/oral	6	0.5, 2, 4, 1, 1, 3	1.9 ± 1.4	1.1 ± 0.2	ND
3 daily doses,	Orpiment	100/oral	6	2, 1.5, 2.5, 2.5, 1.5, 1.5	1.9 ± 0.5	0.9 ± 0.1	ND
CBA mice	Cyclophosphamide	10/oral	3	36.5, 18, 36	30.1 ± 10.5†	0.4 ± 0.2†	ND
Experiment 2	Corn oil	10 mL/oral	5	1.5, 2.5, 0.5, 1.5, 2.5	1.7 ± 0.8	0.9 ±0.1	< 100
1 dose,	Corn oil	10 mL/IP	5	1, 0.5, 2, 0.5, 0.5	0.9 ±0.65	1.0 ± 0.1	< 100
CBA mice	Na arsenite	10/IP	5	1.5, 1.5, 1, 1, 2.5	1.5 ± 0.6	1.0 ± 0.1	< 100
	Na arsenite	*5/IP*	5	3.5, 1.5, 2, 3, 1	2.2 ± 1.0	1.0 ± 0.1	< 100
	Na arsenite	2.5/IP	5	3, 2, 1, 3.5, 2	2.3 ± 1.0	1.1 ± 0.03	< 100
	Orpiment	500/oral	5	0.5, 2.5, 2.5, 1, 1.5	1.6 ± 0.9	1.0 ± 0.1	900
Experiment 3							
1 dose,	Na arsenite	5/IP	3	7.5, 9, 8	8.2 ± 0.8	0.9 ± 0.1	ND
BALB/c mice	(in water)						
Experiment 4	Distilled water	10 mL/IP	4	3, 3.5, 3.5, 2.5	3.1 ± 0.5	1.1 ± 0.2	ND
1 dose,	Na arsenite	5/IP	5	8,4.5,4,4.5	5.25 ± 1.8*	1.1 ± 0.1	ND
BALB/c mice	(in water)	10/IP	5	4, 7, 5, 8.5, 5	5.9 ± 1.8*	0.95 ± 0.2	ND
	Corn oil	10 mL/IP	5	1, 1, 2.5, 2.5, 1.5	1.7 ±0.8	1.15 ± 0.15	ND
	Na arsenite	*10/IP*	5	2, 4, 2.5, 1, 1.5	2.2 ± 1.15	1.1 ± 0.1	ND
Experiment 5	Distilled water	10 mL/IP	6	3, 3, 2, 5, 2.5, 1	2.75 ± 1.3	1.0 ± 0.1	ND
1 dose,	Na arsenite	10/IP	6	14, 13, 9.5, 11, 13.5, 7.5	11.4 ± 2.6†	0.5 ± 0.1†	ND
CBA mice	(in water)						
Experiment 6	Corn oil	10 mL/oral	6	2, 2, 2, 1.5, 2, 1	1.75 ± 0.4	1.1 ± 0.1	< 100
1 dose,	Orpiment[b]	160/oral	6	4, 5, 3, 4.5, 3, 2	3.6 ± 1.1†	1 ± 0.1	390
CBA mice	Na arsenite	10/IP	6	9.5, 6.5, 9, 8, 9, 10	8.7 ± 1.25†	0.6 ± 0.01†	< 100
	(in water)						
Experiment 7	Corn oil	20 mL/oral	5	3.5, 3, 3.5, 2, 2.5	2.9 ± 0.65	1.0 ± 0.2	< 100
1 dose,	Orpiment	500/oral	5	5, 5, 4.5, 3, 2.5	4 ± 1.2	0.8 ± 0.1*	650
CBA mice	Na arsenite	10/IP	5	3, 2.5, 1.5, 1, 2.5	2.1 ± 0.8	1.1 ± 0.2	< 100
	Na arsenite	10/IP	5	8.5, 5, 11, 4.5, 12.5	8.3 ± 3.5†	0.7 ± 0.1*	< 100
	(in water)						
Experiment 8	Distilled water	10 mL/IP	5	1.5, 3, 3.5, 2.5, 4.5	3.0 ± 1.1	0.95 ± 0.1	ND
1 dose,	*(3% EtOH)*						
C57BL6 mice	Na arsenite[c]	10/IP	5	8.5, 15.5, 25, 27.5, 11.5	17.6 ± 8.3†	0.6 ± 0.2†	ND
	(in water)						
	K arsenite	9.8/IP	5	11, 19.5, 15, 14.5, 9.5	13.9 ± 3.9†	0.85 ± 0.2	ND
	(in water)						
	Fowler's solution[c]	7.6/IP	5	10, 15, 13.5, 12, 8.5	11.8 ± 2.6†	1.0 ± 0.1	ND
	(expressed as As_2O_3)						
	Cyclophosphamide	65/IP	3	58.5, 75.5, 89.5	74.5 ± 15.5†	0.9 ± 0.03	ND
	(in water)						

[a]Unless stated otherwise, test agents were dosed in corn oil; water refers to solution in distilled water. MPE, micronucleated polychromatic erythrocytes; PE, polychromatic erythrocytes; NE, normochromatic erythrocytes. MPE/1000 PE based on 2000 PE per animal.

[b]500 mg/kg intended, less suspended than expected.

[c]Each formulation delivering equimolar arsenic as As; 5.76 mg/kg; results statistically insignificant from each other using two-tailed Student's *t*-test.

*$p < 0.05$, one-tailed Student's *t*-test.

†$p < 0.01$, one-tailed Student's *t*-test.

chromatic/normochromatic [PE/NE]), was determined among 1000 erythrocytes (PE and NE).

Determination of Arsenic in Blood

A method was developed to determine arsenic in mouse blood by electrothermal atomic absorption spectroscopy (ETA-AAS). Typical sample preparation procedures involve digestion of the blood with acids (*15*); however, these methods are time consuming and risk contamination. A simple, rapid method has been developed that involves diluting the blood with deionized water and Triton X-100. The direct injection of blood into the graphite furnace results in a buildup of carbonaceous residue and nonspecific absorption during the atomization cycle, and precautions must be taken to efficiently ash the sample without the volatilization of arsenic. The chemical stabilization of arsenic was achieved using nickel nitrate as a matrix modifier (*16*). However, it has been found that the addition of Ni to blood results in coagulation/precipitation of proteins (*17*), therefore a "within-tube" standards-addition procedure was used by adding the As standard and Ni to the sample before the dry/ash/atomization cycle. A L'Vov platform was used (*18*). The introduction of oxygen during the ash stage was evaluated and rejected (*19–21*).

Instrumentation

A Perkin Elmer model 3030 atomic absorption spectrometer equipped with an HGA-500 graphite furnace and an AS-40 autosampler were used. Deuterium arc background correction was used. Absorbance measurements were at 193.7 nm using an argon hollow cathode lamp (Perkin Elmer) and a band width of 0.7 nm. Pyrolytically coated graphite tubes with totally pyrolytic L'Vov platforms were used, and measurements were based on peak height, using a read time of 2.0 sec. Some blood samples contained lumps and these were dispersed using an ultrasonic processor, model W-370 (Heat Systems Ultrasonic Inc., Farmingdale, New York) in two 10-sec sessions.

Sample Preparation

Blood samples from each test group were pooled and diluted 1:4 by pipetting 500 μg of blood and diluting with 500 μL of 0.5%

Table 2. Furnace program.

Phase	Temperature, °C	Ramp	Time/sec
Dry	90	1	30 (300 mL/min Ar)
	130	1	30
Ash	1150	5	60 (300 mL/min Ar)
Atomize	2450	0	3 (0 mL/min Ar)
Clean	2600	1	1 (300 mL/min Ar)

m/V Triton X-100 and 1 mL of deionized water in 30 mL Sterilin Universal plastic containers. Blood samples taken from mice treated with orpiment required a further 10 to 20× dilution.

Arsenic Assays

The Perkin Elmer AS-40 autosampler was used to perform standard additions to the blood within the atomizer tube. A 10 μL volume of blood was dispensed onto the L'Vov platform in the graphite tube, the capillary was automatically washed, and 10 μL of the As standard containing 0.2% m/V nickel nitrate was deposited onto the droplet of blood (*21*). To determine the As content of the blood samples, matrix-matched calibration graphs were prepared using a 1:4 dilution of blood from mice treated with corn oil. The blood from mice treated with orpiment contained higher levels of As and was diluted to achieve an absorbance of 0.1 to 0.2. A standard-additions procedure was used to calculate the As concentration in the blood. The furnace program is shown in Table 2.

Optimum Assay Conditions

A two-stage temperature program was required to dry the sample and to prevent boiling and sputtering of the blood in the graphite tube. Ash/atomization curves for 1.5 ng As in a 1:4 dilution of blood using an Ar ash revealed that addition of nickel nitrate allowed ash temperatures of up to 1200°C without the volatilization of arsenic. Increasing the ash time from 30 sec to 60 sec improved the analyte peak shape and assay sensitivity. The concentration of nickel nitrate was optimized. A standard-additions graph was prepared in a 1:4 dilution of mouse blood to observe any enhancement or suppression of the analyte signal compared with aqueous arsenic standards. It became evident from these graphs that severe suppression of the As signal occurs in the blood matrix. Hence, standard additions or matrix-matched standards were necessary to determine As in the blood samples.

Detection Limit Calculations

A theoretical detection limit was calculated for As in the 1:4 dilution of blood as 35 ng/g. A more realistic detection limit was determined by relating an absorbance, which was just visible above the baseline noise, to the spiked blood arsenic concentration. This was calculated at approximately 100 ng/g and was used as the detection limit for the analytical procedure.

Results

The results of eight micronucleus experiments, together with selected determinations of arsenic in blood, are shown in Table 1 in the order in which they were conducted. The sequence of experiments illustrates the problems that can beset genotoxicity testing. In experiment 1, a negative test response was observed for orpiment. The relatively low dose level used was caused by problems encountered with dosing the suspension, but was offset by the use of a triple-dose protocol. Although the experiment had a positive control (CP), we decided to repeat the experiment using the more appropriate positive control, sodium arsenite (*5*). Confident of a positive response for sodium arsenite, we omitted CP from experiment 2, and this gave completely negative data. Nonetheless, we established that orpiment was absorbed, albeit the level of arsenic in the arsenite-treated animals was below the level of detection of the arsenic assay. In experiment 3, we repeated exactly the experiment reported by Deknudt et al. (IP injection of an aqueous solution using male BALB/c mice) (*5*). The positive responses observed involved changing two variables at once, so in experiment 4 we established that IP injection of an aqueous solution was critical for a positive test response (IP injection in corn oil giving negative results). Experiment 5 confirmed that the strain of mouse was not important, so in experiment 6 we reverted to male CBA animals. In experiment 6, we observed a positive response for sodium arsenite and proof again that orpiment was absorbed into the bloodstream. The orpiment data from experiment 6 was positive when assessed by statistical analysis. However, the magnitude of this response lay within the historical control group mean range, and no such positive effect was observed in experiments 1, 2, or 7. Experiment 7 also confirmed that sodium arsenite was only positive following injection in water. The orpiment response in experiment 7 was elevated but was not statistically significant. In experiment 8, sodium and potassium arsenite and Fowler's solution, each gave a clear positive response; use of a third strain of mouse in this experiment (C57BL6) confirmed that the genotoxicity of arsenite is strain independent.

From these experiments we conclude that sodium and potassium arsenite are clearly positive in the micronucleus assay. Fowler's solution gave a positive response of the same magnitude as that of the equimolar arsenites (experiment 8). Orpiment was absorbed as evidenced by the elevated levels of arsenic in the blood of mice, but an overall negative micronucleus test response was observed. Arsenite is rapidly eliminated when administered by IP injection (~75% in 24 hr) (*4*), thus the failure to detect it in blood at 24 hr indicates a different pharmacokinetic profile from that of orpiment. The elevated blood levels of arsenic in orpiment-treated animals, coupled to the lack of a depression in erythopoiesis (as seen for arsenite) are consistent with a genetically inactive form of arsenic being present in the blood of orpiment-treated mice.

Discussion

The ability of the rodent bone marrow micronucleus assay to detect as positive the large majority of the established human carcinogens qualifies it for use in studying human carcinogens whose exact causative agent is not known. Among the established carcinogenic human environments, the causative agent in cancer induced by derivatives of arsenic is probably the least well defined. Smelting of metal arsenides or arsenic sulfide ores, or purification of arsenic trioxide derived as a byproduct from the smelting of other nonferrous ores, and therapeutic exposure to

Fowler's solution (potassium arsenite) has adequate evidence of human carcinogenicity (*3,4,7,22*). There is, however, only very limited evidence for the carcinogenicity of arsenic derivatives to experimental animals (*4*). Neither is it at all clear how derivatives of arsenic could cause cancer. The ability of some derivatives of arsenic to form pseudo disulfide bonds (As–S) is responsible for a range of enzyme toxicities, and this activity may also lead to the limited evidence for genotoxic activity, most of which is associated with sodium arsenite and its ability to transform cells and damage chromosomes (*in vitro*) (*23,24*). The only positive rodent genotoxicity data reported in the literature for derivatives of arsenic is for sodium arsenite in the mouse bone marrow micronucleus assay (*5*).

The present experiments have confirmed the data of Deknudt et al. (*5*) indicating that sodium arsenite is genotoxic to the bone marrow cells of mice. Similar activity was observed for potassium arsenite, either as the pure chemical or as formed from As_2O_3 in Fowler's solution (Table 1). In contrast, orpiment was concluded to be inactive in this assay despite blood levels of arsenic being at least 10-fold higher in orpiment-treated mice than in those exposed to sodium arsenite. These data suggest that oxygen derivatives of As(III) (*25*) are responsible for the cancer induced in humans. Thus, arsenic trioxide (As_2O_3), which is associated with lung cancer in smelters, is soluble in potassium bicarbonate to yield potassium arsenite, which, in Fowler's solution, induces skin cancer in treated humans. The possibility exists that As_2O_3 absorbed in man forms arsenite by reaction with bicarbonate in blood.

The inactivity of orpiment in the micronucleus assay strengthens the idea that the naturally occurring sulfides of arsenic do not contribute directly to smelters' lung cancer. A corollary of this inactivity is that the relatively high levels of arsenic absorbed by the mice was not transformed into the appropriate As(III) oxide species. This observation is interesting within the context of the closely allied experiments of Arrouijal et al. (*26*) and Hildebrand et al. (*27*) on nickel subsulfide (α-Ni_3S_2), the presumed carcinogenic species in nasal cancer induced among nickel ore roasters (*28*). These investigators demonstrated solubility for α-Ni_3S_2 in assay medium and located nickel-containing deposits within cells treated *in vitro*. This cellular bioavailability of nickel was probably responsible for Arrouijal et al. (*26*) also being able to demonstrate a positive mouse bone marrow micronucleus assay response for nickel subsulfide.

It is proposed that arsenite (AsO_2^-) is the common carcinogenic species in both the lung cancer among smelters and the skin cancers among those exposed to Fowler's solution. The common activity of these separate arsenic derivatives in the mouse micronucleus assay (experiment 8; Table 1) suggests that this test system may be more effective than animal carcinogenicity bioassays for determining the potential carcinogenic hazard of other derivatives of arsenic. The general insensitivity of rodents to the carcinogenicity of derivatives of arsenic remains a mystery. The extent of human exposure to arsenic trioxide was substantial in the last century, the sublimation chambers being referred to as the poison tower (Fig. 1). Obviously, under those conditions of exposure, cancer did not become apparent due to early deaths, as evidenced by the following quotation: "Its manufacture [As_2O_3] has been chiefly confined to Bohemia and Hungary. Persons brought up from their youth in the works live not longer than

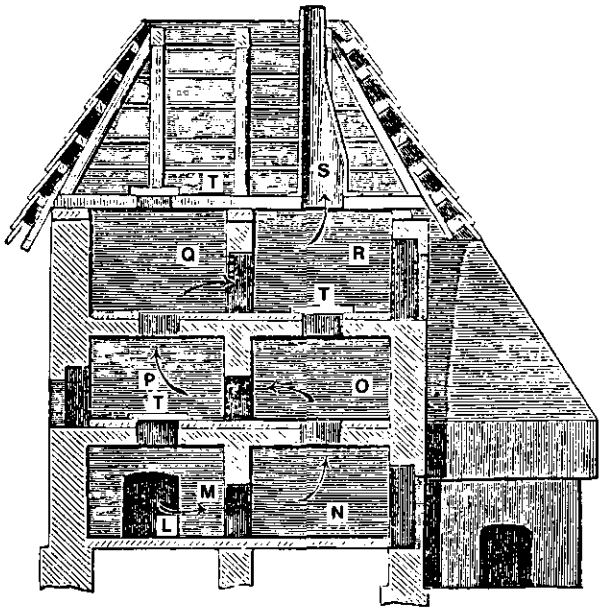

FIGURE 1. Muspratt (*36*) describes the As_2O_3 isolation tower as follows; a section of the "poison tower," where the arsenious acid is condensed in the spaces *M, N, O, P, Q, R*; the fumes enter the lower compartment at *L*, and then follow the direction of the arrows, until finally, the uncondensable vapors depart through the opening *S*. In the flues and first chambers of the tower, the purest arsenic is to be found, that which is deposited in the upper ones being impregnated with sulfur. At the termination of the working of each charge, the covers, *T*, are taken away for the purpose of collecting the whole of the condensed arsenious acid into the lower chamber, which is emptied only about once every 2 months and contains at that period about 25 tons of the impure compound. The arsenious acid procured by the foregoing mode is purified by sublimation before it is sent to market.

to the age of 30 or 35 years. Knowing the deleterious nature of their occupation, they are careless" (*29*).

Obviously, as industrial hygiene increased, a wave of cancer deaths occurred (*4*) and then abated as hygiene improved even further and as usage waned in the 1960s forward (*30*). Thus, there may be a narrow window between toxicity and tolerance where cancer is evidenced, and this may contribute to the difficulty of showing a carcinogenic effect in animals. Some negative rodent cancer bioassay data may, however, be genuine, as for As_2S_3 (*31*), given its inactivity as a genotoxin in the present experiments, but the carcinogenic inactivity of arsenite to rodents is more mysterious (*4*). A further contribution to the problem of demonstrating an effect in animals may be statistical and related to the heterogeneous effects seen for skin cancers induced by arsenite (as Fowler's solution) in man. Thus, the usual dose of 0.2 to 0.5 mL of a 1% solution gives rise to skin cancers in only a susceptible subgroup of humans (*4*). Cuzick et al. (*32*) reported no overall increase in (internal) cancer incidence among a cohort of patients treated orally with Fowler's solution ($\sim$ 1.2 mg/kg/day for 9 months), but an increase in skin cancer was seen among a subgroup showing signs of arsenical poisoning and dermatological changes such as hyperkeratosis. It would obviously be of great value to resolve the carcinogenicity of arsenic trioxide or sodium/potassium arsenite to rodents by the conduct of a full National Toxicology Program-type rodent bioassay. To date, the

only derivative of arsenic tested by the National Toxicology Program is roxarsone (a nitrophenyl arsonic acid), and that gave only equivocal evidence of pancreatic cancer in the male rat (*33*).

Confirmation of activity for arsenite in the mouse bone marrow micronucleus assay, coupled to the demonstration by Arrouijal et al. (*26*) of similiar activity for Ni_3S_2 and our recent observation of activity in this assay for two further human carcinogens [sulfur mustard (*34*) and *N*-(2-chloroethyl)-*N*-(4-methylcyclohexyl)-*N*-nitrosourea (*35*)] brings the number of human carcinogens clastogenic to the rodent bone marrow to 23 (*2*).

H. T. acknowledges financial support by the Laboratory of the Government Chemist.

REFERENCES

1. Shelby, M. D. The genetic toxicity of human carcinogens and its implications. Mutat. Res. 204: 3–15 (1988).
2. Shelby, M. D., and Zeiger, E. Activity of human carcinogens in the Salmonella and rodent bone marrow cytogenetics tests. Mutat. Res. 234: 257–261 (1990).
3. International Agency for Research on Cancer. Arsenic and Arsenic Compounds. IARC Monograph No. 23. IARC, Lyon, 1980, pp. 39–141.
4. International Agency for Research on Cancer. Arsenic and Arsenic Compounds (Group 1). IARC Monograph No. 23, Supplement 7, IARC, Lyon, 1987, pp. 100–106.
5. Deknudt, Gh. Leonard, A., Arany, J., Jenar-Du Buisson, G., and Delavignette, E. *In vivo* studies in male mice on the mutagenic effects of inorganic arsenic. Mutagenesis 1: 33–34 (1986).
6. Poma, K., Degraeve, N., Kirsch-Volders, M., and Susanne, C. Cytogenetic analysis of bone marrow cells and spermatogonia of male mice after *in vivo* treatment with arsenic. Experientia 37: 129–130 (1981).
7. National Academy of Sciences. Arsenic. NAS, Washington, DC, 1977.
8. Van Thoor, J. W. Ed. Chemical Technology, An Encyclopedic Treatment, Vol. 1. Barnes and Noble, New York, 1968, pp. 393–400.
9. Ramsey, W. Modern Chemistry: Systematic. J. M. Dent, London, 1906.
10. Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals, 11th ed. Merck and Company, Inc., Rahway, NJ, 1989.
11. Ashby, J. The efficient preparation of corn oil suspensions. Mutat. Res. 187: 45 (1987).
12. Ashby, J., and Mirkova, E. Re-evaluation of the need for multiple sampling times in the mouse bone marrow micronucleus assays: results for DMBA. Environ. Mol. Mutagen. 10: 297–305 (1987).
13. Tinwell, H., Bandara, L., and Ashby, J. Activity of DMBA, DMH and CP in triple- and single-dose rodent bone-marrow micronucleus assays. Mutat. Res. 234: 195–198 (1990).
14. Tinwell, H., and Ashby, J. Comparison of acridine orange and giemsa stains on several mouse bone marrow micronuclueus assays—including a triple dose study. Mutagenesis 4: 476–481 (1989).
15. Bauslagh, J., Rodziuk, B., Saeed, K., and Thomassen, Y. Reduction of effects of structured non-specific absorption in the determination of arsenic and selenium by electrothermal atomic absorption spectrometry. Anal. Chim. Acta 165: 149–157 (1984).
16. Ediger, R. D. Atomic absorption analysis with the graphite furnace using matrix modification. Atom. Absorp. Newslett. 14: 127–130 (1975).
17. Subramanian, K. S. Determination of arsenic in whole blood by graphite platform in-furnace atomic absorption spectrometry with nitric acid deproteinisation and nickel fortification. J. Anal. Atom. Spect. 3: 111–114 (1988).
18. L'Vov, B. V. Electrothermal atomization—the way towards absolute methods of atomic absorption analysis. Spectrochem. Acta B 33: 153–193 (1977).
19. Eaton, D. K., and Holcombe, J. A. Oxygen ashing and matrix modifiers in graphite furnace atomic absorption spectrometric determination of lead in whole blood. Anal. Chem. 55: 946–950 (1983).
20. Grobenski, Z., Lehmann, R., Tamm, R., and Welz, B. Improvements in graphite-furnace atomic absorption microanalysis with solid sampling. Mikrochim. Acta 1: 115–125 (1982).
21. Stephen, S. C., Ottaway, J. M., and Littlejohn, D. Slurry atomisation of foods in electrothermal atomic absorption spectrometry. Fres. Z. Anal. Chem. 328: 346–353 (1987).
22. Furst, A. A new look at arsenic carcinogenesis. In: Arsenic, Industrial, Biomedical and Environmental Perspectives (W. H. Lederer and R. J. Fensterheim, Eds.), Van Nostrand Reinhold, New York, 1983, pp. 151–165.
23. Leonard, A., and Lauwerys, R. R. Carcinogenicity, teratogenicity and mutagenicity of arsenic. Mutat. Res. 75: 49–62 (1980).
24. Eastmond, D. A., and Tucker, J. D. Identification of aneuploidy-inducing agents using cytokinesis blocked human lymphocytes and an antikinetochore antibody. Environ. Mol. Mutagen. 13: 34–43 (1989).
25. Cotton, F. A., and Wilkinson, G. Advanced Inorganic Chemistry, 4th ed. John Wiley and Sons, New York, 1980, p. 480.
26. Arrouijal, F. Z., Hildebrand, H. F., Vophi, H., and Marzin, D. Genotoxic activity of nickel subsulphide α-Ni_3S_2. Mutagenesis 5: 583–589 (1990).
27. Hildebrand, H. F., Collyn d'Hooghe, M., Shirali, P., Bailly, C., and Kerckaert, J. P. Uptake and biological transformation of β-NiS and α-Ni_3S_2 by human embryonic pulmonary epithelial cells (L312) in culture. Carcinogenesis 11: 1943–1950 (1990).
28. International Agency for Research on Cancer. Nickel and Nickel Compounds (Group 1). IARC Supplement 7, IARC, Lyon, 1987, pp. 264–269.
29. Popular Encyclopedia, Vol. 1. Blackie and Son, Glasgow, 1862, p. 283.
30. Fitzgerald, L. D. Arsenic sources, production and applications in the 1980's. In: Arsenic, Industrial, Biomedical and Environmental Perspectives (H. W. Lederer and R. J. Fensterheim, Eds.), Van Nostrand Reinhold, New York, 1983, pp. 3–9.
31. Pershagen, G., and Bjorklund, N. E. On the pulmonary tumourogenicity of arsenic trisulphide and calcium arsenate in hamsters. Cancer Lett. 27: 99–104 (1985).
32. Cuzick, J., Evans, S., Gillman, M., and Price Evans, D. A. Medicinal arsenic and internal malignancies. Br. J. Cancer 45: 904–911 (1982).
33. National Toxicology Program. Bioassay of Roxarsone in B6C3F$_1$ mice and Fischer 344 rats. Technical Report 345, NTP, Research Triangle Park, NC, 1988.
34. Ashby, J., Tinwell, H., Callander, R. D., and Clare, N. Genetic activity of the human carcinogen sulphur mustard towards Salmonella and the mouse bone marrow. Mutat. Res. 257: 309–313 (1991).
35. Tinwell, H., and Ashby, J. Activity of the human carcinogen MeCCNU in the mouse bone marrow micronucleus assay. Environ. Mol. Mutagen. 17: 152–154 (1991).
36. Muspratt, S. Chemistry, Theoretical, Practical and Analytical, Vol. 1. William Mackenzie, Glasgow, 1860, pp. 213–222.