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Bladder Cancer and Arsenic Exposure: Differences in the Two Populations Enrolled in A Study in Southwest Taiwan

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Objective Analyses of bladder cancer mortality in the Black Foot Disease (BFD) endemic area of southwest Taiwan conducted by Morales *et al.* showed a discontinuity in risk at 400 μg/L arsenic in the drinking water in a stratified analysis and no discontinuity in a continuous analysis. As the continuous analysis presentation had been used by both the NRC and the EPA to assess the carcinogenic risk from arsenic ingestion, an explanation of the discontinuity was sought. **Methods** Review of 40 years of published health studies of the BFD-endemic area of SW Taiwan showed that earlier publications had limited their cancer associations with arsenic levels in artesian well waters and that the reports of Morales *et al.*, NRC, and EPA failed to do so. Underlying data for the Morales *et al.* study were obtained from the appendix to the NRC report. Bladder cancer mortality rates were calculated from case counts and person-years of observation for each study village. Villages were categorized by water source according to the descriptions from the underlying study. Graphic and regression analyses were conducted of the bladder cancer mortality rates using exposure as a continuous variable and simultaneously stratifying by water source. **Results** The median village well arsenic levels ranged from 350 to 934 μg/L for villages solely dependent on artesian well water and from 10 to 717 μg/L for villages not solely dependent on artesian well water. Bladder cancer mortality rates were found to be dependent upon the arsenic level only for those villages that were solely dependent on artesian well water for their water source. Bladder cancer mortality rates were found to be independent of arsenic level for villages with non-artesian well water sources. **Conclusions** The data indicate that arsenic exposure levels do not explain the bladder cancer mortality risk in SW Taiwan among villages not dependent upon artesian well water. The association for villages dependent upon artesian well water may be explained either by arsenic acting as a high-dose carcinogen or in artesian well water as a co-carcinogen with some other aspect of artesian well water (possibly humic acid). Arsenic exposure level alone appears to be an insufficient exposure measure to describe the risk of bladder cancer mortality in the BFD-endemic area. Risk analyses that fail to take water source into account are likely to misrepresent the risk characterization, particularly at low arsenic levels.

Key words: Arsenic ingestion; Bladder cancer; Dose-response; Non-linear; SW Taiwan; Cancer risk

INTRODUCTION

The rural partially-coastal townships of Peimen, Hsue Chia, Putai, and I Chu in southwest

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Taiwan have been studied by public health specialists since the 1950s. Studies of human cancers (skin, lung, bladder, and liver) in this area have served as the basis for the risk assessments of arsenic ingestion by EPA and other non-US agencies[1–5].

These townships are the location of a previously unknown peripheral vascular disease called locally "Wu Chiao Ping", which is literally translated from Chinese as "Blackfoot Disease". The name describes the black color change of an affected foot that occurs in the gangrenous stage of the disease as the affected part became demarcated from the adjacent non-affected part[6]. The characteristic that distinguished villages that had cases of Blackfoot disease (BFD) from those that did not was the predominance of artesian wells as their source of water supply. The artesian wells had been constructed in the 1920s and were used until the 1950s to 1960s, when their use was replaced with piped water.

In 1961, Tseng *et al.* concluded that BFD was related to the water derived from the artesian wells in the area[6], and in 1962, Chen and Wu described the BFD area in five townships as having 109 villages, 39 with only artesian wells, 30 with both artesian and shallow wells, and 40 using only shallow wells or surface water[7]. In the 1960s, high levels of arsenic were found in the artesian well waters and tanks, and cases of chronic arsenicism (including skin cancers) were identified. By 1968, Tseng *et al.* concluded that arsenic in the drinking water (from 110 artesian wells and 4 shallow wells) was the cause of the BFD, the skin cancers, and the chronic arsenicism[8]. In contrast, Lu suggested that the cause of BFD was not the arsenic in the artesian well water but the presence of humic acids in the same wells[9].

In 1985, Chen *et al.* studied the population of 84 villages of four townships in the BFD-endemic area and reported elevated mortality rates of cancers of internal organs in the BFD-endemic area, notably cancers of bladder, kidney, skin, lung, liver, and colon[10]. He demonstrated that the risks were highest in villages that only used artesian wells (n=31), lower in villages that used both artesian and shallow wells (n=27), and lowest in villages that only used shallow wells (n=24) or surface water (n=2), and attributed the increased risks to the high arsenic artesian well water. Using a case-control design, Chen *et al.* demonstrated a three to four fold risk of bladder and lung cancer mortality with use of artesian well water for 40 or more years[11]. The Chen *et al.* case-control and case follow-up studies of BFD resulted in the implication of pathogenic and carcinogenic properties of the artesian well water in the BFD-endemic area[12].

Wu *et al.* reported that they could identify adequate arsenic level in only 27 of the 84 villages and extended the study to include an additional 15 villages in the townships of Yenshi and Hsai Ying, for a total of 42 villages[13]. They reported a dose-response relationship between arsenic concentration intervals (< 300 μg/L, 300-590 μg/L, and 600 (+)μg/L) in well water and mortality from cancers and vascular diseases. These were the same arsenic concentration intervals that Tseng *et al.* had used in their report on BFD, skin cancer, and chronic arsenicism[8].

Chen *et al.* calculated carcinogenicity potency indices for liver, lung, bladder, and kidney cancers using the 42 villages grouped by village median arsenic levels as <100 μg/L (13 villages), 100-290 μg/L (8 villages), 300-590 μg/L (15 villages), and 600 (+)μg/L (6 villages)[14]. The arsenic well values for each well in each of the 42 villages were shown in Table A10-1 of the 1999 report from the National Research Council (NRC) of the US National Academy of Science (NAS)[4]. A summary arsenic exposure value was developed for each village, which was the median of all the well-specific values given in Table A10-1 for that village. These tables also included the number of male and female bladder, lung, and liver cancer deaths for each village as well as the person-years of observation. Thus, village-specific rates could be calculated.

These 42 villages comprise the study population that later provided the database for the Morales *et al.* report[15] and served as the basis for the analyses by both the US Environmental Protection Agency (EPA) and the NRC[3-5]. These reports analyzed the village mortality data using the summary arsenic exposure values from the villages as a continuous variable, based on a single value for each village, instead of a categorical variable. The village data were not grouped within exposure intervals nor were probability distributions assigned to exposures. All of these reports have shown a significant dose-response association between bladder and lung cancer mortality and arsenic exposure measured as a continuous variable.

The above findings present a history of the development of the published studies of cancer in the BFD-endemic areas. They present an interesting conundrum. One set of studies reports that the cancers are related to the use of artesian well water and the other set of studies reports that the cancers are related to the arsenic levels of water used by the village without any mention of the source of water. We have attempted to investigate this conundrum by using both exposure classifications simultaneously. The SW Taiwan study has served as a primary basis for assessing the human carcinogenicity of arsenic exposure through drinking water. In order to determine the generality of the findings, the details of the associations within that study base have to be understood.

METHODS AND MATERIALS

The basic arsenic exposure and cancer outcome data for the 42 villages, published in the NRC report, is publicly available[4]. For each village (identified by a number in the form 3-H), the median arsenic concentration (ppm; mg/L) for each of the wells in that village and the median of those values provide the exposure information. The median was originally used by Wu *et al.*[13] and has been used in the analyses that follow his study database. The person-years of mortality observation (1973-1986) for males and females provide the observation information and serve as the denominator for the rate calculations for each village. The number of deaths from bladder, lung, and liver cancers for males and females are given for each village and serve as the numerator in the rate calculations. (In that particular table, it appears that the mortality counts of the lung cancers and liver cancers had been transposed.)

Chen *et al.* have classified each village's water sources as artesian, mixed, and shallow[10]. The analysis below uses the identical classification. Chen *et al.* states that "the arsenic content of artesian wells in the BFD-endemic areas ranged from 0.35 to 1.14 ppm with a median of 0.78 ppm, while the shallow well water in BFD-endemic areas had arsenic content between 0.00 and 0.30 ppm with a median of 0.04 ppm." We have accepted this determination.

Tseng *et al.* reported that measurements of artesian well waters may be variable over time and measured as low as 0.01 ppm[8]. We make the necessary assumption that Chen *et al.* refer to the median measurement for a particular well. Thus, this paper classifies individual wells as "artesian" if its median measurement in Table A-10 was greater than 0.325 ppm and as "shallow" if its median measurement was less than 0.325 ppm.

In order to classify villages by water source as "shallow", "mixed", or "artesian", we have looked at the set of well medians for each village and classified them as follows:

The bladder cancer mortality rates (per 100 000 PY) were calculated for each village by dividing the number of such cancer deaths in the village by the number of person-years for the village (male and female data combined).

The Wu *et al.* analysis used age-adjusted mortality rates[13], while the Morales *et al.* analyses used age as a parameter in its continuous analysis[15]. Our analysis does not account for age as an influence on the village specific mortality rates since age data on neither the villages nor the cases has been made public.

TABLE 1

Village Classification by Water Sources

Village Water Sources	Criteria	Number
Artesian	All wells with medians greater than 0.325 ppm	14 villages
Mixed	Some wells with medians greater than 0.325 ppm, and other wells with medians less than 0.325 ppm	9 villages
Shallow	All wells with medians less than 0.325 ppm	19 villages

The village-specific mortality rates were displayed in a scatter-plot against the summary arsenic exposure values for the villages (median of the well medians) with separate identification of villages by their water sources (artesian, mixed, or shallow). This provided an opportunity to examine the arsenic exposure dose-relationship for each of the groups of villages simultaneously.

The median of well medians was used as the representative exposure level for each village both because that is what was used in Wu *et al.*[13] and in the analyses based in Wu *et al.* and because no other information was available for an alternative choice. The use of the mean of the well medians would have necessitated the dubious assumption that there was no well preference within the village and that all wells were as likely to be used for drinking water, independent of taste, distance, or other factors. Furthermore, Wu *et al.* had commented that the arsenic concentrations of well water samples in the same village were not normally distributed.

RESULTS

The mortality data was assembled by the Taiwanese researchers based on death certificate information for the years 1973-1986[13]. Taiwan has a death certificate system with mandatory completion that is considered to be as complete and as accurate as those in North America and much of Europe. Cancer death mortality surveillance had been conducted from 1973 through 1986 on this 35 000 person population, for total person-years (PY) of observation count of 490 929 PY. The male to female ratio was 1.08. Village population ranged from about 300 people to 1 800 people with a median of 750. A total of 175 bladder cancer deaths were observed during this period with a range of 0–18 bladder cancer deaths per village (median=3; mean=4). The overall bladder cancer mortality rate was 36 per 100 000 PY (175/490 929 =36 deaths per 10^5 PY).

Well arsenic data in the BFD-endemic area had been collected in the early 1960s[7] and were again collected as part of a national survey in the mid-1970s[16]. These 42 villages had a total of 153 wells with a range of 1-47 per village (median=2; mean=4). The summary arsenic levels of the individual wells ranged between 10 μg/L and 1 411 μg/L. The well water arsenic exposure level for each village had been taken to be the median of the summary values of each of the wells in the village. These medians ranged between 10 μg/L and 934 μg/L (median=283; mean=320).

Fig. 1 shows the distribution of the bladder cancer mortality rates (per 100 000 PY) for the 42 villages by their median well arsenic level. Least-squares linear regression analysis

yields a slope of 0.0424 (95% CI, +0.0053 – +0.0894) bladder cancer deaths per 100 000 person-years per 1 μg/L arsenic with R^2=0.115. The assumption of an average lifetime in Taiwan of 65 years would convert this regression to a slope of 2.76×10^{-5} (95% CI, $+0.35\times10^{-5}$ – $+5.81\times10^{-5}$) lifetime risk of bladder cancer mortality per μg arsenic per liter (lifetime exposure).

Fig. 1 presents the SW Taiwan bladder cancer mortality analytic results using the assumption that the arsenic level is the only exposure metric relevant to bladder cancer mortality risk. It has not given consideration to the observation in Chen *et al.*[10] that water source is also an important factor for bladder cancer risk. Chen *et al.*[10] noted that the studied villages differed by water source in that some villages only had artesian wells, some had both artesian and shallow wells, and some had only shallow wells and no artesian wells. They further demonstrated that the cancer mortality rates were different for these three groups of villages and that the cancer mortality rates were greatest for the villages that were solely dependent upon artesian wells for their water supply.

Fig. 2 has been developed from Chen *et al.*[10] (Chart 4 with data presented on a linear scale). Fig. 2 demonstrates that the arsenic-associated SMRs were greatest for bladder cancer and that the bladder cancer SMR for the areas using only artesian wells was six times

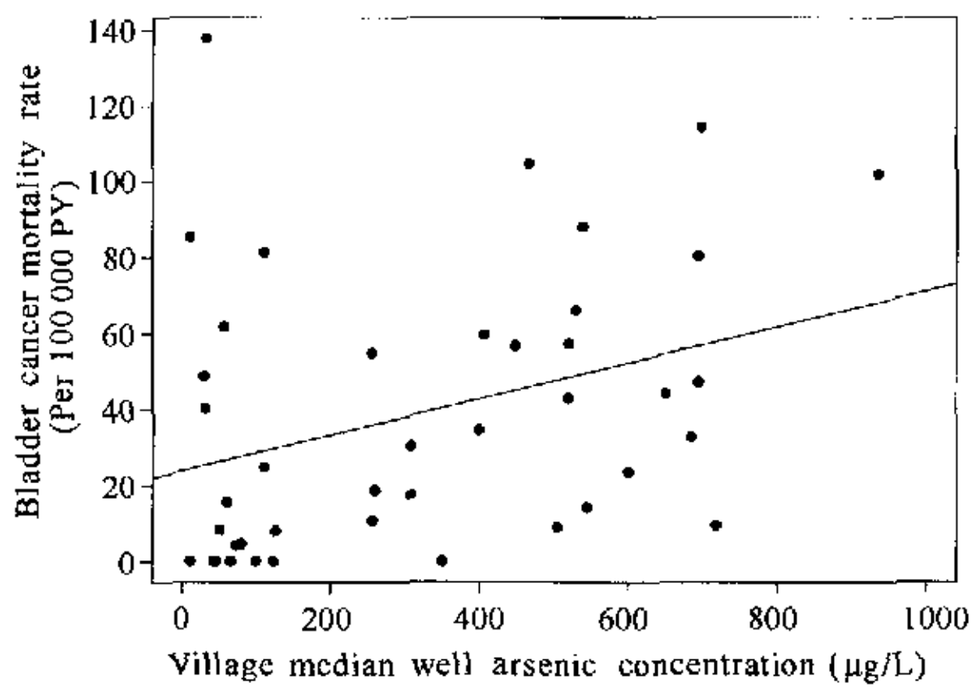

FIG. 1. Bladder cancer mortality rate (per 100 000 PY) for villages by village median well arsenic level (μg/L).

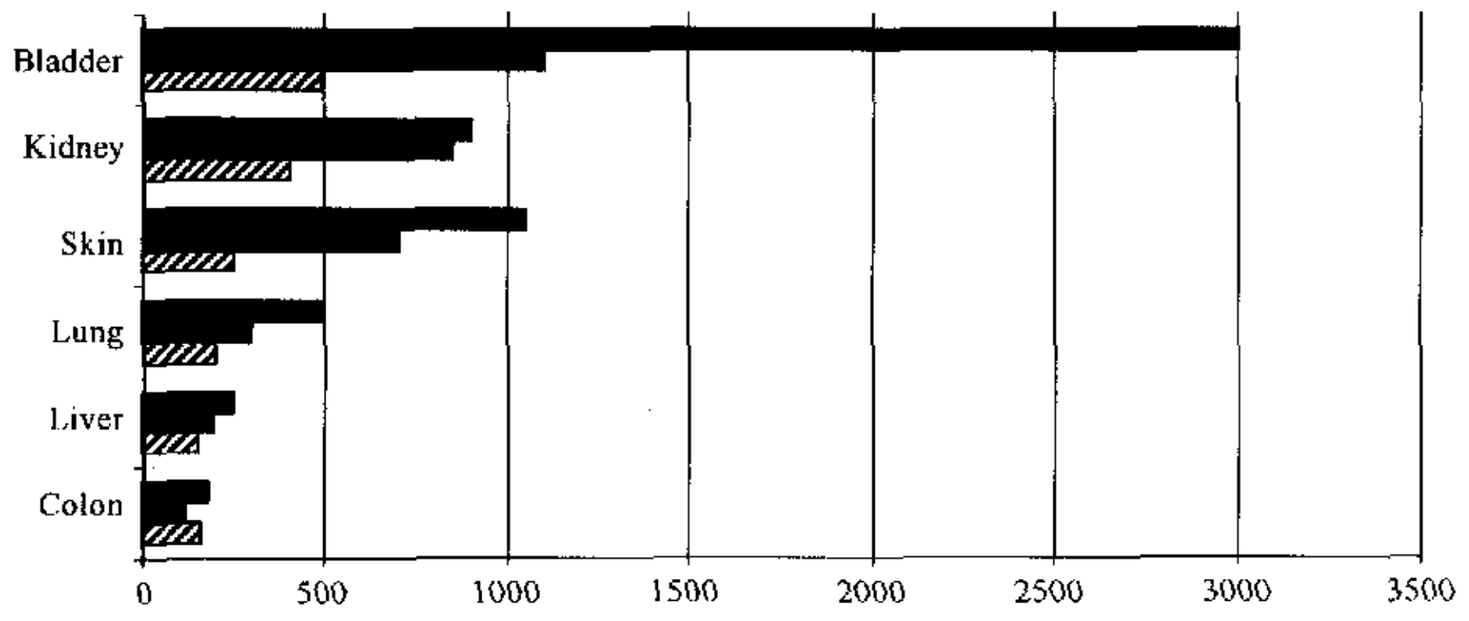

FIG. 2. Standardized mortality ratio for cancers by well types in village (estimated from Chart 4[10]).

greater than that for areas using only shallow wells and three times greater than that for areas using both artesian walls and shallow wells.

Figs. 1 and 2 above demonstrate the bladder cancer mortality risk for SW Taiwan using two different exposure metrics-arsenic level and water source-each separately. Fig. 3 below examines the bladder cancer mortality risk looking at both arsenic level and water source simultaneously.

Fig. 3 presents the same bladder cancer mortality rates and median village arsenic levels in Fig. 1. Here, however, separate regression lines have been calculated for villages that use shallow, artesian, and mixed water sources. This analysis reveals that neither the shallow well villages (β=-0.01071, 90% CI - 0.148 +0.127) nor the mixed well villages (β=-0.03974, 90% CI - 0.105 +0.026) show a positive slope for bladder cancer mortality. In contrast, only the artesian well villages show a positive slope with respect to bladder cancer mortality and village median well arsenic level (β=0.1139, 90% CI +0.006 – +0.222.) These data suggest that the bladder cancer risk is associated with arsenic in artesian wells and not with arsenic in the shallow wells.

Fig. 4 offers a third representation of the village bladder cancer mortality rates, this time contrasting the rates for villages dependent on artesian wells with those for the studied villages in the BFD-endemic area that were not dependent on artesian wells. This presentation reverts back to Chen *et al.*'s original report[10] that the exposure metric of interest was the arsenic level in the artesian wells. This figure separates the villages into the two groups – those dependent on only artesian wells and those that have some non-artesian water source – and examines in each group the relationship between bladder cancer and arsenic level. This analysis finds a positive association between bladder cancer and median well water arsenic level only in the villages that only had artesian wells and not in the villages that had an alternative drinking water source.

Multiple linear regression analysis of the 42 village bladder cancer mortality data produces adequate fitting models whether for the data as presented in Figs. 1, 3, and 4.

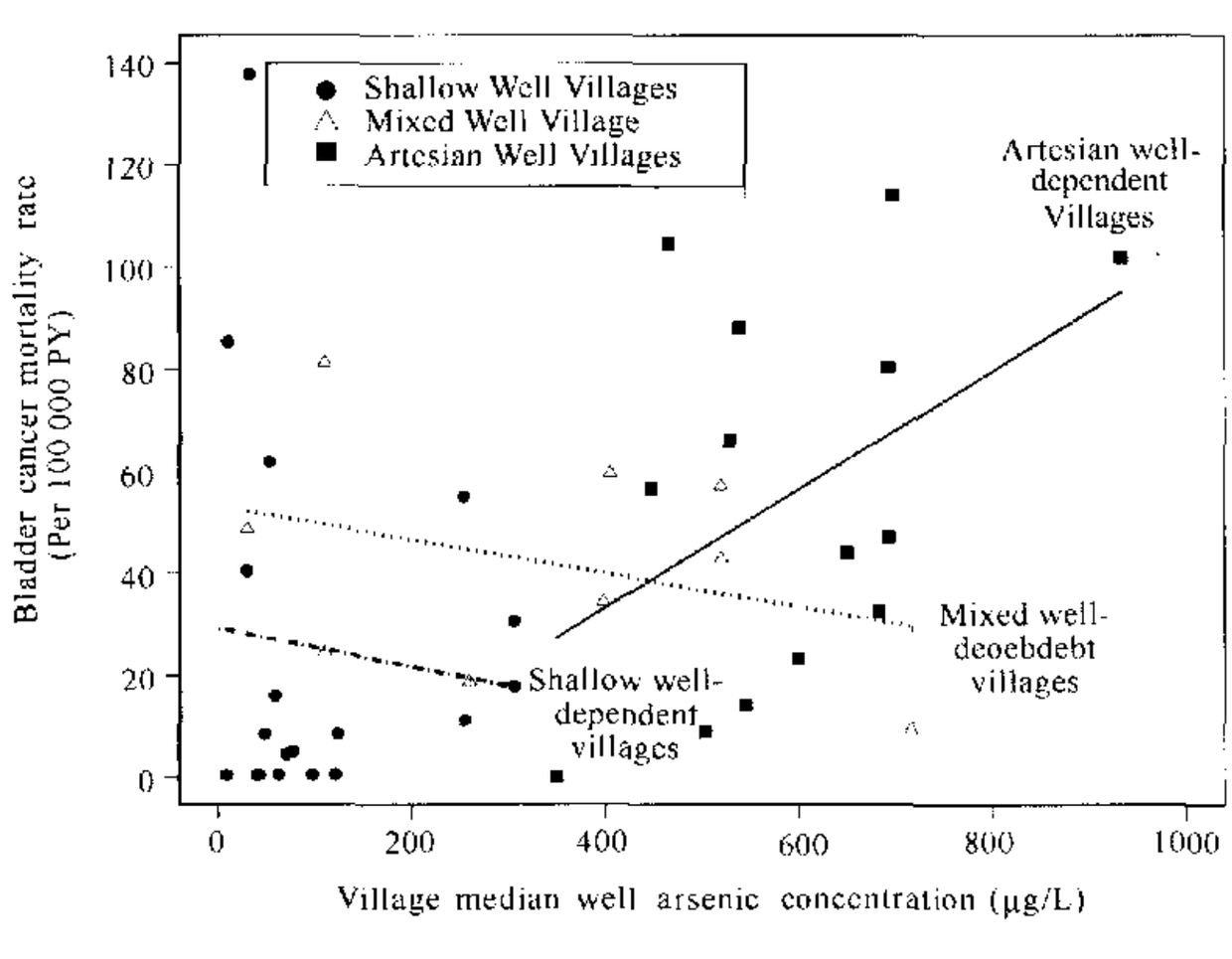

FIG. 3. Bladder cancer mortality rate (per 100 000 PY) for villages by village median well arsenic level (μg/L).

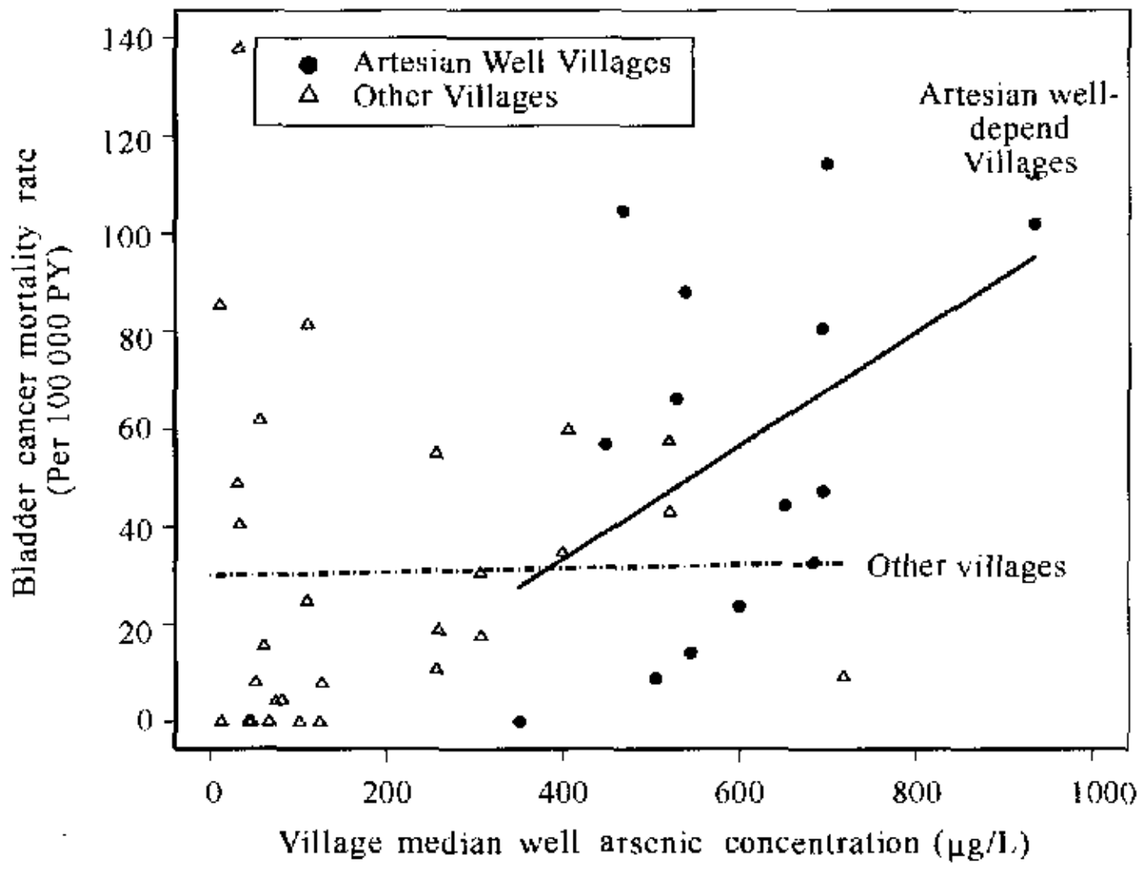

FIG. 4. Bladder cancer mortality rate (per 100 000 PY for villages by village median well arsenic level (μg/L).

TABLE 2

Multiple Linear Regression Analyses

Figure	Description	*P* Value of *F*	Adj R^2	Variable	*P*
1	Arsenic Only	0.03	0.09	Arsenic	0.03
3	Three Strata	0.04	0.11	Artesian Only	0.01
4	Two Strata	0.02	0.10	Artesian Only	0.02

The arsenic level data alone do not appear to have greater ability to predict the bladder cancer mortality rate in these villages than does the classification of villages by water source alone. *Because 20% of the villages that have non-artesian well water sources also have* median arsenic levels comparable to those of water from artesian wells, the results in Table 2 indicate that in the villages studied here, the type of wells supplying a village with drinking water has an effect on that village's bladder cancer mortality rate that is independent of the arsenic level in the water from that well.

DISCUSSION

The Morales *et al.* paper[15] and its underlying data have served as the basis for the quantitative cancer risk assessments for arsenic in drinking water conducted by the NRC and the EPA[3–5]. The Morales *et al.* paper presents the cancer mortality rates both as standardized mortality ratios stratified by arsenic level into eight strata and as a continuous measure based on the age-adjusted mortality rates for each of the 42 villages[15]. Fig. 1 above presented for bladder cancer the crude mortality rates for the 42 villages. Fig. 5 below presents for bladder cancer the standardized mortality ratios stratified by arsenic level into the eight strata of Morales.

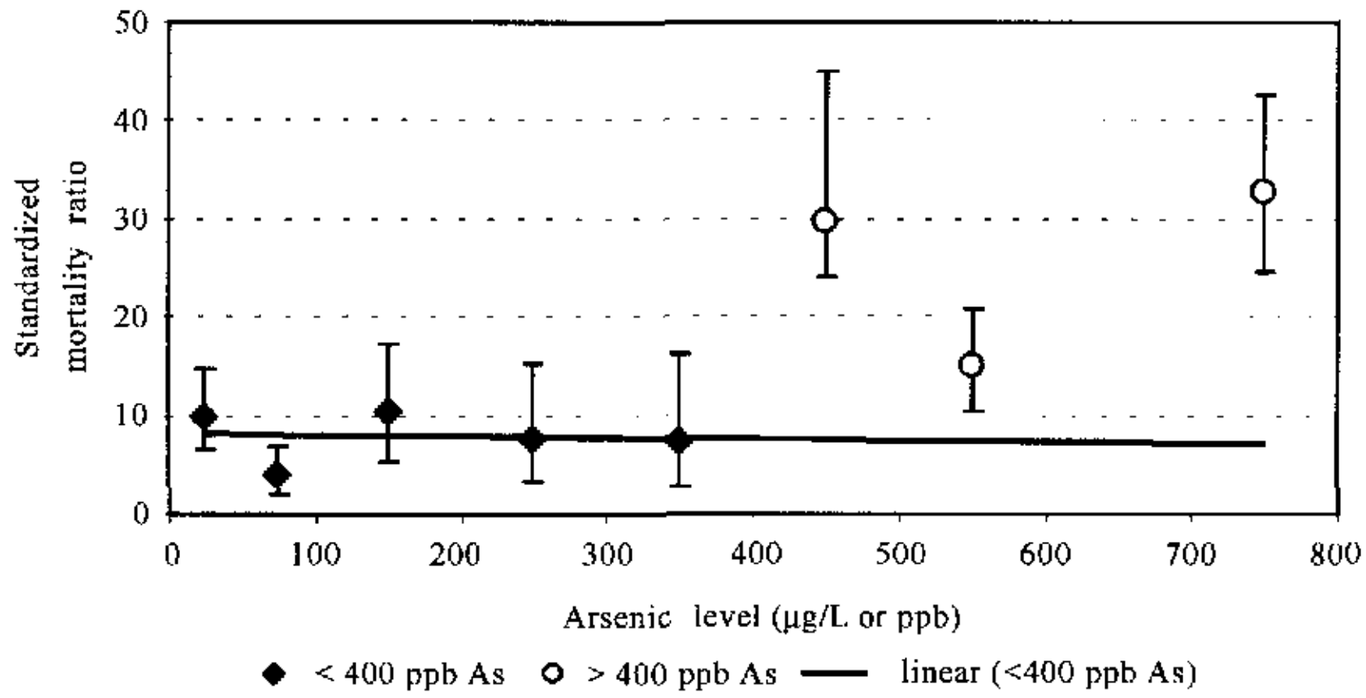

FIG. 5. Morales *et al.* bladder cancer SMRS (and 95% CI) by drinking water arsenic level (interval mid-point).

Fig. 5 is intriguing in that it reveals an unexplained discontinuity in the bladder cancer mortality risk that was not evident in Fig. 1. This stratified analysis showed that while even the SW Taiwan population with the lowest levels of arsenic in their drinking water have bladder cancer mortality rates nearly 10 times higher than the reference population (the rest of Taiwan), there is no change in the bladder cancer mortality rate with respect to village arsenic exposure from 10 to 400 µg/L (β=-0.0015, 95% CI -0.0366 – +0.0336). Further, the stratified analysis shows that the arsenic exposure-bladder cancer mortality relationship seen at exposures in the range of 0 – 400 µg/L did not fit the data from higher exposures, since the 95 % confidence range around each estimate above 400 µg/L excluded the regression line for the data from the lower range. The study population is reasonably balanced at about 400 µg/L as sixty percent of the SW Taiwan study population was exposed to drinking water with arsenic levels below 400 µg/L and forty percent at 400 µg/L or greater. The risk predicted by the first group does not fit the data from the second group.

We propose in our analysis above that the reason for the discontinuity seen in Fig. 5 was not seen in Fig. 1 as a consequence of combining data from different populations of villages. Fig. 4 demonstrates that a distinction between villages dependent upon artesian well water and villages not dependent upon artesian well water would explain the difference between Figs. 1 and 5.

We suggest in addition two possible mechanistic explanations – (1) that arsenic behaves like a high-dose phenomenon with respect to bladder cancer in that it only demonstrates its effect at high arsenic exposure level, or (2) that arsenic behaves like a co-carcinogen, for instance, with some constitutive factor that distinguishes artesian well water from other waters in SW Taiwan.

High-dose Phenomenon

Other authors have also observed a discrepancy in the relationship between arsenic exposure and bladder cancer risk at high and low arsenic levels or that the association is a high dose phenomenon[17]. In a seven-year prospective incidence study from SW Taiwan, Chiou *et al.* found even among artesian well users that a significantly increased risk of bladder cancer only occurred among those with exposures over 700 µg/L[18]. A multivariate adjusted relative risk analysis further showed the absence of a cigarette smoking factor in

SW Taiwan and that a significant increased bladder cancer risk was only associated with a cumulative exposure of 20 000 μg/L×year or greater (about 700 μg/L for 30 years).

Similarly, in a retrospective study of all of Taiwan, Guo and Tseng found both for bladder cancer mortality and bladder cancer incidence that no arsenic-dependent increase with exposure well arsenic levels was seen below 640 μg/L[19].

Elsewhere in Taiwan, Chiou *et al.* assembled in 1991–1994 a cohort of 8 102 residents with well arsenic levels of <0.15 μg/L to 3 590 μg/L for a prospective cancer incidence study in NE Taiwan[20]. Through 1996, they found a not-statistically increased standardized incidence rate for bladder cancer of 1.96 (95% CL, 0.94–3.61). Multivariate-adjusted relative risks for urinary organ cancers (bladder and kidney) and for transitional cell carcinoma (bladder and kidney) are only significantly elevated for those exposed to arsenic levels greater than 100 μg/L. This finding led to a positive trend. Half of the residents with exposures greater than 100 μg/L had exposures of over 300 μg/L and nearly 20% had exposures greater than 600 μg/L. Further follow-up may clarify the dose-response curve further. Its next report should be quite informative.

Studies in other countries showed similar findings. Tsuda *et al.* conducted a 33-year follow-up cohort mortality study of residents of a small town in Japan where a factory had contaminated the local wells[21]. They reported a significant increase in urinary cancer mortality for persons whose drinking water was >1 000 μg/L arsenic for five years and no urinary cancer among those exposed to lower levels.

Cuzick *et al.* reported from England on the mortality follow-up of about 450 patients who had been treated with Fowler's solution (potassium arsenite) for an average of 8-9 months[22]. Treatments had occurred between 1945 and 1969 and followed through 1990. Most treatments were for dermatological conditions, although some were treated for miscellaneous conditions including malaria, anemia, epilepsy, and anxiety. The dosages were reported as cumulative doses ranged from less than 250 mg to over 10 000 mg. Conversion of dosages to average daily doses assuming the mean duration of treatment indicated no increase in bladder cancer risk until exposures exceeded the equivalent of about 1 400 μg/L. While each of these exposures were at high doses for less than lifetime durations, these studies had the advantage that each of the study persons was specifically enumerated in the study and thus no issues of in- or out- migration were present.

Studies have been conducted in Latin America of populations with assumed lifetime exposures. Smith *et al.* reported the bladder cancer mortality experience of an area of Chile[23]. Arsenic concentrations in the drinking water of various towns in region II of northern Chile were known or estimated for the period 1950-1994 and the mortality experience during 1989-1993 was reported. While the arsenic level of the small town of Taltal (1981 population=7 620) was reported as 60 μg/L throughout the period, the town of San Pedro (1981 population=3 070) had levels consistently at 600 μg/L and the capital of Antofagasta (1981 population=219 310) had levels of 870 μg/L for the fifteen years of 1955-1969. Smith *et al.* reported an overall bladder cancer standardized mortality ratio of 6.0 for a population that had a population-weighted average exposure of about 570 μg/L thirty years earlier for about fifteen years. These were apparently lifelong exposures for a population for which the distinction between those who had consistently low arsenic exposures and those that had consistently high exposures cannot be made.

Similarly, Hopenhayn-Rich *et al.* have reported on bladder cancer mortality in rural counties of Argentina (1986-1991) that counties with more arsenic in the drinking water and more evidence of arsenic dermatoses had higher bladder cancer mortality rates[24]. These data are difficult to interpret because both the exposure and outcome data have been

aggregated at the county level and do not reflect known uncertainties at the town levels. The tables in the study show that within the high arsenic exposure area in the two counties the mean arsenic level in towns vary from 40 μg/L to 533 μg/L. Over 50 % of the population of each of these counties comes from towns having average arsenic water levels of 250 μg/L or greater. Furthermore, the authors report that only 20% of the population "may have been exposed" to the high arsenic levels. Thus, it is not possible to determine which bladder cancer deaths were among those who came from towns with high or low arsenic exposure levels or among those 20% who may have drank from the arsenic containing waters or those 80% who did not.

There are a number of studies that have sought to find an increased bladder cancer risk in areas with low arsenic levels but have not found such data. Bates *et al.* found no overall association of inorganic arsenic with risk of bladder cancer among Utah residents of towns with arsenic concentrations of 0.5–160 μg/L (mean=5.0 μg/L) for an average of 35 years[25]. The adjusted odds ratio at the highest exposure level (≥74 mg/liter-year) was not different than the reference group (*OR*=1.00, 90% CI [0.5–2.1]). The cases and controls came from the Utah study area of the National Bladder Cancer Study and included all cases and controls that had spent at least 50 % of their pre-diagnosis lifetime in any of the 88 Utah towns and cities for which water source arsenic levels had been determined. Two exposure indices were developed – cumulative dose in mg arsenic and units of mg/liter*year. Among 15 tests for a dose response relationship, the only positive trend was for ever smokers with exposure measured in mg/L*years at an interval of 10-19 years prior to diagnosis. The authors concluded that "there was no overall association of inorganic arsenic with risk of bladder cancer."

Lewis *et al.* investigated an association of drinking water arsenic and mortality outcome in a study of residents of Millard County, Utah, where median drinking water arsenic concentrations in study towns ranged from 14 to 166 μg/L[26]. The median arsenic level and *the weighted mean arsenic level for the county are approximately* 100 μg/L. Standardized mortality ratio (SMR) results were presented for a wide range of death outcomes, both cancer (including bladder cancer) and non-cancer, for males and for females, and by low, medium, high, and all levels of arsenic exposure in ppb-years. A non-statistically significant deficit of bladder cancer mortality was seen overall (Observed=5; Expected=9.7; SMR= 0.52). These data are sufficiently strong to reject a risk of greater than 1.21 at an exposure of 100 μg/L.

Kurttio *et al.* conducted a case /control study of 844 bladder cancer cases in the Finnish Cancer Registry (1980-1995) where well arsenic levels ranged between 0.05 to 64 μg/L[27]. They initially reported a trend in arsenic exposure (<0.01, 0.1–0.5, and ≥0.5 μg/L) three to nine years prior to diagnosis, but found this was due to the risk for persons who had smoked cigarettes in the 1970s and were in the highest arsenic exposure strata. No change in bladder cancer risk by arsenic exposure was seen among those who were never smokers or ex-smokers in the 1970s.

The only arsenic-associated bladder cancer risk seen in either case/control study was among cigarette smokers[25,27]. Otherwise, there was no bladder cancer risk observed with low drinking water arsenic levels.

The studies in Taiwan cited above indicate that arsenic is a high-dose carcinogen. Chiou *et al.* reported a cut-off of 700 μg/L for artesian well users in the BFD-endemic area[18]. Guo *et al.* reported a cut-off of 640 μg/L[19]. The Chiou *et al.* study is not yet able to distinguish a cut-off at 100 μg/L, 300 μg/L, or 600 μg/L[20]. In non-Taiwan studies, Tsuda *et al.* shows a cut-off at > 1 000 μg/L[21] and Cuzick *et al.* found no excess below the equivalent of 1 400

μg/L[22].

In Latin America, Smith *et al.* reported that in Northern Chile increased bladder cancer mortality was associated with a population at an average of 570 μg/L for fifteen years, thirty years prior to the study[23]. Hopenhayn-Rich *et al.* reported increased bladder cancer rates for rural counties in Argentina with exposures up to 533 μg/L but were unable to link bladder cancer deaths to specific water arsenic concentrations[24].

Finally, in low arsenic exposure studies seeking information on an association with bladder cancer, the only positive findings were with cigarette smoking and no association was found for arsenic exposure independent of cigarette smoking at 0.5-160 μg/L[25], at 14-166 μg/L[26], or at 0.05-64 μg/L[27]. The above studies suggest that bladder cancer is associated with arsenic exposure at high arsenic water concentrations (in hundreds of μg/L) and not a low arsenic exposure levels.

Co-carcinogenic Phenomenon

Taken as a whole, these studies seem to support the concept of a discontinuity in the relationship between arsenic exposure and the risk of bladder cancer, with only high levels of arsenic exposure exhibiting an association with bladder cancer. Our analysis of the bladder cancer mortality data based on the study group of Wu *et al.*[13] and Morales *et al.*[15] showed a discontinuity at 400 μg/L and suggested that this apparent discontinuity might be a consequence of combining the bladder cancer death data from two populations of villages – those dependent upon artesian well water and those not dependent upon artesian well water.

We further proposed that the increased bladder cancer risk in artesian well dependent villages that was not evident in non artesian well dependent villages with similar reported arsenic levels in our study may reflect the consequences of an artesian well co-factor for the carcinogenic effect. Alternative explanations may be that (1) artesian wells contain some bladder carcinogen, other than arsenic, present in concentrations that correlate with the presence of inorganic arsenic, (2) artesian well might contain some substance which interacts with arsenic in inducing bladder cancer, and (3) artesian wells might contain another substance which arsenic transforms into a bladder carcinogen.

Previously, Lu had focused attention on "fluorescent" non-arsenic contaminants in the artesian well waters and proposed that the humic acids found in the artesian well waters might be a causal factor for BFD[9]. It may also be that humic acids are relevant to the issue of bladder cancer risk. Humic acid forms a bridged complex with arsenic and other metals in the presence of a polyvalent cation such as iron. The series of papers entitled "Epidemiological Studies on Blackfoot Disease in Taiwan" showed that the artesian well waters in the BFD-endemic areas also had higher iron levels and noted that "one of the most remarkable differences in physical characteristics between artesian well and shallow well water was the growth of algae in artesian well water."[28] It is clear that the BFD-endemic artesian well water was much more complex than just a high arsenic-containing water.

Other studies that stimulate the interest in humic acid include those of Yu *et al.*[29] and of Tully *et al.*[30]. Yu *et al.* demonstrated that the humic acid from the waters of the BFD-endemic area of Taiwan were mutagenic, whereas the humic acid from the chronic arseniasis area of Inner Mongolia and from the USA were not. The Tully *et al.* paper is interesting because the study was designed to demonstrate whether arsenic and other metals stimulated gene expression employed humic acid as a carrier for the metals. The Tully paper showed that arsenic at 50-250 mol/L affected stress-related promoters specifically, but not promoters related to either the aryl hydrocarbon (Ah receptor) promoters or to DNA-damage promoters.

The humic promoter hypothesis provides one theoretical model for experimental exploration, but other substances also merit attention. For example, Lin and Yang[31] discussed the ability of selenium and fluoride to modify the effects of arsenic. However, as fluoride levels in the BFD-endemic artesian well waters are similar to those in the shallow wells, fluoride is unlikely to explain the SW Taiwan observations but may elsewhere. In 1993, Chiang *et al.* in the British Journal of Urology pointed out that "the high content of arsenic and the high concentration of a "fluorescent substance" in artesian well water in this [BFD] area are the only 2 related factors identified so far" to explain the incidence of bladder cancer in the black foot endemic area in Taiwan[32].

Andrews *et al.* present another theoretical model based on their Dartmouth study of toenails arsenic levels, namely that "inhibition of DNA repair capacity is a potential mechanism for the co-carcinogenic activity of arsenic."[33] Toenail arsenic levels related inversely to the expression of DNA repair genes isolated from the same person's lymphocytes. They suggested cigarette smoking and ultraviolet light as potential cocarcinogenic exposures.

Moore *et al.* have recently demonstrated in bladder cancer patients from Argentina and Chile that bladder tumors in patients with higher levels of arsenic exposure showed higher levels of chromosomal instability, raising the possibility that higher arsenic exposure levels cause bladder tumors to behave more aggressively[34]. Thus, the appearance of excess bladder cancer mortality at higher arsenic exposures may reflect the more aggressive nature of these cancers and their greater likelihood to become clinically apparent. This more aggressive behavior would then be reflected in more aggressive metastatic behavior and tumor progression, not primary tumor initiation.

Smoking may be another potentiating factor. A number of studies have found a bladder cancer risk limited to smokers with higher arsenic exposures. Bates *et al.* noted that the adjusted odds ratio for non-smokers was 0.91 (90% CI 0.3-3.2) and for smokers was 3.32 [90% CI 1.1–10.3] at the highest level of exposure ($\geqslant$74 mg/liter-year)[25]. Their analysis of the window of exposure indicated that the dose-response relationship applied to those exposed between 30 and 39 years prior to diagnosis and was limited to smokers. In contrast, Kurttio *et al.* found an association between cigarette smoking and higher arsenic exposure levels which occurred only for recent (<10 years) arsenic exposure[27]. In a multivariate-adjusted analysis in NE Taiwan, Chiou *et al.* showed that cigarette smoking contributed a relative risk of 11-12 for urinary tract cancer[20]. In analyses of the SW Taiwan data, Chiou *et al.* did not find a cigarette smoking effect on bladder cancer incidence[18], and the design of the Morales *et al.* study did not permit an assessment of smoking as a risk factor for bladder cancer as smoking histories were not part of the Wu *et al.* data base[15]. Thus, smoking may or may not be an additional confounding (or effect modifying) factor or co-factor in the SW Taiwan study. A cigarette smoking effect may combine both the effect of smoking itself and the effect of the arsenic content in cigarette smoke. Doll reported that smokers inhale approximately 7.5 g As_2O_3 (5.5 μg Arsenic) per cigarette[35].

SUMMARY

Re-examination of the bladder cancer mortality data from the southwest Taiwan studies reveals that the assumption that the arsenic level in the drinking water is the only measure of increased bladder cancer risk may be too simplistic and is not the best explanation of the underlying data. Stratification of the data by type of water sources for the villages has as much power to predict the bladder cancer mortality rates as does the measure of arsenic

exposure.

The use of these data without stratifying by water source does not appear to be appropriate in the development of a quantitative risk analysis (QRA) for arsenic ingestion. We propose that the best data from the SW Taiwan data set for QRAs at low arsenic levels would be the data on the villages not dependent on artesian wells or not using artesian wells. Furthermore, QRAs that have used the SW Taiwan data to estimate risk in the USA have introduced further error by attempting to convert Taiwan exposures into US equivalent exposures with extrapolation to the US exposure range and by assuming that health care parameters in rural Taiwan are equivalent to those in the US. The best basis for the calculation of bladder cancer risk in the United States from ingestion of arsenic in drinking water is to use US data. Frost *et al.* have shown that such a study is feasible in the US[36].

This analysis of the SW Taiwan bladder cancer mortality found no increase in bladder cancer risk below 400 μg/L and suggests that this discontinuity in risk may be the consequence of mixing two populations of villages into the analysis. Separation of the data into artesian well dependent villages and non artesian well dependent villages shows an arsenic association only for the artesian well-dependent villages, suggesting potentially an artesian well co-carcinogenic factor, and no arsenic association for villages not dependent upon artesian wells.

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