Arsenic Exposure and Health Effects
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Reproductive and Developmental Effects Associated with Chronic Arsenic Exposure

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ABSTRACT

Chronic exposure to inorganic arsenic is known to cause cancer and non-cancer health effects in humans. The evidence from animal studies clearly shows that arsenic is teratogenic, and the findings of limited human studies suggest that inorganic arsenic may be associated with several reproductive/developmental outcomes, including increased rates of spontaneous abortion, low birth weight, congenital malformations, pre-eclampsia and infant mortality. The city of Antofagasta, located in northern Chile, has a history of high arsenic exposure in drinking water. Due to changes in the sources of water, there were considerably high arsenic levels in the public drinking water supply from 1958 to 1970 (over 800 μg/L), which decreased gradually to the current concentrations close to 50 μg/L. A number of studies have reported various health effects associated with the high exposure period, including skin alterations typically linked to arsenic exposure and increases in bladder and lung cancer. We conducted an ecologic study of infant mortality rates in Chile from 1950 to 1996, comparing Antofagasta to low arsenic exposure areas.Temporal and cross-regional comparisons showed a general steady decline over time in late fetal, neonatal and post-neonatal mortality rates for all locations, consistent with improvements in standard of living and health care. However, comparatively high rates were observed in Antofagasta for the three outcomes studied during the 12-year period of highest arsenic exposure, compared to Santiago and Valparaíso, two locations used as reference groups. While not definitive, these findings support a role for arsenic in the observed increases in mortality rates. Given the worldwide public health concern for arsenic effects, more population studies are needed in the area of human reproductive and developmental effects.

Keywords: arsenic, reproduction, developmental effects, Chile, drinking water, infant mortality, environment

INTRODUCTION

Arsenic is a naturally occurring element present throughout the earth's crust. Both organic and inorganic arsenic are present in the environment, but the inorganic forms are considered much more toxic (henceforth referred to simply as "arsenic", unless otherwise noted). Elevated human exposure to arsenic occurs mainly from mining and smelting of metals, pesticide production and application, medicinal treatments, and ingestion of arsenic-rich water, usually from natural contamination (IARC, 1980; ATSDR, 1993). A number of populations worldwide have been and/or are currently exposed to high arsenic levels in drinking water, and in recent years a growing number of exposed groups have been identified, including populations living in regions of India, Bangladesh, Thailand, Mexico, Chile, Argentina, China, Hungary and Finland. In the United States, an estimated 350,000 people currently receive drinking water containing more than 50 μg/L of arsenic, the current standard set by the U.S. Environmental Protection Agency (EPA, 1987). Although higher exposures are more common in western states of the United States, in recent years concern has grown in other states in areas where private well use is common and arsenic has been more extensively measured and often found to be near the EPA standard or the World Health Organization recommended lower standard of 10 μg/L (e.g. Minnesota, New Hampshire and Michigan) (Small-Johnson et al., 1998; Karagas et al., 1998; Michigan Department of Public Health, 1982).

Chronic arsenic exposure at high doses has neurologic, dermatologic, vascular and carcinogenic effects (IARC, 1980; ATSDR, 1993). Exposure to arsenic from drinking water increases the risks of skin, lung and bladder cancer and possibly other target sites (Wu et al., 1989; Chen et al., 1992; Hopenhayn-Rich et al., 1998; Smith et al., 1998), and also appears to increase the risk of diabetes (Shibata et al., 1994; Rahman et al., 1998).

Despite extensive research on the health risks of arsenic exposure, the potential impact of arsenic on human reproduction has been given minimal attention. However, there is sufficient data from animal studies, supported by *in vitro* and mechanistic information, and limited data from human studies, to indicate that arsenic may be associated with adverse reproductive effects in humans. The purpose of this paper is two-fold: first, to give an overview of existing evidence from the perspective of plausible effects in humans, and second, to present the descriptive results of an ecologic study of early infant mortality and its relationship to arsenic exposure from drinking water.

REVIEW OF THE EVIDENCE FOR REPRODUCTIVE TOXICITY

Human Studies of Arsenic and Pregnancy Outcomes

Several studies from the Ronnskar copper smelter in Sweden reported reproductive effects among female employees and nearby residents (Nordstrom et al., 1978a; Nordstrom et al., 1978b; Nordstrom et al., 1979a; Nordstrom et al., 1979b). For 662 births occurring between 1930 and 1959, the average birth weight of babies born to women employees (3,394 g) was significantly lower than those born to residents from an unexposed town distant from the smelter (3,460 g) ($p < 0.05$), and was lowest for those whose mothers worked in the most highly exposed jobs (e.g. smelting and cleaning operations) (3,087 g) (Nordstrom et al., 1979a). An increasing trend in the rates of spontaneous abortion was observed comparing all women employed at the smelter during pregnancy (14%), those who worked and whose husbands also worked at the smelter (19%) and those in high exposure jobs (28%) (Nordstrom et al., 1979a). A larger epidemiologic study of pregnancy outcomes for all women (N=4427) born after 1929 who lived in four areas of increasing distances from the smelter found a dose–response increase for the risk of spontaneous abortion with residential proximity to the smelter (Nordstrom et al., 1978b). Congenital malformations occurred in 6%

of babies born to female employees who worked during pregnancy, compared to 2% among employees who did not work while pregnant ($p < 0.005$) (Nordstrom et al., 1979b).

Although arsenic exposures in and around the Ronnskar smelter were high, confounding from lead or copper could not be excluded. Moreover, no adjustments were made for the effects of other potential confounding risk factors, such as maternal age, which is known to have a strong relationship with spontaneous abortion and congenital anomalies.

In southeast Hungary, rates of spontaneous abortions and stillbirths for the period 1980–87 were examined in an area with drinking water arsenic levels over 100 μg/L and a control area with low arsenic levels (Borzsonyi et al., 1992). Spontaneous abortions were 1.4-fold ($p < 0.02$) and stillbirths 2.8-fold ($p < 0.02$) higher in the exposed region, but the effect of potential confounders was not assessed. An increased incidence of spontaneous abortions and perinatal death in areas of Argentina with high arsenic in water has been reported, but no further detail was provided (Castro, 1982). In Bulgaria, the incidence of toxemia of pregnancy and mortality from congenital malformations in an area close to a smelter with environmental contamination from various metals were significantly higher than the national rates (Zelikoff et al., 1995).

Three U.S. studies reported adverse reproductive effects associated with relatively low water arsenic levels. An ecologic study examined mortality from cardiovascular-related causes in 30 counties from 11 states which had recorded arsenic measurements in public drinking water supplies greater than 5 μg/L (mean range: 5.4–91.5). This investigation found increases in mortality from congenital anomalies of the heart and other anomalies of the circulatory system over a 16-year period (1968–74) for two Nevada counties classified in the highest exposure group (29 and 46 μg/L average arsenic concentration in water) (Engel and Smith, 1994). In a case-control study conducted in Massachusetts, the adjusted prevalence odds ratio (POR) for congenital heart disease, comparing those whose water supply had arsenic levels above the detection limit (reported as 0.8 μg/L) with those below the limit, was not elevated (Zierler et al., 1988), but when stratified by type of heart disease, the POR for coarctation of the aorta was 3.4 (1.3–8.9). In a study of spontaneous abortions in the same area, Aschengrau et al. reported an adjusted odds ratio of 1.5 for the group with the highest arsenic concentrations: 1.4-1.9 μg/L (Aschengrau et al., 1989). We note that in these studies even the "exposed" areas had arsenic levels that are extremely low and therefore the results are difficult to interpret.

A hospital case-control study investigated the occurrence of stillbirths in relation to residential proximity to an arsenical pesticide production plant in Texas (Ihrig et al., 1998). Exposure was categorized in three groups according to arsenic air levels. An increasing trend in the risk of stillbirths was observed, significant for the high exposure group. When stratified by ethnicity, however, the findings remained significant for Hispanics only. It should be noted that this study was quite small, and more so when stratified by exposure and ethnic sub-groups; additionally, other exposures from the chemical plant were possible and were not measured in the study. Therefore, the results should be considered suggestive but not conclusive.

Overall, the epidemiologic evidence on adverse reproductive outcomes, although it suffers from methodological limitations, suggests positive associations with arsenic exposures.

Animal Teratogenicity

The teratogenicity of arsenic in animals is well-documented (Willhite and Ferm, 1984; Golub, 1994, Golub et al., 1998; DeSesso et al., 1998). Arsenic-induced defects include anophthalmia, exencephaly, and malformations of the genito-urinary, skeletal and cardiovascular systems. In general, there is consistent evidence from numerous animal studies showing that arsenic causes neural tube defects, and mechanistic hypotheses have been proposed (Shalat et al.,

1996). In addition, arsenic induces other forms of developmental toxicity, including death and growth retardation in the fetuses of exposed pregnant laboratory animals. Findings are consistent across studies, with effects found to be dependent on dose, route and timing of administration (Golub et al., 1998).

However, in a recent article, DeSesso et al. (1998) concluded that all previous positive findings of congenital malformations could be dismissed based on weaknesses in study design or interpretation of results. One of their criticisms of earlier work was that the high doses administered to pregnant animals generally caused maternal toxicity. Although many of the studies did find developmental effects at doses high enough to induce maternal toxicity, the existing evidence supports an increase in fetal abnormalities that is not secondary to maternal effects (Golub, 1994). Given the extensive number of peer-reviewed, published papers totaling over 50 studies describing positive, consistent results across different laboratories, using several animal species and modes of exposure, we should consider the results and interpretation provided by DeSesso et al. cautiously. It is also important to point out that arsenic appears to be unique in relation to its effects on humans: while an adequate animal model has not yet been found for the carcinogenicity of arsenic, there is now ample evidence of high cancer risks for several target sites in humans (skin, bladder and lung, and possibly others). It seems that humans have higher susceptibility to the toxic effects of arsenic compared to most commonly used laboratory animals. Therefore, human developmental effects may occur at lower doses than those determined from animal models, and as with cancer and other health outcomes, they will only be identified through epidemiological studies of chronically exposed populations.

Concentration in Tissues and Placental Transfer

Human autopsy studies have found high levels of arsenic accumulation in tissues of cancer target organs associated with arsenic exposure (Dang et al., 1983; Gerhardsson et al., 1988). However, these studies did not report concentrations in reproductive organs. In animal studies, rodents (Danielsson et al., 1984; Calvin and Turner, 1982), rabbits (Vahter and Marafante, 1983) and hamsters (Marafante and Vahter, 1987) have been shown to accumulate arsenic in the testis and epididymis. In female rats, arsenic concentrations in the ovaries were as high as in the liver (Ramos et al., 1995). Sheep fed a mixture of metals from emissions from a copper and zinc plant showed preferential accumulation in different organs by different metals with arsenic showing higher concentrations in ovaries than in kidneys or liver (Bires et al., 1995).

Placental transfer of inorganic arsenic is known to occur in both animals and humans (Ferm, 1977; Squibb and Fowler, 1983; Willhite and Ferm, 1984; Hood et al., 1987; Nicholson et al., 1982; Tabacova et al., 1994). A recent study in an area of Argentina with high arsenic in drinking water (250 μg/L), found a close relation between placental and cord blood arsenic levels, indicating considerable placental transfer of arsenic to the developing fetus during pregnancy (Concha et al., 1998).

Mechanisms

Several possible biological mechanisms can be postulated to support the evidence of arsenic-induced reproductive/developmental effects. Shalat et al. (1996) proposed plausible mechanistic roles for arsenic in the pathogenesis of neural tube defects, based on inhibition and disruption of cell proliferation, cell metabolism and placental/embryonal vascularization. All these processes can be affected by arsenic and in turn can affect neural tube formation.

In humans, methylation of inorganic arsenic appears to be the main detoxification pathway. Glutathione (GSH) and associated enzymes have been found to play a role in several steps of this methylation process (Thompson, 1993; Chiou et al., 1997). Since GSH is

involved in many biological pathways, it has been proposed that high arsenic exposures may decrease the availability of GSH, and perhaps even more in the presence of other contaminants that also use GSH for detoxification (Hopenhayn-Rich et al., 1996). The potential oxidative damage due to depletion of GSH provides possible pathways for reproductive toxicity, such as congenital malformations, pre-eclampsia and abnormal sperm development. Explanted mouse embryos and yolk sacs treated with arsenic showed malformative syndromes in parallel with decreasing levels of GSH, the teratogenicity was dose- and timing-dependent, and it was further enhanced by inhibition of GSH synthesis (Zelikoff et al., 1995). High levels of placental arsenic in women living near a smelter were associated with a lower percentage of placental GSH, and a concomitant increase in lipid peroxides capable of causing oxidative damage to the developing embryo (Tabacova et al., 1994). Arsenic concentrations were also associated with increased lipid peroxidation and lower levels of GSH in female treated rats (Ramos et al., 1995).

Arsenic may also cause male-mediated reproductive effects. GSH is normally found in high levels in rat testis and is believed to play an important role during meiosis in normal spermatogenesis (Calvin and Turner, 1982). In addition, membranes of mature sperm are susceptible to oxidative damage, and GSH forms part of the antioxidant defense systems in sperm (Lai et al., 1994). Therefore, decreased availability of GSH could lead to oxidative stress and lipid peroxidation of the sperm membrane, and to defective sperm function (Grieveau, 1995). Given the strong evidence of arsenic-induced clastogenicity and aneuploidy in other human cells (Nordenson et al., 1981; Moore et al., 1997; Gonsebatt et al., 1997), together with the accumulation of arsenic in the testis where sperm are formed, arsenic may also have a direct effect on human sperm chromosomes. Chromosomal aberrations, aneuploidy or other cytogenetic damage to sperm during spermatogenesis could lead to transgenerational effects including spontaneous abortion or aneuploid offspring.

Summary of the Evidence

It is well established that arsenic is genotoxic and causes cancer at various target sites in humans. It has also been proposed that arsenic is a likely reproductive toxicant. The human evidence, although limited, is consistent with animal, and *in vitro* laboratory results, and is suggestive of effects at various endpoints. Therefore, further studies of arsenic effects on human reproduction are warranted.

ECOLOGIC STUDY OF INFANT MORTALITY

INTRODUCTION

A retrospective ecologic study design was employed to investigate trends in infant mortality during the period 1950 to 1996 for a region of Chile with naturally elevated arsenic exposure in comparison to other regions with low background arsenic levels in the water. In addition, infant mortality rates were examined over time within the presumed high arsenic exposure area with respect to the temporal variation of arsenic levels in the water supply.

The population under study resides in Chile's Region II. This is generally a very dry area, with the Atacama Desert occupying a vast proportion of its territory. The surface water that supplies most of the region comes from rivers originating in the Andes mountains. Antofagasta, the largest city in the region with a current population of around 250,000 people, has a well-documented history of arsenic exposure. In 1958, due to insufficient water supply to serve the growing population and the decrease in water availability, water from the Toconce River was introduced as the main new water source. This river contains naturally occurring arsenic and at the time that inhabitants first used it, arsenic levels in this public water supply were approximately 800 μg/L. An increase in specific health effects in

this population began shortly after the change in water supply. The first reported cases of chronic arsenic poisoning appeared at the regional hospital in Antofagasta in 1962 (Zaldivar, 1980). A number of publications document the effects of exposure to arsenic in Antofagasta during that time (Zaldivar et al., 1981; Borgoño et al., 1977; Arroyo-Meneses, 1991; Smith et al., 1998). After 12 years of exposure to the high concentrations of the Toconce River, an arsenic removal plant was installed at the public water supply company.

METHODS

Vital Statistics Data

We obtained vital statistics data from the Instituto Nacional de Estadísticas (INE) in Chile, which centralizes local and national annual information on population vital statistics and census data. For this study, we obtained natality and mortality data from the period 1950 to 1996 from the yearly published books housed at INE's central office. The information in these reports has been collated from birth and death certificates obtained from civil registration and health department offices from each local government responsible for routinely collecting this data. The INE infant mortality data are classified separately as late fetal deaths (over 28 weeks of gestation, mainly consisting of stillbirths) and infant deaths, the latter being further divided into those under 28 days of age and those 28 days to one year of life. We will refer to these groups as fetal, neonatal and post-neonatal, respectively, according to standard definitions.

Exposure Data

Historical water measurement data from public water supplies for Antofagasta, obtained from a compilation of existing sources (water company records and the regional health service), are given in Table 1. As mentioned previously, water from the Toconce River was introduced as the primary new water source for the entire city in 1958, serving all the population using piped water. Based upon the data collected, the mean arsenic level during the period 1958–1970 was estimated to be 860 μg/L. Before that, arsenic levels in the water averaged 90 μg/L. In 1970, the city installed an arsenic removal plant for this water supply. Subsequent measurements established a decline in the arsenic levels over the next 26 years to the current levels close to 50 μg/L. The primary, extremely high exposure period for Antofagasta was during the period 1958–1970.

To enhance validity, we examined several comparison groups and present results from the following in this report: all of Chile, the metropolitan region of Santiago, which currently concentrates about 40% of the country's population, and the county of Valparaíso. Santiago includes the capital and surrounding areas. Valparaíso, like Antofagasta, is a coastal town and they share similar size and sociodemographic characteristics. Neither Santiago nor Valparaíso have historical evidence of high arsenic contamination, and recent water surveys

TABLE 1

Average arsenic levels in Antofagasta. Data represents an average of existing arsenic water measurements (Pedreros, 1994).

Year	Concentration (μg/L)
1950–1957	90
1958–1970	860
1971–1979	110
1980–1987	70
1988–1996	40

conducted by the National Environmental Commission (FONDEF, 1997) and by our group show arsenic levels to be low (<5 μg/L). In general, aside from Region II, no major populations in Chile have had such high exposures to arsenic from drinking water (Smith et al., 1998).

ANALYSIS

For this study, we retrieved yearly vital statistics information from 1950 to 1996, to examine infant mortality rates in the county of Antofagasta over time, and to compare Antofagasta rates to those in low arsenic areas. Although we used the county as the unit of analysis, for Antofagasta and Valparaíso the majority of their population lives in cities bearing the same names (99% and 97%, respectively).

Changes over time in the number and geographic boundaries of geopolitical localities had to be considered in developing a common geographic unit for the analysis of the data. Birth and mortality data are reported by different aggregate geographic regions. Since 1976, Chile has been stratified into regions, numbered 1 through 12 from north to south (plus the metropolitan area of Santiago which is considered separately). Regions have been further divided into provinces, and then into comunas (equivalent to counties). Prior to 1976, Chile was divided into 25 provinces, and throughout the years, several changes occurred in the number and jurisdiction of geographic delimitations. One of us (C.F.) undertook an in-depth reclassification of geographic areas to achieve compatibility across the entire period. The comuna was the smallest unit of analysis with identifiable vital statistics information, standardized across time.

Further, births and deaths are reported by both place of occurrence and place of maternal residence. Since we were interested in the relationship between infant mortality and arsenic exposure from drinking water (either through maternal or infant ingestion) we used location of maternal residence for coding the birth and death data.

Infant mortality rates were calculated by dividing the number of deaths by the number of live births per location and multiplying by 1000. For late fetal mortality, we divided the number of fatalities by the number of live births + fetal deaths, as is standard practice, to obtain the death rate per total births. After calculating yearly mortality rates for each group, considerable variation was noted from year to year, given the relatively small number of events. Therefore, we grouped the rates into 4-year periods (except for the last period for which we only had 3 years of data), to achieve higher stability and still maintain a distinct period of highest arsenic exposure in Antofagasta.

Since the patterns of mortality in the three outcomes studied did not vary greatly between Santiago and Valparaíso, the two reference groups, we concentrated further analysis in comparing Antofagasta to Valparaíso, given the greater homogeneity found within a county (as opposed to the large Santiago metropolitan area) and the similarities shared by these two counties in particular. For each mortality outcome, we calculated rate differences for each 4-year time period.

RESULTS

The period studied is one of general decrease worldwide in fetal and infant mortality due mainly to improvements in living conditions and health care. Figure 1 shows mortality rates in Chile as a whole for the 3 outcomes studied. Figures 2, 3 and 4 show the 4-year fetal, neonatal and post-neonatal mortality rates, respectively, for Antofagasta, Santiago and Valparaíso. For Antofagasta, the rates were generally higher at the beginning of the study period (1950–57), but a clear pattern of elevated rates in comparison to Santiago and Valparaíso is observed during the high arsenic years (1958–69). After 1970, mortality rates

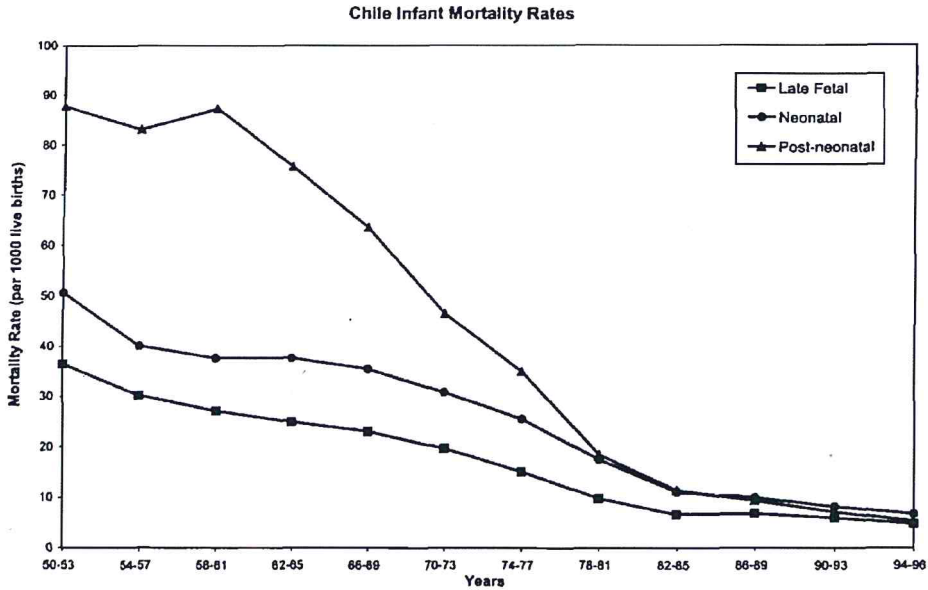

Fig. 1. Late fetal, neonatal and post-neonatal mortality rates for all of Chile, 1950–1996.

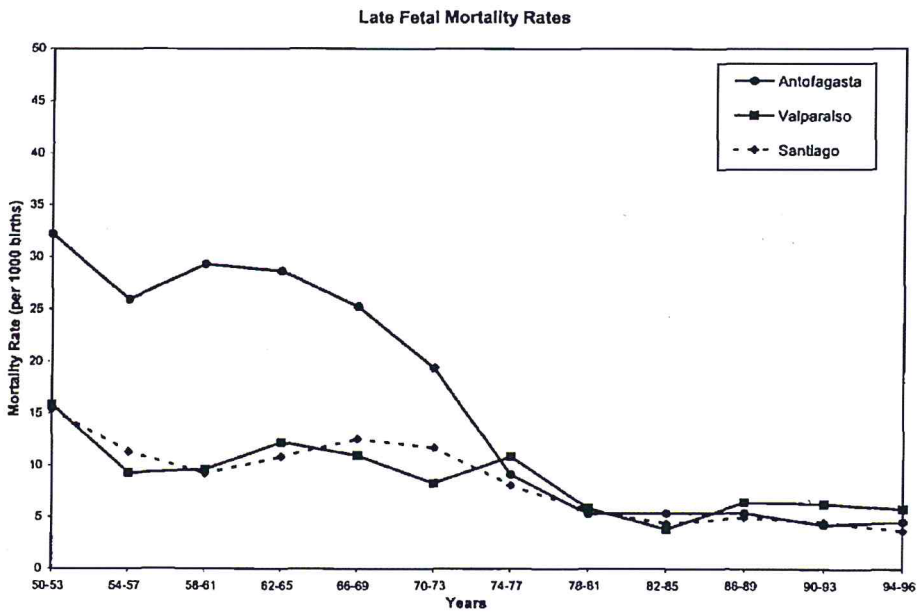

Fig. 2. Late fetal mortality rates for Antofagasta, Santiago and Valparaíso, 1950–1996.

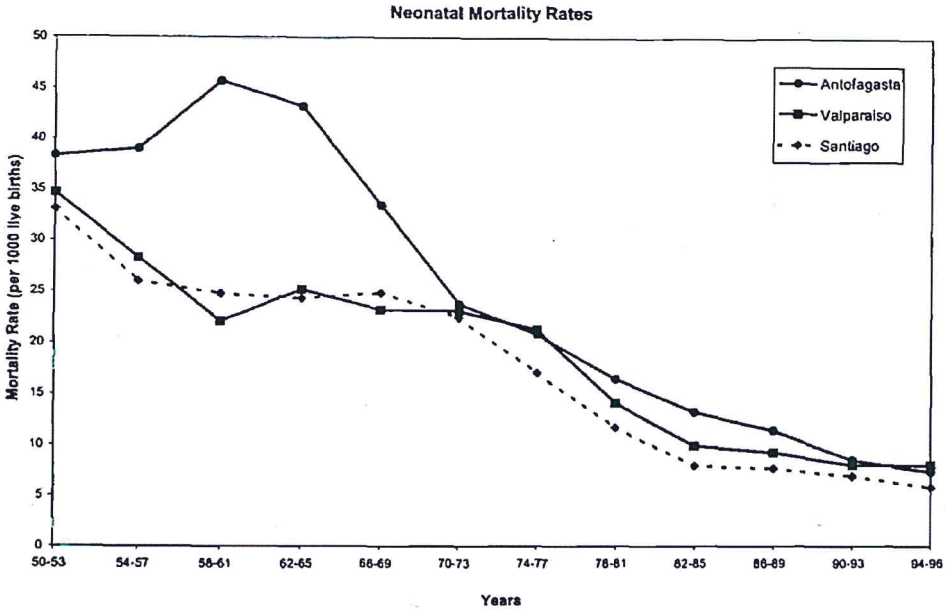

Fig. 3. Neonatal mortality rates for Antofagasta, Santiago and Valparaíso, 1950–1996.

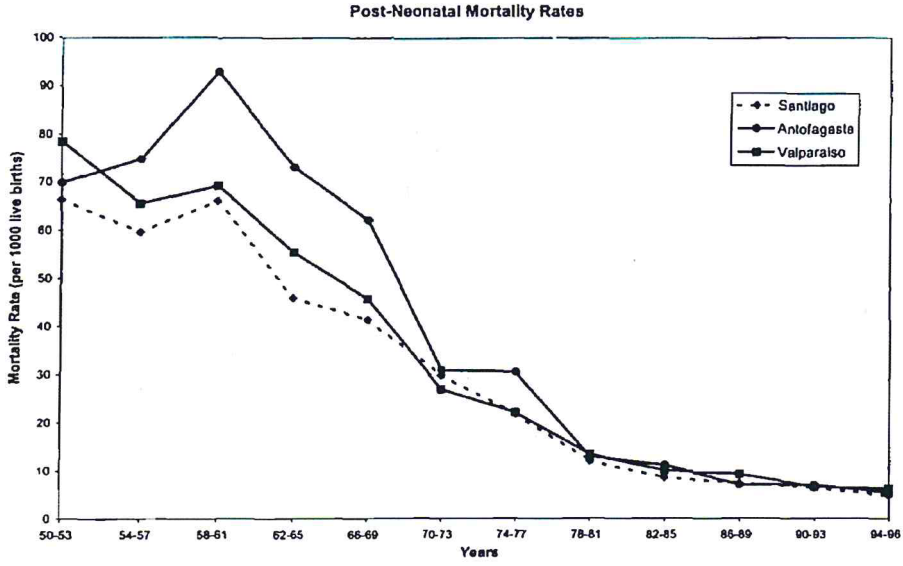

Fig. 4. Post-neonatal mortality rates for Antofagasta, Santiago and Valparaíso, 1950–1996.

TABLE 2

Mortality rates and rate differences for Antofagasta and Valparaiso. Mortality rates were calculated per 1000 live births for neonatal and postneonatal period, and per 1000 total births (live + late fetal) for the late fetal period.

Year	Late Fetal				Neonatal				Post-neonatal			
	ANT.	VALP.	R.D.	95% C.I.	ANT.	VALP.	R.D.	95% C.I.	ANT.	VALP.	R.D.	95% C.I.
1950–53	32.2	15.9	16.3	15.4–17.3	38.4	34.7	3.7	3.0–4.4	69.8	78.4	–8.6	(7.8–9.4)
1954–57	25.9	9.3	16.7	15.8–17.4	39.0	28.3	10.7	9.9–11.5	74.8	65.5	9.2	8.5–10.1
1958–61	29.3	9.6	19.7	18.9–20.5	45.7	22.1	23.6	22.7–24.5	93.0	69.3	23.7	22.8–24.6
1962–65	28.6	12.3	16.4	15.6–17.0	43.2	25.2	18.0	17.2–18.8	73.1	55.4	17.6	16.8–18.6
1966–69	25.2	11.0	14.3	13.5–14.9	33.5	23.2	10.3	9.6–11.0	62.1	45.8	16.3	15.4–17.2
1970–73	19.4	8.3	11.1	10.5–11.7	23.8	23.1	0.7	0.2–1.3	31.0	26.9	4.0	3.5–4.7
1974–77	9.1	10.9	–1.7	(1.3–2.3)	20.9	21.3	–0.5	(0.1–0.9)	30.5	22.2	8.4	7.6–9.0
1978–81	5.3	5.9	–0.5	(0.3–0.9)	16.5	14.1	2.3	1.9–2.9	13.0	13.5	–0.5	(0.03–1.0)
1982–85	5.4	3.8	1.6	1.3–1.9	13.2	9.9	3.3	2.8–3.8	11.2	10.1	1.1	0.7–1.5
1986–89	5.4	6.5	–1.0	(0.7–1.5)	11.4	9.3	2.2	1.7–2.5	7.1	9.3	–2.2	(1.8–2.6)
1990–93	4.3	6.3	–2.1	(1.6–2.4)	8.5	8.0	0.5	0.1–0.9	7.0	6.7	0.3	0.04–0.6
1994–96	4.5	5.8	–1.2	(0.9–1.7)	7.3	8.0	–0.7	(0.3–1.2)	5.4	6.1	–0.8	(0.3–1.1)

Abbreviations: ANT.= Antofagasta; VALP= Valparaíso; R.D.= Rate Difference; C.I.= Confidence Interval.

level off and become very similar to those in the two comparison areas. The increases vary by outcome, but are generally more pronounced in the first 4-year period (1958–61). Table 2 shows the comparison of rates between Antofagasta and Valparaíso. The rate differences, which were reflected in the graphs, peak in the 1958–61 period for all three outcomes.

DISCUSSION

The results of this study show decreasing trends in fetal, neonatal and post-neonatal mortality over time in all of Chile, as well as in Santiago and Valparaíso. These are consistent with observations in many countries in the latter half of this century. The main factors reported to affect infant mortality in Chile are improvement in the standard of living, development of programs for maternal and infant care (prenatal, nutritional supplementation, health education, etc.) and the decrease in birth rate, most noticeable since the 1960s (INE, 1994). The same tendency is observed in Antofagasta, except for increases observed in 1958–61 which decline thereafter but remain elevated relative to Santiago and Valparaíso until 1974–77 for fetal mortality, and until 1970–73 for neonatal and post-neonatal mortality. These results show a generally close temporal relationship with the considerably high arsenic levels present in the city's water supply from 1958 to March of 1970. As in the rest of the country, mortality rates in Antofagasta did decline gradually during this time period probably due to the time trend effect, that is, other, non-arsenic related factors, such as improvement in health care and standard of living, which were improving in Antofagasta alongside the rest of Chile. Moreover, starting in the 1960s, Region II experienced a surge of economic growth due mostly to the great expansion associated with copper mining (mainly in the high desert Chuquicamata mine). The rate of growth in the region as a whole greatly surpassed that of the rest of the country. A study of the development of production by regions showed that adjusted annual per capita income in Region II had the greater increase, by far, than any other region (CIEPLAN, 1994). This regional expansion brought improvements in health care as well, which likely explain, at least in part, the fact that in the period before the arsenic highest years, Antofagasta had higher mortality rates for the three outcomes examined, while after the arsenic removal plant was installed, rates declined to levels similar to those of the comparison regions (as indicated by the rate differences).

Ecologic studies, in which both exposures and outcomes are measured at the group level and not at the individual level, are subject to the bias known as the ecologic fallacy. This problem arises when the exposure does not apply to all persons in the group, such that those experiencing the adverse outcomes might not be the same individuals who are experiencing the exposure. However, since water is consumed by virtually all residents, and in this case the public water supply came from one main source, this bias seems unlikely to have influenced the results of this study. The distinct temporal pattern of Antofagasta rates relative to other regions also argues against this bias. As with other epidemiologic study designs, ecologic studies can be subject to confounding and effect modification. Although it is possible that other factors not accounted for in this analysis could have been present concurrently with the high arsenic that could explain or exacerbate the observed increase in infant mortality, we could not identify any other factors during that time period that would so closely relate to the timing of the arsenic exposure. The change in water supply was an indisputable event, and no other contaminant has been described to account for the many documented health effects suffered by the population of Antofagasta due to arsenic exposure during that time period.

With regards to the validity of these data, the accuracy of birth and death certification is hard to assess. However, there is evidence of under-ascertainment, which has improved over time. In Chile, children whose births are not registered by March of the year after they were born are counted in a separate, late registration category. A study of birth registrations from 1955–1988 showed variations ranging from 11.4% to 4.2% in late birth registrations (INE/CELADE, 1990). Variations were also observed between regions, with the northern-most ones (which include Antofagasta, Valparaíso and Santiago) having the lowest late registration rates. The increase in births that occur in hospitals (42.3% in 1953 versus 99.7% in 1994) and births assisted by medical professionals (57.5% in 1953 vs. 100% in 1994) also impacts completeness of birth registration. Regional statistics show Antofagasta, Santiago and Valparaíso were three of the four provinces with the highest percentage of births assisted by physicians in 1953 relative to the rest of Chile (INE, 1953).

Death registration is also characterized by omissions, especially for newborns that died in the first hours or days after birth. A study conducted in Santiago in 1968–69 showed that over half of the babies born in hospitals who died were not registered (Legarreta et al., 1973). Another study in Santiago found 13% of the deaths of children under the age of five (5) were not registered, and most of this under-registration occurred for deaths in the neonatal period. In Chile, the certification of infant deaths by a physician increased from 55% in 1952 (INE, 1953) to 96% in 1994 (INE, 1994).

The omissions in death reporting and the late birth registrations described above will affect mortality rates, which will be biased up or down depending whether birth or death omissions were higher. However, given the much greater number of births than deaths, under-reporting of deaths will have a much greater effect on the rates. There is no evidence that under-reporting of deaths was significantly different between Antofagasta, Valparaíso and Santiago. Moreover, if the increases in fetal and infant mortality observed in Antofagasta were at least, in part, attributable to the high arsenic water levels during those years, the omissions would underestimate rather than over-estimate the effect.

In this paper, we have reviewed the literature for reproductive effects of arsenic and have found suggestive evidence for arsenic-related human developmental toxicity. Furthermore, we have conducted a study of infant mortality rates in Chile comparing high and low areas of arsenic exposure. The period of highest exposure was accompanied by higher rates in Antofagasta, as compared to the unexposed areas of Santiago and Valparaíso. While not definitive, these findings support a role for arsenic in the increased mortality. If arsenic reproductive toxicity is specific to certain causes of death, then the actual magnitude of the effect would probably be larger than that observed here. Further studies are warranted to

investigate the risks of specific causes of late fetal and infant death in very highly exposed populations, as well as of other less drastic but nevertheless adverse developmental effects at moderately elevated levels.

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REFERENCES

Arroyo-Meneses, A. 1991. Hidroarsenicismo cronico en la region de Antofagasta. *Primera Jornada Sobre Arsenicismo Laboral y Ambiental II Region*, 35–47.

Aschengrau, A., Zierler, S., Cohen, A. 1989. Quality of Community Drinking Water and the Occurrence of Spontaneous Abortion. *Arch. Environ. Health*, 44(5), 283–290.

ATSDR, 1993 Toxicological Profile for Arsenic, Update, Report No. TP-92/102. Agency for Toxic Substances and Disease Registry, U.S. Dept. of Health and Human Services, Atlanta, GA.

Bires, J., Maracek, I., Bartko, P., Biresova, M., Weissova, T. 1995. Accumulation of trace elements in sheep and the effects upon quantitative and qualitative ovarian changes. *Vet. Human Toxicology*, 37 (4), 349–356.

Borgoño, J.M., Vicent, P., Venturino, H., Infante, A. 1977. Arsenic in the drinking water of the city of Antofagasta: epidemiological and clinical study before and after the installation of a treatment plant. *Environ. Health Perspect.*, 19, 103–105.

Borzsonyi, M., Bereczky, A., Rudnai, P., Csanady, M., Horvath, A. 1992. Epidemiological studies on human subjects exposed to arsenic in drinking water in Southwest Hungary. *Arch. Toxicol.*, 66, 77–78.

Calvin, H.I., Turner, S.I. 1982. High levels of glutathione attained during postnatal development of rat testis. *J. Exp. Zool.*, 219, 389–393.

Castro, J.A. 1982. Efectos carcinógenicos, mutagénicos y teratogénicos del arsénico. *Acta Bioquim. Clin. Latinoamericana*, 16(1), 3–17.

Chiou, H-Y., Hsueh, Y-M., Hsieh, L-L., Hsu, L-I., Hsu, Y-H., Hsieh, F-I., Wei, M-L., Chen, H-C., Yang, H-T., Leu, L-C., Chu, T-H., Chen-Wu, C., Yang, M-H., Chen, C-J. 1997. Arsenic methylation capacity, body retention, and null genotypes of glutathione *S*-Transferase M1 and T1 among current arsenic-exposed residents in Taiwan. *Mutat. Res.*, 386, 197–207.

CIEPLAN Corporación de Investigaciones Económicas para Latinoamérica - Ministerio del Interior, Subsecretaría de Desarrollo Regional y Administrativo. 1994. *Evolución del Producto por Regiones 1960–1992*, Santiago, Chile.

Concha, G., Vogler, G., Lexcano, D., Nermell, B., Vahter, M. 1998. Exposure to Inorganic Arsenic Metabolites during Early Human Development. *Toxicol. Sci.*, 44, 185–190.

Dang, H.S., Jaiswal, D.D., Somasundaram, S. 1983. Distribution of arsenic in human tissues and milk. *Sci. Total Environ.*, 29, 171–175.

Danielsson, B.R., Dencker, L., Lindgren, A., Tjalve, H. 1984. Accumulation of toxic metals in male reproduction organs. *Arch. Toxicol.*, Suppl. 7, 177–180.

DeSesso, J.M., Jacobson, C.F., Scialli, A.R., Farr, C.H., Holson, J.F. 1998. An assessment of the developmental toxicity of inorganic arsenic. *Reproduct. Toxicity*, 12(4), 385–433.

Engel, R.E., Smith, A.H. 1994. Arsenic in drinking water and mortality from vascular disease: an ecological analysis in 30 counties in the United States. *Arch. Environ. Health*, 49(5), 418–127.

EPA (Environmental Health Protection Agency) 1987. Estimated national occurrence of arsenic in public drinking water supplies. EPA contract No. 68-01-7166.

Ferm, V.H. 1977. Arsenic as a tetagenic agent. *Environ. Health Perspect.*, 19, 215–217.

FONDEF—Facultad de Ciencias Fisicas y Matemáticas de la Universidad de Chile. 1997. Determinación de línea base de arsénico ambiental, Santiago, Chile.

Gerhardsson, L., Brune, D., Nordberg, G.F., Wester, P.O. 1988. Multielemental assay of tissues of deceased smelter workers and controls. *Sci. Total Environ.*, 74, 97–110.

Golub, M.S. 1994. Maternal toxicity and the identification of inorganic arsenic as a developmental toxicant. In: *Reproductive Toxicology*. pp. 283–295. Elsevier, Amsterdam and New York.

Golub, M.S., Macintosh, M.S., Baumrind, N. 1998. Developmental and reproductive toxicity of inorganic arsenic: animal studies and human concerns. *J. Toxicol. Environ. Health*, Part B, 199–241.

Gonsebatt, M.E., Vega, L., Salazar, A.M., Montero, R., Guzmán, P., Blas, J., Del Razo, L.M., García-Vargas, G., Albores, A., Cebrián, M.E., Kelsh, M., Ostrosky-Wegman, P. 1997 Cytogenetic effects in human exposure to arsenic. *Mutat Res.*, 386, 219–228.

Grieveau, J.F., Dumont, E., Renard, P., Callegari, J.P., Le Lannou, D. 1995. Reactive oxygen species, lipid peroxidation and enzymatic defense systems in human spermatozoa. *J. Reproduct. Fertil.*, 103, 17–26.

Hood, R.D., Vedel-Macrander, G.C., Zaworotko, M.J., Tatum, F.M., Meeks, R.G. 1987. Distribution, metabolism, and fetal uptake of pentavalent arsenic in pregnant mice following oral or intraperitoneal administration. *Teratology*, 35, 19–25.

Hopenhayn-Rich, C., Biggs, M.L., Smith, A.H., Kalman, D.A., Moore, L.E. 1996. Methylation study in a population environmentally exposed to high arsenic drinking water. *Environ. Health Perspect.*, 104, 620–628.

Hopenhayn-Rich, C., Biggs, M.L., Smith, A.H. 1998. Lung and kidney cancer mortality associated with arsenic in drinking water in Córdoba, Argentina. *Int. J. Epidemiol.*, 27, 561–569.

IARC. World Health Organization. 1980. *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Metals and Metallic Compounds*. Lyon, France.

Ihrig, M.M., Shalat, S.L., Baynes, C. 1998. A hospital-based case-control study of stillbirths and environmental exposure to arsenic using an atmospheric dispersion model linked to a geographical information system. *Epidemiology*, 9(3), 290–294.

INE (Instituto Nacional de Estadísticas - Ministerio de Salud. Servicio Registro Civil e Identificación) 1994. *Anuario de Demografía*, Santiago, Chile.

INE (Instituto Nacional de Estadísticas - Ministerio de Salud. Servicio Registro Civil e Identificación) 1953. *Movimiento Demográfico*, Santiago, Chile.

INE/CELADE (Instituto Nacional de Estadísticas y Centro Latinoamericano de Demografía - Ministerio de Economía, Fomento y Reconstrucción) 1990. *Chile, Estimación de la Oportunidad de Inscripción de los Nacimientos—Total País y Regiones 1955–1988*, Santiago, Chile.

Karagas, M.R., Tosteson, T.D., Blum, J., Morris, J.S., Baron, J.A., Klaue, B. 1998. Design of an Epidemiologic Study of Drinking Water Arsenic Exposure and Skin and Bladder Cancer Risk in a U.S. Population. *Environ. Health Perspect.*, 106(4), 1047–1050.

Lai, M-S., Hsueh, Y-M., Chen, C-J., Shyu, M-P., Chen, S-Y., Kuo, T-L., Wu, M-M., Tai, T-Y. 1994. Ingested inorganic arsenic and prevalence of diabetes mellitus. *Am. J. Epidemiol.*, 139(5), 484–492.

Legarreta, A., Aldea, A., López, L. 1973. Omisión del registro de defunciones de niños ocurridas en maternidades, Santiago, Chile. *Bol. Sanit. Panam.*, 75(4), 308–314.

Marafante, E., Vahter, M. 1987. Solubility, retention, and metabolism of intratracheally and orally administered inorganic arsenic compounds in the hamster. *Environ Res.*, 42, 72–82.

Michigan Department of Public Health. 1982. Arsenic in Drinking Water—A Study of Exposure and a Clinical Survey.

Moore, L.E., Smith, A.H., Hopenhayn-Rich, C., Biggs, M.L., Kalman, D.A., Smith, M.T. 1997. Micronuclei in Exfoliated Bladder Cells among Individuals Chronically Exposed to Arsenic in Drinking Water. *Cancer Epidemiol. Biomarkers Prev.*, 6, 31–36.

Nicholson, W.J., Perkel, G., Selikoff, I.J. 1982. Occupational exposure to asbestos: population at risk and projected mortality— 1980–2030. *Am. J. Ind. Med.*, 3, 259–311.

Nordenson, I., Sweins, A., Beckman, L. 1981. Chromosome aberrations in cultured human lymphocytes exposed to trivalent and pentavalent arsenic. *Scand. J. Work Environ. Health*, 7, 277–281.

Nordstrom, S., Beckman, L., Nordenson, I. 1978a. Occupational and environmental risk in and around a smelter in northern Sweden—I. Variations in birth weight. *Hereditas*, 88, 43–46.

Nordstrom, S., Beckman, L., Nordenson, I. 1978b. Occupational and environmental risk in and around a smelter in northern Sweden—III. frequencies of spontaneous abortion. *Hereditas*, 88, 51–54.

Nordstrom, S., Beckman, L., Nordenson, I. 1979a. Occupational and environmental risk in and around a smelter in northern Sweden—V. Spontaneous abortion among female employees and decrease birth weight in their offspring. *Hereditas*, 90, 291–296.

Nordstrom, S., Beckman, L., Nordenson, I. 1979b. Occupational and environmental risks in and around a smelter in northern Sweden—VI. congenital malformations. *Hereditas*, 90, 297–302.

Pedreros, R. 1994. Presencia de Arsénico en II Región y Estudio Retrospectivo de Cohortes Expuestas. Presented at Segundas Jornadas de Arsenicismo Laboral y Ambiental, Servicio Nacional de Salud, Antofagasta, Chile.

Rahman, M., Tondel, M., Ahmad, S.A., Axelson, O. 1998. Diabetes Mellitus Associated with Arsenic Exposure in Bangladesh. *Am. J. Epidemiol.*, 148(2), 198–203.

Ramos, O., Carrizales, L., Yañez, L., Mejía, J., Batres, L., Ortíz, D., Diaz-Barriga, F. 1995 Arsenic Increased Lipid Peroxidation in Rat Tissues by a Mechanism Independent of Glutathione Levels. *Environ. Health Perspect.*, 103 (Suppl.1), 85–88.

Shalat, S.L., Walker, D.B., Finnell, R.H. 1996. Role of arsenic as a reproductive toxin with particular attention to neural tube defects. *J. Toxicol. Environ. Health*, 48, 253–272.

Shibata, A., Ohneseit, P.F., Tsai, Y.C., Spruck, C.H., Nichols, P.W., Chiang, H., Lai, M., Jones, P.A. 1994. Mutational spectrum in the p53 gene in bladder tumors from the endemic area of black foot disease in Taiwan. *Carcinogenesis*, 15(6), 1085–1087.

Small-Johnson, J., Soule, R., Durkin, D., and Minnesota Dept. of Health. Minnesota Arsenic Research Study (MARS): Geochemical Parameters, Human Exposures and Health Effects Associated with Low-Level Arsenic in Drinking Water. Presented at the International Society of Environmental Epidemiology Conference, Boston, Massachusetts, August 1998.

Smith, A.H., Goycolea, M., Haque, R., Biggs, M.L. 1998. Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water. *Am. J. Epidemiol.*, 147(7), 660–669.

Squibb, K.S., Fowler, B.A. 1983. The toxicity of arsenic and its compounds. *Biolog. Environ. Effects Arsenic*, 233–69.

Tabacova, S., Baird, D.D., Balabaeva, L., Lolova, D., Petrov, I. 1994. Placental arsenic and cadmium in relation to lipid peroxides and gluthathione levels in maternal-infant pairs from copper smelter area. *Placenta*, 15(8), 873–881.

Thompson, D.J. 1993. A chemical hypothesis for arsenic methylation in mammals. *Chem. Biol. Interact.*, 88, 89–114.

Vahter, M., Marafante, E. 1983. Intracellular interaction and metabolic fate of arsenite and arsenate in mice and rabbits. *Chem. Biol. Interact.*, 47, 29–44.

Willhite, C.C., Ferm, V.H. 1984. Prenatal and development of toxicology of arsenicals. *Nutritional and Toxicological Aspects of Food Safety*, Ch. 9, pp. 205–228.

Wu, M-M., Kuo, T-L., Hwang, Y-H., Chen, C-J. 1989. Dose–response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. *Am. J. Epidemiol.*, 130 (6), 1123–1132.

Zaldivar, R. 1980. A morbid condition involving cardiovascular, bronchopulmonary, digestive and neural lesions in children and young adults after dietary arsenic exposure. *Zbl. Bakt. I Abt. Orig. B*, 179, 44–56.

Zaldivar, R., Prumes, L., Ghai, G.L. 1981. Arsenic dose in patients with cutaneous carcinomata and hepatic haemangioendothelioma after environmental and occupational exposure. *Arch. Toxicol.*, 47, 145–154.

Zelikoff, J.T., Bertin, J.E., Burbacher, T.M., Hunter, E.S., Miller, R.K., Silbergeld, E.K., Tabacova, S., Rogers, J.M. 1995. Health Risks Associated with Prenatal Metal Exposure. *Fundam. Appl. Toxicol.*, 25, 161–170.

Zierler, S., Theodore, M., Cohen, A., Rothman, K.J. 1988. Chemical quality of maternal drinking water and congenital heart disease. *Int. J. Epidemiol.*, 17(3), 589–594.