Accepted: 10 December 2018
DOI: 10.1002/ajim.22939

RESEARCH ARTICLE

WILEY AMERICAN JOURNAL OF INDUSTRIAL MEDICINE

Long-term effect of arsenic exposure: Results from an occupational cohort study

Emilio Antonio L. Gianicolo PhD[1,2] | Cristina Mangia MSc[3] | Marco Cervino MSc[4] | Antonella Bruni MSc[5] | Maurizio Portaluri MD[6] | Pietro Comba PhD[7] | Roberta Pirastu MSc[8] | Annibale Biggeri[9,10] | Mariangela Vigotti[1] | Maria Blettner[2]

[1] Italian National Research Council, Institute of Clinical Physiology, Lecce, Italy
[2] University of Mainz, Institute of medical Biostatistics, Epidemiology and Informatics, Mainz, Germany
[3] Italian National Research Council, Institute of Atmospheric Sciences and Climate, Lecce, Italy
[4] Italian National Research Council, Institute of Atmospheric Sciences and Climate, Bologna, Italy
[5] Epidemiological and Statistical Unit, Azienda Sanitaria Locale, Taranto, Italy
[6] Department of Radiotherapy, General Hospital, Brindisi, Italy
[7] Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy
[8] Fondazione Sapienza, Università di Roma, Roma, Italy
[9] Department of Statistics, Computer Science, Applications, University of Florence, Florence, Italy
[10] Epidemiologia e Prevenzione Social Enterprise, Torino, Italy

Correspondence
Emilio Antonio L. Gianicolo, University of Mainz, Institute of medical Biostatistics, Epidemiology and Informatics. Obere Zahlbacher Str. 69, 55131, Mainz, Germany.
Emili: emilio.gianicolo@uni-mainz.de

Funding information
Municipality of Manfredonia

Background: In 1976 in Manfredonia (Italy), arsenic was released into the atmosphere due to an accident in a petrochemical plant. We aimed to analyze the mortality of workers involved in the factory for the site cleaning activities.

Methods: The cohort consisted of 1467 workers grouped into contract, fertilizer, and plastic workers. The outcome of interest was mortality for specific causes. Standardized mortality ratios (SMR) and 95% confidence intervals (95%CI) were computed.

Results: For all workers and all causes of death combined, the SMR was less than 1.0. Mortality ratios were increased for malignant neoplasms of the pleura, bone and melanoma of the skin. Contract workers, the group mostly exposed to arsenic, showed statistically significant SMRs for several malignancies, in particular for lung cancer (SMR = 1.26; 95%CI: 1.05-1.54).

Conclusions: Overall, the results reported here on mortality among persons occupationally exposed to arsenic are consistent with the literature and biologically plausible.

KEYWORDS
arsenic, epidemiology of disasters, industrial accident, lung cancer, petrochemical plant

1 | INTRODUCTION

The International Agency for Research on Cancer (IARC) classifies arsenic and inorganic arsenic compounds as carcinogenic to humans (Group 1).[1] Inorganic arsenic compounds cause lung cancer, urinary bladder cancer, and skin cancer. Positive associations have also been observed between exposure to arsenic and cancer of the kidney, liver, and prostate.[1] Evidence is also conclusive for non-neoplastic

Institution at which the work was performed: University Medical Center Mainz, Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI).

conditions such as skin ailments.[2] Evidence of an association with non-cancer outcomes such as diabetes and cardiovascular diseases is still inconclusive.[3–5]

From 1971 to 1994, a chemical plant located about 2 km from the built-up area of Manfredonia (Figure 1) produced caprolactam (plastic) and urea (fertilizers). The initial product for the fertilizer manufacturing was ammonia, obtained from methane gas. In the synthesis of ammonia, achieved in an ammoniac washing column, arsenic compounds were used as a catalyzer for chemical reactions.[6]

On September 26, 1976, a major chemical accident occurred due to the explosion of the ammoniac washing column, and an estimated 10-39 tons of arsenic compounds were released into the atmosphere.[7,8] Emissions following the explosion were of two types:

1. A liquid solution and the solid materials of the column, which descended in the proximity of the plant.
2. A cloud of gas and droplets, rising to 200 meters, which dispersed in the direction of the city of Manfredonia.

In the days after the accident, about 1800 persons were present in the chemical plant, including workers contracted by minor companies (contract workers) who worked in the same plant for the main company and were mainly assigned to manual work. During the first 6 days after the accident, contract workers and workers from the fertilizer area conducted initial cleaning-up activities, consisting of[9]:

1. Using brooms to clean up dust. This was performed by contract workers;
2. Water jet reclamation. This was performed by workers from the fertilizer area's maintenance division;
3. Filling barrels with materials contaminated by arsenic. This activity was performed by workers from the fertilizer area; and
4. Covering barrels inside the factory with cement, an activity performed by contract workers.

In this phase of maximum contamination and exposure, workers were not provided with protective equipment, which was only distributed by the management of the plant 6 days later.[9]

In 1996, 20 years after the explosion and after a formal accusation that malignant tumors in exposed workers could be associated with the arsenic exposure, the Court of Foggia initiated criminal proceedings against ten former managers of the plant and two medical consultants. The trial ended in 2007 with a verdict of absolution for all defendants.[10,11]

The purpose of this cohort study is to evaluate the mortality pattern of workers employed at the time of the accident and involved in site cleaning through an internal comparison with estimated exposure and a comparison with the mortality experience of the general population of the Foggia Province.

2 | METHODS

2.1 | Study design

For this study, a cohort enrolled within the trial initiated by the Court of Foggia was used.[12] It consisted of 1467 workers, grouped in two sub-cohorts, for which different inclusion and exclusion criteria were used.

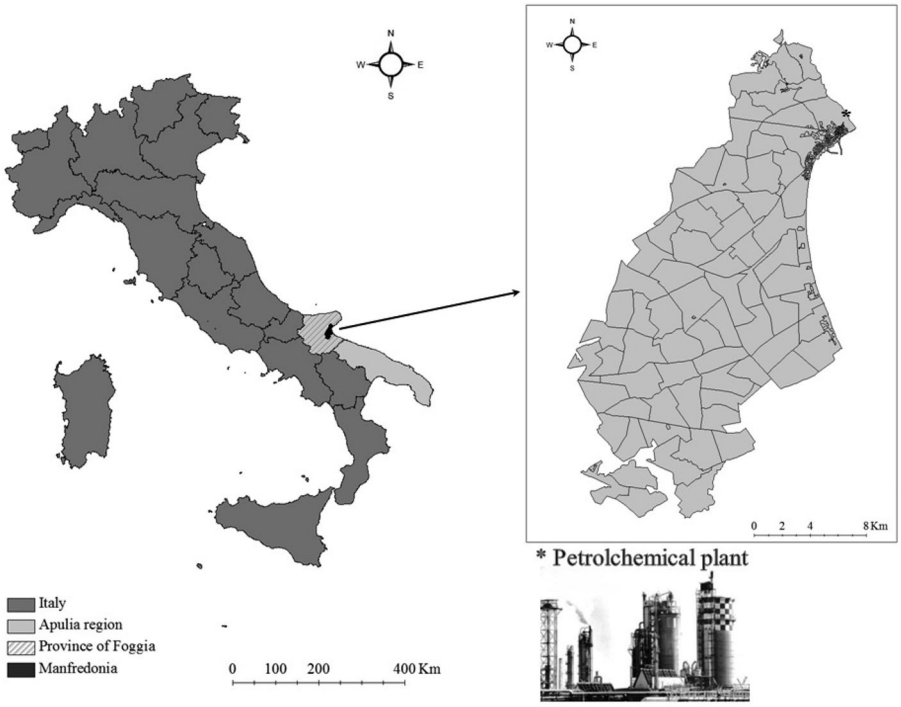

FIGURE 1 Location of the Manfredonia petrochemical plant

Inclusion and exclusion criteria were as follows:

Permanent staff was recruited and consisted of workers employed in the production of caprolactam and urea. For their enumeration, employee registries were used and all workers were included in the cohort.

Contract workers were recruited, for which a disease registry of the Worker's Health and Safety Board was used for the enumeration. Thus, only those who had reported an injury related to the accident or levels of arsenic in urine over the thresholds initially set by the authorities up to 100 μg/L and afterwards to 300 μg/L were entered into the registry and included in the cohort.

For all workers, information about the area of work (fertilizer/plastic), whether the employee was from a contracted company, the date and place of birth, municipality of residence, vital status, and cause of death as of October 31, 2001, were available from the follow up performed within the trial. For all members of the cohort, a new follow up was performed in 2016/7 by contacting General Registries Offices or by using, for those residents in Manfredonia, the General Registry Office of the City. Worker vital status was updated up to March 15, 2016.

The outcome of interest for this study was the overall and cause-specific mortality. Causes of death were coded using the ninth Revision of the International Classification of Diseases (ICD IX). Causes of death were selected a-priori according to the available literature (Table S1 in supplementary material). For workers deceased after October 31, 2001, a request for a copy of the death certificate was made to the Health Authority of the deceased's residence.

2.2 | Statistical analyses

Person-years were calculated for each cohort member from the date of the accident until date of death, date of lost to follow-up, or March 15, 2016, whichever occurred first.

First, crude and age-standardized mortality rates were calculated per 1000 person years. A Poisson regression was then performed and the following factors were considered: exposure in three levels (contract workers were assumed to be the most exposed, fertilizer workers as having average exposure, and plastic workers as the least exposed). We also investigated the interaction between working area and residence in Manfredonia as high levels of urinary arsenic among workers resident in Manfredonia have previously been reported.[13–15]

2.3 | External comparison analyses

The mortality experience of cohort members was compared with the mortality experience of the population of Province of Foggia (630 000 residents and some 5500 deaths observed yearly) using standardized mortality ratios (SMRs).[16]

The expected number of cases was estimated using cause-specific death rates for the calendar year (1976 to 2016) and 5-year age groups (20-85 years) of the Province of Foggia, available from the Italian Institute of Statistics.

In order to take into account missing causes of death, the method proposed by Rittgen and Becker[17] was implemented, and corrected SMRs were computed. 95% confidence intervals (95%CIs) were calculated using exact tests.[18] All tests were two-tailed.

We addressed the issue of correctly defining the latency period by calculating SMRs assuming different latency periods.

2.4 | Internal comparison analysis

A quantitative approach to estimate arsenic exposure, such as arsenic concentrations in urine or the number of days worked during clean-up activities, was not possible because such information was not available for this study. Thus, information based on the literature[15] and reports written for the trial by industrial hygienists[9] were used and an approximate indicator of exposure was adopted. Hence, also for internal comparison purposes, contract workers were assumed to be the most exposed, fertilizer workers as having average exposure, and plastic workers as the least exposed; Cox proportional hazard models were then applied. The time to event, that is, mortality for different causes, was treated as a dependent variable and time in study was taken as the time scale. Working area (assuming work in plastic as the reference category), age at the time of the accident (5-year groups), and residence (Manfredonia or elsewhere) were used as covariates. Hazard ratios (HRs) and 95%CIs were calculated.

2.5 | Sensitivity analysis

In the external comparison analysis, a sensitivity analysis was conducted by comparing the mortality experience of cohort members with the corresponding population of the Apulia Region (about 4 million inhabitants and around 40 000 deaths per year).

3 | RESULTS

Overall, the cohort included 1467 workers; 114 were deceased before 2001 and a further 192 had died as of the second follow up (March 15, 2016). For 93% of all deceased individuals, the cause of death was ascertained.

Of the workers who were alive in 2001, 728 were resident in Manfredonia and follow up was performed through a record linkage with the general registry of the City of Manfredonia. In this subgroup, 8 persons were not identified in the city archives, 2 were lost to follow-up, 619 were alive, and 99 persons were deceased.

Among the additional 625 non-residents in Manfredonia, 205 registrars in Italian municipalities were contacted by mail. In this subgroup, no information on vital status was available for 8 persons, 16 were lost to follow-up, 508 persons were alive, and 93 were deceased.

One thousand four hundred fifty-one persons were included in the analysis. Sixteen persons for whom no vital status information was available were discarded from the analyses. Eighteen lost to follow up

TABLE 1 Cohort size and results of follow-up in a historical cohort study of workers employed in a petrochemical plant in Manfredonia (South Italy), 1977-2016

	Total		Women		Men	
	No.	%	No.	%	No.	%
Total cohort	1467	100.0	39	100.0	1428	100.0
No information on vital status	16	1.1	0	0.0	16	1.1
Included in the analysis	1451	98.9	39	100.0	1412	98.9
Vital status on 15 March 2016						
Alive at follow-up 2016	1127	77.7	39	100.0	1088	77.1
Lost to follow-up by 2016	18	1.2	0	0.0	18	1.3
With follow-up available up to 2001	6	33.3			6	33.3
With data of work cessation available	12	66.7			12	66.7
Deceased	306	21.1	0	0.0	306	21.7
With known cause of death	285	93.1			285	93.1

were registered (Table 1). For 12 of them, the information on vital status was retrieved from the previous follow up. For the remaining six employees, the date of cessation of employment was used as the censoring date.

Further analyses were restricted to male workers, as only 39 female workers were enrolled in the cohort (Table 1). 306 deaths were confirmed in the cohort up to March 15, 2016. The cause of death was ascertained for 285 persons (93.1%). The cohort included 1412 male workers, yielding 51 234 person-years between 1976 and 2016. Almost 62% (n = 885) of persons under study were directly employed by the petrochemical plant. 527 were contract workers, among whom more than half of the total deaths (n = 161) were observed (Table 2).

Table 3 shows cause-specific age-adjusted mortality rates stratified by working area during the accident and residence. In addition, the coefficient of interaction between workers' residence and their working area during the accident are shown. For almost all major categories of causes of death, age adjusted mortality rates were higher among workers living in municipalities other than Manfredonia, and coefficients of interaction not significantly different from zero (Table 3). Conversely, age-adjusted mortality rates for malignant neoplasms of respiratory and intrathoracic organs and for lung cancer were higher among workers living in Manfredonia, and coefficients of interaction positive and statistically significant (Table 3). No such interaction was detected for deaths from diseases of the heart and circulatory system. Given the small number of cases, corresponding observations for cancers other than those in the lung cannot be assessed.

3.1 | External comparisons

Table 4 and Supplemental Tables S2-S5 show the results of the external comparison for the cohort as a whole (Table S2), and separately for the plastic workers (Table S3), fertilizers (Table S4), and contract workers (Table S5).

For all workers and all causes of death combined, the SMR was less than 1.0 (Table 3). The reduction in the general mortality was primary driven by mortality from diseases of the circulatory system, the respiratory system, and the digestive system (Tables 4 and S2). However, the SMR for malignant neoplasm of the pleura

TABLE 2 Persons under study, number of deaths, and person-years according to different characteristics of the cohort

	Persons under study		Number of deaths		Person years	
	n	%	*n*	%	*N*	%
All	1412	100.0	306	100.0	51 234	100.0
Permanent staff	885	62.7	145	47.4	32 811	64.0
Fertilizer area	656	46.5	114	37.3	24 156	47.1
Plastic area	227	16.1	30	9.8	8577	16.7
Other	2	0.1	1	0.3	78	0.2
Contract workers	527	37.3	161	52.6	18 423	36.0
Residence						
Manfredonia	769	54.5	161	52.6	28 128	54.9
Elsewhere	643	45.5	145	47.4	23 107	45.1

Follow up to 15 March, 2016. Analysis restricted to men.

TABLE 3 Age-adjusted mortality rates (per 1000 person years), 95% confidence intervals (95%CI), and interaction terms according to the working area during the accident and residence

	Residence									
	Manfredonia				Elsewhere				Coefficient of interaction	
Cause of death Area of working	Number of deaths	Age standardized mortality rate	95%CI		Number of deaths	Age standardized mortality rate	95%CI		Estimate	Pr > ChiSq
All causes										
Contract workers	100	3.1	2.4	4.1	61	3.9	2.9	5.3	−0.0031	0.9456
Fertilizer	49	2.1	1.6	2.9	65	2.7	2.0	3.6		
Plastic	12	1.7	0.9	3.0	18	2.6	1.6	4.2		
Natural causes										
Contract workers	89	2.6	2.0	3.5	54	3.2	2.3	4.5	−0.0067	0.8882
Fertilizer	45	1.9	1.3	2.6	59	2.3	1.7	3.1		
Plastic	10	1.3	0.7	2.5	15	2.1	1.2	3.5		
Malignant neoplasms										
Contract workers	42	1.3	0.9	1.9	21	1.3	0.8	2.2	0.0189	0.782
Fertilizer	26	1.1	0.7	1.7	29	1.2	0.8	1.8		
Plastic	7	1.0	0.4	2.0	7	1.0	0.5	2.2		
Malignant neoplasm of digestive organs and peritoneum										
Contract workers	13	0.4	0.2	0.8	7	0.5	0.2	1.1	−0.0561	0.6392
Fertilizer	6	0.3	0.1	0.6	12	0.5	0.3	1.0		
Plastic	3	0.4	0.1	1.4	4	0.6	0.2	1.7		
Malignant neoplasm of respiratory and intrathoracic organs										
Contract workers	19	0.6	0.3	1.1	5	0.3	0.1	0.8	0.2487	**0.0305**
Fertilizer	11	0.5	0.2	0.9	5	0.2	0.1	0.5		
Plastic	3	0.4	0.1	1.3	2	0.3	0.1	1.2		
Lung cancer										
Contract workers	18	0.5	0.3	1.1	4	0.2	0.1	0.7	0.3115	**0.0113**
Fertilizer	10	0.4	0.2	0.9	4	0.2	0.1	0.5		
Plastic	3	0.4	0.1	1.3	1	0.1	0.0	1.0		
Diseases of the circulatory system										
Contract workers	33	0.9	0.6	1.5	18	1.0	0.6	1.8	0.0297	0.7214
Fertilizer	11	0.4	0.2	0.8	19	0.7	0.4	1.2		
Plastic	1	0.1	0.0	0.9	5	0.7	0.3	1.6		

P-values <0.05 are in bold.

was 4.20 (95%CI: 1.92-8.52); for the code corresponding to malignant neoplasm of bone, connective tissue, skin, and breast, the SMR was 1.75 (95%CI: 1.23-2.48); for melanoma of the skin, it was 2.17 (95%CI: 1.20-3.83). Furthermore, a remarkable deficit in deaths from injury and poisoning in all groups was observed (Table S2).

Mortality rates differed among subgroups. Plastic workers showed a decrease in mortality risk for all deaths combined and for almost all the investigated causes of death (Table S3).

Fertilizer workers showed statistically significant SMRs for malignant neoplasms of the pleura (SMR = 4.68; 95%CI: 1.25-13.28), for malignant neoplasms of bone, connective tissue, skin, and breast

TABLE 4 Observed and expected number of deaths, standardized mortality ratios (SMR), and 95% confidence interval (95%CI) according to the area of working for selected causes of death among male workers of a petrochemical plant in Manfredonia (South Italy), 1976-2016

Cause of death	Observed number of deaths (*O*)	Corrected[b] observed number of deaths (O_c)	Expected number of deaths (*E*)	SMR[a]	95%CI[a]	
Plastic workers						
All deaths	30	30	49.4	0.61	0.41	0.87
Natural causes of death	25	28.8	44.2	0.65	0.53	0.84
Malignant neoplasms	14	16.2	18.9	0.85	0.65	1.17
Digestive organs and peritoneum	7	8.1	6.5	1.24	0.84	1.89
Respiratory and intrathoracic organs	5	5.8	5.9	0.98	0.61	1.60
Lung	4	4.6	5.4	0.85	0.50	1.47
Diseases of the circulatory system	6	6.9	13.6	0.51	0.33	0.80
Fertilizer workers						
All deaths	114	114	167	0.68	0.56	0.82
Natural causes of death	104	110.8	150.7	0.74	0.67	0.81
Malignant neoplasms	55	58.6	62.7	0.94	0.83	1.07
Digestive organs and peritoneum	18	19.2	21.6	0.89	0.72	1.11
Respiratory and intrathoracic organs	16	17.0	19.8	0.86	0.69	1.09
Lung	14	14.9	18.2	0.82	0.64	1.06
Diseases of the circulatory system	30	32.0	48.1	0.67	0.56	0.79
Contract workers						
All deaths	161	161	179.7	0.90	0.76	1.05
Natural causes of death	143	151.5	166.1	0.91	0.84	0.99
Malignant neoplasms	63	66.7	62.6	1.07	0.95	1.20
Digestive organs and peritoneum	20	21.2	21.3	0.99	0.82	1.22
Respiratory and intrathoracic organs	24	25.4	20.2	1.26	1.05	1.52
Lung	22	23.3	18.5	1.26	1.05	1.54
Diseases of the circulatory system	51	54.0	57.7	0.94	0.83	1.07
All workers						
All deaths	306	306	396.0	0.77	0.69	0.86
Natural causes of death	272	292.0	361.2	0.81	0.76	0.86
Malignant neoplasms	132	141.7	144.3	0.98	0.91	1.06
Digestive organs and peritoneum	45	48.3	49.5	0.98	0.86	1.11
Respiratory and intrathoracic organs	**45**	**48.3**	**46.0**	**1.05**	**0.92**	**1.20**
Lung	**40**	**42.9**	**42.1**	**1.02**	**0.89**	**1.17**
Diseases of the circulatory system	87	93.4	119.4	0.78	0.71	0.86

Reference population: Province of Foggia.

[a]ICD-9, International Classification of Diseases, Ninth Revision; CI confidence interval; SMR, standardized mortality ratio.

[b]Corrected for missing specific information for cause of death (Rittgen & Becker[17]).

(SMR = 2.18; 95%CI: 1.33-3.57), and for melanoma of the skin (SMR = 3.16; 95%CI: 1.44-6.49) (Table S4).

The SMR for contract workers for all deaths is close to 0.90 (95% CI: 0.76-1.05) and was 1.07 (95%CI: 0.95-1.20) for all malignancies combined (Table 3). In addition, an increase in SMRs for malignant neoplasm of respiratory and intrathoracic organs (SMR: 1.26; 95%CI: 1.05-1.52); lung cancer (SMR: 1.26; 95%CI: 1.05-1.54); malignant neoplasm of the pleura (SMR: 4.73; 95%CI: 1.26-13.32); malignant neoplasm of bone, connective tissue, skin, and breast (SMR: 1.85; 95% CI: 1.02-3.27), and genitourinary organs (SMR: 1.33; 95%CI: 1.00-1.78) emerged (Table S5).

3.2 | Latency

SMR calculations for lung cancer (Figure 2) and diseases of the circulatory system (Figure S1) were repeated assuming six different latency periods.

Among contract workers, SMRs were higher than one for latency periods equal to 20 years or less; they reached their maximum for latency periods between 15 and 20 years, and decreased toward the null in the subsequent periods (Figure 2).

For fertilizer and plastic workers, no specific pattern was observed. SMRs were very close to 1.0, and there was no evidence of an excess in lung cancer mortality for any latency period considered (Figure 2). For diseases of the circulatory system, no particular patterns emerged (Figure S1).

3.3 | Internal comparison

Compared to plastic workers, contract workers had hazard ratios of 1.85 for all causes of death (95%CI: 1.25-2.75); 1.95 for natural causes of death (95%CI: 1.36-2.80); 2.78 for diseases of the circulatory system (95%CI: 1.18-6.54); and 2.55 for diseases of the cardiovascular system (95%CI: 0.99-6.53). The HRs for respiratory diseases did not diverge from the null (Table S10). Among fertilizer workers, HRs were greater than one for different causes of death but estimates presented wide uncertainty.

Cox's regression models also showed increases in risk for workers living in Manfredonia (lung cancer, HR: 2.3; 95%CI: 1.1-4.9; malignant neoplasm of respiratory organs, HR: 1.9; 95%CI: 1.0-3.7). For other causes of death, no increases in the hazard ratios were observed (Data not shown in table).

3.4 | Sensitivity analysis

The sensitivity analysis was performed assuming the Apulia Region as the reference instead of just the population of the Province of Foggia. The results were generally similar with those obtained using the provincial population as a reference (Tables S7-S10).

4 | DISCUSSION

The reported cohort of Italian workers exposed to arsenic included 1467 workers followed up to March, 2016, and accounted for more than 51 000 person-years. The observed number of deaths for the entire cohort and for all causes of death combined was less than expected when compared with the reference population. However, SMRs were significantly greater than one for neoplasms of the pleura (based on two cases), malignant neoplasms of the bone, connective tissue, skin, breast (seven cases), and for melanoma of the skin (three cases).

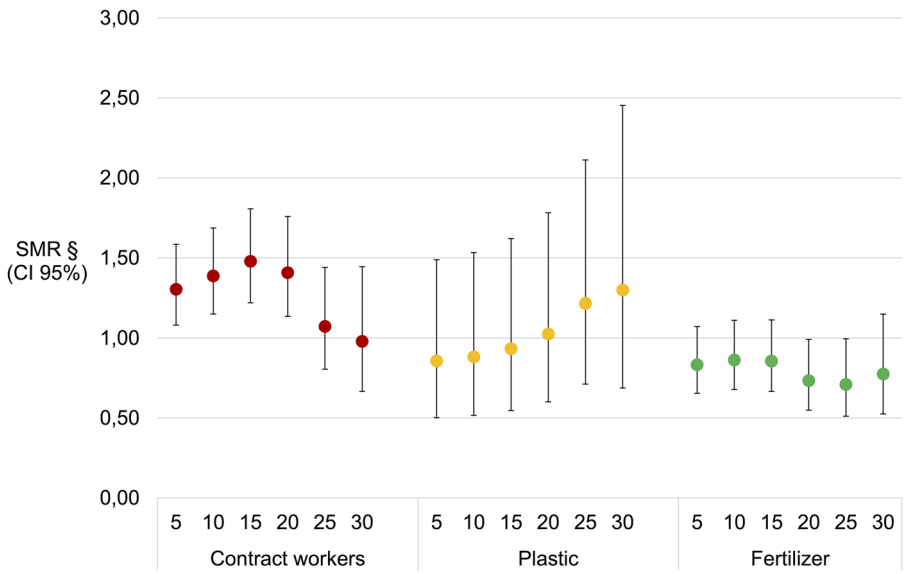

FIGURE 2 Standardized mortality ratios (SMR) and 95% confidence intervals (95%CI) for lung cancer among workers of a petrochemical plant in Manfredonia (South Italy), 1976-2016, according to 5, 10, 15, 20, 25, and 30 years of latency and working area during the accident. Reference population: Province of Foggia. [Color figure can be viewed at wileyonlinelibrary.com]

Internal comparisons showed higher risks for cancer and non-cancer mortality among contract and fertilizer workers rather than plastic workers. However, all of the excesses noted for the fertilizer workers over plastic workers included one in the 95%CI.

Overall, cancer-specific SMRs were generally greater than 1.0 in the sub-cohort of fertilizer workers and especially among contract workers. In the sub-cohort of plastic workers, the SMRs were rarely greater than 1.0.

Workers from the fertilizer area showed elevated SMRs for malignant neoplasms of the pleura, for bone, connective tissue, skin, and breast, and for melanoma of the skin.

Among contract workers, the number of observed cases was significantly higher than expected for several tumors, namely for malignant neoplasms of respiratory and intrathoracic organs, for lung cancer, for malignant neoplasms of the pleura, malignant neoplasms of bone, connective tissue, skin, and breast, and for malignant neoplasms of genitourinary organs.

Our results are generally comparable with those reported in the literature. According to the monograph published in 2012 by the IARC, arsenic and inorganic arsenic compounds are carcinogenic to humans (Group 1).[1] Notably, the workers employed at the time of the accident were exposed to the most toxic arsenic compounds, arsenate (As + 5) and arsenite (As + 3),[19] which indeed can cause lung and non-melanoma skin cancer.

During cleaning up, the cohort in Manfredonia could have been exposed to arsenic through inhalation, ingestion, or dermal contact. The IARC reviewed several occupational cohort studies in which mortality for lung and respiratory malignancies was associated with arsenic exposure via inhalation. An increase in lung and respiratory cancer mortality was observed in different working settings, such as manufacture of arsenic-containing insecticides[20,21] and in a fertilizer plant.[22]

Different investigators have found that the risk of lung cancer mortality increased with long latency periods. In contrast to Marshall et al[23] and to Smith et al,[24] who found in Chile increasing risks even 40 years after exposure reduction to arsenic contaminated water, we observed increased risk for latency periods between 15 and 20 years. This inconsistency could be due to different exposure pathways (ie, ingestion or inhalation of arsenic or dermal contact among workers in Manfredonia versus ingestion of contaminated water among the exposed population in Chile) and exposure durations (massive, acute exposure among workers in Manfredonia versus prolonged exposure to contaminated water among the exposed population in Chile). However, our findings are consistent with those of Järuop and colleagues, who analyzed a cohort of Swedish smelter workers and found higher SMRs when they introduced a 10-year minimum latency period.[25] In a cohort study of Russian fertilizer workers, an excess in mortality for all cancers combined and for lung cancer assuming a latency period longer than 20 years was also observed.[22]

In our study, an excess of mortality from malignancies of the pleura was observed, based on two cases, which may suggest an occupational exposure to asbestos, which was used in the Manfredonia petrochemical plant.[26]

Furthermore, an increase in mortality for lung cancer among workers living in Manfredonia was observed, possibly due to residential exposure to arsenic.[27]

4.1 | Healthy worker effect

In the external comparison analysis, mortality for diseases of the circulatory and respiratory systems was lower than expected.

This may be explained by the healthy worker effect. To address this issue different methods have been proposed in the literature.[28–33] In our study, an internal comparison analysis was performed, and an increase in mortality for all natural causes combined among contract workers was found. In particular, higher hazard ratios in fertilizer and contract workers (compared to plastic workers) were observed for diseases of the circulatory but not for diseases of the respiratory system.

4.2 | Strengths and limitations

To our knowledge, this occupational cohort study has one of the longest follow-up periods of workers acutely exposed to arsenic. The information on vital status and cause of death is almost complete.

In order to describe the mortality experience of those involved in the site cleaning of the petrochemical plant, external and internal approaches were adopted and sensitivity analyses were performed. Furthermore, the potential role of residential exposure to arsenic in enhancing mortality for specific causes of death was tested.

Two different sources were used for cohort enumeration. For permanent staff, plant personnel records were used, and all workers permanently employed in the period during the site cleaning were enrolled. For contract workers, a disease registry managed by the Worker's Health and Safety Board was used in which all those who had reported an injury related to the accident or had urinary arsenic levels over the thresholds set by the authorities were entered. Using this inclusion criterion, some 422 workers who took part in clean-up activities following the accident and were included in an epidemiological surveillance program were not enrolled[13,34] due to a lack of information in the adopted registry. This clinical and biomarker-based exclusion criterion may have elevated the observed risk among contract workers.

Soon after the accident a technical committee decreed that only workers with urinary arsenic levels <100 μg/L were allowed to remain in the plant. The threshold of 100 μg/L was set because the previous controls almost never led to exceedance of this threshold (Figure S3).[13] However, 2 weeks after the accident the technical committee issued a report based on 700 medical examinations performed among workers: 45% of them showed concentrations of urinary arsenic >100 μg/L. Ninety-two workers were admitted to the City Hospital of Manfredonia with levels of urinary arsenic even >3,000 μg/L.[13] On December 1, 1976, the Worker's Health and Safety Board, following the advice of the Institute of Occupational Medicine of Bari University, increased the threshold of urinary arsenic levels below which workers were allowed to remain in the plant to 300 μg/L (Figure S3), and on December 5, 1976, it was proposed to further increase the threshold up to 800 μg/L[7] (Figure S3).

According to the authorities, the decision to raise the return to work threshold was justified by the decreased quantity of arsenic in the plant following the site cleaning activities. In clinical studies conducted on workers involved in clean-up activities, investigators observed higher arsenic concentrations in urine, particularly among workers resident in Manfredonia. Some authors suggested a possible residential exposure to arsenic as a factor associated with the increase in arsenic concentrations in urine.[13,14] According to other authors, the diet, richer in fish and crustaceans, was likely the factor that could have caused increased levels of arsenic in the urine.[15,19] After the accident and during the following weeks there was a ban on the sale of fish products caught in the sea near the petrochemicals of Manfredonia. In fact, a crisis in the fisheries sector arose. It is therefore unlikely that workers increased their fish consumption coincidentally in this period. Thus, increasing values of arsenic concentrations (ie, >1000 μg/L) were unlikely due to diet and more likely due to occupational and residential arsenic exposure.

In summary, entry of contract workers into the registry managed by the Worker's Health and Safety Board and used for the enumeration of the cohort was not based on scientific rationale.

Since in our cohort only 39 persons were females we excluded them from the analysis. Therefore, we could not evaluate possible differential effect of arsenic exposure by sex.

4.3 | Causation

The association between arsenic exposure and lung cancer might be confounded by smoking. However, individual data were not available. In order to address this issue, an indirect adjustment was performed following the method proposed by Axelson and Greenland.[35] Results of indirect adjustments, carried out under different hypotheses of smoking habits among sub-groups of workers differently exposed to arsenic, showed that the confounding by smoking was likely to be minimal (Figure S2 and supplementary note).

Another way to indirectly control for smoking was to analyze other smoking-related diseases.[36] In this study, SMRs for diseases of the respiratory system were less than 1.0, a noteworthy finding. In addition, the internal comparisons did not show any increased risk for respiratory diseases. Thus, it is unlikely that the sub-cohort of contract workers with the greatest exposure to arsenic smoked substantially more than the provincial population or more than the least exposed subgroups of workers.

However, before deducing causation, some additional issues still need to be discussed.[37]

4.3.1 | Strength of the association

Among contract workers, a statistically significant increase in lung cancer mortality was reported. This excess clearly increased with latency periods between 15 and 20 years from the arsenic exposure. However, an excess of lung cancer was already observed 5 years after the explosion. Continuous exposure to arsenic among contract workers therefore cannot be ruled out.

4.3.2 | Consistency

Several studies have reported an association between lung cancer and arsenic exposure, with different study designs and in different occupational settings, including fertilizer production.

4.3.3 | Plausibility

Causation is biologically plausible. Indeed, the arsenic compounds the workers in Manfredonia were exposed to are considered the most toxic forms of arsenic.

Therefore, our findings of excess lung cancer in contract workers support a causal effect.

4.4 | Power

Although the study was large compared to many other studies and the follow-up was long, the number of deaths in different subgroups was relatively small. However, in interpreting differences between different groups or between the cohort and reference populations, we focused more on effect sizes, on patterns, and on the homogeneity of results obtained by assuming different statistical approaches rather than statistical significance. We have also to acknowledge low statistical power for certain rare cancers (eg, neoplasms of genitourinary organs), for which lack of statistical significance does not mean lack of carcinogenicity.

Although *P* values and confidence intervals were presented and the term "statistical significant" was used, no adjustment for multiple comparisons was done. The possibility of chance (false positive) or multiple comparisons to explain some results cannot be ruled out. However, several authors have warned against adjusting for multiple comparisons, preferring to present data consistently with the Bradford-Hill criteria, where other issues, such as consistency with evidence over time/studies or biological plausibility, are given priority.[38,39] Furthermore, in the present study a pre-established hypothesis was formulated before analyzing data using a statistical analysis plan.[40]

In the present study, non-significance therefore does not mean "not important". In addition, since false negative results may also have occurred because of limited power, the absence of evidence should not be interpreted as evidence of absence.[41]

5 | CONCLUSION

Overall, the results reported here on mortality among persons occupationally exposed to arsenic are consistent with the literature and biologically plausible. For lung cancer, a statistically significant excess of mortality was reported in the subgroups of workers most exposed to arsenic.

This study also provides new data suggesting an increased cancer mortality among workers resident in Manfredonia. Thus, the hypothesis that residential exposure plays a role is justified and an epidemiological study on the population residing in Manfredonia is warranted.

Our study shows that occupational data are still a valuable tool for investigating long-term effects of arsenic, especially in case of massive exposures due to an industrial disaster.

AUTHORS' CONTRIBUTIONS

EG and MB conceived of the study. PC and RP designed and conducted the epidemiological study for the Court of Foggia. CM and MC collected information about the explosion and contamination. EG was responsible for primary manuscript writing. All authors contributed to interpretation of results and writing and gave final approval for the manuscript.

ACKNOWLEDGMENTS

We acknowledge Professor Benedetto Terracini (University of Turin), Professor Reyn van Ewijk and Professor Stephan Letzel (University of Mainz) for useful discussions. We wish to thank Kathy Taylor for helping revise and edit the English. This original article is also part of the PhD of Emilio A. L. Gianicolo at the University of Mainz, Institute for Medical Biostatistics, Epidemiology and Informatics.

FUNDING

The study was co-funded by the Italian National Research Council and by the City of Manfredonia.

ETHICS APPROVAL AND INFORMED CONSENT

The research protocol was approved by the Ethics Committee of the University Hospital in Foggia (16/CE/2016).

DISCLOSURE (AUTHORS)

The authors declare no conflicts of interest.

DISCLOSURE BY AJIM EDITOR OF RECORD

Rodney Ehrlich declares that he has no conflict of interest in the review and publication decision regarding this article.

DISCLAIMER

None.

ORCID

Emilio Antonio L. Gianicolo http://orcid.org/0000-0002-3473-0752
Marco Cervino http://orcid.org/0000-0002-4226-8537

REFERENCES

1. IARC. *A review of human carcinogens. Part C: arsenic, metals, fibres, and dusts*. Vol 100C. Lyon, France: Available from: http://monographs.iarc.fr/ENG/Monographs/vol100C/index.php.; 2012.
2. Gupta DK, Soumya C. *Arsenic Contamination in the Environment The Issues and Solutions*. Cham, Switzerland: Springer International Publishing; 2017.
3. Navas-Acien A, Sharrett AR, Silbergeld EK, et al. Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. *Am J Epidemiol*. 2005;162:1037–1049.
4. Maull EA, Ahsan H, Edwards J, et al. Evaluation of the association between arsenic and diabetes: a National Toxicology Program workshop review. *Environ Health Perspect*. 2012;120:1658–1670.
5. Navas-Acien A, Silbergeld EK, Streeter RA, Clark JM, Burke TA, Guallar E. Arsenic exposure and type 2 diabetes: a systematic review of the experimental and epidemiological evidence. *Environ Health Perspect*. 2006;114:641–648.
6. Liberti L, Polemio M. Arsenic accidental soil contamination near Manfredonia. A case history. *J Environ Sci Health C*. 1981;16:297–314.
7. Ambrosi L, Amicarelli V. L'incidente di Manfredonia: la cronistoria degli avvenimenti. *La Medicina del lavoro*. 1982;73:271–275.
8. Mangia C, Cervino M, Gianicolo EAL. Arsenic contamination assessment 40 years after an industrial disaster: measurements and deposition modeling. *Air Qual Atmos Health*. 2018;11:1081–1089.
9. Casciani M, Attias L. *Expert testimony in lawsuit number 8437/96*. Foggia: Court of Law; 1999.
10. De Marchi B, Biggeri A, Cervino M, et al. A participatory project in environmental epidemiology: lessons from the Manfredonia case study. *WHO Public Health Panorama*. 2017;3:321–327.
11. Malavasi G. Manfredonia: catastrofe continuata, cittadinanza ritrovata e rimozione. *Epidemiologia e prevenzione*. 2016;40:389–394.
12. Comba P, Pirastu R. Indagine Epidemiologica di Mortalià dei Lavoratori Presenti nello Stabilimento Enichem di Manfredonia il 26.9.1976 e nel Periodo Successivo. In Procura della Repubblica di Foggia. 2004.
13. Soleo L, Assennato G, Misciagna G, et al. L'incidente di Manfredonia: l'intervento dell primo momento. *La Medicina del lavoro*. 1982;73:309–323.
14. Soleo L, Assennato G, Misciagna G, et al. L'incidente di Manfredonia: l'indagine sanitaria a distanza. *La Medicina del lavoro*. 1982;73:324–335.
15. Bertazzi PA, Metelka L, Riboldi L, Guercilena S, Foa V, Dompe M. Valutazione dell'arsenico urinario totale quale indicatore di esposizione professionale. *La Medicina del lavoro*. 1982;73:353–364.
16. Esteve J, Benhamou E, Raymond L. Statistical methods in cancer research volume IV: descriptive epidemiology. *IARC Sci Publ*. 1994;128:1–302.
17. Rittgen W, Becker N. SMR analysis of historical follow-up studies with missing death certificates. *Biometrics*. 2000;56:1164–1169.
18. Hartung J, Elpelt B, Klösener KH. *Statistik – Lehr- und Handbuch der angewandten Statistik*. München: Oldenbourg. 2005.
19. Foà V, Colombi A, Maroni M, Buratti M, Calzaferri G. The speciation of the chemical forms of arsenic in the biological monitoring of exposure to inorganic arsenic. *Sci Total Environ*. 1984;34:241–259.
20. Sobel W, Bond GG, Baldwin CL, Ducommun DJ. An update of respiratory cancer and occupational exposure to arsenicals. *Am J Ind Med*. 1988;13:263–270.
21. Ott MG, Holder BB, Gordon HL. Respiratory cancer and occupational exposure to arsenicals. *Arch Environ Health*. 1974;29:250–255.
22. Bulbulyan MA, Jourenkova NJ, Boffetta P, Astashevsky SV, Mukeria AF, Zaridze DG. Mortality in a cohort of Russian fertilizer workers. *Scand J Work Environ Health*. 1996;22:27–33.
23. Marshall G, Ferreccio C, Yuan Y, et al. Fifty-year study of lung and bladder cancer mortality in Chile related to arsenic in drinking water. *J Natl Cancer Inst*. 2007;99:920–928.
24. Smith AH, Marshall G, Roh T, Ferreccio C, Liaw J, Steinmaus C. Lung, bladder, and kidney cancer mortality 40 years after arsenic exposure reduction. *J Natl Cancer Inst*. 2018;110:241–249.
25. Jarup L, Pershagen G, Wall S. Cumulative arsenic exposure and lung cancer in smelter workers: a dose-response study. *Am J Ind Med*. 1989;15:31–41.
26. Giua R. Relazione tecnica. Taranto: Regione Puglia Azienda Unita' Sanitaria Locale Servizio di Prevenzione e Sicurezza degli Ambienti di Lavoro; 1996.

27. Mangia C, Cervino M, Bruni A, Gianicolo EA, Delle Noci G. Modelling population exposure to arsenic release during the chemical accident occurred at Manfredonia (Italy) in1976. Paper presented at: 28th Annual Conference International Society for Environmental Epidemiology Old and new risks: challenges for environmental epidemiology; 1–4 September 2016, 2016; Rome.
28. Checkoway H, Pearce N, Kriebel D. *Research Methods in Occupational Epidemiology*, *Second Edition*. New York: Oxford University Press. 2004.
29. Sterling TD, Weinkam JJ. Extent, persistence, and constancy of the healthy worker or healthy person effect by all and selected causes of death. *J Occup Med*. 1986;28:348–353.
30. Arrighi HM, Hertz-Picciotto I. The evolving concept of the healthy worker survivor effect. *Epidemiology* 1994;5:189–196.
31. Arrighi HM, Hertz-Picciotto I. Controlling the healthy worker survivor effect: an example of arsenic exposure and respiratory cancer. *Occup Environ Med*. 1996;53:455–462.
32. Fox AJ, Collier PF. Low mortality rates in industrial cohort studies due to selection for work and survival in the industry. *Br J Prev Soc Med*. 1976;30:225–230.
33. Robins J. A new approach to causal inference in mortality studies with a sustained exposure period—application to control of the healthy worker survivor effect. *Math Model*. 1986;7:1393–1512.
34. Corsi G, Rossi A, Maestrelli P, Bartolucci GB, Picotti G. Rilievi su un episodio di intossicazione subacuta da composti arsenicali inorganici. *La Medicina del lavoro*. 1979;70:282–291.
35. Axelson O, Steenland K. Indirect methods of assessing the effects of tobacco use in occupational studies. *Am J Ind Med*. 1988;13:105–118.
36. Steenland K, Beaumont J, Halperin W. Methods of control for smoking in occupational cohort mortality studies. *Scand J Work Environ Health*. 1984;10:143–149.
37. Hill AB. The environment and disease: association or causation? *Proc R Soc Med*. 1965;58:295–300.
38. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*. 1990;1:43–46.
39. Savitz DA, Olshan AF. Multiple comparisons and related issues in the interpretation of epidemiologic data. *Am J Epidemiol*. 1995;142:904–908.
40. Perneger TV. What's wrong with Bonferroni adjustments. *BMJ*. 1998;316:1236–1238.
41. Altman DG, Bland JM. Absence of evidence is not evidence of absence. *BMJ*. 1995;311:485.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Gianicolo EAL, Mangia C, Cervino M, et al. Long-term effect of arsenic exposure: Results from an occupational cohort study. *Am J Ind Med*. 2019;1–11. https://doi.org/10.1002/ajim.22939