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Strategies for evaluating the environment–public health interaction of long-term latency disease: The quandary of the inconclusive case–control study

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ABSTRACT

Environmental links to disease are difficult to uncover because environmental exposures are variable in time and space, contaminants occur in complex mixtures, and many diseases have a long time delay between exposure and onset. Furthermore, individuals in a population have different activity patterns (e.g., hobbies, jobs, and interests), and different genetic susceptibilities to disease. As such, there are many potential confounding factors to obscure the reasons that one individual gets sick and another remains healthy. An important method for deducing environmental associations with disease outbreak is the retrospective case–control study wherein the affected and control subject cohorts are studied to see what is different about their previous exposure history. Despite success with infectious diseases (e.g., food poisoning, and flu), case–control studies of cancer clusters rarely have an unambiguous outcome. This is attributed to the complexity of disease progression and the long-term latency between exposure and disease onset. In this article, we consider strategies for investigating cancer clusters and make some observations for improving statistical power through broader non-parametric approaches wherein sub-populations (i.e., whole towns), rather than individuals, are treated as the cases and controls, and the associated cancer rates are treated as the dependent variable. We subsequently present some ecological data for tungsten and cobalt from studies by University of Arizona researchers who document elevated levels of tungsten and cobalt in Fallon, NV. These results serve as candidates for future hybrid ecologic case–control investigations of childhood leukemia clusters.

1. Introduction

Disease clusters appear in populations as statistical "outliers"; there is always some random background incidence rate of disease that we have come to regard as normal, but when the rate exceeds some statistical expectation, we become concerned that there is some source that we need to identify and remove. The classic example used in public health education to illustrate this point is the 1854 Asiatic cholera epidemic in London that raised public awareness for the value of epidemiology [1]. Here, a huge cluster of the disease occurred in the St. James Parish but much lower incidence was found in neighboring districts of Charing Cross and Hanover Square. Using a simple precursor to the classic "case–control study design" wherein afflicted and control groups of individuals are compared as to what distinguishes them, Dr. John Snow is credited for deducing that the most important environmental commonality among the afflicted was their source of drinking water; he recommended that the pump handle be removed from a particular well and the outbreak almost immediately subsided. Unfortunately, this success story is not often repeated in investigations of modern cancer clusters.

Specific cancers are characterized as random and generally rare diseases that strike individuals for unknown reasons. We understand that there are likely some gene–environment interactions that affect the probability that someone becomes ill, and that, with exception of known carcinogen exposures like tobacco smoke or asbestos, we all encounter fairly randomly distributed environmental exposures in our daily lives. Therefore, when a local cluster of cancer does occur, we expect that there is a good reason waiting to be found. Furthermore, we tend to put great effort into the investigation of such clusters in the hope that something specific can be learned to prevent future cancer incidence and to intervene immediately on behalf of the general public's health. Like infec-

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tious disease outbreaks, cancer clusters are also investigated using case–control studies, but there is rarely an unambiguous outcome [2]. In this article, we take the example of the Fallon Nevada childhood leukemia cluster and look into the quandary of the inconclusive case–control study from a statistical perspective. We then develop a hypothesis for ecological associations based on environmental measurements made in air, dust, lichens, and trees that showed elevation of tungsten and cobalt with respect to the surrounding areas. We propose that such a result could serve as a first step towards implementing a more detailed, human biomarker based hybrid ecological and case–control approach.

2. Study of a leukemia cluster

Establishing the environmental links to human cancer occurrence is a difficult endeavor and fraught with ambiguity. The environmental exposures are complex, often very low, and variable. Cancer's intrinsic rarity, apparently random nature, and the long latency of onset serve to further obscure the cause-effect links. A common epidemiological approach for such rare diseases is to employ a case–control design to link the disease with historical exposure measurements or other assessments (e.g., records review, questionnaires, and data bases). In short, one identifies as large a group as possible of cancer victims, chooses a matched set of similar people without cancer, and then attempts to determine how the environmental exposures or history differ between the groups.

This basic philosophy was employed for investigation of a cancer cluster. From 1997 to 2001, there were 15 childhood cases of acute lymphoblastic leukemia (ALL) found in Fallon, NV (pop. ~8200), a small community within Churchill County (pop. ~26,000) [3]. The U.S. 2000 census counts about 6700 children (<18 yrs) in Churchill County resulting in a yearly incidence rate of ALL of 560×10^{-6}. Every year there are about 2400 new cases of ALL reported nationwide [4] from a pool of 78.1 million children resulting in a national ALL incidence rate of 30.7×10^{-6}. As such, the Fallon incidence rate represents an 18.2-fold increase over the expected value. This is a very simple estimate that does not include adjustment for other factors such as demographics and age distributions. In a more detailed study, the Fallon cancer cluster was deemed "unlikely due to chance" based on the first 11 cases [5].

Both the State of Nevada State and U.S. Federal Agencies diligently sought an explanation in the hopes of developing an intervention strategy. They employed a case–control strategy that compared the blood, urine, homes, schools, environments, infection status, histories, etc. of the case children to comparable (control) children in the same area. Additionally, they assessed community environmental exposure in a cross-sectional effort and compared these results to national averages. Study compounds were restricted to metals and pesticides of toxicological interest, polychlorinated biphenyls (PCBs), and a few chlorinated and aromatic volatile organic compounds (VOCs). These efforts were largely inconclusive; the only statistically significant results were community wide elevations of environmental tungsten and a larger than expected range of arsenic levels in urine [6]. According to the Centers for Disease Control (CDC), neither of these species has been related to leukemia; in fact, arsenic trioxide was approved by the Food and Drug Administration for treatment of patients with acute promyelocytic leukemia [7].

More recently, a second cluster of childhood ALL has been identified in Sierra Vista, Arizona (pop. 46,000); this has not been investigated yet. From 1995 to 2003, the pediatric leukemia count reached 12 cases [8]. The 2000 census data indicate about 9700 children in Sierra Vista, resulting in an estimated yearly incidence rate of 155×10^{-6}. Compared to the expected value for the nation, this represents a 5-fold increase.

Because both towns are impacted by military bases (Fallon Naval Air Station and Fort Huachuca Army Base, respectively) and both include significant flight operations and an underground jet fuel pipeline, community groups have suggested an environmental connection. There is great concern expressed in the media and on the Internet that there is an association between jet fuel exposures and the occurrence of ALL [9–11]. In the following, we address the methodology for finding an environmental association with cancer occurrence as the first diagnostic step. It is not meant to establish or even imply an unequivocal cause–effect relationship, which can only be developed through subsequent animal, cellular, genetic, and/or molecular level studies.

3. Dissecting the problem

The unsuccessful search for an environmental cause of childhood leukemia in the Fallon case–control study has (at least) three possible explanations:

1. Perhaps there is some other unknown (non-environmental) cause for this cluster, or perhaps the cluster is truly a random effect.
2. Maybe a true environmental link exists but the analytical methodology is not focused on the correct compounds or biomarkers.
3. Possibly the case–control method is somehow statistically flawed or inappropriate.

If the first explanation were true, then no environmental investigation could yield results and leukemia incidence would remain a mystery. Although cancers are often related to external exposures or insults, it may not be true in this case, and the occurrence in any given individual is thus not traceable to such an event. The second suggestion is possible because there is a common theme to the two communities: the military bases. Adding more environmental exposure candidates that might be related to this factor, such as jet fuel/exhaust in air or water, radars and microwave communications, or metals from turbine engines, is easily accomplished. Finally, there may be a weakness in the study design itself. In fairness, from an epidemiological perspective, the case–control philosophy is sound, but there may be underlying assumptions concerning the exposure assessment component that are violated.

Childhood cancers are only partly understood; some suspected external causes for childhood ALL are prenatal exposure to benzene, X-rays, infectious agents, and chemotherapy chemicals. The medical consensus is that there is a 5–40 year latency period for the development of cancers in general, yet ALL is most common at ages 2–3 [12,13]. So, if there is indeed some environmental exposure that increases risk of ALL, the first question to be addressed concerns whether or not the current environment of the child is relevant, or if perhaps the parents' history or the gestational period (mother's pregnancy) is more appropriate for study. It could well be that comparisons between case parents and control parents are more appropriate. The child's current exposure state, especially for a known environmental carcinogen such as benzene [14] with a short half-life, could be totally irrelevant.

Second, the assumption that differential exposure drives cancer risk may not be tested properly with the existing case–control design. Cancer is stochastic in the population; this means it is an apparently random event even though it is expected to have an increasing probability of occurrence with increasing exposure to carcinogens [15,16]. We can consider the hypothetical situation wherein 10,000 children are all living in a town where the average exposure to some environmental chemical is absolutely constant for all of them (e.g., same air, same food and water levels, same breathing and ingestion rates). Because cancer is rare and random,

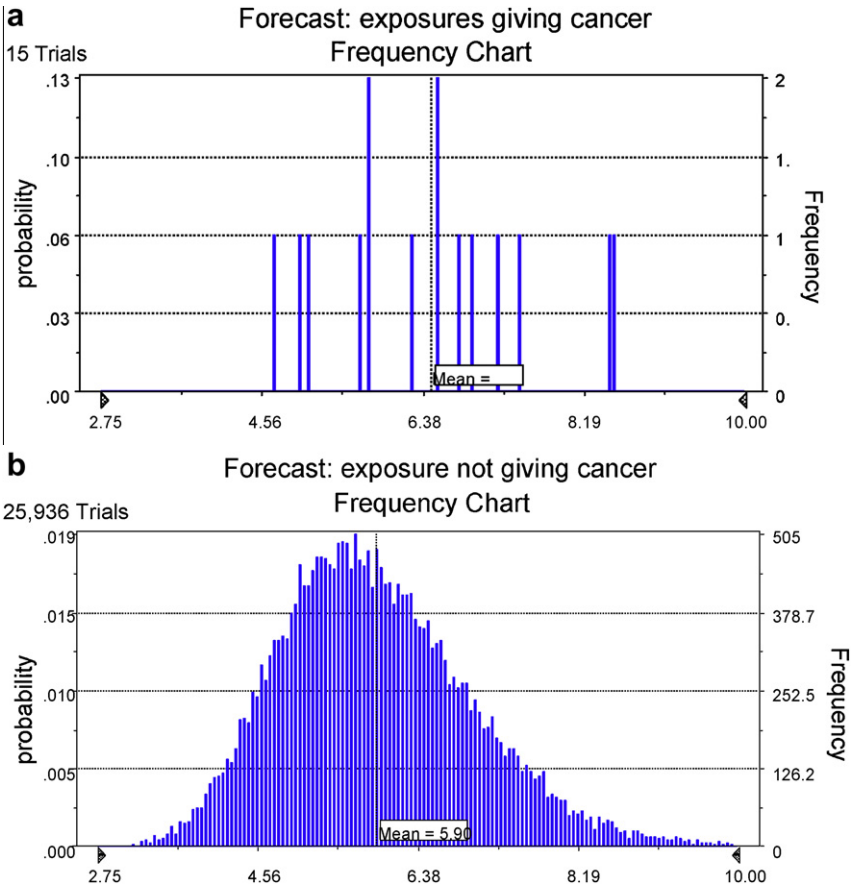

Fig. 1. Frequency distributions for exposures giving rise to cancer cases (a) and controls (b) under the assumption of 15/26,000 (or 1.94×10^{-4}) leukemia risk with log-normal underlying exposure distribution (GSD = 1.2) and log-normal susceptibility distribution (GSD = 3.2). This demonstrates that if the variability of exposure is appreciably lower than that of susceptibility, there is little or no expected difference in exposure between cases and controls, that is, the conventional case–control design gives null results.

we would expect that only a small subset of them (say 15) would develop ALL. If we now attempt the case–control strategy, and compare the exposures of the cancer victims to those of their peers in the town, we would get a null result because they all had the same exposure! Real life is not that simple, but this thought experiment shows that an individual's susceptibility (or the population's variability in susceptibility) must also be considered in the case–control design and that looking only at exposure differences can fail to find the cancer association.

4. The importance of exposure variance within a population

The effect of the respective variabilities of exposure and susceptibility within a population can be demonstrated with some simple Monte Carlo simulations. Suppose the population cancer susceptibility is log-normally distributed with a 100-fold variability range for the middle 95th percentile (2.5–97.5%); this indicates a geometric standard deviation of about 3.2, and is considered fairly conservative for the combined biological processes of DNA repair, metabolic activity, and detoxification where estimates range from 85- to 500-fold [17,18]. Here we explored a low (GSD = 1.2), medium (GSD = 2.0) and high (GSD = 3.2) log-normal distribution for the exposure ranges (corresponding to 2-fold, 16-fold, and 100-fold ranges, respectively). In general, we would expect the 1.2 value to be most likely because cancer effects are related to long time averages rather than brief excursions, and people living in the same small community would be expected to have similar average daily rates of intake (ADRI) at least from environmental sources. ADRI is assumed to be proportional to the dose rate and is used as a surrogate measure of target organ dose in regulatory risk assessment. Typically, ADRI is expressed in units of mg/(kg day) and is calculated as follows:

$$\mathrm{ADRI} = \mathrm{C_{med}} \times \mathrm{IR_{med}} \times \frac{\mathrm{ED}}{(\mathrm{BW} \times \mathrm{AT})}$$

where C_{med} is the concentration of chemical in the medium, IR_{med} the intake rate of medium, ED the exposure duration, BW the body weight, and AT the averaging time. This type of calculation is used for assessing incremental cancer risk over a lifetime [19–21].

Note that this assumes that we exclude subjects with remarkable occupational exposures, as they would obscure the search for an environmental association. Using the linear low dose–response curve model, the probability of cancer (P_{ci}) was estimated as the product of the exposure (E_i) and the susceptibility (S_i) of the ith individual: $P_{ci} = K \times (E_i \times S_i)$ where K is some constant to adjust for the compound's concentration units, ADRI units, and cancer slope factor [16]. The exact choices for numeric values are not relevant as long as they are consistent for these comparisons of statistical behavior.

In our Monte Carlo simulations, each iteration generated a fictitious individual, with the values for E_i and S_i randomly selected from their respective underlying distributions, and the resulting P_{ci} tested against some hypothetical threshold Pth chosen to achieve a certain number of cases. If Pth was exceeded, the individ-

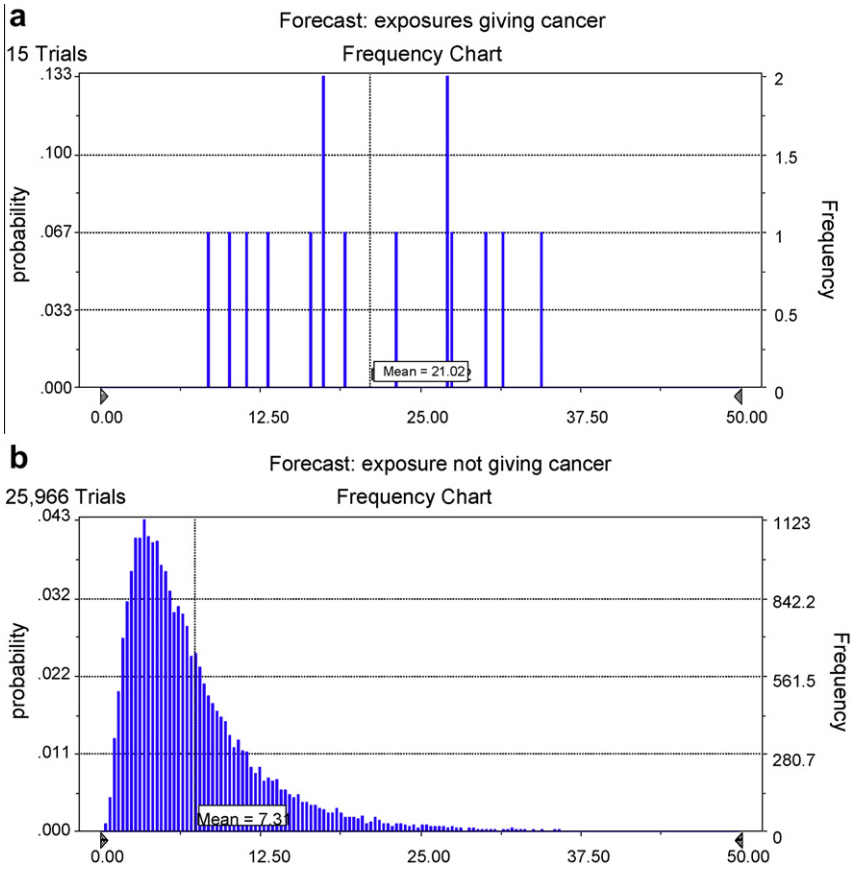

Fig. 2. Frequency distributions for exposures giving rise to cancer cases (a) and controls (b) under the assumption of 15/26,000 (or 1.94×10^{-4}) leukemia risk with log-normal underlying exposure distribution (GSD = 2.0) and log-normal susceptibility distribution (GSD = 3.2). This demonstrates that if the variability of exposure is moderately lower than that of susceptibility, there is some expected differentiation in exposure between cases and controls, but that some fraction of cases are likely to have exposures below the population mean. As such, the conventional case–control design may give an indication of association between exposure and cancer occurrence, but it is not necessarily unambiguous.

ual was designated a cancer case, otherwise, he or she was not. The respective E_i and S_i values were accumulated separately for cases and non-cases.

In total, we generated a fictional population of 26,000 individuals for each of our three exposure scenarios (number of residents of Fallon and surrounding Churchill County, NV) with an expected cancer outcome in each simulation of about 15 cases. Figs. 1–3 illustrate the results, respectively, for the parameters discussed above. In Fig. 1, the results of our first simulation show that when the variability in the exposures was appreciably less in contrast to the susceptibility (GSD = 1.2 vs. 3.2), there was very little difference in the exposure levels that do, or do not, result in a cancer case. Thus, observations of cancer incidence in this simulated population are predominantly driven by S_i.

In the second and third simulations, where the exposure range increased from 2-fold to 16- and 100-fold, respectively, the differences in exposure levels between observed cases and non-cases became more distinct (Figs. 2 and 3). These results suggest that observations of cancer incidence in these simulated populations are more heavily influenced by E_i than by S_i.

Another, more subtle implication is demonstrated in these three simulations; the rarity of the cancer cases (shown in Figs. 1a, 2a, and 3a) does not allow for good estimates of averages or trends. Any given case individual, in any of the three scenarios, could have an exposure lower than a randomly selected control; it is only on average that they show a difference. As such, dealing with only a few cases, rather than larger populations, results in weakening of statistical significance (calculations performed with Crystal Ball 2000.5, Decisioneering, Inc., Denver, CO).

5. Suggestions for epidemiological approach and hypothesis testing

Our first recommendation is to refocus the subject pool to address adults of childbearing age; children can also be tested, but they should remain as separate cohorts. The choice of subject pool will speak to the first issue above concerning whose exposures are relevant.

Resolving the second concern about the statistical problems with linking exposure to effect could require a different strategy. A shift to an "ecologic-like" study design may prove fruitful; in this approach, the definition of a case is changed from an individual with cancer to a location (like Fallon, Nevada or Sierra Vista, Arizona) with higher than expected cancer incidence. A control would be defined as a similar town with similar geographic, economic, and demographic characteristics, but with an average, or below average, cancer incidence. Now, subjects can be chosen without regard to their cancer status and so we have access to as many as necessary to achieve statistical power. Furthermore, this design avoids the issue of the relative distribution variability between exposures and susceptibilities because a "case" is no longer an individual but a whole town. Some concessions are the assumptions that the underlying nature of human cancer susceptibility is not somehow geographically skewed between towns and that the overall environmental character of the towns has not changed in the past few years. Also, there is the concern over ascribing a group trend to any given individual.

It should be noted that cancer epidemiology is a rapidly changing scientific field, and that there are different opinions among ex-

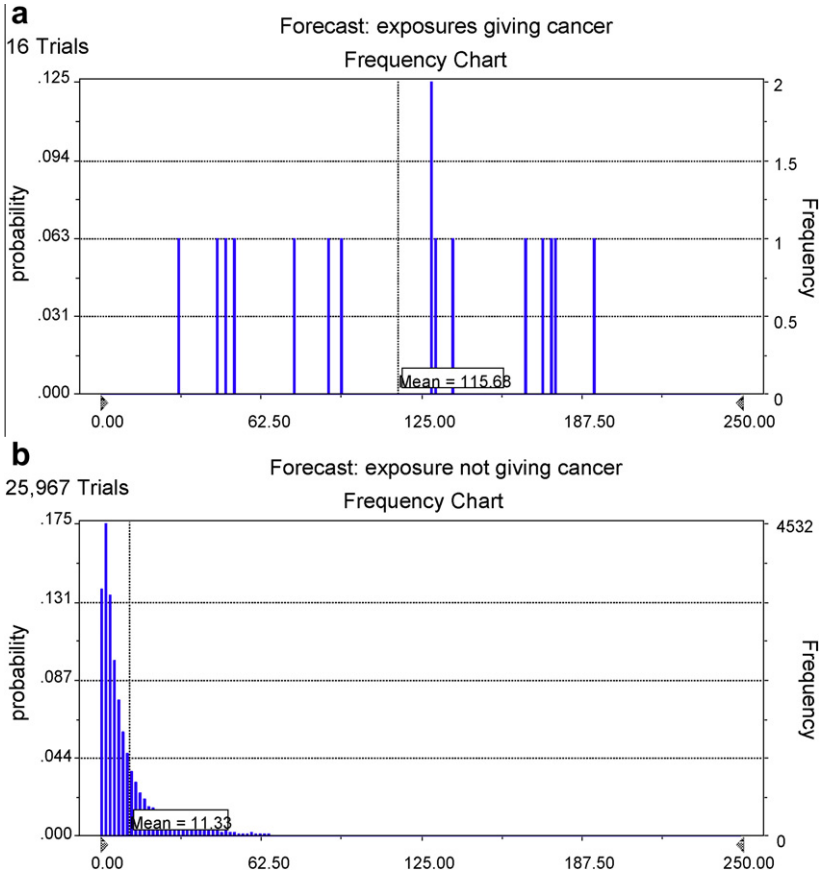

Fig. 3. Frequency distributions for exposures giving rise to cancer cases (a) and controls (b) under the assumption of 15/26,000 (or 1.94×10^{-4}) leukemia risk with log-normal underlying exposure distribution (GSD = 3.2,) and log-normal susceptibility distribution (GSD = 3.2). This demonstrates that if the variability of exposure is similar to that of susceptibility, there is large differentiation in exposure between cases and controls, and that the probability of a case exposure below a control exposure is low. As such, the conventional case–control design is expected to give unambiguous results.

perts as to the relative value of various approaches such as ecologic, time series, case–control, prospective cohort, cross-sectional, case-cohort, case-crossover, or repeat measures designs [22–24]. Here, there is further difficulty in actually naming the study design. In a true ecologic design, both exposure and outcome parameters are population based; in a "semi-ecologic" design, only the outcome is based on individuals. In the above design, the outcome variable is population-based but the exposure population is cross-sectional from specific cohorts of individuals. As such, it is a hybrid among case–control for towns, cohort for exposure, and ecologic for outcome.

In the following discussion, we assume that the major concern is to find an association of environmental exposure to "compound X" with the occurrence of childhood leukemia so that something can be done to protect the public. Under an ecologic type design, this does not demonstrate an exact causal relationship, but at least it provides a potential course of action. As in all successful investigations, the first step is to articulate the null hypothesis (Ho) and the alternative hypothesis (Ha), and to define the statistic with which we decide between them:

Ho: There is no association between population (cross-sectional) exposure to X with recent incidence of childhood leukemia (ALL) in four representative communities that include Sierra Vista, Arizona and Fallon, Nevada.

Ha: There is a linear association, i.e., sufficient evidence to reject Ho at $\alpha = 0.05$.

Test method: Cochran–Mantel–Haenszel (C–M–H) test of linear association (chi-square distribution), continuous data (bracketed into ordinal categories) in a 4×4 contingency table using "table values".

This approach tests for a dose–response type association, however, a less stringent C–M–H test of "row mean scores" could be applied as well.

6. Some practical discussions for gathering data

As mentioned above, the two control communities should be chosen to be as similar as possible to Fallon, NV and to Sierra Vista, AZ except with unremarkable (background level) leukemia incidence. To address public concerns, they should also be similarly impacted by local operations as are their counterparts, that is, near a military facility, airbase, airport, etc., in the event that this becomes an important factor. Guidance for these choices can be gleaned from the literature [5].

If subjects are classified into four categories based on their increasing level of environmental exposures or chemical biomarkers and there are four communities, this results in 16 possible entries in the C–M–H contingency table (Table 1). The numerical ranges for leukemia incidence (rows) and exposure level (columns) are strawmen that would be replaced with actual data. The internal data entries, "n_{ij}" represent the total number of subjects that fall into each table bin, the "totals" row or column notation represents a sum with a subscripted period, that is, "$n_{.2}$" is the total number of

Table 1
Example of a contingency table for Cochran–Mantel–Haentzel test for linear association. Table entries are integers representing the number of subjects falling in the particular bin. Exposure and leukemia incidence values are numerical (continuous variable).

Contingency table for leukemia incidence vs. exposure level					
Towns with leukemia yearly incidence (cases/million children)	Exposure level, compound X, in blood (ng/L)				Row totals
	0–5	>5–10	>10–20	>20	
Control a (23)	*n*11	*n*12	*n*13	*n*14	*n*1.
Control b (31)	*n*21	*n*22	*n*23	*n*24	*n*2.
Sierra Vista (155)	*n*31	*n*32	*n*33	*n*34	*n*3.
Fallon (550)	*n*41	*n*42	*n*43	*n*44	*n*4.
Column totals	*n*.1	*n*.2	*n*.3	*n*.4	*n*.

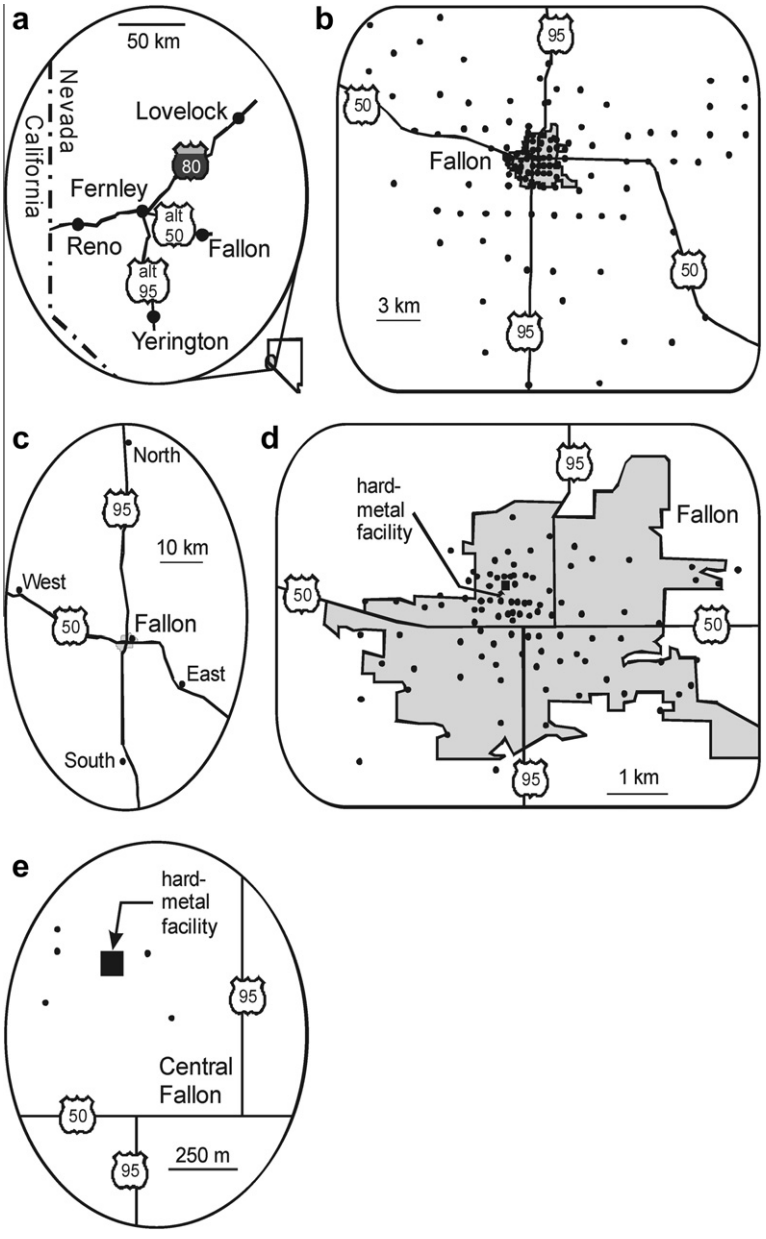

Fig. 4. Maps of (a) west-central Nevada, showing locations of communities in which aerial dust and tree-ring sampling was done, (b) Fallon, showing locations where surface dust sampling was done, (c) Fallon, showing locations where lichen sampling was done, (d) Fallon, showing locations where tree leaf sampling was done, and (e) central Fallon, showing locations where specific tree-ring sampling was done within Fallon.

entries in the second column, "$n_{.}$" represents the total number of subjects, etc. For the C–M–H analysis, we require row totals "$n_{i.}$" > 30, and cannot have more than 20% of the individual entries "n_{ij}" < 5. As such, the absolute minimum number of subjects is 120 total. A good rule of thumb is to at least go 50% higher, say to 180 subjects in total, evenly distributed among the four communities.

The C–M–H test of linear association is a standard method in statistics available in most modern statistical software packages (e.g., SAS Institute, Cary, NC). Briefly, if there were no correlation at all between exposure and occurrence of leukemia, we expect that the "n_{ij}" entries would be randomly (chi-square) distributed with expected values $ne_{ij} = (n_{i.} \times n_{.j})/n.$; as the correlation strengthens, we would expect the highest values clustered along the main diagonal. The test provides a "p-value" for the null hypothesis Ho described above. If the p-value is less than some predetermined level, say $\alpha = 0.05$, then there is sufficient evidence to reject Ho with confidence that there is less than a 5% chance of incorrectly concluding that there is an association between compound X and incidence of leukemia. If multiple tables are created for testing associations for different environmental exposures or biomarkers, the significance level $\alpha = 0.05$ should be corrected (i.e., using Bonferroni's correction, or Scheffe's contrasts) to avoid incorrectly rejecting individual null hypotheses. These are standard statistical techniques found in textbooks [25].

7. Using ecological measurements to develop hypotheses

An initial approach for generating plausible hypotheses that might be tested with the procedures described above involves the broad view across all ecological exposures to see what differences there may be regionally. This differs from the hybrid approach as the locations are also treated ecologically, rather than represented by individuals. We recall that ecological study of places with excessive illness cannot link exposure to environmental contaminants with disease in particular individuals, largely because individual exposure information is not collected [26]. More generally, it is also not possible to assert causal linkage between an environmental contaminant and an illness from ecological data alone. We also recall that in the case–control study of places with excessive illness the number of case subjects available for testing is often very low, even in places with uniquely high occurrence rates of a disease. Furthermore, because many variables interact to confound simple cause-and-effect relationships, the case–control type of study has inherently low odds of concluding a positive association between anything in particular and the illness of interest [27]. In the following discussion, we illustrate an ecological approach to look for an ecological association that could illuminate subsequent investigations using the proposed hybrid (non-parametric) approach.

8. Ecological tungsten exposures

As described earlier, CDC performed a case–control study in Fallon in the search for a cause of the cluster of childhood leukemia. Specifically, blood, urine, and cheek swabs were collected from case subjects as well as from comparison subjects, and indoor air, play yard soil, household dust, and tap water were collected from homes of case families as well as of comparison families [6,28]. Among other results, community-wide exposure to the element tungsten was found, and this exposure was higher than expected. Ultimately, however, no biochemical or exposure based relation between leukemia and individual tungsten exposure was identified, which is not surprising because, as discussed above, the overall exposure averages in a close knit community may have appreciably less variance than between individual susceptibility. This initial result, however, was sufficient to prompt further research by a team of researchers from the University of Arizona who performed multiple ecologic studies of Fallon, all with the underlying intention of uncovering environmental aspects of Fallon that might be distinct compared to other towns of west-central Nevada and/or pristine outlying desert in the surrounding area. Cobalt was also measured in addition to tungsten because airborne cobalt was shown to be elevated within Fallon compared to outside of Fallon in preliminary data collected by others [29]. Various environmental media were chosen to represent different locations and potentially different exposure time frames. Temporally, they ranged from airborne dust collected for 24 h up to tree rings that span many years, and spatially they scaled from sub-kilometer up to 100 km [30].

8.1. Aerial dust [31]

Aerial dust was collected within Fallon and four comparison towns to identify potentially elevated metals concentrations (Fig. 4a). Fallon was found to be distinct from other towns of west-central Nevada by having high levels of airborne tungsten and cobalt particulates (Fig. 5a). Depending on wind velocity on any given day of dust sampling, airborne tungsten and cobalt was as much as many tens of times higher in Fallon than in comparison communities.

8.2. Surface dust [32]

Surface dust was collected in and around Fallon to help discern the source or sources of elevated airborne tungsten and cobalt particulates (Fig. 4b). Based on proximity maps, the source area of these two metals was just north of Highway 50 and west of Highway 95 (Fig. 5b). Peak surface dust concentrations of tungsten and cobalt were up to 100× and 10× higher respectively in this source area than in the periphery of Fallon.

8.3. Lichens [33]

Lichens are known to quantitatively absorb atmospheric constituents over their life spans, which can be up to many decades. Lichen samples were collected within and outside Fallon to supplement the aerial and surface deposit samples and were used to provide time integrated comparison data (Fig. 4c). Again, Fallon was found to be distinct environmentally from outlying desert in elevated tungsten and cobalt levels. Tungsten and cobalt concentrations were up to 12× and 2× higher respectively in lichens located within Fallon than in lichens located in outlying desert (Table 2). This distinction was not due to differences in tungsten or cobalt in rock substrates.

8.4. Leaf surfaces [34]

Tree leaves serve as collection surfaces for dry particle deposition, and leaves were collected in and around Fallon (Fig. 4d). In the absence of rain events, these data represent time frames of multiple days to weeks. Analyses showed elevated levels of tungsten and cobalt deposition in Fallon and again confirmed a suspected source north of Highway 50 and west of Highway 95 (Fig. 5c). Peak leaf surface dust concentrations of tungsten and cobalt were up to 20× and 6× higher respectively in this source area than in the periphery of Fallon.

8.5. Tree rings [35]

For the objective of assessing temporal variability of tungsten and cobalt in Fallon since the late 1980s, that is, since before the onset of the cluster of childhood leukemia, tree rings were collected from the area north of Highway 50 and west of Highway 95 (Fig. 4e) as well as from comparison towns of west-central Nevada (Fig. 4a). Fallon was found to be distinct temporally by increasing tree-ring tungsten beginning by the mid-1990s that was not shown in trees of other communities as well as by elevated

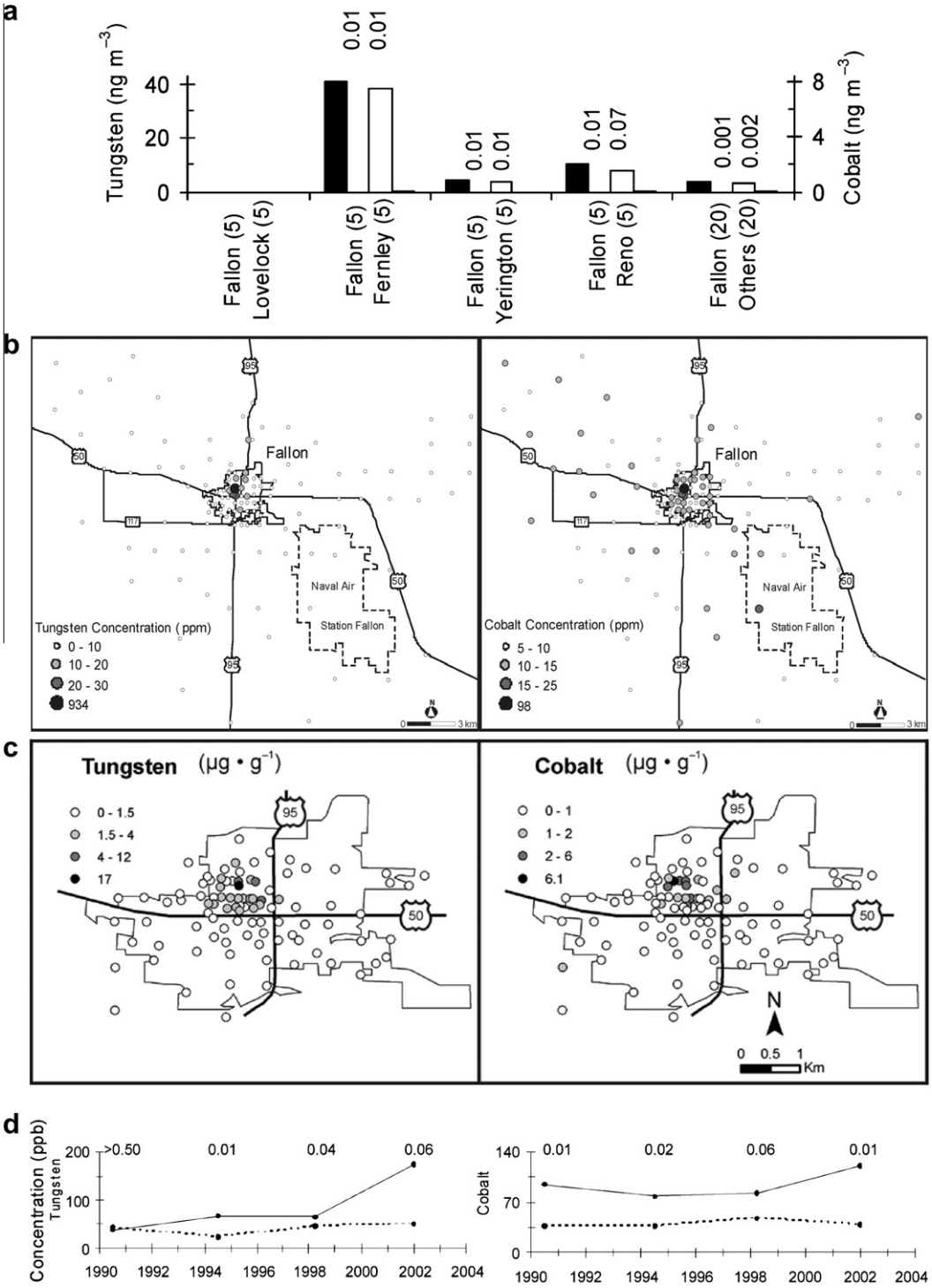

Fig. 5. Representative results from ecologic study of Fallon: (a) tungsten (black bars) and cobalt (white bars) airborne loadings within Fallon and comparison (other) towns for each sampling session. Significance levels (unadjusted for multiple testing) for the Mann–Whitney test of medians between Fallon and its respective comparison town are given above the bars. (b) Surface dust concentrations of tungsten and cobalt. (c) Concentrations of tungsten and cobalt in leaf surface particulates. (d) Median concentrations of tungsten and cobalt in Nevada tree rings through time. In all cases, the solid line indicates Fallon (n = 5 trees) and the dotted line indicates comparison towns (n = 6 trees). Data are plotted using the approximate mid-point of each time period as the x-axis value. P-values of significance from the one-tailed Mann–Whitney tests of medians and/or cumulative distribution functions are given for each time period for each element.

tree-ring cobalt compared to trees of other communities since the early 1990s (Fig. 5d).

8.6. Consistency of results

For all environmental media, regardless of the expected integration times ranging from a day to multiple years, tungsten and cobalt results were remarkably consistent. In all cases, tungsten levels were elevated from 10× to 100× in Fallon over comparison communities or surrounding desert, and cobalt levels were elevated from 2× to 10× in Fallon over comparison communities or surrounding desert. Elevated airborne tungsten and cobalt corresponded to the general area just north of Highway 50 and west of Highway 95, where a hard-metal facility that blends

Table 2
Cobalt and tungsten concentrations and standard errors in lichens and rock substrates.

Element	Lichens				
	Within Fallon (n = 10)		Outside Fallon (n = 20)		Ratio within:outside
	Median ($\mu g\ g^{-1}$)	Standard error	Median ($\mu g\ g^{-1}$)	Standard error	
Cobalt*	3.59	0.73	1.79	0.65	2.01
Tungsten**	24.95	1.91	1.99	0.30	12.54
Element	Rock substrates				
	Within Fallon (n = 3)		Outside Fallon (n = 12)		Ratio within:outside
	Median ($\mu g\ g^{-1}$)	Standard error	Median ($\mu g\ g^{-1}$)	Standard error	
Cobalt	24.00	0.58	23.00	0.47	1.04
Tungsten	2.40	0.17	2.45	0.06	0.98

* Significance at the 0.06 level.
** Significance at the <0.001 level. Differences in medians were tested using the two-tailed Mann–Whitney test of medians.

tungsten carbide and tungsten carbide–cobalt alloys [29] is located.

9. Toxicology of exposure to tungsten and cobalt

As mentioned earlier, ecological evidence alone cannot confirm a cause-and-effect relationship between an environmental exposure and an illness. Rather, ecological evidence identifies unusual environmental exposures that can then be posed as candidates for further biomedical study related to an illness. For Fallon, ecological research provides evidence for a spatial-temporal association between tungsten and cobalt exposure and childhood leukemia. Given these ecological results, further investigation of a biochemical link between exposure to metals and childhood leukemia seems prudent.

Few studies have been published on the carcinogenicity of tungsten, but among such studies, tungsten in drinking water has induced a significant increase in mammary carcinoma in rats [36]; heavy metal tungsten alloys have transformed human osteoblast cells to the tumorigenic phenotype, perhaps due to direct damage to genetic material in the form of increased DNA breakage or chromosomal aberrations [37]; and tungsten pellets embedded intramuscularly into rats have caused tumors, including rhabdomyosarcoma [38]. Additionally, tungsten ore administered to human leukemia cells in the laboratory has increased growth of pre-existing leukemia cells by 170% compared to control samples over a 72-h culture period [39]. In utero exposure by mice to tungsten compounds altered expressions of many genes, one of which functions as a tumor suppressor whose alteration might have consequences with respect to cancer [40]. Tungsten retained by mammals is predominantly stored in the bones [41,42], and given the major role that bone marrow plays in leukemia [43], accumulation of tungsten in the bones could link environmental exposure to tungsten with childhood leukemia.

As for cobalt, lung and other cancers have been noted from exposure to airborne cobalt [44–46]. More importantly, simultaneous exposure to cobalt and tungsten carbide, which might occur as a by-product of hard-metal manufacturing [47], appears to have a synergistic carcinogenic effect [48–52]. For example, the simultaneous exposure of tungsten and cobalt has synergistically activated carcinoma cells in the lab more than individual exposures of each metal [53]. Indeed, the International Agency for Research on Cancer has declared cobalt metal with tungsten carbide as "probably carcinogenic to humans" [54, p. 133]. This allows for a possible linkage between childhood leukemia and concurrent exposure to both tungsten and cobalt and serves as further justification for all the research contained in this special issue dedicated to environmental and biomedical aspects of the cluster of childhood leukemia of Fallon, NV.

10. Reaction to Fallon studies

Over the years, stakeholder reaction to the various Fallon studies has been mixed. The public is eager for answers and assurance that the issues are resolved. Scientists would like to more fully understand the etiology of childhood cancers and the underlying gene–environment interaction and human biochemistry to help prevent future incidence. Parties affected by potential emission controls or regulatory sanctions require hard evidence. Newspapers, the Internet, congressional reports, and weblogs from various affected groups contain a plethora of opinion and discussion regarding the Fallon leukemia cluster specifically as well as issues surrounding inexplicable childhood cancers in general. See Supplemental Information for a collection of media clippings related to science done about the Fallon childhood leukemia cluster and public reaction to it.

11. Conclusions

Because of the difficulty and potential limitations of case–control studies, as well as the ecological study limitations as discussed in this article, there is a prevailing school of thought that the Fallon cancer cluster, and by implication other cancer clusters, defy explanation. Despite a great deal of hard work, CDC authors themselves noted that "...the inability of modern science to identify the role of environmental exposures in leukemia incidence reflects the complexity of defining a relationship between exposure and cancer in a community setting" [24]. More generally, connecting toxic pollution with outbreaks of illness is scientifically difficult [23].

The inconclusiveness of the Fallon case–control study is potentially related to the statistical approaches implemented coupled to the underlying unknown distributions in exposures and susceptibility. The discussion above demonstrates that there is a distinct possibility that the currently employed case–control design can fail due to the differing variance component between the distribution of integrated (long-term) exposures and the inherent individual cancer susceptibility even if exposures are directly associated with the cancer. Unlike the example of the London cholera epidemic, the rarity of the cancer provides too few subjects for study in the general populations. The suggested change in philosophy to a "semi-ecologic" hybrid design, wherein the localities or towns are treated as cases or controls depending on their cancer incidence, has a number of advantages over the current approach using only individuals (or their families) with and without cancer. There is no longer a limit as to the number of subjects, the underlying distribution for inter-individual susceptibility is no longer important, yet rare events can still be studied by using regional cancer incidence as the outcome variable. Furthermore, the C–M–H statistical approach is statistically robust regardless of underlying distributions

of inter-subject exposures, and the results are unambiguous because the number of subjects can be adjusted to satisfy all assumptions. There is an inherent flexibility in the suggested approach in that separate tables for adults and for children (with adequate numbers of subjects) can be quickly developed to help resolve the issue as to the underlying mode of action of exposure and cancer relationship. Also, the tables can be easily expanded to accommodate additional rows and columns as more studies are performed. Finally, any number of environmental exposures, such as jet fuel, tungsten, and/or arsenic can be tested simultaneously.

The ecological exposure evidence for candidate compounds tungsten and cobalt is of interest in that it provides the only statistical results to date that may demonstrate a potential association to the Fallon leukemia cluster. Although the evidence is circumstantial in that there is as yet no plausible biochemical pathway and also no individual biomarker evidence differentiating Fallon from similar communities, the measurements do establish a starting point for developing the non-parametric contingency table suggested herein.

The primary drawback is that neither the ecological data nor the non-parametric C–M–H approach demonstrates a direct cause–effect link; they only suggest associations between exposures and cancer and thus may only indicate a path for future etiologic study. The primary advantage is that the potential relationship between a specific exposure (such as jet fuel, tungsten, or arsenic) and leukemia could be ruled in or out based on defensible statistical grounds. This would certainly improve the current state of inconclusive results and provide suggestions for an immediate intervention strategy if a positive correlation were found.

Conflict of interest statement

Paul R. Sheppard and Mark L. Witten have provided documents, data, and declarations in Cases CV03-03482, Richard Jernee et al. vs Kinder Morgan Energy et al., and CV03-05326, Floyd Sands et al. vs Kinder Morgan Energy et al., Second Judicial District Court of Nevada, Washoe County, which are related to the childhood leukemia cluster of Fallon. In these cases, the law firm of Dunlap and Laxalt, representing the plaintiffs, with full disclosure to all defendants and their counsels, made an unsolicited donation of $15,000 to assist Witten and Sheppard in furthering their research, with a request that defendants provide similar donations. Neither Witten nor Sheppard have profited personally as a result of doing their research in Fallon or from providing material in these cases. Other authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cbi.2011.02.020.

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