DATA ANALYSIS OF CHEMOMETRICS AND HEALTH OUTCOMES: COMMON EPIDEMIOLOGICAL MISTAKES IN DIOXIN RESEARCH

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Introduction

Epidemiologic methods have improved substantially in the last few years. In fact, we appear to be on the threshold of a unified theoretical approach to the design, analysis and interpretation of epidemiologic studies. Here, we review some common epidemiological mistakes identified in dioxin research and discuss how current thinking about epidemiologic methods can be used to improve the quality of epidemiological dioxin studies. In particular, we examine current approaches to accounting for the impact of study imperfections when interpreting study results. We also briefly discuss the role of statistics in the analysis and interpretation of epidemiologic studies.

Results and Discussion:

One of the most conspicuous concerns regarding the epidemiological dioxin literature is the presence, and often lack of acknowledgement, of confounding. More specifically, studies of dioxin exposure and health outcomes do not, and frequently cannot, isolate the true effect of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from the effects of other dioxin-like compounds and/or mixtures of other chemical exposures. Although more recent studies have begun to examine the effect of dioxin mixtures on health outcomes,[1-7] a large majority of epidemiological studies have focused strictly on TCDD as the exposure of interest.[8] For instance, of the 13 studies evaluating cardiovascular disease (CVD) mortality in occupational cohorts, only five assessed the impact of multiple congeners on disease and just three of these estimated exposure using total toxic equivalencies (TEQ), presenting an obvious dilemma when interpreting the relationship between exposure to dioxin and risk of CVD.

In addition, most epidemiological studies of dioxin exposure examine the mortality experience of exposed populations. While these types of studies can be useful, caution should be used when making inferences about the study results, particularly if mortality rates in the population of interest are compared solely to the rates of an external referent population. Specifically, comparison of workers to the general population can result in confounding since employed workers are often healthier and have differing characteristics compared to the general population. Consequently, the use of nested case-control, Poisson or time-dependent proportional hazards regression models is suggested for mortality cohort studies as these methods utilize an internal referent population. Notable examples of these approaches have been employed in only a few dioxin studies,[5,9-15] but are well-founded and suggest these methods can be useful techniques for managing specific aspects of confounding in dioxin research.

Some level of confounding is present in all epidemiological studies; yet it is important to understand that simple adjustment for confounders (either in a model or by stratafication) may not necessarily control confounding. For example, Table 1 presents the distribution for a disease risk factor by exposure and population type, where doomed indicates that a population of individuals will become ill regardless of exposure and immune represents a population of individuals who will never develop the disease regardless of exposure (i.e. exposure has no effect on disease outcome). Although only the 'Total' and 'Incidence' data would be observable in an actual study, the counts and proportions presented demonstrate that the risk factor is related to exposure and predicts disease among unexposed individuals. Yet, if the risk factor is not controlled for, the proportion of diseased individuals in the exposed and unexposed groups is equal, as it should be since exposure does not affect the outcome. On the contrary, adjusting for the risk factor produces estimates that erroneously suggest the risk factor inhibits the development of disease in both strata.

Table 1: Hypothetical Distribution for a Disease Risk Factor by Exposure and Individual Type*

	Risk Factor Present		Risk Factor Absent		Crude	
Individual Sub-type	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed
Doomed†	60	70	40	180	100	250
Immune‡	40	30	60	220	100	250
Total	100	100	100	400	200	500
Incidence	0.60	0.70	0.40	0.45	0.50	0.50

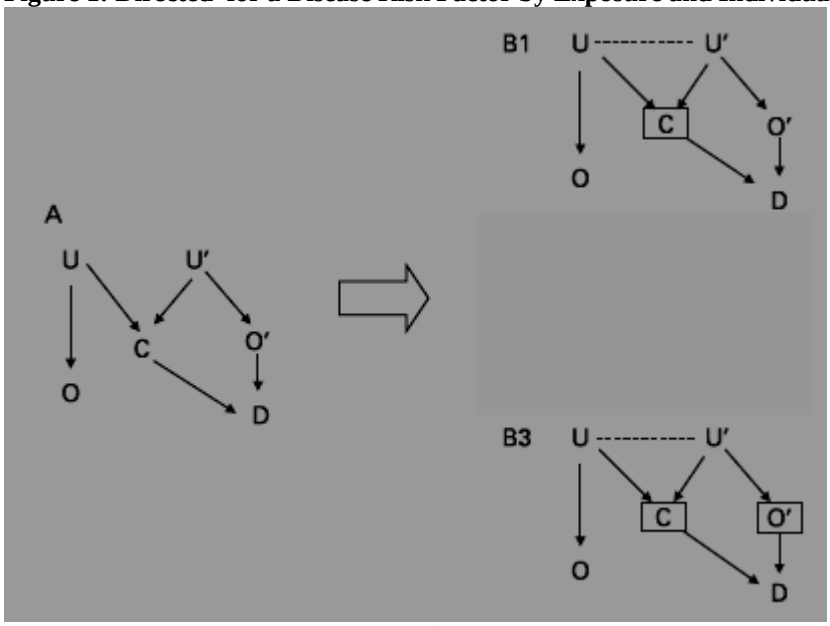
*From Greenland and Robbins (1986)[16]

†Become ill regardless of exposure

‡Are immune to disease regardless of exposure

Additionally, the covariates that can and should be controlled for are frequently not included in most analyses. More importantly, human dioxin studies consistently fail to either adjust for confounders specific to each disease being studied or overadjust for a multitude of covariates. A potential solution to this problem and a method with which to help identify confounders is the use of directed acyclic graphs (DAGs), which are directed graphs that do not contain directed cycles, as a tool to determine which variables to include in disease-specific epidemiological models. In an example adapted from Richiardi et al. (2008),[17] controlling for socioeconomic status potentially addresses the confounding effect of this factor; however, it could also introduce bias if other occupational exposures cause the outcome of interest. More explicitly, in Figure 1, (A) illustrates that occupational exposure to TCDD of chemical production workers (O) is related to socioeconomic status (C) through joint unmeasured causes (U). Likewise, unmeasured factors (U') are also present that define the relationship between SES and working in other occupations (O') involving exposures to CVD risk factors. Finally, low SES (C) is a risk factor for cardiovascular disease (D). In (B1), adjustment for SES (C) eliminates confounding by this covariate, but also results in a spurious association between occupational exposure to TCDD from chemical production work (O) and risk of CVD (D). However, (B3) demonstrates that controlling for SES (C) and other occupations with exposures to risk factors for CVD (O') produces a valid assessment of the relationship between being employed as a chemical production worker (O) and CVD (D).

Figure 1: Directed for a Disease Risk Factor by Exposure and Individual Type



The square around a variable means conditioning for that variable. Dashed lines without arrow-heads are used to connect independent causes of a collider, which has been conditioned on.

Misclassification of both exposures and health outcomes also provides an ever-present challenge when interpreting the dioxin epidemiological literature. With regard to exposure misclassification, it is critical to recognize that in studies with small numbers of unexposed (or exposed) cases, misclassification of even a few individual can result in substantial changes to the estimate of effect. Such an example is demonstrated in Tables 2A and 2B. Given the data in Table 2A, the true estimate of the association between dioxin exposure and endometriosis is OR = (34*3)/(24*8) = 0.53. No assume that three of the cases not exposed, were indeed exposed (Table 2B). Based on the new classification of exposed, the OR is (37*3)/(24*5) = 0.93, quite larger than the true estimate.

Table 2A: True Classification* of Dioxin Exposure by Disease State†

	Endometriosis +	Endometriosis -	Total
Dioxin Exposure	34	24	58
No Dioxin Exposure	8	3	11
Total	42	27	69

*For the purpose of this example, we will assume that the effect measure reported in the study is the "true" effect and that there was no misclassification in the study

†Adapted from Pauwels et al. (1986)[18]

Table 2B: Misclassification of Dioxin Exposure by Disease State

	Endometriosis +	Endometriosis -	Total
Dioxin Exposure	37	24	61
No Dioxin Exposure	5	3	9
Total	42	27	69

Relative to exposure misclassification, the misclassification of outcomes can also bias estimates of effect. Furthermore, it is often assumed that non-differential misclassficiation causes estimates of effect to be biased towards the null. However, exact non-differential misclassification only results in bias toward the null under very specific conditions,[19-20] and non-differential misclassification does not ensure that measures of association will be biased toward the null even if those conditions are met.

Lastly, regarding the use of statistics in epidemiological dioxin studies, it is essential that researchers understand statistics are simply a tool and the results they produce must be evaluated with caution. For example, when examining multiple outcomes, as so many human dioxin studies do, consideration must be given to the number of outcomes evaluated and how many were reported. Reporting only statistically significant results can result in type I error issues. More specifically, if 100 endpoints are examined and a study uses an alpha of 0.05, then we can expect five 'real' results and five spurious results and review of all the results may help determine which results are biologically meaningful. Moreover, and estimate of effect can be elevated but not significant and still represent an important finding that needs to be explored further, whereas some statistically significant results may not be meaningful (e.g. OR = 1.02 and 95% Confidence Interval = 1.01 – 1.03). While over-reliance on statistical significance and p-values is common in the epidemiological dioxin literature, often statistical significance cannot truly be detected as 1) a good mathematical equation for random error has not been established and 2) the calculation of p-values assumes a study has been executed perfectly and the substitute population is exchangeable with the population we would like to compare to the exposed population.

In summary, there are many limitations of the dioxin epidemiological literature. Nonetheless, no study is perfect, nor can it ever be so. Accordingly, it is important to acknowledge the limitations of our studies, but more importantly, make the extra effort to evaluate how these imperfections affect study results. Conducting sensitivity and/or uncertainty analyses are possible solutions to address study shortcomings in addition to using biomonitoring data collected for cohorts to validate exposure classification. Moreover, it is vital that dioxin epidemiological research is not over-reliant on statistical significance as a means of determining the relevancy of study results. Certainly, statistics can be a useful tool in conducting research, but should be considered a means to an end in epidemiology. Rather, evaluation of epidemiological health outcomes research should focus on study bias and methods to evaluate the impact of these limitations.

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