Scientific Research Publishing

Open Journal of Epidemiology, 2024, 14, 312-330
https://www.scirp.org/journal/ojepi
ISSN Online: 2165-7467
ISSN Print: 2165-7459

An Analysis of Specific Categories of Birth Defects and Developmental Disabilities for Children of Participants of the Air Force Health Study

George J. Knafl

School of Nursing, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
Email: gknafl@unc.edu

How to cite this paper: Knafl, G.J. (2024) An Analysis of Specific Categories of Birth Defects and Developmental Disabilities for Children of Participants of the Air Force Health Study. *Open Journal of Epidemiology*, **14**, 312-330.
https://doi.org/10.4236/ojepi.2024.142022

Received: March 3, 2024
Accepted: May 18, 2024
Published: May 21, 2024

Abstract

Background: The Air Force Health Study collected reproductive outcomes for live-born children of male Air Force veterans of the Vietnam War. **Methods**: Dioxin values for participants were obtained from blood samples. Analyses were conducted of occurrence of 16 specific categories of birth defects and developmental disabilities. Children were categorized as conceived before and after the start of participants' Vietnam War service. Children conceived before the start of Vietnam War service were treated as being conceived when their fathers had unquantifiable dioxin values. Children conceived after the start of Vietnam War service for participants with missing dioxin values were excluded from primary analyses, but were used to assess the impact of their exclusion on conclusions. Correlation between values for specific categories for multiple children fathered by the same participant was accounted for. The dose-response relationship was treated as a step function increasing for dioxin values larger than adaptively identified individual thresholds changing with the specific category. **Results**: For 15 of 16 specific categories, the probability of occurrence increased substantially for a sufficiently high dioxin level above identified thresholds. Exclusion of children due to missing dioxin likely did not affect these results. **Conclusions**: Results supported the conclusion of substantial adverse effects on a wide variety of specific categories of birth defects and developmental disabilities due to sufficiently high exposures to dioxin, a toxic contaminant of Agent Orange used for herbicide spraying in the Vietnam War. Results may hold more generally, but might also have been affected by a variety of limitations.

Keywords

Agent Orange, Air Force Health Study, Birth Defects, Developmental

Disabilities, Dioxin, Dose-Response Relationship, Vietnam War

1. Introduction

Data on health, reproductive, and mortality outcomes for male Air Force veterans of the Vietnam War were collected as part of the Air Force Health Study (AFHS). It is also called the Ranch Hand Study after herbicide spraying activities during the Vietnam War named Operation Ranch Hand [1] [2] [3] [4]. Details on study design and subject selection were described in [1]. A purpose of the study was to investigate the effect on these outcomes of dioxin, a toxic contaminant of Agent Orange used for herbicide spraying in the Vietnam War. See [5] for more details on Agent Orange. The conclusion reached in [3] about reproductive outcomes was that there was little or no support for adverse effects due to paternal exposure to dioxin on these outcomes for conceptions and for live-born children of AFHS participants. In 2000, Vietnam Veterans of America obtained reproductive outcome data available at that time from the AFHS which was then approved by the Human Subjects Research Review Committee of the Yale School of Nursing as exempt.

A reanalysis in [6] of several AFHS birth defect outcomes for live-born children concluded that certain modeling decisions made in [3], for example, using 10 parts per trillion (ppt) for a threshold for a high dioxin level, contributed to reaching this conclusion. In contrast, using individualized thresholds for low and high dioxin levels, the probability of a major birth defect, a nonmajor birth defect, and multiple birth defects increased significantly for live-born children of AFHS participants with a high compared to a low dioxin level. Further analyses of a more complete set of general categories of birth defects and developmental disabilities were conducted in [7] using a more complex nonlinear dose-response relationship. Outcomes considered were the occurrence of any birth defect, multiple birth defects, a major birth defect, a nonmajor birth defect, any developmental disability, multiple developmental disabilities, either any birth defect or any developmental disability, as well as both any birth defect and any developmental disability. Adverse effects on these general categories of birth defects and developmental disabilities were identified due to participants' dioxin exposures increasing nonlinearly after an individually assigned threshold. Similar analyses were conducted in [8] of conceptions for AFHS participants identifying adverse effects due to dioxin exposure on the occurrence of non-live born, miscarriage, and preterm conception.

Other than the results of [6] [7] [8], the literature provides only limited support for adverse effects due to paternal Vietnam War service on reproductive outcomes. An extensive discussion of the effects due to exposure of Vietnam veterans to Agent Orange on birth defects is provided in [9]. The Institute of Medicine [10] provides a discussion of the evidence for the effects on reproductive outcomes due to exposure of Vietnam veterans to Agent Orange. In [11], Vietnam veterans had a relative risk of 1.7 compared to non-Vietnam veterans of fathering an infant with one or more major malformations, but this was non-significant. In [12], the risk of having children with moderate-to-severe birth defects was associated with military service in Vietnam, but for mothers and not for fathers. The issue of maternal dioxin exposure effects on birth defects in the offspring of mothers residing in a Vietnam village that was repeatedly and highly sprayed during the Vietnam War was addressed in [13]. Whether the effects due to Vietnam War service may be a consequence of exposures other than dioxin, for example, drug use, was addressed in [14]. On the basis of a systematic review [15], parental exposure to dioxin appeared to be associated with an increased risk for birth defects, but the validity of this review was questioned [16]. On the other hand, the literature review of [17] concluded that offspring are vulnerable to all endpoints of developmental toxicity, including structural and functional abnormalities, as a consequence of paternal, and not just maternal, exposures to chemicals.

While the analyses of [7] identified adverse effects on categories of birth defects and developmental disabilities due to participants' dioxin exposures, those analyses only addressed very general categories. Specific categories of birth defects and developmental disabilities were also available for the live-born children, but most of these were sparsely occurring, and so the specific categories were not considered in the dioxin exposure analyses [7].

Consequently, the objective of this article is to investigate the potential association between paternal exposure to dioxin from Agent Orange during Vietnam War service and the occurrence of specific categories of birth defects and developmental disabilities in their offspring. Such analyses are important to consider since they address which of the specific categories are affected by dioxin exposures and which are not, an issue of potential policy implications. However, the sparse occurrence for these specific categories necessitates an alternate analysis approach.

2. Methods

2.1. Available Data

Data are available for 6922 children with known conception dates for 2613 participants. Participants served in the Air Force in Southeast Asia during the Vietnam War including Ranch Handers involved in herbicide spraying and non-Ranch Handers serving in other capacities. Detailed descriptions of these children and their birth defects and developmental disabilities are provided in [6] [7]. Analyses addressing paternal dioxin exposure effects on categories of birth defects and developmental disabilities are also provided in [6] [7] using the subset of children conceived after the start of Vietnam War service for participants with non-missing dioxin values. However, those analyses only addressed general categories as described in Section 1.

Analyses reported in Section 3 address the occurrence of specific categories of birth defects and developmental disabilities. The specific birth defect categories include the 12 cases of chromosomal; circulatory; digestive; ear, face, or neck; eye; genital; musculoskeletal; nervous; respiratory; skin; urinary; and other birth defects. The specific developmental disability categories include the four cases of developmental delays, emotional disturbance, hyperactivity, and mental retardation. These are the names given to the categories by the AFHS. Different names would likely have been used in some cases had the study been conducted more recently, but the original names have been retained for consistency. While these categories are more specific than the general categories considered in [6] [7], they are not as specific as possible. Most of the specific categories of birth defects and developmental disabilities are sparsely occurring and so an analysis process adjusting for sparsity is used as described in Section 2.5.

2.2. Dose-Response Model

The toxicological dose-response curve used in [7] [8] is assumed to have a threshold below which an effect due to toxic exposure is not observed and then to follow an increasing nonlinear curve after the threshold as supported in [18] [19]. However, a nonlinear relationship might be too complex to estimate for most specific categories of birth defects and developmental disabilities due to being sparsely occurring. For this reason, the less complex dose-response relationship of [6] is used in analyses reported in Section 3. The effect on a specific category due to dioxin exposure is treated as a step function having a constant probability level up to a threshold and changing to a different constant level after that threshold. An adverse effect on a specific category due to dioxin exposure is supported by a positive estimate for the change in the probability of that specific category for a high dioxin level compared to a low dioxin level. To reduce the range of predictor values, the natural log of dioxin values is used instead of the actual dioxin values in ppt.

2.3. The Dioxin-Rated Children Data Set

Analyses of [6] [7] [8] use data for only the children conceived after the start of Vietnam War service for participants with available dioxin values. Using those data for sparsely occurring specific categories would be problematic since these specific categories would have likely only occurred for participants with a restricted range of dioxin values. Estimates of the probability of the occurrence of such specific categories for children rated as having a low dioxin value would then likely be close to or even equal to the unrealistic value of 0. Consequently, analyses reported in Section 3 use a larger subset of children, that is, all children that can be assigned a dioxin value. This dioxin-rated children data set consists of children conceived after the start of Vietnam War service for participants with available dioxin values (as also used in [6] [7] [8]) combined with all children conceived before the start of participants' Vietnam War service. The participants who fathered children conceived before the start of their Vietnam War service are reasonably considered as having had unquantifiable dioxin values (i.e., below the limit of quantifiability) at the time of conception. Estimates of the probability of the occurrence of specific categories for children rated as having a low dioxin value would then have realistic values influenced by the occurrence of specific categories for the children conceived before the start of participants' Vietnam War service. Using this data set has the added benefit of including data for a distinctly larger number of children and participants.

2.4. Statistical Modeling

Outcomes are dichotomous having values 0 or 1 with the value 1 corresponding to the occurrence of the associated specific birth defect and developmental disability category with mean μ equal to the probability of the outcome having value 1. The variance of a dichotomous outcome is a function of the mean, specifically, its variance equals $\mu\cdot(1-\mu)$. The logit link function

$$\text{logit}(\mu) = \log(\mu/((1-\mu))$$

is used to model the mean. This logit function is treated as equaling a general linear function determined by an intercept parameter and/or slope parameters for model predictor variables (called the general linear model).

Since participants could father multiple children, the correlation between outcomes for children of the same participant needs to be addressed. This is accounted for by grouping outcome values into correlated matched sets of children (usually called "subjects") of the same participant indexed by their birth order (called the "within-subject" effect).

The parameters for the means and the correlations are estimated using extended linear mixed modeling (ELMM) [20] by maximizing an associated likelihood function, that is, by solving parameter estimating equations based on partial derivatives of that likelihood. Alternative ELMM models are compared using k-fold likelihood cross-validation (LCV) scores based on combining predictions of k subsets of the data computed with parameters estimated using the rest of the data. A model with a larger LCV score is better than a model with a smaller LCV score, but it is not necessarily substantially better.

LCV ratio tests based on the χ^2 distribution (as for likelihood ratio tests) are used to address this issue based on a cutoff for a substantial percent decrease in the LCV score, decreasing with the number of outcome measurements. A model with a smaller LCV score is substantially improved upon when its LCV score generates a percent decrease greater than the cutoff. Otherwise, it is a competitive alternative to the model with the larger LCV score. LCV ratio tests generate more conservative results than standard tests for zero parameters, that is, removing a parameter generating a significant standard test might not result in a substantial percent decrease in the LCV score.

Individualized thresholds for step function dose-response relationships for the 16 specific categories of birth defects and developmental disabilities are estimated adaptively by maximizing the LCV score (as described in Section 2.5). Effects of a high dioxin exposure level on these specific categories are tested using LCV ratio tests.

These methods have also been used in [7] [8] where more details are provided. They are supported by a SAS® macro available upon request from the author.

This macro supports parameter estimation as well as computation of LCV scores and cutoffs for substantial percent decreases in LCV scores. It also supports identification of nonlinear relationships for a variety of independent and correlated outcome types, not just for correlated dichotomous outcomes.

2.5. Analysis Process

Analyses reported in Section 3 are conducted in the following five steps. Results for these analyses are generated using SAS Version 9.4.

1) Distributions are provided for 16 specific categories including 12 specific categories of birth defects and four specific categories of developmental disabilities as listed in Section 2.1 for the dioxin-rated children described in Section 2.3.

2) Analyses using the methods described in Section 2.4 are conducted of correlated dichotomous outcomes corresponding to the occurrence of each of the 16 specific categories of birth defects and developmental disabilities. Dichotomous outcome values for children of the same participant are treated as correlated within increasing birth order. Based on the assessment given in [7] for general categories of birth defects and developmental disabilities, LCV scores are computed using $k = 5$ folds and the exchangeable correlation structure with a common correlation between outcomes for all pairs of children of the same participant (also called the intra-class correlation).

3) Using data for the dioxin-rated children, analyses are conducted assessing the impact of high versus low dioxin exposure levels on each of the 16 specific categories of birth defects and developmental disabilities. The step function models of Section 2.2 in natural log dioxin are generated for alternative thresholds t for a low versus high dioxin level (as also used in [6]). The selected threshold t^* is the value of t that maximizes the LCV score over alternative values rounded to one decimal digit and ranging from 0 to 2.4 as also used in [7] [8]. The model based on t^* is compared to the model with means constant in dioxin using an LCV ratio test. A substantial effect due to dioxin exposures greater than t^* compared to smaller exposures is indicated by a substantial percent decrease in the LCV score for the model with constant means. When this holds, an increase after t^* in the estimated probability of the associated specific birth defect and developmental disability category for dioxin-rated children supports the conclusion of a substantial adverse effect on that specific category due to increased paternal dioxin exposure for those children.

4) Step 3 analyses do not account for the occurrence of specific categories of birth defects and developmental disabilities for children conceived after the start of Vietnam War service for participants with missing dioxin values. These children are not included in step 3 analyses because they cannot be assigned exact dioxin values. Not being able to account for these children might have a substantial impact on the conclusions reached in step 3 analyses. To assess the impact of not including these children, proportions are imputed for the occurrence of specific categories for these children with associated unquantifiable and quantifiable dioxin values. The observed proportions for the occurrence of specific

categories for children conceived before and after the start of participants' Vietnam War service with missing dioxin values are used to impute proportions of unquantifiable and quantifiable dioxin cases for the subset of these children conceived after the start of participants' Vietnam War service as follows.

Proportions of specific categories for children conceived after the start of participants' Vietnam War service with missing dioxin values having unquantifiable dioxin values are imputed as equal to associated observed proportions for children conceived before the start of participants' Vietnam War service with missing dioxin values. When an observed proportion equals 0, the associated imputed proportion is reset to one assuming that one-half a person has an unquantifiable value so that the proportion is non-zero and an odds ratio can be computed. When one of the imputed proportions is larger than the observed proportion of the specific category for all children conceived after the start of participants' Vietnam War service with missing dioxin values, the imputed proportion is reset to this smaller value (i.e., conservatively treat all observed occurrences of the specific category in this case as occurring for children of participants with unquantifiable dioxin values).

Proportions of specific categories for children conceived after the start of participants' Vietnam War service with missing dioxin values having quantifiable dioxin values are imputed as equal to differences of associated observed proportions for the children conceived after the start of participants' Vietnam War service with missing dioxin values minus associated imputed proportions assigned to children conceived after the start of participants' Vietnam War service having unquantifiable values. Note that this difference can equal 0.

Odds ratios are computed using the above-imputed proportions for children conceived after the start of participants' Vietnam War service with missing dioxin values. Odds ratios are also computed using observed proportions for the dioxin-rated children. Weighted averages of these two odds ratios are used to impute odds ratios for all children using weights based on relative subsample sizes. These weighted average odds ratios estimate the impact of missing dioxin values on the effects due to having a quantifiable compared to an unquantifiable value and provide an indication of the impact of missing dioxin values on analyses based on only measured dioxin values. An odds ratio greater than 1 supports the conclusion that not accounting for children conceived after the start of participants' Vietnam War service with missing dioxin values has likely not substantially affected conclusions about adverse effects based on analysis step 3 results.

5) Including data for children conceived before the start of Vietnam War service provides for a more reliable estimate of the probability of sparsely occurring specific categories. However, their inclusion might affect conclusions had enough data been available for children conceived after the start of Vietnam War service to justify analyzing only those data. This issue is addressed by analyzing data on musculoskeletal birth defects, the most frequently occurring specific category, using just the data for children conceived after the start of Vietnam War service for participants with available dioxin values.

Table 1. Distribution of specific categories of birth defects and developmental disabilities.

category		count	percent (%)[a]
birth defects			
	chromosomal	14	0.22
	circulatory	85	1.33
	digestive	88	1.38
	ear, face, or neck	42	0.66
	eye	34	0.53
	genital	87	1.36
	musculoskeletal	514	8.06
	nervous	25	0.39
	respiratory	11	0.17
	skin	98	1.54
	urinary	83	1.30
	other	15	0.24
	total[b]	1096	
developmental disabilities			
	developmental delays	267	4.19
	emotional disturbance	25	0.39
	hyperactivity	134	2.10
	mental retardation	20	0.31
	total[b]	446	
total[b]		1542	

[a]Out of 6374 children with associated dioxin values for 2423 participants. Children conceived before the start of participants' Vietnam War service assigned an unquantifiable-dioxin value. [b]Accounting for multiple birth defects and/or developmental disabilities for the same child.

3. Results

3.1. Distributions for Specific Categories of Birth Defects and Developmental Disabilities

Dioxin exposure from Agent Orange was not possible before January 18, 1962, the day spraying of Agent Orange started. For this reason, children were considered as conceived after the start of a participant's Vietnam War service when conception occurred after the later of the start date of that participant's first Vietnam War tour and January 18, 1962. There were 6374 dioxin-rated children with 4195 (65.8%) conceived before the start of participants' Vietnam War service. The dioxin-rated children were fathered by 2423 participants with 1680 (69.3%) of these participants fathering a child conceived before the start of their Vietnam War service. Of the 2423 participants, 1270 (52.4%) fathered only children conceived before, and so conceived no children after, the start of participants' Vietnam War service. Of the 6922 children for 2613 participants with available data, 548 (7.9%) children were conceived after the start of participants' Vietnam War service for 216 (8.3%) participants with missing dioxin values, and so only a relatively small number of children and participants were excluded from analyses for the dioxin-rated children. A total of 2200 (84.2%) participants fathered more than one child, indicating the need for accounting for correlation between outcomes for children of the same participant.

Of the 4195 dioxin-rated children conceived before the start of participants' Vietnam War service, 556 (13.3%) had one or more birth defects and developmental disabilities. Of the 2179 dioxin-rated children conceived after the start of participants' Vietnam War service, 608 (27.9%) had one or more birth defects and developmental disabilities, a 109.8% increase in the occurrence of birth defects and developmental disabilities.

Table 1 contains distributions for specific categories of birth defects and developmental disabilities for dioxin-rated children. Percentages for the 12 specific birth defect categories range from 0.17% to 8.06%. Percentages for the four specific developmental disability categories range from 0.31% to 4.19%. Six of the specific birth defect categories and two of the specific developmental disability categories occur for less than 1% of the children. A further five specific birth defect categories occur for less than 2% of the children. Only three other specific categories occur for larger percentages of children. These results indicate the importance of adjusting the analysis approach for the possibility of sparsity.

Table 2. Comparison of constant and low vs. high dioxin models for the occurrence of specific categories of birth defects and developmental disabilities[a].

category		5-fold LCV score		percent decrease[c]
		constant probability	low vs. high dioxin probability[b]	
birth defects				
	chromosomal	4.83286	5.18310	6.76%
	circulatory	2.09992	2.17074	3.26%
	digestive	2.07000	2.11281	2.03%
	ear, face, or neck	2.96054	3.20754	7.70%
	eye	3.28233	3.40408	3.58%
	genital	2.07829	2.12508	2.20%
	musculoskeletal	0.89028	0.93187	4.46%
	nervous	3.79589	3.87029	1.92%
	respiratory	5.75151	6.69988	14.16%
	skin	1.95310	2.03722	4.13%
	urinary	2.11146	2.15948	2.22%
	other	4.79211	4.87584	1.72%
developmental disabilities				
	developmental delays	1.21499	1.29864	6.44%
	emotional disturbance	3.69881	3.74589	1.26%
	hyperactivity	1.68791	1.80527	6.50%
	mental retardation	4.25991	4.25991	0.00%

LCV—likelihood cross-validation

[a]Computed using data for 6374 children with associated dioxin values for 2423 participants. Children conceived before the start of participants' Vietnam War service were assigned an unquantifiable dioxin value. [b]With the probability changing for natural log dioxin greater than compared to less than or equal to the threshold generating the largest 5-fold LCV score. [c]Percent decrease in the LCV score for the constant probability model compared to the high dioxin probability model. The cutoff for a substantial percent decrease for data with 6374 observations is 0.03%.

Dioxin values in parts per trillion (ppt) from blood draws were available for a subset of 2038 participants, 78.0% of the 2613 participants with available data. The analytic method for measuring levels of dioxin, that is, 2,3,7,8-TCDD as well as other TCDD isomers, in human serum was described in [21]. Blood draws were sampled from 9.8 to 30.4 years after the end of participants' last Vietnam War tour. For 115 participants (5.6% of the 2038 participants with available dioxin values), dioxin values were unquantifiable (i.e., below the limit of quantifiability and so too small to be measurable). Otherwise, quantifiable values ranged from 1.25 to 314.2 ppt. Unquantifiable dioxin values were treated in analyses as equaling 1.1 ppt, below the smallest observed quantifiable value of 1.25 ppt, but large enough so that its natural log had a positive value. Lengths for all of participants' Vietnam War tours ranged from 0.1 to 11.8 years, which likely affected their dioxin exposure values.

3.2. Dioxin Effects for the Dioxin-Rated Children

Table 2 provides an assessment of the impact of a high level of dioxin exposure on the occurrence of the 16 specific categories of birth defects and developmental disabilities listed in Table 1. LCV scores are provided for two models, one assuming that the probability of a specific category is constant in dioxin and the other allowing for that probability to change from a low to a high dioxin level determined by an adaptively generated threshold for natural log dioxin. The largest possible threshold 2.4 for a high dioxin level considered in the adaptive search corresponded to the 91.5th percentile of the 6374 dioxin-rated children, and so the range of possible thresholds allowed for a very large number of children to be considered as having a low dioxin level. A total of 4325 (67.9%) of the dioxin-rated children were assigned the unquantifiable value of 1.1 ppt, and so the effect of a low dioxin level was based on large numbers of children for all possible thresholds greater than zero, and so should have provided reliable estimates for low dioxin values. Only 8.5% of the children were assigned dioxin values larger than 2.4. Considering larger thresholds than 2.4 seemed inappropriate since then the number of children considered as having a high dioxin level would likely have been too small to provide reliable estimates.

For 15 of the 16 specific categories of birth defects and developmental disabilities, percent decreases in the LCV score for the constant model compared to the model for an effect to a high dioxin level were substantial (i.e., greater than the cutoff of 0.03% for 6374 observations, and quite a bit larger in all 15 cases). The one exception was the specific developmental disability category mental retardation, for which the probability of a child having mental retardation was reasonably considered to be constant in dioxin.

Table 3 describes the adaptively generated models of Table 2 for the 16 specific categories of birth defects and developmental disabilities. Models are based on step functions in natural log dioxin with the individualized threshold for a high dioxin level adaptively chosen to maximize the LCV score.

Generated thresholds varied from 0 for having the mental retardation developmental disability category to 2.4 for having the nervous birth defect category, covering the complete range of possible thresholds. For the 15 specific categories of birth defects and developmental disabilities with thresholds greater than 0, the probability of the occurrence of the associated specific category increased for high versus low dioxin levels with odds ratios larger than 1 ranging from 1.96 to 6.02. These results supported the conclusion of a substantial adverse dioxin effect on all 12 specific birth defect categories and on three of the four specific developmental disability categories. Estimated exchangeable correlations were not reported in **Table 3** but ranged from −0.006 to 0.099.

Table 3. Generated low vs. high dioxin models for the occurrence of specific categories of birth defects and developmental disabilities[a].

category		threshold[b]	estimated probability		odds ratio
			≤ threshold	> threshold	
birth defects					
	chromosomal	2.0	0.0016	0.0068	4.27
	circulatory	1.8	0.0108	0.0293	2.76
	digestive	0.8	0.0108	0.0209	1.96
	ear, face, or neck	0.9	0.0040	0.0130	3.28
	eye	0.9	0.0038	0.0092	2.43
	genital	1.3	0.0110	0.0226	2.08
	musculoskeletal	0.3	0.0554	0.1345	2.65
	nervous	2.4	0.0034	0.0093	2.75
	respiratory	1.3	0.0008	0.0048	6.02
	skin	0.7	0.0107	0.0260	2.47
	urinary	1.8	0.0111	0.0249	2.27
	other	2.3	0.0019	0.0070	3.70
developmental disabilities					
	developmental delays	0.4	0.0258	0.0760	3.11
	emotional disturbance	0.9	0.0027	0.0070	2.60
	hyperactivity	0.8	0.0131	0.0394	3.09
	mental retardation	0.0	0.0031	0.0031	1.00

[a]With the probability changing for natural log dioxin greater than compared to less than or equal to the threshold. Computed using data for 6374 children with associated dioxin values for 2423 participants. Children conceived before the start of participants' Vietnam War service assigned an unquantifiable dioxin value. [b]The threshold between 0 and 2.4 generating the largest 5-fold likelihood cross-validation (LCV) score.

3.3. Quantifiable Versus Unquantifiable Effects for All Children

Results for the 6374 dioxin-rated children reported in Section 3.2 might have been affected in some way if dioxin values for the other 548 children conceived after the start of participants' Vietnam War service for participants with missing dioxin values had been available. This issue is addressed in Table 4. Imputed odds for a quantifiable dioxin value for these latter 548 children decreased compared to the odds for an unquantifiable value for the eye, musculoskeletal, and urinary birth defect categories (i.e., with odds ratios < 1), were zero for the circulatory and respiratory birth defect categories, and increased for the other seven birth defect categories as well as for all four developmental disability categories. For the three birth defect categories with a decrease, the odds ratios in those cases identified in Table 3 for 6374 dioxin-rated children might have been substantially affected if dioxin values had been available for the other 548 children. However, weighted odds ratios for the combined 6922 children supported the conclusion that, for all 16 specific categories, the odds for a quantifiable dioxin value increased compared to the odds for an unquantifiable dioxin value. Interestingly, this also held for the mental retardation developmental disability category, the only case of Table 3 with no dioxin effect. These results suggested that the adverse effects for the dioxin-rated children identified in Section 3.2 have likely not been substantially affected by missing data.

Table 4. Results for children assigned quantifiable vs. unquantifiable dioxin values for the occurrence of specific categories of birth defects and developmental disabilities[a].

category		conceived after missing dioxin children			dioxin-rated children			all children
		imputed proportion[b]		odds ratio	observed proportion		odds ratio	weighted odd ratio[c]
		unquantifiable	quantifiable		unquantifiable	quantifiable		
birth defects								
	chromosomal	0.0011	0.0044	4.01	0.0014	0.0039	2.79	2.89
	circulatory	0.0073	0.0000	0.00	0.0111	0.0181	1.64	1.51
	digestive	0.0045	0.0101	2.26	0.0109	0.0200	1.85	1.88
	ear, face, or neck	0.0022	0.0051	2.32	0.0042	0.0117	2.81	2.77
	eye	0.0045	0.0010	0.22	0.0039	0.0083	2.14	1.99
	genital	0.0078	0.0141	1.82	0.0109	0.0195	1.80	1.81
	musculoskeletal	0.0431	0.0335	0.77	0.0553	0.1342	2.65	2.50
	nervous[d]	0.0006	0.0012	2.23	0.0039	0.0039	1.00	1.10
	respiratory	0.0018	0.0000	0.00	0.0009	0.0034	3.79	3.49
	skin	0.0100	0.0119	1.19	0.0106	0.0254	2.43	2.33
	urinary	0.0033	0.0003	0.09	0.0116	0.0161	1.39	1.29
	other[d]	0.0006	0.0012	2.23	0.0021	0.0029	1.38	1.45
developmental disabilities								
	developmental delays	0.0156	0.0227	1.47	0.0257	0.0791	3.26	3.11

Continued

emotional disturbance[d]	0.0006	0.0067	12.2	0.0028	0.0063	2.26	3.04
hyperactivity	0.0067	0.0079	1.18	0.0129	0.0381	3.03	2.88
mentalretardation	0.0011	0.0098	8.99	0.0028	0.0039	1.39	2.00

[a]For 6922 children including 548 children with missing dioxin values and conceived after the start of participants' Vietnam War service and 6374 dioxin-rated children. [b]Proportions imputed as follows. For unquantifiable case, use the smaller of 1. observed proportions of specific types for children with dioxin missing and conceived before and 2. observed proportions of specific types for children with dioxin missing and conceived after. For quantifiable case, use the difference in observed proportions of specific types for children with dioxin missing and conceived after minus imputed proportions for children with dioxin missing and conceived before; a 0 difference indicates that all children observed to have the specific type are conservatively imputed as having an unquantifiable value. [c]Odds ratio imputed for all children using odds ratios for the conceived after missing dioxin children and the dioxin-rated children weighted by their relative subsample sizes. [d]None of the children with missing dioxin and conceived before had the associated specific type. To avoid an infinite odds ratio in these cases, the number of these children was set to 0.5 to use in estimating associated proportions for children with missing dioxin and conceived after.

3.4. Special Analysis of Musculoskeletal Birth Defects

Since the musculoskeletal birth defect category is the most frequently occurring at 8.06%, it can reasonably be analyzed using just the data for children conceived after the start of Vietnam War service for participants with available dioxin values. This allows for an assessment of the impact of including data for children conceived before the start of Vietnam War service. Applying analysis steps 2 - 3 to these reduced data, the adaptively generated threshold is 0.8, larger than the value 0.3 of **Table 3**, with LCV score 0.71854. The associated constant model has LCV scored 0.71590 with substantial PD 0.37% (i.e., larger than the cutoff of 0.09% for data with 2179 observations). The low vs. high dioxin model generates an odds ratio 1.59, smaller than the value 2.75 reported in **Table 3**. These results suggest that the inclusion of data for children conceived before the start of Vietnam War service might in some cases have lowered values for the threshold and for the estimated odds ratio, but an adverse effect due to a high dioxin exposure would likely still be identified when it is identified for the dioxin-rated children.

4. Discussion

4.1. Summary of Analyses

Sixteen specific categories of birth defects and developmental disabilities were considered in analyses reported in Section 3 using data for 6374 dioxin-rated children, that is, children of participants who could be assigned a dioxin value at the time of their conception. Specific birth defect categories included the 12 cases of chromosomal; circulatory; digestive; ear, face, or neck; eye; genital; musculoskeletal; nervous; respiratory; skin; urinary; and other birth defects. Specific developmental disability categories included the four cases of developmental delays, emotional disturbance, hyperactivity, and mental retardation. Distributions were presented for these 16 specific categories in **Table 1**. Thirteen of the 16 specific categories occurred in less than 2% of the children with eight of these occurring in less than 1% of the children, indicating the need for adjusting the analysis approach for sparsity.

The analysis approach was adjusted for sparsity in two ways. First, all children conceived before the start of the participants' Vietnam War service were included and assigned an unquantifiable dioxin value to maximize the number of children allocated to a low dioxin level for all possible thresholds to a high dioxin level. The other children were the ones conceived after the start of Vietnam War service for participants with available dioxin values (as also used in corresponding analyses of [6] [7] [8]). Second, a more straightforward step function dose-response relationship than that used in [7] [8] was assumed, constant for a low dioxin level up to a threshold value and changing to a different constant level for a high dioxin level after the threshold. Thresholds were treated as changing with the specific birth defect and developmental disability category under analysis and were adaptively estimated by maximizing the likelihood cross-validation (LCV) score. LCV ratio tests were used to identify the substantial effects due to a high compared to a low dioxin level. Substantial effects were considered adverse effects when estimated probabilities for a high dioxin level were larger than estimated probabilities for a low dioxin level.

Substantial adverse effects due to a high level of dioxin exposure were identified for 15 of the 16 specific categories of birth defects and developmental disabilities (**Tables 2-3**). The exception was the mental retardation developmental disability category, which was reasonably considered not to depend on dioxin exposure. Odds ratios for the 15 adverse effects ranged from 1.96 to 6.02.

The impact of missing dioxin values on the above analyses was addressed by assigning imputed unquantifiable and quantifiable dioxin proportions to the 548 children not used in those analyses. Briefly, the unquantifiable dioxin proportion for a specific category for children conceived after the start Vietnam War service for participants with missing dioxin values was imputed using the associated proportion for the children conceived before the start of Vietnam War service for participants with missing dioxin values. The quantifiable dioxin proportion was imputed as the amount that the observed proportion for these children exceeded the imputed unquantifiable dioxin proportion. These imputed proportions were used to impute odds ratios for these children for a quantifiable compared to an unquantifiable dioxin value for each of the 16 specific categories. These imputed odds ratios were combined with associated observed odds ratios for the 6374 dioxin-rated children, weighting them by their relative subsample sizes, to generate weighted odds ratios for all 6922 children. These weighted odds ratios were all greater than 1, indicating that only a quantifiable dioxin value may be needed for an adverse dioxin exposure effect although not necessarily a substantial adverse effect. These results supported the conclusion that results of substantial adverse dioxin effects on 15 specific categories for the dioxin-rated children had likely not been affected by missing dioxin values.

Results for the specific musculoskeletal birth defect category suggested that the inclusion of data for children conceived before the start of Vietnam War service might in some cases have lowered values for the threshold and for the estimated odds ratio. However, an adverse effect to a high dioxin exposure would likely still have held for children conceived after the start of Vietnam War service when such an effect was identified for the dioxin-rated children.

4.2. Prior Analyses of AFHS Reproductive Outcomes

Results reported in Section 3 are consistent with results reported in [6] [7] [8] but not with results reported in [3], raising the question of why [3] reached different conclusions. The difference in conclusions was due to differences in the data used in analyses and in how those data were analyzed. These issues have also been addressed in more detail in [7] [8], and so have only been summarized here.

Only conceptions occurring after the start of Vietnam War service were considered in [3] while the analyses of Section 3 also considered conceptions occurring before the start of Vietnam War service.

A fixed threshold of 10 ppt was used in analyses of [3] while the analyses of Section 3 used adaptively generated thresholds varying with the specific birth defect and developmental disability category under analysis. Non-Ranch Hand participants were excluded in [3] when they had dioxin values greater than 10 ppt and so then they might be more likely to father children with birth defects and developmental disabilities, but their data were included in reported analyses of Section 3. The children of participants with unquantifiable dioxin values who might have been less likely to father children with birth defects and developmental disabilities were excluded in [3], but were included in analyses reported in Section 3.

After these exclusions, [3] analyzed data for 1,773 children of 1,024 participants. In contrast, analyses reported in Section 3 used much more extensive data for 6374 children, or 260% more, of 2423 participants, or 137% more. Correlation within outcome values for children of the same participant was not accounted for in [3], but was in reported analyses of Section 3. Analyses of [3] focused on differences between Ranch Hand and non-Ranch Hand participants with the latter treated as a comparison group. This issue was addressed in [7] demonstrating that involvement in the herbicide spraying activities of Operation Ranch Hand was an effective surrogate measure for having a high dioxin exposure level, but was not as effective a measure of the impact of dioxin exposure as using the actual observed dioxin values. The analyses reported in Section 3 used actual observed dioxin values to measure having a high dioxin exposure level independent of Operation Ranch Hand participation.

4.3. Limitations

Results for the dioxin-rated children might have been affected by not including data for children conceived after the start of Vietnam War service for participants with missing dioxin values. However, imputation of having unquantifiable and quantifiable dioxin values for these latter children combined with observed dioxin values for the dioxin-rated children allowed for computation of weighted odds ratios for effects due to quantifiable versus unquantifiable dioxin values on the 16 specific categories for these two groups of children combined. These weighted odds ratios supported the conclusion that substantial adverse dioxin effects previously identified for 15 of the specific categories would likely have still held if dioxin values had been available for all participants.

Results for the dioxin-rated children may have been affected by including data for children conceived before the start of participants' Vietnam War service. However, analyses of the most frequently occurring specific category of a musculoskeletal birth defect indicated that, although estimated values of the threshold and of the odds ratio for an adverse dioxin exposure effect might have been affected, the conclusion of an adverse effect would likely have also held if sufficient data for children conceived after the start of Vietnam War service for participants with available dioxin values had been available for all the specific categories.

Analyses assumed a step function dose-response relationship with probabilities of specific categories increasing from low to high constant levels after a threshold, but the actual relationship would likely be more complex [18] [19]. The dose-response relationship was assumed in [7] [8] to be constant up to a threshold and then monotonic afterward, but that relationship was sometimes estimated to be close to a step function. It was possible in the analyses of [7] [8] to obtain reliable estimates of this more complex relationship because outcomes were frequently occurring. Unreliable estimates of such complex relationships were likely for sparsely occurring outcomes like the specific categories so that the simpler step function relationship was preferable. In any case, the existence of an adverse step function relationship would suggest that, if a more general monotonic relationship also existed beyond the threshold, it would likely be increasing and so also adverse.

The analysis process was adjusted to offset sparsely occurring outcomes including incorporating data for children conceived before the start of participants' Vietnam War service and using a step function dose-response relationship. However, the data are still primarily sparsely occurring and this might have had unidentifiable effects on conclusions.

Reported results held only for children of Air Force veterans of the Vietnam War. However, this held for both Ranch Hand and non-Ranch Hand Air Force veterans. The distribution of dioxin values for the non-Ranch Hand Air Force veterans would likely be similar to the distribution for veterans of the other Armed Forces who served in Southeast Asia during the Vietnam War, suggesting that similar adverse effects would hold as well for the children of these non-Air Force veterans.

Analyses addressing adverse dioxin effects on specific categories did not account for characteristics such as child sex, birth weight, paternal and maternal age at conception, participant age at start of Vietnam War service, the length of all Vietnam War tours combined, and non-dioxin paternal and maternal toxic exposures (e.g., drugs, alcohol, tobacco, and other confounding substances), among others. Models for a dioxin effect on specific categories also accounting for effects due to child/participant characteristics could be degenerate as a consequence of the sparsity of those specific categories. If not degenerate, the strength of previously identified adverse effects might be reduced by accounting for such covariates, and some identified adverse effects might no longer be substantial. However, the issue of covariate effects was addressed in [6] for general birth defect categories in participants' children and in [8] for reproductive outcomes for participants' conceptions. Both studies concluded that consideration of covariates likely did not affect their conclusions about adverse dioxin effects, suggesting that this might also hold for specific categories of birth defects and developmental disabilities.

The fact that dioxin exposures were measured many years after the end of participants' last Vietnam War tour could have affected in some way results for reported analyses addressing adverse effects on specific categories due to dioxin exposure. However, [8] concluded that dioxin decay might not have substantially affected their conclusions of adverse effects on reproductive outcomes for AFHS participants' conceptions, suggesting this could hold as well for specific categories of birth defects and developmental disabilities.

Participation might have influenced the results of reported analyses. Participants having higher dioxin exposures and/or those having more children with birth defects and developmental disabilities might have been more likely to participate. While these issues were not assessable, identified adverse effects held for substantive numbers of children of AFHS participants, suggesting that identified effects would likely hold for a more extensive group of children of Vietnam War veterans if not for all of them.

4.4. Conclusions

Results for analyses of the 16 specific categories of birth defects and developmental disabilities supported the conclusion of substantial adverse effects on 15 of those specific categories, including all 12 specific birth defect categories and three of the four of the developmental disability categories, to sufficiently high levels of dioxin exposures for male Air Force veterans of the Vietnam War. Substantial adverse effects were identified for the chromosomal; circulatory; digestive; ear, face, or neck; eye; genital; musculoskeletal; nervous; respiratory; skin; urinary; and other birth defect categories as well as for the developmental delays, emotional disturbance, and hyperactivity developmental disability categories. The one exception was the mental retardation developmental disability category. Children conceived after the start of participants' Vietnam War service for participants with missing dioxin values were excluded from these analyses, but evidence was provided that this likely did not affect conclusions of substantial adverse effects. Children conceived before the start of participants' Vietnam War service were included in these analyses, which might have affected results, but evidence was provided that this likely did not affect conclusions of substantial adverse effects.

In summary, results support the conclusion of substantial adverse effects due to dioxin exposure on specific categories of birth defects and developmental disabilities for children of the male participants of the AFHS. These conclusions might hold as well for larger groups of Vietnam War veterans. On the other hand, these conclusions might have been affected by a variety of limitations. Future research is needed to address these issues using other dioxin exposure data sets.

Acknowledgements

Grants R01 AI57043 from the National Institute of Allergy and Infectious Diseases and R03 MH086132 from the National Institute of Mental Health provided partial support for the development of the SAS macro used to conduct reported analyses. No external funding supported work on this article.

Conflicts of Interest

The author declares the following possible conflict of interest: funding by a grant in 2000 from the Vietnam Veterans of America, Linda S. Schwartz, Principal Investigator to conduct statistical analyses of Air Force Health Study reproductive outcome data.

References

[1] Wolfe, W.H., Michalek, J.E., Miner, J.C., Rahe, A.J., Silva, J., Thomas, W.F., Grubbs, W., Lustig, M.B., Karrison, T.G., Roegner, R.H. and Williams, D.E. (1990) Health Status of Air Force Veterans Occupationally Exposed to Herbicides in Vietnam: I. Physical Health. *Journal of the American Medical Association*, **264**, 1824-1831. https://doi.org/10.1001/jama.1990.03450140046032

[2] Michalek, J.E., Wolfe, W.H. and Miner, J.C. (1990) Health Status of Air Force Veterans Occupationally Exposed to Herbicides in Vietnam: II. Mortality. *Journal of the American Medical Association*, **264**, 1832-1836. https://doi.org/10.1001/jama.1990.03450140054033

[3] Wolfe, W.H., Michalek, J.E., Miner, J.C., Rahe, A.J., Moore, C.A., Needham, L.L. and Patterson Jr., D.G. (1995) Paternal Serum Dioxin and Reproductive Outcomes among Veterans of Operation Ranch Hand. *Epidemiology*, **6**, 17-22. https://doi.org/10.1097/00001648-199501000-00005

[4] The Air Force Health Study Assets Research Program. https://www.ncbi.nlm.nih.gov/books/NBK286036/

[5] https://en.wikipedia.org/wiki/Agent_Orange

[6] Knafl, G.J. (2018) A Reassessment of Birth Defects for Children of Participants of the Air Force Health Study. *Open Journal of Epidemiology*, **8**, 187-200. https://doi.org/10.4236/ojepi.2018.84015

[7] Knafl, G.J. (2023) An Analysis of Birth Defects and Developmental Disabilities for Children of Participants of the Air Force Health Study. *Reproductive Toxicology*, **117**, Article 108355. https://doi.org/10.1016/j.reprotox.2023.108355

[8] Knafl, G.J. (2023) An Analysis of Reproductive Outcomes for Conceptions of Participants of the Air Force Health Study. *Reproductive Toxicology*, **119**, Article

108413. https://doi.org/10.1016/j.reprotox.2023.108413

[9] Mekdecki, B. Agent Orange and Birth Defects. https://birthdefects.org/agent-orange/#:~:text=These%20included%20spina%20bfida%2C%20oral,higher%20in%20Vietnam%20veterans'%20children

[10] IOM (Institute of Medicine) (2014) Veterans and Agent Orange: Update 2012. The National Academies Press, Washington. https://www.ncbi.nlm.nih.gov/books/NBK195090/pdf/Bookshelf_NBK195090.pdf

[11] Aschengrau, A. and Monson, R.R. (1990) Paternal Military Service in Vietnam and the Risk of Late Adverse Pregnancy Outcomes. *American Journal of Public Health*, **80**, 1218-1224. https://doi.org/10.2105/AJPH.80.10.1218

[12] Kang, H.K., Mahan, C.M., Lee, K.Y., Magee, C.A., Mather, S.H. and Matanoski, G. (2000) Pregnancy Outcomes among US Women Vietnam Veterans. *American Journal of Industrial Medicine*, **38**, 447-454. https://doi.org/10.1002/1097-0274(200010)38:4<447::AID-AJIM11>3.0.CO;2-J

[13] Phuong, N.T.N., Thuy, T.T. and Phuong, P.K. (1989) An Estimate of Reproductive Abnormalities in Women Inhabiting Herbicide Sprayed and Non-Herbicide Sprayed Areas in the South of Vietnam, 1952-1981. *Chemosphere*, **18**, 843-846. https://doi.org/10.1016/0045-6535(89)90206-3

[14] Stanton, M.D. (1976) Drugs, Vietnam, and the Vietnam Veteran: An Overview. *The American Journal of Drug and Alcohol Abuse*, **3**, 557-570. https://doi.org/10.3109/00952997609014295

[15] Ngo, A.D., Taylor, R., Roberts, C.L. and Nguyen, T.V. (2009) Association between Agent Orange and Birth Defects: Systematic Review and Meta-Analysis. *International Journal of Epidemiology*, **35**, 1220-1230. https://doi.org/10.1093/ije/dyl038

[16] Fraser, F.C. (2009) Does Paternal Exposure to Agent Orange Cause Birth Defects? *American Journal of Medical Genetics Part A*, **149A**, 835-836. https://doi.org/10.1002/ajmg.a.32798

[17] Mattison, D.R. (2010) Environmental Exposures and Development. *Current Opinions in Pediatrics*, **22**, 208-218. https://doi.org/10.1097/MOP.0b013e32833779bf

[18] Brent R.L. (2001) Addressing Environmentally Caused Human Birth Defects. *Pediatrics in Review*, **22**, 147-179. https://doi.org/10.1542/pir.22.5.153

[19] Roberts L. (1991) Dioxin Risks Revisited. *Science*, **251**, 624-626. https://doi.org/10.1126/science.1846976

[20] Knafl, G.J. (2023) Modeling Correlated Outcomes Using Extensions of Generalized Estimating Equations and Linear Mixed Modeling. Springer Nature Switzerland AG, Switzerland. https://doi.org/10.1007/978-3-031-41988-1

[21] Patterson Jr., D.G., Hampton, L., Lapeza Jr., C.R., Belser, W.T., Green, V., Alexander, L. and Needham, L.L. (1987) High-Resolution Gas Chromatographic/High-Resolution Mass Spectrometric Analysis of Human Serum on a Whole-Weight and Lipid Basis for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin. *Analytic Chemistry*, **59**, 2000-2005. https://doi.org/10.1021/ac00142a023