Diabetes and Cancer in Veterans of Operation Ranch Hand After Adjustment for Calendar Period, Days of Spraying, and Time Spent in Southeast Asia

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Background: *The Air Force Health Study was launched in 1980 as part of a Federal effort to resolve the Agent Orange issue.* ***Objectives:*** *To study diabetes and cancer with additional adjustment for days of spraying, calendar period of service, and time spent in Southeast Asia (SEA).* ***Methods:*** *This was a longitudinal study of veterans of Operation Ranch Hand, the unit responsible for spraying Agent Orange and other 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-contaminated herbicides in Vietnam from 1962 to 1971.* ***Results:*** *Associations between TCDD and diabetes and between TCDD and cancer in Ranch Hand veterans are strengthened after adjustment for calendar period of service, days of spraying, and, for cancer, time spent in SEA.* ***Conclusions:*** *Calendar period of service, days of spraying, and time spent in SEA are important confounders in the Air Force Health Study.* (J Occup Environ Med. *2008;50:330–340*)

The herbicide Agent Orange was a 1:1 mixture of 2,4-dichloro-phenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and was contaminated, from less than 0.05 to almost 50 parts per million, with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).[1] A review by the National Academy of Sciences concluded that there is sufficient evidence of an association between exposure to herbicides and TCDD and type 2 (adult onset) diabetes, soft tissue sarcoma, non-Hodgkin lymphoma and Hodgkin disease, and limited/suggestive evidence of an association between TCDD or herbicide exposure and cancer of larynx, bronchus, prostate, and multiple myeloma.[2]

Herbicides were used in Vietnam by the US Air Force Operation Ranch Hand to defoliate, by aerial spraying from C-123 aircraft. From 1962 through 1965 small quantities of Agent Purple (2,4-D; 2,4,5-T), Blue (Cacodylic acid), Pink (2,4,5-T), and Green (2,4,5-T) were sprayed. From 1965 through 1970 more than 11 million gallons of Agent Orange (2,4-D; 2,4,5-T), and smaller quantities of White (2,4-D; picloram) and Blue were sprayed; from 1970 through 1971 only Agents White and Blue were used for defoliation.[1]

This report summarizes a study of diabetes and cancer in veterans of Operation Ranch Hand, the unit responsible for the aerial spraying of herbicides, including Agent Orange, in Vietnam from 1962 to 1971, and

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in a Comparison cohort of other Air Force veterans who served in the Southeast Asia (SEA) region during the same period that the Ranch Hand unit was active but who did not spray herbicides. These results were accumulated during the postservice period from each veteran's departure from SEA to 2004 (cancer to September 30, diabetes to December 31) in men participating in the Air Force Health Study (AFHS), a 20-year prospective study of the health, mortality, and reproductive outcomes of Ranch Hand veterans. This report updates our previous diabetes[3] and cancer[4] studies, but differs by, 1) addressing the number of days of actual herbicide spraying, calendar period of service and, for cancer, time spent in SEA, 2) stratifying to focus our investigation on subgroups that we expected to be at increased risk (regardless of the significance of interaction terms in a statistical model), 3) choosing cut points for stratification based on external information rather than statistical significance and not requiring the same cut points for diabetes and cancer.

Materials and Methods

The study, named the AFHS, sought to determine whether veterans of Operation Ranch Hand experienced adverse health and whether those health effects, if they exist, could be attributed to exposure to herbicides or their TCDD contaminant. The details of the study design and subject selection are published elsewhere.[5] The study compared the health and cumulative mortality experience of Ranch Hand veterans with a comparison group of other Air Force veterans who served in SEA during the same period (1962 to 1971) that the Ranch Hand unit was active and who were not involved with spraying herbicides. Comparisons were matched to Ranch Hands on age, race, and military occupation. The study included periodic analyses of noncombat mortality, in-person interviews, physical examinations, and reproductive outcomes. Physical examinations were conducted in 1982, 1985, 1987, 1992, 1997, and 2002.[6,7] Veterans who participated in at least one of the first five examinations were included. The study protocol[8] was reviewed and approved by the Institutional Review Boards of the participating organizations. Participation was voluntary and consent forms were signed at the examination site prior to study.

In 1987, blood from willing participants was collected and assayed for TCDD in parts per trillion (ppt) on a lipid weight basis. Veterans with no quantifiable TCDD result in 1987, those who refused in 1987, and subjects new to the study were also asked to give blood for the assay at the 1992, 1997, and 2002 examinations. Consistent with the 2002 AFHS report,[7] for those veterans whose TCDD was not measured in 1987, the subsequent measure was extrapolated to 1987 using a first-order kinetics model with a constant half-life of 7.6 years. Nondetectable (nonquantitable) TCDD levels were replaced by the value of the limit of detection (limit of quantitation) divided by $\sqrt{2}$.[9]

Diabetes cases included for analysis were diagnosed during the post-Vietnam period from the end of the tour of duty that qualified a veteran for inclusion in the study to December 31, 2004. We report cumulative postservice diabetes. Each case was verified from medical records and may represent a diagnosis at any of the six physical examinations. Every veteran who attended at least one of the first five examinations, regardless of his vital status, was considered for inclusion in the analysis. We excluded from all analyses of diabetes those veterans with a history of diabetes before service in SEA, and those with no TCDD measurement.

AFHS staff reviewed medical records and laboratory results to determine diabetic status and time to diabetes onset. Veterans who attended at least one examination and had a verified history of diabetes by medical diagnosis or exhibited a 2-hour postprandial glucose laboratory value of 200 mg/dL or greater were classified as diabetic. Veterans not meeting these criteria were defined as nondiabetic.

We estimated the initial TCDD dose at the end of the tour of duty in Vietnam in Ranch Hands having 1987 TCDD levels above background using a constant half-life of 7.6 years and assigned each veteran to one of four exposure categories, named "Comparison," "Background," "Low," and "High," according to his group, 1987 TCDD level, and initial TCDD level. The Background category was comprised of Ranch Hands with 1987 TCDD $\leq$10 ppt. The cut point separating the Low and High categories (91 ppt) was the median initial TCDD level among all Ranch Hands having TCDD levels measured in 1987 greater than 10 ppt. The category labels "Low" and "High" and the category definitions coincide with those used in previously published analyses; veterans assigned to the "Low" and "High" TCDD exposure categories had elevated TCDD levels relative to Comparisons.

In veterans with diabetes, we defined time to onset as the number of years between the end of the tour of duty that qualified a veteran for inclusion in the AFHS and the date of first diagnosis of diabetes. In veterans without diabetes, time to onset was the number of years between the end of the tour of duty that qualified a veteran for inclusion in the study and December 31, 2004.

For each veteran, tour dates, the number of days spent in Vietnam, and the number of days spent in SEA, and for each Ranch Hand veteran the date of the last day of service in the Ranch Hand unit, and for each Comparison veteran the date of the last day of service in SEA were extracted from military records. Days of spraying were computed based on tour dates and "Herbs Tape" data.[8]

We assigned each veteran to one of the four TCDD exposure catego-

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ries. We estimated body mass index (BMI) as weight (kg) divided by the square of height (m). We used Cox proportional hazards models to contrast the background, low, and high categories with the Comparison category with regard to cumulative postservice diabetes. All analyses were adjusted for BMI at the TCDD blood draw, smoking history in 1982 (pack-years),[10] family history of diabetes (yes, no), BMI during the SEA tour that qualified veterans for inclusion in the study, year of birth, last year of service in the Ranch Hand unit or SEA, the ratio of the number of days spent in Vietnam to the number of days spent in SEA, and military occupation (officer, enlisted flyer, enlisted ground). We tested for trend by assessing the significance of the coefficient of log(TCDD) in a fully adjusted Cox proportional hazards model of time-to-diagnosis in the combined cohort with log(TCDD) entered into model as a continuously distributed variable.

Cancer incidence data was obtained from medical records. Malignancies were coded following the rules and conventions of the International Classification of Diseases, 9th Edition, Clinical Modification.[11] Malignancies discovered at death were coded from the underlying causes of death on death certificates. We defined "all-site SEER cancer" as a malignancy included in any of the National Cancer Institute Surveillance Epidemiology and End Results (SEER) anatomical category definitions.[12] All-site SEER cancer included the first diagnosed cancer for each individual with any cancer diagnosis.

The analyses were based on cumulative all-site SEER cancer incidence from January 1, 1982 to September 30, 2004. We used Cox proportional hazards models to contrast the background, low, and high categories with the Comparison category with regard to cancer incidence. All analyses were adjusted for year of birth, BMI at the qualifying tour, and military occupation, skin reaction to sun exposure (burns painfully or freckles with no tan, burns or tans mildly, tans deep brown), eye color (brown, hazel/green, gray/blue), race (black, nonblack), and smoking history in 1982 (pack-years). We tested for trend by assessing the significance of the coefficient of log(TCDD) in a fully adjusted Cox proportional hazards model of time-to-diagnosis in the combined cohort with log(TCDD) entered into model as a continuously distributed variable. All cancer analyses adjusted for race by restricting to white veterans because approximately 94% of the cohort was white. Rounding sometimes prevents a strict concordance between the confidence interval (CI) and the *P* value; in these cases the *P* value is the determinant of statistical significance.

For analyses of diabetes, calendar period of service was defined as "≤1969" if the last day of service in the Ranch Hand unit or SEA was during or before calendar year 1969 and to ">1969" otherwise. For analyses of cancer, calendar period of service was defined as "≤1968" and ">1968". For diabetes, days of spraying was dichotomized as ≤90 days of spraying and >90 days of spraying. For cancer, days of spraying was dichotomized as ≤30 days and >30 days during or before 1967. We hypothesized that herbicides were more heavily contaminated early in the war than late in the war, and that the number of days of spraying was an important determinant of exposure in the Ranch Hand cohort and stratified by calendar period of service and days of spraying. The stratum of interest for diabetes was calendar period of service ≤1969 and >90 days of spraying and we hypothesized that a sharper association between the TCDD biomarker and diabetes would be found in this stratum than in that previously published.[3]

Because all-site SEER cancer risk increased with years of service in SEA among Comparison veterans, we further stratified the analysis of cancer by years of service in SEA (≤2 years, >2 years). The stratum of interest in analyses of cancer was calendar period of service ≤1968 and >30 days of spraying during or before 1967 and ≤2 years of service in SEA and we hypothesized that all-site SEER cancer would be associated with the TCDD biomarker in this stratum.

Results

Diabetes

Table 1 shows the sample reduction, beginning with the total number of veterans either fully or partially compliant to at least one of the first five AFHS physical examinations (n = 3049). After excluding those with diabetes prior to service in SEA

TABLE 1
Sample Reduction

Status	Comparison	Ranch Hand	Total
Total*	1853	1196	3049
Sample reduction for diabetes			
Pre-SEA diabetes	(2)	(3)	(5)
Not compliant†	(284)	(86)	(370)
Missing TCDD	(118)	(87)	(205)
Net	1449	1020	2469
Sample reduction for cancer			
Prebaseline cancer	(47)	(46)	(93)
Not compliant†	(209)	(80)	(289)
Missing TCDD		(84)	(84)
Net	1597	986	2583

*Fully or partially compliant to any physical examination.
†Not fully compliant to at least one physical examination of the first five physical examinations.

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TABLE 2
Demographics*—Diabetes

	Calendar Period of Service (≤1969†)				Days of Spraying		
	Comparison		Ranch Hand			Ranch Hand	
	Yes	No	Yes	No	Comparison	≥90 d	<90 d
N	648	801	657	363	1449	909	111
Median birth year	1940	1939	1937	1942	1939	1939	1939
Median pack-yr	12.1	10	13.4	9.3	11	12.7	10.5
Median TCDD	4.0	3.9	12.6	10.6	3.9	13.3	5.9
Median tour end year	1968	1970	1968	1970	1968	1969	1970
Mean tour BMI	24.8	25.1	24.7	25	24.9	24.8	24.6
Median days SEA	455	761	0	0	578	0	0
Median days Vietnam	93	207	386	396	161	395	334
Black (%)	6.6	6.2	5.9	6.1	6.4	6.5	1.8
Family history of diabetes (%)	30.4	30.2	31.1	28.1	30.3	29.5	34.2
Officer (%)	32.4	42.8	37.6	38	38.2	38	36
Enlisted flyer (%)	13.9	17.2	15.8	19.6	15.7	17.3	16.2
Enlisted ground (%)	53.7	40	46.6	42.4	46.1	44.8	47.7

*Up to and including the 1997 physical examination.
†During or before 1969.

($n = 5$), noncompliance with the first five physical examinations ($n = 370$), and those without a TCDD measurement ($n = 205$), 2469 remained for inclusion in statistical analyses of diabetes. Veterans who served during or before 1969 were similar with regard to birth year, pack years of smoking, and nine other covariates, to those whose last year of service was after 1969 (Table 2). The median TCDD in Ranch Hand veterans among those whose last year of service was during or before 1969 (12.6 ppt) was increased relative to those whose last year was after 1969 (10.6 ppt). Stratification by days of spraying (<90 days, ≥90 days), showed an increased median TCDD in Ranch Hands with at least 90 days of spraying (13.3 ppt) relative to those with less than 90 days of spraying (5.9 ppt); the other covariates did not vary appreciably with stratum of days of spraying.

After adjustment for age, BMI at tour, and last calendar year in Vietnam, the mean TCDD (in log units) was significantly increased in Ranch Hands whose last year of service was during or before 1969 (mean = 2.8) relative to those whose last year was after 1969 (mean = 2.2); difference = 0.5, 95% CI = 0.4 to 0.7, $P < 0.001$ (Table 3). Similar increases were found in enlisted ground ($P < 0.001$) and enlisted flyers ($P < 0.001$), but not officers ($P = 0.31$). A greater variance in the mean TCDD (in log units) was found with regard to stratum of days of spraying (<90 days, ≥90 days) than with calendar period; the mean TCDD was increased in Ranch Hands with at least 90 days of spraying (mean difference = 0.7, 95% CI = 0.5 to 0.9, $P < 0.001$) and similar increases were found in enlisted ground ($P < 0.001$), enlisted flyers ($P = 0.02$), and officers ($P < 0.001$).

TABLE 3
Ranch Hand TCDD by Calendar Period of Service and Days of Spraying—Diabetes

Occupation	*N*	Service ≤1969*	Days of Spraying†	Mean‡	Difference§	95% CI	*P*¶
Enlisted ground	341	Yes		3.2	0.5	(0.2, 0.7)	<0.001
	119	No		2.8			
	407		High	3.2	1.0	(0.7, 1.3)	<0.001
	53		Low	2.2			
Enlisted flyer	128	Yes		3.0	0.7	(0.4, 0.9)	<0.001
	47	No		2.3			
	157		High	2.8	0.6	(0.1, 1.0)	0.02
	18		Low	2.3			
Officer	290	Yes		2.0	0.1	(–0.1, 0.2)	0.31
	95	No		1.9			
	345		High	2.0	0.4	(0.2, 0.6)	<0.001
	40		Low	1.6			
All	759	Yes		2.8	0.5	(0.4, 0.7)	<0.001
	261	No		2.2			
	909		High	2.7	0.7	(0.5, 0.9)	<0.001
	111		Low	2.0			

*During or before 1969.
†High: ≥90 days, Low: <90 days.
‡Log units.
§Yes minus No and High minus Low, computed from raw data and then rounded.
¶Adjusted for age, BMI at tour, last calendar year in Vietnam.

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When contrasting veteran's groups without including TCDD in the analysis, without stratification (not shown in any table), the prevalence of diabetes among Ranch Hands (180 cases of 1020 at risk or 17.7%) and Comparisons (259 of 1449 or 17.9%) was similar and the adjusted relative risk (RR) of diabetes was not significantly increased (RR = 1.21, $P = 0.16$). Stratification by calendar period of service revealed a significant increase in the risk of diabetes among Ranch Hands who served during or before 1969 (Ranch Hand: 130 of 657 [19.8%], Comparison: 105 of 648 [16.2%]; RR = 1.65, $P = 0.005$) but not among those whose last year of service occurred after 1969 (Ranch Hand: 50 of 363 [13.8%], Comparison: 154 of 801 [19.2%]; RR = 0.85, $P = 0.45$). Stratification by days of spraying (<90 days, ≥90 days) revealed a significant increase in the risk of diabetes among Ranch Hands with at least 90 days of spraying (Ranch Hand: 170 of 909 [18.7%], Comparison: 259 of 1449 [17.9%]; RR = 1.32, $P = 0.04$) but not among those with less than 90 days of spraying (Ranch Hand: 10 of 111 [9%], Comparison: 259 of 1449 [17.9%], RR = 0.57, $P = 0.12$).

Analysis by TCDD exposure category without stratification (Table 4) revealed a significantly increased risk of diabetes with log(TCDD) in the combined cohort (test for trend: $P < 0.001$) and significant increases in risk in the Low (RR = 1.45, $P = 0.03$) and High (RR = 1.68, $P = 0.002$) categories. Stratification by calendar period of service (Table 4) showed a significant increase in the risk of diabetes with log(TCDD) in the combined cohort ($P < 0.001$) and increased Ranch Hand risk in the Background (RR = 1.26), Low (RR = 1.87), and High (RR = 1.97) categories among those whose last year of service was during or before 1969 with the increases reaching statistical significance in the Low ($P = 0.005$) and High ($P = 0.003$) categories; no significant increases were found in any of the three TCDD exposure categories in the complement calendar period stratum. Stratification by days of spraying (Table 4), revealed a significant increase in the risk of diabetes with log(TCDD) in the combined cohort ($P < 0.001$) and significant increases in Ranch Hand risk on the Low (RR = 1.45, $P = 0.03$) and High (RR = 1.58, $P = 0.01$) categories among those with at least 90 days of spraying; no significant increases were found in any of the three TCDD categories in the complement stratum. Stratifying by both calendar period of service and days of spraying (Table 4) revealed a significant increase in the risk of diabetes with log(TCDD) in the combined cohort ($P < 0.001$) and significantly increased Ranch Hand risk in the Low (RR = 1.99, $P = 0.002$), and High (RR = 2.12, $P < 0.001$) categories among those whose last year of service was during or before 1969 and who experienced at least 90 days of spraying; no increases in Ranch Hand risk were found in any TCDD exposure category in the complement stratum.

Cancer

After excluding those with cancer before the baseline examination ($n = 93$), noncompliance ($n = 289$), and those without a TCDD measurement ($n = 84$), 2583 remained for inclusion in statistical analyses of cancer (Table 1). Table 5 summarizes sample sizes by stratum of calendar period of service (≤1968, >1968) and years served in SEA (≤2 years, >2 years). Three hundred ninety-four Comparisons and 394 Ranch Hands had their last year of service during or before 1968. Six hundred forty Comparisons and 596 Ranch Hands experienced at most 2 years of service in SEA. Two hundred sixty-eight Comparisons had their last year of service during or before 1968 and served at most 2 years in SEA and 262 Ranch Hands had their last year of service during or before 1968 and served at most 2 years in SEA and experienced at least 30 days of spraying during or before 1967.

Demographic information and TCDD are summarized by TCDD category in the stratum defined by last year of service during or before 1968 and Ranch Hands with at least 30 days of spraying during or before 1967 and its complement (Table 6). There were no appreciable differences between these strata with regard to any of the variables listed.

Ranch Hand mean TCDD (in log units) was significantly increased among those whose last year of service was during or before 1968 (Table 7) relative to the complement stratum (last year ≤1968: mean = 2.7, last year >1968: mean = 2.5, 95% CI = 0.07 to 0.3, $P = 0.001$). The mean log(TCDD) was significantly increased in enlisted ground ($P = 0.005$) and enlisted flyers ($P < 0.001$) but not officers ($P = 0.79$) among those whose last year of service was during or before 1968. The mean TCDD (in log units) was significantly increased in the second ($P < 0.001$) and third ($P < 0.001$) tertiles of days of spraying in enlisted ground, enlisted flyers, and officer Ranch Hands.

Without stratification, there was no significant increase in the risk of cancer with log(TCDD) in the combined cohort ($P = 0.24$) and no significant increase in the risk of all-site SEER cancer in any of the three Ranch Hand TCDD exposure categories (Table 8). The RR was significantly increased in the Low category (RR = 1.7, 95% CI = 1 to 2.9, $P = 0.03$) and the risk of cancer increased significantly with log(TCDD) in the combined cohort ($P = 0.01$) after restriction to those whose last year of service was during or before 1968 (Table 8); there was no significant increase in the risk of cancer with log(TCDD) and no significant increased risk in any of the Ranch Hand TCDD exposure categories in the complement stratum. With restriction to veterans who served at most 2 years in SEA (Table 8), the risk of cancer increased with

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TABLE 4
Diabetes by TCDD Exposure Category and Stratum

Stratum	Trend*	Comp†	Ranch Hand		
			Bkg‡	Low	High
Unstratified					
N	2469	1449	447	286	287
Diabetic (%)	439 (17.8)	259 (17.9)	49 (11.0)	62 (21.7)	69 (24.0)
RR§	1.29		0.86	1.45	1.68
P	<0.001		0.40	0.03	0.002
Stratified by calendar period of service					
≤1969¶					
N	1305	648	274	176	207
Diabetic (%)	235 (18)	105 (16.2)	39 (14.2)	40 (22.7)	51 (24.6)
RR§	1.37		1.26	1.87	1.97
95% CI	1.2–1.56		0.8–1.98	1.21–2.89	1.26–3.06
P	<0.001		0.33	0.005	0.003
>1969‖					
N	1164	801	173	110	80
Diabetic (%)	204 (17.5)	154 (19.2)	10 (5.8)	22 (20)	18 (22.5)
RR§	1.18		0.47	1.08	1.22
95% CI	0.99–1.42		0.23–0.93	0.63–1.85	0.67–2.24
P	0.07		0.03	0.79	0.52
Stratified by days of spraying					
≥90 d					
N	2358	1449	364	269	276
Diabetic (%)	429 (18.2)	259 (17.9)	42 (11.5)	60 (22.3)	68 (24.6)
RR§	1.28		0.97	1.45	1.58
95% CI	1.15–1.42		0.66–1.43	1.04–2.02	1.12–2.24
P	<0.001		0.9	0.03	0.01
<90 d					
N	1560	1449	83	17	11
Diabetic (%)	269 (17.2)	259 (17.9)	7 (8.4)	2 (11.8)	1 (9.1)
RR§	1.22		0.59	0.73	0.35
95% CI	0.97–1.52		0.26–1.32	0.17–3.05	0.05–2.65
P	0.09		0.20	0.66	0.31
Stratified by calendar period of service and days of spraying					
≤1969 and ≥90 d					
N	1267	648	246	172	201
Diabetic (%)	229 (18.1)	105 (16.2)	34 (13.8)	40 (23.3)	50 (24.9)
RR§	1.39		1.37	1.99	2.12
95% CI	1.21–1.58		0.85–2.22	1.28–3.09	1.36–3.3
P	<0.001		0.2	0.002	<0.001
Complement#					
N	1202	801	201	114	86
Diabetic (%)	210 (17.5)	154 (19.2)	15 (7.5)	22 (19.3)	19 (22.1)
RR§	1.13		0.48	0.98	0.98
95% CI	0.95–1.36		0.26–0.87	0.58–1.67	0.54–1.79
P	0.18		0.02	0.94	0.96

*Based on a proportional hazards model of time-to-diagnosis in the combined cohort with log(TCDD) entered into model as a continuously distributed variable.

†Comparisons.

‡Ranch Hand Background.

§Based on a proportional hazards model, adjusted for BMI at blood draw, smoking history (pack-year), family history of diabetes, BMI at tour, year of birth, last calendar year of service in Southeast Asia (Comparison) or Vietnam (Ranch Hand), Vietnam days/SEA days, occupation.

¶During or before 1969.

‖Not during or before 1969.

#Not during or before 1969 and at least 90 days of spraying.

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TABLE 5
Analysis Sample Sizes*—Cancer

Calendar Period of Service	Years in Southeast Asia	Comparison	Ranch Hand			
			Bkg†	Low	High	Total
≤1968‡		394	167	88	139	394
>1968§		994	232	145	103	480
	≤2	640	282	142	172	596
	>2	748	117	91	70	278
≤1968	≤2	268	120¶	49¶	93¶	262¶

*White. Prebaseline cancer, Ranch Hands with missing TCDD excluded ($N = 2262$).
†Bkg indicates Ranch Hand background.
‡During or before 1968.
§Not during or before 1968.
¶At least 30 days of spraying during or before 1967.

log(TCDD) in the combined cohort ($P = 0.05$). With restriction to the stratum of interest (Table 8), the risk of cancer increased significantly with log(TCDD) in the combined cohort ($P = 0.005$) and the RR was significantly increased in the High category (RR = 2.2, 95% CI = 1.1 to 4.4, $P = 0.03$).

Conclusion

The stratum of interest in the analyses of diabetes was comprised of those with early tours and increased spraying, and for cancer it was comprised of those with early tours, increased spraying, and at most 2 years of service in SEA. With restriction to the stratum of interest for diabetes (Table 4), the association between the TCDD biomarker and diabetes was strengthened relative to the results without restriction (Table 4) and relative to a previous publication.[3] After stratification by calendar period of service and days of spraying, the risk of diabetes was increased among Ranch Hand veterans who served during or before 1969 and who experienced at least 90 days of spraying. With regard to cancer, although the sample sizes were approximately halved due to the additional stratification on time served in SEA (Table 5), cancer risk increased significantly with log(TCDD) in the combined cohort and the RR of all-site SEER cancer was significantly increased in the High exposure category (Table 8), whereas no association was found without stratification (Table 8) and none was found in a previous analysis on the entire cohort without restriction.[4]

The calendar period adjustment was made to address the hypothesis that Agent Orange and other phenoxy herbicides were more contaminated early in the war than later in the war. In a survey of 129 samples representing 17 different types of pesticides manufactured from chlorophenols, Woolson et al[13] found TCDD primarily in 2,4,5, T samples.

TABLE 6
Demographics—Cancer

	≥30 pre-1967 Spray Days, Service ≤1968*				Complement†			
	Ranch Hand				Ranch Hand			
	Comparison ($N = 452$)	Bkg‡ ($N = 182$)	Low ($N = 101$)	High ($N = 161$)	Comparison ($N = 1145$)	Bkg‡ ($N = 256$)	Low ($N = 169$)	High ($N = 117$)
Birth year§	1939	1935	1935	1942	1939	1941	1938	1947
Smoking¶	12.4	19.2	20.4	11.3	10.3	7.2	8	8.6
TCDD‖	3.9	5.2	14.8	47.9	3.8	5.8	15.5	47.2
Last year#	1967	1967	1967	1967	1969	1969	1970	1969
BMI**	24.9	24.1	25.2	25.2	25.0	24.1	25.2	25.3
Days SEA††	427	0	0	0	700	0	0	0
Days VN‡‡	75	393.5	395	395	200	393	395	394
Black (%)	5.8	4.9	8.9	6.8	5.8	4.9	8.9	6.8
Officer (%)	30.3	58.8	44.6	3.7	30.3	58.8	44.6	3.7
Enlisted flyer (%)	14.4	10.4	18.8	21.7	14.4	10.4	18.8	21.7
Enlisted ground (%)	55.3	30.8	36.6	74.5	55.3	30.8	36.6	74.5

*Excluding prebaseline cancer, noncompliant, missing TCDD (Net [last row], Table 5).
†The Boolean complement (<30 pre-1967 d of spraying or last year of service after 1968).
‡Bkg indicates Ranch Hand background.
§Median.
¶Median pack-year measured from 1982.
‖Median in parts per trillion.
#Median last calendar year of service in Southeast Asia (Comparison) or Vietnam (Ranch Hand).
**Mean body mass index during qualifying tour of duty in Southeast Asia.
††Median days spent in Southeast Asia.
‡‡Median days spent in Vietnam.

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TABLE 7
Ranch Hand TCDD by Calendar Period of Service and Days of Spraying*

Occupation	N	Service ≤1968†	Days of Spraying‡	Mean§	Difference¶	95% CI	P‖
Enlisted ground	218	Yes		3.3	0.3	(0.09, 0.48)	0.005
	232	No		3.0			
	131		0–289	2.5			
	135		290–381	3.3	0.7	(0.49, 1)	<0.001
	184		382–1050	3.4	0.8	(0.57, 1.07)	<0.001
Enlisted Flyer	75	Yes		3.0	0.5	(0.24, 0.71)	<0.001
	96	No		2.6			
	72		0–289	2.4			
	60		290–381	2.9	0.5	(0.22, 0.74)	<0.001
	39		383–1050	3.2	0.8	(0.52, 1.13)	<0.001
Officer	169	Yes		1.9	0.0	(-0.15, 0.12)	0.79
	196	No		2.0			
	130		0–289	2.2			
	136		290–381	2.7	0.5	(0.4, 0.68)	<0.001
	99		383–1050	2.9	0.7	(0.55, 0.84)	<0.001
All	462	Yes		2.7	0.2	(0.07, 0.3)	0.001
	524	No		2.5			

*Net (last row of Table 1) after sample reduction, $N = 986$.
†Last calendar year of service during or before 1968.
‡Days, tertiles.
§Log units.
¶Yes minus No or 3rd tertile minus first, 2nd tertile minus first, computed from raw data and then rounded.
‖Adjusted for age, BMI at tour, last calendar year in Vietnam.

Of the 42 samples containing 2,4,5-T, one of the components of Agent Orange, 22 samples contained less than 0.5 parts per million (ppm) of TCDD. Of the 20 samples containing more than 0.5 ppm, 15 were obtained from the yearly survey of one manufacturer. The samples were taken from 1966 to 1970, with four samples usually collected each year. Woolson et al reported a 10-fold drop in TCDD content by this manufacturer between 1968 and 1969,[13] nevertheless they did not report the actual values or the name of the manufacturer. The calendar period strata and results (Tables 3 and 7) are consistent with the drop in TCDD contamination between 1968 and 1969 reported by Woolson et al.

The adjustment for days of spraying was made to address the hypothesis, supported by these data (Tables 3 and 7) that TCDD exposure increased with days of spraying in Ranch Hand veterans. The adjustment was made excluding those with a small number of days of spraying, to increase the Mahalanobis distance with respect to TCDD from the Comparison group.[14] Adjustment for calendar period of service also increased the distance because veterans with early tours had higher TCDD levels. The increased TCDD levels in Ranch Hands with early tours was both significant and opposite to the expected pattern based on whole-body elimination. Based purely on whole-body elimination and a first-order pharmacokinetic model, one would expect lower TCDD levels in Ranch Hands with early tours relative to those with later tours, opposite to the pattern in Tables 3 and 7.

The consequences of stratification based on a P value are well described. A P value driven approach will increase the number of false positive results.[15] We attempted to avoid this pitfall by using external information rather than P values to hypothesize strata of interest. Cut points for strata definition were generally based on external information and the magnitude and variance of the RR. With regard to days of spraying, some men worked in the Ranch Hand unit on a temporary duty assignment and these were typically, but not always, 90 days. Available herbs tape data gave days of spraying on a monthly basis, leading us to consider 30, 60, and 90 days as cut points for days of spraying. We had no preconceived preference for any of these three cuts and used the cut for each disease that appeared to give the strongest relation (in terms of the magnitude of the RR) with TCDD. For diabetes, the analysis was not particularly sensitive to the choice of these three cut points except that the use of any of the cuts gave stronger results than not considering days of spraying at all. The results of the cancer analysis were sensitive to the cut point for days of spraying and to the stipulation that the spraying had to occur during or before 1967. We speculated that this sensitivity meant that the herbicides were more contaminated before 1967 and that higher levels of TCDD were apparently required to express cancer but not required to express diabetes.

The cut point for calendar period of service was selected to approximate the break point described by Woolson et al to have occurred during 1968, but was allowed to vary with disease. For diabetes we used >1969 and ≤ 1969, and for cancer we used >1968 and ≤ 1968. We made the choice for the cut separately by disease because these choices, 1969 for diabetes and 1968 for cancer, appeared to reflect an inflection point in the magnitude of the RR in the High TCDD exposure category. Both cuts were influenced by the magnitude of the RR estimate and its variance. As the cut was moved backward in time the variance increased because the sample size decreased. For both diseases, we evaluated the RR in the High TCDD exposure category using 1966, 1967, 1968, and 1969 as the cut. The RR of cancer in the High category increased using 1966 and 1967 as the cut, but the variance increased because of a decrease in the number of

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TABLE 8
SEER Cancer by TCDD Exposure Category and Stratum

Stratum	Trend*	Comp†	Ranch Hand		
			Bkg‡	Low	High
Unstratified§					
N	2262	1388	399	233	242
Number (%)	347 (15.3)	212 (15.3)	64 (16)	46 (19.7)	25 (10.3)
RR¶	1.1		1	1.3	0.9
95% CI	1–1.2		0.8–1.4	1–1.8	0.6–1.4
P	0.24		0.89	0.1	0.65
Stratified by calendar period of service‖					
≤1968#					
N	788	394	167	88	139
Number (%)	118 (15)	55 (14)	20 (12)	22 (25)	21 (15.1)
RR¶	1.2		0.7	1.7	1.5
95% CI	1.0–1.5		0.4–1.3	1–2.9	0.9–2.6
P	0.01		0.26	0.03	0.14
Complement**					
N	1474	994	232	145	103
Number (%)	229 (15.5)	157 (15.8)	44 (19)	24 (16.6)	4 (3.9)
RR¶	1		1.3	1.1	0.4
95% CI	0.8–1.1		0.9–1.9	0.7–1.7	0.1–1
P	0.65		0.11	0.7	0.05
Stratified by yrs served in Southeast Asia††					
≤2 yr					
N	1236	640	282	142	172
Number (%)	135 (10.9)	56 (8.8)	36 (12.8)	24 (16.9)	19 (11)
RR¶	1.2		1.1	1.6	1.6
95% CI	1–1.4		0.7–1.7	1–2.5	0.9–2.8
P	0.05		0.71	0.08	0.11
Stratified by calendar period of service, days of spraying, yrs in Southeast Asia‡‡					
≤1968 and ≥30 d pre-1967 and ≤2 yr					
N	530	268	120	49	93
Number (%)	67 (12.6)	30 (11.2)	10 (8.3)	12 (24.5)	15 (16.1)
RR¶	1.4		0.5	1.7	2.2
95% CI	1.1–1.7		0.2–1.1	0.8–3.5	1.1–4.4
P	0.005		0.09	0.14	0.03

*Based on a proportional hazards model of time-to-diagnosis in the combined cohort with log(TCDD) entered into model as a continuously distributed variable, adjusted for year of birth, military occupation, smoking history (pack-year), skin reaction to sun (burns painfully or freckles with no tan, burns or tans mildly, tans deep brown), eye color (brown, hazel/green, and gray/blue), year served in Southeast Asia.

†Comparisons.

‡Bkg indicates Ranch Hand background.

§Analysis sample sizes (Table 5 first two rows combined).

¶Based on a proportional hazards model of time-to-diagnosis, adjusted for year of birth, military occupation, smoking history (pack-year) skin reaction to sun (burns painfully or freckles with no tan, burns or tans mildly, tans deep brown) , eye color (brown, hazel/green, and gray/blue), years served in Southeast Asia.

‖Analysis sample sizes (Table 5, first two rows).

#During or before 1968.

**Not during or before 1968.

††Analysis sample sizes (Table 5, 3rd and 4th rows).

‡‡Analysis sample sizes (Table 5, last row).

men on the job between 1962 and 1967. Using 1967 as the cut, the RR of cancer in the High TCDD exposure category ($N = 84$) was 2.2 (95% CI = 1 to 4.9, $P = 0.05$) based on 15 cases. Using 1966 as the cut, the RR in the High TCDD exposure ($N = 28$) category was 4.3 (95% CI = 1.1 to 16.6, $P = 0.04$) based on 7 cases. Corresponding results for diabetes are not available. The cut point for time spent in SEA (2 years) was the approximate median number of years of service in SEA among Comparisons; the actual median was 2.1 year. The approximation was used to simplify the presentation. The same results would have been obtained with the actual median, which differed from the approximation by about 1 month.

Another reason for our consideration of calendar period and days of spraying was the serum TCDD mea-

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surement itself. The measurement was specific to the individual and correlated with days of spraying and calendar period of service suggesting that the TCDD measurement was plausibly reflecting actual exposure in Vietnam during the war, but the serum measurement was made in most participants in 1987, approximately 20 years after the peak spraying in Vietnam. Furthermore, although we have used a one-compartment pharmacokinetic model to estimate the initial dose,[16] TCDD elimination actually follows an age-dependent multicompartment model.[17] In the one-compartment model whole-body elimination depends on BMI, with heavier people eliminating more slowly than lean people. The accuracy of the one-compartment model we used was unknown because there was no dosimetry in Vietnam and so the actual initial dose cannot be known. We hypothesized that by stratifying by factors directly related to the spray operations during the war (days of spraying) and the levels of TCDD contamination in the herbicides (reflected by calendar period of service) that some of the exposure classification error caused by variation in TCDD elimination would be decreased.

The results presented here suggest that earlier AFHS results may have been biased toward the null due to misclassification on exposure, and with regard to cancer, in addition, biased toward the null due to lack of adjustment for years served in SEA, a confounder. In retrospect, the cohorts should have been matched on the number of years spent in SEA and calendar period of service, and all analyses should have been adjusted for the number of days of spraying. Nevertheless, no one could have known in 1978 that in 2005 cancer risk would increase in the Comparison cohort with years spent in SEA.[18] Further confounding, not addressed here, derives from the observation that all-site SEER cancer risk increased with TCDD in the Comparison cohort, independently of the increase with time spent in SEA.[18]

The methods shown here can be improved. Days of spraying was derived from an early version of the herbs data summarized in the study protocol.[8] Recently, researchers have retrieved the daily records kept by the Ranch Hand unit during the war. These records give details regarding the actual herbicide formulations sprayed each day together with the number of gallons loaded and the number of gallons remaining in the tanks of the Ranch Hand aircraft after each mission and are the records we sought but could not find in 1978. With these daily records and related documents, researchers have corrected the herbs tape data.[19–21] Access to the corrected herbs tape data and the original daily Ranch Hand records would provide an improvement over the days of spraying reported here.

In addition, congener-specific analyses of sera collected from participants of 2002 AFHS physical examination have been ongoing at the Centers for Disease Control and Prevention and were summarized in presentations at Dioxin Symposia and in published papers.[22,23] A limitation of this study is that although one of us (M.P.) had access to these congener-specific data, we no longer had access to the diabetes and cancer datasets and were not allowed to use congener-specific data in analyses of health outcomes due to the closing of the AFHS and the transfer of the data and specimens to the Medical Follow-up Agency (MFUA) (Public Law 109-364). The MFUA is the new custodian of the AFHS data and we hope that the data will be made publicly available in the future. Total dioxin toxic equivalents could then be used in statistical analyses of health outcome data instead of just using TCDD.[24] Repeated measurements of TCDD, for a larger number of veterans than that previously published,[16] would also allow new estimates of TCDD half-life and the calculation of TCDD body burden.

In the current analysis, we failed to find a covariate adjusted model for either diabetes or cancer, to convincingly explain the results shown here, despite the fact that, with regard to cancer, the majority of the cancer cases in the high exposure category were retained after stratification. Stratification could have been avoided by computing RR at each point on a transect through the multivariate space defined by these variables. For example, RR for all-site SEER cancer could have been computed yearly along the time line from 1971 to 1962.

In conclusion, adjustment for days of spraying, calendar period of service revealed an increased Mahalanobis distance and sharpened associations between the TCDD biomarker and diabetes in Ranch Hand veterans. Stratification by time spent in SEA revealed a significant trend of increased all-site SEER cancer risk and a significantly increased risk in the highest Ranch Hand TCDD exposure category. These data suggest that calendar period of service, days of spraying, and time spent in SEA are important confounders in the AFHS.

References

1. Institute of Medicine. *Veterans and Agent Orange*. Washington DC: National Academy Press; 1994.
2. Institute of Medicine. *Veterans and Agent Orange. Update 2004*. Washington DC: National Academy Press; 2005.
3. Henriksen GL, Ketchum NS, Michalek JE, Swaby JA. Serum dioxin and diabetes mellitus in veterans of Operation Ranch Hand. *Epidemiology*. 1997;8:252–258.
4. Ketchum NS, Michalek JE, Burton JE. Serum dioxin and cancer in veterans of Operation Ranch Hand. *Am J Epidemiol*. 1999;149:630–639.
5. Wolfe WH, Michalek JE, Miner JC, et al. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. I. Physical health. *JAMA*. 1990;264: 1824–1831.
6. Lathrop GD, Wolfe WH, Albanese RA, Moynahan PM. *The Air Force Health Study: An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides, Baseline Morbidity Study Results*. Springfield: National Technical Information Service; 1984.

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7. Michalek JE, Robinson J, Fox K, Elequin V, et al. *The Air Force Health Study: An Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides. 2002 Follow-Up Examination Results*. Springfield: National Technical Information Service; 2005.
8. Lathrop GD, Wolfe WH, Albanese RA, Moynahan PM. *Epidemiologic Investigation of Health Effects in Air Force Personnel Following Exposure to Herbicides: Study Protocol*. San Antonio: USAF School of Aerospace Medicine; 1982.
9. Hornung RW, Reed DR. Estimation of average concentration in the presence of nondetectable values. *Appl Occup Environ Hyg*. 1990;5:46–51.
10. Eliasson B. Cigarette smoking and diabetes. *Prog Cardiovasc Dis*. 2003;45:405–413.
11. Public Health Service. *International Classification of Diseases, 9th Revision, PHS 80–1260.* Washington DC: US Department of Health and Human Services; 1980.
12. *SEER Cancer Statistics Review, 1975–2000.* Bethesda: National Cancer Institute; 2003.
13. Woolson EA, Thomas RF, Ensor PD. Survey of polychlorodibenzo-p-dioxin content in selected pesticides. *J Agric Food Chem*. 1972;20:351–354.
14. Efron B. The efficiency of logistic regression compared to normal discriminant analysis. *J Am Stat Assoc*. 1975;70:892–898.
15. Lagakos SW. The challenge of subgroup analyses—reporting without distorting. *N Engl J Med*. 2006;354:1667–1669.
16. Michalek JE, Tripathi RC. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 15-year follow-up. *J Toxicol Environ Health A*. 1999;57:369–378.
17. Aylward LL, Brunet RC, Starr TB, et al. Exposure reconstruction for the TCDD-exposed NIOSH cohort using a concentration- and age-dependent model of elimination. *Risk Anal*. 2005;25:945–956.
18. Pavuk M, Michalek JE, Schecter A, Ketchum NS, Akhtar FZ, Fox KA. Did TCDD exposure or service in Southeast Asia increase the risk of cancer in air force Vietnam veterans who did not spray agent orange? *J Occup Environ Med*. 2005;47:335–342.
19. Stellman JM, Stellman SD, Weber T, Tomasallo C, Stellman AB, Christian R Jr. A geographic information system for characterizing exposure to Agent Orange and other herbicides in Vietnam. *Environ Health Perspect*. 2003;111:321–328.
20. Stellman JM, Stellman SD, Christian R, Weber T, Tomasallo C. The extent and patterns of usage of Agent Orange and other herbicides in Vietnam. *Nature*. 2003;422:681–687.
21. Stellman SD, Stellman JM. Exposure opportunity models for Agent Orange, dioxin, and other military herbicides used in Vietnam, 1961-1971. *J Expo Anal Environ Epidemiol*. 2004;14:354–362.
22. Pavuk M, Patterson DG Jr, Turner WE, Needham LL, Ketchum NS. Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) in the serum of US Air Force veterans in 2002. *Chemosphere*. 2007;68:62–68.
23. Pavuk M, Patterson DG Jr, Turner WE, Ketchum N, Needham LL. PCDDs, PCDFs, PCBs, and organochlorine pesticides in 800 Air Force veterans in 2002. *Organohalogen Compd*. 2006;68:756–759.
24. Van den BM, Birnbaum LS, Denison M, et al. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci*. 2006;93:223–241.

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