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The Air Force Health Study: An Epidemiologic Retrospective

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In 1979, the U.S. Air Force announced that an epidemiologic study would be undertaken to determine whether the Air Force personnel involved in Operation Ranch Hand–the program responsible for herbicide spraying in Vietnam–had experienced adverse health effects as a result of that service. In January 1982 the Air Force Health Study (AFHS) protocol was approved and the 20 year matched cohort study consisting of independent mortality, morbidity and reproductive health components was initiated. This controversial study has been criticized regarding the study's potential scientific limitations as well as some of the administrative aspects of its conduct. Now, almost 30 years since the implementation of the AFHS and nearly a decade since the final follow up examinations, an appraisal of the study indicates that the results of the AFHS do not provide evidence of disease in the Ranch Hand veterans caused by their elevated levels of exposure to Agent Orange.
Ann Epidemiol 2011;21:673–687.

KEY WORDS: Air Force Health Study, Ranch Hand, Agent Orange, Dioxin, Dosimetry, Epidemiology, Exposure, Cancer, Diabetes, Multiple Comparisons.

INTRODUCTION

January 2012 will mark the 50th anniversary of the start of Operation Ranch Hand, the U.S.-Vietnam allied program for the aerial application of herbicides during the Vietnam War to clear jungle vegetation and thus disrupt enemy combat operations (1–3). Herbicides were also used for crop destruction to deprive the enemy of potential food sources (2, 3). Several herbicides were used during the Vietnam War, but Agent Orange (AO), a 50:50 mixture by weight of the n-butyl esters of two phenoxy acids: 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), was used most extensively. The 2,4,5-T component was contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, also referred to as TCDD or as dioxin (4).

Almost from the outset, the use of herbicides in Vietnam was controversial (2–5). Anti-herbicide spraying articles and stories appeared in both print and broadcast media as early as 1963 when a series of newspaper articles were published charging the United States and South Vietnam with using "dirty war" tactics against the Viet Cong, including spraying "poison" from Ranch Hand planes to destroy rice fields and roadside ambush cover (6).

Scientific organizations such as the Federation of American Scientists expressed concerns about the use of herbicides in Vietnam whereas the Council of the American Association for the Advancement of Science (AAAS) urged Secretary of Defense Robert S. McNamara to investigate the population and environmental implications of using the herbicides in this manner. In 1967, greater than 5000 scientists signed a petition to President Lyndon B. Johnson urging the cessation of herbicide use in Vietnam (2, 4, 5, 7). In 1965, Bionetics Research Laboratory (Maryland) received a contract from the National Cancer Institute to study the possible teratogenic effects in mice and rats of exposure to several pesticides and herbicides, including 2,4-D and 2,4,5-T (4, 5). The 1968 Bionetics report, which was made public in 1969, stated that administration of 2,4,5-T in high doses in mice was teratogenic, causing malformations and stillbirths. They also stated that 2,4-D was potentially "harmful" (4). Bionetics then reanalyzed these 2,4,5-T data and announced that 2,4,5-T was not teratogenic and that the effects observed were caused by the contaminant TCDD (7). Based on these findings, the military suspended the use of AO in April 1970 (2, 4, 5). Later that year, the announcement was made that herbicide operations in Vietnam would be phased out; on January 16, 1971, the Department of Defense ordered the immediate termination of all crop destruction missions (2).

By the mid-1970s, Vietnam veterans were questioning the possible link between exposure to herbicides, mainly AO, in Vietnam and health conditions and/or diseases they were experiencing (5). By the end of the decade, Vietnam veterans took their concerns to Congress (4, 8).

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Received November 16, 2010. Accepted February 1, 2011. Published online March 26, 2011.

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1047-2797/$ - see front matter
doi:10.1016/j.annepidem.2011.02.001

Selected Abbreviations and Acronyms

AO = Agent Orange
2,4-D = 2,4-dichlorophenoxyacetic acid
2,4,5-T = 2,4,5-trichlorophenoxyacetic acid
TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Congressional response included numerous public hearings and legislative initiatives. In 1983, PL 98-181 appropriated money to the Centers for Disease Control (CDC) to conduct research on the health risks of Vietnam veterans to Agent Orange. The Veterans' Dioxin and Radiation Exposure Compensation Standards Act of 1984 (PL 98-542) was passed to address the issue of compensation for disabilities that might have resulted from exposure to Agent Orange in Vietnam. The Agent Orange Act of 1991 (PL 102-4) directed the Secretary of Veterans Affairs to request the National Academy of Sciences to conduct an "independent review and evaluation" of the available scientific and medical literature regarding the health effects of exposure to herbicides used during the Vietnam War (9).

Both the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) have classified TCDD as a human carcinogen (10, 11). In its 2003 Dioxin Reassessment (12–14), EPA determined that TCDD was "best characterized" as carcinogenic to humans. However, a committee of the National Academy of Sciences charged with reviewing EPA's Dioxin Reassessment called the EPA designation "somewhat subjective" because the classification depends "largely on the definition and interpretation of the criteria" used (15).

THE AIR FORCE HEALTH STUDY

In response to Congressional concerns, the U.S. Air Force announced in 1979 that it would undertake an epidemiologic study to determine whether "those servicemen involved in the spraying of herbicides in Vietnam during Operation Ranch Hand experienced adverse health effects as a result of their participation in that program" (16). Ranch Hand personnel were selected for study because they were considered to have had the greatest potential for herbicide exposure (5, 16, 17).

The crews assigned to Operation Ranch Hand consisted of both officers and enlisted personnel. Because of the hazards from enemy gunfire due to the low, slow-flying Ranch Hand missions, some of the early members of the group were volunteers (16).

The flight crew consisted of three officers—a pilot, a copilot, and a navigator—and a spray equipment console operator (enlisted personnel) who was positioned in the rear of the C-123 aircraft. The navigator flew in the lead aircraft (1–3, 5, 16, 18). On the ground, the nonflying (maintenance) personnel were responsible for loading the herbicide into the planes, cleaning the spray equipment post mission and maintaining and repairing the aircraft (2, 3, 5). A typical herbicide mission took approximately 1 hour to reach the assigned spray area and the spraying lasted 5–10 minutes. The aircraft returned to base and often turned around and completed a second spray run (19). The average tour of duty in Ranch Hand was 1 year and the men routinely worked 12–15-hour shifts without a change of clothing (19).

The basic strategy for the AFHS study was approved by the United States Air Force Surgeon General in early 1979 and protocol development was undertaken by the United States Air Force School of Aerospace Medicine, Brooks Air Force Base, San Antonio, Texas. The draft protocol underwent multiple internal and external protocol reviews. Table 1 provides a synopsis of the protocol review process along with some of the major recommendations. Each review was independent of the other reviews and approval of one version of the protocol did not mean that that panel approved the final protocol (16). Some of the concerns expressed during the initial reviews continued to be raised throughout the course of the study such as the lack of power to detect rare diseases or the validity and reliability of the dosimetric assessments—both the exposure index and the blood serum measurements. In January 1981, The Advisory Committee on Special Studies Relating to the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants (Ranch Hand Advisory Committee) was appointed to advise the Secretary and the Assistant Secretary for Health on oversight of the conduct of the Ranch Hand Study (20). The final AFHS protocol was completed in January 1982 (16). This protocol used a matched nonconcurrent (retrospective) cohort design in a prospective setting consisting of independent mortality, morbidity, and reproductive health components (16). The primary focus of this review is the morbidity follow-up study.

THE MORBIDITY STUDY

The AFHS protocol defines the population for the morbidity component as "all living Ranch Handers and their first randomly selected, alive and compliant comparison" (17). The initial study population included 1261 Ranch Hand veterans (Table 2) and 19,101 comparison veterans (17). The number of study participants cited in the AFHS reports, especially in the early reports, varies over the course of the study from report to report but also within the report itself. This is due to the reclassification of study participants. For example, some of the comparison veterans initially selected for the study were determined to be ineligible (17). Other reasons for reclassification included changes in exam status

TABLE 1. Evolution of the AFHS protocol

Protocol version	Date	Major suggested changes
1	June 1979	Population groups for the study should be fully ascertained
		Sources of potential bias must be carefully addressed
		Advantages of in-person vs. telephone interviewing
2	July 1979	Expanded discussion of epidemiologic design
		Expanded statistical analytic strategy
		Consideration of bias sources
		In-person interviews recommended
3	July 1979	Discussion of exposure index
		Development of survival analysis techniques
		Expanded discussion of physical examination procedures
4	August 1979	Expanded discussion of exposure concepts
		Expand the number of controls to three per Ranch Hand death for the mortality study
		Establish an independent monitoring panel to oversee the conduct of the study
		Further expansion of physical examination procedures
5	October 1979	Expand the number of controls to five per Ranch Hand death for the mortality study
		Single center examinations
		Discussion of methodology for replacement of controls
6	November 1979	Expanded exposure index discussion
		More detailed discussion of statistical analytic strategy
7	October 1980	Increased emphasis on fertility and reproductive endpoints
		Enlarged discussion of the mortality analysis
		Enlarged discussion of statistical power
		Discussion of quality control methods
8	November 1980	Presentation of refined data on study population demographic characteristics
9	June 1981	Discussion of matching procedures
		Consideration of time-in-study effects
10	September 1981	Expanded discussion of matching procedures and results
11	January 1982	Refinement of the exposure index
		Presentation of modified performance schedules

AFHS = Air Force Health Study.
Adapted from the Air Force Health Study (16).

("refused" to "partially compliant") or vital status (alive to deceased). Even as recently as the final follow-up exam in 2002, an additional Ranch Hand subject was identified (21). The comparison group included veterans whose training and background as well as military occupational group (pilot, co-pilot, etc.) were similar to their Ranch Hand counterparts.

The comparison veterans, who were characterized as non-risk taking, non-volunteers and non-herbicide exposed, (16) flew C-130 aircraft in Southeast Asia (SEA), an aircraft that was never used in the herbicide operations (16). Comparison veterans had multiple tours of duty in SEA, but spent less than 30% of their SEA service in Vietnam and were stationed mostly in Taiwan, the Philippines, Guam, Japan, and Thailand (16, 17, 21, 22). The use of comparison veterans with SEA experience was anticipated to take into account "combat induced physiologic, psychophysiologic and other related morbidity and mortality disorders" as well as the "effects of alcohol consumption, the use of chemoprophylactic and/or illicit drugs, and the acquisition of tropical diseases associated with life in SEA" (16).

A minimum of 10 comparison veterans were matched to each Ranch Hand veteran on three characteristics and remained matched to that veteran for the duration of the study. These characteristics were:

1. Age: year of birth and closest month possible was used to control for clinical symptoms and signs associated with "advancing age."
2. Air Force Specialty Code (also referred to as occupational category): used to control for officer/enlisted status and crew member/noncrew member status. This matching factor was considered a surrogate for education, socio-economic status and slightly linked to age.
3. Race: used as a surrogate for chronic disease development and socio-economic background.

Because the match between Ranch Hand veterans and their comparison veterans was "very nearly identical," a replacement strategy for noncompliant comparison veterans (e.g., members of the comparison group who left the study for some reason) was developed to ensure adequate numbers of comparison participants. Noncompliant comparison veterans were replaced by the first randomly selected willing comparison veteran from the AFHS database matched to the Ranch Hand veteran who had the same self-perception of health as

TABLE 2. Military status of Ranch Hand personnel identified for the Air Force Health Study as of September 1981

	Officer	Enlisted	Total
Active duty	122	112	234
Retired	214	306	520
Reserve/Air Guard	38	27	65
Separated	74	311	385
Known deceased	13	25	38
Killed in action	13	6	19
Total	474	787	1261

Adapted from the Ranch Hand II Health Study documentation (57).

reported during the initial telephone interview (16, 17, 22). Matching on "self-perception of health" was an attempt to minimize bias due to differential compliance (22).

The morbidity study protocol called for periodic follow-up over a 20-year period and consisted of a baseline examination and five subsequent physical examinations (17, 21–25). Two reports were produced using data from the 1987 follow-up physical examination. The second report (26) presented, for the first time, the comparison of analyses for the serum dioxin measurements obtained during the 1987 examination cycle with the health outcome data from the physical examination.

The morbidity follow-up examinations included sequential questionnaires, medical record reviews, and physical examinations (21). Participation was entirely voluntary and initial contact with study participants was initiated in November 1981. The investigators estimated that 65% of Ranch Hand veterans would respond to the questionnaire and that 60% of respondents would actually participate in the physical examination (17). In actuality, 87% of the 1,206 Ranch Hand veterans contacted were termed "fully compliant" having completed both the interview and the physical examination; only 3% refused to participate in both the interview and physical examination (17).

The initial questionnaire, administered in person, was designed to obtain baseline personal and medical data as well as information on the subject's health perceptions. In addition the questionnaire was designed to provide a cross-reference for data obtained during the physical examination (16). For those entering the study in 1987 or later, the baseline questionnaire was administered at the physical examination. For deceased Ranch Hand and comparison veterans, health histories were obtained through an interview with next-of-kin. Medical records for study participants were obtained (with the subject's consent) to validate the self-reported interview information. An interval questionnaire, for subsequent follow-up examinations, included new questions as well questions from previous questionnaires enabling the collection of longitudinal data (21).

Comprehensive physical examinations were conducted at a single civilian medical center using a standardized protocol. The 2.5-day evaluation included an extensive physical examination, collection of medical history data, and numerous laboratory tests. Serum, urine, and semen samples were obtained and stored for possible future analysis (16). The physical examination and laboratory data were cross-referenced with the interview data. Medical personnel were prohibited from asking or knowing the exposure status of the participants to ensure that blinding of medical personnel to exposure status was maintained.

The number and characteristics of the veterans participating in each examination cycle varied. When possible, noncompliant veterans were encouraged to re-enter the study. "Passive refusals" from either Ranch Hand or comparison veterans, were contacted and encouraged to participate in subsequent examinations, whereas no further contact was made with "hostile refusals." Throughout the study, a comparison veteran lost to follow-up was replaced with a consenting comparison veteran randomly selected from those eligible. If a participating Ranch Hand veteran was lost to follow-up, their matched comparison veteran was retained. If a comparison veteran re-entered the study, both the original and the replacement comparison veterans were followed for the remainder of the study (17, 21–25).

EXPOSURE ASSESSMENT

A major concern during the initial development of the AFHS protocol was the development of objective exposure data (16, 17). Based on anecdotal information, the major route of exposure for Ranch Hand personnel was thought to be direct skin contact (although inhalation was not ruled out) over a "long" period of time. Because the herbicides used in Vietnam were not known at that time to be toxic to either animals or to humans, today's recommended handling practices were not used in Vietnam (16).

Consideration of crew activities suggested that different jobs might have quite different exposure profiles. The pilot and co-pilot remained in the cockpit during herbicide missions. The pilot's duties were to insure that the aircraft was in the proper position in relation to other aircraft, had sufficient maneuvering room, spot targets, hold the proper spray altitude, and turn the spray on and off with a switch mounted on the control yoke. The co-pilot was responsible for the engines, maintaining prescribed airspeed to achieve the desired herbicide application rate, checking the terrain and/or formation spacing to anticipate necessary power changes, anticipating "pull-ups" at the end of each spray run, and applying the necessary power for a turn. The navigator and the flight engineer (mechanic) were free to move around the plane before spraying. After the navigator directed the aircraft to the target, he positioned himself between the pilot and co-pilot and was responsible for

insuring that the spray was dispensed on target and remained within the target area. He was also responsible for a cross-check of the spray and the general condition of the aircraft and assisting during an emergency (2, 3, 18, 27).

The flight engineer was located at the back of the plane near the 1000 gallon herbicide tank and was responsible for the operation of the spray system and for dumping the spray load when directed by the pilot. Because of his proximity to the herbicide tank, the flight engineer "could be covered" with AO from tubing or pipes leaking or breaking or when the tubes or pipes "were punctured by enemy fire" (5, 19). The flight engineer also monitored the aircraft and engines and reported malfunctions.

Maintenance of the Ranch Hand aircraft was carried out within a stepwise organizational structure (16). Primary or routine daily maintenance was conducted by flight line support personnel who were often dedicated exclusively to Ranch Hand operations. Secondary or more extensive maintenance was carried out by consolidated support units at the base level who were responsible for both Ranch Hand and non-Ranch Hand aircraft. Major aircraft overhauls and modifications were carried out at Clark AFB, Philippines. "Adequate" identification of maintenance workers in the secondary units from "available records" was "not feasible" and these men were not included in the AFHS (16). The study protocol suggests that ascertainment was incomplete even for primary maintenance personnel for the period August 1964 to December 1966 (16).

One maintenance assignment that was accomplished with "as little clothing as possible because of the extreme heat" involved greasing an emergency dump valve inside the spray tank. Access was through a hatch on the top of the tank and the grease was applied to a valve at the bottom of the tank "which contained at least 2 inches of herbicide" (19, 23). The herbicide also dissolved the rubber hoses of the aircraft's spray system that caused the spray nozzles to block. Maintenance personnel were frequently "sprayed" with herbicide during tank cleaning as a result of the pressure remaining in the system (19). Herbicide was also used to remove grease from the skin and to clean hands (19).

Initially, investigators classified Ranch Hand personnel into five job title categories—officer pilot, officer navigator, officer other, enlisted flying, and enlisted ground. For analytic purposes the five categories were reduced to three categories based on their likely potential for exposure to herbicides.

PHASE I: THE AFHS EXPOSURE ASSESSMENT

Initially an individual-specific exposure index or estimate was envisioned. The design depended on the availability of operations records containing individual flying time data as well as aircraft maintenance records containing names of ground crew personnel. The individual exposure assessment would be calculated by evaluating "known factors" that would have influenced exposure—date(s) of Ranch Hand tour in Vietnam; number and length of tours in Vietnam with Operation Ranch Hand; number of herbicide spray missions (as reflected by flying hours and air medals); the herbicides sprayed each month and year and crew position (16). The individual exposure index was calculated as the product of the quantity of TCDD-containing herbicide sprayed from aircraft assigned to an individual Ranch Hand's base during his assignment in Vietnam multiplied by the length of that individual's tour of duty. After determining that "objective" data were "lacking" and validated individual estimates were "infeasible," study investigators examined a "more generalized" exposure index that involved a "base-specific" exposure index (16).

The base-specific index was based on the assumption that all personnel at a given base shared equally in the work load. It was anticipated that records would provide a quantitative measure of the number of missions and the amount and type of herbicides sprayed from each base. However, this concept was abandoned when information could not be obtained to link a specific air base to the spray aircraft that were assigned to that base. In addition, military records did not "definitively specify the exact duty locations of all personnel" (16).

THE AFHS EXPOSURE INDEX

Study investigators then turned to the development of an exposure index that, although "less refined" than either the individual or base exposure indices, was considered "feasible" and could "adequately support the analytic strategy of the study design" (16, 17, 22). The AFHS exposure index was designed to distinguish between groups of individuals rather than as an individual measure of exposure (16, 17, 23). This exposure index was applied to all exposed subjects regardless of their job title. For example, all enlisted flyers serving a similar tour of duty received the same exposure classification. The index was based on: (1) the amount of dioxin disseminated throughout Vietnam on a monthly basis for the period January 1962 through April 1970 as represented in the Herbicide Reporting System (HERBS) tapes; and (2) estimates of the TCDD content of 2,4,5-T over time. The exposure index was an estimate or surrogate indicator of potential exposure to any of the four TCDD-containing herbicides (Orange, Purple, Pink, and Green) sprayed from fixed wing aircraft. However, because the actual concentration of TCDD in the herbicides "varied from lot to lot" individual assessments of actual body burden "could not be made" (16, 17, 23). The exposure index for a given subject was defined as

TABLE 3. Numbers of compliant Ranch Hand veterans by exposure index category by examination period

Military occupation	Exposure index category	Effective herbicide orange gallons corresponding to exposure index category	Baseline exam (*n*)	First follow-up exam (*n*)	1987 Follow-up exam (*n*)	1987 Follow-up serum dioxin results (*n*)
Officer	Low	≤35,000	140	127	130	109
	Medium	35,0000–70,000	150	130	124	104
	High	>70,000	151	123	125	106
Enlisted-flying	Low	≤50,000	67	55	55	43
	Medium	50,0000–85,000	70	65	63	57
	High	>85,000	66	57	53	48
Enlisted-ground	Low	≤20,000	185	154	147	127
	Medium	20,000–27,000	186	163	158	139
	High	>27,000	207	142	140	133
Total			1222	1016	995	866

Adapted from the Air Force Health Study: Table VIII-2 (17); Table 8-2 (22); Table 8-1 (23); and Table 3-1, (26).

$$E_i = \{\text{TCDD weighting factor}\} \times G_i \times 1/A_i,$$

where G_i is gallons of TCDD-containing herbicide sprayed in Vietnam during the i^{th} Subject's tour and A_i is the number of airmen with the subject's duties in the Vietnam Theater during the i^{th} subject's tour.

The TCDD weighting factor was an estimate of the relative concentrations of TCDD in the sprayed herbicides and was designed to distinguish those who served in Vietnam before July 1965 (a period in which TCDD levels in herbicides Green, Pink, and Purple were known to be higher) from those who served after that time period. Archived samples of herbicide Purple indicated a mean TCDD concentration of approximately 33 ppm, whereas the mean TCDD concentration in herbicide Orange was 2 parts per million (ppm). Because herbicides Pink and Green were estimated to contain twice as much 2,4,5-T as herbicide Purple, the mean TCDD concentration in these two herbicides was estimated to be approximately 66 ppm (16, 17).

Using historical data from the HERBS tape, Contemporary Historical Evaluation and Combat Operations (CHECO) Reports, and quarterly operations reports, the number of gallons of TCDD-containing herbicide sprayed each month during the war was reconstructed. The exposure index was designed to reflect "the effective number of gallons of Herbicide Orange to which the airman was potentially exposed, where exposure to the higher TCDD-containing herbicides (Purple, Pink, and Green) has been properly weighted to place them on the same footing as Herbicide Orange" (16, 17). Using data based on the number of gallons of herbicides (Green, Pink, and Purple) that were procured and sprayed, an estimated mean concentration of TCDD for the period before July 1, 1965 was determined to be 48 ppm. Herbicides Green, Pink, and Purple gallons were converted to "Herbicide Orange equivalent gallons" by dividing the mean concentration of 48 ppm of TCDD for the three herbicides (Green, Pink, and Purple) by 2 (the mean concentration of Herbicide Orange). This resulted in a weighting factor of 24 for the period before July 1, 1965. Because available documentation indicated that Agent Orange was the only TCDD-containing herbicide that was sprayed by Ranch Hand as of July 1, 1965 a weighting factor of 1 was used after that date (16, 17). Young (28) estimates the dioxin level in AO stocks used in Vietnam at about 1.88 ppm, so the de facto assumption of 2 ppm used by AFHS investigators in the exposure index calculation is in good agreement.

This numeric exposure index was then divided into three levels (low, medium, high) using a different calculation for each of the three Ranch Hand occupational categories (16, 17). The occupational category and dates of each subject's tour(s) in Vietnam were determined by a manual review of military records. Tour date was calculated to the nearest month to obtain the individual's exposure index in "effective or equivalent" herbicide Orange gallons (16, 17). Exposure scores of zero were assigned to the "officer other" category (primarily administrators) and to "enlisted ground" crew with administrative duties (16, 17). The exposure index also assumed that each individual assigned a specific duty in Vietnam, carried out his share of the workload. This "experience factor" was created by dividing the total number of herbicide spray missions flown during a veteran's tour of duty in Vietnam by the number of individuals performing comparable duties during the period of his tour. The AFHS Exposure Index categorization for compliant Ranch Hand veterans by examination period is shown in Table 3.

PHASE II: THE AFHS SERUM DIOXIN ASSAY

When the AFHS was undertaken, measuring the amount of 2,3,7,8-TCDD in the human body required a fairly invasive surgical procedure to remove adipose tissue (29). This obstacle was overcome when a reliable method to measure

2,3,7,8-TCDD in serum (blood) was developed by the Centers for Disease Control (CDC) (29, 30). A study comparing serum and fat TCDD levels showed that TCDD levels in serum were highly correlated with TCDD levels in fat tissue and provided a valid measure of TCDD levels in the human body (29, 31). A second question related to the half-life of TCDD. Observations in animals suggested a TCCD half-life of 1 year that would have resulted in the nearly complete elimination of the substance from the body within 7 years (<1% remaining). However, other studies suggested that 5–8 years was a "more reasonable" estimate in humans (29).

In conjunction with the 1987 follow-up physical examinations, a collaborative study was initiated between the Air Force and the CDC to measure dioxin levels in the serum of both Ranch Hand and comparison veterans (26). Of the 995 Ranch Hand veterans who were fully compliant for the 1987 physical exam, 932 had serum specimens analyzed by the CDC. After the exclusion of 66 samples, 742 Ranch Hand veterans had current dioxin levels that exceeded 5 parts per trillion (ppt) and 521 Ranch Hand veterans had current dioxin levels exceeding 10 ppt (26).

The results of the CDC-AF dioxin serum analyses indicated that:

- Comparison veterans had background TCDD levels (<10 ppt);
- Ranch Hand veterans had higher current TCDD levels than comparison veterans;
- Non-flying Ranch Hand enlisted personnel had the highest TCDD levels; and
- Ranch Hand officers had the lowest TCDD levels (26).

Blood was obtained for dioxin serum assays at each subsequent physical examination. A majority of participants (76%) taking part in the 2002 follow-up examination had completed a serum dioxin assay in 1987 during either the pilot study or the 1987 follow-up examination. That year was selected as the reference point for post-SEA serum dioxin levels, termed either "current dioxin" or "1987 dioxin" depending on the report (21).

If a participant had multiple assays, priority was assigned first to the 1987 pilot study results, second to the 1987 physical examination results, and third to subsequent exam results. For those participants who were first assayed post-1987, dioxin levels greater than 10 ppt were extrapolated to 1987 levels using a first order decay model with a half-life that was specific for a given analysis. For example, a half-life of 8.7 years was used with the 1997 data, whereas a 7.6 half-life was used in the analyses of the 2002 data (21, 25).

According to the 2005 AFHS report, approximately 40% of Ranch Hand veterans were found to have serum dioxin levels of less than 10 ppt (the 98th percentile of the comparisons' lipid-adjusted dioxin distribution) (25). Lipid-adjusted dioxin levels less than 10 ppt are referred to as "background" levels (21, 25).

Serum dioxin results for Ranch Hand veterans by military occupation and follow-up examination are presented in Table 4. When the results from the first follow-up examination were published, evaluation of the accuracy of the AFHS Exposure Index (not based on serum dioxin) was regarded as "only fair" because the index, for the most part, "…failed to display consistent and/or meaningful dose-response relationships" (26). In fact, when Ranch Hand TCDD body burden levels (current or initial) were compared to the AFHS Exposure Index, the correlation was "weak" even though statistically significant.

ANALYSIS OF THE AFHS DATA

The analysis of data from the baseline and the first two follow-up physical examinations used the Air Force exposure index described above (17, 22, 23). After the development of methods to measure serum dioxin levels and the collection of serum-based data from study participants, data analysis for the remaining follow-up cycles used models to assess dose-response relations between dioxin and the health-related data (21). AFHS reports published since the availability of serum dioxin levels have used up to six statistical models to analyze examination results (21, 24–26). Although the 2005 AFHS report states that the statistical models used

TABLE 4. Lipid-adjusted serum dioxin results for Ranch Hand veterans by military occupation and follow-up examination

	1987 Follow-up exam			1992 Follow-up exam			1997 Follow-up exam			2002 Follow-up exam		
	Sample size	Median (ppt)	Range (ppt)	Sample size	Median (ppt)	Range (ppt)	Sample size	Median (ppt)	Range (ppt)	Sample size	Median (ppt)	Range (ppt)
Military occupation												
Officer	319	7.8	0.0–42.6	348	7.7	0.0–36.0	337	7.4	0.0–36.0	307	7.26	0.42–35.95
Enlisted flyer	148	18.1	0.0–195.5	150	17.8	0.0–195.5	151	16.4	0.0–195.5	132	16.03	0.42–195.45
Enlisted ground crew	399	24.0	0.0–617.8	396	24.1	0.0–617.8	375	24.0	0.0–617.8	337	24.03	0.64–617.75
Total	866	12.8	0.0–617.8	894	12.5	0–617.8	863	11.6	0.0–617.8	776	11.43	0.42–617.75

ppt = parts per trillion.
Adapted from the Air Force Health Study: Table 2-4 (26); Table 2-6 (24); Table 2-8 (25); and Table 2-4 (21).

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September 2011: 673–687

TABLE 5. Description and assumptions of the statistical models used in the analysis of data from the AFHS 2002 physical examination

Model 1: Comparison of Ranch Hand (exposed) and comparison veterans (unexposed) without regard to the magnitude of the exposure. Analysis was conducted within each military occupational category (officers, enlisted flyers, and enlisted ground crew).

Assumptions:

- Ranch Hand veterans were exposed; comparison veterans were not exposed
- Enlisted ground crew was more heavily exposed than enlisted flyers; enlisted flyers were more heavily exposed than officers

Model 2: Uses an extrapolated initial dioxin measure for Ranch Hands who had a 1987 dioxin measurement >10 ppt. Initial dioxin was calculated by extrapolating the 1987 dioxin level back in time to the end of the Ranch Hand's tour of duty that qualified him for inclusion in the AFHS. If a Ranch Hand did not have a 1987 dioxin level, then the first dioxin measured (from a subsequent follow-up examination) was used to estimate the initial dioxin level. Ranch Hand veterans with a level ≤10 ppt were excluded from statistical analyses.

Body mass index at the time the serum dioxin sample was taken was included in this model to account for body mass index-related differences in elimination rate.

Assumptions:

- Ranch Hands received a single dioxin dose in Vietnam and background exposure thereafter.
- Ranch Hands experienced first-order dioxin elimination.

Model 3: The health outcomes in each Ranch Hand veteran exposure category are compared with the health outcomes in the comparison veterans. Ranch Hands were divided into three categories: background (serum dioxin levels ≤10 ppt); low (serum dioxin levels >10–118 ppt); and high (>118 ppt). The low and high Ranch Hand groups were combined into the "low + high" group. For those Ranch Hand veterans who did not have a 1987 dioxin measurement, the first measured dioxin level was used.

Ranch Hand veterans with no dioxin measurements were excluded from the analysis.

Body mass index at the time the serum dioxin sample was obtained included in the model to account for body mass index-related differences in elimination rate.

Assumptions:

- Dioxin body burden is eliminated following a first-order model.

Model 4: Uses the 1987 dioxin levels in all Ranch Hand veterans with a dioxin measurement. If a 1987 dioxin measurement was not available, the first dioxin level obtained was extrapolated to the date of the 1987 physical examination. If the first dioxin level was not obtained in 1987 and was ≤10 ppt, it was not extrapolated to 1987 level, but was used at the measured value.

Ranch Hand veterans with no dioxin measurement were excluded from the analysis.

Assumptions:

- Ranch Hand veterans received a single dioxin dose in Vietnam and background exposure thereafter.

AFHS = Air Force Health Study; ppt = parts per trillion.
Adapted from the Air Force Health Study: Tables 7-1, 7-2, 7-3, and 7-4 (21).

in the study are "intentionally consistent" with those used in previous reports, it should be noted that there are differences in the models reported during the course of the study. For example, the value of the dioxin half-life has varied by report (exam periods 1987 and 1992: $t_{½}$ = 7.1 years; exam period 1997: $t_{½}$ = 8.7 years; and exam period 2002: $t_{½}$ = 7.6 years) (21, 24–26). Another revision in the analysis of the 2002 follow-up physical examination data involves the cut points used in Model 3. The high end of the low exposure category was increased from 94 ppt initial dioxin (1997 follow-up examination) to 118 ppt initial dioxin based on a half-life of 7.6 years (21). In addition, some analyses in published studies used analytic methods and methods of confounder adjustment that are different from those presented in the AFHS reports.

The four models used in the analysis of the 2002 physical examination data are a "legacy" from previous AFHS reports and are described in Table 5 (21). Model 1 compares Ranch Hand and comparison veterans using three military occupation categories as a surrogate for exposure to herbicides. Models 2, 3, and 4 incorporate serum dioxin measurements (21). Models 2 and 4 used $\log_2$ (serum dioxin) as the measure of dioxin dose. Analysis of the 2002 examination data were conducted both adjusted and unadjusted for covariates. Variables adjusted in the models included, but were not limited to, age, race, military occupation, body mass index (weight in kg/height in m^2), lifetime cigarette smoking (pack-years) and alcohol consumption (self-reported current alcohol use in drinks/day), lifetime alcohol history (drink/years), current wine use (drinks/day), and lifetime wine history (wine/years).

RESULTS: AFHS 2002 FOLLOW-UP EXAMINATION RESULTS

The AFHS baseline morbidity study, which included data for 13 clinical areas, showed "a few differences" between Ranch Hand and comparison veterans (17). Twenty years later, the final physical examination cycle included 1951 Ranch Hand and comparison veterans. Of 1043 eligible Ranch Hand veterans, 777 (74.5%) participated whereas 737 (67.4%) of 1093 eligible original comparison veterans and 437 (46.0%) of the 951 eligible replacement comparison veterans participated (21).

In the final AFHS report (21), analyses were conducted on over 300 health-related endpoints in 12 clinical areas: general health, neoplasia, neurology, psychology, gastrointestinal, dermatology, cardiovascular, hematology, renal, endocrine,

TABLE 6. Number of endpoints (health outcomes and lab tests) analyzed in the AFHS baseline and follow-up examinations

Follow-up report	PI, year published and reference	Number of models used in analysis	Endpoints (*n*)
Baseline	Lathrop, 1984 (17)	N/A	>190 dependent variables
1985 Follow-up	Lathrop, 1987 (22)	N/A*	150 dependent variables
1987 Follow-up (serum dioxin analysis)	Roegner, 1991 (26)	3	300 endpoints in 12 clinical areas
1992 Follow-up	Grubbs, 1995 (24)	6	~300 endpoints in 12 clinical areas
1997 Follow-up	Michalek, 2000 (25)	4	266 endpoints in 12 clinical areas
2002 Follow-up	Michalek, 2005 (21)	4	>300 endpoints in 12 clinical areas

AFHS = Air Force Health Study; PI = principal investigator.
*Comparisons between Ranch Hand veterans and the comparison veterans, plus tests for interaction, and multiple contrasts in the low, medium, and high exposure categories in the exposure index analysis.

immunology, and pulmonary health (21). The number of health and laboratory endpoints by examination cycle is shown in Table 6. The results from the final examination cycle were consistent with the results of the five prior physical examinations in concluding that the results "did not reveal major differences in the health status of Ranch Hands and comparisons since 1982" (21). The results of the analyses from the final AFHS examination cycle for chloracne, cancer, diabetes and cardiovascular disease are discussed below. For additional information on the analysis for each of the clinical areas, see Appendices G and H of the 2005 report that present the results of the exposure analysis for each of the four models and a summary of the statistically significant results ($p \leq 0.05$) from the adjusted analyses for the four models.

Chloracne

Chloracne is a skin condition observed commonly in humans who have been exposed to high levels of TCDD (32, 33). Chloracne appears after a short interval of high levels of dioxin exposure (comparable to those seen in production workers, i.e., 100–400 ppt), has a relatively short latency period and usually persists for 2–3 years. No evidence of clinically verified chloracne, as defined by the occurrence of secondary lesions, such as scarring, hyperpigmentation, and depigmentation, was observed in either the Ranch Hand or comparison veterans by either examination or from medical record reviews (17, 21).

Cancer

Assessment of cancer was based on questionnaires, clinical assessments (skin neoplasms), and chest x-ray films taken during the 2002 physical examination cycle. In addition, medical records were reviewed to confirm reported cancers and to identify unreported cancers (21). The evaluation "distinguished between skin and systemic neoplasms." Neoplasms were evaluated as all neoplasms, malignant neoplasms, benign neoplasms, and neoplasms of an unspecified nature. Malignant systemic neoplasms were analyzed according to specific sites (21). All neoplasms diagnosed after SEA service were included. Data on neoplasms collected at the 2002 follow-up examination were combined with data from previous examinations for a given individual to develop a cancer history for each study participant. Neoplasms were based on the number of participants with a neoplastic diagnosis and not on the number of neoplasms reported (21).

In the final AFHS report statistically significant findings were observed in the low and/or background dioxin exposure category for several cancers—lung/bronchus, colorectal, prostate, urinary tract, and basal cell carcinoma of the skin. However, these associations were not observed in the high dioxin category. As the report notes, the associations were "not supportive of a dose-response relationship between dioxins and cancer" but may be "driven by factors other than dioxin" (21).

Fifty-three cases of prostate cancer were observed among Ranch Hand veterans versus 67 cases among the comparison veterans. No statistically significant differences were observed between Ranch Hand and comparison veterans overall or when analyzed by occupational category. After adjusting for covariates, a statistically significant inverse association observed between initial dioxin level and prostate cancer was no longer significant. No association between 1987 dioxin serum levels and prostate cancer risk was observed. Although Ranch Hand veterans in the low dioxin category had a significantly increased risk of prostate cancer, this elevation was not seen in the high dioxin category. In addition, no significant association was observed between PSA levels and dioxin serum levels (21).

Skin neoplasms were analyzed by behavior type and cell type. The prevalence of skin cancers in Ranch Hand veterans was higher than in comparison veterans (54.2% vs. 47.8%). The increase was related specifically to basal cell carcinomas and was observed primarily in officers, the military occupational group with the lowest dioxin levels (21). No significant associations with herbicide exposures were noted for either squamous cell carcinoma or melanoma. The investigators concluded that the data "did not support a dose-response relation between dioxin serum levels and development of non-melanoma skin cancers" (21).

There were several site-specific cancers where the number of observed cases in either or both the Ranch

Hand and comparison veterans were "limited" in number (<5 cases). Among these cancers were connective and other soft tissue cancers (1 case vs. 6 cases for Ranch Hand and comparison veterans, respectively). There were no cases of Hodgkin disease, lymphoma, non-Hodgkin lymphoma, or multiple myeloma in Ranch Hand veterans whereas there were several cases in the comparison veterans: one case of Hodgkin disease, two cases of lymphomas, one case of non-Hodgkin lymphoma, and one case of multiple myeloma (21).

Diabetes

The authors of the final AFHS report concluded that diabetes "represents the most important dioxin-related health problem seen in the AFHS" (21). Noting that the "clinically significant" finding for diabetes was "consistent" with previous AFHS reports they concluded that there was a "meaningful adverse relation between type 2 diabetes and exposure to dioxin" (21). The report states that the finding was supported by a "dioxin-related increase in disease severity, a decrease in the time from exposure to first diagnosis, and an increase in fasting glucose and hemoglobin A1c with dioxin in Ranch Hands" (21). Ranch Hand enlisted ground crew and those in the low and high dioxin exposure categories also experienced an increased risk of abnormally high triglycerides that increased with initial dioxin level suggesting, according to the report that, "a subtle effect of dioxin on lipid metabolism cannot be excluded" (21).

Cardiovascular Disease

The most recent *Veterans and Agent Orange, Update 2008* recommended that Ischemic Heart Disease be moved from the "inadequate or insufficient" category into the "limited or suggestive" category with the caveat that "issues of bias and confounding could not be ruled out entirely" (9). The AFHS analyzed a wide variety of cardiovascular endpoints. Only one observation—an increased proportion of Ranch Hand veterans in the high dioxin category with abnormally high diastolic blood pressure—provided any evidence of a dose-response effect in relation to dioxin body burden. The authors concluded that the "prevalence of cardiovascular disease was not increased in the Ranch Hand cohort" (21).

DISCUSSION

Throughout its 20-year history, the AFHS has been criticized regarding scientific limitations as well as some administrative aspects of its conduct (2, 4, 7, 34–38). Even after the study's formal conclusion, the Institute of Medicine Committee charged with determining the disposition of the data collected in the AFHS, enumerated various limitations of the study (38).

We believe that the AFHS, although confronted with numerous challenges, was very well designed and thoroughly conducted. It provided an opportunity to address questions regarding Agent Orange exposure and health effects by comparing an exposed veteran group with nonexposed or very low exposed groups. Qualitative evidence suggested that some Ranch Hand members had experienced substantial exposure to dioxin, however estimating individual exposure levels was challenging. When attempts were made to develop dioxin exposure models, AFHS investigators discovered that the military record systems of the Vietnam era were not sufficient to support such efforts. Nevertheless, because veteran groups could be classified by potential exposure levels based on knowledge of activities and service venues, these studies did provide useful information on the relationship between Agent Orange and a number of disease endpoints.

One criticism of the AFHS relates to the use of serum dioxin levels as the exposure metric, particularly the assumptions made about the backward extrapolation of the measurement with respect to the decay model used and the half-life calculation. Young showed that average levels of dioxin in AO were about 2 ppm but also indicated that there was considerable variation in dioxin levels across different batches (Figure 5.5) (28).

The ability to obtain serum dioxin measurements dramatically changed the dosimetric picture because they *objectively* measured an individual's dioxin exposure level allowing the identification of more highly exposed individuals in the study group. Importantly, the serum-based measurements confirmed some of the qualitative impressions of the earlier interview-based exposure measures. The study's authors, however, reported that although there were statistically significant differences in dioxin blood levels among job categories, the statistical correlations between job category and serum dioxin levels were poor.

Because serum dioxin levels were measured many years after exposure ceased, the levels reported in the 1987 AHFS follow-up study may be as much as six- to eight-fold less than the peak levels that existed at the end of service. That is, exposure for most veterans ended in the late 1960s, and if a half-life of 7.6 years (21) is assumed, roughly 2.5–3 half-lives have elapsed. Applying an eight-fold multiplier to 1987 serum dioxin levels, the dioxin levels in the Ranch Hand veterans are in the low end of exposures measured in selected residents of Seveso, Italy (39) and mid-range of the measurements reported for several of the industrial cohorts included in the IARC study (Table 7) (40).

This observation is critical. As noted above, some investigators questioned the accuracy of the AFHS Exposure Index because it failed to identify exposure-response relationships for disease (26). Although there was a lack of

TABLE 7. Serum dioxin levels from studies of Vietnam veterans, industrial cohorts, and Seveso, Italy

Reference	Study population (*n*)	Range	Mean
Centers for Disease Control (56)	Vietnam ground combat troops with service in heavily sprayed areas (646)	ND–45	4.2
	Non-Vietnam veterans (97)	ND–15	4.1
Air Force Health Study (26)	Ranch Hand veterans (866)	0–617.8	—
	Comparisons (804)	0–54.8	—
Mocarelli et al. (39)	Zone A with Chloracne (10)	828–56,000	19,144
	Zone A without Chloracne (9)	1770–10,400	5240
	Non-ABR zone (10)*	ND–137	—
Fingerhut et al. (41)	Exposed workers (253)	2–3400	—
	Unexposed workers (79)	<20	7
	Workers with ⩾1 yr exposure (119)	—	418
Ott et al. (58)	Workers involved in clean-up after reactor accident (138)	<1–553.0	15.4
	External referents (102)	0.6–9.1	3.0
Kogevinas et al. (adapted from Table 2) (40)	Australian sprayers (37)	2–34	—
	Austrian production workers (9)	98–659	389
	Dutch production workers (31)	1.9–194	53
	New Zealand sprayers (9)	3.0–131	53.3
	Swedish production workers (5)	9–37	17
	German production workers (19)	1.3–6.49	3.2
	German production workers (190)	3–2252	141
	German production workers (20)	23–1935	401.7
	American production workers (253)	2–3400	233
Flesch-Janys et al. (59)	Male production workers (236)	2.0–2252.0	108.3
	Female production workers (39)	6.0–1439.0	110.5
	Production workers: males and females combined (275)	2.0–2252	108.6

ND = non-detect.
*Reference 39 defines 4 zones, A,B,R and Non-ABR which is the unexposed zone.

concordance of the AFHS Exposure Index with measured dioxin body burdens, the body burdens indicated that whereas the Ranch Hand cohort is a high exposure group relative to other Vietnam veterans, it is a relatively low exposure group when compared to heavily exposed individuals in manufacturing (40–42) and community exposures from unplanned environmental releases (43, 44). Though a few statistically elevated disease endpoints have been reported in these more highly exposed cohorts, no disease endpoints are consistently elevated. We conclude that these more highly exposed cohorts show no pervasive evidence of long term adverse health effects caused by exposure to TCDD other than chloracne. The most recent AFHS follow-up failed to show pervasive evidence of any health effect associated with serum dioxin levels and restated that no cases of chloracne were observed in any Ranch Hand veteran (21). Given the levels of direct body burden measurements, a lack of pervasive exposure-related health effects is not unexpected and is consistent with epidemiologic studies of other dioxin exposed cohorts. This observation underscores the important role of direct body burden measurements in the assessment and interpretation of results from occupational epidemiology studies.

Comparison of Ranch Hand with the comparison veterans could reveal health effects resulting not only from dioxin exposure, but also from other unrecognized risk factors associated with the herbicide application process or from nonmilitary risk factors that may differentiate the two groups. In fact, this is not the case. The health status of the comparison and the Ranch Hand veterans is comparable (21). But, if no such comparable veteran group was available one might question whether the health status of the Ranch Hand veterans was so poor that differences among herbicide exposure categories could not be detected. Because the Ranch Hand cohort was in fact compared to a group of veterans with similar characteristics, the absence of a dose-related response for the range of health outcomes assessed is reassuring.

Assumptions related to the validity of dioxin elimination by first-order pharmacokinetics and the consistency of dioxin half-life in Ranch Hand veterans have also been cited as areas of concern (21, 24–26). In the final follow-up analysis approximately 40% of Ranch Hand veterans had serum dioxin levels less than 10 ppt. The question that cannot be addressed (because of lack of data) is whether the Ranch Hand veterans with serum dioxin levels less than 10 ppt may never have been exposed or whether their presumably elevated levels declined to background levels between the time of exposure and the measurement of serum dioxin levels. Therefore, the extent of exposure misclassification of Ranch Hand veterans remains unknown.

Four similar, but not identical, statistical models have been used over the course of the study to analyze the data obtained by physical examination. All four models were analyzed as both "unadjusted" and "adjusted" incorporating a varying number of covariates, some of which are disease

specific. For the final report, data for 12 clinical areas were analyzed resulting in thousands of comparisons. In these circumstances, a likely scenario is that some number of positive results could have resulted by chance, i.e., 1 in 20 or 5%, given statistical testing at a probability level of 0.05.

The results for diabetes, which the AFHS investigators termed the "most important dioxin related health problem seen in the AFHS," provide an example (21). In the analysis of data from the 2002 physical examination, over 200 statistical comparisons for diabetes-related endpoints were conducted. In these analyses, Models 2 and 4 used $\log_2$ (serum dioxin) as the measure of dioxin exposure. Because Ranch Hand veterans are a "low exposure cohort" relative to workers exposed in industrial or environmental settings, and the higher dioxin exposed cohorts did not show an excess risk of diabetes, the adequacy of this dose metric is questioned, as well as the lack of control for multiple comparisons.

Ginevan and Watkins (45) note that logarithmic transformations of dose should be "used with caution" in epidemiologic studies. To illustrate their point they use the AFHS study results as one example. It is notable that in the AFHS most of the significant results reported for diabetes-related endpoints result from the continuous forms of Models 2 and/or 4 that use this log transformation. It is also notable that Model 3 defines high exposure as more than 118 ppt, whereas Model 4 (also based on 1987 dioxin levels) defines high exposure as more than 19.2 ppt, almost five-fold less than the definition of high exposure in Model 3 (21).

If the statistical associations between serum levels of TCDD and diabetes in Ranch Hand veterans actually resulted from a causal process, the evidence of increased diabetes in cohorts whose levels of TCDD were much higher than the TCDD levels in Ranch Hand veterans should have been extensive and obvious. No such results are seen. Although an excess of diabetes-related mortality was observed in females in all exposure zones in the 25-year mortality update of the Seveso accident, no elevated mortality risk was found among males (42). The study's authors noted that this is in contrast to the results from the AFHS.

Steenland et al. (46) explored whether the conflicting Ranch Hand and industrial worker data regarding diabetes could be understood through a joint analysis of the combined data from the Ranch Hand veteran cohort and the NIOSH cohort of chemical workers. In the combined analysis, the prevalence of diabetes did not differ significantly between the combined exposed groups and the combined non-exposed groups, nor was there evidence of a significant difference in mean fasting serum glucose between the two groups. The authors concluded that differences in prevalence of diabetes and fasting serum glucose level between the exposed and unexposed groups in both cohorts combined were negligible. However, their dose-response analysis to examine a trend of increased diabetes with increased TCDD levels found different responses for the Ranch Hand and the NIOSH cohort. Although there was a significant increasing trend in prevalence of diabetes with increasing TCDD level in the Ranch Hand cohort, the trend was not observed in the more highly exposed NIOSH cohort. The authors stated that it was "not clear" as to why the NIOSH analysis did not show an increased risk and concluded that "Due to this heterogeneity of findings, it is impossible to draw any firm conclusions about the relation between concentration of exposure to TCDD and diabetes from these results" (46).

Given the strength of associations of diabetes with age and other known risk factors, adjustment for important covariates to reduce or eliminate confounding is a key consideration. Variables adjusted for in the models were age, race, military occupation, body mass index (weight in kg/height in m^2), lifetime cigarette smoking history (pack-years), the ratio of the waist measurement to the hip measurement at the 2002 physical examination, and family history of diabetes (21). The selection of covariates for the analyses is notable for two reasons. First, military occupation was included as a main predictor variable in Model 1 and a covariate in the other models. A concern with Model 1 is that there is no evidence that military occupation is a risk factor for diabetes, although dioxin levels differ by military occupation category in the Ranch Hand veterans. Consequently, over-adjustment in Model 1 may result in a distortion of the results (47). Second, when age was included as a covariate it was used as a dichotomous variable (birth date $\geqslant$1942 or birth date $<$1942) thereby allowing for the likelihood of residual confounding. This age cut-point is equivalent to an age cut-point of under and above age 60. Using a single cut-point for age in this model may not adequately control for age effects for a disease such as Type 2 diabetes where incidence and prevalence increase substantially with age.

The report also incorporated two definitions of diabetes. The first (AFHS 2002 definition) was formulated using recent American Diabetes Association guidelines whereas the second definition (pre-2002 AFHS diabetes definition) used older diagnostic criteria to achieve comparability with previous analyses. However, these two definitions are sufficiently similar that analyses using both criteria would include two highly dependent or correlated variables, which is not only inefficient, but may distort the study results (47). Furthermore, the possibility of confounding in the association due to the interrelationship between serum dioxin levels, serum lipid levels, serum lipid profiles, diabetes, or other potential risk factors cannot be excluded.

Concerns have been raised regarding the significance of false positive results from the large number of hypothesis

tests carried out in many epidemiologic studies (48, 49). Approaches to address the problem of false positive results are discussed by Katki (50), Wacholder et al. (51), and Ioannidis (52). The large number of statistical tests for the comparisons made in the AFHS, plus the observation that some of the reported significant findings are counter to what would be expected if dioxin was causally associated with an increase in disease (e.g., diabetes), raise concern about the multiple comparisons generated in the analyses of these data. It is highly probable that the large number of comparisons assessed and the resulting likelihood of false positive results could explain most, if not all, of the significant findings in the most recent AFHS report.

A number of studies published in the peer reviewed literature based on data from the AFHS deviate from the original study design and protocol. For example some studies incorporate all study participants who attended at least one of the six physical exams (53), whereas others have elected to use different comparison populations, such as the U.S. general population, which may not be adequate to control for the potential confounders identified when selecting the AFHS comparison group (54). Consequently, results from studies using less representative reference groups and comparisons between studies should be interpreted cautiously.

CONCLUSIONS

The results of the AFHS do not provide evidence consistent with a conclusion that exposure to Agent Orange is causally associated with disease in Ranch Hand veterans – the most heavily exposed veterans of the Vietnam War. The results are particularly persuasive given that the AFHS is the most relevant and potentially informative population for the study of disease and exposure to herbicides, notably AO, of all the veterans studies conducted. A number of features of the AFHS support this conclusion:

- The AFHS was an investigation of the most highly exposed Vietnam veterans to Agent Orange—the Ranch Hand veterans.
- Serum TCDD testing of the Ranch Hand veterans provides objective evidence that they were exposed to TCDD at levels that could be measured 10–15 years later. Measured serum TCDD levels have confirmed that the median level of serum TCDD in Ranch Hand veterans remained significantly elevated even at the time of the 2002 medical examination, 30–40 years after their exposure. Although U.S. Army Chemical Corps veterans, as a group, also have elevated serum TCDD levels, the levels are substantially lower than the levels in Ranch Hand veterans (55). In contrast, serum TCDD levels in U.S. ground troops who served in heavily sprayed areas of Vietnam have consistently been in the range of levels seen in troops with no service in Vietnam or even the general public (56).
- The original AFHS design relied on estimates of exposure based on job assignment, volume of spraying, and similar factors, because serum testing for TCDD was not feasible when the study was initiated. The 1987 serum TCDD testing was regarded as a superior method for estimating actual doses of TCDD experienced by veterans from Agent Orange. Studies estimating exposure from self-reports and/or models of spraying and troop position data are potentially of value in some epidemiologic settings, but should be given considerably less weight relative to results from the AFHS based on serum levels of dioxin determined using an objective and validated biomarker of exposure.
- Comparison veterans were matched with Ranch Hand veterans based on age and other potential confounders to maximize the ability of the study to detect independent health effects of Agent Orange exposure while controlling for potential health effects of Vietnam military experience or SEA military service.
- The broad nature of veteran concerns resulted in the AFHS investigating over 300 health endpoints on multiple occasions. This increased the likelihood that some statistically significant study results linking diseases with exposure to Agent Orange would be expected due to chance as a function of the multiple testing conducted.
- The data accumulated during six comprehensive follow-up medical examinations over 20 years increased the likelihood that even diseases with a long latency would be identified. The number of Ranch Hand veterans available was not sufficient to detect moderate excesses of rare diseases unless the excesses were large. Nevertheless, the AFHS had the power to detect relative risks of 2.0 or more for many diseases—such as heart disease and basal cell carcinoma—occurring at a prevalence of at least 5% in the unexposed population (21).
- Although studies of health effects of Vietnam service on veterans have found increased risks for some diseases, these studies provide no evidence that veterans with these diseases were exposed to Agent Orange or that Agent Orange exposure was causally associated with these diseases.
- The lack of a clear dose-response relationship of TCDD with studied health outcomes in the AFHS argues against a causal relationship.
- The results of the AFHS suggests caution in interpreting associations of health effects with low or minimal TCDD exposure because these associations are not observed in well-designed studies of persons with high or considerably higher TCDD exposures.
- If the reported statistical associations between TCDD levels and diabetes-related endpoints in Ranch Hand

veterans actually result from a causal relationship, it could be argued that an increased risk of diabetes should be seen in industrial workers with much higher TCDD levels, but this has not been observed.

Given the lack of evidence of disease or health-related endpoints associated with exposures to TCDD (Agent Orange) in the Ranch Hand veterans, the available evidence does not seem to be consistent with a causal relationship between dioxin and the health endpoints studied in the AFHS.

This work has been carried out independently and no review by representatives of either sponsoring company has taken place. Drs. Buffler and Mandel served as consultants to the USAF School of Aerospace Medicine, Brooks Air Force Base, San Antonio, TX in 1979 for the development of the initial Scope of Work for the Air Force Health Study of Ranch Hand Veterans.

The Dow Chemical Company and Monsanto Company provided financial support for this independent scientific review.

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