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Original Contributions

Dioxins and Dibenzofurans in Blood and Adipose Tissue of Agent Orange–Exposed Vietnam Veterans and Matched Controls

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Vietnam veterans who were heavily exposed to Agent Orange exceeded matched control subjects in both blood and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) but not in the levels of the 12 other 2,3,7,8-substituted dioxins and dibenzofurans that were detected. Since only TCDD among these compounds was present in Agent Orange but all are present in the population of the industrialized world, it is likely that the elevated TCDD levels arose from wartime exposure. The high correlation ($r = +.89$) of blood with adipose tissue level suggests that there may be a mobile equilibrium between them and that blood measurement could replace adipose tissue measurement of TCDD levels, making the collection of human data less invasive.

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ESTABLISHING the existence or absence of health effects in populations that are exposed to low levels of toxic chemicals is made difficult by a number of factors. Among these classification of study subjects into exposed and unexposed groups often is a major problem, which is compounded by the fact that exposure may have occurred long before the study commences. Since classification errors dilute the effects one seeks to measure, negative epidemiologic results leave open the question of whether the correlation between exposure and toxic end point is absent or whether classification errors made it undetectable. Studies of Vietnam veterans,[1,2] some of whom were exposed to the defoliant mixture known as "Agent Orange," which was contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), during the manufacture of its 2,4,5-trichlorophenoxyacetic acid herbicide component, may very well suffer from this problem.

With the discovery by Rappe et al[3,4] that survivors of the Yusho accident[5] in Japan had detectable traces of dibenzofurans in their blood 11 years after consuming contaminated cooking oil, it became apparent that chlorinated multiring aromatic compounds can have long half-lives in human tissues. (In Japan in 1968, rice oil, which is used in cooking, became contaminated during processing by a polychlorinated biphenyl–based heat exchange fluid. The fluid, in turn, contained small amounts of chlorinated dibenzofurans, leading to a total dibenzofuran content in the rice oil of 5 ppm.) The results suggested that measurement of tissue levels in persons who were exposed in the distant past might confirm exposure status.

In early 1984, Rappe et al[6] reported 14 polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in samples of human adipose tissue from northern Sweden. The compounds had between four and eight chlorines, and all were substituted at the 2, 3, 7, and 8 positions. Laboratories on three continents have since confirmed these observations, and it is now accepted that there is a background of these compounds in the population of the industrialized world.[6-11] Among these substances, however, only 2,3,7,8-TCDD was present in Agent Orange so that isomer-specific measurement of the entire series should provide us with both positive and negative controls in correlating tissue levels with exposure status, as only 2,3,7,8-TCDD levels would be expected to be elevated in herbicide-exposed men.

Halogenated dibenzodioxins and dibenzofurans are lipid soluble and tend to accumulate in adipose tissue. Most

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studies have therefore analyzed fat, which requires obtaining the sample by surgical biopsy. A less invasive assay would allow rigorous epidemiologic examination of the relationship between low-level exposure and subsequent health effects in a large number of people, as suggested by Schecter et al.[11]

A program to compare exposure of Vietnam veterans to Agent Orange with their 2,3,7,8-TCDD body burdens and with their health statuses has therefore been undertaken by the State of New Jersey and is known as the "Pointman Project." The present pilot study was designed primarily to determine, by isomer-specific analysis of all tetrachlorinated to octachlorinated dibenzodioxins and dibenzofurans, whether there is a relationship between exposure and 2,3,7,8-TCDD levels in Vietnam veterans who were exposed to Agent Orange 15 to 20 years ago and whether blood and adipose tissue levels are correlated.

PROCEDURES

Study Groups

Twenty-seven men were studied. Ten were heavily exposed Vietnam veterans, nine of whom handled herbicides regularly while in Vietnam. We emphasized spray handlers at this stage of the investigation rather than ground troops who served in sprayed areas to maximize the likelihood of finding 2,3,7,8-TCDD. Ten Vietnam veterans who had little or no exposure served as Vietnam control subjects. Seven veterans who served during the time of the Vietnam War but who did not go to Southeast Asia served as Vietnam-era control subjects. Era control subjects were included to ensure that if something in Vietnam other than herbicide exposure led to elevated 2,3,7,8-TCDD levels we would be able to detect it.

Each control subject was matched against an exposed veteran for the following four factors in addition to sex: age at time of entry into the study ± 36 months, dates of military service ± 36 months, race/ethnicity (black, white, or Hispanic), and rank (all participants were enlisted men). There was thus one Vietnam control subject for each exposed subject and one era control subject for each of seven of the exposed men. We were unable to find suitable matched era control subjects for three of the exposed men.

Subject selection was initiated with a broad request for potential participants through the mass media, veterans' service organizations, and church and community groups. There were approximately 2700 respondents who completed a short questionnaire that provided age, dates of service, dates in Vietnam, race, branch of service, military unit, and limited information about exposure. From this group we identified 100 men who seemed likely to have handled herbicides during a tour of duty in Vietnam. These men were sent a 30-page questionnaire detailing all military assignments in and out of Vietnam, all civilian occupations, hobbies, diet, and other activities that might influence exposure. Eighty-four questionnaires were returned. The returned questionnaires were reviewed by a selection committee that included the principal investigators (a biochemist and an occupational physician), a psychologist, and two veterans with broad knowledge of conditions in Vietnam. The committee excluded those with confounding exposures and selected those who seemed to have the highest likelihood of exposure in Vietnam, but not elsewhere. We then sent the same questionnaires to potential Vietnam and era control subjects from among the original 2700 respondents who were appropriate matches for the exposed men.

We used the ten men with the highest exposure for whom we could obtain Vietnam control subjects, and for these we found suitable era control subjects for seven. Informed consent was obtained from all subjects after the nature of the study and its possible consequences had been explained in detail.

Military exposure status was confirmed for Vietnam veterans (both exposed men and Vietnam control subjects) by the US Army/Department of Defense Joint Environmental Support Group. The Environmental Support Group confirmed exposure status by ascertaining whether the veteran had a job title that entailed potential exposure as a handler of herbicides. In the one case of an infantryman who was not a spray handler, his presence in areas sprayed by known herbicide missions was verified by the Environmental Support Group. Partly because of the emphasis on spray handlers and partly because of the methods of subject selection, this pilot study is not representative of all Vietnam veterans. Because exposure status was verified, however, we believe that recall bias does not affect the correlation between exposure status and body burden as found by chemical analysis, as both are objectively determined.

Five of the men were members of Operation Ranch Hand, the Air Force unit that flew fixed-wing aircraft on spray missions. Three of these were ground personnel who maintained the aircraft and spray equipment and who filled the tanks with herbicide. The other two operated the spray equipment in flight. A sixth man was an Air Force freight handler who handled drums of defoliants. Two men were Army chemical corps specialists. One man was an Army helicopter crew chief who filled defoliant tanks and flew spray missions. The tenth man was an army light infantry combat soldier with a reported history of herbicide exposure.

Groups of three to six subjects were admitted to the hospital for 3½ days of medical testing, during which they were examined by specialists in occupational and internal medicine, dermatology, neurology, psychology, and psychiatry. A battery of clinical tests also was done, including a study of immune function, and samples of adipose tissue and blood were taken for analysis, as described later herein. The results of the medical testing will be published separately.

Tissue Sampling

Ten to 20 g of subcutaneous tissue were biopsied by liposuction and transferred immediately to hexane-washed polypropylene containers, which were stored frozen. To increase the likelihood of finding 2,3,7,8-TCDD and its congeners in the blood, each subject fasted for 24 hours in an attempt to mobilize TCDD from stored fatty reserves. Following the fast, 300 to 400 mL of blood were drawn into heparin as anticoagulant (see later herein). Serum fatty acids were monitored at 12-hour intervals during the fast as an index of fat mobilization.

Care was taken to minimize the time that blood was in contact with the plastic blood-drawing apparatus to minimize leaching of plasticizer. The standard hospital blood bags that were used had been preloaded with anticoagulant solution, which we removed, using the first 10 to 15 mL of blood as rinse. The rinse was discarded. Fresh heparin was added, and collection continued. The blood was transferred promptly from the bags to hexane-washed glass containers that were stored on ice during transport to the laboratory in New Brunswick, NJ. Red blood cells were separated by centrifugation at 4°C, yielding plasma samples of 150 to 160 mL for chemical analysis. The plasma was placed in hexane-washed polypropylene containers and stored frozen until analyzed.

Sample Coding and Handling

An independent referee team of two people who were not associated with any of the institutions or individuals participating in the study coded and relabeled the samples and returned them to the investigators for shipment

Table 1.—Blood Validation Test Analyses (Levels in Picograms of Analyte per Gram of Blood Fat)

Isomer*	Mean	SEM
2,3,7,8-TCDF†	3.67	0.38
2,3,7,8-TCDD†	4.50	0.26
2,3,4,7,8-PeCDF	28.42	0.89
1,2,3,7,8-PeCDD†	12.42	0.43
1,2,3,4,7,8-HxCDF†	5.50	0.29
1,2,3,6,7,8-HxCDF	4.75	0.28
1,2,3,7,8,9-HxCDF	2.65	0.31
1,2,3,4,7,8-HxCDD	15.08	0.77
1,2,3,6,7,8-HxCDD†	56.50	1.54
1,2,3,4,6,7,8-HpCDF†	13.67	0.67
1,2,3,4,6,7,8-HpCDD	56.73	2.30
OCDD†	395.08	19.67

*TCDF indicates tetrachlorodibenzofuran; TCDD, tetrachlorodibenzo-p-dioxin; PeCDF, pentachlorodibenzofuran; PeCDD, pentachlorodibenzo-p-dioxin; HxCDF, hexachlorodibenzofuran; HxCDD, hexachlorodibenzo-p-dioxin; HpCDF, heptachlorodibenzofuran; HpCDD, heptachlorodibenzo-p-dioxin; and OCDD, octachlorodibenzo-p-dioxin.

†All samples were spiked with the seven carbon 13–labeled standards that are marked. The table, however, shows levels of the unlabeled compounds originally present in the sample and detected in the validation analysis.

Table 2.—Means and SEMs for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Levels

	Groups			
	Exposed Subjects	Vietnam Control Subjects	Era Control Subjects	Pooled Control Subjects
	Adipose Tissue Levels, pg/g Tissue Wet Weight			
No. of subjects	10	10	7	17
Mean	41.7	5.1	3.2	4.3
SEM	16.8	1.4	0.5	0.9
Median	15.4	5.4	3.5	4.1
	Blood Plasma Levels, pg/g Blood Fat			
No. of subjects	9	10	7	17
Mean	46.3	6.6	4.3	5.6
SEM	19.1	0.9	0.9	0.7
Median	25.1	5.3	3.6	4.6

to the University of Umeå in Sweden, where the chemical analyses were performed. There was no relationship between blood and adipose tissue sample numbers. The analytic laboratory and the principal investigators were thus blind to the exposure statuses of the men from whom the samples came. All analytic results were deposited with the referee team before the code was revealed. A consequence of the coding was that no analyses could be repeated, and there was not enough material for replicates.

Chemical Analysis

Adipose Tissue.—Sample extraction and containment enrichment were by the method of Stalling et al[12] and Smith et al,[13] with minor modifications.[14]

The analytic method has been shown in an interlaboratory study[14] to have a high degree of quantitative and qualitative reliability. It also has been validated by analyzing fish, cow's milk, and human milk fortified at different levels with known amounts of carbon 13–labeled standards[15] (C. Rappe, P.-A. Bergqvist, R. Andersson, et al, unpublished data, 1986).

High-resolution gas chromatography/mass spectrometry conditions were as described.[3,16] In the present study, selectivity was enhanced over the interlaboratory study[14] by using a double-focusing mass spectrometry instrument (model 70-250, VG Limited, Manchester, England) operating at a resolution of 6000 to 8000 daltons. The background reduction produced by this instrument also results in higher sensitivity. Isomer separation was achieved by using 60-m columns (model SP2330 or SP2331, Supelco Inc, Bellefonte, Pa).[17] Every third sample was a system blank. All samples were run in electron impact mode with selective ion monitoring.[18]

Blood.—The extraction of blood plasma was based on partitioning in a system of chloroform, methanol, and water. The procedure is a modification of the method of Bligh and Dyer.[19] Solvent was evaporated from a known amount of extract and the lipid that remained was weighed to determine the fat content of the plasma. The fat was removed on a silica column that was eluted with hexane and the extract was cleaned on a carbon column (Carbopack C, Supelco Inc, Bellefonte, Pa) that adsorbs the PCDDs and PCDFs. They were eluted from the carbon column in toluene. High-resolution gas chromatography/mass spectrometry conditions were the same as for adipose tissue.

Validation of Blood Plasma Analysis.—A pooled plasma sample from the Regional Hospital in Umeå was used for a validation study. The sample was divided into 12 aliquots that were analyzed individually. Each was fortified with a standard mixture containing equal amounts of seven $^{13}C_{12}$-labeled PCDDs and PCDFs, which are marked with a dagger in Table 1. On a plasma basis, four spiking levels were used: 0.018 parts per trillion (ppt), 0.058 ppt, 0.18 ppt, and 0.58 ppt of each analyte in the mixture. Three aliquots were spiked at each level, yielding triplicate analyses. For each sample the recovery of the standards was used to compute the levels of the "natural" carbon 12–labeled PCDDs and PCDFs, resulting in 12 readings for each compound. The gas chromatography/mass spectrometry analyses were performed in the same way as for adipose tissue. The analyses agree with one another quite well (Table 1).

Surrogates for Unknowns.—For the veterans' samples two ^{13}C-labeled standards were used: $^{13}C_{12}$-labeled 2,3,7,8-TCDD and octachlorodibenzo-*p*-dioxin. The protocol was fixed as of December 1984, at the time that the first men entered the hospital. At that time other ^{13}C-labeled surrogates were not readily available. Although they have since become available, we thought it better not to change the protocol once analyses had commenced.

The results have been corrected for recovery of the labeled surrogates. $^{13}C_{12}$-labeled 2,3,7,8-TCDD in the extract from plasma was normally 60% to 90%. The recovery of $^{13}C_{12}$-labeled octachlorodibenzo-*p*-dioxin from plasma was somewhat lower, being about 20% in some of the samples. Recoveries of both standards from adipose tissue ranged from 65% to 74%. Blood levels are expressed per gram of blood lipid. Adipose tissue levels are presented per gram of wet tissue. The detection limit for both blood and adipose tissue was 1 ppt or less per gram of fat. In blood, which is approximately 1% fat, this corresponds to a detection limit of 0.01 pg/mL of whole blood. The analytic methods for blood are described in greater detail by Nygren et al.[20]

RESULTS

The levels of 2,3,7,8-TCDD in the exposed men exceeded those in the pooled control subjects by a wide margin in both blood ($P<.01$) and adipose tissue ($P<.001$) (Table 2). The Mann-Whitney U test was used to compare the exposed with the pooled control subjects. Although TCDD levels in the Vietnam control subjects exceeded those in the Vietnam-era control subjects, the difference was small and not significant.

In adipose tissue, the 2,3,7,8-TCDD levels in the exposed men exceed the levels in their matched Vietnam control subjects in nine of the ten pairs (Fig 1, left). The binomial probability of finding nine of ten subjects with an a priori probability of .5 is less than .01.

Because of technical problems with the blood sample from the exposed individual in trio 5 in Fig 1, [illegible] only nine pairs comprising an exposed [illegible]

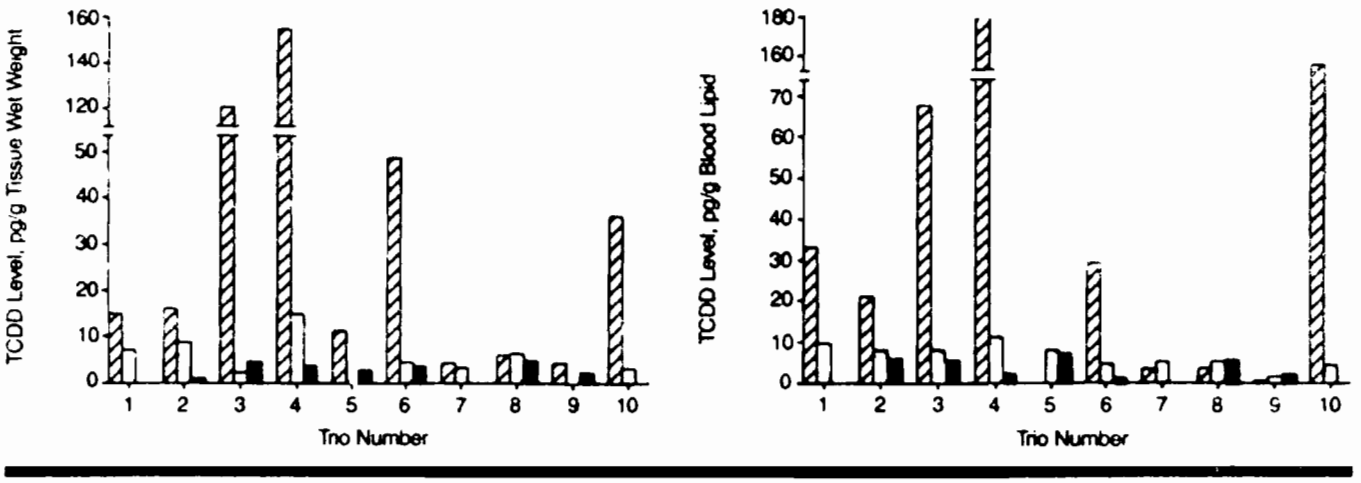

Fig 1.—Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) levels in adipose tissue (left) and blood plasma (right). Each bar represents a sample from one man. Note absence of Vietnam-era control subjects (closed bars) in trios 1, 7, and 10. Broken bars indicate broken Y axis, continuous bars, continuous part of axis; slashed bars, exposed individuals; and open bars, Vietnam control subjects. No levels in blood plasma are presented for exposed individual No. 5.

and a Vietnam control subject were suitable for comparison. In six of these the exposed men exceeded their Vietnam control subjects in 2,3,7,8-TCDD level ($.10>P>.05$).

Figure 2 presents the mean ± 1 SEM for each of the analytes in fat and blood by exposure status. In both fat and blood there is a strikingly higher level of 2,3,7,8-TCDD in the exposed men (Table 2). None of the other compounds exhibited this pattern in fat. In blood, other compounds seem to be somewhat higher in the exposed men than in one or both groups of control subjects. While the origin of this effect was not identified with certainty, some of it, at least, was due to recovery problems in the ^{13}C-labeled surrogates. This is especially likely for octachlorodibenzo-*p*-dioxin, for which the blood recoveries were low, as mentioned previously herein. In any case none of these differences are as striking as that found for 2,3,7,8-TCDD.

Figures 3 and 4 compare blood and fat levels of 2,3,7,8-TCDD. As mentioned previously herein, only seven trios could be completed due to lack of a sufficient number of era veterans who were acceptable as matches, who had no evidence of civilian or military exposure, and who were available. There were thus 17 control subjects and, because of the failure of the analysis on the blood of one exposed individual, nine exposed veterans who were suitable for the comparison. In Fig 3 all 17 control subjects fall in the domain bounded by blood and fat levels of 15 pg/g. Only three of the nine exposed men were in this range. The result is significant (Fisher's exact test, $P = .001$).

The data for the group as a whole, exposed and control subjects taken together, are not normally distributed, so a logarithmic transformation was used. The correlation coefficient for the transformed data was .72 for all 26 points. Because untransformed values close to zero are sensitive to variation in laboratory analysis and have a large effect on the log value, Fig 4 shows the logarithmic regression for men with both blood and fat levels greater than or equal to 1 pg/g. The correlation coefficient for these 22 points was .89. The least squares regression yields the following:

$$\log(\text{adipose tissue level}) = 0.86 \times \log(\text{blood fat level}) + 0.10$$

The SEs for the slope and intercept are 0.10 and 0.25, respectively. The slope is significantly different from zero at the $P = .0001$ level but not different from 1.0, and the intercept does not differ significantly from zero. Some of the scatter in the figure is probably due to the presence of nonlipid components in the adipose tissue samples. The 2,3,7,8-TCDD levels in fat and blood are similar if the blood levels are expressed on a per gram of blood fat basis.

COMMENT

One of the most striking results is that Vietnam veterans who were heavily exposed to Agent Orange exceed matched control subjects in 2,3,7,8-TCDD levels 15 to 20 years after exposure (Table 2 and Fig 1). A comparison of their 2,3,7,8-TCDD levels with the levels found for other isomers (Fig 2), moreover, makes it highly likely that the elevated TCDD levels in the exposed men are due to their wartime exposure to the defoliant. It is thus clear that the compound has an extremely long half-life in human beings, considerably longer than the one-month half-life found in laboratory rodents.[21]

Gross et al[22] measured adipose tissue levels of three Vietnam veterans designated as heavily exposed and of several control veterans. Theirs was a feasibility study whose object was to determine the range of levels that might exist in the men, so the control subjects were not matched individually to exposed subjects. Although the nature of the heavy exposure was not stated, they found a range of 20 to 173 pg of 2,3,7,8-TCDD per gram of adipose tissue in the exposed men and much lower levels in the control subjects. Their highest control level coincided with the lowest value in a heavily exposed subject. The range of levels observed by Gross et al in their exposed men is nearly identical to ours, although their samples were taken approximately ten years after exposure while ours were taken after 15 to 20 years, suggesting slow excretion. Unfortunately, there are no data that allow us to compare exposures between their subjects and ours, so a more detailed comparison of the TCDD levels is impossible.

Nygren et al,[16] in addition, have reported an adipose tissue value of 100 pg/g in a German chemical worker whose exposure occurred in a factory accident in 1953 and who had had no known subsequent exposure. A biopsy sample of his tissue was taken in 1984, 31 years later. Schecter and Ryan[23] and Rappe et al,[24] studying other workers from the same accident, found similar levels.

The only published estimate of the

Agent Orange exposure that might yield a given tissue level of TCDD after a ten-year period is that of Weerasinghe and Gross.[28] They based their estimates on a one-year half-life from primate data,[26] on 100% absorption of the applied dose, and on daily exposure for an entire year.

The studies cited previously herein suggest that TCDD has a longer half-life in humans than in rodents[21] or perhaps primates.[26] However, there has been little direct quantitative work on either half-life itself or on the question of whether elimination over a long time is, in fact, a first-order process. Poiger and Schlatter[27] provided the first direct human half-life measurement. Poiger consumed a small amount of radiolabeled 2,3,7,8-TCDD and monitored its excretion, finding a half-life of approximately five years. A person who was exposed in 1966 and found in 1986 to carry 50 pg/g, which is close to the average (41.7 pg/g, Table 2) in the exposed group, would thus have had an adipose or blood fat burden of 800 pg/g immediately after exposure.

More recently, Pirkle et al[29] have reported blood measurements in Air Force veterans involved in herbicide spraying. Samples drawn in 1987 were compared with samples drawn in 1982 and yielded a half-life corrected for background of 7.1 years. Using this number, back calculation for a person carrying 50 pg/g in 1986 would yield 350 pg/g as the body burden in 1966.

All these calculations, including those of Weerasinghe and Gross,[28] assume, of course, that long-term elimination is governed by a single first-order process. Although the data of Poiger and Schlatter[27] are consistent with first-order kinetics, their experiment ran for only a few years. The question remains open for long-term excretion.

Adipose tissue and blood levels are highly correlated (Figs 3 and 4), making it likely that blood sampling will ultimately replace the fat biopsy in tests of body burden. This is the progression that was followed for measurements of human polychlorinated biphenyl levels,[29] the desire to replace invasive procedures by less drastic methods being the motivating factor in both cases. While we are optimistic that blood measurements will become the norm in the near future, we caution that a few unanswered questions remain. How essential is fasting before sampling? The Red Cross recommends against even a 12-hour fast before blood donation, a precaution that is aimed at avoiding untoward side effects on the donor. This, however, does not speak to the question of the effect of fasting on the blood level

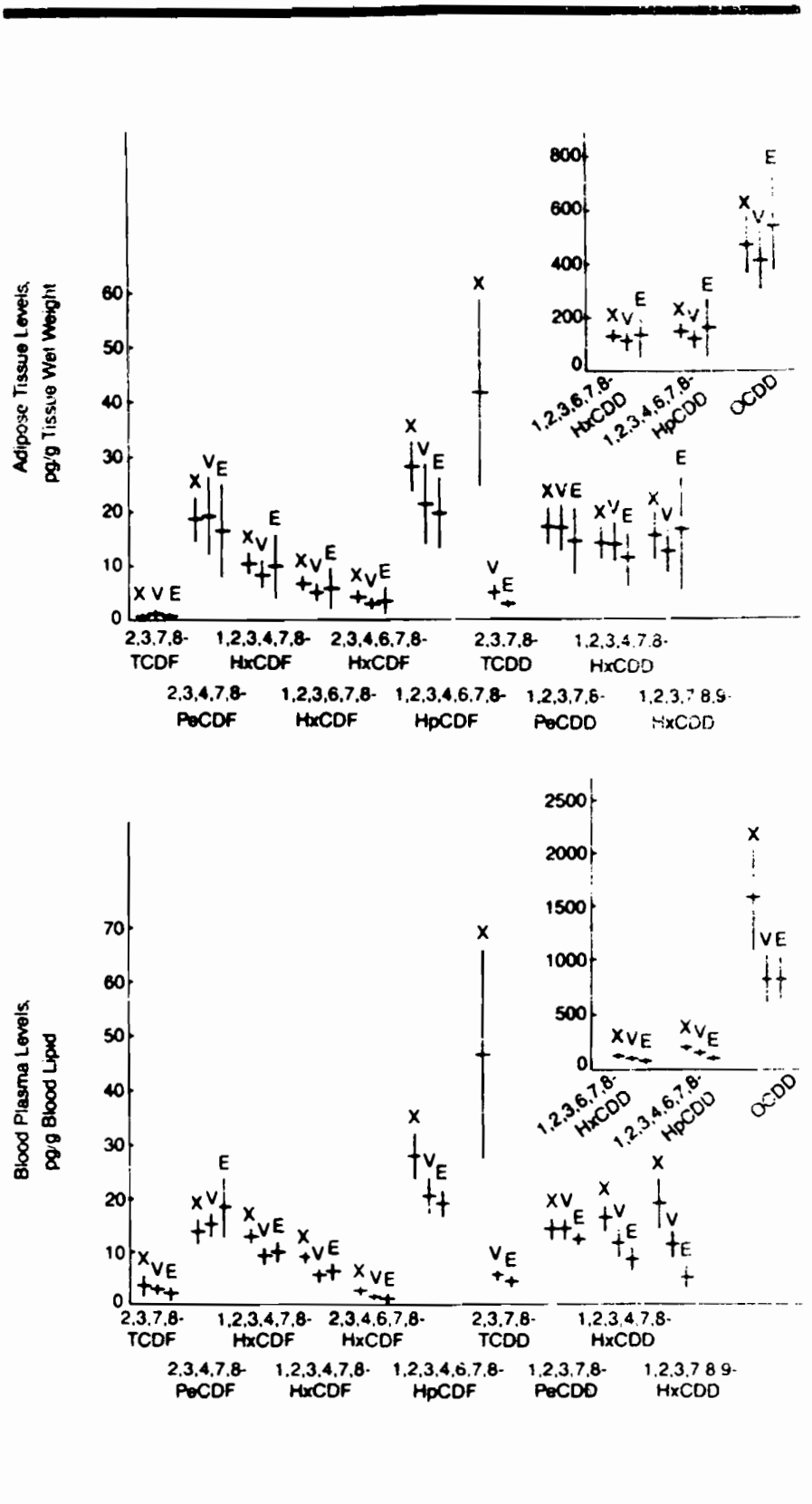

Fig 2.—Mean ± SEM for all 13 congeners found in adipose tissue (top) and blood plasma (bottom). Insets contain three compounds graphed with an ordinate that differs from that of other ten compounds. X indicates exposed subjects (ten); V, Vietnam control subjects (ten); E, era control subjects (seven). HxCDD, hexachlorodibenzo-*p*-dioxin; HpCDD, heptachlorodibenzo-*p*-dioxin; OCDD, octachlorodibenzo-*p*-dioxin; TCDF, tetrachlorodibenzofuran; PeCDF, pentachlorodibenzofuran; HxCDF, hexachlorodibenzofuran; HpCDF, heptachlorodibenzofuran; TCDD, tetrachlorodibenzo-*p*-dioxin; and PeCDD, pentachlorodibenzo-*p*-dioxin.

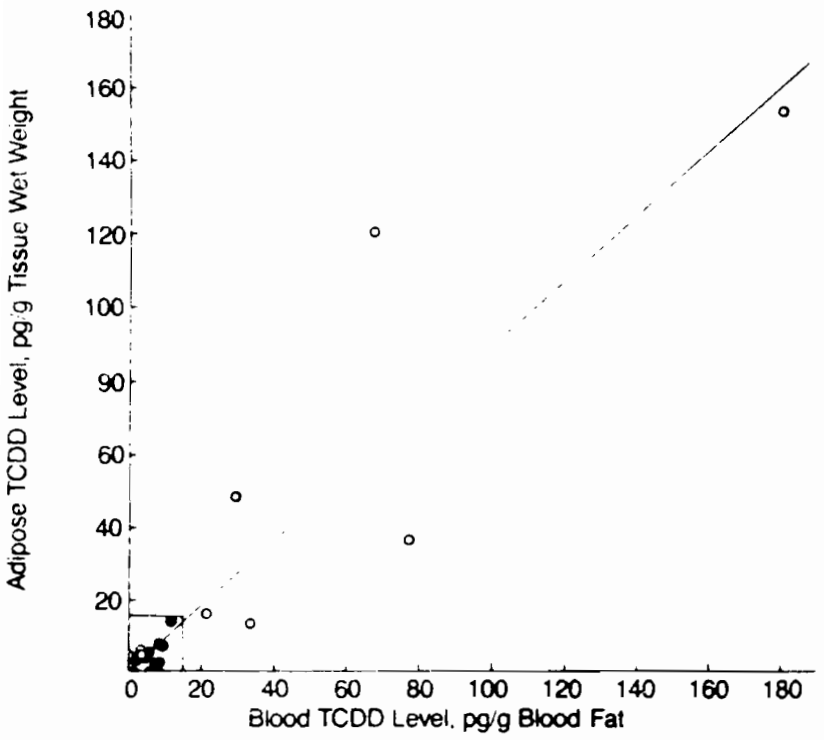

Fig 3.—Comparison of blood fat with adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Least squares regression line is drawn. Box near origin is drawn at 15 pg/g on both axes. It contains all 17 control subjects (closed circles), some atop one another, and three of nine exposed men (open circles) for whom comparison is possible.

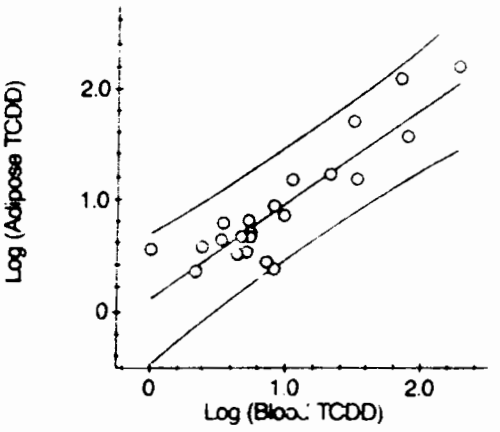

Fig 4.—Regression of adipose tissue on blood fat levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) after logarithmic transformation of both axes. Least squares line (middle line) and 95% confidence limits (top and bottom lines) are shown. Plot contains 22 of 26 points from Fig 3 but with no distinction between exposed men and control subjects. Points indicate blood and adipose tissue samples from all men for whom comparison was possible and from those whose TCDD levels were greater than 1 pg/g.

of dioxin. In the parallel study of Patterson et al,[30] subjects consumed a restricted diet prior to sampling, and the blood-adipose correlation for TCDD was excellent. This does not answer the question, however, of whether fasting changes the blood level; adipose and blood levels could correlate in both situations but with different constants in the logarithmic regression equation given previously herein. A test of the effects of fasting is therefore in progress.

An additional point that should be considered concerns the use of adipose tissue measurements as a kind of "gold standard" for body burdens of lipophilic compounds such as dibenzodioxins. These and similar compounds accumulate in adipose tissue because of their hydrophobicity. It is usually assumed that the larger the overall body burden, the larger will be the amount stored in the fat. This has not been tested adequately. Second, once stored in adipose tissue, toxic compounds may be less likely to cause health problems than material in, for example, the liver or the central nervous system. We are thus left with the question of whether a well-validated blood analysis might, in fact, be a better index of potential health threat than would an adipose tissue measurement, as it is blood rather than fat that circulates to vital organs. Should it turn out, however, that the equilibrium between blood and fat is rapid with respect to the excretion rate, then they would constitute a single-body "compartment," and measurement of either would suffice.

The results support the statement that men with high levels were heavily exposed and can be distinguished from others by both blood and adipose tissue testing. In Fig 3 all six of the men whose 2,3,7,8-TCDD levels exceeded 15 pg/g were in the exposed group; none of the 17 control subjects exceeded that level. It seems reasonable that with further work it will become possible to use blood measurements as an aid in establishing cohorts of exposed and unexposed men for epidemiologic study of health effects, although we recommend strongly against the use of such measurements as the sole basis for classification (see later herein). In using body burden data, one may prefer to be conservative and set a cutoff for a designation of heavy exposure somewhat above 15 pg/g, but it is clear that an acceptable value can be established. Using 15 pg/g gives a specificity (true negatives/all unexposed) of 100% and a sensitivity (true positives/all exposed) of 70%.

In Fig 3 three of the 20 points within the box bounded by 15 pg/g are from exposed men. The probability that a person whose level falls below this might be heavily exposed is thus 15%. It is of interest, however, that at least [illegible] of the three men in the exposed group whose levels are less than 15 pg/g may not have had the heavy exposure that characterizes the rest of the group. One, subject No. 9 in Fig 1, was a ground soldier whose presence in sprayed areas for several months was verified but who was not a spray handler. An accurate knowledge of exposure in such cases is problematic. Another, subject No. 7, found early in his tour that the defoliants made him acutely ill, and after a few missions he ceased to handle them. His exposure could thus have been quite low. Thus, although a low blood or fat level does not necessarily imply the complete absence of exposure, it would seem that heavy exposure does leave sufficient levels of TCDD for the fact of *heavy* exposure to be detected in nearly all cases.

In our experience minimally exposed men can be identified with considerable rigor by a combination of techniques, including questionnaires, military records, interviews in cases where needed, and measurements of TCDD body burden. Although none of these data sources is completely free of error, their combination is far less likely to result in misclassification than any one or two of them. Such a classification procedure should, in our opinion, be used for the exposed population as well, all available

data having to be consistent with exposure or the lack of it to include a prospective subject in an epidemiological study. If a less rigorous procedure is to be used, then a carefully constructed validation will be essential.

It is particularly interesting that two of the three men having the highest TCDD levels in this study were not members of Operation Ranch Hand, the Air Force unit that operated the fixed-wing spray aircraft. They were Army chemical corps specialists. We feel that chemical corps personnel constitute a neglected and useful research resource.

Finally, although we can clearly distinguish heavily exposed men from others, the present data do not speak to the question of identifying persons whose exposures are moderate and who constitute the bulk of the population, both military and civilian, who may have been exposed to greater than background levels. We focused this work on heavily exposed men because we were uncertain of detecting TCDD in them in amounts exceeding those found in the control subjects. Having found it, the next step is to examine men of more moderate exposure. Such a study is in progress.

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