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Dioxin in Body Health and Health Status:

A Feasibility Study

L. B. Hobson, L. E. Lee, M. L. Gross, and A. L. Young

Agent Orange, the defoliant used in Vietnam, contained an average of about 2 parts per million of 2,3,7,8-tetrachlorodibenzo-para-dioxin or TCDD. Since it is extremely toxic for some animal species, it has been blamed for a wide variety of medical problems by veterans who served in Vietnam.

It was suggested that TCDD might remain concentrated in the body's fat long after contact with it and serve both as a cause of ill effects and as a marker to prove prior exposure to Agent Orange. It also became evident that improved methods of gas chromatography and mass spectrometry would allow detection of TCDD in amounts as small as a few parts per trillion, a few picograms per gram of human fat.

In order to test the feasibility of using such assays, the Veterans Administration undertook a pilot study with Dr. Michael L. Gross of the Department of Chemistry, the University of Nebraska in Lincoln. Three groups of men volunteered to provide specimens of their fat: twenty men, believing that they had been exposed to Agent Orange in Vietnam and--except for one man--experiencing medical problems submitted to surgical biopsy of the abdominal wall. A second group of

ten veterans who were having operations anyway provided specimens of fat from the body wall as control samples since none had been in Vietnam nor had contact with Agent Orange. A third group of three Air Force officers had worked with TCDD or Agent Orange within the prior two years and one had also been exposed to the defoliant in Vietnam. They also were biopsied.

To do this, VA surgeons removed 10 to 30 grams of fat from the abdominal wall of the volunteers of the first and last group using local anesthesia. In all instances precautions were taken to avoid contaminating the specimens with TCDD and to deliver them frozen to the assay laboratory.

I am not competent to discuss the methods of extraction, gas chromatography and high resolution mass spectrometry used by Dr. Gross. Nor can I describe in detail the techniques used by the chemists of the Environmental Protection Agency who assayed 15 of the specimens to confirm Gross' findings. I am quite willing to believe that the methods are delicate, exacting, and time-consuming.

Each volunteer had a medical work-up at the VA medical center. The military record of each "exposed" man was examined to evaluate his exposure before the assay results were available. The assays were conducted "blind" as well since the chemist had only a code number for each specimen.

Estimating exposure to Agent Orange is generally a less

than satisfactory procedure and the results must be viewed sceptically. We did divide, as best we could, the 20 "exposed" men into three subgroups: the most likely exposed, the least likely exposed, and an intermediate group with possible but less definite exposure.

The results showed that it is indeed possible to detect and quantify to some degree, at least, 2,3,7,8-TCDD in human fat. To be specific, it was present in fat from 13 of the 20 exposed veterans, from 9 of the 10 controls, and from all three Air Force officers. The amounts ranged from 3 to 99 parts per trillion with the two highest values in "most likely exposed" veterans. The third veteran in that group, however, had no detectible TCDD and the heavily exposed Air Force officer had only 6 parts per trillion.

It will be easier to review the results in graph form (Slide I) than in a large table. The front row of cubes indicates the results from the most likely exposed volunteers, the rear row of cubes refers to controls, the other two portray the possibly exposed and least likely exposed groups. Each cube stands for the highest value obtained for one volunteer. Only three individuals had more than 15 parts per trillion and one of these was a control. Most values were below 10 parts per trillion.

It is difficult in so small a sample to evaluate the significance of an "outlier" such as the 99 parts per

trillion, especially because of the intralaboratory, as well as the interlaboratory, variability on replication. It is best to say only that a larger series would have to be tested with better documentation of exposure before any firm conclusion can be drawn. This is especially true because two recent small series, one in Canada and another in the United States have found TCDD in fat samples from civilians, each with a mean value of about 10 parts per trillion. In Canada one "outlier" of 130 ppt was discarded to arrive at that mean. The mean of our controls was 5.7 ± 3.1 ppt, the mean of the Vietnam veterans (except the "outlier") was 8.3 ± 6.9 .

In considering the clinical situations of the 20 "exposed" veterans, it must be kept in mind that all but one volunteered because they had medical problems. Hence they are a self-selected sample and are not representative of Vietnam veterans. The 19 men had a total of 36 diagnoses ranging from the grave to the trivial. They are reasonably typical of what can be seen in a large clinic serving a truly sick population. Thus, 8 men had minor skin problems, 4 had blood vessel problems of high blood pressure or varicose veins, 3 each had gastro-intestinal disorders, neuro-psychiatric problems, benign tumors, joint diseases, or reproductive disorders. However, one of the benign tumors was inside the skull, one man had sarcoidosis and another a cancer, one was schizophrenic. All of these are serious

diseases.

I will show you the complaints, the diagnoses and the TCDD values of only the most and least heavily exposed groups in order to give you an idea of the results (Slide II). First, please, notice the column of TCDD concentrations. You can readily see, especially for subject number 10, that the assay presented some problems. The "ND" indicates that no TCDD was detected.

"S" stands for skin, "G-I" for gastrointestinal, "V" for vascular, and so on. Without going into detail, the variability and the lack of relation to TCDD values is obvious for both complaints and diagnoses. One serious illness--schizophrenia--was diagnosed in a man with 7 ppt in his fat, the other--a cancer--was in a veteran with no detectible TCDD. Even a small series such as our 20 veterans and 10 controls should reveal some correlation if these levels of TCDD were associated with some one or two diseases or groups of diseases. There is no evidence of this.

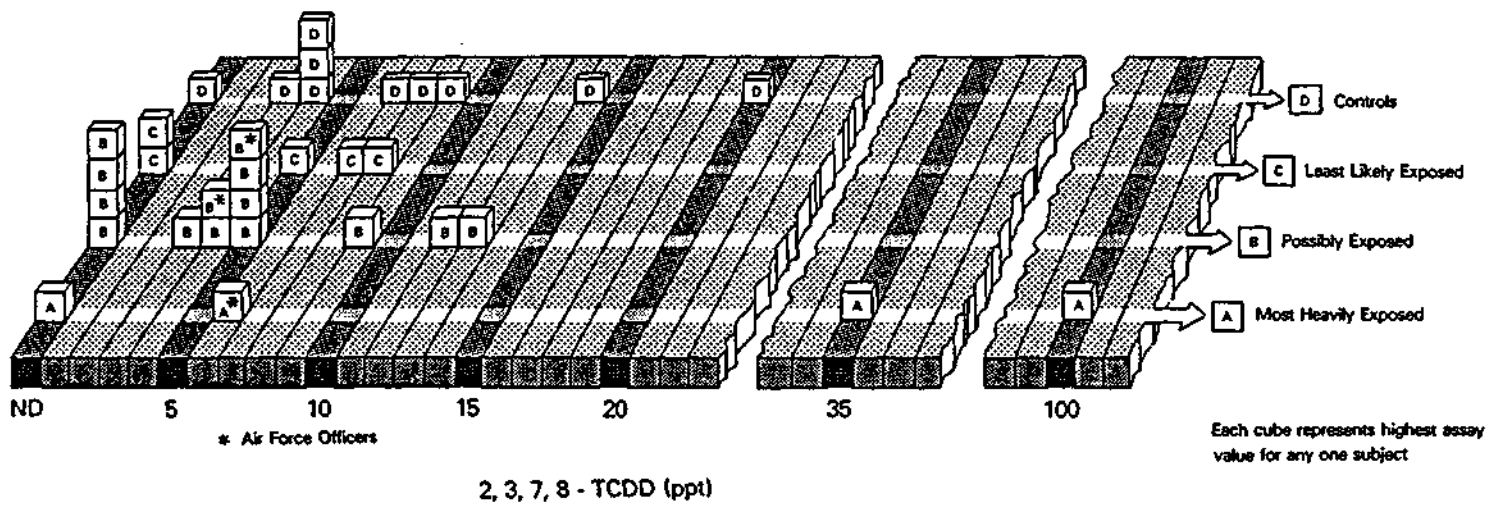
There remains the question of whether the TCDD in the body fat poses a threat to health, for example, if it were all released into the blood within a very short time. It is most unlikely that there is any danger from this source. TCDD concentrates in fat but even if all our soft tissues contained 100 ppt, the blood concentration would only be 0.1

microgram per kilogram of body weight, far below a dose expected to endanger health.

At present, we do not recommend or provide an assay of body fat for TCDD in order to determine whether a veteran was exposed to Agent Orange or to evaluate his medical condition. An operation is necessary to obtain a fat specimen, not always a trivial undertaking. The assay is difficult and therefore expensive and, most important, the results of our pilot study give no hope that useful information will be obtained.

The assay unquestionably has value in research. It would be most desirable to follow the course of TCDD's disappearance from fat after a known exposure to it. It may also help us detect suspected sources of TCDD in our civilian environments but only on a very selective basis. It is by no means a practical screening procedure for occupational or public health studies.

Adipose Tissue Content of 2, 3, 7, 8 - TCDD



Fat Assay Results, Complaints, and Diagnoses of Vietnam Veterans

Subject Code	2,3,7,8-TCDD (ppt)	Complaints	Diagnoses
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Most heavily exposed

26	63,99	S	S
10	12,16,23,35	G-I	S
4*	6	None	None
19	ND,ND	V	V

Least heavily exposed

28	7,8	None	None
15	7	N-P, R, V, S	<input type="checkbox"/> N-P S
34	5	N-P, S, V	B-T
1	ND	CA	<input type="checkbox"/> CA
13	ND	N-P	N-P, B-T

* - Air Force Officer
 - Serious Diagnosis

ND - Not detected

hand 10

Dioxin in Body Fat and Health Status: A Feasibility Study

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Since 1978 there has been mounting concern over human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Much of this concern has been expressed by veterans of the Vietnam war who believe that they were exposed to the TCDD-contaminated herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), an ingredient of Agent Orange, the major defoliant used by the United States Armed Forces in Vietnam.

During a 5-year period from 1965 to 1970, the U.S. Air Force applied more than 40 million liters of Agent Orange containing more than 92 kg TCDD in South Vietnam. Some two million American military personnel served 1-year tours during the same period. Many veterans of that era have reported medical problems that they ascribe to exposure to Agent Orange. Their complaints have ranged from tingling in the extremities to rare forms of cancer. Some veterans have fathered children with birth defects and have suggested that the TCDD is the cause. Accordingly, the Veterans Administration (VA) has initiated extensive health studies of veterans exposed to Agent Orange and its dioxin contaminant during the Vietnam Conflict.

Since TCDD is known to accumulate preferentially in the adipose tissue of certain species of laboratory animals, it was suggested, early in the history of the Agent Orange issue, that the analysis of human fat for TCDD might provide a way to determine earlier Agent Orange exposure. A relation between the presence of this substance and health problems had also been suggested. Although methods for TCDD analysis have improved in recent years, no such study had been carried out in humans with known exposure to herbicides containing this toxic contaminant. Consequently the VA embarked on a small feasibility study to test the methodology and to determine whether conclusions might be drawn regarding the significance of the results. The study was carried out in three groups of adult males as follows:

- (1) Twenty Vietnam veterans all but one of whom claimed health problems related to Agent Orange exposure and who volunteered for the fat biopsy.
- (2) Three U.S. Air Force officers with known heavy and relatively recent exposure in connection with herbicide disposal operations but who did not serve in Vietnam.
- (3) Ten veterans with no service in Vietnam and no known exposure to herbicides who were undergoing elective abdominal surgery and who volunteered to serve as controls.

Methods: The procedure called for the removal of 10-30 grams of subcutaneous adipose tissue from the abdominal wall. This was accomplished surgically under local anesthesia. Precautions were taken before, during and after the procedure

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to avoid contamination by products, e.g., hexachlorophene, that could contain TCDD. Specimens were collected in glass containers previously rinsed with acetone and dried before use. All tissues were refrigerated during shipment to the assay laboratory. Each of the volunteers had a medical history, physical examination, and routine clinical chemistry. The details of military service in Vietnam from the volunteer's report and his service record were examined to evaluate his potential exposure to military herbicides using the dates, location and nature of his service. From these a rough estimate of the likelihood of exposure to TCDD was made without knowledge of the assay results.

The extraction and assay of all samples for TCDD were conducted at the University of Nebraska, Midwest Center for Mass Spectrometry, Lincoln, Nebraska. Approximately 5 to 10 grams of tissue were used, when available, for each analysis. A known amount, generally 2 ng, of internal standard (either ³⁷C-2,3,7,8-TCDD or ¹³C-2,3,7,8-TCDD) was added to the adipose tissue. The sample was digested in alcoholic potassium hydroxide followed by extraction with hexane to remove TCDD. The hexane extract was washed with concentrated sulfuric acid, neutralized, dried and concentrated. The final stage of preparation involved the use of three short-column liquid chromatography steps (silica gel, alumina and Florisil). Gas chromatography/high resolution mass spectrometry was employed for quantitation of 2,3,7,8-TCDD and coeluting isomers. Signal profiles were obtained at a mass resolution of 10,000 for m/z 321.8936, the most abundant molecular ion for TCDD, and for the internal standard mass by signal averaging for approximately 100 sec. commencing with the appearance of the co-eluting internal standard 2,3,7,8-TCDD.

Extracts which contained materials giving signals greater than 2.5 times noise at the exact mass of TCDD (i.e. 321.8936 + 0.0020) over the integration period discussed above were reanalyzed. For the second analysis, signal profiles of m/z 321.8936 and m/z 319.8965 were monitored over the elution period of 2,3,7,8-TCDD (determined by injection of standard solutions). A positive detection was reported if signals were observed above the detection limit (2.5 times noise) and if their intensity ratio was 1.0:0.78 + 0.10, which is consistent with the presence of four chlorine atoms in the molecule. Samples meeting all criteria except the correct isotope intensity ratio have been considered to contain "not detectable" levels of TCDD. For these samples, it was judged that the presence of TCDD is not disproved by the observation of an incorrect isotope ratio at these low concentrations; rather, the presence of TCDD is not confirmed.

Results: Of the twenty veterans who served in Vietnam, seven had no detectible TCDD with the limit of detection at 2 to 6 part per trillion (ppt). Another two had detectible material that could not be validated as TCDD and the results for one was considered equivocal because the measured value was only questionably above the detection limit. Five of the ten remaining Vietnam veterans had TCDD in amounts from 5 ppt to 7 ppt. Three Vietnam veterans had TCDD in the amounts from 9 ppt to 13 ppt. One individual had 63 and 99 ppt (average = 81 ppt) and another had 23 and 35 ppt (average = 29 ppt).

Of the ten control ("unexposed") veterans, four had TCDD identified in their fat (6,7,7 and 14 ppt). Two other veterans had values low enough to be considered equivocal and in three instances the detected material was not validated as TCDD. The remaining veteran had no detectible TCDD.

One of the three Air Force officers with known exposure had no identified TCDD in his fat. The unidentified substance in his case and the TCDD measured in the other two officers was never more than 3 ppt above the limit of detection.

The clinical data were reviewed, seeking correlations between complaints or diagnoses and the assay results. Within the group of twenty Vietnam veterans, seven of them reported some health problems beginning in or during a tour of duty in Vietnam. No two, however, reported the same symptoms. The remaining thirteen veterans reported no illness in Vietnam although only one reported good health at all times. Among the nineteen veterans with current medical complaints, symptoms and diagnoses varied widely without any common pattern. Five had mental problems ranging from nervousness to schizophrenia and three had experienced difficulties of reproduction, namely, spontaneous abortion by the wives of two and congenital heart disease in the son of a third.

Four of the seven veterans who reported difficulties while in Vietnam had no TCDD detected. The five veterans with mental problems included three without detectible TCDD and three whose assays results were 5 to 7 ppt. One of three veterans reporting reproductive problems had no detected TCDD, one had doubtful level and one had 7 ppt. Detectible TCDD in the body fat could not be correlated with clinical chemistry findings. Similar results applied to the controls i.e., veterans who did not serve in Vietnam.

The highest values for TCDD in adipose were found in two Vietnam veterans. Both men had military records substantiating duties that involved the use of TCDD-contaminated herbicides in Vietnam. Other individuals, however, who claimed extensive handling of herbicide in base perimeter operations had little or no TCDD in their adipose.

Discussion: TCDD was found in some persons who reported exposure to Agent Orange and in others who were never in Vietnam. On the other hand, some veterans from Vietnam had no detectible TCDD and the same is true of veterans who were never in Vietnam. The low level of TCDD in two Air Force officers and its absence in another is of special interest since their exposure to TCDD is certain and more recent than that of the veterans.

Among the twenty Vietnam veterans there was no uniformity of symptoms, either immediately after exposure, at the time of biopsy, or during the intervening period. No one symptom or group of symptoms was common to veterans with detectible TCDD in their fat. The presence of TCDD did not mean ill health nor did its absence indicate good health. No detailed statistical analysis was attempted of this small pilot series.

The TCDD content in the fat was very small, not exceeding 100 ppt. Since TCDD accumulates in fat more than in other tissue, the average body concentration would be much lower. Even if all tissues contained 100 ppt, however, the concentration would be less than 0.1 microgram per kilogram of body weight, a value below the demonstrated toxic dose to threaten health.

Conclusions: The present assay for TCDD in fat tissue does not offer a satisfactory routine test for exposure to Agent Orange, providing neither clear evidence of contact with that specific defoliant nor of absence of such contact. Moreover, the technique cannot be used to correlate body levels of TCDD and medical conditions. The assay technique is difficult and not readily available, fat samples must be obtained by surgical biopsy and the source of any TCDD detected is uncertain. The current assay method does provide a research tool under proper conditions and for specific purposes, e.g., for determining the rate of disappearance of TCDD after known exposure.