The New Jersey State Commission

On Agent Orange

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The Measurement of Blood Dioxin Levels in Vietnam Veterans Who Were Heavily Exposed to Agent Orange during the War: A Pilot Project

(Lay language description)

A project of the New Jersey State Agent Orange Commission

under the supervision of its scientific subcommittee:

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PREFACE

The protocol for this project is in two parts, an extended lay language description and a detailed technical proposal. This is the lay language description.

A. OBJECTIVES

The principal objective of this work is to determine whether or not 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, or dioxin) can be detected at levels exceeding controls in the blood of a small number of Vietnam veterans who were heavily exposed during the war to the defoliant mixture commonly known as Agent Orange.

The analytical methods to be used are both too difficult and too expensive ever to be used on a large scale for mass screening. For this reason an additional objective is to identify secondary medical indicators for the presence of TCDD, indicators that could be determined inexpensively at regional medical centers.

B. BACKGROUND

For the past several years considerable public attention has been given to the question of whether or not a firm scientific connection exists between exposure of troops to defoliant chemicals during the war in Vietnam and subsequent medical problems either for the veteran or for his children. There is certainly enough evidence to make one suspicious of such a connection. The dioxin that contaminated Agent Orange is one of the most toxic substances known. The symptoms which veterans exhibit, although variable from man to man, when taken together are strikingly similar to those seen in persons exposed to dioxins in industrial accidents.

The persistence of symptoms over many years has also been noted in the medical literature, although there is great variability in the duration of toxic effects. Dioxin is known to be an animal carcinogen, and recent Swedish research, in which persons exposed through industrial and agricultural activity to recurrent low levels of dioxin contaminated materials have an abnormally high incidence of certain cancers, is disturbing. The Swedish work is supported by studies of soft tissue sarcoma incidence in American factory workers. There are enough reports of cancer among veterans to make one concerned that there may be a problem.

Lastly, dioxin is a teratogen. The evidence establishing teratogenicity, however, comes from studies in which the chemical was administered to pregnant **female** animals and the effect on their offspring noted. For dioxin to be the cause of birth abnormalities in the children of Vietnam veterans would be unusual in that the effect, obviously, is through the father. There is very little work in this area, unfortunately, but one should not dismiss the possibility out of hand simply because it is unusual. Science has produced stranger surprises. Mercury, for example, has been shown to cause chromosome aberrations in men exposed in the course of work to quantities sufficient to produce detectable levels in the blood but insufficient to cause clinical symptoms.

As plausible as all these connections may be, however, and as suspicious as the many instances of illness among veterans may make us, suspicion is not proof. In the absence of clear

evidence one way or the other, moreover, public policy concerning Vietnam veterans cannot be formulated, and public discussion becomes increasingly acrimonious and sterile. The need for research work is therefore clear.

C. RATIONALE

Until recently there was no evidence that it would be possible at this late date to detect dioxin in the blood of Vietnam veterans. The 2,3,7,8-TCDD that was present in Agent Orange is biologically active in such small quantities, and it is so many years since exposure ceased that the prospects of a study such as this one appeared dim. Preliminary data presented initially in June, 1981, and published recently, however, show that with advanced techniques (see below) dibenzofurans, which are close chemical relatives of the dioxins (Fig. 1) were detectable at the parts per trillion level (ppt) in blood samples drawn from survivors of the 1968 "Yusho" accident in Japan. In that incident rice oil, used in cooking, became contaminated in the processing factory by the circulating heat exchange fluid that leaked undetected from pin-holes in a heat exchanger used to heat the oil. The heat exchange fluid was a polychlorinated biphenyl (PCB) mixture (see Fig. 1) which, when newly synthesized, was free of significant contamination. Years of heating and cooling in the heat exchanger, however, led to the formation of polychlorinated dibenzofurans (PCDF's) at a level of five parts per million (ppm) in the rice oil. Some 1200 people became severely ill over the spring and summer months of 1968, mostly from the PCB's and partly from the PCDF's. Much of the

lingering illness in survivors is thought to be related to the PCDF's.

The toxic effects of PCDF's are very similar to those of the dioxins, with the 2,3,7,8-tetrachloro compounds in each case being the most toxic isomers. The most toxic isomers are also known to be the least readily metabolized isomers and are thus most likely to persist in human tissues.

The level of exposure to PCDF's in the Yusho incident, in addition, is much closer to the Vietnam experience than is the case for factory accidents, from which most of our knowledge of the medical effects of chlorinated multiring aromatic hydrocarbons comes. Depending on the batch and the manufacturer, the defoliants used in Vietnam contained between 0.1 part per million (ppm) and 47 ppm of 2,3,7,8-TCDD with a median of 2 ppm. The 5 ppm value for PCDF's found in Yusho oil is thus of the same order of magnitude as the dioxin contamination of Agent Orange.

Although the primary route of exposure in Vietnam was through the skin, while for Yusho it was by ingestion, the fact that PCDF's could be detected in the blood of Yusho patients twelve years after the cesation of exposure makes it rational to look for dioxin in the blood of the most heavily exposed veterans of Vietnam.

D. PROCEDURE

We plan to locate fifty men who were heavily exposed to Agent Orange during service in Vietnam. By heavy exposure we mean men who handled spray directly and regularly as ground rig spraymen, crews of spray aircraft, and others. Men who simply

walked through previously sprayed areas are not, for this purpose, considered to be heavily exposed, nor is present illness to be taken as an indicator of heavy exposure. In all cases the fact of heavy exposure will be verified by a search of military records.

Each heavily exposed veteran will be matched against two controls. Both controls will also be veterans and will be matched to an exposed man for age, race, dates of military service, branch of service, and enlisted versus officer status. One set of controls will be Vietnam veterans whose tour of duty did not entail known herbicide exposure. The other set will not have served in Vietnam[1] and will be presumed to have experienced minimal military exposure to herbicides. All participants will have been questioned in detail to ascertain whether or not other forms of substantial exposure may have occurred. Persons, for example, who have worked in the chemical industry, in certain agricultural occupations, and in other occupations with known potential exposure to dioxin will be excluded for the obvious reason that any dioxin found in them could have arisen from occupational rather than military exposure.[2]

The matching planned here allows a high degree of statistical significance to be achieved with a relatively small

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[1] Nationally only 28% of those who served in the military during the time of the war actually went to Vietnam. The others went to Europe, Japan, Korea, and other places or stayed in the U. S.

[2] We are mindful of the fact that some men who never left the United States were exposed to substantial amounts of defoliant chemicals while in service. Such men will not be used as controls.

number of men. The use of small numbers of men - fifty cases and one hundred controls - is necessitated, in turn, by the nature of the chemical analysis (see below).

It should be emphasized that this is a pilot project rather than a full scale epidemiplogical study. For it to be the latter would require that we identify all those men who fit our definition of heavy exposure as described above. From these we would then have to select randomly a representative group to study. This procedure is impossible for us, for there is no way to identify all those who fit our definition. Our research subjects will be volunteers located through veterans' service organizations and through other channels, with the result that we cannot be absolutely sure that they are representative of all those who are heavily exposed. This in no way detracts from the study's rigor as a pilot project, and we proceed on that basis.

Once the study participants have been selected, each man will be admitted to hospital for three days during which he will receive a thorough physical examination of our design. In addition to the usual features of a physical examination, we shall look for objective signs and symptoms which are attributed to the toxic effects of dioxins and related chlorinated aromatics. Chloracne, for example, arises from exposure to such substances and is widely believed to be a characteristic manifestation of toxicity. Although marked chloracne is unlikely to be present in our subjects, a rigorous dermatological examination will be conducted of all men. Nerve conduction rates, additionally, are known to be depressed by exposure to

these chemicals, although other factors such as high alcohol consumption may have this effect, as well, which will necessitate appropriate controls. The examination will also include a battery of physiological and biochemical tests. The choice of these, too, will be based on what we know about the effects of the dioxins. The "T" cell mediated immune response, for example, is known to be inhibited by them.

Lastly, it should be noted that among the toxic effects attributed to chlorinated aromatics such as TCDD are psychopathological changes. These behavior manifestations, unfortunately, resemble those of "post traumatic stress disorder," which is the delayed manifestation of repressed effects arising from the protracted life threatening stresses of combat in Vietnam. To some degree post traumatic stress potentially affects virtually all combat veterans of the war. Faced with a man who has problems, a clinician has, at present, no way of knowing whether those problems arise from post traumatic stress, from chemical poisoning, or from both together. We shall therefore examine each man for psychological attributes. These will later be correlated with the other medical data and with the chemical results in an effort to sort out these two potential sources of psychological change.

With all these data in hand, blood samples will be drawn for dioxin analysis. Dioxin accumulates primarily in fatty tissues and in the liver, with material in the blood being in equilibrium with stored material. All men will therefore fast for twenty-four hours prior to the drawing of blood. This will draw down

stored dioxin into the blood, enhancing the prospect of finding it. The analyses will be done in Dr. Christoffer Rappe's laboratory in Umea, Sweden. Dr. Rappe developed the testing procedure for blood and did the Yusho work mentioned above.

Before being sent to Sweden, however, the samples, which will be in identical containers, will be handed over to a third party whom we are calling the Impeccable Referee. This person will be someone with expertise in the field but who is not associated with either the New Jersey group or the Swedish group. He or she will remove our labels from the samples, replacing them with a new set and keeping a key to the label numbers. No one but the Referee will then be able to distinguish samples drawn from cases from those drawn from controls. The analytical chemistry will thus be done in a blind fashion. This unusual step is necessary because of the politically and emotionally charged atmosphere surrounding the Agent Orange question. It is essential to avoid any possible accusation of biasing the results by communicating to the analytical chemists either inadvertently or deliberately which samples are which. The Impeccable Referee will thus be a person of unimpeachable reputation and integrity.

The analytical dioxin results will be returned to the Referee, who will break the code, and the statistical analysis will then proceed. In the analysis we shall look for a correlation between heavy exposure to Agent Orange and blood dioxin levels elevated above those of the controls.

If such a correlation is found, attention will then shift to the development of secondary indicators for the presence of dioxins. The analysis of dioxins at the parts per trillion level

requires the use of a specialized high resolution gas chromatograph whose effluent is passed directly into a mass spectrometer. Often more than one kind of mass spectrometry must be done, and the whole operation requires computer control and data analysis. The procedures, including sample work-up, are technically demanding, time consuming, and expensive. The running costs per sample are approximately $1,000, including the costs of synthesizing and purifying individual dioxin isomers for use as instrument calibration standards. One hundred fifty samples (fifty cases and one hundred controls), if received all at once, would occupy Dr. Rappe's laboratory for three solid months, and there are, at best, only a dozen or so laboratories in the world that are capable of doing such analyses. The procedure, therefore, cannot be used to screen many men, and the development of methods that are both ecomonically reasonable and can be used locally will thus be necessary.

We plan three approaches to this problem. In the first we shall use the data from the physical examinations and from the physiological, biochemical and other tests. A cluster analysis will be performed to see if any subset of these data correlate with the presence of dioxin. Should such a cluster be found, the tests in it could be administered locally and relatively inexpensively to identify men who are potentially at risk and whose health should be watched carefully.

In the second we take advantage of the fact that whatever else it may do, dioxin is a liver toxin. The plasma proteins, of which human beings have a great many circulating in the blood,

are made in the liver. It is a reasonable hypothesis that even a sub-clinical disturbance of liver metabolism will alter the plasma protein profile. Standard enzyme function tests, unfortunately, examine only a few of these proteins, and it is quite possible that a biological effect would be missed as a result. If all or nearly all of the plasma proteins could be screened, the likelihood of finding an effect would be greatly enhanced.

Fortunately, a technique exists to do this: two dimensional polyacrylamide gel electrophoresis. This is a biochemical procedure that separates proteins initially on the basis of molecular electrical charge and thereafter on the basis of molecular size. One obtains on a rectangular transparent gel slab a two dimensional pattern of spots, each spot corresponding usually to a single protein or, at most, to a few proteins. Each spot is characterized by its position on the gel and by its intensity, which is a measure of the amount of the protein present in the sample.

It is likely that within experimental error most of the spots will be the same for cases and controls. The same cluster analysis that was mentioned above, however, can be applied here to see if any set of spots correlates with dioxin content in the blood.

In addition to serum proteins, we shall examine the proteins in white cells, as it has been found that treatment of these in the laboratory with chemicals having biological effects similar to those of dioxin alters their protein distributions markedly.

The procedure will be validated by a study of laboratory

animals which will be treated with known amounts of dioxin.

If the electrophoretic procedure can be developed into a successful test, it will have several advantages. It is relatively rapid, requiring a few days. It uses only a small amount of blood. It could be done in any diagnostic laboratory, and its cost would be relatively low.

The third approach to the development of secondary indicators makes use of dioxin's property as an inducer of mixed function oxidase (MFO) activity. MFO is a system of several enzymes whose function is the metabolism of drugs and poisonous chemicals. It is normally present in very small amounts, if at all, and is made (induced) in response to a toxic chemical entering the body. MFO activity is not narrowly specific to the inducing substrate. Among the substrates that are sufficiently similar in shape to the dioxins to be potential additional substrates in its presence are the polychlorinated biphenyls (PCB's), of which there are 209 possible isomers. Residents of advanced industrial countries all carry body burdens of various PCB isomers in sufficient amounts that their analysis is far easier than that of the dioxins. Our hypothesis, therefore, is that persons exposed to dioxins will have produced a type of MFO activity that differs from the norm, and that one manifestation of the difference will be a reduction in some of the PCB isomers relative to the levels in controls who were not exposed to dioxin. Support for this comes from recent work in which it was found that even thirteen years after exposure to dibenzofurans, survivors of the Yusho accident differ in their PCB isomer

distribution from local controls. Two isomers present in the controls are absent in the furan exposed cases. We shall therefore analyze for PCB's as well as for dioxin.

Partly because of the complexity of the study and partly for reasons of expense, we have divided it into two stages. In Stage 1 we shall examine ten exposed men and their associated twenty controls. Stage 2 comprises the remaining forty exposed and eighty controls. If the results of the first stage are promising, we will continue on to the second. If not, the project will be dropped or modified.

In an effort to extract as much useful information from the data as possible, the cluster analyses mentioned above will be carried out with respect to the fact of exposure and with respect to objective signs of illness regardless of the dioxin results.

E. CONCLUDING REMARKS

We are aware that should these experiments succeed two objections may well be raised. The first is that the presence of dioxin does not prove that it is harmful at the levels found. The Veteran's Administration, for example, concedes that all men who served in Vietnam may be presumed exposed. The VA denies, however, that exposure has any deleterious effects at all. The second potential objection is that data obtained on the most heavily exposed men are not necessarily "useful" considering the lesser exposure of most men.

These questions are addressed, at least in part, by other work which the Commission and others have in progress. For the present we respond that one must ask one clear question at a time that is susceptible of an experimental answer. That being done,

one then formulates another question.

In an interim report to the Governor and the Legislature the Commission ended the section on research activity with the following statement:

> "The Commission is mindful of the intense emotional and political factors that surround its work. We expect that our results will produce controversy regardless of whether or not we can establish a firm link between exposure to defoliants and subsequent medical problems. If the work is to have any value at all, it must withstand searching and possibly hostile scrutiny. The scientific plans must therefore be very carefully laid. The Commission is unanimous in its refusal to do its work any other way."

We would appreciate readers' comments, for it is our experience that useful suggestions come not only from scientific colleagues but from interested lay people as well.

Appendix I

The Biopsy of Fat Tissue for Dioxin Analysis

Dioxin accumulates in fat, and we would be more likely to find it if we could analyse fat tissue. Until recently the only way to obtain fat tissue, however, was by abdominal surgery, which we ruled out as too drastic a procedure for research use.

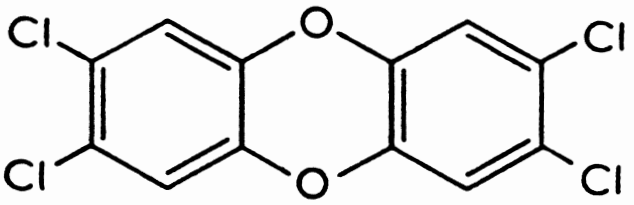
After the study protocol was written, it came to our attention that a much less drastic procedure exists for the removal of subcutaneous fat. It is used by plastic surgeons to contour the body for cosmetic purpose. When adapted for research use, it consists of making a small incision at the crease line between the buttock and upper leg. A thin, hollow steel tube to which suction is applied is inserted under the skin, and the fat is drawn out through the tube.

Although the patient is under general anesthesia, the anesthesia is such that recovery is rapid. The wound does not require stitches but is closed instead by a pressure bandage. There is some discomfort for several days, and vigorous activity is not permitted for a few weeks. A stringent informed consent procedure is followed.

The taking of fat will increase greatly the rigor of our dioxin measurements. By taking both fat and blood from the same people, moreover, we hope to be able to establish the ratio of dioxin in blood to that in fat. If the ratio is constant from person to person, the need to take fat in the future will be greatly diminished, for the amount there will then be obtainable from blood measurements, which are far simpler and less drastic.

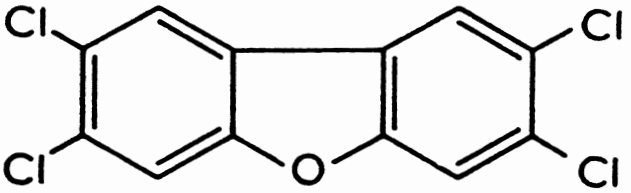
The fat samples will also be re-coded by the Impeccable Referee, and the code numbers will not be the same as those used for the blood samples from the same person.

FIGURE 1



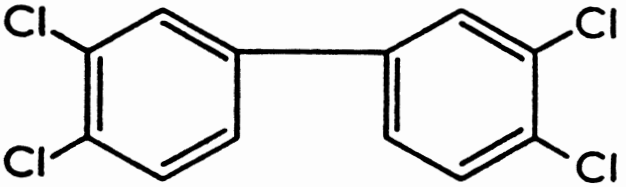
2,3,7,8-tetrachlorodibenzo-p-dioxin

(2,3,7,8-TCDD, "TCDD", "dioxin" - one of 75 possible polychlorinated dibenzodioxins or "PCDD's")



2,3,7,8-tetrachlorodibenzofuran

(2,3,7,8-TCDF, "TCDF" - one of 135 possible polychlorinated dibenzofurans or "PCDF'S")



3,4,3',4'- tetrachlorobiphenyl

(one of 209 possible polychlorinated biphenyls or "PCB's")

O, Cl: Atoms of oxygen and chlorine respectively. Line segments represent chemical bonds. Carbon atoms occur where line segments meet and are not explicitly represented. Parallel line segments represent double bonds. All carbon atoms are involved in four bonds to other atoms. Where only three bonds appear, the fourth is to an atom of hydrogen, which, by convention, is not shown.