

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Levels in Adipose Tissue of Exposed and Control Persons in Missouri

An Interim Report

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The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, commonly known as dioxin, was measured in the adipose tissue of 39 persons with a history of residential, recreational, or occupational exposure in Missouri and in 57 persons in a control group. All participants had detectable levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in their adipose tissue, but the exposed group had significantly higher levels. Levels of six of the exposed persons were more than five times greater than the level of the highest control. Measuring 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in adipose tissue provides a much improved index of exposure, an important advance for research studies evaluating the possible health effects of this compound.

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COMMONLY known as dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is created as an unwanted by-product during the synthesis of 2,4,5-trichlorophenol, a precursor of a number of commercial formulations,¹ which include 2,4,5-trichlorophenoxyacetic acid and hexachlorophene. In 1971, industrial sludge waste from a hexachlorophene production facility in Verona, Mo, was mixed with waste oil and sprayed for dust control on residential, commercial, and recreational areas of eastern Missouri near St. Louis. As of April 1986, forty sites that were related either to the contaminated oil or to other waste products generated at the

Verona chemical facility have been found to be contaminated at levels equal to or greater than one part of TCDD per billion parts of soil.²

Since TCDD is extremely toxic to certain animal species, epidemiologic studies were undertaken to determine whether exposure to TCDD in Missouri has resulted in adverse health effects to humans. An important component of these studies is the estimate of exposure to TCDD. Individuals are generally classified as exposed to TCDD if they were physically located at or near a contaminated site. More refined estimates of exposure require information on the length of time the individual was at a contaminated site, the concentration of TCDD at the site, the type of activity at the site, and the amount of TCDD entering humans through ingestion, inhalation, and dermal routes. These refined estimates require information that is either difficult or impossible to obtain on an individual basis.

Consequently, a potential major advance in estimating exposure would be the direct measurement of TCDD in the body. In the last few years, methods based on gas chromatography-mass spectrometry have been developed to measure TCDD in human adipose tissue.³ Although a few such measurements have been made on individuals with a history of exposure to TCDD, most measurements reported are from samples of adipose tissue obtained at autopsy from individuals with no known TCDD exposure.

The purposes of this study were to determine (1) whether concentrations of TCDD in adipose tissue differ in exposed and control groups and (2) whether TCDD levels are associated with specific demographic and exposure characteristics. We report herein the results for 39 persons with a history of exposure to TCDD and 57 persons with no known exposure. This is an interim report, because the investigation is still in progress.

METHODS

Study Design

The study was a cross-sectional comparison of TCDD levels in an exposed group and a control group from Missouri. From January 1983 to November 1985, the Missouri Department of Health compiled a central listing of individuals who believed they had been exposed to TCDD. The listing consisted of persons who volunteered to complete a questionnaire concerning their medical history, health habits, work history,

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and potential exposure to TCDD. Based on questionnaire responses, the eligible exposed group for this study consisted of approximately 400 persons who were (1) exposed to areas with TCDD levels in soil between 20 and 100 parts of TCDD per billion parts of soil (ppb) for two or more years or (2) exposed to TCDD levels greater than 100 ppb for at least six months. These soil levels were determined by the Environmental Protection Agency and reported to the State of Missouri in 1983.² Persons who met these criteria were classified as having one of three types of exposure: (1) residential, ie, either living in close proximity to areas with TCDD-contaminated soil or having evidence of contamination inside the home; (2) recreational, ie, riding or caring for horses in TCDD-contaminated stable arenas at least one time per week; or (3) occupational, ie, working either in a hexachlorophene production facility or at truck terminals where the grounds had been sprayed with TCDD-contaminated waste oil.

Each exposed participant donated approximately 20 g of subcutaneous adipose tissue from the anterior abdominal wall. A plastic surgeon administered local anesthesia, removed the tissue, and provided follow-up surgical care. Every eligible person who volunteered was included in the study, except one individual who was taking corticosteroids and was advised by his physician not to participate.

The control group was selected from persons undergoing elective abdominal surgery in one of three hospitals located in St Louis, Kansas City, and Springfield, Mo. Each participant in the control group donated approximately 20 g of subcutaneous adipose tissue from the anterior abdominal wall during an elective surgical procedure. When the study is completed, it is expected that the control group will be composed of persons from a broad age range, from both sexes, from white and other than white races, and from both urban and rural residence locations.

Based on responses from a questionnaire, individuals were excluded from the control group who had (1) more than one month of potential direct soil contact at a confirmed TCDD-contaminated site in Missouri;² (2) a history of military duty in Southeast Asia at any time from 1962 through 1970, or (3) occupational or commercial contact with trichlorophenol or its derivatives. Also excluded were persons who (1) had abnormal fat metabolism or were in a catabolic state (eg, had diabetes, lipodystrophies, excessive weight loss, or cancer); (2) were at increased risk of

complications from the biopsy (eg, had a bleeding disorder or immunosuppression); (3) were known to have acquired immunodeficiency syndrome, hepatitis, or active tuberculosis, thus imposing unacceptable risks to laboratory personnel processing the specimens; or (4) were part of a high-risk special study population (eg, children, pregnant women, and prisoners).

All adipose tissue samples were collected from July 1985 through November 1985. All participants were white. Informed consent was obtained from all participants after the nature of the procedure had been fully explained.

The data were analyzed by using standard programs from a statistical analysis system (Statistical Analysis System, Version 5 program package). The TCDD levels were approximately log-normally distributed. Regression analyses were performed with the natural log of TCDD as the dependent variable and age, sex, and exposure status as independent variables. Nonlinear and interaction terms were also tested in the multiple regression models, but these terms were not statistically significant ($P > .05$).

Laboratory Analyses

The adipose tissue specimens were collected, frozen under dry ice, and shipped to the Centers for Disease Control. Levels of TCDD in 10 g of adipose tissue were measured by high-resolution gas chromatography-high-resolution mass spectrometry and are reported on a whole-weight basis.^{4,5} The detection level of TCDD in 10 g of adipose tissue was one part of TCDD per trillion parts of tissue (ppt). The analyst was blind to whether the sample was from an exposed or a control individual. The analyst used two quality-control pools that had been previously characterized by this method and had been found to contain 1.6 and 8.4 ppt of TCDD. A sample of the 1.6-ppt quality-control pool and four adipose samples from Missouri made up an analytical run. Every fourth run, however, consisted of a sample of the 8.4-ppt quality-control pool, a duplicate fat sample selected from a preceding run, two more adipose samples from Missouri, and a reagent blank. An analytical run was considered out of control and was rerun if any one of the following conditions occurred: (1) the analytical standard was outside its 95% control limits, (2) the quality-control pool sample was outside its 99% control limits, or (3) two consecutive results from the quality-control pool were outside the 95% control limits.

RESULTS AND COMMENT

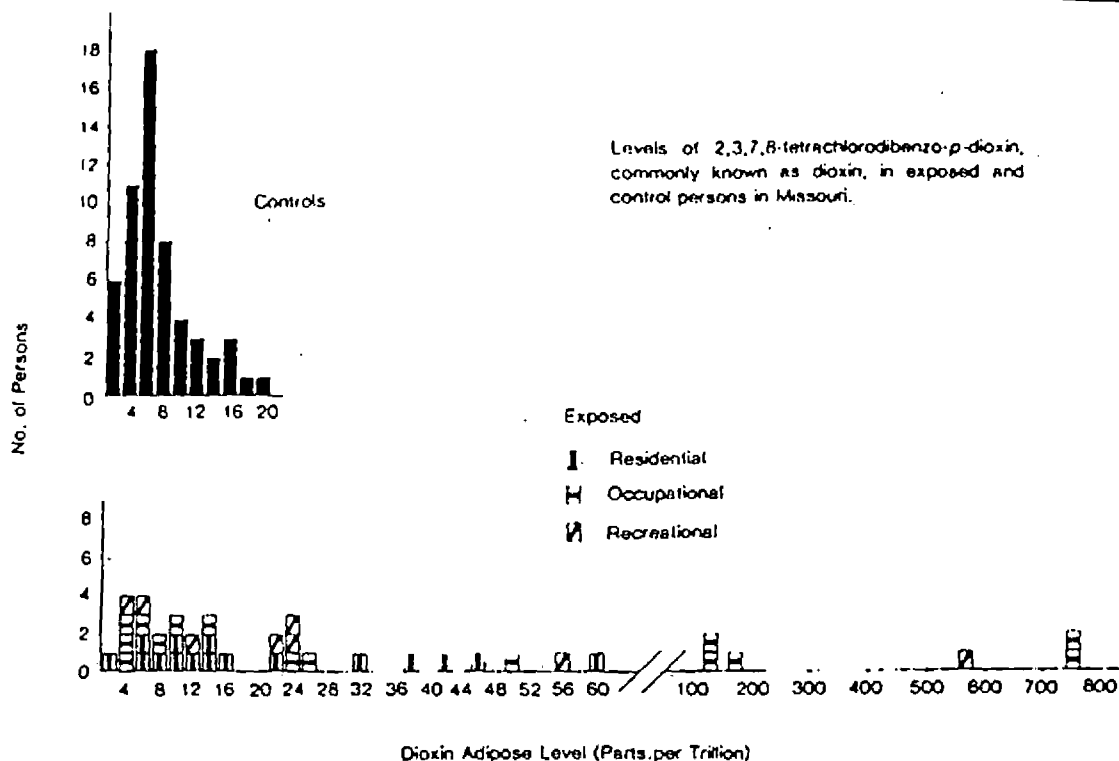
All persons in both the exposed and control groups had detectable levels of TCDD in their adipose tissue, as shown in the Figure and described in Table 1. Nineteen (49%) of the 39 exposed persons had measurements higher than the highest TCDD level (20.2 ppt) of the 57 controls. Six (15%) of the 39 exposed persons had TCDD levels greater than 100 ppt, which was five times higher than the level of the highest control. On the other hand, there were persons from all three exposure subgroups (residential, recreational, and occupational) with TCDD levels within the range of the control group. Levels in the occupational group were, in general, higher than those in the residential group.

The control group was analyzed to determine how age and sex influenced the TCDD levels. Regression analysis showed age to be a significant predictor of TCDD level ($P < .001$), with TCDD increasing approximately 1.3 ppt per decade. After adjustment for age differences, women had slightly higher TCDD levels that were of borderline statistical significance ($P = .05$). In Table 2, TCDD levels in the control group of this study are compared with levels for persons with no known exposure reported from four other studies. The mean values and ranges of values among these studies are similar.

The exposed and control groups differed in mean age and percentage of men (Table 1). After controlling for these differences by using multiple regression, exposure status was a significant predictor of TCDD levels ($P < .001$). Even after persons with TCDD levels greater than 100 ppt were excluded, TCDD levels in the exposed group were significantly higher than those in the controls ($P < .001$). The geometric mean for the exposed group was more than twice that of the controls.

As shown in the Figure, five of the six values greater than 100 ppt were from persons exposed to TCDD during the production of hexachlorophene. The other high value (577 ppt) was found in a man exposed to TCDD while horseback riding in a contaminated arena. Prior to this report, the two highest levels of TCDD in adipose tissue were (1) 1840 ppt, in a specimen obtained at autopsy from a 55-year-old woman who had been exposed to TCDD in Seveso, Italy,⁶ and (2) 130 ppt, in a specimen obtained at autopsy in Canada.⁷

Information on the length of time since last exposure is important for determining the half-life of TCDD in



humans. The half-life of TCDD in primates has been estimated to be about one year.⁸ Recently, an investigator in Switzerland voluntarily ingested radiolabeled TCDD, and from subsequent measurements on urine and feces, he estimated the half-life to be 4.95 years.⁹ Nygren et al¹⁰ measured TCDD in the adipose tissue of a man 31 years after he was exposed in an industrial accident and found a TCDD level of 100 ppt. Assuming first-order kinetics and a one-year half-life, the original level of TCDD in this man would have been 200 million ppb, or 20% of his fat mass—clearly impossible. However, if the half-life were eight years, the original level of TCDD in the subject of Nygren et al would have been 1600 ppt—a more plausible number based on our findings. In the present study, the individuals with TCDD levels of 122 ppt, 166 ppt, and 745 ppt gave a history of last exposure to TCDD from 12 to 14 years before their biopsy. Again, a one-year half-life leads to unacceptably high estimates of original TCDD levels in these men, supporting the contention that the half-life of TCDD in human adipose tissue is longer than one year. These data are consistent with five to eight years.

The exposed participants were probably not a representative sample of the

Table 1.—Comparison of the Levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (Parts per Trillion) in Adipose Tissue of Exposed and Control Groups

Variable	Controls	Exposed			
		Total	Recreational	Residential	Occupational
N	57	39	8	16	15
Arithmetic mean	7.4	79.7	90.8	21.1	136.2
Median	6.4	17.0	23.5	14.5	24.7
Range	1.4-20.2	2.8-750	5.0-577	2.8-59.1	3.5-750
Geometric mean	6.4	21.8	24.8	15.3	29.8
Mean age, y (SD)	52.6 (15.7)	44.3 (13.7)	42.1 (14.7)	39.7 (14.9)	50.3 (9.8)
% Men	35.1	61.5	37.5	43.8	93.3

Table 2.—Comparison of the Adipose Tissue Concentrations of TCDD in Populations With No Known TCDD Exposure*

Source of Specimens	N	Mean, Parts per Trillion	Range, Parts per Trillion
Present study: elective surgical patients in Missouri	57	6.4†	1.4-20.2
Autopsy specimens from Ohio ¹¹	6	...	5-12
Autopsy specimens from St Louis ¹² and Canada ⁷ and adipose from veterans ¹³	61†	7.5	1-15
Autopsy specimens from sudden deaths in St Louis ¹⁴	35	7.2†	...
Autopsy specimens from Georgia ⁸ and Utah ¹⁵	35	7.1†	2.7-19.0

*TCDD indicates 2,3,7,8-tetrachlorodibenzo-p-dioxin.

†Geometric mean.

‡Combination of results from references 7, 12, and 13. Three results from persons known to have had exposure to 2,4,5-trichlorophenol were excluded.

total exposed population. Because of the selection biases of volunteering, first for the central listing and second for excisional adipose tissue biopsy, true population means and ranges of persons exposed to TCDD in Missouri cannot be inferred from these data. We cannot predict the direction and magnitude of these biases. Likewise, the control group is drawn from hospitalized surgical patients and is a nonrandom sample. As a result, the values for the control group must be viewed as a reference range, not as the normal range for the general population of Missouri.

We conclude that the measurement of TCDD in adipose tissue is a much improved exposure index for studies evaluating the possible health effects of TCDD. Unfortunately, the specimen

must be obtained by a surgical procedure, albeit minor, thus precluding its widespread use. This problem could be overcome either by the development of a method for measuring TCDD levels in a more easily obtained specimen, such as blood, or by mathematical modeling of predictors of adipose TCDD levels that would permit construction of an accurate, noninvasive exposure index. Completion of this study will provide additional information on the epidemiologic correlates of exposure and on the range of TCDD values in a broad sample of the Missouri population.

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