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Determination and Measurement of Human Exposure to the Dibenzo-P-dioxins

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One of the major problems in the conduct of epidemiologic studies involving dioxins is in the identification of an exposed population. This is true whether one is talking about Vietnam veterans, Times Beach residents, children of Seveso, Italy, or individuals who consume dioxin-contaminated fish in Michigan or New York. Because dioxin, especially 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), occurs as an environmental contaminant only in trace quantities, its measurement in environmental substrates and human tissue requires complicated extraction procedures and highly sophisticated instrumentation, namely high resolution gas chromatography and high resolution mass spectrometry. To properly address the issue of documenting dioxin exposure, a review of literature is necessary.

Accidental exposure to TCDD. Confirmation that TCDD accumulates in human tissues was documented by Facchetti et al. (1981). This was the first case of human exposure to TCDD in which an analysis was made of cadaveric tissue to detect and study the distribution of dioxin. The subject of the study was a 55-year-old woman who had died from a pancreatic adenocarcinoma seven months after the ICMESA accident in Seveso, Italy in July, 1976. Although the cancer was not a result of the exposure to TCDD, the woman was significantly exposed to the toxic cloud. During the passage of the toxic cloud, the woman was eating a meal in her home with doors and windows open. In the four days after the event, the woman consumed vegetables from the garden attached to her home. Animals reared by the woman's family in an area adjacent to the home began to die over a period of 15 days after the event. The woman was evacuated from her home and associated area after 16 days. Subsequent tests for TCDD indicated that the subject had lived in a sector of zone A which had a mean soil concentration of 185 ug/m^2. On the basis of the circumstances recorded, it was presumed that the woman absorbed toxic substances contained in the cloud by inhalation, ingestion and contact. Although she did not develop toxic symptoms from the exposure, two young nephews living with her at the time of the accident devloped serious chloracne. The results (means of three independent determinations) for the GC-MS analysis for 2,3,7,8-TCDD in selected human tissues are shown in Table 1.

Table 1. Tissue concentrations, parts per trillion, of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the organs of a woman[a] who died of cancer seven months after exposure, Seveso, Italy

Sample	2,3,7,8-TCDD/wet tissue (ppt)
Fat	1840[b]
Pancreas	1040[bc]
Liver	150[bc]
Thyroid	85[c]
Brain	60[b]
Lung	60[b]
Kidney	40[b]
Blood	6[c]

[a] Data from Fachetti et al., 1981

[b] Values obtained at a resolution of 2,500

[c] Values obtained at a resolution of 10,000

As noted, 2,3,7,8-TCDD was present in all of the tissues analyzed. On the basis of concentrations observed, it was possible to distinguish four groups of tissues: adipose tissue and pancreas tissue with levels between 1,000 and 2,000 parts per trillion (ppt); liver tissue with levels between 100 and 200 ppt; other tissues (thyroid, brain, lungs, kidneys) with levels between 10 and 100 ppt; and blood with levels less than 10 ppt. The levels in the pancreas may have been abnormally high due to the presence of cancerous cells.

The data suggest that blood levels are one-tenth the level of TCDD in liver tissue and one one-hundredth the level in adipose tissue. Reggiani (1981), estimated that the TCDD body burden in the above subject at the time of death was 40 ug. Because human milk has a high fat content where TCDD could reside, Reggiani examined TCDD levels in human milk from those exposed to TCDD while living in Zones A and B of Seveso at the time of and immediately after the release of three deaths of lung cancer by 1967. In any event, not one of the 87 died of lung cancer. When they died, their lungs were examined and were found to be full of asbestos. They did not die of lung cancer. On the other hand, instead of three deaths among the cigarette smokers, there were 24. So the problem came up of multiple factor interaction.

When we did the study of the 17,800 men in the asbestos union, we again took smoking histories. For those who did not smoke and did not work with asbestos, the rate was 11 per 100,000 per year. For those who worked with asbestos but did not smoke, it was five times as much, 58. Five times a very low figure is still very little. On the other hand, for those who smoked but did not work with asbestos it was 122, and in those who smoked and worked with asbestos it was

601 per 100,000, an this extraordinary increase. These rates, by the way, are different for every tissue. It was true for lung cancer, for the esophagus and for the larynx. It was not true for mesothelioma and it was not true for the remainder of the gastrointestinal with levels between 1,000 and 2,000 parts per trillion (ppt); liver tissue with levels between 100 and 200 ppt; other tissues (thyroid, brain, lungs, kidneys) with levels between 10 and 100 ppt; and blood with levels less than 10 ppt. The levels in the pancreas may have been abnormally high due to the presence of cancerous cells.

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Table 2. TCDD levels, parts per trillion, in human milk from breastfeeding mothers exposed to TCDD in Seveso, Italy and South Vietnam

Sample Location	2,3,7,8-TCDD Level (Whole Milk Basio) (ppt)	Reference
Seveso (Zones A & B)	2.3 - 28.0	Reggiani, 1981
South Vietnam (Area sprayed with Agent Orange)	40.0 - 50.0	Baughman, 1976

Environmental Exposure to TCDD. The Environmental Protection Agency (EPA) has analyzed human adipose tissue for TCDD. Kutz (1981) reported on six specimens of human adipose tissue collected from residents of an urban Ohio county to serve as control specimens for some analytical studies done bythe EPA Dioxin Monitoring Program. These specimens were excised during post-mortem examinations from individuals with no recorded or known exposure to 2,4,5-T or Silvex. Subsequently, they were analyzed in duplicate following the EPA Dioxin Monitoring Program protocol. Instrumental determinations were conducted at two independent laboratories.

The results, shown in Table 3, demonstrated that all specimens contained residues of 2,3,7,8-TCDD. Levels ranged between five and 12 ppt, with a detection limit below 5 ppt. Kutz emphasized that all studies conducted to date, including this one, have been accomplished utilizing small sample sizes and deliberate specimen selection criteria. Consequently, these few data cannot be construed as being representative of the general population.

Table 3. Levels of 2,3,7,8-TCDD ppt, in human adipose tissue

Source	Total Number of Samples[a]	Number Positive	Range (ppt)	Mean + SD[b]	Reference
EPA Ohio Monitoring Program	6	6	5-12		Kutz
Great Lakes Area, Canada	23	22	4.1-21.8,130	10.7 + 5.4 (n = 21)	Ryan and Williams
			Kingston	12.4 + 5.8 (12)	
			Ottawa	8.6 + 4.4 (9)	
U.S. Veterans Administration	33	25	3-29,99	7.7 + 5.5 (24)	Hobson et al.
			Vietnam Experience	8.3 + 6.9 (13)	
			No Vietnam Experience	5.7 + 3.1 (11)	
Total	62	53	3-30	10 ppt	

[a] Detection limits defined in text

[b] Excluding outlying high samples

Recently, the Canadian scientists Ryan and Williams (1983) released data on the analysis of human fat tissue from the Great Lakes Area for 2,3,7,8-TCDD residues. The fat samples (10 to 20 g) were obtained from deceased elderly hospital patients from the communities of Kingston and Ottawa, Ontario. The results obtained by Ryan and Williams are also shown in Table 3. Levels of 2,3,7,8-TCDD were found in 22 of 23 samples analyzed. Values ranged from 4.1 to 130 ppt. Excluding the one outlying high sample, average values found were 10.7 + 5.4 (n = 21) with the highest value being 21.8 ppt. Grouping of the 22 samples which had been analyzed in a blind fashion with regard to origin showed that the 12 Kingston samlpes had an average of 12.4 + 5.8 ppt (n = 12) and the Ottawa samples 8.6 + 4.4 ppt (n = 9) but the difference was only significant at about P = 0.1 level. Ryan and Williams concluded from these data it would appear that most human fat tissues from older patients in the Great Lakes Area have low but measurable amounts of TCDD.

The issue of Agent Orange and the Vietnam veteran prompted a study of TCDD in human adipose tissue collected from U.S. Vietnam-Era veterans. This study, reported by Hobson et al. (1983), was initiated in 1979 with with the selection of two groups of adult males: (1) 21 Vietnam veterans, all but two of whom claimed health problems related to Agent Orange exposure, and who volunteered for the fat biopsy; and (2) 12 veterans with no service in Vietnam. Ten of the latter group had no exposure to any herbicides, were undergoing elective abdominal surgery and volunteerred to serve as controls. The other two individuals were active duty U.S. Air Force officers with known heavy and relatively recent exposure in connection with herbcide disposal operations. 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 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 results of this study are also shown in Table 3. Fourteen of the 21 Vietnam veterans had levels of TCDD in their adipose tissue at or above the detection limit. Three of these men had detectible material that could not be validated as TCDD or the measured value was only questionably above the detection limit. Six Vietnam veterans had TCDD in amounts from 5 to 7 ppt. Three Vietnam veterans had TCDD in amounts from 9 to 13 ppt. One veteran had 63 and 99 ppt, and another had 23 and 35 ppt.

Of the 13 individuals who had never served in Vietnam, five had TCDD identified in their fat (4, 6, 7, 7 and 14 ppt). Six had values low enough to be considered equivocal or the detected material was not validated as TCDD. The remaining veteran had no detectible TCDD. In the two Air Force officers with known heaviest exposure, TCDD measured was never more than 3 ppt above the limit of detection.

Among the 21 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 of this small pilot series was attempted.

Hobson et al. (1983) concluded that the results of the very complex and technically difficult analysis indicated that very low levels of TCDD, believed to be 2,3,7,8-TCDD, could be detected in human adipose tissue in the range 3-99 ppt. The levels, however, did not correlate well with known exposure and nonexposure, and there was no correlation with health status. The study results did indicate that the assay method was feasible, but would serve no clinically or administratively useful purpose until additional data are available on background levels of TCDD in the general United States population.

The available data on TCDD residues in human tissue suggest that TCDD can be distributed throughout body tissues following accidental exposures. In such situations, a major site for storage of TCDD in the human body is in the adipose tissue. However, significant levels of residue (albeit, much lower than in adipose tissue) may be found in the liver and human milk. The monitoring of persons not involved in episodic events involving TCDD suggests that adult human adipose tissue may have a "background" level of TCDD that may reflect a generalized contaminaion of our environment. This "background" level may be very low (approximately 10 ppt) and detectible only through the use of sophisticated analytical methods.

Intentional Exposure to TCDD. In a review of 23 industrial episodes involving human exposure to TCDD, the one consistent medical finding was "chloracne" (Young, 1978). Chloracne is a skin reaction characterized by an acniform dermatitis with comedones (blackheads) and inclusion cysts or papules, and frequently pustules so severe that they cause permanent scarring. Morphologically it is similar to adolescent acne, but it is usually more severe particularly on the upper face, ears and neck. Active chloracne lesions have been reported many years after exposure to TCDD, but the condition usually clears up spontaneously in a few months. The confirmation that the chloracnigen encountered in the industrial production of trichlorophenol was 2,3,7,8-TCDD involved the dermal testing of TCDD on humans.

Bauer et al. (1961) reported on an experiment in which an investigator applied a 0.01 percent solution containing TCDD to his forearm and developed lesions characteristic of chloracne. Mild dermatitis developed in two days, followed by comedones several days later.

In the mid-1960s, prisoners (volunteers) at Holmesburg Prison in Philadelphia were dermally exposed to 2,3,7,8-TCDD in an experiment designed to determine the dose required to induce chloracne. The dermatologist that administered the material, Dr. Albert Kligman,

never published the results, but the Dow Chemical Company, sponsor of the studies, released them in testimony in the EPA 2,4,5-T/Silvex Cancellation Hearing (Towe 1980). A one percent suspension of TCDD in alcohol/chloroform was applied to the backs of 10 subjects on alternate days for one month. This protocol resulted in the application of a cumulative dose of 7,500 ug of TCDD. Eight of the 10 subjects developed chloracne, which lasted four to seven months. No other adverse health effects were noted from urinalysis or chemical tests monitoring blood, liver and kidney function. A cumulative dose of 16 ug applied in the same manner was ineffective; no intermediate doses were tested. Asssuming that the entire quantity of TCDD was absorbed by the skin and if the average volunteer weighed 70 kg, then the dose of TCDD dermally-applied required to produce chloracne would be approximately 100 ug TCDD/kg body weight.

Earlier it was noted that the amount of TCDD in the women at Seveso (whose organs were analyzed at autopsy) was approximately 40 ug. She did not have chloracne but the blood and adipose tissues contained detectable levels of TCDD.

Discussion and Conclusion. There can be no doubt that exposure to TCDD occurred to individuals involved in the trichlorophenol industrial episodes where chloracne has been reported. Data by Rowe (Kligman Report) and Reggiani suggest that there is a threshold level at which chloracne occurs. Although there are no TCDD data on human tissue levels from individuals currently having chloracne, data by Rappe et al. (1983) suggest that the chloracnigens 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran persist for many years in body tissues. For the epidemiologist, it is important to know how long these chemnicals persist after acute and chronic exposures and what tissues should be monitored. The removal of adipose tissue during autopsy permits retrospective study, but similar surgical procedures for prospective studies may require the resolution of a series of medico-legal issues.

Blood analysis for TCDD has been effectively employed by Rappe et al. and Facchetti (1981), but Bickel et al. (1983) has pointed out that "fat storage is the chief contributor to the body burden of the polychlorinated xenobiotics." Characteristically, the concentrations of polychlorinated compounds in adipose tissue may be 1000 fold greater than in plasma. This suggests that the plasma levels of TCDD in individuals reported earlier by Hobson et al. and Ryan and Williams would be in the femtogram (1 X 10-15 gram) concentration, a level below the current state of the art. Although the analytical chemist may soon perfect techniques to measure such low concentrations, the biologist, physician and epidemiologist will likely conclude that it has no biologic significance in view of the overwhelming effects of life style and other chemicals in our environment.

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