

CHAPTER 2

DIOXIN ASSAY

SAMPLE ACQUISITION

Blood for the serum dioxin assay was drawn on the morning of the second day of the physical examination in 1987. Participants who volunteered to give blood for the dioxin assay fasted after midnight (water was allowed). Blood was drawn from the participants with a 15-gauge needle into a blood pack unit without anticoagulant. The blood pack units had been tested previously by the Centers for Disease Control (CDC) and were found to be free of dioxin contamination. Participants selected for the immunology studies had 250 ml of blood drawn; all others had 350 ml of blood drawn. After drawing, the bags were clamped, labeled, placed upright at room temperature, and allowed to clot for 7 hours. Appendix B-1 contains the Scripps Clinic and Research Foundation's (SCRF) procedure for the dioxin blood collection and processing.

The unit bags were centrifuged for 15 minutes at 4500 RPM at a temperature of 4°C to 10°C. The serum was then transferred to transfer packs (also dioxin-free) from the spun unit bag by a plasma extractor. The transfer packs were spun for 15 minutes at 4500 RPM. The serum was then placed into four Wheaton bottles: two 4-ounce bottles for the serum dioxin analysis, a 5 ml bottle for the lipid profile, and a 10 ml bottle for reserve serum. Samples were logged and stored at -20°C or less until shipment. Frozen samples, packed in dry ice in styrofoam boxes, were shipped twice weekly from SCRF, La Jolla, California, to Brooks Air Force Base, Texas. At Brooks Air Force Base, inventory was taken and the specimens were stored at -70°C until shipment to the CDC. All samples were coded so that the CDC was blinded to the group status (Ranch Hand, Comparison) of each specimen.

ANALYTICAL METHOD

The serum samples were analyzed for dioxin in analytical runs that consisted of a method blank, three unknown samples, and a quality control pool sample (1, 2). Cholesterol esters, triglycerides, and high-density lipoprotein cholesterol were determined in duplicate by standard methods. Total phospholipids were determined in duplicate by modifying (3) the Folch et al. procedure (4). Fresh cholesterol was determined in duplicate by an enzymatic method (5). For each analysis, the results of the duplicate analyses were averaged and the mean was used. These results were used to calculate the concentrations of (a) total lipids using the summation method (6), (b) low-density lipoprotein cholesterol, and (c) very low-density lipoprotein cholesterol (7).

QUALITY CONTROL

Quality assurance was maintained with matrix-based materials that are well characterized for dioxin concentration and isotope ratios to ensure that the analytical system was in control. Quality control (QC) charts were maintained for each of these materials (five serum pools). The concentration in the QC sample from each analytical run must be within 99 percent confidence limits established for the QC material (8, 9). The unlabeled and carbon-13 labeled internal standard isotope ratios must be within 95 percent confidence limits. All analytical runs for the dioxin and lipid measurements were in control. No dioxin was detected

TABLE 2-1.
Report Field Definition

Report Field Value	Definition
G	Good result
GML	Good result, missing lipids
GND	Good result, below limit of detection
GNQ	Good result, below limit of quantitation
NR	No result

in the blanks (on-column injection of 100 femtograms from a standard solution produces detectable signals that are greater than three times the background noise).

DATA DELIVERED TO THE AIR FORCE BY THE CENTERS FOR DISEASE CONTROL

The dioxin data used in this report were derived from a data base of results on 932 Ranch Hands and 888 Comparisons delivered by the CDC in January 1990. The CDC sent data on whole-weight and lipid-weight dioxin concentrations to the Air Force together with the total sample weight, weights of lipid fractions, total lipid weight, the detection limit, quantitation limit, and all associated QC information, including results from blank samples. Table 2-1 defines a "report" field in the data base.

Some participants (150 Ranch Hands and 50 Comparisons) participated in a pilot dioxin study in April 1987 (8). Four of these (three Ranch Hands and one Comparison) had a missing dioxin result (report=NR), the rest had good results (report=G). The remaining 147 Ranch Hands and 49 Comparisons were included in the dioxin data base from which the analysis data set for this report was derived. Of these, 145 Ranch Hands and 48 Comparisons were also fully compliant to the 1987 physical examination. Forty-seven of the pilot study participants (43 Ranch Hands and 4 Comparisons) also had blood drawn for the dioxin assay at the 1987 physical examination (May 1987 through March 1988). If a participant was assayed during the pilot study but not at the 1987 physical examination, or if he was assayed at the pilot study and at the 1987 physical examination, then his pilot study assay was used.

Table 2-2 shows counts of study participants by group, report, and compliance to the 1987 physical examination.

TABLE 2-2.
Sample Sizes by Group, Report, and Compliance to the
1987 Physical Examination

Report	Ranch Hand		Comparison	
	Fully Compliant	Noncompliant	Fully Compliant	Noncompliant
G	858	2	761	1
GML	0	0	1	0
GND	8	0	43	0
GNQ	20	0	51	0
NR	44	0	31	0
Total	930	2	887	1

Missing dioxin results (report=NR or GML) and nonquantifiable dioxin results (report=GNQ) were excluded from analysis in this report. The resulting effective sample sizes (866 Ranch Hands and 804 Comparisons) were determined by the condition that the participants were fully compliant to the 1987 physical examination. Table 2-3 summarizes this sample size reduction.

TABLE 2-3.
Sample Sizes Used in This Report

		Ranch Hand	Comparison
Fully compliant to 1987 physical examination and assayed for dioxin		930	887
Less	Report		
	GNQ	(20)	(51)
	NR	(44)	(31)
	GML	(0)	(1)
Total		866	804

TABLE 2-4.

Dioxin Result Summary of 866 Ranch Hands and 804 Comparisons

Stratum	Ranch Hands			Comparisons		
	n	Median	Range	n	Median	Range
Officer	319	7.8	0-42.6	291	4.7	0-18.5
Enlisted Flyer	148	18.1	0-195.5	127	4.0	0-12.8
Enlisted Groundcrew	399	24.0	0-617.8	386	4.0	0-54.8
Total	866	12.8	0-617.8	804	4.2	0-54.8

Table 2-4 summarizes, by military occupation and group, the dioxin results among the 866 Ranch Hands and 804 Comparisons whose results were used in analyses of dioxin versus health in this report.

The 95th, 98th, and 99th percentiles of the Ranch Hand dioxin distribution were 110.8, 168.0, and 211.0 ppt; the corresponding Comparison percentiles were 8.3, 10.2, and 14.2 ppt.

CDC subsequently provided 314 Comparison dioxin results after January 1990 (the beginning date for statistical analyses involving Comparison data). Of these 314 dioxin results, 253 had a report field value of G or GND, 24 had a report field value of GNQ, and 37 had a report field value of NR (no result). Of the 253 Comparisons, the median current dioxin result was 4.1 ppt, the range of levels was between 0 ppt and 13.6 ppt, and the first and third quartiles were 2.9 ppt and 5.8 ppt. The percentages of the 253 Comparisons and of the 804 Comparisons analyzed in this report, having levels less than 10 ppt, were 97.8 and 97.6, respectively. A statistical contrast of the dioxin distributions of these 253 and the 804 Comparisons included in this report revealed no significant difference ($p=0.15$), as expected.

The phrase "serum dioxin" is used throughout this report and is defined as the serum lipid-weight concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Its relationship with dioxin concentrations in other compartments, such as adipose tissue, is a subject of continuing research. The lipid-weight dioxin measurement, also called "current dioxin body burden" in this report, is a derived quantity calculated from the formula $\text{ppt} = \text{ppq} \cdot 102.6 / W$, where ppt is the lipid-weight concentration, ppq is the actual weight of dioxin in the sample in femtograms, 102.6 corrects for the average density of serum, and W is the total lipid weight of the sample (9). The correlation between the serum lipid-weight concentration and adipose tissue lipid-weight concentration of TCDD has been observed to be 0.98 in 50 persons from Missouri (10). Using the same data, Patterson et al. calculated the partitioning ratio of dioxin between adipose tissue and serum on a lipid-weight basis as 1.09 (95% C.I.: [0.97,1.21]). On the basis of these data, a one-to-one partitioning ratio of dioxin between lipids in adipose tissue and the lipids in serum cannot be excluded. Measurements of dioxin in adipose tissue generally have been accepted as representing the body burden concentration of dioxin. The

high correlation between serum dioxin levels and adipose tissue dioxin levels in their study suggests that serum dioxin is also a valid measurement of dioxin body burden.

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