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2 THE DIOXIN ASSAY

2.1 PARTICIPANTS SELECTED FOR DIOXIN MEASUREMENT

The eligibility for participants at the 1997 physical examination to have a blood measurement of dioxin was determined by assignment to one of three categories: (a) previous participants with a quantitative dioxin result who were selected for an additional blood measurement of dioxin to advance pharmacokinetic studies (1), (b) previous participants returning to the 1997 physical examination with no prior dioxin blood measurement or no previously quantitative dioxin results, and (c) first-time participants. Of the 2,121 participants at the 1997 follow-up examination, a total of 594 participants were asked to provide a blood sample for use in analysis of serum dioxin levels. Table 2-1 shows the number of participants selected for the 1997 dioxin blood measurement belonging to each category by exposure group (Ranch Hand, Comparison). Table 2-1 also gives the number of actual dioxin assay results obtained that belonged to each category by exposure group.

Table 2-1. Participants with a 1997 Blood Measurement of Dioxin

| Category | Number Eligible | | | Number of Results | | |
|---|-----------------|------------|------------|-------------------|------------|------------|
| | Ranch Hand | Comparison | Total | Ranch Hand | Comparison | Total |
| Returning participants with a previous quantitative dioxin result selected for another blood measurement of dioxin to advance pharmacokinetic studies | 430 | 0 | 430 | 421 | 0 | 421 |
| Returning participants who either attended the 1987 or 1992 follow-ups but had no previous dioxin blood measurement or no previous quantitative dioxin result | 18 | 42 | 60 | 17 | 40 | 57 |
| Participants who were selected for a dioxin blood measurement for the first time | 11 | 93 | 104 | 5 | 80 | 85 |
| Total | 459 | 135 | 594 | 443 | 120 | 563 |

Table 2-2 displays the reasons why blood samples from 31 participants were not obtained. Nine participants were medically deferred because of pending surgery or a low hemoglobin level, and 22 participants refused the blood measurement of dioxin. Samples for the remaining 563 participants were shipped to the Centers for Disease Control and Prevention (CDC) for analysis.

Table 2-2. Participants Eligible for the 1997 Blood Measurement of Dioxin and Reasons for Participant Sample Exclusions

| Distribution of Sample Exclusion | Ranch Hand | Comparison | Total |
|--|------------|------------|-------|
| Total Eligible for Blood Measurement of Dioxin | 459 | 135 | 594 |
| Less: | | | |
| Medically Deferred | (7) | (2) | (9) |
| Refused | (9) | (13) | (22) |
| Total Specimens Sent to CDC | 443 | 120 | 563 |

2.2 SAMPLE ACQUISITION

Following a CDC protocol, blood was drawn from consenting participants for the serum dioxin assay on the morning of the second day of the 1997 physical examination. The participants were instructed to fast after midnight (water was allowed), and samples were drawn with a 15-gauge needle into a blood pack unit without anticoagulant. CDC purchased blood bags in lots of 1,200, packaged in 50 boxes of 24 bags per box, and tested one bag per box to assess dioxin contamination. If the tested bag was found to be free of dioxin contamination, the box of 24 bags was shipped to the Air Force for use in the study.

Participants had 280 ml of blood drawn. After the draw, the bags were clamped, labeled, placed upright, and the samples were allowed to clot at room temperature for 7 hours.

The clotted samples were centrifuged for 15 minutes at 4,500 revolutions per minute between 4° and 10 °C. The serum was then transferred from the spun unit bag by a plasma extractor to transfer packs that also were tested and found to be free of dioxin. The transfer packs were then spun for 15 minutes at 4,500 revolutions per minute. The serum was 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 the reserve serum. Samples were catalogued and stored at -70 °C or colder until shipment. Appendix A contains the detailed procedures used by Scripps Clinic for the dioxin blood collection and processing. Frozen samples were packed in dry ice in Styrofoam boxes and shipped weekly from Scripps Clinic in 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 CDC. All samples were coded so that the CDC staff was blinded to the exposure group status (Ranch Hand, Comparison) of each specimen.

2.3 ANALYTICAL METHOD

The serum samples were analyzed for dioxin in groupings consisting of a method blank, three unknown samples, and a quality control (QC) pool sample (2, 3). Cholesterol esters, triglycerides, and high-density lipoprotein cholesterol were determined in duplicate by standard methods. Total phospholipids were determined in duplicate by modifying the Folch, et al., procedure (4, 5). Free cholesterol was determined in duplicate by an enzymatic method (6). For each analysis, the mean result of duplicate analyses was used to calculate the concentrations of total lipids using the summation method (7), low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol (8).

2.4 QUALITY CONTROL

Quality assurance was maintained with matrix-based materials well characterized for dioxin concentration and isotope ratios to ensure that the analytical system was in control. QC charts were maintained for each of these materials (five serum pools). The concentration in the QC sample from each analytical run was

required to be within established 99-percent confidence limits (9, 10). The unlabeled and carbon-13 labeled internal standard isotope ratios were required to be within 95-percent confidence limits. All analytical runs for the dioxin and lipid measurements were in control. No dioxin was detected in the blanks (on-column injection of 100 femtograms from a standard solution produces detectable signals greater than three times the background noise).

2.5 DATA DESCRIPTION

CDC delivered whole-weight and lipid-adjusted dioxin concentrations to the Air Force, together with the total sample weight, weights of lipid fractions, total lipid weight, detection limit, quantitation limit, and all associated QC information, including results from blank samples. The lipid-adjusted dioxin concentration was calculated using the whole-weight dioxin concentration and the total lipid weight. Details of the calculation are discussed subsequently in this chapter. Table 2-3 provides the results of the 1997 physical examination blood measurements of dioxin by exposure group and result comment. Result comments are based on whether the result was measurable, or good, (G); measurable, but below the limit of detection (GND) or below the limit of quantitation (GNQ); or no result was obtained (NR).

Table 2-3. Result Comments for the 1997 Blood Measurements of Dioxin

| Result Comment | Ranch Hand | Comparison | Total |
|--|------------|------------|-------|
| Good Result (G) | 430 | 82 | 512 |
| Good Result, Below Limit of Detection (GND) | 11 | 35 | 46 |
| Good Result, Below Limit of Quantitation (GNQ) | 0 | 0 | 0 |
| No Result (NR) | 2 | 3 | 5 |
| Total | 443 | 120 | 563 |

Note: The two Ranch Hands with no result at the 1997 follow-up examination had a good result at a previous follow-up examination.

The Air Force Health Study (AFHS) dioxin database is a combination of the dioxin assay results from the 1987, 1992, and 1997 examinations. Table 2-4 shows the number of blood measurements of dioxin by year and illustrates the high percentage of study participants who have had dioxin measurements. Of the 2,121 fully compliant participants for the 1997 study, 2,101 (99.1%) had blood measurements of dioxin in 1997 or in a previous study.

Table 2-4. Dioxin Results for 1997 Physical Examination Participants

| Years of Dioxin Blood Measurement Result | Ranch Hand | Comparison | Total |
|--|------------|------------|-------|
| No Dioxin Blood Measurement | 6 | 14 | 20 |
| 1987 Only | 297 | 865 | 1,162 |
| 1992 Only | 56 | 118 | 174 |
| 1997 Only | 12 | 93 | 105 |
| 1987 and 1992 | 68 | 134 | 202 |
| 1987 and 1997 | 153 | 6 | 159 |
| 1992 and 1997 | 5 | 7 | 12 |
| 1987, 1992, and 1997 | 273 | 14 | 287 |
| Total | 870 | 1,251 | 2,121 |

Note: 1987 includes participants from both the 1987 pilot study and the 1987 follow-up physical examination.

Participants may have been assayed during any combination of four events: the pilot study conducted in April 1987 (9), the 1987 follow-up examination (May 1987 to March 1988), the 1992 follow-up examination (May 1992 to March 1993), or the 1997 follow-up examination (May 1997 to April 1998). The majority of participants had an assay in 1987, through either the pilot study or the 1987 follow-up examination. Consequently, 1987 was designated as the reference point for post-Southeast Asia (SEA) serum dioxin levels, termed “current dioxin” in previous AFHS reports and “1987 dioxin” subsequently in this report.

Each participant with a good (G or GND) dioxin result was given a “reference” dioxin assay result derived from the good result. When a participant had multiple assay results, first priority was given to the 1987 pilot-study dioxin results, second priority was given to results derived from serum collected at the 1987 physical examination, third priority was given to the 1992 results, and fourth priority was given to the 1997 results. Figure 2-1 outlines this decision process and shows that the first quantitative result was used.

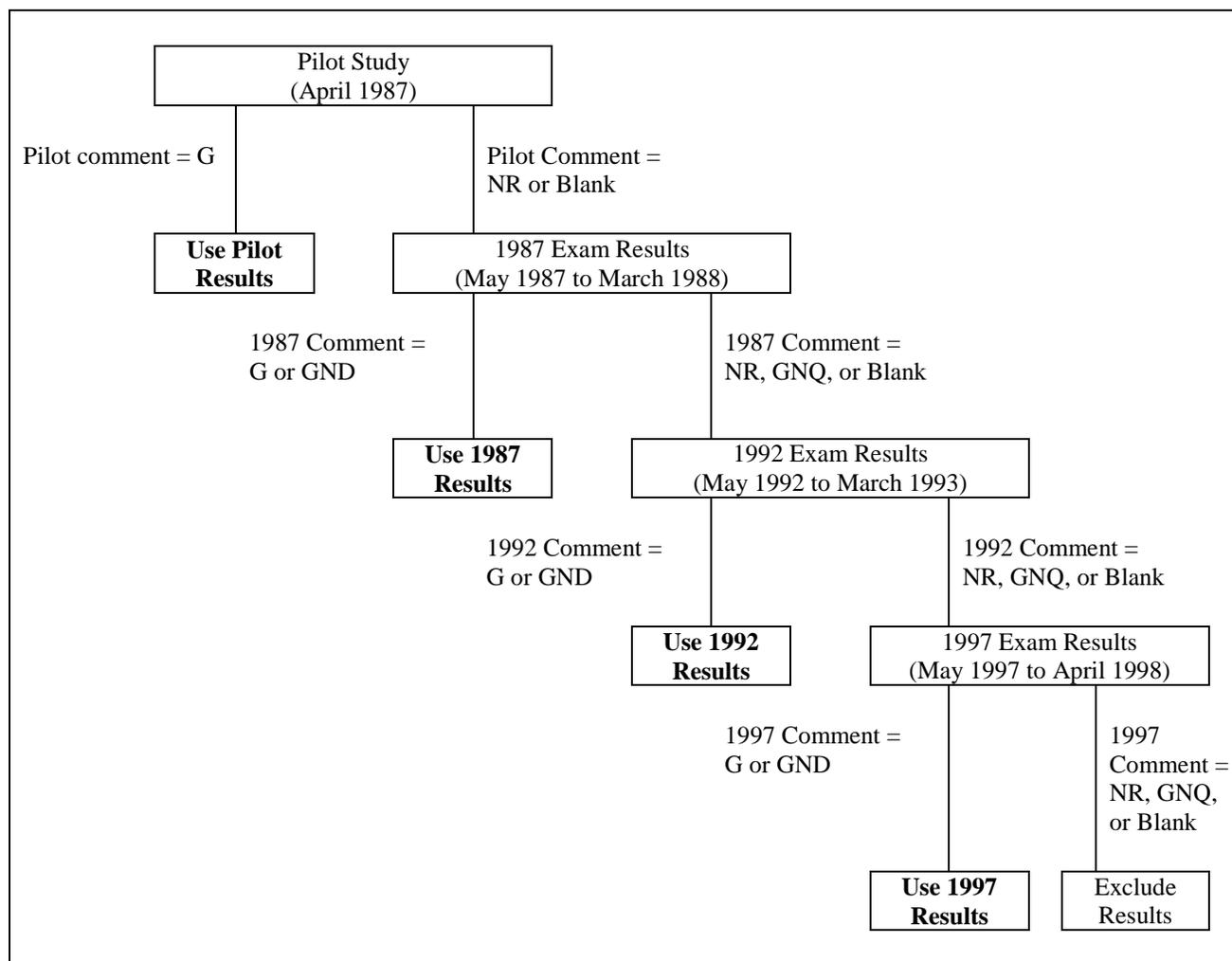


Figure 2-1. Decision Process for Determination of Dioxin Results for Analysis

Of the 2,121 fully compliant participants at the 1997 physical examination, 870 were Ranch Hands and 1,251 were Comparisons. Of the 2,121 participants, 20 had never had blood measured for dioxin. Six participants had missing dioxin results (result comment = NR) or nonquantitative dioxin results (result comment = GNQ). A total of 2,095 participants, consisting of 863 Ranch Hands and 1,232 Comparisons, had quantitative dioxin measurements. Table 2-5 summarizes the sample sizes by exposure group. The six participants with missing or nonquantitative dioxin results are cross-classified in Table 2-6 by result comment and exposure group.

Table 2-5. Results from Blood Measurements of Dioxin

| Summary of Sample Size Reduction | Ranch Hand | Comparison | Total |
|--|------------|------------|-------|
| 1997 Follow-up Participants | 870 | 1,251 | 2,121 |
| Less: No Blood Measurement of Dioxin at any Physical Examination | (6) | (14) | (20) |
| 1997 Follow-up Participants with a Dioxin Assay | 864 | 1,237 | 2,101 |
| Less: Missing or Nonquantitative (Good Result, but Below Limit of Quantitation or No Result) | (1) | (5) | (6) |
| 1997 Follow-up Participants with Quantitative Dioxin Results | 863 | 1,232 | 2,095 |

Table 2-6. Results from Blood Measurements of Dioxin with Missing or Nonquantitative Results

| 1987 Assay | 1992 Assay | 1997 Assay | Ranch Hand | Comparison | Total |
|------------|------------|------------|------------|------------|-------|
| GNQ | | | 1 | 1 | 2 |
| GNQ | GNQ | | 0 | 1 | 1 |
| GNQ | | NR | 0 | 1 | 1 |
| | | NR | 0 | 2 | 2 |
| Total | | | 1 | 5 | 6 |

Note: GNQ = Good result, below level of quantitation.
NR = No Result.

If the 1987 pilot study or follow-up measurement was not used, the 1987 dioxin level was derived for each participant in the following manner. If the 1992 measurement was used, the level was extrapolated to 1987 levels when the 1992 dioxin concentration surpassed 10 parts per trillion (ppt). These extrapolated lipid-adjusted dioxin values were calculated using a first-order elimination model with a half-life of 8.7 years and a background level of 4 ppt. Levels at or below 10 ppt were not extrapolated because the first-order elimination model was not considered to be valid at background levels (lipid-adjusted 1987 dioxin levels ≤ 10 ppt). If the 1997 measurement was used, the level was extrapolated to 1987 levels when the 1997 dioxin concentration surpassed 10 ppt. Details on the extrapolation method are given in Chapter 7, Statistical Methods. A summary detailing the year the measurement was used and whether the dioxin level was extrapolated to 1987 dioxin levels is provided in Table 2-7 by exposure group.

Table 2-7. Summary of Number of Assays Used for 1997 Follow-up Participant Dioxin Measures

| Study | Ranch Hand | Comparison | Total |
|--------------------------|------------|------------|-------|
| Pilot (1987) | 127 | 44 | 171 |
| 1987 Follow-up | 615 | 858 | 1,473 |
| 1992 Follow-up | 99 | 213 | 312 |
| Extrapolated to 1987 | 35 | 0 | 35 |
| Not Extrapolated to 1987 | 64 | 213 | 277 |
| 1997 Follow-up | 22 | 117 | 139 |
| Extrapolated to 1987 | 4 | 0 | 4 |
| Not Extrapolated to 1987 | 18 | 117 | 135 |
| Total | 863 | 1,232 | 2,095 |

2.6 LIPID-ADJUSTED AND WHOLE-WEIGHT CURRENT DIOXIN MEASUREMENTS

Serum dioxin is defined as the serum concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). It can be expressed as a lipid-adjusted or a whole-weight measurement. The lipid-adjusted dioxin measurement, also called “current dioxin body burden,” is a derived quantity calculated from the formula $ppt = ppq \cdot 102.6/W$, where ppt is the lipid-adjusted concentration, ppq (parts per quadrillion) is the actual weight of dioxin in the sample (also known as whole-weight dioxin) in femtograms, 102.6 corrects for the average density of serum, and W is the total lipid weight of the sample (10).

The correlation between the serum lipid-adjusted concentration and adipose tissue lipid-adjusted concentration of dioxin has been observed to be 0.98 in 50 persons from Missouri (11). Using the same data, Patterson, et al., calculated the partitioning ratio of dioxin between adipose tissue and serum on a lipid-adjusted basis as 1.09 (95% confidence interval: [0.97,1.21]). On the basis of these data, a one-to-one partitioning ratio of dioxin between lipids in adipose tissue and 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 the study by Patterson, et al., suggests that serum dioxin is also a valid measurement of dioxin body burden.

Figures 2-2 and 2-3 show the distribution of serum lipid-adjusted dioxin for the 863 Ranch Hands and 1,232 Comparisons whose results were used in analyses of 1987 dioxin versus health in this report. Figure 2-4 compares distributions of serum lipid-adjusted dioxin concentrations for Ranch Hands and Comparisons on the same scale (parts per trillion). Figure 2-5 compares distributions of the logarithm (base 2) of serum lipid-adjusted dioxin concentrations for Ranch Hands and Comparisons on the same scale.

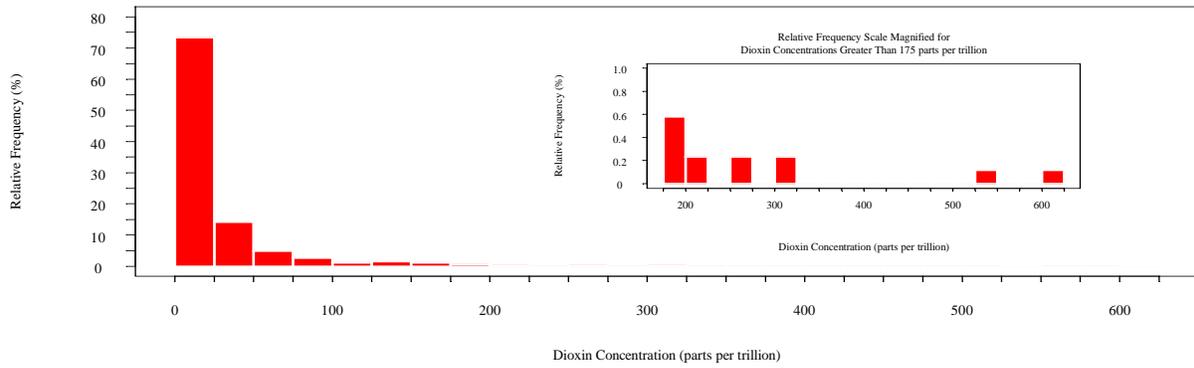


Figure 2-2. Relative Frequency Distribution of Lipid-adjusted Dioxin Concentrations for 863 Ranch Hands

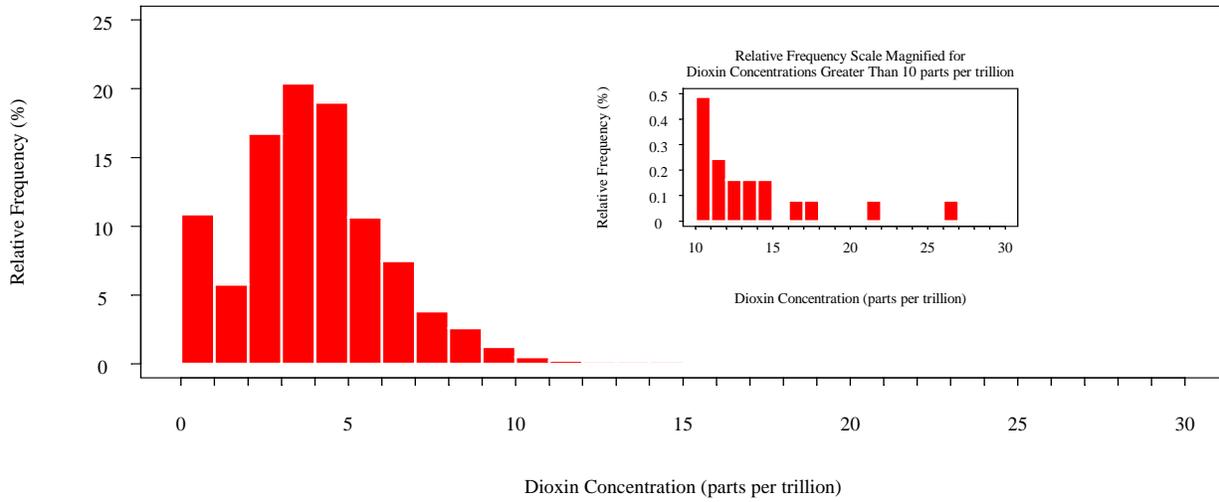


Figure 2-3. Relative Frequency Distribution of Lipid-adjusted Dioxin Concentrations for 1,232 Comparisons

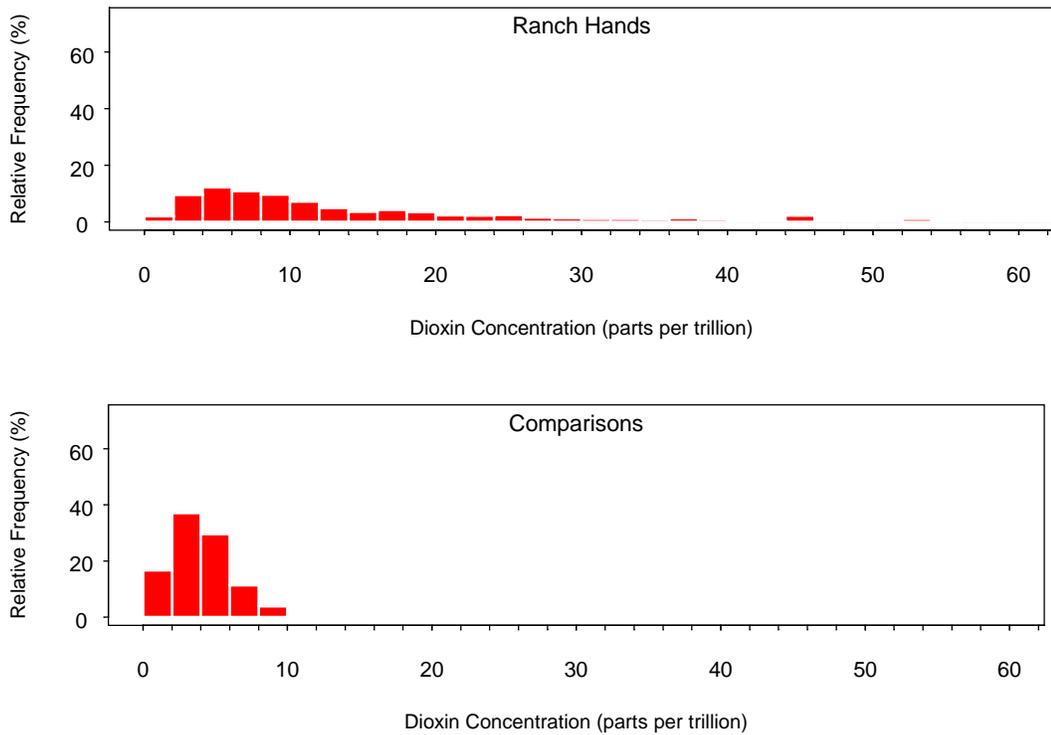


Figure 2-4. Relative Frequency Distribution of Lipid-adjusted Dioxin Concentrations

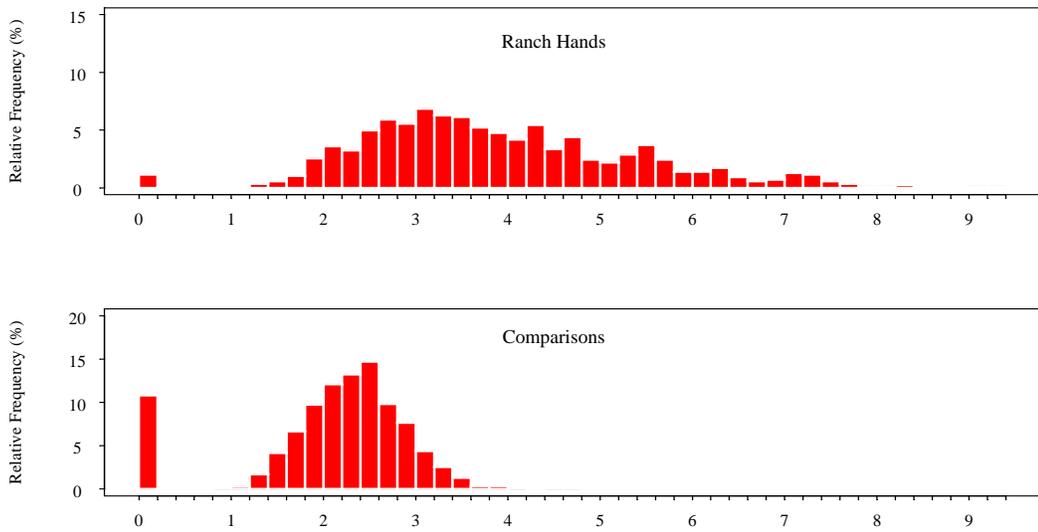


Figure 2-5. Relative Frequency Distribution of the Logarithm (Base 2) of Lipid-adjusted Dioxin Concentrations

Table 2-8 summarizes, by military occupation and exposure group, the serum lipid-adjusted dioxin results among the 863 Ranch Hands and 1,232 Comparisons whose results were used in the analyses of dioxin versus health in this report. For Ranch Hands, the median level was greatest for enlisted groundcrew and least for officers.

Table 2-8. Lipid-adjusted Dioxin Result Summary

| Military Occupation | <u>Ranch Hand</u> | | | <u>Comparison</u> | | |
|---------------------|-------------------|--------------|-------------|-------------------|--------------|-------------|
| | n | Median (ppt) | Range (ppt) | n | Median (ppt) | Range (ppt) |
| Officer | 337 | 7.4 | 0-36.0 | 486 | 4.0 | 0-17.3 |
| Enlisted Flyer | 151 | 16.4 | 0-195.5 | 186 | 3.8 | 0-12.8 |
| Enlisted Groundcrew | 375 | 24.0 | 0-617.8 | 560 | 3.6 | 0-26.6 |
| Total | 863 | 11.6 | 0-617.8 | 1,232 | 3.8 | 0-26.6 |

Note: ppt = parts per trillion.

2.7 SUMMARY

In summary, serum was collected for dioxin analysis for 563 participants at the 1997 follow-up at Scripps Clinic. The serum was shipped from Scripps Clinic to Brooks Air Force Base to CDC according to rigid protocols. The data collected from the 1997 follow-up assays were combined with data from the 1987 pilot study, 1987 follow-up examination, and 1992 follow-up examination for use in pharmacokinetic studies and for determining post-SEA dioxin levels. After combining data from this and previous follow-ups, a total of 863 of the 870 Ranch Hands (98.5%) and 1,232 of the 1,251 Comparisons (99.1%) attending the 1997 follow-up examination had quantitative dioxin assay results.

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