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## **4 PHYSICAL EXAMINATION METHODOLOGY**

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The 1997 follow-up examination was given to 2,121 invited and scheduled participants, who traveled to the examination site at Scripps Clinic in La Jolla, California. The examination consisted of the following major elements:

- Adipose tissue extraction
- Laboratory testing
- Medical outbriefings
- Physical examination
- Psychological testing
- Specialized testing (e.g., phlebotomy for measurement of serum dioxin).

The Combat Experience Questionnaire and skin, hair, and eye color determinations (components of the 1985 follow-up examination) were administered to all participants who did not attend the 1985, 1987, and 1992 follow-up examinations.

The Air Force carefully prescribed the details of the above examination elements in the Examiners' Handbook, provided in Appendix B. All physical examination procedures were approved by the Air Force Research Laboratory Institutional Review Board (IRB) at Brooks Air Force Base and by the Scripps Clinic IRB. Clinical variations were neither desired nor authorized; all proposed examination procedural changes were reviewed in detail by Air Force technical and contractual personnel prior to the start of the examinations. An important objective of the entire physical examination process was to ensure that bias was not created by any procedural change. This objective was carried out successfully.

The requirement to maintain blind examinations was particularly stringent. The clinical staff was prohibited from knowing or seeking information as to the group identity (i.e., Ranch Hand, Comparison) of any participant. At the end of his examination, each participant was asked to note on the critique form whether such information was sought by any member of the clinical or paramedical staff. In 1997, nine participants indicated that an examining physician had asked them about specific duties in Southeast Asia (SEA). Two of these participants later stated that they had answered erroneously. Three participants stated that they had not been questioned but rather had volunteered information in casual conversation. The balance of the nine participants could not be identified because they chose to remain anonymous. In all known cases, the physician or technician involved was reminded to be more careful in his or her conversations.

### **4.1 EXAMINATION CONTENT**

The examination content, as designed by the Air Force, emphasized detection of medical endpoints suspected of being associated with exposure to phenoxy herbicides, chlorophenols, or dioxin. In each follow-up study, the Air Force has used findings from the previous examination to refine the current examination.

The general content of the 1997 physical examination and psychological test battery is shown in Table 4-1. The complete laboratory test series accomplished at Scripps Clinic is displayed in Table 4-2.

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**Table 4-1. Elements of the 1997 Follow-up Physical Examination**

Elements	Remarks
Adipose Tissue Extraction	313 Participants
Chest X Ray	Radiologist
Dermatologic Examination	Dermatologist
Doppler	Technician; Caffeine and Nicotine Abstinence
Electrocardiogram	Caffeine and Nicotine Abstinence
General Physical Examination	Internist
Immunologic Studies	40% Random Sample
Neurological Examination	Neurologist
Patient Outbriefing	Internist, Medical Diagnostician
Psychological Evaluation: Symptom Checklist 90-Revised (SCL-90-R) Jenkins Activity Survey	
Pulmonary Function	Internist with Subspecialty in Pulmonary Disease
Vibrotactile Threshold	Technician

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**Table 4-2. Laboratory Test Procedures Performed at Scripps Clinic**

<b>Chemistry</b>	
2-hour Postprandial Glucose (mg/dl)	Gamma Glutamyl Transferase (GGT) (U/l)
Alanine Aminotransferase (ALT) (U/l)	Glycated Hemoglobin (percent)
Alkaline Phosphatase (U/l)	High Density Lipoprotein (HDL) Cholesterol (mg/dl)
Amylase (U/l)	Serum Creatinine (mg/dl)
Aspartate Aminotransferase (AST) (U/l)	Serum Insulin ( $\mu$ IU/ml @ 2 hours after fasting glucose)
Cholesterol (mg/dl)	Total Bilirubin (mg/dl)
Creatine Kinase (U/l)	Total Lactic Dehydrogenase (LDH) (U/l)
Direct Bilirubin (mg/dl)	Triglycerides (mg/dl)
Fasting Glucose (mg/dl)	
<b>Coagulation</b>	
Patient Prothrombin Time (seconds)	
<b>Hematology</b>	
Absolute Bands (thousand/mm <sup>3</sup> )	Differential Segs (percent)
Absolute Basophils (thousand/mm <sup>3</sup> )	Erythrocyte Sedimentation Rate (mm/hr)
Absolute Eosinophils (thousand/mm <sup>3</sup> )	Hematocrit (percent)
Absolute Lymphocytes (thousand/mm <sup>3</sup> )	Hemoglobin (gm/dl)
Absolute Monocytes (thousand/mm <sup>3</sup> )	Mean Corpuscular Hemoglobin (MCH) (pg)
Absolute Reactive Lymphs (thousand/mm <sup>3</sup> )	MCH Concentration (MCHC) (gm/dl)
Absolute Segs (thousand/mm <sup>3</sup> )	Mean Corpuscular Volume (MCV) (cubic micra)
Differential Bands (percent)	Platelet Count (thousand/mm <sup>3</sup> )
Differential Basophils (percent)	RBC Morphology
Differential Cells Counted	Red Blood Cell (RBC) Count (million/mm <sup>3</sup> )
Differential Eosinophils (percent)	White Blood Cell (WBC) Count (thousand/mm <sup>3</sup> )
Differential Lymphs (percent)	WBC Morphology
Differential Monocytes (percent)	Platelet Observation
Differential Reactive Lymphs (percent)	

**Table 4-2. Laboratory Test Procedures Performed at Scripps Clinic (Continued)**

<b>Immunology</b>	
Anti Delta Total Antibody	Hepatitis B Surface Antigen
Anti-Thyroid Antibody	Hepatitis B Surface Antigen Confirmatory
Hepatitis A Total Antibody	Hepatitis C Virus Antibody
Hepatitis B Core Antibody	
<b>Lupus Panel</b>	
Anti-Mitochondrial Antibody	Anti-Smooth Muscle Antibody
Anti-Nuclear Antibody	Latex Rheumatoid Factor (IU/ml)
Anti-Parietal Cell Antibody	Thyroid Microsomal Antibody
<b>Fecal Studies</b>	
Fecal Occult Blood	
<b>Protein Profile</b>	
$\alpha$ -1-Acid Glycoprotein (mg/dl)	Haptoglobin (mg/dl)
$\alpha$ -1-Antitrypsin (mg/dl)	IgA (mg/dl)
$\alpha$ -2-Macroglobulin (mg/dl)	IgG (mg/dl)
Albumin (mg/dl)	IgM (mg/dl)
Apolipoprotein B (mg/dl)	Prealbumin (mg/dl)
C3 Complement (mg/dl)	Transferrin (mg/dl)
C4 Complement (mg/dl)	
<b>Radioimmunoassay</b>	
Estradiol (pg/ml)	Prostate-Specific Antigen (ng/ml)
Follicle Stimulating Hormone (FSH) (mIU/ml)	T <sub>4</sub> ( $\mu$ g/dl)
Free Testosterone (pg/ml)	Thyroid Stimulating Hormone (TSH) ( $\mu$ IU/ml)
Luteinizing Hormone (mIU/ml)	Total Testosterone (ng/dl)
<b>T &amp; B Lymphocytes and Subsets (special immunology testing performed on 818 participants)</b>	
CD20+ Cells (B cells) (percent)	Absolute CD16+56+ Cells (Natural Killer Cells) (per mm <sup>3</sup> )
CD3+ Cells (T cells) (percent)	Absolute CD20+ Cells (B Cells) (per mm <sup>3</sup> )
CD4+ Cells (Helper T Cells) (percent)	Absolute CD3+ Cells (T Cells) (per mm <sup>3</sup> )
CD3+CD4+ Cells (Helper T Cells) (percent)	Absolute CD4+ Cells (Helper T Cells) (per mm <sup>3</sup> )
CD8+ Cells (Suppressor T Cells) (percent)	Absolute CD3+CD4+ Cells (Helper T Cells) (per mm <sup>3</sup> )
CD3+CD8+ Cells (Suppressor T Cells) (percent)	Absolute CD8+ Cells (Suppressor T Cells) (per mm <sup>3</sup> )
CD45 Total Lymphs (Common Leukocyte Antigen) (percent)	Absolute CD3+CD8+ Cells (Suppressor T Cells) (per mm <sup>3</sup> )
Lymphs (percent)	Absolute Lymphocytes (per mm <sup>3</sup> )
	CD16+56+ Cells (Natural Killer Cells) (percent)
<b>Urinalysis</b>	
2-hour Postprandial Urine Glucose (g/dl)	Urinary Glucose (g/dl)
Leukocyte Esterase	Urinary Ketones (mg/dl)
Urinary Bacteria (per high-powered field)	Urinary Mucus (per high-powered field)
Urinary Bilirubin	Urinary Nitrites
Urinary Blood	Urinary pH
Urinary Casts (per low-powered field)	Urinary Protein (mg/dl)
Urinary Clarity	Urinary RBC (per high-powered field)
Urinary Color	Urinary WBC (per high-powered field)
Urinary Comment	Urine Specific Gravity
Urinary Crystals (per high-powered field)	Urobilinogen (Ehrlich unit/dl)
Urinary Epithelial Cells (per high-powered field)	

## **4.2 ADIPOSE TISSUE EXTRACTION**

The follow-up results of the 1987 and 1992 Air Force Health Study (AFHS) showed a rise in the incidence of pre-diabetic indicators of type 2 diabetes, non-insulin-dependent diabetes mellitus (NIDDM), in the participants exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). To examine the relation between dioxin exposure and glucose transporting activity in human adipose tissue cells, 313 participants volunteered to participate in a separate sub-study of the AFHS in which approximately 10 grams of adipose tissue were removed by liposuction and preserved for laboratory analysis. The information derived from the adipose tissue sub-study may help explain the positive association between dioxin body burden and diabetes mellitus in veterans of Operation Ranch Hand.

The Air Force designated 650 potential participants for adipose extraction by a random selection process within classifications of exposure, age, body fat, and diabetes. A consent form was provided to each adipose tissue-designated participant at the evening orientation. Over the course of the 1997 physical examination, a board-certified plastic surgeon extracted an adipose sample from 313 participants. The procedure lasted 30 minutes and required the use of a local anesthetic. The adipose tissue specimens were shipped to Brooks Air Force Base weekly for storage. The results of this study will be summarized in a separate report.

## **4.3 QUALITY CONTROL**

As in the baseline and the 1985, 1987, and 1992 studies, quality control (QC) requirements for both laboratory testing and clinical procedures were extensive. Although details are provided in Chapter 6, the following categories summarize the extent of the emphasis on quality. For laboratory testing, Westgard rules (1<sub>2s</sub>) were used throughout the study. Single reagent lots and control standards were used when practical, duplicate specimens were routinely and blindly retested, and testing overlaps were mandatory when test reagent lots were changed.

The Scripps clinical team was instructed to ensure clinician consistency. In total, 18 board-certified physicians in internal medicine, neurology, and dermatology participated in the general, specialty, and diagnostic examinations. In addition, 12 radiologists, 5 pulmonologists, and 4 cardiologists performed tests and interpreted results. To reduce observer variability, turnover in the clinical and paramedical staffs was minimized during the 11 months of examinations. One Scripps Clinic physician served as the Project Medical Director, responsible for the scheduling, conduct, and QC of the examinations. All examining physicians reviewed the mark-sense examination forms prior to a pre-examination test. To minimize recording errors, the layout of the form was designed to parallel the flow of the clinical examination. Because data transcription was not permitted, each physician was responsible for filling in the bubbled form. To a large extent, the use of these mark-sense forms and subsequent QC measures were the primary reason for a clean clinical data set. A complete set of forms is provided in Appendix B. Additional QC included the following elements:

- A detailed onsite quality control process was employed by Scripps Clinic, Science Applications International Corporation (SAIC), and Air Force physicians and personnel.
- Clinical quality assurance meetings were conducted to detect and correct problems.
- Examiners were unaware of the exposure status of the participants.
- Automated blood pressure recording was performed.

#### 4.4 CONDUCT OF EXAMINATIONS

All examinations, from May 1997 to April 1998, were conducted in accordance with the Examiners' Handbook. Excluding weeks with national holidays, two groups of participants, averaging approximately 25 per group, were examined weekly.

A demanding logistics effort was required to contact, transport, and examine the 2,121 study participants. Pre-examination contact consisted of making telephone calls to recruit participants, determine special requirements (e.g., wheelchair assistance), and arrange transportation. Once scheduling was reasonably firm, the SAIC logistics coordinator sent each participant a detailed information package outlining dietary requirements, a stool occult blood testing kit (Hemoccult<sup>®</sup>), inbriefing schedules, important telephone numbers, a request for medical records, and local maps designating examination site dining and recreational facilities.

To encourage participation in future follow-up studies, some activities were continued in 1997. These included participant critique forms, an informational meeting open to any accompanying family members and friends, and preventive medicine examinations such as human immunodeficiency virus and prostate-specific antigen testing. Proctosigmoidoscopy, as well as treadmill tests, were made available to participants for a nominal fee. Accompanying family members also were offered the opportunity to use the clinic facilities at a discounted rate.

Each morning of the examinations, the current group of participants was transported to the Scripps Clinic, having fasted and abstained from nicotine and caffeine since midnight the previous evening. In addition, alcohol was strictly prohibited from 24 hours before the first day of the examination through the second day of the examination. On the first day, each participant was given an individualized 2-day schedule outlining his medical, interviewing, and laboratory appointments. The schedule carefully noted the specific required periods of caffeine and nicotine abstinence for generalized periods in relation to electrocardiograph testing. Although the clinic schedules generally were assigned at random, consideration was given to smokers and diabetics because of the fasting and abstinence restrictions. Figure 4-1 shows a typical 2-day schedule prepared for a participant. The participant depicted in this schedule was in good self-reported health, was a smoker, and was asked to participate in the blood measurement of dioxin on Day 2.

As in the previous examinations, schedules were printed with specific directions to aid participants in locating clinic departments, although for many tests, participants were escorted from the waiting room. Throughout the examination day, time was provided for waiting-room activities (i.e., renewal of past friendships, discussions of experiences in SEA, consumption of refreshments when permitted, and completion of paperwork). On the second day of the examination, the participants completed testing and examinations and received outbriefings from a medical diagnostician.

The psychological tests (the SCL-90-R and the Jenkins Activity Test) were self-administered and reviewed by a Scripps Clinic psychologist. If a problem was indicated, the participant was advised of the issue during his medical debrief. Upon completion of these debriefings, the participants were paid their stipend and reimbursed for travel expenses.

##### 4.4.1 Blood Collection

On the first examination day, each participant had 160 ml of blood collected. Detailed immunology testing (see Table 4-2) was conducted on approximately 40 percent of the participants. These

# AIR FORCE HEALTH STUDY

Participant Schedule for: Monday, May 05, 1997 and Tuesday, May 06, 1997

Case Number – group #

## *Participant's Full Name*

Day: 1 Monday, May 05, 1997

Start Time	End Time				
0600		Meet in Hotel Lobby	Shuttle Bus	Transfer to Scripps	
0615		Bus to Scripps			
0630		Orientation and signing of consent forms	Green 2 N	Waiting Room	
0645	TBA*	Blood Draw 1 and 2	Green 2 W	Room W263 A	
0800		Physical Exam	AOP 3 A	Internal Medicine	Dr. Sargeant
0845		Dermatology	AOP 1 B	Dermatology	Dr. Cornell
1100		Chest X Ray	Green 1	Radiology	Please sign in
1200		Spirometry/ECG	Green 2 W	Room 264	
1300		Psychology Exam	Green 2 N	Room 231	
1415		Vibrotactile	AOP 3 A	Vascular Lab	Please sign in
1430		Doppler Exam	AOP 3 A	Internal Medicine	
1545		Bus to Hotel	Green 3 W	Outside Fountain	

TBA\* = BLOOD DRAW 2 SCHEDULED 2 HOURS AFTER DRINKING GLUCOLA

NO FOOD, CAFFEINE, OR NICOTINE PRIOR TO BLOOD DRAWS 1 OR 2 ON DAY 1

NO CAFFEINE OR NICOTINE WITHIN 4 HOURS PRIOR TO DOPPLER EXAM, ECG, OR SPIROMETRY

MT01

smoker

Good

**Figure 4-1. Typical 2-Day Clinic Schedule**

# AIR FORCE HEALTH STUDY

Participant Schedule for: Monday, May 05, 1997 and Tuesday, May 06, 1997

Case Number – group #

## *Participant's Full Name*

Day: 2    Tuesday, May 06, 1997

Start Time	End Time				
0615		Board Shuttle Bus	Hotel		
0630		Bus to Scripps			
0700		Blood Draw 3	Green 2 W	Room W263 A	
0800		Neurology Exam	AOP 3 A	Neurology – CHECK IN	Dr. Otis
0830		NORC Interview	Green 2 N	Room CP228	
1015		NIDR Dental Exam	Green 2 W	Room 213	
1315		Debriefing	AOP 3 A	Internal Medicine	Dr. Moore
1330		Exit Interview	Green 2 N	Waiting Room	Rita Taliaferro
1400		Bus to Hotel	Green 3 W	Outside Fountain	

**TBA\* = BLOOD DRAW 2 SCHEDULED 2 HOURS AFTER DRINKING GLUCOLA**

**NO FOOD, CAFFEINE, OR NICOTINE PRIOR TO BLOOD DRAWS 1 OR 2 ON DAY 1**

**NO CAFFEINE OR NICOTINE WITHIN 4 HOURS PRIOR TO DOPPLER EXAM, ECG, OR SPIROMETRY**

MT01

smoker

Good

**Figure 4-1. Typical 2-Day Clinic Schedule (Continued)**

participants were identified by the last digit of their participant study identification number used for previous testing, thus establishing a longitudinal connection between examinations. The immunologic tests were subjected to highly structured QC procedures set forth by the Air Force. Participants chosen for immunology testing had an additional 30 ml of blood collected. An additional blood collection of 10 ml was taken 2 hours after the first blood collection to assess 2-hour postprandial glucose and insulin. Blood bank chairs were used for maximum comfort and total body support in the event of a reaction. These chairs were selected because they could be shifted easily into the Trendelenburg position if a participant felt faint. Out of the 160 ml of blood collected from each participant, the Air Force was provided 40 cc of serum for archival purposes as well as human immunodeficiency virus and syphilis testing.

On the second day of the group examination, 563 participants were invited and provided a second blood collection for dioxin analysis at the Centers for Disease Control and Prevention. A total of 280 ml of blood was collected for these participants, unless the participant had blood collected for immunology testing the previous day. In this case, only 250 ml of blood was collected.