APPENDIX A

SAMPLE COLLECTION PROTOCOL

Attachments	Topic
A-1	Scope of Work--Surgeon and Hospital Coordinator
A-2	Medical Exclusion Screening Form
A-3	Information for and Consent by Hospital Participants Who Are Between 12 and 21 Years of Age
A-4	Information for and Consent by Hospital Participants Who Are 21 Years of Age or Older
A-5	Specimen Collection and Shipping Protocol
A-6	Dioxin and Furan Exposure-Related Survey
A-7	Example of Hospital Recruitment Letter

ATTACHMENT A-1

SCOPE OF WORK--SURGEON AND HOSPITAL COORDINATOR

1. The hospital surgeon or hospital coordinator shall submit the demographic data regarding the age, sex, race, and geographic location of surgical patients at the hospital during the preceding year. This shall include the total number of surgical patients in a particular category as well as the percentage of the total that are in a category. Geographic location need be no more specific than by county (although zip code would be preferable); ages may be grouped, e.g., 12-34, 35-49, and 50 plus years.

2. The hospital coordinator shall be given specifications for the age, sex, and residence status of potential tissue contributors and shall be responsible for identifying eligible elective (i.e., surgery scheduled more than 48 hours in advance) surgical candidates by reviewing upcoming surgical operating schedules.

3. The hospital coordinator shall then contact the participant's surgeon to explain the project and determine by interview with the patient, chart review, or discussion with the surgeon if the participant has any of the following characteristics which would exclude the subject from the study:

   a. Pregnancy
   b. Malignancy, excluding nonmalignant melanoma skin cancers (areas of the body not immediately adjacent to the site of the malignancy can be sampled)
   c. Insulin-dependent diabetes
   d. Immunosuppression caused by either a disease process or therapeutic medications
   e. History of unintentional weight loss greater than 10 lb in preceding 6 months
   f. Bleeding disorder
   g. Infectious or serum hepatitis, active tuberculosis, or acquired immune deficiency syndrome
   h. Children less than twelve (12) years of age

   A short form for recording the above exclusion screening information will be provided by Midwest Research Institute (MRI) and must be completed by the hospital coordinator or the surgeon prior to each participant's surgery.

4. The surgeon and hospital coordinator shall decide who will explain the study purpose to the patient, obtain written informed consent, and then administer the questionnaire prior to surgery. These activities will be done either by the surgeon, or at the surgeon's request, delegated to the hospital coordinator.

5. Following the interview with the study participant, the hospital coordinator shall review the questionnaire responses and validate the questionnaire data.

6. The hospital coordinator and surgeon must be responsible for ensuring the

adipose tissue is collected in sufficient quantities (at least 5 g; 10-20 g preferred) and properly stored in the provided containers until shipment.

7. The hospital coordinator shall be responsible for shipping all specimens to MRI. MRI will provide the hospital coordinator with the specimen containers, shipping materials, and shipping instructions, and will pay for shipping costs.

8. The hospital shall submit a resume for the prospective coordinator. The coordinator should have experience interacting with and interviewing hospital patients due to the sensitive nature of this study.

9. The hospital shall submit a description of who in the hospital organization will supervise the coordinator and how the coordinator will interact with various hospital departments such as the surgery department, pathology department, nursing department, the laboratory, and the hospital tissue committee.

10. Preparation of final reports shall be the responsibility and prerogative of the State of California Air Resources Board (ARB) and MRI. Individuals, institutions, or agencies working on contract will be acknowledged in reports and subsequent publications.

11. The hospital surgeon and hospital coordinator are responsible for ensuring that the patient identification remains confidential. The questionnaire data shall be submitted to MRI under the hospital patient identification code. The hospital shall also retain the informed consent forms as part of the hospital study records to ensure that confidentiality has been maintained.

12. All information, with the exception of patient identification, obtained in this study will become the property of the ARB and MRI and will be considered confidential, and will not be released to any individual, institution, organization, or agency without their prior written approval. This clause will be binding on MRI in perpetuity.

ATTACHMENT A-2

MEDICAL EXCLUSION SCREENING FORM FOR USE BY HOSPITAL COORDINATOR

Name of potential study participant: _____

In the list below, check the appropriate line next to any condition for which the potential study participant has been diagnosed within the past two years, currently has, or is currently undergoing medical treatment. Check "NONE" if appropriate.

_____Pregnancy (Check only if now pregnant)

_____Age less than 12 years

_____Infectious or serum hepatitis

_____Active tuberculosis

_____AIDS (acquired immune deficiency syndrome)

_____Bleeding disorders

_____Immunosuppressed state, either due to a disease process or therapeutic medications

_____History of unintentional weight loss greater than 10 pounds in preceding 6 months

_____Malignancy, excluding nonmalignant melanoma skin cancers (areas of the body not immediately adjacent to the site of the malignancy can be sampled)

_____Insulin-dependent diabetes

_____NONE OF THE ABOVE

Signature of Hospital Coordinator: _____

Date: _____

Note: In order to maintain patient identification as confidential information, this form is to be retained in the hospital study files and should not be forwarded to Midwest Research Institute or the Air Resources Board of the State of California.

ATTACHMENT A-3

INFORMATION FOR AND CONSENT BY HOSPITAL PARTICIPANTS WHO ARE BETWEEN 12 AND 21 YEARS OF AGE

DETERMINATION OF BODY BURDEN LEVELS OF POLYCHLORINATED DIBENZO-*p*-DIOXINS AND DIBENZOFURANS OF CALIFORNIA RESIDENTS

Dear Volunteer:

1. You have been asked to participate in a study to determine the levels of the compounds, polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), that may be present in the body burdens of the general California population. This study is being conducted by Midwest Research Institute of Kansas City, Missouri, for the Air Resources Board of the State of California. Specifically, we are studying how likely it is that these compounds which are present in the environment are taken up in body fat.

2. We would like for you to give consent for your child to take part in this research. We are approaching your child to be included in the group of people who are representative of the general California population.

3. The hospital coordinator for this hospital, _____, has received verbal consent from your child's physician, _____, before coming to you now to ask your child to participate in this study.

4. Your child's participation would be as follows:

   a. During the course of the operation, permit the same surgeon who will perform the scheduled operation to collect a sample of fat from just under the skin. (The fat will be removed from the incision made for the scheduled operation.)

   b. Allow the hospital coordinator for this hospital to ask you and your child several questions about your child's possible exposure to chemicals and, if applicable, past work experience.

   c. Permit the hospital coordinator for this hospital to review your child's hospital medical chart.

5. There is no basis for expecting your child's participation to expose him or her to additional risk of serious harm or discomfort beyond the risks of the surgery for which he or she was admitted to this hospital.

6. There is no other way to get the information required for this part of our research.

7. The only benefit to expect from taking part in this research is the satisfaction of having added to our knowledge of the background levels of PCDDs and PCDFs on the body burden of the general California population.

8. You may end your child's participation in this research at any time, and this will have no effect on any care or treatment which he or she is receiving or entitled. The coordinator for this hospital or your child's physician may end participation of your child at any time either with or without your consent.

9. Results of this research may be published, but your child's name will not be used and he or she will not be identified in any such publication.

10. In the event that you believe participation in this research study has led to injury, contact _____ or _____ at _____ to identify the resources which may be available to you. You should understand that the Midwest Research Institute and the State of California do not have any program to provide compensation for persons who may experience injury while participating in research projects when the injury is not due to the fault of the investigators.

11. Please indicate, by marking the appropriate box below, whether you have any questions or concerns about the project or how it will affect your child. If you or your child has further questions, you or your child may choose to receive written answers to all of them.

_____ I have no further questions.
(Check or initial here.)

_____ I have the following question(s):
(Check or initial here.)

_____

_____

_____

_____

_____

_____ Signature of Hospital Coordinator: _____
Date

I have explained to my child what his/her participation involves in this study, and he/she has given me his/her affirmative agreement to participate.

I, hereby, consent to allow my child to take part in research directed by John S. Stanley, Ph.D. The research is being done for the State of California Air Resources Board by Midwest Research Institute. I understand other persons may assist Dr. Stanley or be associated with him. Consent for my child is given freely and with full knowledge of the nature and purpose of this project, the risks that may be involved, the alternative available, and the benefits that may be expected.

Name of Minor: _____
(print or type)

Check, Initial, or Signature of Minor: _____

Signature of Parent/Guardian: _____

Date: _____

Witness: _____

ATTACHMENT A-4

INFORMATION FOR AND CONSENT BY HOSPITAL PARTICIPANTS WHO ARE 21 YEARS OF AGE OR OLDER

DETERMINATION OF BODY BURDEN LEVELS OF POLYCHLORINATED DIBENZO-p-DIOXINS AND DIBENZOFURANS OF CALIFORNIA RESIDENTS

Dear Volunteer:

1. You have been asked to participate in a study to determine the levels of the compounds, polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), that may be present in the body burdens of the general California population. This study is being conducted by Midwest Research Institute of Kansas City, Missouri, for the Air Resources Board of the State of California. Specifically, we are studying how likely it is that these compounds which are present in the environment are taken up in body fat.

2. We will appreciate it if you will volunteer to take part in this research. We are approaching you to be included in the group of people who are representative of the general California population.

3. The hospital coordinator for this hospital, _____, has received verbal consent from your physician, _____, before coming to you now to ask you to participate in this study.

4. Your participation would be as follows:

   a. During the course of the operation, permit the same surgeon who will perform your scheduled operation to collect a sample of fat from just under the skin. (The fat will be removed from the incision made for the scheduled operation.)

   b. Allow the hospital coordinator for this hospital to ask you several questions about your past work experience, education, and possible exposure to chemicals.

   c. Permit the hospital coordinator for this hospital to review your hospital medical chart.

5. There is no basis for expecting your participation to expose you to additional risk of serious harm or discomfort beyond the risks of the surgery for which you were admitted to this hospital.

6. There is no other way to get the information required for this part of our research.

7. The only benefit to expect from taking part in this research is the satisfaction of having added to our knowledge of the background levels of PCDDs and PCDFs on the body burden of the general California population.

8. You may end your participation in this research at any time, and this will have no effect on any care or treatment which you are receiving or entitled. The coordinator for this hospital or your physician may end your participation at any time either with or without your consent.

9. Results of this research may be published, but your name will not be used and you will not be identified in any such publication. Your name will remain confidential with the hospital that collects the adipose specimen.

10. In the event that you believe participation in this research study has led to injury, contact _____ or _____ at _____ to identify the resources which may be available to you. You should understand that the Midwest Research Institute and the State of California do not have any program to provide compensation for persons who may experience injury while participating in research projects when the injury is not due to the fault of the investigators.

11. Please indicate, by marking the appropriate box below, whether you have any questions or concerns about the project or how it will affect you. If you have further questions, you may choose to receive written answers to all of them.

_____ I have no further questions.
(Check or initial here.)

_____ I have the following question(s):
(Check or initial here.)

_____

_____

_____

_____

_____

_____ Signature of Hospital Coordinator: _____
Date

Name of Participant: _____
(print or type)

I, hereby, consent to take part in research conducted by John S. Stanley, Ph.D. The research is being done for the State of California Air Resources Board by Midwest Research Institute. I understand other persons may assist Dr. Stanley or be associated with him. My consent is given freely and with full knowledge of the nature and purpose of this project, the risks that may be involved, the alternative available, and the benefits that may be expected.

Signature of Participant: _____

Witness: _____ Date: _____

ATTACHMENT A-5

SPECIMEN COLLECTION AND SHIPPING PROTOCOL

A. Introduction

The proper collection, processing, storage, and shipment of physiologic specimens from participants in the State of California Air Resources Board and Midwest Research Institute (MRI) study is critical to the success of the study. The following sections describe the procedures which must be followed for all specimen collections. These procedures must be strictly adhered to in order to avoid contamination, loss, or degradation of the specimens. Please familiarize yourself with the study protocol and make sure that you understand the concept of the study, the role of all the personnel involved, and your own role.

It is extremely important that all records associated with each subject be maintained in an organized and complete manner to ensure that all information is properly collected and accurate. Specimens should be labeled promptly and processed as a unit, and precautions must be taken to avoid patient-specimen-record mixups. This type of error is usually the most common error in the laboratory setting, but careful planning and a well-organized work area will keep such errors to a minimum. Some of the information required for the specimen label and shipping list will be collected at the time of specimen collection. Problems in collection should be noted in a sample log and in the comments section of the shipping list.

B. Supplies

The supplies needed for adipose specimen collection and shipment are included in the kit provided by MRI. The kit includes:

- Clean, screw-cap glass jars with foil-lined caps
- Precleaned aluminum foil squares
- Specimen labels
- Styrofoam® insulated shipping box
- Zip-lock bags
- Bubble-pack packing material
- "ORM-A" dry ice labels
- Envelope for questionnaires
- Preaddressed mailing label
- Federal Express return labels, preaddressed by MRI

C. Procedure for Collecting and Processing Specimens

1. Adipose tissue obtained surgically from subcutaneous tissue in the anterior abdominal wall is the preferred tissue for 2,3,7,8-TCDD analysis. A minimum of 5 grams of tissue is required for analysis; 10 to 20 grams is preferred, if available. Needle-biopsy is inadequate because it generally yields a maximum of half a gram of tissue.

2. To ensure that an adequate amount of tissue is collected, the specimen may be weighed in the precleaned foil squares included in the supplies kit. Dispose of each square after use.

3. Place the adipose specimen in a specimen jar. To one of the pre-printed labels, add the patient's identification code, the date collected, and your facility ID number, and attach to the jar. As an extra precaution, secure the label with a strip of cellophane tape.

4. Freeze the tissue immediately, preferably at a temperature of -20°C or lower, within at least 30 minutes of collection.

5. Do not collect pathological tissue.

6. DO NOT ADD PRESERVATIVES, SALINE, WATER, OR FIXATIVE SUBSTANCES TO THE TISSUE.

D. Shipment Procedures and Information Required

1. Assemble shipper, dry ice, and packing materials. Work quickly, so that the frozen tissues will not be exposed to ambient temperatures for more than 5 to 10 minutes. It is imperative that the tissue specimens be kept in a hard frozen state.

2. Wrap each specimen jar with a folded paper towel or a strip of bubble-pack packing material and secure with tape or a rubber band. If more than two jars of tissue are to be shipped, return each wrapped jar to the freezer until all are wrapped.

3. Place paper towels or bubble-pack material in the bottom of the shipper. Place the wrapped specimen jars in the zip-lock bag and place on the packing material.

4. On top of the specimen jars, place several additional layers of paper towels or bubble-pack material.

5. Fill the shipper with dry ice, close it, and secure the top with the strap. Label appropriately for shipment via FEDERAL EXPRESS such that specimens will be received at MRI within 24 hours. Affix the preprinted mailing label and an "ORM-A" label to the outside of the aluminum box.

6. SHIP ONLY ON MONDAYS THROUGH WEDNESDAYS, and never the day before a holiday.

7. Use the preaddressed Federal Express forms to ship. Telephone the laboratory at (816) 753-7600 the day the shipment is sent. Ask for Ms. Kay Turman or Dr. John Stanley.

8. In the envelope supplied, place the questionnaires for the specimens and attach securely to the inside of the lid of the aluminum box. Be sure all the required information has been filled in on each questionnaire.

E. Shipment of Specimens to MRI, Kansas City, Missouri

1. Beginning of study and general instructions.

- Determine from the local office the times FEDERAL EXPRESS packages are picked up in order to connect with the best flights to Kansas City, Missouri. Shipments to Kansas City should be scheduled only Monday through Wednesday mornings. IMPORTANT: Since the materials packed in accordance with the instructions below will remain frozen only about 2½ days, shipments should not arrive in Kansas City on weekends or on federal holidays.

- Inquire about regulations in your area concerning shipment of adipose specimens with dry ice and the quantity of dry ice allowed per shipper. Also, make sure the specimens will be received at MRI in Kansas City within 24 hours.

- Telephone the laboratory at MRI the day the shipment is mailed (816) 753-7600. Speak with Ms. Kay Turman, Mr. Michael McGrath, or Dr. John Stanley.

- For all shipments, do not pack the shippers with frozen specimens and dry ice until just before transport to the Federal Express pick-up point.

- Maintain a supply of dry ice from a local supplier for shipping specimens. A block should be sawed at the plant into 1-in slabs. Then each of these should be sawed lengthwise. A 7-in x 10-in slab would fit easily into the shipper without having to break the slab. (Large pieces are preferable to small chunks since they do not volatilize as rapidly.)

2. Paper Work

For each shipment, fill out the Specimen Shipping List provided by MRI. Please give the following information in the spaces provided:

- Page number, e.g., 1 of 4
- Shipment number--number shipments sequentially starting with 1
- Number of frozen shippers--total number of shippers (containing frozen adipose specimens) which are being mailed in this shipment
- Type of specimens--adipose
- Number of specimens--number of specimens shipped
- Name, title, signature, and phone number of person sending shipment
- Date shipped
- Specimen ID for each participant
- Date collected, e.g., 051088

Comments: Specify any deviations from collection, storage, and shipment protocols, and date of occurrence. If the number of specimens in a shipment is too large to fit on one page of the Shipping List, please use the continuation sheets provided. Attached is an example of a completed Specimen Shipping List. Photocopy two extra copies. The original will be shipped with the specimens, a copy mailed to MRI in a separate envelope, and a copy for your records.

SPECIMEN INFORMATION SYSTEM
FORM 1
STATE OF CALIFORNIA AIR RESOURCES BOARD AND MIDWEST RESEARCH INSTITUTE STUDY
SPECIMEN SHIPPING LIST

Case : 88-8941

Shipment Number : _____

No. Frozen Shippers: _____

Type of Specimens : Adipose

No. Specimens : _____

Shipped by: _____
Name Title

_____
Signature

_____
Date Shipped Phone No.

Received by: _____ of MRI
Name

_____
Signature Date Received

Patient's Hospital ID No.	Tissue Type	Date Collected MM-DD-YY	Comments (Specify any deviation from collection, storage, and shipment protocols, and date of occurrence.)
_____	Adipose	05-10-88	Adipose frozen 40 min post collection
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

ATTACHMENT A-6

DRAFT QUESTIONNAIRE

DIOXIN AND FURAN EXPOSURE-RELATED SURVEY

(Please use ball point pen or dark pencil)

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XX XX
XX XX
XX CONFIDENTIALITY XX
XX XX
XX YOU WILL NOT BE ASKED FOR YOUR NAME OR ADDRESS. XX
XX INSTEAD,THE HOSPITAL WILL ASSIGN YOU A CONFIDENTIAL XX
XX IDENTIFICATION NUMBER. XX
XX XX
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

QUESTIONS 1 THROUGH 11 ARE TO BE COMPLETED BY HOSPITAL PERSONNEL

1. Hospital Code _____

2. Patient's Hospital ID# _____

3. Date when sample was taken ___/___/___

4. Reason for patient's surgery (diagnosis) _____

5. Anatomical origin of adipose tissue sample _____

6. Sex of patient: Male ¦_¦ Female ¦_¦

7. Race of patient:
¦_¦ White (non-Hispanic)
¦_¦ Black (non-Hispanic)
¦_¦ Hispanic
¦_¦ American Indian or Alaskan Native
¦_¦ Asian or Pacific Islander
¦_¦ Unknown

8. Age Category: 12-34 ¦_¦ 35-49 ¦_¦ 50 and above ¦_¦

9. Weight: _____ lbs or _____ kg

10. Height: _____ ft _____ in or _____ cm

11. Caliper measurement on upper arm: _____ mm

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QUESTIONS 12 THROUGH 32 ARE TO BE COMPLETED BY THE PATIENT

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QUESTIONS 12 THROUGH 17 PERTAIN TO YOUR RESIDENTIAL HISTORY

12. In what Zip Code do you live now? _____

13. How many years have you been living there? _____

14. Do you <u>currently</u> live near (within 5 miles) or work at any of the following facilities:

	Live near			Work at	
	Yes	No	Don't Know	Yes	No
Municipal waste incinerator	☐	☐	☐	☐	☐
Sewage sludge incinerator	☐	☐	☐	☐	☐
Hospital	☐	☐	☐	☐	☐
Wire reclamation incinerator	☐	☐	☐	☐	☐
Hazardous waste site	☐	☐	☐	☐	☐
Wood treatment facility	☐	☐	☐	☐	☐

15. In what Zip Code did you live previously? _____

16. How many years did you live at that location (Q #14)? _____

17. Did you <u>previously</u> live near (within 5 miles) and/or work at any of the following facilities:

	Lived near			Worked at	
	Yes	No	Don't Know	Yes	No
Municipal waste incinerator	☐	☐	☐	☐	☐
Sewage sludge incinerator	☐	☐	☐	☐	☐
Hospital	☐	☐	☐	☐	☐
Wire reclamation incinerator	☐	☐	☐	☐	☐
Hazardous waste site	☐	☐	☐	☐	☐
Wood treatment facility	☐	☐	☐	☐	☐

==========

QUESTIONS 18 THROUGH 25 ARE RELATED TO POTENTIAL CHEMICAL EXPOSURE

18. In the last 5 years, did or does your job involve the manufacture, analysis, use, transport or disposal of chemicals (such as pesticides, fertilizers, petroleum products, etc.) ?

Yes ☐ No ☐ Don't know ☐

19. In the last 5 years, did or does your job involve the manufacture and use of electrical equipment (such as transformers, capacitors, etc.)?

Yes ☐ No ☐ Don't know ☐

20. In the last 5 years, did or does your job involve the incineration of plastic or wood materials?

Yes ☐ No ☐ Don't know ☐

21. Have you served in the U.S. Armed Forces in Vietnam ?

Yes ☐ No ☐

If you answered 'Yes' on Question #21 please answer Questions a) and b) below, otherwise continue with Question #22 on the next page.

a) When did you serve in Vietnam (month/year)?

from /___//___/ (mo yr) to /___//___/ (mo yr)

and from /___//___/ (mo yr) to /___//___/ (mo yr) if interrupted service

b) Were you involved in defoliant (herbicide spraying) operations or did you operate in defoliated areas in Vietnam?

Yes ☐ No ☐ Don't know ☐

22. Have any termite and pest control services (such as ACE, Orkin, Terminix, etc.) been used inside and/or outside your home in the last 5 years? (Include previous home(s) if less than 5 years at present address).

Yes ☐ No ☐ Don't know ☐

23. Has your household, or landlord, subscribed to any lawn and garden spraying services in the last 5 years? (Include previous home(s) if less than 5 years at present address).

Yes ☐ No ☐ Don't know ☐

24. Have you used any wood preservatives (such as Penta) in the last 5 years?

Yes ☐ No ☐ Don't know ☐

25. In the last 5 years have you used any of the following products during your leisure activities such as lawn care, gardening, woodworking, etc ?

	Yes	No	Don't Know
Herbicides	☐	☐	☐
Pesticides	☐	☐	☐
Wood preservatives (such as Penta)	☐	☐	☐

==========

QUESTIONS 26 THROUGH 32 PERTAIN TO YOUR OCCUPATIONAL HISTORY

26. What is your current employment status?

- [ ] Working full time
- [ ] Working part time
- [ ] Homemaker
- [ ] Retired and not working
- [ ] Retired and working part time
- [ ] Retired but working full time
- [ ] Student
- [ ] Unemployed

27. During the last 5 years, what has been your usual or main occupation? (If retired or not currently working, enter your occupation while employed.)

Occupation _____

28. How many years have you been employed (were you employed) in this occupation? _____

THE FOLLOWING IS A LIST OF TYPES OF OCCUPATIONS THAT ARE RELEVANT TO THIS SURVEY. THIS LIST DOES NOT INCLUDE ALL POSSIBLE OCCUPATIONS. PLEASE CHECK ALL OF THE BOXES THAT DESCRIBE AN OCCUPATION WHICH YOU HAVE HAD IN THE LAST 5 YEARS.

29. PROFESSIONAL OCCUPATIONS

a ☐ Professional WITHOUT chemical exposure
b ☐ Professional WITH chemical exposure, such as:

- ☐ Chemist, chemical engineer
- ☐ Medical profession, clinical chemist, pharmacist
- ☐ Teacher--Chemistry, biology, agriculture
- ☐ Other occupation in that category _____

c ☐ Not Applicable

If you checked Question 29 b), please indicate the names of the chemicals to which you were exposed:

Names: _____
_____

☐ Don't know

30. OCCUPATIONS IN TRANSPORTATION

- ☐ Mechanic--automobile, aircraft, heavy equipment
- ☐ Driver--commercial transportation
- ☐ Attendant--garage, parking lot, service station
- ☐ Traffic policeman
- ☐ Other occupation in that category _____

☐ Not Applicable

31. SKILLED LABORERS (other than in transportation)

a ☐ Laborer WITHOUT chemical exposure
b ☐ Laborer WITH chemical exposure, such as:

- ☐ Carpentry, woodshop, furniture making, lumber mill
- ☐ General building construction, electrician, etc.
- ☐ Road construction
- ☐ Electronic technician (TV, radio, etc), machine repairman
- ☐ Printer
- ☐ Foundry worker
- ☐ Fireman
- ☐ Lineman (electric, telephone)
- ☐ Other occupation in that category _____

c ☐ Not Applicable

If you checked Question 31 b), please indicate the names of the chemicals to which you were exposed:

Names: _____
_____

☐ Don't know

32. NON-PROFESSIONALS OR UNSKILLED LABORERS

a ☐ Non-professional or unskilled laborer WITHOUT chemical exposure
b ☐ Non-professional or unskilled laborer WITH chemical exposure, such as:

- ☐ Custodial
- ☐ Farmer, groundsman, nursery man, grove laborer
- ☐ Pest exterminator, lawn care services
- ☐ Other occupation in that category _____

c ☐ Not Applicable

If you checked Question 32 b), please indicate the names of the chemicals to which you were exposed:

Names: _____
_____

☐ Don't know

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XX XX
XX THANK YOU VERY MUCH FOR YOUR PARTICIPATION! XX
XX XX
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

ATTACHMENT A-7

EXAMPLE OF HOSPITAL RECRUITMENT LETTER

January 1, 1988

Dr. John Doe
General Hospital
Somewhere, USA 99999

Dear Dr. Doe:

Midwest Research Institute (MRI), under contract to the State of California Air Resource Board (ARB), is conducting a study to determine the current body burden levels of polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) in the general California population. The compounds are classified as toxic organic chemicals and are associated with emissions from major stationary combustion sources (municipal incinerators, hazardous waste incinerators, etc.). These compounds are also unwanted by-products in certain commercial chemical products (chlorophenol-based). The most toxic of these compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is the compound associated with Agent Orange.

These compounds are known to accumulate in biological systems, particularly in fatty tissue. Several studies have been conducted to date to address the relationship between specific exposure (for example, in Vietnam veterans and Times Beach, Mo. residents) and body burden levels of these compounds. The objective of the study funded by ARB is to estimate the current body burden levels and determine the potential impact on these levels from the release of these compounds from various incineration sources.

In order to obtain the current body burden data, MRI will analyze human adipose tissue from a random sample of the California population. Human adipose tissue will be sampled from cross sections of California residents stratified by age, sex, and area of residence within the state. Sampling will take place during surgical procedures performed in hospitals in Los Angeles and San Francisco. This letter is our invitation to you to participate in this important study.

Your role, as the surgeon, would be to select a staff member to assist you in identifying appropriate cases, obtaining consent from the patients, and completing questionnaires with the patients. You would then collect the sample during the surgery and store it frozen until all samples have been collected. The samples will then be shipped frozen to MRI. We will furnish all supplies, and your organization will be remunerated for your services at the rate of $100 per acceptable sample.

We have enclosed some background information, procedures for collection of the adipose samples, a quota sheet with the sample distribution, and a sample consent form and questionnaire for the patient. Please take a moment to look

over the enclosures. We will telephone you in a week or two to answer any questions you might have and to further discuss the study. We hope you will assist us and the State of California ARB in providing selected adipose samples for this study.

Sincerely,

MIDWEST RESEARCH INSTITUTE

John S. Stanley, Ph.D.
Principal Chemist

APPENDIX B

ANALYTICAL PROTOCOL FOR DETERMINATION OF PCDDs AND PCDFs IN HUMAN ADIPOSE TISSUE

(The analytical protocol cited is the detailed procedure developed for the USEPA and the Veterans Administration (Ref. Stanley et al., 1986, EPA 560/5-86-020). Modification to this method for analyses conducted on ARB Contract No. A6-195-33 has been described in Section 3.3 of the technical report.

TABLE OF CONTENTS

Section	Description	Page
1	Scope and Application	B-3
2	Summary of Method	B-3
3	Definitions	B-6
4	Interferences	B-7
5	Safety	B-7
6	Apparatus and Equipment	B-8
7	Reagents and Standard Solutions	B-11
8	High Resolution Gas Chromatography/Mass Spectrometry Performance Criteria	B-13
9	Quality Control Procedures	B-31
10	Sample Preservation and Handling	B-33
11	Sample Extraction	B-33
12	Cleanup Procedures	B-35
13	Analytical Procedures	B-38
14	Data Reduction	B-43
15	Reporting and Documentation	B-50

ANALYTICAL PROTOCOL FOR DETERMINATION OF PCDDs AND PCDFs IN HUMAN ADIPOSE TISSUE

1. SCOPE AND APPLICATION

1.1 This method provides procedures for the detection and quantitative measurement of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) at concentrations ranging from 1 to 100 pg/g for the tetrachloro congeners up to 5 to 500 pg/g for the octachloro congeners in 10-g aliquots of human adipose tissue.

1.2 The minimum measurable concentration is estimated to range from 1 pg/g (1 part per trillion) for 2,3,7,8-TCDD and 2,3,7,8-TCDF up to 5 pg/g for OCDD and OCDF. However, these detection limits depend on the kinds and concentrations of interfering compounds in the sample matrix and the absolute method recovery.

1.3 The method will be used to determine PCDDs and PCDFs, particularly congeners with chlorine substitution in the 2,3,7,8 positions. Table 1 lists the specific PCDDs and PCDFs and target method detection limits.

2. SUMMARY OF METHOD

Figure 1 presents a schematic of the analytical procedures for determination of PCDDs and PCDFs in human adipose tissue. The analytical method requires extraction and isolation of lipid materials from human adipose samples. This is accomplished using sample sizes ranging up to 10 g. The tissue is spiked with known amounts of the carbon-13 labeled PCDDs and PCDFs (e.g., 500 pg of $^{13}C_{12}$-TCDD/F to 2,500 pg of $^{13}C_{12}$-OCDD/F) as internal quantitation standards. Extraction and homogenization are accomplished using methylene chloride and a Tekmar Tissuemizer®. The extract is filtered through anhydrous sodium sulfate to remove water. The extraction procedure is repeated (three to five times) until the tissue sample has been thoroughly homogenized. The final extract is adjusted to a known volume (100 mL) and the extractable lipid is determined using a minimum of 1% of the final volume. The methylene chloride in the remaining extract is concentrated until only an oily residue remains. The residue is diluted with hexane (~ 200 mL), and 100 g of sulfuric acid modified silica gel (40% w/w) is added to the solution with stirring. The mixture is stirred for approximately 2 h, and the supernatant is decanted and filtered through anhydrous sodium sulfate. The adsorbent is washed with at least two additional aliquots of hexane.

The combined hexane extracts are eluted through a column consisting of a layer of sulfuric acid modified silica gel, and a layer of unmodified silica gel. The eluate is concentrated to approximately 1 mL and added to a column of acidic alumina. The PCDDs and PCDFs are eluted from the alumina using 20% methylene chloride/hexane. This eluate is concentrated to approximately 0.5 mL and is added to a 500-mg Carbopak C/Celite column. The PCDDs and PCDFs are eluted from the column using 20 mL of toluene.

Table 1. Target PCDD and PCDF Congeners and Target Method Detection Limits

Compound	CAS no.[a]	Target method detection limit (pg/g)[b]
2,3,7,8-TCDD	1746-01-6	1.0
2,3,7,8-TCDF	51207-31-9	1.0
1,2,3,7,8-PeCDD	40321-76-4	1.0
1,2,3,7,8-PeCDF	57117-41-6	1.0
2,3,4,7,8-PeCDF	57117-31-4	1.0
1,2,3,4,7,8-HxCDD	39227-28-6	2.5
1,2,3,6,7,8-HxCDD	57653-85-7	2.5
1,2,3,7,8,9-HxCDD	19408-74-3	2.5
1,2,3,4,7,8-HxCDF	70648-29-9	2.5
1,2,3,6,7,8-HxCDF	57117-44-9	2.5
1,2,3,7,8,9-HxCDF	72918-21-9	2.5
2,3,4,6,7,8-HxCDF	60851-34-5	2.5
1,2,3,4,6,7,8-HpCDD	35822-46-9	2.5
1,2,3,4,6,7,8-HpCDF	67562-39-4	2.5
1,2,3,4,7,8,9-HpCDF	55673-89-7	2.5
OCDD	3268-87-9	5.0
OCDF	39001-02-0	5.0

[a] Chemical Abstract Services number.
[b] pg/g = parts per trillion.

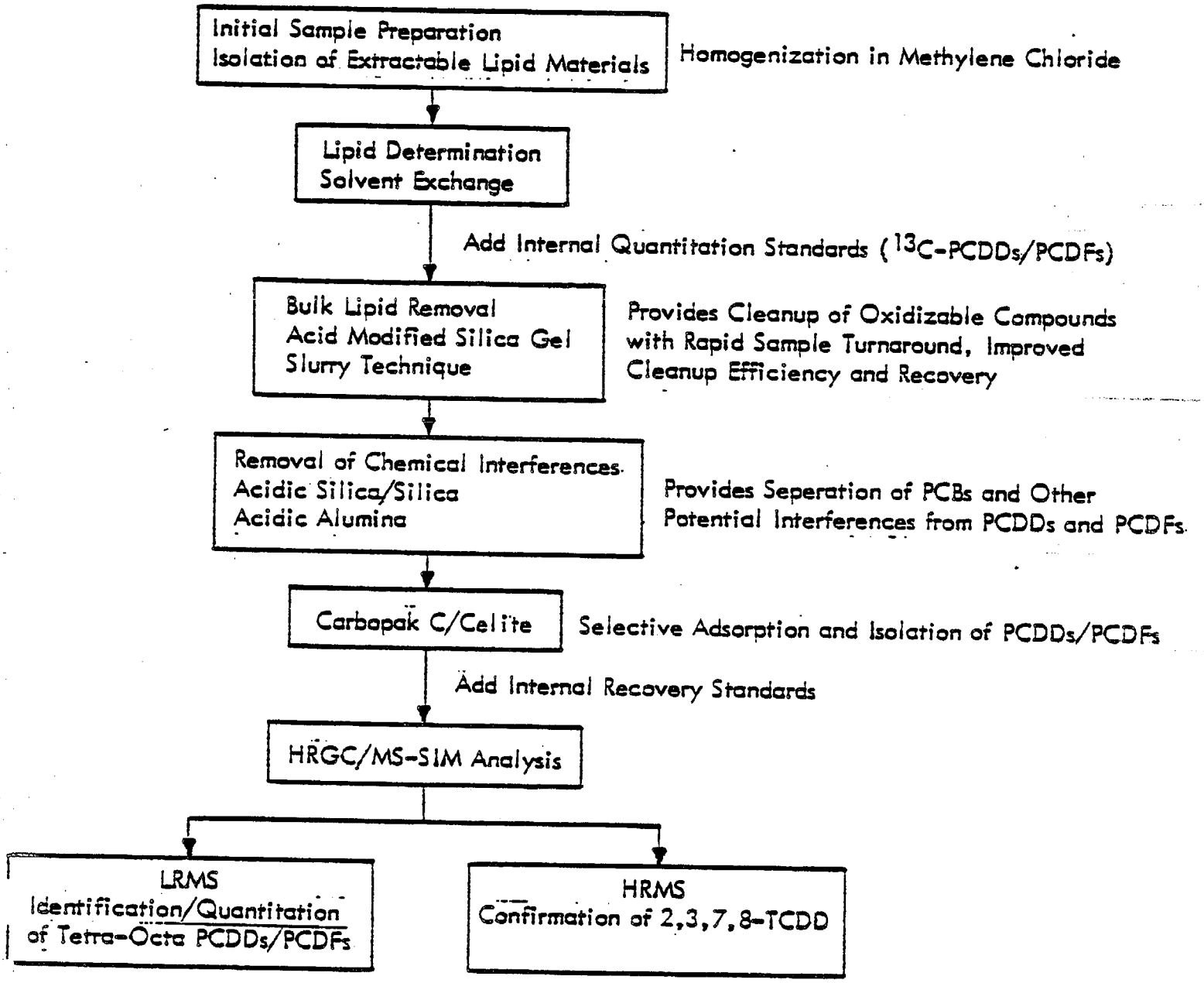

Figure 1. Schematic of the sample preparation and instrumental analysis procedures for determination of PCDDs and PCDFs in human adipose tissue.

The toluene is concentrated to less than 1 mL and transferred to conical vials. Tridecane (10 µL) containing 500 pg of an internal recovery standard is added as a keeper, and the extract is concentrated to final volume.

The HRGC/MS analysis is completed in the selected ion monitoring mode (SIM). Analysis of the tetra- through octachloro PCDD and PCDF congeners is achieved using low resolution mass spectrometry. Separation of the tetra- through octachloro PCDD and PCDF congeners is achieved using a 60-m DB-5 column. Verification of the 2,3,7,8-TCDD is achieved using either a 50-m CP Sil 88 column or 60-m SP-2330 column and HRGC/MS-SIM analysis in the high resolution mode (R = 10,000).

3. DEFINITIONS

3.1 Concentration calibration solutions -- Solutions containing known amounts of the native analytes (unlabeled 2,3,7,8-substituted PCDDs and PCDFs), the internal quantitation standards (Carbon-13 labeled PCDDs and PCDFs), and the recovery standard, $^{13}C_{12}$-1,2,3,4-TCDD. These calibration solutions are used to determine instrument response of the analytes relative to the internal quantitation standards and of the internal quantitation standards relative to the internal recovery standard.

3.2 Internal quantitation standards -- Carbon-13 labeled PCDDs and PCDFs, which are added to every sample and are present at the same concentration in every method blank and quality control sample. These are added to the adipose tissue and are used to measure the concentration of each analyte. The concentration of each internal quantitation standard is measured in every sample, and percent recovery is determined using the internal recovery standard.

3.3 Internal recovery standard -- $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD which is added to every sample extract just before the final concentration step and HRGC/MS-SIM analysis.

3.4 Laboratory method blank -- This blank is prepared in the laboratory through performing all analytical procedures except addition of a sample aliquot to the extraction vessel. A minimum of one laboratory method blank will be analyzed with each batch of samples.

3.5 HRGC column performance check mixture -- A mixture containing known amounts of selected TCDD standards; it is used to demonstrate continued acceptable performance of the capillary column, to separate (≦ 25% valley on a 50-m CP Sil 88 or 60-m SP-2330 HRGC column and 30 to 60% for a 60-m DB-5 HRGC column) 2,3,7,8-TCDD isomer from all other 21 TCDD isomers, and to define the TCDD retention time window.

3.6 Relative response factor -- Response of the mass spectrometer to a known amount of an analyte relative to a known amount of an internal standard (quantitation or recovery).

3.7 Mass resolution check -- Standard method used to demonstrate static resolution of 10,000 minimum (10% valley definition).

3.8 Sample batch -- A sample batch consists of up to 10 human adipose tissue samples, one method blank, 2 internal quality control (QC) samples (spiked and unspiked), and an external performance audit sample (blind spike).

4. INTERFERENCES

Chemicals which elute from the HRGC column with ± 10 scans of the internal and/or recovery standards and which produce within the retention time window ions at any of the masses used to detect or quantify PCDDs, PCDFs, or the internal quantitation and recovery standards are potential interferences. Most frequently encountered potential interferences are other sample components that are extracted along with the PCDDs and PCDFs, e.g., PCBs, chlorinated methoxybiphenyls, chlorinated hydroxydiphenyl ethers, chlorinated benzylphenyl ethers, chlorinated naphthalenes, DDE, DDT, etc. The actual incidence of interference by these chemicals depends also upon relative concentrations, mass spectrometric resolution, and chromatographic conditions. Because very low levels (pg/g) of PCDDs and PCDFs are anticipated, the elimination of interferences is essential. High purity reagents and solvents must be used and all equipment must be scrupulously cleaned. Laboratory method blanks must be analyzed to demonstrate absence of contamination that would interfere with measurement of the PCDDs and PCDFs. Column chromatographic procedures are used to remove coextracted sample components; these procedures must be performed carefully to minimize loss of PCDDs and PCDFs during attempts to increase their concentration relative to other sample components.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. The 2,3,7,8-TCDD is a known teratogen, mutagen, and carcinogen. Ingestion of microgram quantities can result in toxic effects. The other 2,3,7,8-substituted PCDDs and PCDFs may exhibit teratogenic, mutagenic, and carcinogenic effects. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. Only experienced personnel will be allowed to work with these chemicals.

5.2 All laboratory personnel will be required to wear laboratory coats or coveralls, gloves, and safety glasses. The neat standards, stock, and working solutions will be handled only in a Class A fume hood or glove box. When manipulating stock standards or working solutions, the analyst is advised to place the solution vials in a secure holder (sample block or glass beaker) to prevent accidental spills.

5.3 If these standards are spilled, absorb as much as possible with absorbent paper and place in a container clearly labeled as PCDD or PCDF waste. Solvent-wash all contaminated surfaces with toluene and absorbent paper followed by washing with a strong soap and water solution. Dispose of all contaminated materials in sealed steel containers labeled as contaminated with PCDD and/or PCDF residue and indicate the approximate level of contamination. As a final precaution, prepare a wipe sample of the exposed surface area and include the wipe as part of the sample analysis batch. This will be used to confirm that the work area is free of contamination.

5.4 If handling of these compounds results in skin contact, immediately remove all contaminated clothing and wash the affected skin areas with soap and water for at least 15 min.

5.5 Disposal of laboratory wastes -- All laboratory wastes (solvents and absorbents) will be disposed of as hazardous wastes. The laboratory personnel should take care to dispose of the sodium sulfate, silica gel, and alumina in separate containers. Excess solvents should be disposed of in gallon polyethylene jugs containing a layer of activated charcoal. Excess solvent that is known to be contaminated with PCDDs or PCDFs should be kept at a minimum by evaporating the solvent with a stream of air.

6. APPARATUS AND EQUIPMENT

6.1 High Resolution Gas Chromatograph/Mass Spectrometer/Data System (HRGC/HRMS/DS)

6.1.1 The GC must be equipped for temperature programming, and all required accessories must be available, such as syringes, gases, and a capillary column. The GC injection port must be designed for capillary columns. The use of splitless injection techniques is recommended. When using this method, a 1-μL injection volume is used. The injection volumes for all extracts, blanks, calibration solutions, and the performance check sample must be consistent.

6.1.2 High Resolution Gas Chromatograph-Mass Spectrometer Interface

The HRGC/MS interface is directly coupled to the mass spectrometer ion source. All components of the interface should be glass or glass-lined stainless steel. The interface components should be compatible with 300°C temperatures. The HRGC/MS interface must be appropriately designed so that the separation of the PCDDs and PCDFs which is achieved in the gas chromatographic column is not appreciably degraded. Cold spots and/or active surfaces (adsorption sites) in the HRGC/MS

interface can cause peak tailing and peak broadening. It is recommended that the HRGC column be fitted directly into the MS ion source. Graphite ferrules should be avoided in the HRGC injection port since they may absorb PCDDs or PCDFs. Vespel or equivalent ferrules are recommended.

6.1.3 Mass Spectrometer

The mass spectrometer must be capable of maintaining a minimum resolution of 10,000 (10% valley) for high resolution confirmation analysis. The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with total cycle time (including voltage reset time) of 1 s or less.

6.1.4 Data System

A dedicated hardware or data system is required to control the rapid multiple ion monitoring process and to acquire the data. Quantification data (peak areas or peak heights) and SIM traces (displays of intensities of each m/z (characteristic ion) being monitored as a function of time) must be acquired during the analyses. Quantifications may be reported based upon computer-generated peak areas or upon measured peak heights.

6.2 HRGC Columns

For isomer-specific determinations of 2,3,7,8-TCDD, the following fused silica capillary columns are recommended: a 50-m CP-Sil 88 column and a 60-m SP-2330 (SP-2331) column. However, any capillary column which separates 2,3,7,8-TCDD from all other TCDDs may be used for such analyses, provided that the minimum acceptance criteria in Section 8 are met.

6.3 Miscellaneous Equipment

6.3.1 Nitrogen evaporation apparatus with variable flow rate.

6.3.2 Balance capable of accurately weighing to ± 0.01 g.

6.3.3 Balance capable of accurately weighint to ± 0.0001 g.

6.3.4 Water bath -- equipped with concentric ring cover and capable of being temperature-controlled.

6.3.5 Stainless steel spatulas or spoons.

6.3.6 Magnetic stirrers and stir bars.

6.3.7 High speed tissue homogenizer -- Tekmar Tissuemizer® equipped with an EN-8 probe or equivalent.

6.3.8 Vacuum dessicator.

6.4 Glassware

6.4.1 Erlenmeyer flask -- 500 mL.

6.4.2 Kuderna-Danish apparatus -- 500-mL evaporating flask, 15-mL graduated concentrator tubes with ground-glass stoppers, and three-ball macro Snyder column (Kontes K-570001-0500, K-503000-0121, and K-569001-0219 or equivalent).

6.4.3 Minivials -- 1-mL borosilicate glass with conical-shaped reservoir and screw caps lined with Teflon®-faced silicone disks.

6.4.4 Powder funnels -- glass.

6.4.5 Chromatographic columns for the silica and alumina chromatography -- 1 cm ID x 10 cm long and 1 cm ID x 30 cm long with 250-mL reservoir and equipped with TFE stopcocks.

6.4.6 Chromatographic column for the Carbopak cleanup -- disposable 5-mL graduated glass pipets, 6 to 7 mm ID.

6.4.7 Glass rods.

6.4.8 Carborundum boiling chips -- Extracted for 6 hr in a Soxhlet apparatus with benzene and air dried.

6.4.9 Glass wool, silanized (Supelco) -- Extract with methylene chloride and hexane and air dry before use.

6.4.10 Glassware cleaning procedure -- All glassware used for these analyses will be cleaned via the following procedure. Wash the glassware in soap and water, rinse with copious amounts of tap water, distilled water, and distilled-in-glass acetone, in that order. Immediately prior to use, the glassware should be rinsed with distilled-in-glass quality solvents: methylene chloride, toluene, and hexane. The glassware should be allowed to dry fully.

As an added precuation, all glassware will be marked with a unique code that should be noted in the extraction and cleanup procedures for each sample. This glassware tracking will allow background results from specific glassware to be documented.

After use, each piece of glassware should be rinsed with the last solvent used in it, followed by a rinse with toluene, then acetone, before transferring it to the glassware washing facility.

7. REAGENTS AND STANDARD SOLUTIONS

7.1 Column Chromatography Reagents

7.1.1 Alumina, acidic (Biorad, AG-4) -- Extract the alumina in a Soxhlet apparatus with methylene chloride for 18 h (minimum of two cycles per hour). Air dry and activate it by heating in a foil-covered glass container for 24 h at 190°C.

7.1.2 Silica gel -- High purity grade, type 60, 70-230 mesh; extract the silica gel in a Soxhlet apparatus with methylene chloride for 10 h (minimum of 2 cycles per hour). Air dry and activate it by heating in a foil-covered glass container for 24 h at 130°C.

7.1.3 Silica gel impregnated with 40% (by weight) sulfuric acid -- Add two parts (by weight) concentrated sulfuric acid to three parts (by weight) silica gel (extracted and activated) (e.g., 40 g of H_2SO_4 plus 60 g of silica gel) in a glass screw-cap bottle. Tumble for 5 to 6 h, shaking occasionally until free of lumps.

7.1.4 Sulfuric acid, concentrated -- ACS grade, specific gravity 1.84.

7.1.5 Graphitized carbon black (Carbopack C, Supelco), surface of approximately 12 m^2/g, 80/100 mesh -- Mix thoroughly 3.6 g of Carbopack C and 16.4 g of Celite 545® in a 40-mL vial. Activate at 130°C for 6 h. Store in a desiccator.

7.1.6 Celite 545® (Fischer Scientific), reagent grade, or equivalent.

7.2 Desiccating agents -- Sodium sulfate; granular, anhydrous. Before use extract with methylene chloride for 16 h (minimum of two cycles per hour), air dry and then muffle for ≧ 4 h in a shallow tray at 400°C. Let it cool in a desiccator and store in oven at 130°C.

7.3 Solvents -- High purity, distilled in glass: methylene chloride, toluene, benzene, cyclohexane, methanol, acetone, hexane; reagent grade: tridecane. High purity solvents are dispensed from Teflon® squirt bottles.

7.4 Concentration Calibration Solutions (Table 2)

Eight tridecane solutions containing native calibration standards, $^{13}C_{12}$-labeled internal quantitation standards, and two internal recovery standards are required. The complete compound list is

Table 2. Concentration Calibration Solutions

Compound	Concentration in calibration solutions in pg/μL							
Native	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8
2,3,7,8-TCDD	200	100	50	25	10	5	2.5	1
2,3,7,8-TCDF	200	100	50	25	10	5	2.5	1
1,2,3,7,8-PeCDD	200	100	50	25	10	5	2.5	1
1,2,3,7,8-PeCDF	200	100	50	25	10	5	2.5	1
2,3,4,7,8-PeCDF	200	100	50	25	10	5	2.5	1
1,2,3,4,7,8-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,6,7,8-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,7,8,9-HxCDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,6,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,7,8,9-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5
2,3,4,6,7,8-HxCDF	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,6,7,8-HpCDD	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,6,7,8-HpCDF	500	250	125	62.5	25	12.5	6.25	2.5
1,2,3,4,7,8,9-HpCDF	500	250	125	62.5	25	12.5	6.25	2.5
OCDD	1,000	500	250	125	50	25	12.5	5
OCDF	1,000	500	250	125	50	25	12.5	5
Internal Quantitation Standards								
$^{13}C_{12}$-2,3,7,8-TCDD	50	50	50	50	50	50	50	50
$^{13}C_{12}$-2,3,7,8-TCDF	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeCDD	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeCDF	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	125	125	125	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	125	125	125	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	125	125	125	125	125	125	125	125
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	125	125	125	125	125	125	125	125
$^{13}C_{12}$-OCDD	250	250	250	250	250	250	250	250
Internal Recovery Standard								
$^{13}C_{12}$-1,2,3,4-TCDD	50	50	50	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	125	125	125	125	125	125	125	125

given in Table 2. The native 2,3,7,8-TCDD is supplied as a certified standard solution from the U.S. EPA QA Reference Materials Branch. All other native compounds were supplied in crystalline form by Cambridge Isotope Laboratories (Woburn, MA). $^{13}C_{12}$-Labeled internal quantitation standards were supplied in solution in n-nonane by Cambridge Isotope Laboratories. Portions of the native standards were accurately weighed to the nearest 0.001 mg with a Cahn 27 electrobalance and dissolved in toluene.

7.5 Column Performance Check Mixture

The column performance check mixture consists of several TCDD isomers which will be used to document the separation of 2,3,7,8-TCDD from all other isomers. This solution will contain TCDDs (A) eluting closely to 2,3,7,8-TCDD, and the first- (F) and last-eluting (L) TCDDs.

Analyte	Approximate amount per ampule
Unlabeled 2,3,7,8-TCDD	10 ng
$^{13}C_{12}$-2,3,7,8-TCDD	10 ng
1,2,3,4-TCDD (A)	10 ng
1,4,7,8-TCDD (A)	10 ng
1,2,3,7-TCDD (A)	10 ng
1,2,3,8-TCDD (A)	10 ng
1,3,6,8-TCDD (F)	10 ng
1,2,8,9-TCDD (L)	10 ng

7.6 Spiking Solutions

Three solutions are prepared using the same stock as in Section 7.4. A native standard solution and a $^{13}C_{12}$ internal quantitation standard solution are prepared in isooctane (Tables 3 and 4). A recovery standard solution is prepared in tridecane (Table 4). Samples are spiked with 100 µL of internal quantitation standard solution and final sample extracts are spiked with 10 µL of internal recovery standard solution.

8. HIGH RESOLUTION GAS CHROMATOGRAPHY/MASS SPECTROMETRY PERFORMANCE CRITERIA

Samples and standards are analyzed by using a Carlo Erba MFC500 gas chromatography (GC) coupled to a Kratos MS50TC double-focusing mass spectrometer (MS) to be operated in the electron impact mode. The HRGC/MS interface is simply a direct connection of the fused silica HRGC column to the ion source of the MS via a heated interface oven. Data acquisition and processing are controlled by a Finnigan-MAT Incos 2300 data system.

Table 3. Native Spiking Solution[a]

Compound	Concentration (pg/μL)
2,3,7,8-TCDD	5
2,3,7,8-TCDF	5
1,2,3,7,8-PeCDD	5
1,2,3,7,8-PeCDF	5
2,3,4,7,8-PeCDF	5
1,2,3,4,7,8-HxCDD	12.5
1,2,3,6,7,8-HxCDD	12.5
1,2,3,7,8,9-HxCDD	12.5
1,2,3,4,7,8-HxCDF	12.5
1,2,3,6,7,8-HxCDF	12.5
1,2,3,7,8,9-HxCDF	12.5
2,3,4,6,7,8-HxCDF	12.5
1,2,3,4,6,7,8-HpCDD	12.5
1,2,3,4,6,7,8-HpCDF	12.5
1,2,3,4,7,8,9-HpCDF	12.5
OCDD	25
OCDF	25

[a]Prepared in isooctane.

Table 4. Internal Standard Spiking Solutions

Compound	Concentration (pg/μL)
Internal Quantitation Standards[a]	
$^{13}C_{12}$-2,3,7,8-TCDD	5
$^{13}C_{12}$-2,3,7,8-TCDF	5
$^{13}C_{12}$-1,2,3,7,8-PeCDD	5
$^{13}C_{12}$-1,2,3,7,8-PeCDF	5
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	12.5
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	12.5
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	12.5
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	12.5
$^{13}C_{12}$-OCDD	25
Internal Recovery Standard[b]	
$^{13}C_{12}$-1,2,3,4-TCDD	50
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	125

[a]Prepared in isooctane.
[b]Prepared in tridecane.

Table 6. Ions Monitored for HRGC/MS of PCDD/PCDF

Descriptor	ID	Mass	Nominal dwell time (sec)
A1	TCDF	303.902	0.090
		305.899	0.090
	$^{13}C_{12}$-TCDF	315.942	0.090
		317.939	0.090
	TCDD	319.897	0.090
		321.894	0.090
	$^{13}C_{12}$-TCDD	331.937	0.090
		333.934	0.090
	HxCDPE	373.840	0.090
	PFK (lock mass)	380.976	0.090
A2	TCDF	303.902	0.045
		305.899	0.045
	TCDD	319.897	0.045
		321.894	0.045
	PeCDF	337.863	0.045
		339.860	0.045
	$^{13}C_{12}$-PeCDF	349.903	0.045
		351.900	0.045
	PeCDD	353.858	0.045
		355.855	0.045
	$^{13}C_{12}$-PeCDD	365.898	0.045
		367.895	0.045
	PFK (lock mass)	380.976	0.035
	HpCDPE	407.801	0.035
A3	HxCDF	373.821	0.080
		375.818	0.080
	PFK (lock mass)	380.976	0.080
	$^{13}C_{12}$-HxCDF	385.861	0.080
		387.858	0.080
	HxCDD	389.816	0.080
		391.813	0.080
	$^{13}C_{12}$-HxCDD	401.856	0.080
		403.853	0.080
	OCDPE	443.759	0.080

Table 6 (continued)

Descriptor	ID	Mass	Nominal dwell time (sec)
A4	PFK (lock mass)	380.976	0.040
	HxCDD	389.816	0.040
		391.813	0.040
	HpCDF	407.782	0.040
		409.779	0.040
	$^{13}C_{12}$-HpCDF	419.822	0.040
		421.819	0.040
	HpCDD	423.777	0.040
		425.774	0.040
	$^{13}C_{12}$-HpCDD	435.817	0.040
		437.814	0.040
	$^{37}Cl_4$-HpCDD	429.768	0.040
		431.765	0.040
	NCDPE	477.720	0.040
A5	PFK (lock mass)	380.976	0.06
	OCDF	441.743	0.07
		443.740	0.07
	$^{13}C_{12}$-OCDF	453.783	0.07
		455.780	0.07
	OCDD	457.738	0.07
		459.735	0.07
	$^{13}C_{12}$-OCDD	469.779	0.07
		471.776	0.07
	DCDPE	511.681	0.06

8.1.3 HRGC Column Performance (60-m DB-5)

The HRGC column performance must be demonstrated at the start of each 12-h analysis period.

8.1.3.1 Inject 1 µL of the column performance check solution (Section 7.5) and acquire selected ion monitoring (SIM) data for m/z 320, 322, 332, and 334.

8.1.3.2 The chromatographic peak separation between 2,3,7,8-TCDD and the peaks representing any other TCDD isomers should be resolved with a valley of 30-60%, where

$$\text{Valley \%} = (x/y)(100)$$

x = measured height of the valley between the chromatographic peak corresponding to 2,3,7,8-TCDD and the peak of the nearest TCDD isomer; and

y = the peak height of 2,3,7,8-TCDD.

Figure 2 is an example of the separation of a TCDD isomer mixture and the calculation of isomer resolution.

It is the responsibility of the laboratory to verify the conditions suitable for the appropriate resolution of 2,3,7,8-TCDD from all other TCDD isomers. The column performance check solution also contains the TCDD isomers eluting first and last under the analytical conditions specified in this protocol, thus defining the retention time window for total TCDD determination. Any individual selected ion current profile or the reconstructed total ion current (m/z 320 + m/z 322) consititutes an acceptable form of data presentation.

8.1.4 Initial Calibration for PCDD/PCDF Analysis

Initial calibration is required before any samples are analyzed for PCDD/PCDF. Initial calibration is also required if any routine calibration does not meet the required criteria listed in Section 8.1.7.

8.1.4.1 Tune and calibrate the instrument with PFK as outlined in Section 8.1.1.

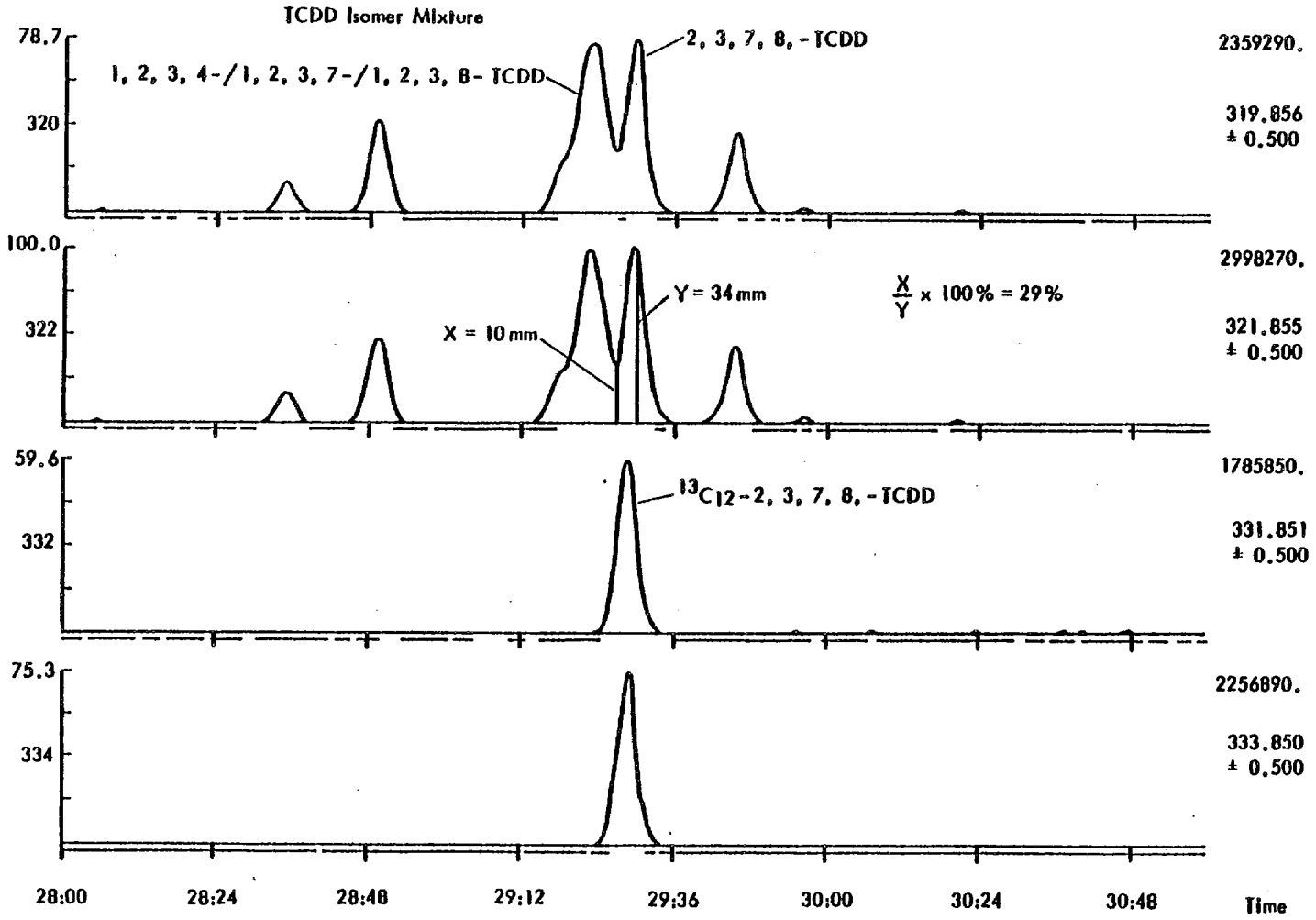

Figure 2. Example of the separation of 2,3,7,8-TCDD from other TCDD isomers on a 60 m DB-5 column.

8.1.4.2 Six of the eight concentration calibration solutions listed in Table 2 will be analyzed for the initial calibration phase. These must include solutions CS4 through CS8 (Table 2). The analyst may select any of the remaining solutions for demonstrating calibration at the upper concentration range.

8.1.4.3 Using the HRGC and MS conditions in Table 5 and the SIM monitoring descriptors in Table 6, analyze a 1-µL aliquot of each of the six concentration calibration solutions in triplicate.

8.1.4.4 Compute the relative response factors (RRFs) for each analyte in the concentration calibration solution using the criteria for positive identification of PCDD/PCDF's given in Section 14.1 and the computational methods in Section 14.2.

8.1.4.5 Compute the means and their respective relative standard deviations (% RSD) for the RRFs from each triplicate analysis for each analyte in the standard.

8.1.4.6 Calculate the grand means ($\overline{RRF}$) and their respective RSDs using the six mean RRFs for each analyte.

8.1.5 Criteria for Acceptable Initial Calibration

8.1.5.1 The % RSD for the response factors for each triplicate analysis of a single concentration calibration standard for each analyte must be less than ± 30% except for the TCDD and TCDF, which must be less than ± 20%.

8.1.5.2 The variation of the mean RRFs for the six concentration calibrated standards (Section 8.1.5.1) must be less than 30% except for the TCDD and TCDF which must be less than 20%.

8.1.5.3 The SIM traces for all ions used for quantitation must present a signal-to-noise (S/N) ratio of ≧ 2.5. This includes analytes and isotopically labeled standards.

8.1.5.4 Isotopic ratios must be within ± 20% of the theoretical values (see Table 7).

NOTE: If the criteria for acceptable calibration listed above have been met, the RRF can be considered independent of the analyte quantity for the calibration concentration range. The grand mean RRF from the initial calibration for unlabeled PCDD/PCDFs and for the isotopically labeled standards will be used for all calculations until routine calibration criteria (Section 8.1.7) are no longer met. At such time, new mean RRFs will be calculated from a new set of six triplicate determinations.

8.1.6 Routine Calibrations

Routine calibrations must be performed at the beginning of every day before actual sample analyses are performed and as the last injection of every day.

8.1.6.1 Inject 1 µL of the concentration calibration solution CS 7 (see Table 2) as the initial calibration check on each analysis day. It is recommended that the analyst select a concentration calibration solution that brackets the sample concentrations observed on a single analysis date as the last injection of each analysis date.

8.1.6.2 Compute the RRFs for each analyte in the concentration calibration solution using the criteria for positive identification of PCDD/Fs given in Section 14.1 and the computational methods in Section 14.2.

8.1.7 Criteria for Acceptable Routine Calibration

8.1.7.1 The measured RRF for all analytes must be within ± 30% of the grand mean values established by triplicate analysis of the calibration concentration solutions, except for TCDD and TCDF, which must be within ± 20% of the mean values established in the initial calibration step.

8.1.7.2 Isotopic ratios must be within ± 20% of the theoretical value for each analyte and isotopically labeled standard (see Table 7).

Table 7. Ion Ratios for HRGC/LRMS Analysis of PCDD/PCDF

Compound	Ions monitored	Theoretical ratio	Acceptable range
TCDF	304/306	0.76	0.61 - 0.91
$^{13}C_{12}$-TCDF	316/318	0.76	0.61 - 0.91
TCDD	320/322	0.76	0.61 - 0.91
$^{13}C_{12}$-TCDD	332/334	0.76	0.61 - 0.91
PeCDF	338/340	0.61	0.49 - 0.73
$^{13}C_{12}$-PeCDF	350/352	0.61	0.49 - 0.73
PeCDD	354/356	0.61	0.49 - 0.73
$^{13}C_{12}$-PeCDD	366/368	0.61	0.49 - 0.73
HxCDF	374/376	1.22	0.98 - 1.46
$^{13}C_{12}$-HxCDF	386/388	1.22	0.98 - 1.46
HxCDD	390/392	1.22	0.98 - 1.46
$^{13}C_{12}$-HxCDD	402/404	1.22	0.98 - 1.46
HpCDF	408/410	1.02	0.82 - 1.22
$^{13}C_{12}$-HpCDF	420/422	1.02	0.82 - 1.22
HpCDD	424/426	1.02	0.82 - 1.22
$^{13}C_{12}$-HpCDD	436/438	1.02	0.82 - 1.22
OCDF	442/444	0.87	0.70 - 1.04
$^{13}C_{12}$-OCDF	454/456	0.87	0.70 - 1.04
OCDD	458/460	0.87	0.70 - 1.04
$^{13}C_{12}$-OCDD	470/472	0.87	0.70 - 1.04

8.1.7.3 If any of the above criteria is not met, a second attempt may be made before repeating the entire initialization process.

8.2 HRGC/HRMS Analysis (Isomer Specific TCDD Analysis)

Isomer specific analysis for 2,3,7,8-TCDD is carried out with the instrumental conditions and parameters shown in Table 8. In addition to monitoring the masses of the most abundant molecular ions of TCDD, an ion corresponding to the loss of COCl from the molecular ion is monitored for verification purposes. Mass spectrometer resolution is maintained at or above 10,000 (10% valley definition) in order to increase the specificity of the analysis.

8.2.1 Tuning and Mass Calibration

8.2.1.1 The mass spectrometer must be operated in the electron (impact) ionization mode. Static resolving power of at least 10,000 (10% valley) must be demonstrated before any analysis of a set of samples is performed. Static resolution checks must be performed at the beginning and at the end of each 12-h period of operation. However, it is recommended that a visual check (i.e., not documented) of the static resolution be made before and after each analysis.

8.2.1.2 The MS shall be tuned daily using PFK to yield a resolution of at least 10,000 (10% valley) and optimal response at m/z 254.986. This step is followed by calibration of an accelerating voltage scan of PFK beginning at m/z 254 (typical calibration range is 255 to 493 amu). Other voltage scans from the same data file are used to establish and document both the resolution at m/z 316.983 and the mass measurement accuracy at m/z 330.979.

8.2.1.3 Following calibration, the SIM experiment descriptor is updated to reflect the new calibration. Six masses (see Table 8) are monitored by scanning ∿ m/10,000 amu (atomic mass units) over each mass. The total cycle time is kept to 1 s. The m/z 280.983 ion from PFK is used as a lock mass because it is the most abundant PFK ion within the range of m/z 255 to 334 and therefore permits the use of low partial pressures of PFK, which minimizes PFK interferences at the analytical masses.

Table 8. HRGC/HRMS Operating Conditions

Mass spectrometer

Accelerating voltage:	8,000 V
Trap current:	500 µA
Electron energy:	70 eV
Electron multiplier voltage:	2,000 V
Source temperature:	280°C
Resolution:	10,000 (10% valley definition)

SIM Parameters

Identity	Mass	Nominal dwell times (s)
TCDD-COCl	258.930	0.15
TCDD	319.897	0.15
TCDD	321.894	0.15
$^{13}C_{12}$-TCDD	331.937	0.15
$^{13}C_{12}$-TCDD	333.934	0.15
PFK (lock mass)	280.983	0.10

Overall SIM cycle time = 1 s

Gas chromatograph

Column coating:	CP-Sil 88
Film Thickness:	0.2 µm
Column dimensions:	50 m x 0.22 mm ID
Helium linear velocity:	~ 25 cm/s
Helium head pressure:	1.75 kg/cm^2 (25 psi)
Injection type:	Splitless, 45 s
Split flow:	30 mL/min
Purge flow:	6 mL/min
Injector temperature:	270°C
Interface temperature:	240°C
Injection size:	2 µL
Initial temperature:	200°C
Initial time:	1 min
Temperature program:	200°C to 240°C at 4°C/min

8.2.2 Mass Measurement and Resolution Check

Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10% valley) at m/z 254.986 (or any other mass reasonably close to m/z 259). Calibrate the voltage sweep at least across the mass range m/z 259 to m/z 334 and verify that m/z 330.979 from PFK (or any other mass close to m/z 334) is measured within ± 5 ppm (i.e., 1.7 mmu, if m/z 331 is chosen) using m/z 254.986 as a reference. Documentation of the mass resolution must then be accomplished by recording the peak profile of the PFK reference peak m/z 318.979 (or any other reference peak at a mass close to m/z 320/322). The format of the peak profile representation must allow manual determination of the resolution; i.e., the horizontal axis must be a calibrated mass scale (amu or ppm per division). The results of the peak width measurement (performed at 5% of the maximum which corresponds to the 10% valley definition) must appear on the hard copy and cannot exceed 100 ppm (or 31.9 mmu if m/z 319 is the chosen reference ion).

8.2.3 HRGC Column Performance (50-m CP Sil 88/60-m SP-2330)

Prior to any HRGC/HRMS analysis of calibration solutions or samples for 2,3,7,8-TCDD, the resolution of the HRGC columns must be documented to be within allowable limits in order to provide conditions adequate for unambiguous isomer-specific analysis of 2,3,7,8-TCDD. This column performance check must be demonstrated at the start of each 12-h analysis period.

8.2.3.1 Inject 2 µL of the column performance check solution and acquire selected ion monitoring (SIM) data for m/z 258.930, 319.897, 321.894, 331.937, and 333.934 within a total cycle time of ≦ 1 s (Table 8).

8.2.3.2 The chromatographic peak separation between 2,3,7,8-TCDD and the peaks representing any other TCDD isomers must be resolved with a valley of ≦ 25%, where

$$\text{Valley \%} = (x/y)(100)$$

x = measured height of the valley between the chromatographic peak corresponding to 2,3,7,8-TCDD and the peak of the nearest TCDD isomer; and

y = the peak height of 2,3,7,8-TCDD.

8.2.3.3 If the above resolution requirement is not met, corrective action must be taken and acceptable resolution documented prior to any further analyses. Corrective action may include removal of the first meter of the HRGC column, replacement or clearing of the injector port, or complete replacement of the GC column.

8.2.3.4 The column performance check solution also contains the TCDD isomers eluting first and last under the analytical conditions specified in this protocol, thus defining the retention time window for total TCDD determination. The peaks representing 2,3,7,8-TCDD and the first and the last eluting TCDD isomer should be labeled and identified as such on the chromatograms (F and L, respectively). Any individual selected ion current profile or the reconstructed total ion current (m/z 259 + m/z 320 + m/z 322) constitutes an acceptable form of data presentation.

8.2.4 Initial Calibration for HRGC/HRMS 2,3,7,8-TCDD Analysis

Initial calibration is required before any samples are analyzed for 2,3,7,8-TCDD. Initial calibration is also required if any routine calibration does not meet the required criteria listed in Section 8.2.6.

8.2.4.1 At least six of the concentration calibration solutions listed in Table 2 must be utilized for the initial calibration. These must include solutions CS4 through CS8. The analyst may select any of the remaining solutions for demonstrating calibration at the upper concentration range.

8.2.4.2 Tune and calibrate the instrument with PFK as described in Section 8.2.1.

8.2.4.3 Inject 1 μL of the column performance check solution (Section 8.2.3) and acquire SIM mass spectra data for m/z 258.930, 319.897, 321.894, 331.937, and 333.934 using a total cycle time of ≦ 1 s (see Table 8). The laboratory must not perform any further analysis until it has been demonstrated and documented that the criterion listed in Section 8.2.3.2 has been met.

8.2.4.4 Using the same GC and MS conditions (Table 8) that produced acceptable results with the column performance check solution, analyze a 1-µL aliquot of each of the six concentration calibration solutions in triplicate.

8.2.4.5 Calculate the RRFs for unlabeled 2,3,7,8-TCDD relative to $^{13}C_{12}$-2,3,7,8-TCDD and the RRF for $^{13}C_{12}$-2,3,7,8-TCDD relative to $^{13}C_{12}$-1,2,3,4-TCDD using the criteria for positive identification of TCDD by HRGC/HRMS given in Section 14.1 and the computational methods in Section 14.2.

8.2.4.6 Calculate the six means (RRFs) and their respective relative standard deviations (% RSD) for the response factors from each of the triplicate analyses for both unlabeled and $^{13}C_{12}$-2,3,7,8-TCDD.

8.2.4.7 Calculate the grand mean RRFs and their respective relative standard deviations (% RSD) using the six mean RRFs.

8.2.5 Criteria for Acceptable Initial Calibration

The criteria listed below for acceptable calibration must be met before analysis of any sample is performed.

8.2.5.1 The percent relative standard deviation (RSD) for the response factors from each of the triplicate analyses of a single concentration calibration standard for both unlabeled and $^{13}C_{12}$-2,3,7,8-TCDD must be less than 20%.

8.2.5.2 The variation of the mean RRFs from the six concentration calibration standards unlabeled and $^{13}C_{12}$-2,3,7,8-TCDD must be less than 20% RSD.

8.2.5.3 SIM traces for 2,3,7,8-TCDD must present a signal-to-noise ratio of ≧ 2.5 for m/z 258.930, m/z 319.897, and m/z 321.894.

8.2.5.4 SIM traces for $^{13}C_{12}$-2,3,7,8-TCDD must present a signal-to-noise ratio ≧ 2.5 for m/z 331.937 and m/z 333.934.

8.2.5.5 Isotopic ratios for 320/322 and 332/334 must be within the allowed range (0.61 to 0.91).

NOTE: If the criteria for acceptable calibration listed above have been met, the RRF can be considered independent of the analyte quantity for the calibration concentration range. The grand mean RRF from the initial calibration for unlabeled 2,3,7,8-TCDD and for $^{13}C_{12}$-2,3,7,8-TCDD will be used for all calculations until routine calibration criteria (Section 8.2.6) are no longer met. At such time, new mean RRFs will be calculated from a new set of six triplicate determinations.

8.2.6 Routine Calibrations

Routine calibrations must be performed at the beginning of a 12-h period after successful mass resolution and HRGC column performance check runs and before analysis of actual samples. The response factor calibration must also be verified at the end of each analysis date.

8.2.6.1 Inject 1 µL of the concentration calibration solution (CS7, Table 2) which contains 2.5 pg/µL of unlabeled 2,3,7,8-TCDD, 50.0 pg/µL of $^{13}C_{12}$-2,3,7,8-TCDD, and 50 pg/µL of $^{13}C_{12}$-1,2,3,4-TCDD. Using the same HRGC/MS/DS conditions as used in Table 8, determine and document acceptable calibration as provided below.

8.2.7 Criteria for Acceptable Routine Calibration

The following criteria must be met before further analysis is performed. If these criteria are not met, corrective action must be taken and the instrument must be recalibrated.

8.2.7.1 The measured RRF for unlabeled 2,3,7,8-TCDD must be within 20% of the mean values established in the initial calibration by triplicate analyses of concentration calibration solutions.

8.2.7.2 The measured RRF for $^{13}C_{12}$-2,3,7,8-TCDD must be within 20% of the mean value established by triplicate analysis of the concentration calibration solutions during the initial calibration.

8.2.7.3 Isotopic ratios must be within the allowed range (0.61 to 0.90).

8.2.7.4 If one of the above criteria is not satisfied, a second attempt can be made before repeating the entire initialization process.

NOTE: An initial calibration must be carried out whenever the routine calibration solution is replaced by a new one from a different lot.

9. QUALITY CONTROL PROCEDURES

9.1 Summary of QC Analyses

9.1.1 Initial and routine calibration and instrument performance checks.

9.1.2 Analysis of a batch of samples with accompanying QC analyses:

Sample batch -- 10 NHATS adipose tissue samples plus additional QC analyses including 1 method blank, a control tissue and a spiked tissue sample.

"Blind" QC (external QC) samples may be submitted by an external source (quality assurance group or independent laboratory) and included among the batch of samples. Blind samples include spiked samples, unidentified duplicates, and performance evaluation samples.

9.2 Performance Evaluation Solutions -- Included among the samples in every third batch will be a solution provided by the quality control coordinator containing known amounts of unlabeled 2,3,7,8-TCDD and/or other PCDD/PCDF isomers. The accuracy of measurements for performance evaluation samples should be in the range of 70-130%.

9.3 Column Performance Check Solutions

9.3.1 At the beginning of each 12-h period during which samples are to be analyzed, an aliquot of the HRGC column performance check solution shall be analyzed to demonstrate adequate HRGC resolution for selected TCDD isomers.

9.4 Method Blanks

9.4.1 A minimum of one method blank is generated with each batch of samples. A method blank is generated by performing all steps detailed in the analytical procedure using all reagents, standards, equipment, apparatus, glassware, and solvents that would be used for a sample analysis, but omit addition of the adipose tissue.

9.4.1.1 The method blank must contain the same amounts of Carbon-13 labeled internal quantitation standards that are added to samples before bulk lipid cleanup.

9.4.1.2 An acceptable method blank exhibits no positive response for any of the characteristic ions monitored.

9.4.1.2.1 If the above criterion is not met, solvents, reagents, spiking solutions, apparatus, and glassware are checked to locate and eliminate the source of contamination before any samples are extracted and analyzed.

9.4.1.2.2 If new batches of reagents or solvents contain interfering contaminants, purify or discard them.

9.5 Control Samples -- Control samples are prepared from a bulk sample(s) of human adipose tissue or similar matrix (e.g., porcine fat). This material is prepared by blending the tissue with methylene chloride, drying the extract by eluting through anhydrous sodium sulfate, and removing the methylene chloride using rotoevaporation at elevated temperatures (80°C). The evaporation process should be extended to ensure all traces of the extraction solvent have been removed. The resulting oily matrix (lipid) is subdivided into 10-g aliquots which are analyzed with each sample batch. The results of the individual analysis will be used to give a measure of precision from batch to batch over an entire program. Sufficient tissue should be extracted to provide a homogeneous lipid matrix that can be used over the total analysis program. Enough lipid matrix is necessary to prepare the spiked samples describe in Section 9.6.

9.6 Spiked Samples -- Spiked lipid samples are prepared using a portion of the homogenized lipid described in Section 9.5. Sufficient spiked lipid matrix is prepared to provide a minimum of one spiked sample per sample batch. It is recommended that a minimum

of three spiked levels of the matrix are prepared ranging from 10 to 50 times the estimated limit of detection for each compound. Each analysis of spiked sample must be accompanied by analysis of a control sample in order to make the necessary corrections for background contribution before determining the accuracy of the method (Equation 9-1).

$$\text{Accuracy (\%)} = 100\% \times \frac{\text{Conc. spiked sample-conc. control sample}}{\text{Spike level}} \qquad \text{Eq. 9-1}$$

9.7 Duplicate Sample Analysis -- When possible a duplicate analysis of specific samples is included in the sample batch as an additional measure of method precision. It is suggested that the total tissue sample is extracted to isolate lipids material and then subdivided for duplicate analysis. Precision is calculated as relative percent difference (RPD) where the differences in the duplicate measurements (for each analyte) is divided by the average of the two measurements and multiplied by 100%.

9.8 External Samples -- Samples submitted as blinds to the analyst may consist of either performance solutions of PCDD and PCDF congeners or spiked sample matrices. These performance solutions or samples should be submitted by a source external to the analytical program (QA unit of analysis laboratory or independent laboratory). Performance audit solutions are intended to evaluate instrument calibration and quantitation procedures. Spiked blind samples must be accompanied by the corresponding unspiked samples to correct concentrations for background concentration. The blind spiked samples are intended to evaluate the total analytical procedure. The analyst must keep in mind that it is necessary to compare differences in standard sources for each type of external sample.

10. SAMPLE PRESERVATION AND HANDLING

All adipose tissue samples must be maintained at less than -20°C from time of collection. The analyst should instruct the collaborator collecting the sample(s) to avoid the use of chlorinated materials. Samples are handled using stainless steel forceps, spatulas, or scissors. Aliquots of samples removed from sample bottles not used for analysis are disposed rather than returned to the sample vial. All sample bottles (glass) are cleaned as specified in Section 6.4.10. Teflon®-lined caps should be used. As with any biological sample, the analyst should avoid any undue exposure.

11. SAMPLE EXTRACTION

11.1 Extraction of Adipose Tissue

11.1.1 Accurately weigh to the nearest 0.01 g a 10-g portion of a frozen adipose tissue sample into a culture tube (2.2 x 15 cm).

Note: Sample size may be smaller, depending on availability.

11.1.2 Addition of internal quantitation standards

Allow the adipose tissue specimen to reach room temperature and then add the carbon-13 internal quantitation spiking solution (Section 7.6) such that it delivers 500 to 2,500 pg of each of the surrogates specified in Table 4 in a 100-µL volume.

11.1.3 Add 10 mL of methylene chloride and homogenize the mixture for approximately 1 min with a Tekmar Tissuemizer®.

11.1.4 Allow the mixture to separate and decant the methylene chloride extract from the residual solid material using a disposable pipette. The methylene chloride is eluted through a filter funnel containing a plug of clean glass wool and 5 to 10 g of anhydrous sodium sulfate. The dried extract is collected in a 100-mL volumetric flask.

11.1.5 A second 10-mL aliquot of methylene chloride is added to the sample and homogenized for 1 min. The methylene chloride is decanted, dried, and transferred to the 100-mL volumetric flask as specified in Section 11.1.3

11.1.6 The culture tube is rinsed with at least two additional aliquots (10 mL each) of methylene chloride, and the entire contents are transferred to the filter funnel containing the anhydrous sodium sulfate. The filter funnel and contents are rinsed with additional methylene chloride (20 to 40 mL). The total eluent from the filter funnel is collected in the 100-mL volumetric flask. Discard the sodium sulfate.

11.1.7 The final volume of the extract for each sample is adjusted to 100 mL in the volumetric flask using methylene chloride.

11.2 Lipid Determination

11.2.1 Preweigh a clean 1-dram glass vial to the nearest 0.0001 g using an analytical balance tared to zero.

11.2.2 Accurately transfer 1.0 mL of the final extract (100 mL) from Section 11.1.7 to the 1-dram vial. Reduce the volume of methylene chloride from the extract using a water bath (50-60°C) gentle stream of purified nitrogen until an oil residue remains.

11.2.3 Accurately weigh the 1-dram vial and residue to the nearest 0.0001 g and calculate the weight of lipid present in the vial based on difference. Nitrogen blow-down is continued until a constant weight is achieved.

11.2.4 Calculate the percent lipid content of the original sample to the nearest 0.1% as shown in Equation 11-1.

$$\text{Lipid content, LC (\%)} = \frac{W_{LR} \times V_{EXT}}{W_{AT} \times V_{AL}} \times 100\% \qquad \text{Eq. 11-1}$$

where: W_{LR} = weight of the lipid residue to the nearest 0.0001 g calculated from Section 11.2.3;

V_{EXT} = total volume of the extract in mL from Section 11.1.6 (100.0 mL);

W_{AT} = weight of the original adipose tissue samples to the nearest 0.01 g from Section 11.1.1; and

V_{AL} = volume of the aliquot of the final extract in mL used for the quantitative measure of the lipid residue (1.0 mL).

11.2.5 Record the lipid residue measured in Section 11.2.3 and the percent lipid content calculated from Section 11.2.4.

11.3 Extract Concentration

11.3.1 Quantitatively transfer the remaining extract volume (99.0 mL) to a 500-mL Erlenmeyer flask. Rinse the volumetric flask with 20 to 30 mL of additional methylene chloride to ensure quantitative transfer.

11.3.2 Place the Erlenmeyer flask on a hot plate at 40°C to remove solvent until an oily residue remains.

12. CLEANUP PROCEDURES

12.1 Bulk Lipid Removal

12.1.1 Add a total of 200 mL of n-hexane to the spiked lipid residue in the 500-mL Erlenmeyer flask.

12.1.2 Slowly add, with stirring, 100 g of the 40% w/w sulfuric acid impregnated silica gel (Section 7.1.3). Stir with a magnetic stir-plate for 2 h.

12.1.3 Allow solids to settle and decant liquid through a powder funnel containing 20 g of anhydrous sodium sulfate and collect in a 500-mL sample bottle.

12.1.4 Rinse solids with two 50-mL portions of hexane. Stir each rinse for 15 min, decant, and dry by elution through sodium sulfate combining the hexane extracts from Section 12.1.3.

12.1.5 After the rinses have gone through the sodium sulfate, rinse the sodium sulfate with an additional 25 mL of hexane and combine with the hexane extracts from Section 12.1.4.

12.1.6 Prepare an acidic silica column as follows: Pack a 1 cm x 10 cm chromatographic column with a glass wool plug, add approximately 25 mL of hexane, add 1.0 g of silica gel (Section 7.1.2) and allow to settle, then add 4.0 g of 40% w/w sulfuric acid impregnated silica gel (Section 7.1.3) and allow to settle. Pack a second chromatographic column (1 cm x 30 cm) with a glass wool plug, add approximately 25 mL of hexane, add 6.0 g of acidic alumina (Section 7.1.1), and allow to settle and then top with a 1-cm layer of sodium sulfate (Section 7.2). Elute the excess hexane solvent through the columns until the solvent level reaches the top of the chromatographic packing. Inspect columns to ensure they are free of channels and air bubbles. Wash the alumina column with 40 mL of 50% v/v methylene chloride/hexane. Remove the methylene chloride from the adsorbent by eluting the column with an additional 100 mL of hexane. Elute the excess solvent from the column until the solvent level reaches the top of the sodium sulfate layer.

12.1.7 Quantitatively transfer the hexane extract from the Erlenmeyer flask (Sections 12.1.3 through 12.1.5) to the silica gel column reservoir. Allow the hexane extract to percolate through the column and collect in a KD concentrator.

12.1.8 Complete the elution of the extract from the silica gel column with 50 mL of hexane in the KD concentrator. Concentrate the eluate to approximately 1.0 mL, using nitrogen blow-down as necessary.

Note: If the 40% sulfuric acid/silica gel is noted to be highly discolored throughout the length of the adsorbent bed it is necessary to repeat the cleaning procedure beginning with Section 12.1.1.

12.2 Separation of Chemical Interferences

12.2.1 Transfer the concentrate (1.0 mL) to the top of the alumina column. Rinse the K-D assembly with two 1.0-mL portions of hexane and transfer the rinses to the top of the alumina column. Elute the alumina column with 18 mL of hexane until the hexane level is just below the top of the sodium sulfate. Discard the eluate. Columns must not be allowed to reach dryness (i.e., a solvent "head" must be maintained).

12.2.2 Place 30 mL of 20% (v/v) methylene chloride in hexane on top of the alumina and elute the TCDDs from the column. Collect this fraction in a 50-mL culture tube.

12.2.3 Prepare an 18% Carbopak C/Celite 545® mixture by thoroughly mixing 3.6 g of Carbopak C (80/100 mesh) and 16.4 g of Celite 545® in a 40-mL vial. Activate at 130°C for 6 h. Store in a desiccator. Cut off a clean 5-mL disposable glass pipet (6 to 7 mm ID) at the 4-mL mark. Insert a plug of glass wool and push to the 2-mL mark. Add 500 mg of the activated Carbopak/Celite mixture followed by another glass wool plug. Using two glass rods, push both glass wool plugs simultaneously towards the Carbopak/Celite mixture and gently compress the Carbopak/Celite plug to a length of 3 to 3.5 cm. Pre-elute the column with 2 mL of toluene followed by 1 mL of 75:20:5 methylene chloride/methanol/ benzene, 1 mL of 1:1 cyclohexane in methylene chloride, and 2 mL of hexane. The flow rate should be less than 0.5 mL/min. While the column is still wet with hexane, add the entire eluate (30 mL) from the alumina column (Section 12.2.2) to the top of the column. Rinse the culture tube which contained the extract twice with 1 mL of hexane and add the rinsates to the top of the column. Elute the column sequentially with two 1-mL aliquots of hexane, 1 mL of 1:1 cyclohexane in methylene chloride, and 1 mL of 75:20:5 methylene chloride/methanol/benzene. Turn the column upside down and elute the PCDD/PCDF fraction with 20 mL of toluene into 6-dram vial.

12.2.4 Using a stream of nitrogen, reduce the toluene volume to approximately 1 mL. Carefully transfer the concentrate into a 1-mL minivial and reduce the volume to about 200 µL using a stream of nitrogen.

12.2.5 Rinse the concentrator tube with three washings using 500 µL of 1% toluene in methylene chloride. Concentrate to 200-500 µL and add 10 µL of the tridecane solution containing the internal recovery standard and store the sample in a refrigerator until HRGC/MS analysis.

12.2.6 Immediately prior to analysis, using a gentle stream of nitrogen at room temperature, remove toluene and methylene chloride. Submit sample to HRGC/MS once a stable 10 µL volume of tridecane is attained.

13. ANALYTICAL PROCEDURES

13.1 HRGC/MS Analysis for PCDD/PCDF

13.1.1 Once routine calibration criteria are met, the instrument is ready for sample analysis. Prior to the first sample, a blank injection of tridecane should be analyzed to document system cleanliness. If any evidence of system contamination is found, corrective action must be taken and another tridecane blank analyzed.

The typical daily sequence of injections is shown in Table 9 and Figure 3.

Note: Syringe Technique -- Congeners of PCDD/PCDF in the syringes used for HRGC/MS analysis can be problematic unless the syringes are properly handled between samples. The following procedure has been found to be very effective for PCDD/PCDF removal from contaminated syringes and will be used throughout these analyses.

- Rinse the syringe 10 times with isooctane.
- Fill the syringe with toluene and sonicate syringe and plunger in toluene for 5 min and repeat at least twice.
- Rinse the syringe 10 times with tridecane and pull up 1 µL of clean tridecane.
- Syringe is ready for use.

At no time should air be introduced into the HRGC column by using an air plug in the syringe. The oxygen present in the air plug will quickly degrade a nonbonded GC phase.

13.1.2 Inject a 1-µL aliquot of the extract into the GC, operated under the conditions previously used (Section 8.1) to produce acceptable results with the performance check solution.

Table 9. Typical Daily Sequence for PCDD/PCDF Analysis

1.	Tune and calibrate mass scale versus perfluorokerosene (PFK).
2.	Inject column performance mixture.
3.	Inject concentration calibration solution 2.5 to 12.5 pg/µL (CS-7) solution.
4.	Inject blank (tridecane).
5.	Inject samples 1 through "N".
6	Inject concentration calibration solution 2.5 to 12.5 pg/µL (CS-7) solution or other concentration calibration solutions CS1 to CS8 to bracket observed sample concentration.

INSTRUMENTAL ANALYSIS

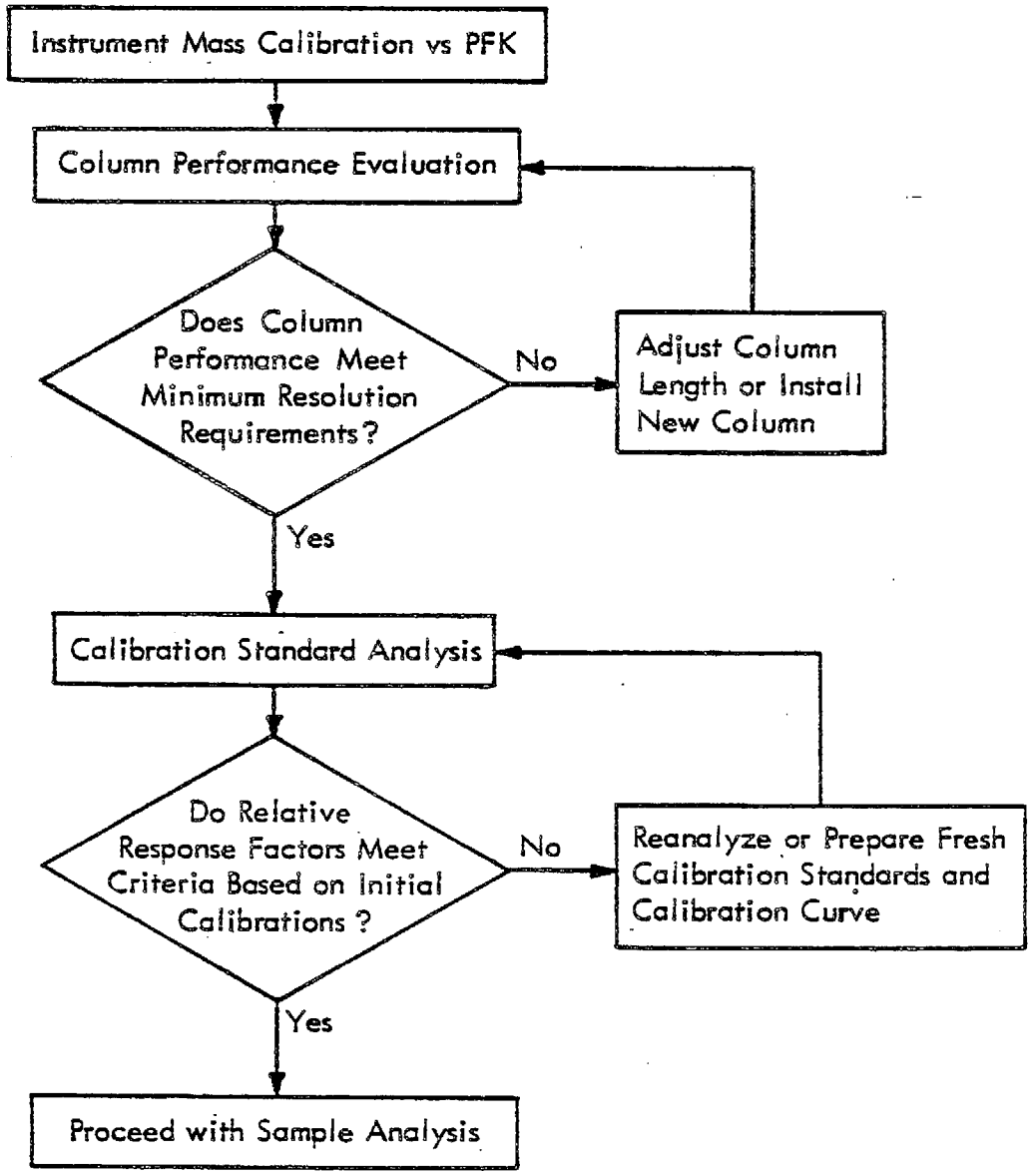

Figure 3. Daily QA procedures for proceeding with sample analysis.

13.1.3 Acquire SIM data according to the same acquisition and MS operating conditions previously used (Section 8.1) to determine the relative response factors.

13.1.3.1 Acquire SIM data for the characteristic ions designated in Table 6.

13.1.3.2 Instrument performance shall be monitored by examining and recording the peak areas for the recovery standard, $^{13}C_{12}$-1,2,3,4-TCDD. If this area should decrease to less than 50% of the calibration standard, sample analyses shall be stopped until the problem is found and corrected.

13.2 HRGC/HRMS Confirmation of 2,3,7,8-TCDD

The presence of 2,3,7,8-TCDD observed through the general PCDD and PCDF procedure should be confirmed using HRGC/HRMS (resolution 10,000).

13.2.1 Once the daily criteria of mass calibration, mass resolution, HRGC performance, and routine calibration are met and documented, the instrument is ready for sample analysis. Prior to the first sample, a blank injection of tridecane will be made to document system cleanliness.

The typical daily schedule for HRGC/HRMS analysis of TCDD is shown in Table 10 and Figure 3.

13.2.2 Inject a 1-µL aliquot of the extract into the GC, operated under the conditions previously used (Section 8.2) to produce acceptable results with the column performance check solution.

13.2.3 Acquire SIM data according to Section 8.2.4.3. Use the same acquisition and MS operating conditions previously used to determine the relative response factors.

13.2.3.1 Acquire SIM data for the following selected characteristic ions:

m/z	Compound
258.930	TCDD - COCl
319.897	Unlabeled TCDD
321.894	Unlabeled TCDD
331.937	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD
333.934	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD

Table 10. Typical Daily Schedule for HRGC/HRMS Analysis of TCDD

1.	Tune and calibrate mass scale.
2.	Perform mass measurement check and mass resolution check.
3.	Inject column performance check solution.
4.	Inject the routine concentration calibration solution (CS7) and confirm response factor consistency.
5.	Inject tridecane blank.
6.	Inject samples 1 through "N".
7.	Inject concentration calibration solution and confirm response factor consistency.
8.	Mass resolution check.

14. DATA REDUCTION

In this section, the procedures for the data reduction are outlined for the analysis of data from both the HRGC/MS method for PCDD/PCDF and the HRGC/HRMS method for 2,3,7,8-TCDD. Figure 4 presents a schematic of the qualitative criteria for identifying PCDDs and PCDFs.

14.1 Qualitative Identification

14.1.1 The ion current responses for each mass for a particular PCDD/PCDF analyte must be within ± 1 s to attain positive identification of that analyte. For example, m/z 338 and m/z 340 must have maximum peak responses that are within ± 1 s to be positively identified as a pentachlorodibenzofuran.

14.1.2 The ion current intensities for a particular PCDD/PCDF must be ≧ 2.5 times the noise level (S/N ≧ 2.5) for positive identification of that isomer.

14.1.3 The integrated ion current ratios of the analytical masses for a particular PCDD/PCDF must fall within the ranges shown in Table 7.

14.1.4 The recovery of the internal quantitation standards should be between 50 and 115%.

14.2 Quantitative Calculations

14.2.1 Relative response factors for native PCDD and PCDF analytes (RRF). RRFs are calculated from the data obtained during the analysis of concentration calibration solutions using the following formula:

$$RRF = \frac{A_{STD} \cdot C_{IS}}{A_{IS} \cdot C_{STD}} \qquad \text{Eq. 14-1}$$

where A_{STD} = the sum of the areas of the integrated ion abundances for the analyte in question. For example, for TCDD, A_{STD} would be the sum of the integrated ion abundances for m/z 320 and 322;

A_{IS} = the sum of the areas of the integrated ion abundances for the labeled PCDD/F used as the internal quantitation standard for the above analyte. For example, for $^{13}C_{12}$-2,3,7,8-TCDD, A_{IS} would be the sum of the integrated ion abundance for m/z 332 and 334.

C_{STD} = concentration of the analyte in pg/μL;

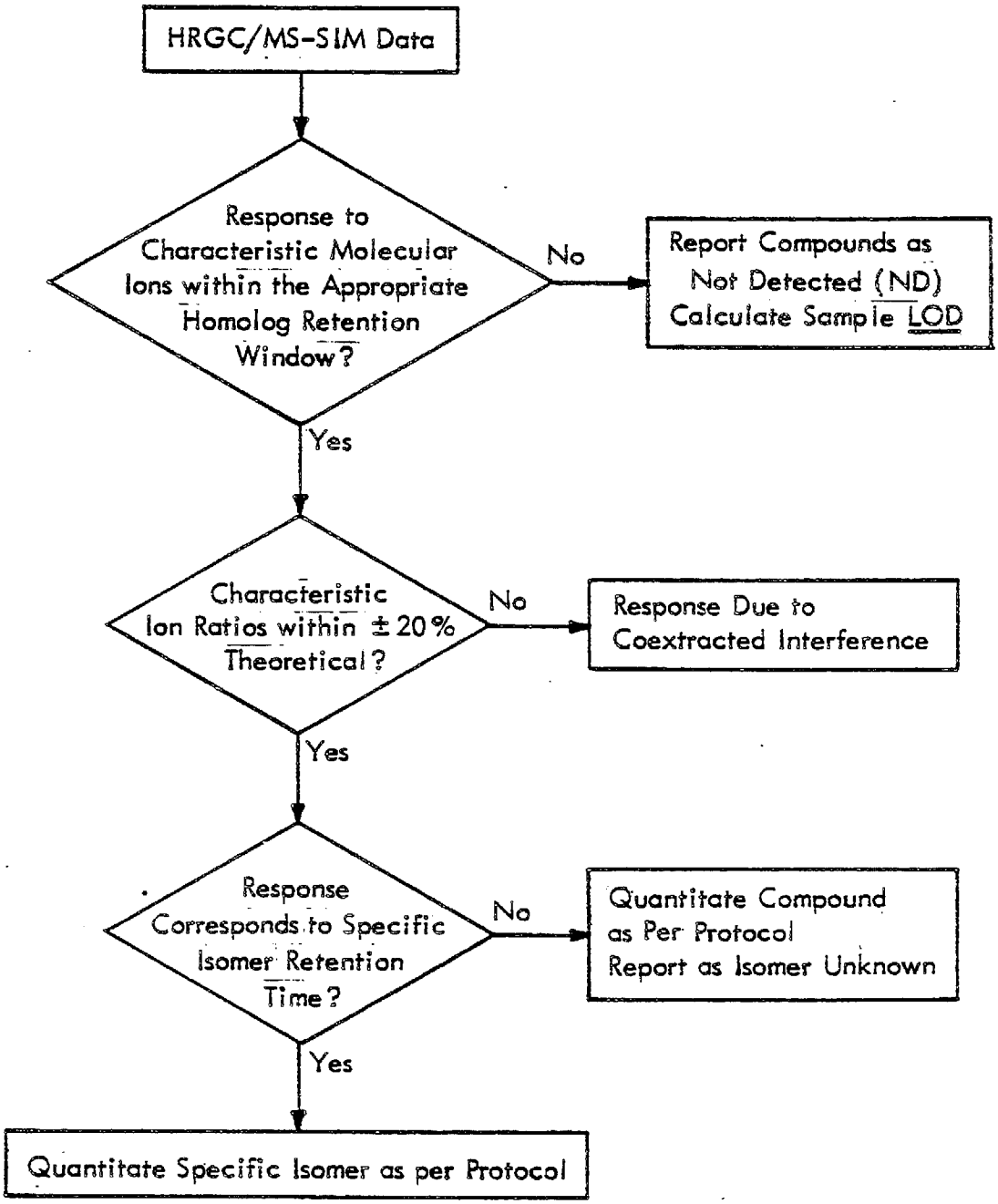

Figure 4. Qualitative criteria for identifying PCDDs and PCDFs.

C_{IS} = concentration of the internal quantitation standard in pg/μL; and

Table 11 provides the pairing of target analytes to internal quantitation standards for determining RRF values for PCDD and PCDF compounds.

14.2.2 Relative response factors for the internal quantitation standards (RRF_{IS}). The RRF_{IS} values are calculated from data obtained during the analysis of concentration calibration solutions using the following formula.

$$RRF_{IS} = \frac{A_{IS} \times C_{RS}}{A_{RS} \times C_{IS}} \qquad \text{Eq. 14-2}$$

where A_{IS} and C_{IS} are defined as given in Section 14.2.1 and

C_{RS} = concentrations of the internal recovery standard in pg/μL; and

A_{RS} = the sum of the areas of the integrated ion abundances for the labeled PCDD ($^{13}C_{12}$-1,2,3,4-TCDD or $^{13}C_{12}$-1,2,3,7,8,9-HxCDD). For example, for $^{13}C_{12}$-1,2,3,4-TCDD, A_{RS} would be the sum of the integrated ion abundance for m/z 332 and 334.

Refer to Table 11 for pairing of the internal quantitation standards with the appropriate internal recovery standard.

14.2.3 Concentrations of sample components. Figure 5 presents a schematic for quantitation of PCDDs and PCDFs which meet the criteria specified in Section 14.1. Calculate the concentration of PCDD/Fs in sample extracts using the formula:

$$C_{sample} = \frac{A_{sample} \cdot Q_{IS} \cdot 100}{A_{IS} \cdot RRF \cdot W_{AT} \cdot LC} \qquad \text{Eq. 14-3}$$

where C_{sample} = the lipid adjusted concentration of PCDD or PCDF congener in pg/g;

A_{sample} = sum of the integrated ion abundances determined for the PCDD/PCDF in question;

A_{IS} = sum of the integrated ion abundances determined for the labeled PCDD/F used as the internal quantitation standard for the above analyte;

Table 11. Target Analyte/Internal Quantitation Standard and Internal Quantitation Standard/Internal Recovery Standard Pairs

Target analyte	Internal standards	
	Quantitation	Recovery
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
OCDF	$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
OCDD	$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD

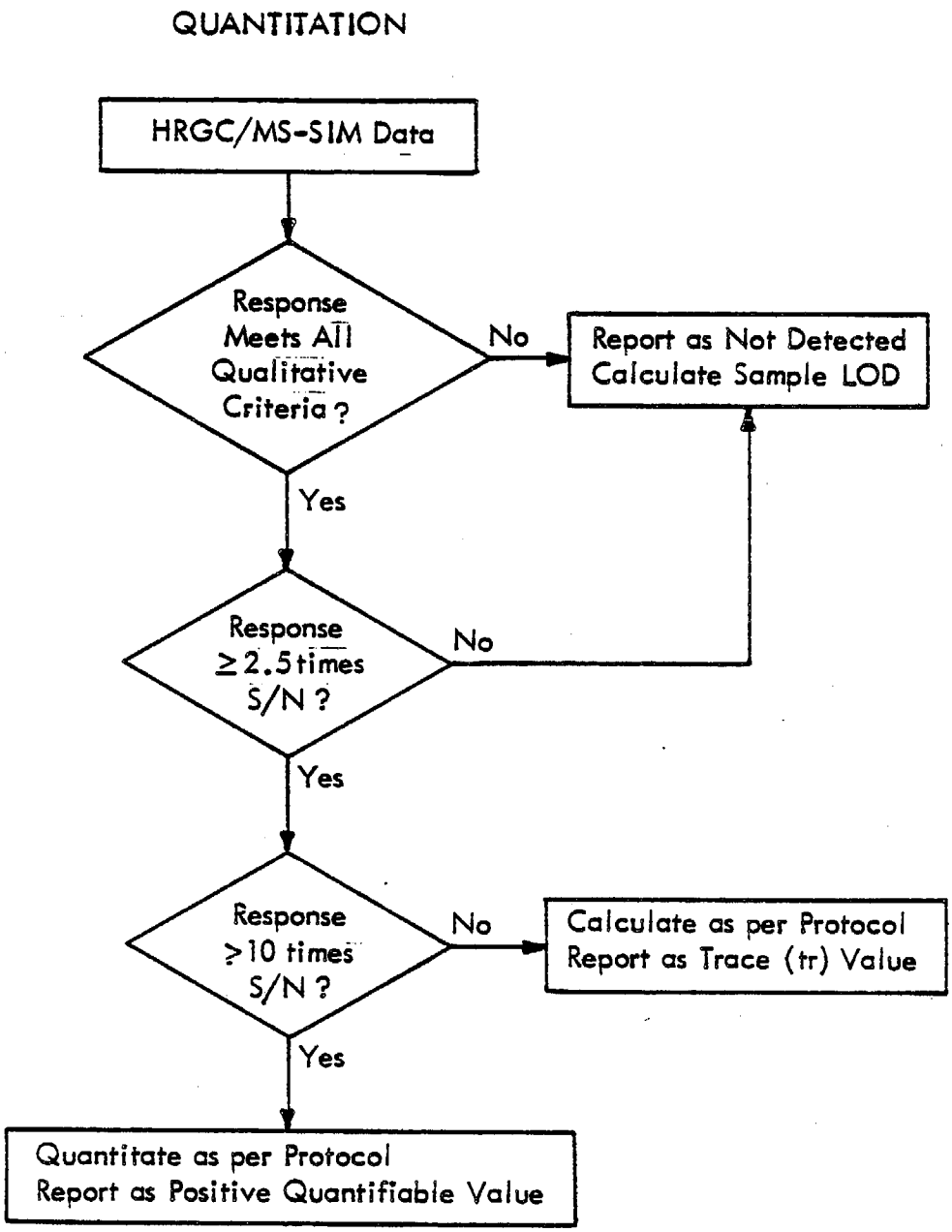

Figure 5. Procedure for quantitation of PCDDs and PCDFs in human adipose tissue.

Q_{IS} = the amount (total pg) of the labeled internal quantitation standard added to the sample prior to extraction;

RRF = relative response factor of the above analyte relative to its labeled internal quantitation standard determined from the initial triplicate calibration;

W_{AT} = weight (g) of original adipose tissue sample; and

LC = percent extractable lipid determined from Eq. 11-1.

Refer to Table 11 for pairing of target analytes with the appropraite internal quantitation standard.

Quantitative data should be classified to indicate the intensity of the signal response. Suggested qualifiers include: not detected, ND (signal-to-noise ratio is less than 2.5); trace, TR (signal-to-noise ratio is greater than or equal to 2.5 but less than 10); and positive quantifiable, PQ (signal-to-noise ratio is greater than or equal to 10).

14.2.4 Recovery of internal quantitation standards. Calculate the recovery of the labeled internal quantitation standards measured in the final extract using the formula:

$$\text{Internal Quant. Std. Percent Recovery} = \frac{A_{IS} \cdot Q_{RS}}{A_{RS} \cdot Q_{IS} \cdot RRF} \cdot 100 \qquad \text{Eq. 14.4}$$

where A_{IS} = sum of the integrated ion abundances determined for the labeled PCDD/PCDF internal quantitation standard in question;

A_{RS} = sum of the integrated ion abundances determined for m/z 332 and m/z 334 of $^{13}C_{12}$-1,2,3,4-TCDD or m/z 390 and m/z 392 of $^{13}C_{13}$-1,2,3,7,8,9-HxCDD (recovery standards)

Q_{RS} = amount (pg) of the respective recovery standard, added to the final extract;

Q_{IS} = amount (pg) the labeled internal quantitation standard added to the sample prior to extraction; and

RRF_{IS} = relative response factor for the labeled internal quantitation standard in question relative to the internal recovery standard. This value shall be the RRF determined from the initial calibration.

Refer to Table 11 for pairing of the internal quantitation standards with the appropriate target analytes.

Note: The result of calculations as presented in Section 14.2 may be off by as much as 1% due to the fact that 1 mL of the final 100 mL volume from the extraction was used for lipid determination.

14.3 Estimated Method Detection Limit

Estimated method detection limits must be calculated in situations where (1) no response is noted for a specific congener; (2) a response is noted but ion ratios are incorrect; and (3) where a response is quantitated as a trace value.

14.3.1 For samples in which no unlabeled PCDD or PCDF is detected, calculate the estimated minimum detectable concentration. The background area is determined by integrating the ion abundances for the characteristic ions in the appropriate region and relating the product area to an estimated concentration that would produce that product area.

Use the formula:

$$C_E = \frac{2.5 \cdot A_{sample} \cdot Q_{IS}}{A_{IS} \cdot \overline{RRF} \cdot W_{AT}} \qquad \text{Eq. 14-5}$$

where C_E = estimated concentration of unlabeled PCDD or PCDF required to produce A_{sample};

A_{sample} = sum of integrated ion abundances or peak heights for the characteristic ions of the unlabeled PCDD or PCDF isomer in the same group of ≧ 5 scans used to measure A_{IS}; and

A_{IS} = sum of integrated ion abundances for the appropriate ions characteristic of the respective internal quantitation standard.

Q_{IS}, $\overline{RRF}$, and W_{AT} retain the definitions previously stated in Section 14.2. Alternatively, if peak height measurements are used for quantification, measure the estimated detection limit by the peak height of the noise in the 2,3,7,8-TCDD RT window.

14.3.2 For samples for which a response at the retention time of a specific PCDD or PCDF congener is noted, but the qualitative criteria for ion ratios are outside the acceptable range (Table 7), the estimated detection level is calculated as given in Eq. 14.3 except the values are qualified as not detected, ND, and the concentration is reported in parenthesis.

14.3.3 If a response for a specific PCDD or PCDF congener is qualified as a trace, TR, value (signal to noise is greater than or equal to 2.5 but less than 10) the analyst must also provide an estimated method detection limit. This is accomplished by using the observed signal to noise on either side of the response and calculating as given in Eq. 14-5.

15. REPORTING AND DOCUMENTATION

All data should be reported on an individual sample basis using the data report format shown in Figure 6. The analyst is required to maintain all raw data, calculations, and control charts in a format as to allow a complete external data review. Suggested data formats for tracing calculations are provided in Figure 7.

State of California Air Resources Board Sacramento, CA 95812	ARB Body Burden Study PCDD/F Analysis Form
Sample Code ... Lab Number Batch Number Wet Tissue Weight (g) % Extractable Lipid	Analysis Date LRMS / / 87 Analysis Date HRMS / / 87 Reviewed by:

Native Compounds	Data Qualifier (1)	LOD (pg/g) (2)	Concentration (pg/g) (2)	Internal Quantitation Standard	Spiked Level (pg)	Percent (%) Recovery
2378-TCDF (3)				13C12-2378-TCDF	500	
2378-TCDD (3)				13C12-2378-TCDD	500	
12378-PeCDF				13C12-12378-PeCDF	500	
23478-PeCDF				13C12-12378-PeCDD	500	
12378-PeCDD				13C12-123478-HxCDF	1250	
123478-HxCDF				13C12-123678-HxCDD	1250	
123678-HxCDF				13C12-1234678-HpCDF	1250	
234678-HxCDF				13C12-1234678-HpCDD	1250	
123789-HxCDF				13C12-OCDD	2500	
123478/123678-HxCDD						
123789-HxCDD						
1234678-HpCDF						
1234789-HpCDF						
1234678-HpCDD						
OCDF						
OCDD						

Remarks

(1) ND - Not detected above Limit of Detection (LOD); TR - Trace; PQ - Positive Quantifiable.
(2) Concentration based on total extractable lipid (g).
(3) From High Resolution Mass Spectrometry Data

Figure 6. Analysis report form.

RAW DATA SUMMARY FOR DETERMINATION OF 1,2,3,7,8-PeCDD IN HUMAN ADIPOSE TISSUE

Sample no.	Sample weight (xx.xx g)	Extractable lipid content (xx.x %)	Analysis date	Amount $^{13}C_{12}$-PeCDD (pg)	$^{13}C_{12}$-PeCDD m/z 332	$^{13}C_{12}$-PeCDD m/z 334	Ion ratio 366/368	1,2,3,7,8-PeCDD m/z 354	1,2,3,7,8 m/2 356	Ion ratio 354/356	1,2,3,7,8-PeCDD conc.[a] (pg/g)

[a] Value reported as concentration in extractable lipid.

Figure 7. Example of raw data summary format for the determination of 1,2,3,7,8-PeCDD in human adipose tissue.

APPENDIX C

QUALITY ASSURANCE PROGRAM PLAN (QAPP)

ARB Contract No. A6-195-33
Task No. I-C

SECTION 1.0

DETERMINATION OF CURRENT BODY BURDENS FOR POLYCHLORINATED DIBENZO-*p*-DIOXINS AND DIBENZOFURANS IN CALIFORNIA RESIDENTS

SECTION 2.0

TABLE OF CONTENTS

Section	Heading	Pages	Revision	Date
1.0	Title Page	1	0	02/12/88
2.0	Table of Contents	1	0	02/12/88
3.0	Project Description	2	0	02/12/88
4.0	Project Organization and Management	4	0	02/12/88
5.0	Personnel Qualifications	3	0	02/12/88
6.0	Facilities, Equipment, Consumables, and Services	4	0	02/12/88
7.0	Data Generation	7	0	02/12/88
8.0	Data Reduction and Analysis	4	0	02/12/88
9.0	Data Quality Assessment	4	0	02/12/88
10.0	Corrective Action	2	0	02/12/88
11.0	Documentation and Reporting	2	0	02/12/88

SECTION 3.0

PROJECT DESCRIPTION

The State of California, through the Air Resources Board (ARB), has expressed a need to develop a preliminary estimate of the current body burdens of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDF) in California residents. The impetus for this program arises from the classification of PCDDs and PCDFs as toxic air contaminants. The ARB has identified potential PCDD and PCDF source categories that currently exist in the State of California and has developed an estimate of relative emissions from each of 11 different point source categories. The ARB has also projected a number of additional point source operations that have been proposed for the State of California.

As of this date, body burden levels of PCDDs and PCDFs in California residents have not been established. In order to fully assess the impact of PCDDs and PCDFs as toxic air contaminants, it is necessary to determine the extent of uptake and exposure to the general California population. Preliminary estimates of the body burden levels of PCDDs and PCDFs in the general California population can be achieved through the analysis of human biological samples such as adipose tissue. Body burden levels determined at this time can be used by the ARB in future studies to assess the overall impact of airborne releases of PCDDs and PCDFs from combustion sources.

The objectives of the program are to provide the ARB with a preliminary estimate of the current body burden levels for PCDDs and PCDFs in a representative sample of the California population. This will be accomplished through a program of field sampling and laboratory analysis of human adipose tissue samples.

The chemical analysis of the human adipose tissue samples will be conducted for specific PCDD and PCDF congeners (tetra- through heptachloro congeners substituted in the 2,3,7,8-position). These data will be used by the ARB to estimate health risks from the dioxins and furans designated as toxic air contaminants, and to compare with source-specific isomers ("fingerprint" isomers) detected in future monitoring studies.

The data will be used to determine if any correlation exists between body burden levels and lifestyle factors such as age, occupation, and residence history. The survey design and chemical analysis will be conducted in a manner which ensures the quality and reliability of the data. Of considerable importance is the need to establish data of known quality that can be compared to other existing data bases or that can be used for comparison in future studies focused on body burden levels of these compounds.

This QAPP addresses the quality control procedures and criteria that will be

implemented throughout the analysis program. The objective of the program is to achieve accurate measurements of the residue levels of the 2,3,7,8-substituted PCDD and PCDF congeners in the adipose tissues from donors selected according to a specific experimental design. Specific QA criteria that are addressed in this document are (1) consistency of calibration (±20% variability of response factors for TCDD and TCDF, and ±30% for all other congeners); (2) absolute method recoveries for all internal quantitation standards versus internal recovery standards (50-115%); (3) precision through the analysis of a control tissue matrix and duplicate analysis of specific design samples; and (4) accuracy through the analysis of spiked tissue samples.

SECTION 4.0

PROGRAM ORGANIZATION AND MANAGEMENT

The technical team will be led by Dr. John Stanley, who will serve as program manager. For management purposes, the program will be assigned to the Analytical Chemistry Section, John Hosenfeld, Head, of the Chemical Sciences Department, Dr. John E. Going, Director. Together they will ensure that all necessary resources are available, ensure that the program quality assurance coordinator is fully informed and involved in the program, and critically review all progress reports and interim or final reports to ARB.

4.1 Program Manager

The program team will be managed by Dr. John S. Stanley. Dr. Stanley has the responsibility and authority to execute the program activities that are in compliance with contractual agreements. He is MRI's principal contact with the ARB project officer. He is responsible for:

- coordinating all phases of this sampling and analysis program,
- reviewing and approving all reports before submission to the ARB,
- assuring technical quality and performance,
- monitoring progress and adherence to schedules,
- monitoring expenditures in comparison to budgets and funding,
- interacting with MRI's Accounting and Contract Departments to ascertain that program cost accounting and contract requirements are fulfilled,
- addressing problems and taking corrective action in a timely and effective manner,
- assuring that all procedures and results are documented appropriately, and
- reporting regularly to MRI management.

As program manager, Dr. Stanley has the authority to direct all technical support activities toward completing the assigned work.

4.2 Task Leaders

Each project will be managed by a task leader. The task leaders will coordinate the activities with the MRI program manager. Each task leader is responsible for:

- planning activities to complete specific tasks,
- preparation of reports and protocols,
- ensuring the technical quality of the project,
- monitoring progress and adherance to schedules,
- monitoring expenditures in comparison to budgets and funding,
- reporting regularly to the MRI program manager, and
- addressing problems and taking corrective action in a timely and effective manner.

Each task leader has the authority to direct technical activities toward completing the assigned work.

The task leaders will report regularly to the program manager on the technical and financial status of active tasks. These weekly briefings will include the potential for problems with schedule, staffing, technical progress, or other areas which may affect the project.

Ms. Karin Bauer is the task leader responsible for developing the survey design and completing the necessary statistical analysis. As part of her responsibilities, Ms. Bauer will:

- design and implement the survey for the collection of samples,
- develop and test the questionnaire to gather demographic data,
- statistically analyze the results from the chemical analyses, and
- provide input to the project leader for the monthly and final reports.

Ms. Kay Turman is responsible for recruitment of the cooperating collection agencies and collection of the necessary samples. Ms. Turman will:

- identify collection sites that meet the requirements of the survey design,
- recruit the necessary medical professionals to assist in sample collection,

- identify the sample collection procedures,
- prepare detailed sample collection protocols,
- provide the necessary sampling and shipping materials, and
- ensure that the samples collected meet the requirements of the survey design.

Dr. John Stanley will oversee the work for the preparation of samples for analysis by HRGC/MS. He will:

- be responsible for staff training and documentation,
- ensure that all analytical protocols are followed and documented,
- take corrective action for any problems and communicate action in writing to the QAC and department management,
- be responsible for sample custody,
- be responsible for document control, and
- be responsible for data traceability.

Dr. Stanley will be assisted in the sample preparation activities by Mr. Randy Ayling, Mr. Mike McGrath, and Mr. Mark Ross. Mr. Ayling, Mr. McGrath, and Mr. Ross have considerable experience in the preparation of biological samples (specifically adipose tissues) for PCDDs and PCDFs.

Dr. Tom Sack will oversee the HRGC/MS analysis of the sample extracts for PCDDs and PCDFs. He will:

- ensure that all equipment calibration and maintenance procedures are followed,
- ensure that all analysis protocols are followed and documented,
- take corrective action for any problems and communicate action in writing to the program manager, QAC, and department management, and
- be responsible for sample custody and data traceability.

Dr. Sack will be assisted in the HRGC/MS analysis of the adipose tissue samples by Mr. Kelly Thornburg, Mr. David Mills, and Mr. Rick Robson.

In addition to the key technical staff, MRI supports the involvement of a quality assurance coordinator to assess the quality of data. As demonstrated in the program organizational structure (Figure 4-1), a quality

assurance coordinator (QAC) will be involved in this program. His primary lines of communication are to the Chemical Sciences Department Director, Dr. John E. Going.

Mr. Thomas Dux is the project QAC. He will:

- assure that all sampling and analysis protocols are fully detailed,
- work with the individual task leaders to ensure that all aspects of the QAP are addressed,
- conduct performance and system audits throughout the duration of the project,
- examine data books, records, forms, and any other hard-copy information, and
- report audit findings to department management and to the program manager.

SECTION 5.0

PERSONNEL QUALIFICATIONS

Midwest Research Institute's program team is comprised of professionals with demonstrated experience in the areas of survey design, statistical analysis of multivariate data, recruitment of medical professionals for collection of biological samples, coordination of biological sample analysis activities, and analysis of biological samples for ultratrace (1 to 10 parts per trillion) levels of PCDDs and PCDFs. The key technical staff available for this program are identified in Figure 4-1. This team of experienced professionals will ensure that the qualified resources are available to provide the necessary timely and thorough completion of the proposed program. Brief synopses on the specific areas of expertise for each of the key individuals are presented below.

Dr. John S. Stanley, Program Manager, has expertise in trace organic analysis using high resolution capillary gas chromatography, gas chromatography/mass spectrometry analysis, and high resolution mass spectrometry/selected ion monitoring for PCDD, PCDF, and PCB analyses.

Since joining MRI in 1981, Dr. Stanley has been project leader and task leader on several major analytical and environmental programs. Recently, he has directed a program to assess levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in human adipose tissues under a cooperative agreement between the U.S. Environmental Protection Agency (EPA) and the Veterans Administration (VA). The EPA/VA cooperative program has involved developing detailed analytical protocols and a quality assurance program plan for determination of PCDDs and PCDFs at concentrations of 1 to 10 pg/g (parts per trillion).

Dr. Stanley has also directed a series of Special Analytical Services projects under EPA's Contract Laboratory Program to provide analysis of soils, sediments, and water for low parts-per-trillion to parts-per-quadrillion levels of PCDDs. Dr. Stanley has also evaluated an HRMS method for TCDD determination in soil and water at low parts-per-billion to parts-per-quadrillion levels, conducted a method validation study of EPA Method 613, and prepared a literature review and recommendations for PCDD and PCDF analysis in biological matrices for EPA and the VA.

Another major research area has involved evaluating analytical protocols for polyhalogenated dibenzo-p-dioxins and dibenzofurans and PCBs in commercial products, waste products, air and flue gas emissions, and wastewaters. Dr. Stanley also coordinated laboratory analysis for a comprehensive assessment of PAHs, PCDDs, and PCDFs from various combustion sources (coal-fired utility boilers, municipal waste incinerators, hazardous waste incinerators, and hospital incinerators).

Mr. John M. Hosenfeld, Section Head, Analytical Chemistry, directs a staff of more than two dozen analytical chemists involved in complex multimedia sampling and analysis programs. The section works extensively in analytical method development, modification, and validation for both long-term and quick-response programs.

Mr. Hosenfeld's analytical work has been directed toward the determination of trace quantities of pollutants in environmental and human media, along with the development, modification, and validation of methods. Mr. Hosenfeld has been a lead person in several MRI programs requiring collection of biological sample matrices. Specifically, he has been responsible for operation of the U.S. EPA's National Human Adipose Tissue Survey (NHATS), has coordinated and maintained activities with EPA in soliciting the cooperation of national blood collection organizations to participate in EPA's proposed National Blood Network, and has coordinated the collection and analysis of body fluid samples (blood and urine) from residents of pentachlorophenol (PCP) treated log homes.

The analysis of complex data sets obtained from instrumental equipment and environmental survey results has been an ongoing activity for Mr. Hosenfeld. He provided lead expertise at MRI for applying chemometrics and pattern recognition techniques for examining data obtained from chromatographic, plasma emission, atomic absorption, and clinical chemistry instruments as well as environmental data bases.

Mr. Thomas P. Dux, Chemist, serves as quality assurance coordinator (QAC) for various MRI programs involving analytical and environmental chemistry. He is responsible for auditing and validating laboratory analytical data to ensure high technical accuracy and QA compliance; performing QA and editorial reviews of analytical reports, sampling and analysis plans, and project QA plans; conducting system audits of laboratory operations both at MRI and subcontractor facilities; preparing QA performance audit samples; instructing staff in QA/QC theory and practice; and developing and implementing project-specific QA plans and procedures. Currently, Mr. Dux is QAC on two projects, an installation restoration project under USATHAMA QA regulations and an EPA project for the Office of Solid Wastes. These projects require high-volume, fast-turnaround sample analysis and reporting, primarily using GC/MS, ICP, and GFAA techniques. They also require certification of USATHAMA methods plus modification and implementation of SW-846 protocols. Other assignments concern hazardous waste incinerator trial burns, engineering performance testing, and various EPA projects for the Office of Toxic Substances.

Karin M. Bauer, Senior Statistician, provides statistical and computational expertise in support of MRI research programs in such diverse fields as analytical chemistry, air and water quality assessment, microbiology, bio-organics, and traffic engineering. Her responsibilities include design and analysis of experiments, survey design and data reduction, preparation of statistical reports, and the development and implementation of quality assurance plans. Ms. Bauer is experienced in the use of statistical package programs such as SAS, BMDP, and SPSS and in the development of computer programs in Basic and Fortran. In recent years, she has been instrumental in

developing a capability in pattern recognition at MRI in conjunction with MRI chemists.

Project activities have also involved multivariate statistical analysis of airborne emissions of PCDDs and PCDFs from municipal waste incinerators. Statistical methods such as pattern recognition techniques were used to explore relationships between emissions of specific organic pollutants and key combustion indicators. In the context of the National Human Adipose Tissue Survey, for which MRI is conducting broad scan chemical analyses of adipose tissue specimens, Ms. Bauer was instrumental in identifying the need for automated data transfer from the mass spectrometer onto the EPA mainframe. A majority of the work is being performed using in-house microcomputers with available software packages and customized supplemental software. In addition, various chemometric techniques will be identified and incorporated into the laboratory's QA program so that higher quality data may be produced.

Ms. Kay Turman, Chemist, has been a lead person in the collection of biological samples for MRI programs. Specifically, she has been the project leader for a program to operate and maintain EPA's National Human Adipose Tissue Survey (NHATS). Ms. Turman's responsibilities include coordination of the sample collection process, recruitment of the cooperating physicians and medical examiners, development of the necessary tracking documentation, archival of the NHATS repository, and interaction with the analytical laboratories.

Dr. Thomas M. Sack, Senior Mass Spectrometrist, has considerable experience with high resolution mass spectrometry (HRMS) and its use in chemical analysis. He is responsible for the operation and maintenance of a Kratos MS50TC high performance mass spectrometer as well as methods development for new applications. Dr. Sack is skilled in the application of many mass spectrometric techniques including GC/MS, HRMS, and alternate ionization techniques such as chemical ionization and desorptive ionization. His knowledge extends to the use of Finnigan/Incos and Kratos data systems for sophisticated data reduction and manipulation.

Since joining MRI in 1985, Dr. Sack has directed the implementation of pyrolysis GC/MS instrumentation and has been a key figure in its use for a wide range of experiments. Dr. Sack has coauthored a method for the determination of 2,3,7,8-TCDD by HRGC/HRMS and has acted as the mass spectrometry task leader for several projects involving the trace analysis of chlorinated dibenzodioxins and dibenzofurans. He is currently involved in the use of HRGC/HRMS to determine total tetrachlorodibenzodioxins, HRGC/LRMS to simultaneously determine tetra-octa chlorodioxins and furans in various matrices, pyrolysis GC/MS to study the thermal degradation of transformer utility materials, and FAB-MS to analyze polar and nonvolatile materials.

SECTION 6.0

FACILITIES, EQUIPMENT, CONSUMABLES, AND SERVICES

6.1 Facilities and Equipment

6.1.1 Evaluation

This project will require the use of general trace organic laboratory facilities and the mass spectrometry facility. These facilities were described in detail in the MRI proposal.

Sample preparation activities (dishwashing, compositing, extraction, and sample cleanup) will be completed in a laboratory (MRI, Lab 332-W) that has been designated for ultratrace analysis work only. This laboratory is equipped with five Class A hoods, one walk-in hood, and a canopied wash area.

6.1.2 Inspection and Maintenance

MRI's maintenance program consists of both scheduled (or preventive maintenance) and nonscheduled maintenance procedures. Records of maintenance performed on the instruments are maintained in the respective instrument logbooks. In addition, any instrument repair not performed by the laboratory personnel is handled by the Instrument Services Department, which also adheres to a record-keeping program.

The scheduled maintenance program involves the service performance of certain instruments at regular intervals. The type of services included in this program is presented in Table 6-1.

The nonscheduled maintenance program involves the necessary servicing of equipment on an "as needed" basis. This can include items in the scheduled maintenance program but most often involves the type of service listed in Table 6-2.

6.1.3 Calibration Procedures and Reference Materials

6.1.3.1 GC/MS/Data System Calibration and Evaluation

Standard operating procedures (SOPs) for GC/MS data system (DS) calibration and evaluation have been prepared for each of the GC/MS/DS instruments that will be used for analysis for PCDDs and PCDFs in human adipose tissue. These SOPs will be available from MRI upon request.

Table 6-1. Scheduled Maintenance

Equipment	Service	Frequency
Kratos MS-50TC	Check/change forepump oil	1 yr/as needed
	Check/change turbopump oil	1 yr/as needed
Finnigan-MAT 311A	Change forepump oil	1 yr/as needed
	Check/change turbopump oil	1 yr/as needed

Table 6-2. Nonscheduled Maintenance

Instrument	Service	Frequency of repair
All mass spectrometers	Ion source cleaning	As needed
	Vacuum chamber bake-out	As needed
	Electronic component repair	As needed
	Replace or repair jet separator	As needed
All gas chromatographs	Electronic component repair	As needed
	Pneumatics repair/replacement	As needed
All computer data systems	Alignment of disk drives	As needed
	Repair/replacement of electronic components	As needed

6.1.3.2 Calibration

MRI will use PCDD and PCDF calibration standards that have been previously used on existing adipose tissue programs. The calibration of the instruments will be conducted as described in "Routine Calibration," Section 8.2.6 of the attached protocol. If successful calibration is not achieved, MRI will conduct the following calibration procedure.

6.1.3.2.1 Calibration for quantitative measurements will be conducted with standards at a minimum of six concentration levels in the linear range of the instrument.

6.1.3.2.2 Standards will be run in triplicate at the beginning of the project and compared to an analysis of the blank samples. The results of the multipoint calibration curve will be used to establish the initial relative response factor (RRF) control charts for each analyte.

6.1.3.2.3 Following the multilevel calibration, analysis of samples will be initiated.

6.1.3.2.4 Single-point calibrations will be performed at the beginning and end of each working day to assure the instruments' stability. The RRF values from these single-point calibration curves will be appended to the RRF control charts for each target analyte.

6.1.3.2.5 The relative response factors (RRF) from the single-point calibration will be checked with the average RRFs from the multilevel calibration. The RRFs must agree within ±20% for the 2,3,7,8-TCDD and 2,3,7,8-TCDF and ±30% for the penta- through octachloro PCDDs and PCDFs. If the criterion is not met, the calibration standard must be reanalyzed or the calibration curve must be rerun. The HRGC/MS analyst is responsible for documenting the RRF values on a daily basis. The RRFs will be summarized and reported with the data from each sample batch.

6.1.3.3 Calibration Standards

Calibration standards have been obtained from all available sources including the EPA reference materials

repository. Noncertified compounds or solutions have been characterized for purity and interferences. Most of the PCDD and PCDF standards and internal standards have been acquired from Cambridge Isotope Laboratories (Woburn, Massachusetts). MRI recently participated in an interlaboratory comparison of the PCDD and PCDF standards. The results demonstrated that MRI's standards are in good agreement with other laboratories participating in that study. Additional detail is provided in Section 7.0 of this QAPP. Stock calibration standards will be prepared prior to sample analysis. Calibration over a defined concentration range will require serial dilution of the highest concentration standard to the required final concentration.

6.2 Consumables

All reagents including adsorbents, solvents, and other expendable reagents will be screened as blanks to check for impurities that might lead to false positive identification in actual tissue samples. Solvents will be purchased as distilled-in-glass pesticide quality.

Where possible, standards will be obtained from the EPA reference materials repository. Supporting documentation for the stated purity of all standard compounds will be requested as necessary and will be compared with in-house evaluations.

SECTION 7.0

DATA GENERATION

7.1 Experimental Design

The experimental design requires the collection of adipose tissue samples based on a stratified sampling design. The strata considered are:

1. Geographic location within California
2. Age of donor
3. Sex of donor

7.1.1 Geographical Strata

Two urban areas, Los Angeles and San Francisco, were selected for this study. This selection was based on two criteria: (a) potential exposure to existing or planned emission sources and (b) high population densities. It is our understanding based on the ERT report (Ambient Concentrations of PCDDs/PCDFs in the South Coast Air Basin, Document No. P-E509-400-01, June 1987) that the San Francisco area is not and will not be affected by numerous emission point sources as is the case with the Los Angeles area. Hence San Francisco will serve as the control urban area. Los Angeles was selected based on its location within the South Coast Air Basin and the concern for the air quality in this area due to existing and planned emission sources.

7.1.2 Age Strata

Previous studies have shown that body burden levels of PCDDs and PCDFs increase with age. Thus stratification by age is important to obtain independent concentration estimates within each age group. Three age groups have been chosen. These are 18-34 years, 35-49 years, and 50-plus years. Age groups below 18 years have been excluded as a result of difficulties anticipated in receiving consent from parents or guardians. Also, it is our understanding that the number of elective surgeries in this age group would be very minimal, and hence it would be difficult to fulfill the quota.

7.1.3 Sex of Donor Strata

The literature review has also shown a slight difference in PCDD and PCDF residue levels between males and females. Thus sex has been selected as an additional important factor in this study.

7.1.4 Sample Size and Quotas

MRI plans to collect and analyze a total of 60 adipose tissue specimens. The quotas within the 12 strata described above (2 geographical locations x 3 age groups x 2 sex groups) have been determined. The restrictions on the allocation of donors to the strata are as follows:

- At least two donors per stratum
- Equal allocation to each age group
- Equal allocation to each sex
- 40 donors in Los Angeles and 20 donors in San Francisco

The allocations and quotas are summarized below:

	Los Angeles		San Francisco	
	Male	Female	Male	Female
18-34	6	6	2	2
35-49	7	7	4	4
50 and above	7	7	4	4
Total	20	20	10	10

Given the small sample sizes within each group, only small deviations from the design can be tolerated. However, variations of ±2 donors within categories of more than 2 will be accepted as long as the total sample size is maintained.

To provide the medical facilities with some flexibility, and also as a backup plan in case some strata are difficult to fill, the medical personnel can oversample up to two more donors in a given category should the opportunity arise.

7.2 Sample Tracking

The adipose tissue samples will be analyzed in four batches of 15 design samples each. Each sample batch will also include a laboratory method blank, a control tissue (or lipid) QC sample, and a spiked tissue (or lipid) sample. Up to 15% of the design samples will be analyzed in duplicate to provide within-batch precision estimates. Additional detail on each of the sample types is presented later in this section.

All design samples will be organized into batches of 15 upon receipt. The analyst will assign laboratory codes (MRI Lab No.) to each sample.

The laboratory code is explained in the following example:

8941A06 - 01 - 11

MRI Project No./ARB Task No. - Batch No. - Sample Sequence No. Please note that Task 06 is equivalent to the ARB Task II-B as specified in the MRI proposal.

7.3 Laboratory Analytical Procedures

Appendix B to the Phase I interim report provides the detailed analytical method for analysis of human adipose tissue for PCDDs and PCDFs. This protocol describes procedures for glassware preparation, sample handling, extraction cleanup, isolation of PCDDs and PCDFs, instrument analysis, and reporting.

7.4 Internal Quality Control Checks

The method accuracy and precision will be assessed by analyzing specific QC samples with each batch of samples. Other quality control checks will be routinely included to document instrument performance. These QC activities are described below.

7.4.1 Method Blank

A method blank or procedural blank will be prepared with each sample batch. The method blank will be treated exactly as a sample although no sample matrix will be present. Method blanks will serve as indicators as to presence of artifacts from the sample preparation scheme. A positive identification of a target analyte in a method blank will require further evaluation of glassware, solvents, chromatographic reagents, etc., to isolate the material responsible for artifact contribution.

7.4.2 Replicate Samples

As stated previously, at least 15% of the design specimens will be included for replicate sample analysis to demonstrate the precision of the analytical method. Inclusion of replicates will be accomplished by selecting a sample of sufficient mass (15 to 20 g) and splitting to provide two approximately equal aliquots. Each aliquot will be carried through the entire analytical procedure (extraction, cleanup, and instrumental analysis). In addition to replicate analyses to determine within-batch precision, a "control" tissue or lipid matrix will be analyzed for between-batch precision. This tissue sample or lipid matrix will be prepared by extracting a large tissue sample (~ 100 g) and isolating the lipid materials. This homogenized lipid matrix will be aliquotted into approximately 10-g portions for use as the control

sample and for preparation of spiked samples as described below (Section 7.4.3).

7.4.3 Spiked Samples

A spiked sample will be included with each sample batch to demonstrate method accuracy. Samples spiked with target PCDDs and PCDFs will be prepared at two concentration levels to document method performance over the working range. The spiked samples will be prepared from the same batch of homogenized tissue (lipid matrix) used to prepare the control samples. This will provide a consistent sample background for all the spiked samples.

7.4.4 Internal Standards

Stable isotope labeled PCDDs and PCDFs will be used for quantitation of the target analytes and to assess method performance on a per-sample basis.

7.4.4.1 Quantitation Standards

Stable isotope labeled PCDDs and PCDFs will be added to each sample prior to sample preparation for use as internal quantitation standards. Since these compounds are taken through all method procedures, the data will reflect method recovery. As noted in Table 7-1, carbon-13 labeled internal quantitation standards may be available for each PCDD and PCDF homolog and for specified congeners. Pairing of the specific congeners and carbon-13 analogs provides accurate measurements of the PCDD and PCDF levels in human adipose tissue.

7.4.4.2 Recovery Standards

Additional stable isotope labeled PCDDs and PCDFs, $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD, will be added to the final extracts prior to HRGC/MS analysis to provide an accurate measurement of the method recovery for each of the internal quantitation standards.

7.4.5 Calibration Standards

Calibration standards will be analyzed at the beginning and end of each day's run to document response factor variability and instrument sensitivity. This will be accomplished using a low level

Table 7-1. Calibration/Internal Standards

Calibration standard	Internal standards	
	Quantitation	Recovery
PCDD		
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	
1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	
OCDD	$^{13}C_{12}$-OCDD	
PCDF		
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	
1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	
1,2,3,4,7,8-HxCDF 1,2,3,4,8,9-HxCDF 1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	
1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	
OCDF		

calibration standard ranging in concentration from 2.5 pg/µL each of 2,3,7,8-TCDD and 2,3,7,8-TCDF up to 12.5 pg/µL each of OCDD and OCDF (standard CS-7, Table 2, Appendix B) as the first standard of each day. The standard analyzed at the end of the analysis day will be selected from the remaining five calibration standards.

7.4.6 Control Charts

Control charts will be used to demonstrate the consistency of individual target analyte RRF values over time.

7.4.7 Reagent Blanks

Reagent blanks will be analyzed to identify sources of background if PCDDs and PCDFs are detected in method blanks.

7.5 Performance and Systems Audits

7.5.1 Performance Audits

An audit will be conducted by the quality assurance coordinator (QAC) prior to analysis, if the procedure or instrument has changed, if analytical problems are suspected, and when requested. The audit will consist of:

- Issuing at least one performance audit sample with the first sample batch to the HRGC/MS analyst. The HRGC/MS analyst will analyze the sample as received and will report the concentration of the sample to the QCC. If the results are within 70 to 130% of the concentration submitted, the analysis of samples will proceed. If the results do not meet this criterion, the calibration curve must be reestablished and the analysis of a second performance audit sample must be successfully completed.

- Preparing and submitting a report of the audit results to the program manager, applicable section heads, and the department director.

7.5.2 Systems Audits

An audit will be conducted a minimum of one time by the QAC. The audit will, where appropriate, include:

- Reviewing actual practices versus the protocol and reporting deviations from protocol.

- Inspecting calibration and maintenance records.

- Inspecting QC practices.

- Preparing and submitting a report to the program manager, applicable section heads, and the department director.
- Reviewing the reports to determine if QA objectives were met.
- Tracing selected analytical data to verify the calculations and the analytical results.
- Conducting additional audits as directed by the program manager or department director.

SECTION 8.0

DATA REDUCTION AND ANALYSIS

8.1 Collection

Data collection will utilize both manual and computerized acquisition systems. All activities will be legibly recorded using permanent ink in the project notebook or on worksheets. Each person who records data will sign and date each sheet. Strip charts, magnetic tapes, etc., will be labeled with a format identifier, project number, date, the ID(s) of the instrument, and the name of the person responsible for the data recording equipment. Custody of the original data media will be the responsibility of assigned project staff until archived.

8.2 Data Reduction

Standard data reduction procedures with built-in checks will be used. For example, if an integrator or computer is used to calculate concentrations, the standards used to generate the curve musts be back-calculated using the curve to ensure satisfactory curve-fitting over the anticipated range. In addition, all sample manipulations (e.g., weighing, dilution, concentrations, etc.) must be clearly documented. One example of data reduction for HRGC/MS includes:

- Searching for the target compounds of interest using a computer automated search routine.
- Visually inspecting the quantitation report from the search to ensure that the internal standard was found by the search routine.
- Determining the relative response factor (RRF) for each of the native PCDD and PCDF analytes to the designated internal quantitation standard by using:

$$RRF = \frac{A_{STD} \cdot C_{IS}}{A_{IS} \cdot C_{STD}}$$

where A_{STD} = the sum of the areas of the integrated ion abundances for the analyte in question. For example, for TCDD, A_{STD} would be the sum of the integrated ion abundances for *m/z* 320 and 322;

A_{IS} = the sum of the areas of the integrated ion abundances for the labeled PCDD/F used as the internal quantitation standard for the above analyte. For example, for $^{13}C_{12}$-2,3,7,8-TCDD, A_{IS} would be the sum of the integrated ion abundances for *m/z* 332 and 334;

C_{STD} = concentration of the analyte in pg/µL; and

C_{IS} = concentration of the internal quantitation standard in pg/µL.

- Determining the relative response factor for the internal quantitation standard (RRF_{IS}) for the data obtained during the analysis of the concentration calibration standard by using:

$$RRF_{IS} = \frac{A_{IS} \times C_{RS}}{A_{RS} \times C_{IS}}$$

where A_{IS} and C_{IS} are defined as given above;

C_{RS} = concentrations of the internal recovery standard in pg/µL; and

A_{RS} = the sum of the areas of the integrated ion abundances for the labeled PCDD ($^{13}C_{12}$-1,2,3,4-TCDD or $^{13}C_{12}$-1,2,3,7,8,9-HxCDD). For example, for $^{13}C_{12}$-1,2,3,4-TCDD, A_{RS} would be the sum of the integrated ion abundance for *m/z* 332 and 334.

Refer to Table 11, Appendix B, for pairing of the internal quantitation standards with the appropriate internal recovery standard.

- Confirming that responses for characteristic ions of PCDDs and PCDFs meet the qualitative criteria based on ion ratios and relative retention time (RRT) for specific 2,3,7,8-substituted congeners.

- Calculating the amounts of the target compounds found in the extract using:

$$C_{sample} = \frac{A_{sample} \cdot Q_{IS} \cdot 100}{A_{IS} \cdot RRF \cdot W_{AT} \cdot LC}$$

where C_{sample} = the lipid-adjusted concentration of PCDD or PCDF congener in pg/g;

A_{sample} = sum of the integrated ion abundances determined for the PCDD/PCDF in question;

A_{IS} = sum of the integrated ion abundances determined for the labeled PCDD/F used as the internal quantitation standard for the above analyte;

Q_{IS} = the amount (total pg) of the labeled internal quantitation standard added to the sample prior to extraction;

RRF = relative response factor of the above analyte relative to its labeled internal quantitation standard determined from the initial triplicate calibration;

W_{AT} = weight (g) of original adipose tissue sample; and

LC = percent extractable lipid.

Refer to Table 11, Appendix B, for pairing of target analytes with the appropriate internal quantitation standard.

8.3 Data Validation

The data validation process will include:

- Validating all equations and computer programs and documenting the validation.
- Confirming that raw areas for internal recovery standards and calibration standards are near the expected value.
- Validating and checking electronic data transfer.
- Verification of all data transfers.
- Checking calculations.
- Reporting of all associated blank, standard, and QC data along with results for analyses of each batch of samples.
- Maintaining records of reviewing, proofing, and validation.
- Reviewing and approving all data by the project staff.
- Reporting protocol deviations and assumptions with the results.

8.4 Storage

Raw data will be documented in laboratory notebooks, on printer paper, as strip chart recordings, or may be stored on magnetic tape or disk. All data will be archived according to the existing MRI SOP (QA-7, Record Retention and Archiving).

8.5 Data Analysis

Following the chemical analysis of all the adipose tissue samples, the data will be gathered from the laboratory notebooks and the MS quantitation reports. These data will be entered into Lotus spreadsheets using personal computers. If feasible, data will be automatically transmitted from one system to another. The questionnaire data on lifestyle factors will be entered manually. The specific PCDD and PCDF isomer data will be presented by geographical location (i.e., Los Angeles and San Francisco), age, and sex categories. Basic statistics such as average concentrations, percentiles, standard deviations, and percent relative deviations (coefficient of variation) will be computed for each geographic location, age, and sex category stratum as defined by the sampling design, and the results will be presented in tabular form. Confidence limits for the estimated PCDD and PCDF selected isomer levels will be computed based on a preassigned confidence level and the probability distribution of such concentrations in the population. Correlations between body burden levels and lifestyle factors will be computed and tested for statistical significance provided that a reasonable amount of data is available from the questionnaire. All quality assurance/quality control data such as calibration results, replicate and duplicate analysis results, spiked and blank sample concentrations, and other QA/QC-related results obtained during the chemical analysis phase of the project will be documented and analyzed appropriately.

Readily available statistical software will be used for all computations. The statistical basis for all reported estimations of concentrations of the analytes of interest will be documented as well as their mathematical derivations.

SECTION 9.0

DATA QUALITY ASSESSMENT

9.1 Precision

The data from the analysis of the adipose tissue samples will provide several measures of method precision. These data will result from analysis of replicate samples (spiked and unspiked). These replicates will provide a measurement of batch-to-batch method performance as well as method reproducibility over the entire study. Method precision can also be assessed for each PCDD or PCDF homolog for a specific sample batch from the absolute recoveries of the internal quantitation standards in each sample. These data can also be assessed to provide additional precision estimates for the entire analysis program.

The measurement for precision of the replicates (greater than 2) will be the standard deviation (S.D.) and/or the relative standard deviation (RSD):

$$S.D. = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \overline{X})^2}{n - 1}} \qquad RSD = \frac{100\% \times S.D.}{\overline{X}}$$

where n = number of replicate determinations;

X_i = an individual data point; and

$$\overline{X} = \text{mean} = \frac{\sum_{i=1}^{n} X_i}{n}$$

9.2 Accuracy

9.2.1 Performance Audit Samples

The accuracy for the performance audit samples will be assessed as percent recovery (R) as demonstrated below.

$$R = \frac{\text{Amount Found}}{\text{Amount Prepared}} \times 100$$

9.2.2 Spiked Adipose Tissue Samples

Accuracy of the HRGC/MS-SIM method for each PCDD and PCDF congener will be determined by analyzing the spiked and unspiked samples with each batch of samples. The measurement of accuracy for each spiked congener will be percent recovery (R).

$$R = \frac{\text{Spiked Sample Value - Unspiked Sample Value}}{\text{Amount Spiked}} \times 100$$

9.3 Quality Assurance Objectives

The quality assurance objectives are summarized in Table 9-1.

Table 9-I. QC Procedures and Criteria Analysis of Human Adipose Tissue Samples for PCDDs and PCDFs

Analysis event	Frequency	QC criteria	Corrective actions	Responsibility
Instrument mass calibration	Daily; real-time interpretation	Must demonstrate accurate mass calibration using perfluorokerosene (PFK). First activity of analysis day.	Recalibration. If criteria not achieved, do not proceed with analysis.	MS analyst
• PCDD/PCDF analysis (low resolution MS)	First event of analysis day	Using PFK, tune to a minimum resolution of 3,000 (10% valley) and optimal response and peak shape m/z 381. Adjust magnetic field to pass m/z 300 at accelerating voltage. Introduce PFK through direct inlet and acquire accelerating voltage scans from 8000 to 4000 V using Incos Data System. Lockmass (m/z 381) identified in PFK spectrum used to update. Mass calibration ranges from 301 to 593 amu.	Refer to tuning and mass calibration procedure (Section 8.1.1 in Appendix B). If criteria cannot be achieved, instrument may require maintenance.	MS analyst
• 2,3,7,8-TCDD confirmation (high resolution MS)	First and last events of analysis day	Using PFK, tune to minimum resolution of 10,000 (10% valley) and optimal response for m/z 254.986.	Refer to tuning and mass calibration procedure (Section 8.2.1 in Appendix B). If criteria cannot be achieved, instrument may require maintenance.	MS analyst
Column performance check	Daily; real-time interpretation	Must demonstrate isomer specificity for 2,3,7,8-TCDD before proceeding with analysis of calibration standard • 60-m DB-5 column, 30-60% resolution (Section 8.1.3, Appendix B) • 50-m CP Sil 88/60-m SP-2330, $\leq$ 25% resolution (Section 8.2.3, Appendix B)	Adjust column length and reanalyze performance mixture. If necessary, install a new HRGC column and evaluate performance.	MS analyst
Calibration standards				
• Initial calibration	Precedes initial sample analysis	Triplicate analysis of six concentration calibration standards. % RSD of RRF for triplicate analyses ±30% for PCDD/PCDF, ±20% for TCDD/TCDF; % RSD of RRF for mean RRF for all standards ±30% for PCDD/PCDF, ±20% for TCDD/TCDF (Sections 8.1.4, 8.1.5, 8.2.4, and 8.2.5 (Appendix B).	Prepare fresh concentration calibration standard.	MS analyst Task leader
• Routine calibration	Precedes sample analysis on daily basis. Also must demonstrate calibration as last injection of each analysis day.	Measured RRF values for solution CS-7 (Appendix B, Table 2) must be within ±30% for PCDD/PCDF and ±20% for TCDD/TCDF.	Reanalyze solution CS-7 or repeat the initial calibration sequence. (Sections 8.1.6, 8.1.7, 8.2.6, and 8.2.7, Appendix B). If calibration criteria are not met at the end of the day, all samples are subject to reanalysis by HRGC/MS.	MS analyst Task leader

Table 9-1 (continued)

Analysis event	Frequency	QC criteria	Corrective actions	Responsibility
Tridecane blank	Precedes sample analysis following calibration standards; real-time daily	Blank run should not demonstrate positive responses (> 2.5 times S/N) for the (Section 13.1.1, Appendix B).	Clean injection syringe, repeat blank analysis.	MS analyst
Samples/QC samples				
◦ Analysis	As submitted in sample batch; real-time	See Sections 13.1 and 13.2, Appendix B. Document response of internal recovery standard(s) and compare to daily calibration standard. Internal recovery standard responses must be within 50% of response noted for calibration. Standard used to verify RRF values. Samples submitted as blinds to MS analyst.	If internal recovery standard noted to be < 50% of calibration standard, reanalyze and/or check mass calibration.	MS analyst
• Data interpretation	Following analysis of sample batch	See Sections 9.0 and 14.0, Appendix B.		
- Performance evaluation samples	Real-time	Check solutions provided by QAC for measurement of accuracy, 70-130%. Do not proceed with sample analysis until notified of acceptable performance by the QAC.	Reanalyze solution and/or calibration standard(s).	MS analyst QAC Task leader
- External (blind)/ Internal (QC samples)		Accuracy should be within 50-130% of spike level. Recovery of internal quantitation standards should be within 50-115%.	Reanalyze solution and/or calibration standards.	MS analyst QAC Task leader
- Tissue samples		Recovery of internal quantitation standards should be within 50-115%.	Reanalyze solution and/or calibration standards.	MS analyst QAC Task leader

SECTION 10.0

CORRECTIVE ACTION

The program manager or appropriate task leader has primary responsibility for taking corrective action; if he is unavailable, the QAC will be contacted for instructions. Any problems resulting in loss of data or data integrity will be reported to the program manager. Some of the types of problems and corrective actions to be taken are listed below.

10.1 System Audits and Performance Audits

If problems are detected during any audit:

- The QA coordinator (QAC) will immediately notify the program manager or appropriate task leader of the problem(s) and any action(s) he has taken. Notification can be verbal, followed by an audit memo.
- The program manager or task leader and the QAC will then collectively decide on the appropriate action. The program manager or task leader will implement the corrective action, then prepare and send a memo of the corrective action taken to the QAC.
- The ARB project officer will be notified of any unresolved problems by the program manager prior to submission of any data package.

10.2 Data Outside Control Limits

At any time the data fall outside previously designated limits, the following corrective action is applied:

- Where appropriate, samples should be reanalyzed to bring data into control limits. This may constitute repreparation of the samples in the laboratory and then redetermination with the appropriate instrument, if sufficient sample is available, or simply redetermination of the sample extracts previously prepared.
- If data are marginal or other reasons prevent reanalysis, the MRI task leader or program manager will consult with the QAC for other appropriate action. If data outside the control limits are to be reported, the reason for the action must be documented and the report carefully annotated.
- If data are consistently out of the control limits, corrective action should be taken by the appropriate staff and documented in the

notebook. The QAC will be notified of the corrective action by the program manager or appropriate task leader.

10.3 Loss of Data

The MRI program manager will investigate the problem, then perform one or more of the following actions:

- If the problem is correctable, the problem/action-taken is documented in the project records. If necessary, the program manager then prepares and sends a problem/action-taken memo to the QAC. Corrective action may include reanalysis of samples.

- If the problem is not correctable, the MRI program manager will assess the impact of the data loss and discuss it with the ARB project officer.

SECTION 11.0

DOCUMENTATION AND REPORTING

11.0 Documentation

All manual documentation will be performed as follows: all information will be entered in a bound laboratory book or established forms. All informaion will be recorded using permanent ink.

Where signatures are required, the following information will be entered in project records: printed name, signature, and written initials. The traceable initials may be used in place of the signature.

Manual and computerized documentation will include the following:

- Project identifiers (project number, task number, etc.)
- Staff identifiers (signature, printed name, or traceable initials)
- Equipment identifiers (type/model/serial number/etc.)
- Computer program identifiers (name of program/revision date/author).
- Subject identifiers (type/number/code/etc.)
- Date (month/day/year)

Manual corrections of original data/information will be performed as follows: draw a line through the erroneous information, leaving the original information legible. Add the correct information, sign (traceable initials are permitted), and date the correction. Explain the correction; use codes if explained in project records. Some code examples are: EE (entry error--transposition error, wrong page used, etc.); CE (calculation error--used wrong numbers, wrong program, etc.); TE (transposition error). Do not superimpose numbers; use error handling instead.

Corrections in computerized records must be traceable (i.e., initials/ date/reason). The original value must be retained in the records.

Manual additions to original data/information must be signed (or initialed) and dated.

11.2 Record Keeping

The following records will be maintained as a minimum requirement:

- Equipment/instrument calibration/maintenance records.
- All information related to the project: technical plans, QA plans, additional protocols, raw data, source of data, methods of computation, validation data, final data, deviations from protocol, reports, communications, etc.

11.3 Reporting

Sample analysis data will be reported to the ARB project officer on a batch basis. The sample batch data will include all QC results (recovery data, accuracy of spiked sample analyses, method precision, etc.). All data (standards and samples) will be archived such that verification of calculations can be accomplished for each analysis event. The analytical method describes the calculations required to achieve RRF values and final lipid-adjusted concentrations. The ARB project officer will be provided examples of raw data and calculations on request. The final report to ARB will contain a summary of all design sample analyses and cumulative QA/QC data.

APPENDIX D

RELATIONSHIP BETWEEN PERCENT BODY FAT AND ANTHROPOMETRIC DATA IN HUMANS

Subcontract No. 255-8941-1

Technical Assistance Services for Determination of Current Body Burdens for PCDDs and PCDFs in California Residents

Monthly Progress Report No. 3
for the Period
1-31 July, 1988

to

Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

Attention: Dr. John S. Stanley
Program Manager

8 August 1988

1940 Fifth Avenue, Suite 200, San Diego, CA 92101 (619) 531-0092

Relationship Between Percent Body Fat and Anthropometric Data in Humans

1.0 Introduction and Background

The purpose of the literature analysis was to derive estimates of proportion of body fat based on triceps skinfold measurements. If triceps skinfold measurements were not available, then a method was sought to estimate triceps skinfold measurements from weight and height.

Communication with recognized researchers in health and nutrition statistics (Ref. No. 14) and IWG personnel's experience in the field are reflected in the bibliography of relevant literature shown on page A3-14.

Data for estimating triceps skinfold from weight and height may be found the NHANES II data set developed by the National Center for Health Statistics. (Ref. No. 13). NHANES II provides height, weight, and triceps skinfold for white, black, and all other races; for male and female; and for six age categories. As shown below, estimates are developed independently for male and female and for each of the six age categories. All races are combined because of the paucity of data on races other than white and black.

The overall two-step procedure for estimating proportion of body fat from weight and height is based on linear relationships. Lampman et al. (Ref. Nos. 8, 9, 10) all indicate fundamentally linear relationships between body fat, weight and height.

Durnin and Womersley (Ref. No. 3) calculate body fat based on tricep skinfold measurements and an intermediate estimate of body density. Mellits and Cheek (Ref. No. 12) calculate body fat directly from weight or height with an intermediate estimate of total body water. The method of Durnin and Womersley is used in our analyses because of the information available in NHANES II on the triceps skinfold intermediate variable; there are no such data immediately available on total water with which to verify the Mellits and Cheek analysis.

A number of other papers in the bibliography provide background information on the occurrence of various elements and compounds based on age, weight, and sex of the subjects. A wide range of the overall population is covered in these papers producing a rather disparate array of relationships among age, weight, sex, and body burdens of various materials. The papers by Lampman el al. cited above fall in this category, as does Ellis et al. (Ref. No. 4). Estimates from these papers are not

used directly in our analyses; however, they do provide the basis for the choice of linear relationships by convincing us that strong, consistent linear relationships do in fact exist. The papers also convince us that the best estimates are predicated on both weight and height and not weight alone or height alone, as in Mellits and Cheek (Ref. No. 12).

2.0 Development of Estimates

2.1 Regression Coefficients

The following regression coefficients were calculated for Triceps Skinfold vs weight and height from NHANES II data (Ref. No. 13).

REGRESSION COEFFICIENT SUMMARY FOR TRICEPS SKINFOLD VS. WEIGHT AND HEIGHT

	regression coefficients	male	female
18-24 Years all races	Bo	-273.352844	-14.263807
	B_1	-0.469455	0.787458
	B_2	1.805642	-0.078067
25-34 Years all races	Bo	-190.690170	-22.926670
	B_1	-0.075465	0.624502
	B_2	1.185791	0.039446
35-44 Years all races	Bo	13.968863	-19.885094
	B_1	0.719409	0.736984
	B_2	- 0.331078	- 0.200647
45-54 Years all races	Bo	-6.583013	-143.316047
	B_1	0.531508	0.246848
	B_2	-0.130799	0.954931
55-64 Years all races	Bo	-37.319698	-175.031281
	B_1	0.442006	0.115544
	B_2	0.090326	1.214152
65-74 Years all races	Bo	-151.554108	-108.771423
	B_1	-0.070613	0.347319
	B_2	0.989702	0.704237

The above regression estimates are the linear coefficients of equations in the form:

$$S = Bo + B_1 \cdot X_1 + B_2 \cdot X_2 \quad (1)$$

where

S = triceps skinfold measurement (mm),
X_1 = weight (kg), and
X_2 = height (cm).

Age and sex categories are as indicated. The purpose of the equations is to estimate the triceps skinfold measurements when only weight and height data are available.

The following is the second step in estimating proportion of body fat from the triceps skinfold estimate when the triceps skinfold measurement is estimated from height and weight. The following is also the entry point for estimating the proportion of body fat when the triceps skinfold measurement is available. The regression coefficients are taken from Durnin and Wormersley (Ref. No. 3).

REGRESSION COEFFICIENT SUMMARY FOR BODY DENSITY VS. TRICEPS SKINFOLD

	regression coefficients	male	female
16-19 Years all races	Bo	1.1252	1.1159
	B_1	0.0625	0.0648
20-29 Years all races	Bo	1.1131	1.1319
	B_1	0.0530	0.0776
30-39 Years all races	Bo	1.0834	1.1176
	B_1	0.0361	0.0686
40-49 Years all races	Bo	1.1041	1.1160
	B_1	0.0609	0.0762
50+ Years all races	Bo	1.1143	1.1278
	B_1	0.0618	0.0775

The above regression estimates are the linear coefficients of equations in the form:

$$D = Bo + B_1 \cdot LOG(S) . \quad (2)$$

where

D = body density (10^3 kg/m^3) and
S = triceps skinfold (the dependent variable from equation 1)

The purpose of these equations is to estimate proportion of body fat (1) from measured triceps skinfold, or (2) from triceps skinfold estimated from equation 1. Proportion of body fat is then calculated from the following relationship.

$$F = \frac{4.95}{D} - 4.5 \qquad \text{[Ref. 3]} \qquad (3)$$

where

F = proportion of body fat, and
D = body density (the dependent variable from equation 2)

The actual age of the subject should be acquired and used to choose the correct age category independently for equation 1 and equation 2. This method is preferable to combining non-matching age categories between the two equations. Combining age categories would only result in an increased error variance for estimated proportion body fat.

The width of a confidence interval on the estimate of the proportion of body fat has a minimum of approximately 0.049 or 4.9% near the mean values of weight and height and a maximum of 0.091 or 9.1% near the extremes of the weight and height. The probability of the F ratios for all regression coefficients in the two-step computation procedure is greater than 0.99 (note that equation 3 is a deterministic relationship).

Following are two example analyses, calculated and compared to data from the literature:

EXAMPLE ONE

25 year old male, weight 80kg, height 175cm

Triceps skinfold = -190.690 - 0.075 Wt + 1.186 Ht
= 11.157mm

Body Density = 1.1131 - 0.0530 log (11.157)
= 1.0576

$$\text{Proportion Body Fat} = \frac{4.95}{10.576} - 4.5$$
$$= 0.181 \ (18.1\%)$$

Durnin and Womersley (Table 9, Ref. No. 3) predict a Fat value between 17.7 and 17.9%.

Mellits and Cheek predict 18.2% Fat.

EXAMPLE TWO

57 year old female, weight 65 kg, height 160cm

Triceps skinfold = 175.031 + 0.1155 Wt + 1.2142 Ht
= 26.743

Body Density = 1.1278 - 0.0775 log (26.743)
= 1.00725

$$\text{Proportion Body Fat} = \frac{4.95}{1.023} - 4.5$$
$$= 0.414 \ (41.4\%)$$

Durnin and Womersley (Table 9, Ref. No. 3) predict a Fat value between 41.2 and 41.9%

Mellits and Cheek predict 41.9% Fat.

2.2 Regression Analysis Summaries

The following Tables 1 through 12 are a complete summary of the regression analysis used in deriving the tables of coefficients given above for equation 1. Definitions used in the analysis are:

- the dependent variable is Triceps Skinfold
- the independent variables are Wt (B1) and Ht (B2).
- The method is linear regression with no raw data transformations.

The data used for the analyses, which were derived from the NHANESII database, are shown in Table 13.

3.0 Additional Comments

3.1 Advantages of using Actual Age rather than Age Categories

Actually, the questionnaire should have asked for age and not just age category. In the first place, age is not a difficult value to get from hospital or other records. In the second place, that type of information should never be given up in lieu of recording age category only. The importance of recording age is apparent when it is seen that the coefficient tables for equations 1 and 2 and the experimental design for the study are all three based on different age categories.

Table 1. MALE (all races): 18-24 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	987	39311.37			
Regression	2	39032.31	19516.16	68885.9255	0.999999
B1	1	78.22	78.22	276.0918	0.999999
B2	1	328.49	328.49	1159.4628	0.999999
Residual	985	279.06			

Variable	Value	Width of Conf. Interval
B0	-273.352844	0.005199
B1	-0.469455	0.054713
B2	1.805642	0.102689

Table 2. MALE (all races): 25-34 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	1066	50710.72			
Regression	2	50087.82	25043.91	42778.5191	0.999999
B1	1	1.54	1.54	2.6278	0.999999
B2	1	97.95	97.95	167.3159	0.999999
Residual	1064	622.90			

Variable	Value	Width of Conf Interval
B0	-190.690170	0.006579
B1	-0.075465	0.090143
B2	1.185791	0.177509

Table 3. MALE (all races): 35-44 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	744	35604.88			
Regression	2	35198.74	17599.37	32152.7865	0.999999
B1	1	216.72	216.72	395.9299	0.999999
B2	1	15.59	15.59	28.4733	0.999990
Residual	742	406.15			

Variable	Value	Width of Conf. Interval
B0	13.968863	0.007596
B1	0.719409	0.070043
B2	-0.331078	0.120201

Table 4. MALE (all races): 45-54 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	689	30661.12			
Regression	2	30468.93	15234.46	54455.2333	0.999999
B1	1	213.27	213.27	762.3183	0.999999
B2	1	2.60	2.60	9.286	0.997208
Residual	687	192.20			

Variable	Value	Width of Conf Interval
B0	-6.583013	0.005853
B1	0.531508	0.037299
B2	-0.130799	0.083167

Table 5. MALE (all races): 55-64 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	1226	45719.99			
Regression	2	45650.65	22825.33	402948.9513	0.999999
B1	1	79.69	79.69	1406.8317	0.999999
B2	1	1.01	1.01	17.8763	0.999876
Residual	1224	69.33			

Variable	Value	Width of Conf. Interval
B0	-37.319698	0.002155
B1	0.442006	0.022815
B2	0.090326	0.041361

Table 6. MALE (all races): 65-74 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	1198	42900.56			
Regression	2	42724.81	21362.41	145379.6614	0.999999
B1	1	5.30	5.30	36.0674	0.999998
B2	1	306.41	306.41	2085.2565	0.999999
Residual	1196	175.74			

Variable	Value	Width of Conf Interval
B0	-151.554108	0.003583
B1	-0.070613	0.022764
B2	0.989702	0.041962

Table 7. FEMALE (all races): 18-24 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	1065	76193.10			
Regression	2	75868.31	37934.15	124151.3421	0.999999
B1	1	104.73	104.73	342.7777	0.999999
B2	1	0.33	0.33	1.0940	0.703967
Residual	1063	324.80			

Variable	Value	Width of Conf. Interval
B0	-14.263807	0.003878
B1	0.787458	0.082358
B2	-0.078067	0.144522

Table 8. FEMALE (all races): 25-34 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	1169	105109.33			
Regression	2	104980.65	52490.33	476043.7219	0.999999
B1	1	277.60	277.60	2517.5919	0.999999
B2	1	0.20	0.20	1.8136	0.825159
Residual	1167	128.68			

Variable	Value	Width of Conf Interval
B0	-22.926670	0.001983
B1	0.624502	0.024098
B2	0.039446	0.056711

Table 9. FEMALE (all races): 35-44 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	843	86803.80			
Regression	2	86531.74	43265.87	133742.6200	0.999999
B1	1	561.64	561.64	1736.1301	0.999999
B2	1	6.94	6.94	21.4413	0.999952
Residual	841	272.06			

Variable	Value	Width of Conf. Interval
B0	-19.885094	0.003739
B1	0.736984	0.034260
B2	-0.200647	0.083931

Table 10. FEMALE (all races): 45-54 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	762	75050.99			
Regression	2	74796.08	37398.04	111502.1261	0.999999
B1	1	70.02	70.02	208.7749	0.999999
B2	1	190.71	190.71	568.5928	0.999999
Residual	760	254.91			

Variable	Value	Width of Conf Interval
B0	-143.316047	0.004095
B1	0.246848	0.033096
B2	0.954931	0.077580

Table 11. FEMALE (all races): 55-64 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	1328	115356.78			
Regression	2	115246.95	57623.48	695753.0741	0.999999
B1	1	11.81	11.81	142.5838	0.999999
B2	1	237.50	237.50	2867.6311	0.999999
Residual	1326	109.82			

Variable	Value	Width of Conf. Interval
B0	-175.031281	0.001640
B1	0.115544	0.018732
B2	1.214152	0.043893

Table 12. FEMALE (all races): 65-74 Years

Source of Variation	Degrees of Freedom	Sums of Squares	Expt Mean Squares	F-Ratio	Pr(f>F)
Total	1415	119903.87			
Regression	2	119659.76	59829.88	346328.7917	0.999999
B1	1	68.84	68.84	398.4921	0.999999
B2	1	62.27	62.27	360.4646	0.999999
Residual	1413	244.10			

Variable	Value	Width of Conf Interval
B0	-108.771423	0.002324
B1	0.347319	0.033680
B2	0.704237	0.071803

Table 13. Data Derived From NHANES II Data Base

Age	Sex	n	Weight Mean	Weight SD	Height Mean	Height SD	Triceps Skin Fold Mean	Triceps Skin Fold SD
18 - 24	M	988	73.8	12.7	177.0	7.1	11.6	6.5
25 - 34	M	1067	78.7	13.7	176.7	6.7	12.9	7.0
35 - 44	M	745	80.9	13.4	176.3	7.3	13.8	7.1
45 - 54	M	690	80.9	13.6	175.2	6.6	13.5	6.7
55 - 64	M	1227	78.8	12.8	173.7	6.9	13.2	6.3
65 - 74	M	1199	74.8	12.8	171.3	7.1	12.7	6.1
18 - 24	F	1066	60.6	11.9	163.4	6.6	20.7	8.6
25 - 34	F	1170	64.2	15.0	163.1	6.3	23.6	9.9
35 - 44	F	844	67.1	15.2	162.8	6.3	26.3	9.8
45 - 54	F	763	68.0	15.3	161.3	6.4	27.5	9.7
55 - 64	F	1329	67.9	14.7	160.1	6.4	27.2	9.5
65 - 74	F	1416	66.6	13.8	158.1	6.2	25.7	9.0

3.2 Comparison Between Estimated versus Actual Triceps Skinfold Data

The second comment involves the method for converting weight, height, and triceps skinfold into proportion of body fat. In order to fine-tune the method developed above, an estimate of triceps skinfold should be calculated for all subjects. Where actual skinfold measurements are available, the estimated values should be compared to the measured values in order to derive an estimate of within-subject error variance for the estimation procedure.

3.3 Advantages of Obtaining Additional Skinfold Measurements

If it were possible, both triceps and subscapular skinfold measurements should be taken. From Durnin and Womersley (Ref. No. 3), estimates based on the sum of both measurements produce a significantly lower variance of estimation than do estimates based on the measurement of triceps skinfold only.

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APPENDIX E

METHOD RECOVERIES FOR THE INTERNAL QUANTITATION STANDARDS (IQS)

Table E-1. Method Recoveries (%) for Internal Quantitation Standards
CARB Body Burden Study--Batch 1

	SEX	Female	Male	Female	Female	Female	Female	Male	Male	Female	Female
	AGE	59	50	42	52	59	44	49	33	88	46
	CITY	LA	LA	LA	LA	LA	LA	LA	LA	LA	LA
COMPOUND											
13C12 TCDF		68	51	63	59	65	57	63	58	58	49
13C12 TCDD		83	74	105	72	75	69	75	66	75	64
13C12 PeCDF		110	83	101	92	113	87	114	87	96	92
13C12 PeCDD		153	109	137	122	142	111	171	129	133	118
13C12 HxCDF		68	74	65	65	61	63	58	65	62	66
13C12 HxCDD		62	81	64	64	80	61	59	64	66	65
13C12 HpCDF		76	69	66	66	70	66	63	65	67	61
13C12 HpCDD		111	107	93	83	99	77	98	88	107	102
13C12 OCDD		149	120	92	122	148	108	139	115	142	127

Table E-1 (continued)

	SEX	Female	Male	Female	Male	Female	Male	Male
	AGE	66	12-34	53	33	30	27	33
	CITY	LA	LA	LA	LA	LA	LA	LA
COMPOUND								
13C12 TCDF		65	59	55	55	56	58	66
13C12 TCDD		79	68	72	76	66	82	79
13C12 PeCDF		110	101	87	98	94	83	107
13C12 PeCDD		143	141	100	131	117	110	123
13C12 HxCDF		78	78	87	59	68	55	76
13C12 HxCDD		70	78	84	58	65	53	72
13C12 HpCDF		79	68	73	62	75	50	74
13C12 HpCDD		131	91	91	87	103	78	97
13C12 OCDD		170	126	110	136	147	93	128

Table E-2. Method Recoveries (%) for Internal Quantitation Standards
CARB Body Burden Study--Batch 2

	SEX	Male	Male *	Male	Male	Male *	Male	Male	Male
	AGE	50 +	50 +	50 +	50 +	50 +	50 +	12-34	35-49
	CITY	LA	LA	LA	LA	LA	LA	LA	LA
COMPOUND									
13C12 TCDF		77.5	73.6	74.0	72.0	70.8	83.9	63.6	68.4
13C12 TCDD		73.5	67.8	62.9	65.5	65.8	70.8	64.4	69.7
13C12 PeCDF		81.5	78.2	81.5	77.4	74.0	80.0	63.7	69.0
13C12 PeCDD		96.3	89.4	90.3	84.5	80.0	99.3	78.3	85.3
13C12 HxCDF		91.3	79.9	67.1	75.9	77.7	173	82.1	79.5
13C12 HxCDD		88.8	86.1	84.8	76.8	77.6	72.3	81.5	85.8
13C12 HpCDF		90.5	88.0	78.1	79.3	82.8	49.0	72.6	86.7
13C12 HpCDD		86.2	82.3	71.2	78.0	79.8	135	72.2	89.9
13C12 OCDD		91.6	72.6	68.1	77.2	97.6	342	68.9	91.5

* - mean of duplicate sample preparation analyses

Table E-2 (continued)

SEX	Male	Male	Female	Male	Female	Male	Male	Male
AGE	42	42	51	50	52	58	76	55
CITY	LA	SF	SF	SF	SF	SF	SF	LA
COMPOUND								
13C12 TCDF	58.5	67.9	62.5	63.6	65.6	65.1	61.3	63.0
13C12 TCDD	59.2	67.0	45.2	63.7	66.3	63.5	55.7	65.9
13C12 PeCDF	53.0	65.1	60.7	61.4	61.8	62.8	54.1	57.2
13C12 PeCDD	68.2	82.7	74.9	68.1	76.6	63.8	63.7	71.2
13C12 HxCDF	68.0	75.9	69.6	73.7	75.8	70.0	70.9	73.9
13C12 HxCDD	80.9	80.9	74.9	78.3	79.9	78.4	74.1	79.4
13C12 HpCDF	60.4	82.6	61.8	62.7	83.0	69.5	71.7	68.7
13C12 HpCDD	68.1	81.5	71.3	61.2	78.9	69.6	73.1	66.8
13C12 OCDD	67.3	82.9	70.4	53.9	74.6	60.6	65.5	64.4

Table E-3. Method Recoveries (%) for Internal Quantitation Standards
CARB Body Burden Study--Batch 3

	SEX	Female	Male	Female	Female	Male	Male	Female *
	AGE	37	39	43	43	65	65	65
	CITY	SF	SF	SF	SF	SF	SF	SF
COMPOUND								
13C12 TCDF		76.8	72.3	71.3	78.0	64.8	85.6	72.7
13C12 TCDD		71.8	76.8	74.1	79.1	66.1	91.8	75.0
13C12 PeCDF		80.2	69.7	70.2	69.0	61.9	89.1	72.0
13C12 PeCDD		92.8	88.4	82.9	88.0	80.6	107	88.0
13C12 HxCDF		77.1	80.9	68.4	77.6	68.5	68.1	68.0
13C12 HxCDD		86.9	92.5	80.7	93.5	81.3	82.5	83.1
13C12 HpCDF		82.4	87.7	78.1	78.4	73.2	74.9	78.2
13C12 HpCDD		86.4	93.8	85.5	82.6	76.8	87.2	90.2
13C12 OCDD		85.5	91.4	92.8	78.4	72.9	92.3	97.7

*-mean of duplicate sample preparations and analyses

Table E-4. Method Recoveries (%) for Internal Quantitation Standards
CARB Body Burden Study--Batch 4

COMPOUND	SEX AGE CITY	Female * 35-49 SF	Female 44 SF	Female 35-49 SF	Female 35-49 SF	Female 63 SF	Male 50 + SF	Male 35-49 SF	Male 50 + LA	Male 50 + LA
13C12 TCDF		82.1	87.1	77.0	77.3	92.4	88.5	78.7	81.0	93.9
13C12 TCDD		87.5	65.2	65.2	65.1	74.3	72.6	65.3	66.6	77.3
13C12 PeCDF		78.8	77.7	72.6	71.7	84.1	83.9	75.9	73.7	93.5
13C12 PeCDD		84.8	90.3	118	73.8	103	94.4	87.8	85.0	108
13C12 HxCDF		74.8	84.6	162	75.2	84.0	80.4	72.0	77.9	92.8
13C12 HxCDD		74.8	85.2	100	77.0	86.6	81.0	75.7	85.9	91.9
13C12 HpCDF		78.4	88.4	166	72.8	109	97.3	98.1	93.9	111
13C12 HpCDD		81.0	106	258	65.4	115	102	97.3	92.4	102
13C12 OCDD		73.3	106	199	48.5	124	118	92.3	98.8	95.9

*-mean of duplicate sample preparations and analyses

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Table E-5. Method Recoveries (%) for Internal Quantitation Standards
CARB Body Burden Study--Batch 5

	SEX	Male	Male	Female	Female	Female	Female	Female	Female*
	AGE	50+	50+	32	42	39	35-49	50 +	50 +
	CITY	LA	LA	SF	SF	SF	SF	SF	SF
COMPOUND									
13C12 TCDF		71.7	77.0	70.8	77.2	71.7	73.4	80.2	73.6
13C12 TCDD		63.1	62.1	69.1	68.7	68.8	70.3	73.6	72.9
13C12 PeCDF		82.3	80.9	69.6	79.6	74.7	72.1	72.7	55.0
13C12 PeCDD		120	118	83.5	102	93.9	86.1	92.2	72.6
13C12 HxCDF		72.2	80.0	83.7	82.0	78.9	80.2	78.8	74.1
13C12 HxCDD		81.5	85.8	86.7	89.7	82.7	86.7	85.9	90.2
13C12 HpCDF		74.4	101	92.2	94.6	80.2	85.9	73.8	73.7
13C12 HpCDD		82.7	104	102	98.6	94.3	97.8	91.9	88.0
13C12 OCDD		60.8	102	84.2	88.8	91.3	93.0	94.6	83.9

*-mean of duplicate sample preparations and analyses

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