

## Appendix A – 2001 Exposure Investigation Protocol

### Biological and Residential Environmental Sampling Protocol

#### Exposure Investigation

#### Calcasieu Estuary

(a.k.a. Mossville)

Lake Charles, Calcasieu Parish, Louisiana

CERCLIS No. LA0002368173

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### Purpose

The purpose of this Exposure Investigation is to further characterize dioxin exposures previously identified in Calcasieu Parish, Louisiana. Serum dioxin levels will be retested in participants of ATSDR's 1998 Exposure Investigation and in those individuals evaluated in the 1998 Health Consultation. Additional environmental samples will also be collected.

### Introduction

In 1998, the Region VI U.S. Environmental Protection Agency (EPA) and a Calcasieu community group, the Calcasieu League for Environmental Action Now (CLEAN), requested that the Agency for Toxic Substances and Disease Registry (ATSDR) evaluate dioxin<sup>\*</sup> levels reported in human blood samples, area sediment samples, and a composite clam sample. Subsequently, the ATSDR issued a health consultation and concluded that the serum dioxin levels were elevated<sup>i</sup>. ATSDR recommended that the source(s) of dioxin exposures be identified.

In response to this recommendation, the ATSDR conducted an exposure investigation (EI) in the Mossville community in December 1998. The purpose of this EI was to "determine if there was evidence for increased exposure to dioxins in residents of Mossville"<sup>ii</sup>. This investigation collected blood samples from 28 residents; surface soil samples from 3 residences; and chicken eggs raised at one residence. All samples were analyzed for dioxins. Several residents had blood serum dioxin levels above a comparison population's range. However, the soil and egg samples did not contain dioxins at levels of health concern. Two recommendations documented in the 1998 EI were to 1) evaluate potential pathways for human exposure to dioxin from environmental and dietary sources and 2) evaluate strategies to assess past exposures to dioxin.

The health significance of the dioxin blood levels measured in the 1998 EI is unclear. Dioxin is known to be extremely toxic in some animals. However, humans appear to have more resistance to these effects. The primary clinical effects of exposure to high levels of dioxin in humans include chloracne and transient, mild hepatotoxicity<sup>2</sup>. Since the 1998 EI, EPA, the state of

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\* Dioxin(s) refers to chlorinated dibenzodioxins (CDDs), chlorinated dibenzofurans (CDFs), and coplanar polychlorinated biphenyls (PCBs)

Louisiana, and local industry have been conducting sampling investigations in Calcasieu Parish to determine dioxin levels in the environment (soil, surface water, sediment, biota, and air). The ATSDR and the state of Louisiana have been conducting health education (community and health professional) and environmental data evaluations.

The Mossville Environmental Action Now (M.E.A.N.) group has made repeated requests that ATSDR initiate further investigation and conduct follow-up on individuals who participated in the first EI. Community members are concerned about potential sources of exposure to dioxin in their environment and how that relates to the levels of dioxin found in their blood.

No obvious source of dioxin exposure has been identified through environmental sampling, including the limited sampling conducted in the first EI. More comprehensive residential sampling in the proposed follow-up EI will include indoor dust, surface soil, and private well sampling for dioxin. In addition, surface dust sampling will be conducted in attics as a preliminary investigation technique in determining the potential for historical air dioxin deposition (the potential for past dioxin air emissions from industry cannot be ruled out as a factor in the elevated serum dioxin levels found in the community).

Food is the most common source of dioxin exposure. Therefore, we will collect samples representative of the participant's diet including locally raised chicken eggs and locally raised or caught fish and crawfish. Although EPA has tested fish in area waters, the literature indicates that in some communities, farm-raised fish fed with dioxin-contaminated feed produce elevated dioxin levels<sup>iii,iv</sup>. This may be a significant dietary source for the Mossville community. To determine the magnitude of these occurrences, the U.S. Food and Drug Administration is currently conducting a nationwide survey of dioxin levels in farm-raised fish and crustaceans<sup>v</sup>. By collecting samples of fish that EI participants have either caught or bought, it is anticipated that a distinction both by dioxin concentration and by congener pattern can be made that may assist in determining if their diet has an impact on serum dioxin levels and congener patterns.

#### Initial Contacts

ATSDR has consulted with representatives of the Mossville Environmental Action Now (MEAN), CLEAN, Louisiana Department of Health, Louisiana Department of Environmental Quality, and Region VI EPA. In addition, ATSDR's Division of Health Studies, Division of Health Education and Promotion, Division of Toxicology, Office of Regional Operations, and Office of Urban Affairs have participated in the development of this EI and/or have reviewed this protocol.

Representatives of ATSDR (Exposure Investigation Section, Community Involvement Branch, Petition Response Section, and Office of Regional Operations) will be responsible for contacting and scheduling the biological and environmental sampling. This EI should not interfere with other ongoing health and environmental investigation activities. It may provide information on sources of exposure that will be of assistance in the upcoming ATSDR Division of Health Study investigation.

#### Target Areas

The individuals who participated in the two previous rounds of serum dioxin testing are the primary focus of this investigation. Eleven community members participated in the first round of testing in 1997 and 28 members participated in the initial ATSDR exposure investigation conducted in 1998. Individuals with possible occupational exposure to dioxins were excluded in

the previous exposure investigation. Therefore, up to 39 individuals and their residences may be sampled. Participation is expected to be lower, as some participants have relocated to other areas and some have died.

## Biological Sampling

### Objective

Biological sampling may assist in determining whether individuals are still being exposed to dioxins in the Mossville area. If exposure to dioxin occurred primarily in the past, participants' dioxin levels should be decreasing at a predictable rate. However, if participants have been exposed since their initial sampling, blood levels may not be decreasing at these expected rates. Comparison of previous blood dioxin levels with current levels will address temporal (historic vs. current) patterns of exposure.

The 11 individuals who initially provided blood dioxin data to ATSDR through CLEAN as well as the 28 individuals who participated in the first exposure investigation will be recruited to determine their current blood dioxin levels. Data on the half-life of dioxins in humans will be used along with previous sampling results and dates of collection to generate expected blood dioxin levels. These expected levels will be compared with current blood dioxin levels gathered through biological sampling. This comparison will address whether participants have been exposed to elevated levels of dioxins in the last three years. A survey will also be completed on participants to gather information on demographics, residential and occupational histories, potential dietary sources of dioxins, as well as data on weight changes over the last three years (significant weight gain/loss can directly impact blood dioxin levels). The results obtained in this exposure investigation will be applicable only to this site and are not generalizable to other populations.

### Participants

Biological sampling will be restricted to the initial two groups for whom ATSDR has prior blood dioxin data. Participants will be recruited from this combined group of 39 individuals. There are no children in this group. Potential participants will be excluded if they are pregnant or if they have a medical condition such as anemia that precludes them from being able to safely donate a minimum 50-ml blood sample. Individuals must currently reside in the Mossville/Calcasieu area.

### Consent Forms

Prior to collecting any biologic samples, the participants will be asked to provide consent for this testing. Each participant must sign an informed consent form.

### Sample Collection and Analysis

A licensed phlebotomist will collect a maximum of 100-ml blood sample by venipuncture from all consenting participants. Blood samples will be collected in 10-ml glass Vacutainer® tubes. After collection, blood samples will be held at room temperature for 1-2 hours and allowed to clot. The samples will then be stored on ice and delivered to the Centers for Disease Control and Prevention, National Center for Environmental Health (NCEH) Laboratory in Atlanta, Georgia, for analyses.

Serum samples will be analyzed for dioxins at the NCEH lab using gas chromatography/isotope dilution-high resolution mass spectroscopy<sup>vi</sup>. The blood serum samples will also be analyzed for total lipid content so the results can be expressed in blood lipid concentration.

For those 11 individuals who provided blood dioxin data to ATSDR through CLEAN, blood samples will be split and analyzed by both the NCEH laboratory and the Hamburg, Germany laboratory (Ergo Forschungsgesellschaft mbH) that initially provided dioxin analyses. This split sampling will help address any inconsistencies between analytic methods among the two labs and enable a comparison between historic and current blood dioxin levels for these eleven individuals.

## **Environmental Sampling**

### *Objective*

Results of this sampling effort are intended to determine whether dioxins are present in the current living environments of individuals who participated in the 2 previous blood dioxin investigations. One part of the investigation will assist in determining if current exposure to contamination is possible. This part of the investigation will include sampling the following:

- surface (top 1 inch) soil in the yard from
  - main residential entry way
  - an area of high use (e.g., garden)
- indoor surface (floor) dust from
  - main entryway
  - a frequently used living area (e.g., play room, tv room)
- private well water (if applicable)
  - eggs from chickens raised on the property (if applicable)
  - fruits/vegetables raised on the property
  - Catfish/crawfish from the resident's refrigerator/freezer

This information will be used in determining if a possible source of dioxin exposure is present in the residential environment. If one or more sources are found, recommendations will be made to mitigate or to stop exposures.

The second part of the environmental investigation is a preliminary attempt in determining if attic surface dust can be used as an indicator of relative air dioxin deposition. Calcasieu Parish contains a large number of chemical manufacturing plants that produce chemicals such as chlorinated hydrocarbon solvents, vinyl chloride monomer, and petroleum-based chemicals. Chemical wastes from some of these operations are burned in hazardous waste incinerators operated by industries in the area.<sup>2</sup> Small amounts of dioxins can be produced in the incineration process. Therefore, possible patterns of attic surface dust levels may provide information on relative air dioxin deposition in the past. It cannot be used to estimate the resident's exposure to dioxin (past or present), or to make health risk calculations, or predict potential health effects.

Property owners and occupants must give their consent for access prior to being selected as a sampling location.

## **Consent Forms**

Prior to collecting any environmental samples, the resident/property owner will be asked to provide consent for access and sampling. They will be requested to sign a consent form.

## ***Sample Collection***

Up to two surface soil samples will be collected from each individual's yard: one sample will be a composite of surface soil collected near the entryway used most; another sample will be a composite of surface soil in a high-use area (e.g., play area where adults may congregate with children, garden area). These areas will be identified by the resident.

Two indoor floor dust samples will be collected inside of each home following EPA Standard Operating Procedure<sup>vii</sup>. A Nilfisk<sup>TM</sup> vacuum equipped with a HEPA filter will be used to vacuum the marked area. A minimum of 10 grams of dust is needed to yield an analytical detection limit of 1 part per trillion (ppt or ng/kg). If the sample bag does not appear to have collected enough dust from the square-meter area, then another adjacent square-meter area will be vacuumed. The surface vacuumed can include wood, tile, carpet, etc. Both the surface type and the total area of sample collection will be recorded. One sample will be collected from the main entryway. The other sample will be collected from an area where the resident spends the bulk of their time. One attic dust sample will be collected from each home. A square-meter will be measured and marked using masking tape.

- If the resident uses or has used private well water, a tap water sample will be collected.
- If the resident raises chickens on his/her property, 1-3 eggs will be collected.
- If the resident raises vegetables or fruits on their property, samples will be collected.
- If the resident has fresh or frozen catfish or crawfish available, samples will be collected.

Minimum sample size requirements:

- 2 4-ounce jars for soil
- 1 4-ounce jar for dust
- 1 1-liter jar for water

## ***Sampling Handling and Storage***

Samples will be handled, stored, and shipped in accordance with applicable EPA and DOT guidelines.

## **Chemical Analysis of Samples**

Through an Interagency Agreement with the Division of Federal Occupational Health, water, soil, surface, and attic dust samples will be analyzed for dioxins using EPA Methods 8082 and 8290, or equivalent.

Prior to sample extraction dust samples will be sieved through a 150  $\mu\text{m}$  screen (EPA SOP<sup>7</sup>).

Egg, fish fillets, crawfish, and edible plant samples will be analyzed by the Environmental Chemistry Laboratory (Stennis Space Center, St. Louis, MS), using EPA Method 1613 as amended.

## ***Quality Assurance/Quality Control***

Laboratory analysis will be conducted with method-specific QA/QC requirements.

## **Biological and Environmental Results**

### ***Presentation of Results***

A health interpretation of biological and environmental results will be provided to each participating individual/household. Attic dust results will also be provided, but without a health interpretation. If dioxin is found at levels of potential health significance in biological or environmental samples, the appropriate federal, state, and local health or environmental agencies will be informed. Individual test results will not be made available to the public. Confidentiality will be protected according to Federal and State laws. If applicable, any impacted residents will be informed on methods to avoid or reduce potential exposures. A summary of all results will be provided in an Exposure Investigation Report.

### ***Follow-Up Activities***

Follow-up health activities may include health education for residents and their health care providers. If elevated levels of dioxins are detected, recommended follow-up may also include additional environmental sampling and analysis.

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<sup>i</sup> Agency for Toxic Substances and Disease Registry; Health Consultation for Calcasieu Parish; October 16, 1998.

<sup>ii</sup> Agency for Toxic Substances and Disease Registry; Exposure Investigation for Calcasieu Estuary; November 19, 1999.

<sup>iii</sup> H. Fiedler et al.; PCDD, PCDF, and PCB in farm-raised catfish from Southeast United States – concentrations, sources, and CYP1A Induction; *Chemosphere* 37:9-12 (1998).

<sup>iv</sup> E. Jensen and M. Bolger; Exposure assessment of dioxins/furans consumed in dairy foods and fish; *Food Additives and Contaminants* 18(5):395-403 (2001).

<sup>v</sup> Personal communication, L. Wilder (ATSDR) R. Canady (FDA), July 2001.

<sup>vi</sup> DG Patterson, SG Isaacs, LR Alexander, et al.; Method 6: Determination of specific polychlorinated dibenzo-p-dioxins and dibenzofurans in blood and adipose tissue by isotope dilution-high-resolution mass spectroscopy; *Environmental Carcinogen Method of Analysis and Exposure Measurement* 299-342 (1991).

<sup>vii</sup> U.S. Environmental Protection Agency, Environmental Response Team (ERT); Collection of indoor dust samples from carpeted surfaces for chemical analysis using a Nilfisk GS-80 vacuum cleaner; 2000.