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The Gulf Long-Term Follow-Up Study (GuLF STUDY): Biospecimen Collection at Enrollment

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Abstract

The 2010 *Deepwater Horizon* (*DWH*) explosion in the Gulf of Mexico led to the largest ever marine oil spill by volume. The GuLF STUDY is investigating possible human health effects associated with oil spill activities. One objective of the study is to utilize biological specimens from study participants to investigate spill-related health effects. This paper describes the methods for collecting, processing, shipping, and storing specimens during the enrollment phase of the study. GuLF STUDY participants living in Gulf states (Alabama, Florida, Louisiana, Mississippi, and eastern Texas) were eligible to complete a home visit at enrollment, one to three years after the *DWH* explosion. During this visit, blood, urine, toenail and hair clippings, and house dust samples were collected. Specimens were shipped overnight to a central processing laboratory in containers with cold and ambient temperature compartments. Most blood and urine specimens were then aliquoted and stored in liquid nitrogen vapor or at −80°C, with some samples stored at −20°C. A total of 11,193 participants completed a home visit, and over 99% provided at least one biospecimen. Most participants provided blood (93%), urine (99%), and toenail clippings (89%), and 40% provided hair. Nearly all participants (95%) provided house dust samples. Most samples were received by the lab one (58%) or two (25%) days after collection. These biospecimens enable investigation of a range of biomarkers of spill-related health effects, and possibly some biomarkers

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of spill-related exposures. The biospecimen collection, handling, and storage protocols were designed to maximize current and future scientific value within logistical and budgetary constraints and can serve as a template for future studies conducted in similar time-critical and geographically dispersed settings.

Keywords

biospecimens; blood; urine; toenail; hair; house dust; field study

Introduction

The explosion of the *Deepwater Horizon (DWH)* drilling rig in the Gulf of Mexico on April 20, 2010 resulted in the release of an estimated 4.9 million barrels of crude oil into the Gulf of Mexico (National Commission on the Deepwater Horizon Oil Spill 2011). This was the largest marine oil spill by volume in history and resulted in the oiling of over 1,100 linear miles of coastline (Michel et al. 2013). An estimated 110,000–140,000 workers and volunteers participated in spill-related activities (Kwok et al., in press). These individuals were charged with stopping the oil release, drilling relief wells, and flaring oil; cleaning the waters (searching for and collecting oil, handling boom, burning oil), cleaning beaches, marshes and shoreline structures (e.g., jetties); decontaminating vessels, boom, other equipment, personnel, and wildlife of oil; and providing support by moving equipment in the ports, feeding, housing and transporting personnel, providing security, and addressing safety, medical, industrial hygiene, and environmental issues.

Crude oil contains a number of chemicals with known or suspected human toxicity. These include volatile organic compounds, including benzene, toluene, ethylbenzene, and xylene (BTEX) (Costantini et al. 2008; Steinmaus et al. 2008; IARC 2012; Lim et al. 2014; Chang et al. 2010; Kirkeleit et al. 2006), n-hexane (Neghab et al. 2012), polycyclic aromatic hydrocarbons (PAH) (ATSDR 1995), and heavy metals (ATSDR 2005; 2007; 2008). Specific to the *DWH* disaster, flaring of captured oil and gas and *in situ* burning of oil on the ocean surface produced particulates (Middlebrook et al. 2012), and *in situ* burns also generated small amounts of dioxins (Aurell et al. 2010).

The dispersants Corexit 9500 and Corexit 9527, used to break up the oil in the water, contain various respiratory irritants, including 2-butoxyethanol, propylene glycol, and dioctyl sodium sulfosuccinate (Roberts et al. 2011; EPA 2011). Evidence from rat models suggests possible neurological (Sriram et al. 2011), immunological (Anderson et al. 2011), and cardiovascular (Krajnak et al. 2011) effects of Corexit 9500. In addition to chemical and physical hazards (e.g., high heat and humidity), the widespread economic disruption produced by the oil spill may have led to increased stress in the Gulf population.

Previous studies of populations involved in oil spills point to a number of effects detectable with biomarkers. These include increased DNA damage (Perez-Cadahia et al. 2006; 2008; Aguilera et al. 2010; Laffon et al. 2016), chromosomal abnormalities (Rodriguez-Trigo et al. 2010), and altered hormone levels (Perez-Cadahia et al. 2007; 2008; Laffon et al. 2013). Some of these effects, including chromosomal alterations, elevated plasma cortisol levels,

and decreased serum kynurenine levels, persisted for 2 years (Rodriguez-Trigo et al. 2010) or more (Laffon et al. 2013; 2016) after exposures lasting 3–9 months on average. These findings are consistent with studies of other populations showing increased DNA damage in relation to low level occupational or ambient benzene (Fracasso et al. 2010; Maffei et al. 2005) and PAH (Gamboa et al. 2008; Novotna et al. 2007) exposure. Benzene exposure, even at relatively low levels, has also been associated with hematotoxicity and immunotoxicity (Lan et al. 2004) and shorter leukocyte DNA telomere length (Hoxha et al. 2009). Other biomarkers of potentially adverse biological effects include aberrant epigenetic profiles and alterations in gene expression.

The GuLF STUDY was initiated to investigate the possible adverse health effects associated with working on *DWH* oil spill response and clean-up (Kwok et al., in press). This investigation focused on oil spill workers rather than the general population because the former were likely to have higher and better characterized spill-related exposures. In the broadest sense, “exposure” here encompasses oil, oil-related byproducts (including weathered oil and oil combustion products), and dispersants, as well as the adverse working conditions (high temperature and humidity, long work shifts, few days off), and the psychological and socioeconomic impacts of the spill.

The GuLF STUDY collected biospecimens to investigate relationships between exposures and biologic effects. The aim of this paper is to describe the collection, processing, shipping, and storage of samples in the GuLF STUDY. It explains some of the challenges of conducting such a large study in multiple states over a relatively short time period and provides the rationale for certain methodological decisions.

Methods

The design of the Gulf STUDY is described in detail elsewhere (Kwok et al., in press). In brief, persons who were involved in any aspect of *DWH* oil spill work and/or completed worker safety training in anticipation of performing spill-related work were identified from administrative records. Potential participants were recruited by telephone and invited to complete a 30–60 minute telephone enrollment interview. Participants were required to be at least 21 years of age at enrollment. Interviews were conducted in English, Spanish, or Vietnamese. Enrollment interviews were conducted from March 2011 to March 2013. Individuals who completed the telephone interview constitute the GuLF STUDY Cohort (N=32,608). All cohort members who lived in one of the Gulf States (Alabama, Florida, Louisiana, Mississippi, and east Texas) and spoke English or Spanish were invited to participate in a home visit. Home visits were conducted from May 2011 to May 2013. The Home Visit Subcohort is composed of the 11,193 cohort members who completed a home visit. Only 136 (1.2%) home visit participants were known to be still engaged in spill-related work at the time of their home visit, with a median of 21 months and maximum of 35 months since the end of spill-related work among all home visit participants. Finally, a stratified random sample of Home Visit Subcohort members residing in selected coastal and adjacent counties in Louisiana and Alabama and oversampled for oil spill workers constitute the Biomedical Surveillance Subcohort (N=4,050; 91% workers). This subcohort was established for characterization of blood parameters (in assays requiring fresh blood), for

detailed urinalysis at the time of the home visit, and for possible invitation to periodic follow-up clinical examinations depending on proximity to subsequently established clinics in those two states.

Certified Medical Assistants (CMA), who have been trained in phlebotomy and other clinical procedures as part of their professional certification, served as field agents and underwent extensive additional training and certification by the GuLF STUDY research team prior to conducting the home visits. They were also subjected to regular quality control review and day-to-day oversight by the GuLF STUDY research team central field manager and a field manager located in each state.

During the home visit, field agents obtained informed consent, collected several physiologic and anthropometric measures, conducted a pulmonary function test, administered an additional questionnaire, and collected biological specimens and house dust samples. The three hour home visits were conducted 7 days per week, from early morning into the early evening. Whenever possible, home visits for members of the Biomedical Surveillance Subcohort were scheduled early on Monday through Thursday to facilitate overnight shipping of specimens. An average of 119 home visits per week was conducted.

Characteristics of the Home Visit Subcohort are shown in Table 1.

Pre-visit instructions

Home Visit Subcohort members were sent preparatory instructions approximately one week before the visit. They were instructed not to clip their toenails or cut their hair or to dust around doors or windows prior to the visit. They were requested, on the day of the home visit, to do the following: 1) not eat or drink anything except water for 8 hours prior to the visit, if possible; 2) collect a first morning void urine sample, as described below; 3) remove any toenail polish; and 4) wash their hair, but not use any conditioner or hair styling products.

Sample collection, processing in the home, and shipment to the central processing laboratory

Prior to each home visit, field agents were sent customized specimen collection kits containing a dual-chamber shipping kit, supplies needed for specimen collection and limited processing, cold packs for shipping, and pre-printed specimen and shipping labels. These kits were produced and shipped from a central location to ensure standardization.

Field agents collected blood, toenail clippings, and hair samples (Table 2 and Table 3) and retrieved urine samples from consenting participants. To minimize variability in sample handling, the minimum necessary specimen processing was performed in the participant's home prior to shipping the samples by overnight carrier to a central lab for final processing, aliquotting, and long-term storage and/or analysis. All samples were labeled with unique specimen barcodes linked to the participant's study ID.

Blood—Blood was collected only from participants with systolic blood pressure less than 180, diastolic blood pressure less than 110, and heart rate between 40 and 120. A total of

52.5 ml venous blood was collected from each participant into 8 Becton Dickinson (BD) Vacutainer® tubes. Different tube types were used to allow for a range of future analyses. The following tubes were collected: two 10 ml red-top serum tubes; one 10 ml, one 6 ml, and one 2 ml lavender-top EDTA tube; one 6 ml yellow-top Acid Citrate Dextrose Solution B (ACD-B) tube; one 6 ml royal blue-top EDTA trace metals tube; and one 2.5 ml PAXgene RNA tube. As a compromise between *i*) collecting tubes in an order that minimized contamination of tubes with additives from previous tubes and *ii*) maximizing the range of tube types from subjects for whom it was not possible to collect all 8 tubes, the following order of collection was used: 2×10 ml red-top, 10 ml lavender-top, 6 ml yellow-top, 6 ml royal blue-top, 2 ml lavender-top, 6 ml lavender-top, 2.5 ml PAXgene.

The blood in the red-top tubes was allowed to clot for 30 min, before being centrifuged at 1300x g for 15 min using a portable centrifuge in the participant's home. The serum and clots were separated and retained. The 10 ml and 6 ml lavender-top tubes were also centrifuged at this time, separating and retaining the plasma and the packed cells. All other blood specimens remained unprocessed and in their original tubes for shipment to the central processing lab, where some underwent processing and/or aliquotting prior to storage.

Urine—The pre-visit packet mailed to each participant contained a 90 ml sterile urine collection cup and lid, together with detailed instructions for collecting and refrigerating a clean catch first morning void (FMV) on the day of the home visit. The participant was called the day before the visit and reminded to collect the FMV. If participants did not collect a FMV on the day of the home visit, they were asked to provide a clean catch random urine sample during the home visit.

During the home visit, a small amount of urine was removed from the collection cup and applied to a Diastix® reagent strip for urinalysis to measure glucose concentration, which was reported to the participant in an individualized report. Collections from members of the Biomedical Surveillance Subcohort underwent additional, more detailed urinalysis in a CLIA-compliant clinical lab. For these subjects, the field agent transferred some of the urine to a BD urinalysis preservative tube with a preservative containing 94% sodium propionate, 5.6% ethyl paraben, and 0.4% chlorhexidine.

To minimize the risk of assay interference by preservatives and/or contaminants in the preservative, the remainder of the urine sample was initially shipped unpreserved to the central processing lab for aliquotting and long-term storage. However, in early quality control testing, some collections were found to have bacterial overgrowth. Consequently, as a compromise to address both bacterial contamination and concerns regarding assay interference, subsequent urine collections were split, with 16 ml shipped in the BD urinalysis preservative tubes and the remainder shipped unpreserved.

Saliva—For all participants who were unable to provide a blood sample during the home visit, a saliva specimen was collected for DNA extraction using an Oragene OG-250 DNA Self-Collection kit (DNA Genotek Inc., Kanata, Ontario, Canada).

Other biospecimens—Each participant was asked to provide a toenail clipping from each toe (after removing any nail polish with acetone-based pads), unless contraindicated by a medical or physical condition. If toenail clippings could not be collected during the home visit, the participant was provided instructions and a prepaid, self-addressed envelope and asked to collect and mail toenail clippings to the central processing lab at a later date.

In addition, an attempt was made to collect a hair sample approximately 7 mm thick and as close as possible to the scalp at the nape of the neck; the minimum required hair length for collection was changed from 10 cm to 1 cm midway through the study. Most of this cohort is male (78%) and most had short hair, precluding collection of an adequate sample from a large proportion of participants.

House dust—The field agent collected house dust samples by using 6 alcohol pads to wipe dust from the top of one door frame, window frame, picture frame, or bookshelf/cabinet (2 pads per top surface) in each of three rooms in the participant's home. The wipes were sealed in a zip-lock pouch for shipment and storage. In selected counties/parishes in Alabama and Louisiana, the field agent also collected a house dust sample using a standard vacuum collection protocol (NHANES 2006). Specifically, the field agent collected vacuumed dust samples using a Eureka Mighty Mite and DUSTREAM© collection system (Indoor Biotechnologies). The participant was asked to identify the bed (or other sleeping location) and the side of the bed where s/he typically slept, as well as the surface on which s/he typically slept (e.g., the bottom sheet). The field agent then vacuumed, in a standardized manner, a 0.84 m^2 (1 square yard) area both where the participant slept and the floor immediately next to where s/he slept for 60 sec each. The dust filter was removed and transferred to a plastic transport bag for shipment to the central processing lab for long-term storage. Temperature and humidity were measured and recorded during collection of the vacuumed dust samples.

Collection of Quality Assurance/Quality Control (QA/QC) samples—Substantial volumes of biospecimens are often needed for QA/QC and assay validation. To meet this need, extra blood and urine were collected, with the participants' consent, from 98 (2.4%) participants in the Biomedical Surveillance Subcohort and 114 (1.6%) additional home visit participants. The QA/QC samples included 4 tubes of blood (one 10 ml red-top, one 10 ml lavender-top, one 6 ml yellow-top tube, and one 6 ml royal blue-top), and additional urine if less than 50 ml had been produced for the original collection. These specimens were processed, shipped, and stored in the same manner as the corresponding main study specimens.

Specimen shipment to the central processing lab—Specimens were shipped by priority overnight service to the central processing lab in a two-compartment shipper specifically designed for this study and insulated with 1-inch high-density encapsulated polystyrene foam. During shipment, the ACD-B tube and 2 ml lavender-top tube were placed in the top compartment, at ambient temperature, and other specimens in the bottom compartment, near, but not against, frozen 500 g phase-change foam bricks (Tables 2 & 3). Specimens that were collected too late to ship the same day were stored overnight, with all tubes except the ACD-B tube and 2 ml lavender-top tube chilled with freezer bricks, by the

field agent. After insertion of fresh freezer bricks, these specimens were shipped the next day by priority overnight service. Because weekend shipments were prohibitively expensive, specimens collected on the weekends or too late on Friday evening were held by the field agent, with daily changes of freezer bricks, and shipped on Monday by priority overnight service.

Centralized specimen processing and storage

Specimens were processed at the central processing lab on day of receipt, following standardized protocols. Some specimens had alternate processing protocols for a fraction of participants (Tables 2 & 3). The 2 ml lavender-top tube from Biomedical Surveillance Subcohort members was promptly delivered to a CLIA-compliant lab and analyzed for complete blood count (CBC) with white blood cell differential; for all other participants, this specimen was stored as whole blood at −80°C. The 6 ml ACD-B tube from Biomedical Surveillance Subcohort members underwent discontinuous density gradient centrifugation to isolate mononuclear cells, which were mixed with 10% DMSO, aliquoted (1 ml), step frozen, and stored in liquid nitrogen vapor; for the remaining participants, the whole blood was mixed with 10% DMSO, aliquoted (1 ml), step frozen, and stored in liquid nitrogen vapor. All blood and urine specimens were aliquoted (blood: 1 ml; urine: 2 ml and 7 ml) into sealed cryovials and stored at −20°C, −80°C, or in liquid nitrogen vapor, except for the glass royal blue-top trace metal tubes and PAXgene tubes (Tables 2 and 3), which were stored in their original tubes, placed at a 45° angle to prevent breakage during freezing and storage, at −20°C. The saliva specimens were also stored in their original collection container at −20°C. Aliquots of each blood and urine specimen from each participant were separately stored in at least two freezers at the NIEHS Repository to reduce the risk of complete loss due to freezer failure.

Toenail specimens were stored in a sealed manila envelope containing a silica gel desiccant packet at ambient temperature (~20°C) in a humidity-controlled environment at the Repository. Hair and house dust samples were stored at −20°C.

Both the central processing lab and Repository have automatic temperature monitoring and power backup systems that ensure maintenance of appropriate storage temperatures and conditions and specimen integrity during temporary and long-term storage. The central processing lab and the Repository jointly maintain an inventory control system that provides annotation and history of all stored specimens, including (1) the condition of specimens upon receipt, (2) storage location of specimens, and (3) number of specimens used and number available for analysis.

Quality Assessment

All specimens underwent visual inspection and temperature assessment upon receipt at the central processing lab, with any anomalies recorded in the specimen database. To assess the impact of collection and handling procedures on the integrity of the biospecimens, a small quality assessment study was performed on a random sample of biospecimens, with particular focus on the effect of time from collection to processing. The ability to grow EBV-immortalized lymphocyte cultures was determined from cryopreserved peripheral blood

mononuclear cell (PBMC) pellets (N=6) and from cryopreserved whole blood (ACD-B) aliquots (N=9), stored for an average of 115.8 days (range: 78–155) and 117.1 days (range: 92–147), respectively. Bacterial overgrowth was evaluated by urine culture, separately for first morning voids (N=18) and spot urine samples (N=18), to assess differences due to time between collection and field processing of the sample.

Results

Sample collection

Over 99% of participants provided at least one biospecimen. Urine was obtained from 11,061 (98.8%) participants, one or more blood specimens from 10,389 (92.8%), toenail clippings from 9,993 (89.3%), a hair sample from 4,428 (39.6%), and house dust from 10,615 (94.8%) (Table 2 and Table 3). Approximately 32.2% of the participants provided FMV urines. Among participants providing a random, rather than FMV, urine specimen, collection times ranged between approximately 8 am and 9 pm, with a median collection time of approximately 1 pm. A saliva sample was collected from 613 (76.2%) of the 804 participants from whom a blood sample was not obtained. Among the 10,389 participants who provided one or more blood specimens, 99.2% provided urine, 89.4% provided urine and toenail clippings, and 85.9% provided urine, toenail clippings, and house dust samples.

Approximately 58% of samples were processed by the central processing lab within one day of collection. Approximately 25% of samples were collected too late on a Monday through Thursday to be shipped that day or were collected on a Sunday and, consequently, were received and processed up to two days later. Approximately 16% of samples were collected too late on Friday or on Saturday and were received and processed 3–4 days later.

Rigorous staff training and oversight enabled us to minimize other issues associated with specimen collection, processing, and shipping. Although, overall, 1.0% of blood and urine specimens that were shipped chilled arrived warm at the central processing lab, only 0.3% of specimens received at the lab within one day were warm upon receipt. In addition, 3.2% of serum specimens appeared to be hemolyzed upon receipt. This hemolysis was not related to transport time, but appeared to be slightly more common among specimens collected in winter.

Quality assurance of biospecimens

DNA yields from packed cells averaged 23.7 μg/ml for samples processed within one day of collection and 19.0 μg/ml for samples processed within 4 days of collection. EBV-immortalized lymphocyte cultures were successfully grown from 100% of the cryopreserved PBMC pellets (N=6) and cryopreserved whole blood (N=9) aliquots. Among the PBMC pellets, the mean % viability of lymphocytes was 60.9 and 78.6% for samples processed within 1 day and 2 days of collection, respectively. After EBV-transformation and cryopreservation, the % viability of the resulting cells was 93.3 and 91.8% for samples processed within 1 day and 2 days of collection, respectively. Among the whole blood samples, the mean % viability of lymphocytes was 61.7, 67.4, and 58.6% for samples processed within 1 day, 2 days, and 3–4 days of collection, respectively. After EBV-

transformation and cryopreservation, the % viability of resulting cells was 91.2, 92.9, and 92.6% for samples processed within 1 day, 2 days, and 3–4 days of collection, respectively.

Lastly, bacterial overgrowth was examined in 36 urine samples by whether they were first morning void or random urine samples and by time from collection to processing. After a 48-hr culture, among the samples overall, 36.1% showed no growth, 36.1% had <10,000 colony-forming units (CFU)/ml, and the remainder (27.8%) had up to 100,000 CFU/ml. There was no clear pattern in either the proportion showing growth or in the number of colony-forming units by time from collection to processing (data not shown). First morning voids were moderately more likely than randomly collected urines (70.6 vs. 57.9%, respectively) to demonstrate growth. In response to these findings, urine collection procedures were modified to split each sample at the time of the home visit and to add preservative to one fraction.

Discussion

A range of biospecimens and environmental samples was successfully collected from 11,193 participants in the ongoing GuLF STUDY, with one or more samples collected from over 99% of them. These included blood, urine, toenail clippings, hair, house dust, and, from a small proportion of participants, saliva. All specimens were collected using standardized protocols, with all non-urgent processing performed in a central processing lab. Most samples arrived at the central processing lab within one day, and most samples that were delayed arrived in good condition.

Quality assurance testing indicated that the lymphocytes remained viable for EBV-immortalization in all cryopreserved samples, including the cryopreserved PBMC pellets and cryopreserved whole blood aliquots prepared from participant samples, even among samples that were not processed within one day. As expected, DNA yields were good across the range of times from blood collection to processing, although results suggested somewhat higher yields for samples with shorter processing times. To address the problem of bacterial overgrowth in urine samples while minimizing the potential interference of additives on future analyses of these samples, a portion of each urine sample was preserved at the time of the home visit and the remainder left unpreserved.

These samples will allow us to assess a range of biomarkers of effect in relation to the oil spill clean-up experiences of this cohort. These effects include potential cytogenetic alterations, such as chromosomal aberrations and micronuclei (Zijno et al 2007; Fenech 2002), in the cryopreserved peripheral lymphocytes. These alterations have been observed previously among oil spill clean-up workers (Perez-Cadahia et al. 2008b; Rodriguez-Trigo et al. 2010; Perez-Cadahia et al. 2007; Laffon et al. 2016) and benzene-exposed workers (McHale et al. 2008), including in blood samples collected at least two years after exposure (Rodriguez-Trigo et al. 2010). The samples may also be useful in examining possible endocrine effects, such as altered plasma levels of cortisol and prolactin, which were previously noted among oil spill clean-up workers (Perez-Cadahia et al. 2008a; Perez-Cadahia et al. 2007); such analyses will need to consider time of collection to account for circadian rhythms. Such effects were also observed in relation to certain metals commonly

found in crude oil (Vrabie et al. 2011; Lafuente 2013), although metal concentrations in the *DWH* oil were relatively low (Liu et al. 2012). In addition, the blood specimens may be examined for certain immune effects such as altered cytokine levels. Other possible biomarkers of interest include liver enzymes and inflammatory markers. The specimens collected in the PAXgene blood RNA tubes enable us to evaluate gene expression in relation to the spill. The DNA from the stored blood clots or packed cells may be analyzed for methylation patterns or telomere shortening. The urine may be analyzed for a number of potential biomarkers of effect, including markers of oxidative stress, kidney damage, inflammation, and metabolic abnormalities.

The cryopreserved lymphocytes may be useful in evaluating certain types of disease susceptibility via assays such as the comet assay and host cell reactivation assay (Chang et al. 2006). Disease susceptibility (and gene-environment interactions) might also be examined via genotyping of selected genes, such as those encoding relevant metabolizing enzymes and DNA repair proteins, using DNA extracted from the packed cells, blood clots, or saliva samples.

Because of the many scientific, legal, administrative, sociological, and logistical hurdles to conducting a study of this magnitude and complexity, it took 11 months to begin enrollment and collection of biospecimens and another two years to complete this phase of the study. Most individuals were no longer actively engaged in *DWH* oil spill activities by the time they provided the biospecimens. Many spill-related exposures, including BTEX, n-hexane, and PAHs, have short biological half-lives measured in hours to days (ATSDR 1995; 1999; 2007a; 2007b; 2015) and would be non-detectable in most participants if occurring from the spill. Medium-term biomarkers of exposure, such as PAH adducts of DNA or protein, would also be undetectable from the spill in most participants, and stable long-term biomarkers of these exposures are lacking. Consequently, these biospecimens are of limited value for measuring most spill-related chemical exposures. This is a typical limitation of epidemiologic studies of disasters, which often experience substantial delays getting into the field. Therefore, such studies often rely on retrospective reconstruction of exposures, as is being done in the GuLF STUDY. Nonetheless, we may be able to measure certain heavy metals in the whole blood collected in trace metals tubes or in the toenail clippings or hair (ATSDR 2007b; 2008; 2005). Importantly, the GuLF STUDY exposure reconstruction effort will result in quantitative estimates for a number of important spill-related chemicals, based upon extensive personal and area air monitoring data collected on a subset of workers during spill-related activities, together with detailed interview data from all GuLF STUDY participants. Such exposure estimates represent a substantial improvement over the crude measures of exposure used in most previous studies of oil spill health effects (Aguilera et al. 2010; Laffon et al. 2016) and enable more effective investigation of the relationships between exposures and biomarkers of health effects.

These specimens are expected to be used primarily in nested studies of cases vs. non-cases or, based upon air or dermal exposure estimates, more highly exposed vs. lower or non-exposed participants, in order to cost-effectively maximize their scientific value. One concern that is likely to arise in some of these studies is the impact of transit time on the biomarkers of interest. This will need to be assessed on a case-by-case basis, as some

analytes are more stable than others. Season of collection and, as noted above, time of day may also affect levels of certain biomarkers and will need to be considered. Selection of samples for a given study might depend on the characteristics of the analyte and of the assay.

As part of the GuLF STUDY, 3,403 cohort members living within 60 miles of clinics established in Mobile, Alabama and New Orleans, Louisiana (including 86.2% of the Biomedical Surveillance Subcohort) completed clinical exams with additional biospecimen collection between August 2014 and June 2016, and may be asked to complete periodic subsequent exams with repeat collection of all or most specimens. This will allow longitudinal evaluation of markers of interest..

The delays in getting some specimens to the central lab for processing and long-term storage resulted from the need to balance conflicting scientific, logistical, and financial considerations. Because the GuLF STUDY was in response to a specific event of limited duration, it was important to complete the baseline data and specimen collection as quickly as possible. Many study participants were unavailable during normal business hours. Participants were spread out over a large geographic area encompassing hundreds of square miles, and many had limited access to health care, precluding our use of central facilities or personal physicians to collect specimens. Consequently, field agents went to participants' homes, with many of these visits conducted in the evenings and on weekends, delaying transport of specimens to the central processing lab. Nonetheless, most specimens were received by the central processing lab within one day.

High rates of bacterial contamination of urine have been observed in other studies (Leisure et al, 1993; Prandoni et al. 1996), although some studies reported lower rates (Lipsky et al. 1984; Lohr et al, 1986). The impact of this contamination generally depends upon the particular analyte being measured. The impact of the urine preservatives that are used similarly depends upon the particular analyte being measured and/or assay being considered. The GuLF STUDY investigators attempted to maximize the utility of these urine samples by splitting them at the time of the home visit and preserving one portion while leaving the other unpreserved.

Specimens and study data will be made available to other researchers for scientifically valid collaborative research that addresses questions relevant to the impact of the oil spill on human health. Information on, and procedures for requesting access to, study resources may be found on the study website at www.gulfstudy.nih.gov. Priority for biomarker-based studies will be given to research that can best—or only—be carried out using the unique resources of the GuLF STUDY, which are limited and non-renewable.

CONCLUSIONS

The GuLF STUDY successfully collected a range of biospecimens and environmental samples at enrollment from a large majority of home visit participants. These specimens, as well as repeat specimens that will be collected in future phases of this prospective study, will be used primarily for analysis of markers of effect from the oil spill clean-up. Some specimens may also be used to assess the magnitude of certain oil spill-related exposures,

although many oil constituents of interest are not persistent and, therefore, cannot be measured in these specimens. An overarching objective for this component of the GuLF STUDY was to collect a wide range of specimens and to process and store them such that, to the extent feasible, analysis options were maximized. Despite the challenges of conducting a large-scale cohort study in the wake of a complex environmental disaster, this cohort is the largest and most extensively characterized cohort of oil spill workers, especially in terms of spill-related exposures and available biospecimens.

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Table 1

Selected characteristics at enrollment of the Home Visit Subcohort. GuLF STUDY 2011–2013.

Characteristic	N (%) (n=11,193)
Gender	
Male	8,752 (78.2)
Female	2,441 (21.8)
Age (years)	
21–29	1,973 (17.6)
30–39	2,363 (21.1)
40–49	2,780 (24.8)
50–59	2,754 (24.6)
≥ 60	1,316 (11.8)
Missing	7 (0.1)
Race	
White	6,106 (54.6)
Black	3,881 (34.7)
Asian	76 (0.7)
Other	1,094 (9.8)
Missing	36 (0.3)
Ethnicity	
Hispanic	676 (6.0)
Non-Hispanic	10,487 (93.7)
Missing	30 (0.3)
State of residence	
Alabama	2,956 (26.4)
Florida	3,216 (28.7)
Louisiana	2,742 (24.5)
Mississippi	1,936 (17.3)
Texas	343 (3.1)
Educational attainment	
< High school	2,378 (21.3)
High school or GED	3,789 (33.9)
> High school	4,991 (44.6)
Missing	35 (0.3)
Body mass index (BMI, kg/m^2)	
< 18.5	92 (0.8)
18.6–24.9	2,752 (24.6)
25.0–29.9	4,253 (38.0)
≥ 30	3,978 (35.5)
Missing	118 (1.1)
Cigarette smoking status	
Never	4,733 (42.3)

Characteristic	**N (%) (n=11,193)**
Former	2,321 (20.7)
Current	4,089 (36.5)
Missing	50 (0.5)
Worked on spill at least one day	8,968 (80.1)
Still working on spill at time of home visit	103 (0.9)

Table 2

Blood specimen collection and processing. GuLF STUDY 2011–2013.

Blood collection tube	Order of draw	N (%) of participants (n=11,193)	Processing in home	Disposition[a]	Shipment temperature	Long-term storage temperature[b]
Red top tube (2×10 mL)	1, 2	10,383 (92.8)	Centrifuge	Serum	4°C	LN2
				Clot	4°C	−80°C
Lavender top EDTA tubes						
10 mL	3	10,281 (91.9)	Centrifuge	Plasma	4°C	LN2
				Packed cells	4°C	LN2
2 mL	6	10,171 (90.1)	None	HVS: Stored	Ambient	LN2
				BSS: CBC analysis	Ambient	N/A
6 mL	7	10,154 (90.7)	Centrifuge	Plasma	4°C	LN2
				Packed cells	4°C	LN2
Yellow top ACD-B tube (6 mL)	4	10,247 (91.5)	None	HVS: 10% DMSO added, step frozen	Ambient	LN2
				BSS: lymphocytes isolated, 10% DMSO added, step frozen	Ambient	N/A
Royal blue top EDTA tube (6mL)	5	10,212 (91.2)	None	Stored	4°C	−20°C
PAXgene RNA tube (2.5 mL)	8	10,079 (90.0)	None	Stored	4°C	−20°C

[a] HVS=Home Visit Subcohort; BSS=Biomedical Surveillance Subcohort

[b] LN2=vapor phase of liquid nitrogen

Table 3

Non-blood biological and environmental sample collection and processing at home visit in the GuLF STUDY (n=11,193)

Specimen	N (%) of participants	Processing in home	Disposition[a]	Shipment temperature	Long-term storage temperature[b]
Urine	11,061 (98.8)	Reagent strip urinalysis on small sample; 16 mL transferred to preservative tubes; 24 mL left unpreserved	HVS: All stored	4°C	LN2 and −80°C
			BSS: Urinalysis of ~8 mL; remainder stored	4°C	LN2 and −80°C
Toenail clippings (n=10)	9,993 (89.3)	None	Stored	Ambient	Ambient
Hair	4,428 (39.6)	None	Stored	Ambient	−20°C
Saliva (for DNA)[c]	613 (76.2)	None	Stored	4°C	−20°C
House dust wipes	10,615 (94.8)	None	Stored	Ambient	−20°C
Vacuum dust	176	None	Stored	Ambient	−20°C

[a] HVS=Home Visit Subcohort; BSS=Biomedical Surveillance Subcohort

[b] LN2=vapor phase of liquid nitrogen

[c] Among participants who refused or were unable to provide blood samples