Federal seafood safety response to the Deepwater Horizon oil spill

Gina M. Ylitalo1, Margaret M. Krahns, Walton W. Dickhoffs, John E. Steinb, Calvin C. Walkerb, Cheryl L. Lassitterb, E. Spencer Garretts, Lisa L. Desfosseb, Karen M. Mitchellf, Brandi T. Noblec, Steven Wilsonb, Nancy B. Becke, Ronald A. Benners, Peter N. Koufopouloss, and Robert W. Dickeys

1 Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA 98112; 2 National Seafood Inspection Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Pascagoula, MS 39567; 3 Seafood Inspection Program, National Marine Fisheries Service, Silver Spring, MD 20910; Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, DC 20503; 4 Division of Seafood Science and Technology, Gulf Coast Seafood Laboratory, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, Dauphin Island, AL 36528; 5 Division of Seafood Safety, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, MD 20740

Edited by Marcia K. McNutt, US Geological Survey, Reston, VA, and approved January 11, 2012 (received for review June 2, 2011)

Following the 2010 Deepwater Horizon oil spill, petroleum-related compounds and chemical dispersants were detected in the waters of the Gulf of Mexico. As a result, there was concern about the risk to human health through consumption of contaminated seafood in the region. Federal and Gulf Coast State agencies worked together on a sampling plan and analytical protocols to determine whether seafood was safe to eat and acceptable for sale in the marketplace. Sensory and chemical methods were used to measure polycyclic aromatic hydrocarbons (PAHs) and dispersant in >8,000 seafood specimens collected in federal waters of the Gulf. Overall, individual PAHs and the dispersant component diocetyl sodium sulfosuccinate were found in low concentrations or below the limits of quantitation. When detected, the concentrations were at least two orders of magnitude lower than the level of concern for human health risk. Once an area closed to fishing was free of visibly floating oil and all sensory and chemical results for the seafood species within an area met the criteria for reopening, that area was eligible to be reopened. On April 19, 2011 the area around the wellhead was the last area in federal waters to be reopened nearly 1 y after the spill began. However, as of November 9, 2011, some state waters off the Louisiana coast (Barataria Bay and the Delta region) remain closed to fishing.

On April 22, 2010, 2 d after the explosion on the Deepwater Horizon (DWH) drilling platform, the rig collapsed and the wellhead failed. The explosion resulted in the loss of human life and the uncontrolled release of >200 million gallons of Louisiana light crude oil occurring ~5,000 feet below the sea surface. DWH was declared a Spill of National Significance on April 29, 2010 and became the largest oil spill in US history (1). Among the significant human and environmental impacts of the spill, marine fisheries and supporting marine and estuarine ecosystems were subjected to contamination by crude oil, compromising the safety of seafood resources (1). An immediate and coordinated federal and state response ensued to safeguard seafood safety. Federal and state agencies mobilized personnel and resources to begin sampling seafood on April 28, 2010. Federal and state fishery closures were guided by observations of where oil was seen and forecasted to spread on the basis of climatic and hydrographic models (2). Seafood was collected around the periphery of the closed areas and from dockside and seafood market outlets across the Gulf coast and analyzed for oil-spill related contaminants to assess the effectiveness of the fishery closures. When the flow of oil was stopped on July 15, 2010 and the oil began to dissipate, sampling and analyses were conducted to determine whether seafood from previously closed areas was safe for harvest and human consumption. Sampling of reopened areas in federal waters continued through June 2011. We describe how federal agencies, working with the states, developed seafood safety criteria and protocols. In addition, sampling schemes, analyses, and data reporting for seafood safety efforts conducted in federal waters are provided. Results of testing the seafood collected in federal waters are also discussed.

Collaboration Among Federal and State Agencies to Develop a Protocol

The US Food and Drug Administration (FDA) operates a mandatory hazard analysis and critical control point (HACCP) safety program for all fish and fishery products under the provisions of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.), the Public Health Service Act (42 U.S.C. 201 et seq.), and related regulations. The National Oceanic and Atmospheric Administration (NOAA), under provisions of the Magnuson–Stevens Fishery Conservation and Management Act (16 U.S.C. 1801 et seq.), has the authority to close and open, with concurrence of the FDA, federal waters for seafood harvest. Marine resource and public health agencies of states bordering the Gulf of Mexico retain jurisdiction and responsibility for the health and safety of fish and fishery products within their respective territorial waters (3 miles from coastline except for Florida, which is 9 miles from coastline). Under these guidelines, should an oil spill occur, federal and state agencies, including the NOAA and the FDA, determine whether seafood is at risk for contamination and, if so, when seafood from a previously contaminated area may once again be safe for harvest and human consumption.

As the oil spread and fisheries were closed as a result of the spill, scientists and risk managers from all of the affected state and federal agencies convened to develop a comprehensive protocol to ensure the safety of Gulf seafood before impacted areas could be reopened for harvest for the American public. The NOAA publication titled Managing Seafood Safety After an Oil Spill provides agencies guidance in such situations (3). This guidance and current information from the FDA, the NOAA, the Environmental Protection Agency (EPA), the Centers for Disease Control and Prevention, and counterpart Gulf state agencies were used to establish a unified DWH seafood protocol (4). The DWH seafood safety risk assessment, an integral component of the protocol, was built upon an approach taken by the FDA in 1990 after the Exxon Valdez oil spill in Prince William Sound, Alaska (3). The protocol was implemented, by agreement of all federal and state authorities, in the reopening of commercial and recreational fisheries in both federal and state waters.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

1To whom correspondence should be addressed. E-mail: gina.ylitalo@noaa.gov.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1108886109/-/DCSupplemental.
Selection of Toxic Crude Oil Chemical Indicators

Crude oil is composed of a complex mixture of hundreds of compounds. Among them, polycyclic aromatic hydrocarbons (PAHs) are recognized internationally as the oil constituents of greatest human health concern because of their persistence in the environment and their potential for toxic or carcinogenic effects in humans (5). Consequently, 13 PAHs and their alkylated homologs were selected as the appropriate chemical indicators of the human health risk posed by crude oil residues in seafood (see SI Text, List of Polycyclic Aromatic Hydrocarbon (PAH) Chemical Indicators).

Development of Health Risk Assessment

A human health risk assessment of PAHs in seafood was undertaken to develop maximum exposure levels that are believed to be safe or associated with negligible risk. Uncertainty is inherent in any risk assessment process due to interspecies, intraspecies, or high-to-low dose extrapolations required for risk estimation. Various assumptions must be made to extrapolate data from animal or human studies using models to estimate human population risks. Some subpopulations, such as young children, may be at higher risk due to higher seafood consumption per unit body weight. Other subpopulations, such as elderly people with certain diseases, may be more susceptible to PAH effects from seafood consumption due to compromised health (3). However, many of the inputs used in this health risk assessment were derived to be overly protective to account for uncertainty and variability (e.g., upper 95% confidence bound on cancer risk values and inter- and intraspecies uncertainty factors on reference doses for noncarcinogenic PAHs).

Of the 13 PAHs and alkylated homologs selected for critical analysis of potentially impacted seafood, the criteria agreed upon for 7 PAHs with cancer endpoints provided conservative estimates of contamination levels and consumption rates that, if sustained for a period of 5 y, may result in an upper bound consumer lifetime cancer risk of $1 \times 10^{-5}$. The agreed-upon criteria for 6 PAHs with noncancer endpoints were derived from EPA reference doses of daily exposure expected to have no significant risk of adverse effect during a lifetime of exposure (4). The EPA reference doses are derived to be protective of the human population, including sensitive subgroups such as children and pregnant women (see http://www.epa.gov/iris/help_gloss.htm for reference value definitions).

Values for event-specific variables were determined for seafood consumption, average body weight, exposure duration, and averaging time. Seafood consumption data specific only to Gulf Coast populations were not available during development of the protocol and therefore the National Health and Nutrition Examination Survey (NHANES) 90th percentile consumption data for national seafood eaters only were used and were adjusted for consumption frequency (6). Meal portion and frequency (16.4 seafood meals per month) were converted to annualized daily equivalents. The average US adult body weight of 80 kg (7) and averaging time of 78 y (8) were assumed. For purposes of risk assessment, average adult body weight may be viewed as an estimate of average lifetime body weight—and averaging time as average life span—of people composing a population. An exposure duration of 5 y was selected in consideration of the nature of the spilled oil (i.e., light crude), physical conditions (e.g., 29.5 °C water temperature), spill location (50 miles offshore), and metabolic capacities of seafood species likely to be impacted.

Sampling Scheme for Federal Waters

At the greatest extent of the oil spill, most state waters extending from Louisiana to the panhandle of Florida were closed to fishing. By June 2, 2010, 2010 ~37% (88,522 square miles) of federal waters in the Gulf Exclusive Economic Zone were closed (Fig. 1) (9). Commercial, recreational, and subsistence harvest areas in federal waters that were likely to be in the path of the spilled oil were selected for sample collection in the NOAA National Marine Fisheries Service seafood sampling plan (10). Initially, the survey design for reopening the closed fishing areas was based on selection of random stations in preoil spill nearshore waters along the coastlines of Louisiana, Mississippi, Alabama, Texas, and Florida. However, a more formal survey design was developed and implemented in May 2010 due to the increased area closed to fishing. In this design, the closed region in federal waters was separated into three broad areas on the basis of the extent of cumulative oil inundation from the initial days of the spill. A fourth “perimeter” area was also established to account for uncertainty in the extent of the spill and fish movement and to provide a baseline of samples from preoil conditions for comparison. Each area was further divided into 30×30 nautical mile “grids” extending from the state–federal boundary to the outer boundary of the closed area (Fig. 2) (10).

Grids exhibiting heavy cumulative oil inundation were targeted for more intensive sampling than areas with minimal and

Fig. 1. Gulf of Mexico map showing the extent of oiling that occurred from April 22 through August 21, 2010 after the Deepwater Horizon platform explosion occurred on April 20, 2010. The black-and-white circle indicates the wellhead location. The orange shaded areas show the cumulative National Environmental Satellite, Data and Information Service (NESDIS) satellite footprints of the oil. The yellow polygon overlay shows how the federal fisheries closure areas aligned with the oil distribution.
moderate cumulative oil inundation (10). Sampling intensity was measured as the minimum number of stations successfully sampled per grid that then relates to the target sample size. Two stations were successfully sampled in grids with minimal oil inundation whereas five stations were successfully sampled in grids with heavy oil inundation. Stations were generally chosen by random selection for all vessels, with the exception of the pelagic longline sampling, which targets highly migratory species. Due to the amount of area (10–20 miles) covered by the longline gear and the large migratory range of these species within the Gulf, sampling by random stations in a grid was not relevant. For reopening closed areas in federal waters, the number of days sampling by random stations in a grid was not relevant. For and the large migratory range of these species within the Gulf, 20276

Materials and Methods

Sampling Scheme for Federal Waters. In response to the DWH oil spill, seafood sampling in the Gulf started on April 28, 2010 and included collection of a number of sample types described in the sampling plan (10). The seafood included surveillance, surveillance-perimeter, surveillance-closed, surveillance-reopened, reopening, and dockside surveillance samples (see definitions in SI Text, Sample Classifications for Federal Waters).

Seafood Collection Criteria for Sensory Testing and Chemical Analyses. The criteria described in the protocol (4) for reopening samples subjected to sensory testing and chemical testing included collecting three to six subsamples per seafood type (e.g., oysters, shrimp, crabs, and finfish) at each sample location (SI Text, Seafood Collection Criteria for Sensory Testing and Chemical Analyses).

Sensory Testing and Chemical Analyses. A panel of seven NOAA- and FDA-trained sensory experts performed sensory testing of seafood samples for abnormal odor or taste (known as “taint”). Samples were sequentially evaluated for raw odor, cooked odor, and cooked flavor (SI Text, Sensory and Chemical Testing).

Seafood PAH analyses were conducted using both a detailed gas chromatography/mass spectrometry (GC/MS) method (11) and a more rapid “screening” liquid chromatography/fluorescence detection (LC-FLD) method (12). Gulf seafood was also analyzed for the dispersant component diocyl sodium sulfosuccinate (DOSS) using a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method (13, 14) that was developed and validated by the FDA and the NOAA (SI Text, Sensory and Chemical Testing).

Risk Assessment. To establish the safety of seafood following the DWH oil spill, standard FDA and EPA risk assessment methods for carcinogenic and noncarcinogenic PAHs were used. A toxic equivalency approach was used to estimate the cancer risk for individual PAHs likely to be found in Gulf light crude oil. Tissue levels of carcinogenic PAHs were multiplied by their respective toxicity equivalency factor (relative to benzo(a)pyrene (BaP)) and added to the BaP level to determine the total BaP equivalent concentration. These data were used together with other factors (e.g., body weight, averaging time, seafood consumption rate, and cancer slope factor) to determine the human level of concern (LOC) for each carcinogenic PAH (SI Text, Cancer risk).

Noncancer risks were determined on the basis of the concentrations of anthracene, phenanthrene, fluoranthene, fluorene, and anthracene/phenanthrene were summed with the parent compounds and compared with the appropriate toxicity criterion. The LOC values for noncarcinogenic PAHs were estimated using reference dose, body weight, consumption weight, and a conversion factor (SI Text, Noncancer risks).
Results

Sensory Testing for Seafood from Federal Waters. Federal and state agencies used a tiered strategy to ensure that seafood from the Gulf was safe for consumption. Initially, if oil was visibly found on the water surface, a fishery was recommended for closure, which included a precautionary zone around the visible surface sheen. Once no visible floating oil was observed on the sea surface, this strategy combined sensory testing—expected to detect a broad array of potential contaminating chemicals—with sensitive analytical methods that measured chemical contaminants. For a closed area to be eligible for reopening, the following criteria had to be met (these criteria were based on past oil spill information and provided a high level of confidence that the seafood was not tainted by oil) (4). A minimum of 70% (five of seven) of the expert assessors had to have found no detectable petroleum or dispersant odor or flavor from each subsample. If a subsample failed, then the sample location failed, which in turn failed the grid. Second, if the area passed the sensory test, then samples would undergo chemical analyses. In federal waters 0.16% (6 of 3,810) seafood samples failed sensory testing. This low failure rate was expected because an area was not sampled for reopening testing until there was no visible oil with minimal risk of reoiling.

Chemical Analyses for Seafood from Federal Waters. The GC/MS method measured individual PAHs at detection levels (<1 ng/g) and provided data on alkylated homologs. The LC-FLD screening method is six times faster than GC/MS and provided reliable measurements of the targeted carcinogenic and noncarcinogenic PAHs in seafood, but did not have the specificity of the GC/MS method to confirm the PAH analyte identity. Thus, if a sample analyzed by screening had a PAH level approaching the LOC for that compound, then the sample was reanalyzed using GC/MS to confirm this finding. Although the LC-FLD method increased laboratory capacity, the method provided limited information on the levels of certain alkylated PAH homologs. Therefore, subsets of seafood samples were selected to be reanalyzed by GC/MS to confirm the PAH results and provide more information on alkylated homologs.

Overall, individual carcinogenic PAHs (Fig. 3) and noncarcinogenic PAHs (Fig. 4) were found at low concentrations or below the limits of quantitation. When detected, the PAH concentrations were at least two orders of magnitude lower than the corresponding LOC for each PAH. Furthermore, DOSS was found only at low levels (ranging from 0.05 to 0.29 μg/g) or below the limits of quantitation. Once all sensory and chemical results for the grids within a seafood harvesting area met the criteria for reopening, that area was eligible to be reopened. For all seafood samples collected in a closure area that passed sensory testing, the concentration of each PAH must be below the FDA LOC as determined using the chemical analytical methods for the closed harvest area to be eligible for reopening (4). All seafood samples collected in federal waters met this criterion established in the protocol (4).

Discussion

No two oil spills are exactly alike and thus sampling protocols and fisheries reopening criteria to ensure seafood safety must be modified from previously established guidelines for each particular event (3). More than 4.9 million barrels of oil were spilled over the course of almost 3 mo before the capping of the DWH wellhead (1), so an immediate and well-coordinated seafood safety response was the highest priority for the NOAA, the FDA, and other responding agencies. Priority issues were (a) closing seafood harvesting areas affected by the spill, (b) developing criteria for reopening areas closed to harvesting, (c) planning for full-scale sampling and testing of important commercial and recreational seafood species from the closed areas and from reference areas for spill-related chemicals to determine whether the seafood had been contaminated by spill-related compounds, and (d) conveying the information on testing results to the public and issuing any health advice related to the consumption of seafood potentially contaminated with spill-related chemicals. It was essential that regulators and scientists work together to identify key questions that must be answered and to develop criteria for data that would be collected.

Following the establishment of the protocol (4), a seafood safety sampling plan that contained all relevant information for sampling in federal waters was developed (10). Important...
commercial and recreational seafood species (including nearshore, reef, and pelagic species) and primary harvesting areas were identified to target sampling in federal waters. The criteria established in the protocol (4) specified the minimum numbers of samples required to be tested for sensory testing and chemical analyses for an area to be considered for reopening. Details on seafood sampling for reopening oil-impacted areas are publicly available online at http://www.fda.gov/Food/ucm217601.htm and information on fishery closure boundaries in federal waters can be found at http://sero.nmfs.noaa.gov/ClosureInformation.htm. In addition, oversampling was recommended so that a portion of each sample could be archived for future studies. Information on seafood sampling, including gear used, species collected, number of individuals sampled, and time and location of collection, was recorded with chain of custody protocols and transferred to a database for access by end users (e.g., federal managers).

Discussions among representatives from responding federal agencies (e.g., NOAA, FDA, and EPA) and the affected Gulf states led to identification of the appropriate compounds to target in analyzing seafood. PAHs were selected as oil-related chemicals of concern due to their toxic and carcinogenic properties as well as their ability to persist in the environment (5). One unusual aspect of the DWH oil spill was the extensive use of dispersant (e.g., Corexit 9500 and Corexit 9527) that was applied onto surface waters as well as applied directly to the waters near the wellhead. DOSS was selected as the best target component of the Corexit dispersant due to its bioactivity, extremely low volatility, and potential to persist in the environment longer than other dispersant components.

Initially, a detailed GC/MS method was used to determine levels of PAHs in seafood but it became apparent soon after testing began that a more rapid method was needed to increase the capacity of the seafood safety program. As a result, a method to rapidly measure PAHs in seafood was developed and validated by the FDA as part of the response to the DWH spill. Because the PAH screening method was much more rapid than the comprehensive GC/MS method, larger numbers of samples were analyzed in less time; however, the GC/MS method remained the standard for measuring PAH levels (including alkylated homologs) in seafood and was also used to confirm PAH screening results.

Published research on the persistence of dispersant chemicals in the environment and their toxicity suggested that potential contamination of seafood would pose a low risk to consumers (see ref. 14 for references). However, public concern about dispersant use led to a collaborative effort between the FDA and the NOAA to develop and validate a rapid, sensitive chemical method for measuring DOSS from the dispersant in a wide array of edible seafood. A description of the chemical method can be found at the Web site http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM231510.pdf, and results of dispersant testing of seafood collected in federal waters as part of the reopening can be obtained at the Web site http://sero.nmfs.noaa.gov/PreviousReopenings.htm. Once the DOSS method was developed and validated, up to 50% of seafood samples previously analyzed for PAHs by GC/MS were subsequently analyzed for DOSS and all subsequent samples for reopening were analyzed for DOSS.

Analyses for spill-related contaminants proceeded in a timely manner, especially considering the large number of samples tested and the nearly 90,000 square miles of federal waters of the Gulf of Mexico that were closed. For example, sensory results were available within 4 h of sample analysis and the chemical data for a sample batch containing 14 field samples were quality assured and finalized within 48 h (LC-FLD PAH results) to 96 h (GC/MS PAH results) postreception.

It has been well established in the scientific literature that all teleost fish (modern bony fishes, e.g., grouper, snapper, and tuna) have a well-developed capacity to metabolize and eliminate PAHs and other oil constituents such as aliphatic hydrocarbons (15). Because of this efficient metabolism, there is a very low potential for PAHs to accumulate in muscle and consequently a low potential for transfer of PAHs up the food chain to human consumers. However, efficient PAH metabolism by species other than teleosts is not universal (3). For example, bivalves (e.g., oysters and clams) have a lower capacity to metabolize PAHs, whereas crustaceans such as shrimp have an intermediate metabolic capacity. In the current study, concentrations of individual PAHs measured in seafood collected in federal waters were, in many cases, below the limit of detection (LOD) of the sensitive gas chromatography/mass spectrometry (GC/MS) instrumentation used; (Right) circles and triangles indicate samples where the levels are above the GC/MS LOD. Abbreviations: ANT/PHN, anthracene + phenanthrene; FLA, fluoranthene; FLU, fluorene; NPH, naphthalene; PYR, pyrene.
quantitation or, when detected, were at least two orders of magnitude lower than the FDA LOC for the particular compound (Figs. 3 and 4). Although scientists were confident that high levels of PAHs would not be found in edible seafood tissues, it was necessary to test seafood to assure the public of its safety. Without an extensive, real-time sampling and analysis protocol, there would be little to no ability to convince the public that Gulf seafood was safe to eat following this major oil spill.

The PAH concentrations determined by various federal and state agencies to be of concern for human health were based on a projected daily level of consumption (grams per day of finfish, mollusks, crabs, and shrimp) and an acceptable risk (e.g., 1/100,000 risk for cancer) for 5 y (SI Text, Cancer risk). For the LOC calculations, to ensure a health protective approach (in addition to using cancer and noncancer values that are designed to be conservative values), the FDA used the 90th percentile of the national consumption data reported in the National Health and Nutrition Examination Survey for fish, shrimp, and shellfish for calculating risk and then adjusted the 90% meal size to account for the number of meals eaten by a 90th percentile consumer to ensure protection for low- through high-level consumers. Nevertheless, some concerns were raised with regard to the seafood consumption rates used by the FDA to calculate the PAH LOCs for the DWH seafood assessment (16). For example, a 2010 National Resources Defense Council survey of 547 people living in Louisiana, Alabama, Mississippi, and Florida showed that the average shrimp median daily consumption rate among residents was 3.6 times higher (48 g/d) than the FDA estimated shrimp consumption rate of 13 g/d (17). On the basis of the information collected on PAH levels in various seafood species from the Gulf, risk assessors can calculate the seafood consumption rates that are allowable without being a health concern for Gulf seafood. The Louisiana Department of Wildlife and Fisheries and the Louisiana Department of Health and Hospitals determined that the average consumer could eat ~63 pounds of peeled shrimp, 5 pounds of oyster meat, or 9 pounds of finfish every day for 5 y and have minimal risk of health effects (18). The concentrations of PAHs measured in Gulf seafood were very low or were below the levels of quantitation, and thus these compounds did not appear to pose a health risk, even when higher consumption values are applied to the risk model.

In summary, scientists and regulators from federal and state agencies worked together to develop a seafood sampling plan and analytical protocols to determine whether seafood was safe to eat and acceptable for sale in the marketplace in response to the 2010 DWH oil spill. Approved methods for sensory testing and chemical analyses were used to detect PAHs and the dispersant component DOSS in >8,000 seafood specimens collected in federal waters of the Gulf. Overall, a low percentage (0.16%) of these samples failed sensory testing, which was expected as sampling of an area commenced only when the risk of oil being present was negligible. We also found that the concentrations of individual PAHs and the dispersant component DOSS measured in Gulf seafood were below the limits of quantitation or, when detected, were at least two orders of magnitude lower than the FDA LOC for each compound. Once an area closed to fishing was free of visibly floating oil and all sensory and chemical results for the seafood species within an area met the criteria for reopening, that area was eligible to be reopened. Since July 2010, ~88,500 square miles of federal waters have been reopened, with the area nearest the wellhead being reopened nearly 1 y (April 2011) after the spill began. As of November 9, 2011, a few areas off the Louisiana coast (Barataria Bay and the Louisiana Bight) remain closed because of residual oil and because the species at risk for exposure, such as oysters, have low metabolic capacity and a higher risk of accumulation of PAHs.

ACKNOWLEDGMENTS. We thank the vessel captains, their crew, and observers for their support in the field operations during sample collection. We specially thank the NOAA and FDA sensory experts for providing rapid results for the thousands of seafood samples tested for oil contamination. We appreciate the guidance provided by Usha Varanasi and the timely chemical and data analyses provided by Bernadita Anulacion, Daryle Boyd, Dennis da Silva, Catherine Sloan, and their colleagues at the Northwest Fisheries Science Center and by Laura Falks, Nina Crain, Lisa Price, Leland Davis, Katie Scott-Westfall, and their colleagues at the National Seafood Inspection Laboratory. We further acknowledge the many Food and Drug Administration consumer safety officers for field inspections and sample collection; Office of Regulatory Affairs and Food Emergency Response Network scientists for chemical and data analyses; and Center for Food Safety and Applied Nutrition scientists for response planning, logistical support, and data review.

Supporting Information

Ylitalo et al. 10.1073/pnas.1108886109

SI Text

List of Polycyclic Aromatic Hydrocarbon (PAH) Chemical Indicators. Thirteen toxicologically representative PAHs and alkylated homologs were selected for critical analysis of potentially impacted seafood (1). They included 6 noncarcinogenic PAHs (naphthalene and C1, C2, C3, and C4 alkylated naphthalene homologs; fluorene and C1, C2, and C3 alkylated fluorene homologs; anthracene/phenanthrene and combined C1, C2, C3, and C4 alkylated anthracene/phenanthrene homologs; pyrene; and fluoranthene) and 7 carcinogenic PAHs [chrysene, benzo(k) fluoranthene, benzo(b)fluoranthene, benzo(a)anthracene, indeno (1,2,3-cd)pyrene, dibenz(a,h)anthracene, and benzo(a)pyrene]. The PAH levels of concern, and factors for their derivation, were developed specifically for this particular oil spill event.

Sample Classifications for Federal Waters. Since the initiation of seafood safety sampling on April 28, 2010, a variety of samples have been collected and they are described in the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service Seafood Sampling Plan (2):

Surveillance samples are collected in an area before closure. These samples help provide a baseline of preoil conditions for comparison of seafood analyzed for chemical analyses. Surveillance-perimeter samples are collected outside the original closed area. These samples are used to provide supplemental information on the perimeter of the closed area and to account for fish movement outside the grids. Surveillance-closed samples are collected within a closed harvest area but are not used for the purposes of reopening and are used to monitor seafood contamination within a closed harvest area before reopening. Surveillance-reopened samples are collected in areas previously closed to harvest but subsequently reopened. Sampling is conducted ~1 wk after reopening and continues through two 7-d sampling periods, separated by at least 1 wk. The purpose of this sampling is to ensure the continued safety of seafood marketed from these open harvest areas. Reopening samples are collected within each grid in a closed fishing area for both sensory and chemical analyses and are used specifically in reopening grids closed to harvest. Dockside surveillance samples are purchased by the NOAA Fisheries Southeast Fisheries Science Center port samplers in major ports in Louisiana, Mississippi, Alabama, and Florida and transported to the National Seafood Inspection Laboratory for analysis. These samples help to minimize the risk of tainted seafood reaching the market.

Seafood Collection Criteria for Sensory Testing and Chemical Analyses. For a closed area to be considered for reopening, the criteria described in the protocol (1) for sensory testing included collecting up to six subsamples per seafood type (three subsamples for oysters) for each targeted depth location at each sample location in the area under consideration for reopening. A subsample consists of individual organisms for legal size finfish and multiple organisms for shrimp and shellfish depending on the seafood type (e.g., 6 blue crabs, 10 oysters, and 0.5 pound of shrimp).

For chemical analyses, the criteria listed in the protocol (1) require collecting a minimum of 15 oysters, 0.5 pound of shrimp, and up to six finfish per species (multiple species of fish were collected from some sites, in particular in areas of heavy oiling) at or near each sample location. A sample of edible crab tissue includes collecting a minimum of 10 legal size organisms from each crab sampling location for these analyses.

Sensory and Chemical Testing. Using glass Pyrex bowls with lids, raw samples were presented as caught for smaller species and fillets for larger pelagic species. A portion of raw sample was placed into a glass-covered Pyrex bowl and transferred to a microwave oven for cooking. The sample was fully cooked and presented to the panel again with the top on the bowl. In this way as much moisture would remain to keep the sample as warm as possible throughout the test. Crabs, on the other hand, were evaluated only in the cooked state. They were brought in live and then steamed prior to evaluation. Sensory testing of seafood collected in federal waters was performed at the NSIL.

The gas chromatography/mass spectrometry (GC/MS) method (3) used to measure PAHs in seafood is a reliable and sensitive analytical method that has been used to measure these compounds in seafood and other marine organisms collected after previous oil spills and natural disasters (4, 5). For the GC/MS method (3), seafood samples were extracted with dichloromethane using an accelerated solvent extractor. Polar compounds were removed from the extracts using a gravity flow silica/alumina column and followed by separation of PAHs from interfering biogenic compounds using liquid chromatography (LC) with size exclusion chromatography. PAHs were then measured on a low-resolution quadrupole GC/MS system. To increase laboratory capacity for analysis for PAHs in seafood, a method was developed by FDA to rapidly measure PAHs using liquid chromatography/fluorescence detection (LC-FLD) (6). PAHs were extracted from seafood with acetonitrile/water using a QuEChERS (i.e., quick, easy, cheap, effective, rugged and safe) extraction procedure. Each sample extract was passed through a 0.20 μm filter and was subsequently analyzed using LC-FLD.

The method to measure the dispersant component dioctyl sodium sulfosuccinate (DOSS) in seafood used the same rapid QuEChERS extraction procedure used for PAHs followed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis (7, 8). All chemical analyses of seafood collected in federal waters were performed at either the Northwest Fisheries Science Center in Seattle, WA or at the NSIL. Laboratories conducting chemical analyses of Gulf seafood used a number of quality assurance measures, including analyses of method blanks, National Institute of Standards and Technology Standard Reference Materials (when available), matrix spikes, incurred chemical contaminant residues in laboratory-exposed seafood, and continuing calibration verification standards, to ensure that instruments were in excellent operating condition and that the chemical data were of known and acceptable quality.

Cancer risk. To estimate the cancer risk for individual PAH compounds likely to be found in the Gulf of Mexico light crude oil, a toxic equivalency (TEQ) approach was used. TEQ approaches are often used when determining health risks associated with exposure to mixtures of compounds with similar chemical structures and biological activities (9). The concentration of benzo(a)pyrene equivalents (BaPE) is considered the most valid measure of the carcinogenic potency of a complex mixture of PAHs. For the Deepwater Horizon (DWH) oil spill, the carcinogenic activity for each PAH relative to benzo(a)pyrene (BaP) was estimated as a toxicity equivalency factor (TEF) (10). Using this method, tissue concentrations of carcinogenic PAHs (other...
than BaP) were multiplied by their respective TEF and added to the BaP concentration to determine the total BaPE concentration. The following TEF values were used: chrysene, 0.001; benzo(k)fluoranthene, 0.01; benz(a)anthracene, 0.1; indeno(1,2,3-cd)pyrene, 0.1; benzo(b)fluoranthene, 0.1; and dibenz(a, h)anthracene, 1.

The following equation was used to determine the public health levels of concern (LOC) (in micrograms per gram or milligrams per kilogram equaling parts per million wet weight) for carcinogenic PAH compounds (BaPE) potentially found in seafood:

\[ \text{LOC(BaPE)} = (\text{RL} \times \text{BW} \times \text{AT} \times \text{CF}) / (\text{CSF} \times \text{CR} \times \text{ED}) \]

Definitions, assumptions, and specific factors used in the above equation are described below.

**LOC**: Level of concern.

**BaPE**: Benzo(a)pyrene equivalent.

**Risk level (RL)**: Risk-based criteria were selected to prevent consumers from being exposed to the carcinogenic components of crude petroleum in doses that exceed a RL of 1 x 10^-5 (1 in 100,000). This RL is within the acceptable range of risks (1 x 10^-4 to 1 x 10^-6) used by the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) in regulatory criteria for food and drinking water (11) and is provided as an example of an acceptable risk level in the US EPA Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories (12).

**Body weight (BW)**: The average adult body weight, 80 kg, was adopted from the most recent CDC National Health Statistics Report (13).

**Averaging time (AT)**: The averaging time, 78 y, was adopted from the most recent CDC National Vital Statistics Report (14). Conversion factor (CF): Unit conversion factor (1,000 μg/mg). Cancer slope factor (CSF): The upper-bound estimate of the probability that an individual will develop cancer over a lifetime as a consequence of exposure to a given dose of a specific carcinogen. For the DWH seafood risk assessment, the EPA current BaP CSF value of 7.3 (mg-kg-d)^-1 was adopted (15).

**Consumption rate (CR)**: Consumption rates for shrimp and crab (13 g/d), oysters (12 g/d), and finfish (49 g/d) were adopted from the 2005–2006 National Health and Nutrition Examination Survey (NHANES) data for high-level (90th percentile) seafood consumers adjusted for consumption frequency. The FDA adjusted the 90% meal size to account for the number of meals consumed by a 90th percentile consumer to determine the appropriate seafood consumption rate for high-level consumers. For 90th percentile consumption values, data from the 2005–2006 NHANES 2-d recall survey were used. To determine the average daily rate for these consumers, the 2005–2006 NHANES 30-d recall survey was used to determine frequency of seafood meals eaten by 90th percentile consumers.

**Grams of seafood per day = [meal frequency/30 d in a month] x meal size**

where

- **Meal frequency** = 9.1 meals per month for finfish, 2.9 meals for oysters, and 4.4 for shrimp/crab;
- **Meal size** = 160 g for finfish, 120 g for oysters, and 90 g for shrimp/crab;
- **Grains of seafood per day** = 49 g for finfish, 12 g for oysters, and 13 g for shrimp/crab.

**Exposure duration (ED)**: The exposure duration was assumed to be 5 y. This is a conservative estimate of the potential re-}

Using the assumptions and equation shown above, the levels of concern for each of the seven carcinogenic PAHs for shrimp and crabs, oysters, and finfish are presented in Fig. 3. Concentrations of alkylated homologs of the carcinogenic PAHs listed above were excluded as they are found in very low levels in the Louisiana light crude oil.

**Noncancer risks**. Noncancer risks were also determined on the basis of the concentrations of anthracene, phenanthrene, fluorene, naphthalene, and pyrene measured in seafood. Alkylated homologs of naphthalene, fluorene, and anthracene/phenanthrene were summed with the parent compounds and compared with the appropriate toxicity criterion. The alkylated homologs of pyrene and fluoranthene were not included due to the very low levels found in the Louisiana light crude oil. The following equation was used to set the public health protective LOC (micrograms per gram or milligrams per kilogram equaling parts per million wet weight) for these noncarcinogenic PAHs potentially found in seafood:

\[ \text{LOC} = (\text{RfD}) (\text{BW}) (\text{CF}) / \text{CR} \]

The following specific factors and assumptions were used in the above equation:

**Reference dose (RfD)**: An estimate of daily human exposure to a chemical that is likely to be without significant risk of adverse effects during a lifetime, in milligrams per kilogram per day. RfDs for selected PAH compounds were obtained from the US EPA’s Integrated Risk Information Service (IRIS) database (accessed June 2010; see IRIS database for specific chemicals). The RfD for anthracene was used as a surrogate for phenanthrene.

**BW**: The average adult body weight, 80 kg, was adopted from the most recent CDC National Health Statistics Report (13).

**CA**: Unit conversion factor (1,000 μg/mg).

**CR**: Consumption rates for shrimp and crab (13 g/d), oysters (12 g/d), and finfish (49 g/d) were adopted from the 2005–2006 NHANES data for high-level (90th percentile) seafood consumers adjusted for consumption frequency as described above.

Using the above equation and assumptions, the noncancer public health levels of concern for individual PAHs were calculated and are shown in Fig. 4.


