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Environmental stress in the Gulf of Mexico and its potential impact on public health

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A B S T R A C T

The Deepwater Horizon (DWH) oil spill in the Gulf of Mexico was the largest maritime oil spill in history resulting in the accumulation of genotoxic substances in the air, soil, and water. This has potential far-reaching health impacts on cleanup field workers and on the populations living in the contaminated coastal areas. We have employed portable airborne particulate matter samplers (SKC Biosampler Impinger) and a genetically engineered bacterial reporter system (umu-ChromoTest from EBPI) to determine levels of genotoxicity of air samples collected from highly contaminated areas of coastal Louisiana including Grand Isle, Port Fourchon, and Elmer’s Island in the spring, summer and fall of 2011, 2013, and 2014. Air samples collected from a non-contaminated area, Sea Rim State Park, Texas, served as a control for background airborne genotoxic particles. In comparison to controls, air samples from the contaminated areas demonstrated highly significant increases in genotoxicity with the highest values registered during the month of July in 2011, 2013, and 2014, in all three locations. This seasonal trend was disrupted in 2012, when the highest genotoxicity values were detected in October, which correlated with hurricane Isaac landfall in late August of 2012, about five weeks before a routine collection of fall air samples. Our data demonstrate: (i) high levels of air genotoxicity in the monitored areas over last four years post DWH oil spill; (ii) airborne particulate genotoxicity peaks in summers and correlates with high temperatures and high humidity; and (iii) this seasonal trend was disrupted by the hurricane Isaac landfall, which further supports the concept of a continuous negative impact of the oil spill in this region.

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1. Introduction

The 2010 Deepwater Horizon (DWH) oil spill in the Gulf of Mexico is the largest environmental disaster and remediation effort in the U.S. history. Approximately 800 million liters of crude oil were spilled and about 7 million liters of chemical dispersant were applied along the northern Gulf of Mexico, resulting in over 1600 km of the shoreline contaminated with weathered oil (Barron, 2012; Kleindienst et al., 2015). The closing of almost 50 million acres to fishing, hunting, and tourism, displacement of residents, and the arrival of workers recruited to participate in mitigation efforts, all contributed to the overall impact of the spill (Barron, 2012; Fisher et al., 2014). A large number of governmental and non-governmental agencies were involved in environmental relief efforts and interventions to alleviate both the environmental and economic impacts of the spill. Since the beginning of the spill, cleanup workers have been reporting acute symptoms of sickness including skin and eye irritation, respiratory problems, and headaches (D’Andrea and Reddy, 2013; King and Gibbins, 2011; Rotkin-Ellman et al., 2010). In addition, long-term health problems are also anticipated, however a direct cause and effect relationship between chronic exposure to weathered oil and health require further investigation (Diaz, 2011). Multiple chemicals and physical factors associated with the oil spill are known to cause both acute and chronic health problems. These chemicals include volatile organic compounds (VOCs): benzene, toluene, xylene, and ethyl

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benzene; semi-volatile compounds: polycyclic aromatic hydrocarbons (PAHs), higher molecular weight alkanes, and particulate matter (PM 2.5 and PM 10.0). In addition oil mist, carbon monoxide, gasoline and diesel engine exhausts were all present during the cleanup efforts (McCoy and Salerno, 2010).

Importantly, there was an increase of over 10-times in the concentration of the relatively low molecular weight, volatile components, including naphthalene and C-1-naphthalenes, and in higher molecular weight analytes between September 2010 and October 2012 in heavily oiled marsh in Louisiana (Turner et al., 2011). Although these volatile components are well known to trigger both immune responses, and cause DNA damage in affected organisms including humans (Alpha et al., 2015; Tagiyeva and Sheikh, 2014; Wang et al., 2013; Wang et al., 2014), air sampling and the evaluation of airborne particulates have not been used in the assessment of health risks associated with oil spills. Our research team collected and analyzed airborne particulate matter (PM) samples from three areas of coastal Louisiana, which according to the U.S. Environmental Protection Agency (EPA) demonstrated heavy contamination with the natural crude oil post DWH oil spill. These include Grand Isle, Port Fourchon, and Elmer’s Island. The PM samples were collected in the spring, summer and fall of 2011, 2012, 2013 and 2014. Our data demonstrate: (i) persistent air genotoxicity in the monitored areas of coastal Louisiana over the last four years; (ii) air genotoxicity peaks in the summers, which correlated with high temperatures and high air humidity; and (iii) this seasonal trend was disrupted by the hurricane Isaac landfall, which caused a further increase of air genotoxicity in the fall of 2012. In conclusion, our data indicate a continuous negative health impact of DWH oil spill, which persists in the monitored regions of the Gulf, and supports the need for a continuous monitoring of air quality in this region.

2. Materials and methods

2.1. Air sample collection and storage

BioSampler Impinger (SKC, Inc.) with sonic flow sample pumps were used according to manufacturer recommendations. Briefly, particulate matter (PM) recovered from size-segregated air samples were collected from oil-impacted beaches at Port Fourchon, Elmer’s Island, and Grand Isle, Louisiana. These air samples were collected during 8-h sampling campaigns, which occurred on consecutive days over weekends in March, July, and October of 2011, 2012, 2013 and 2014. Airborne PM samples collected from a non-contaminated area (Sea Rim State Park, TX) were used as controls. The samples were then stored at −80 °C until further analyses. The selection of oil impacted sites was made by using air quality data gathered through the U.S. Environmental Protection Agency (EPA) in response to the DWH oil spill to identify the most heavily impacted regions in the Gulf (2011 United States Environmental Protection Agency (EPA, 2011)). Permits were aquired from the Louisiana State Park Service to collect samples in Grand Isle State Park, one of the most adversely affected coastal shorelines of Louisiana.

2.2. Genotoxicity of air samples

A standard bacterial reporter assay (umu-ChromoTest from EBPI) was used to determine the genotoxicity of the collected PM samples. In this assay, DNA damage induces activity of the reporter gene, umuC, as a part of genotoxic response. In this system, the umuC gene is fused to lacZ, thus its activation results in the production of ß-galactosidase, measured using a standard colorimetric reaction and UV–vis plate reader (FilterMax F5, Molecular Devices). The results are expressed as Induction Ratios, which were calculated using the formula recommended by the manufacturer in which the positive control is the response to the known genotoxic compound [4-nitroquinoline 1-oxide (4NQO)], and the negative control is the response to the elution buffer. Accordingly, the Induction Ratio of 1.5 was determined as the baseline for the genotoxicity. In particular, a series of dilutions of the air samples were tested to select a dilution, which is not cytotoxic to bacteria, and still triggers high genotoxic response.

2.3. Meteorological data

Meteorological data were logged (ACU > RITE Professional Weather Center-5-in-1 wireless sensor) at the collection sites during PM sampling campaigns. The data include: temperature (F), humidity (%), wind direction, wind speed (mph), heat index (F), dew point (F), and wind chill (F).

2.4. Statistical analysis

Statistical evaluation was based on one-way ANOVA with Sattherthwaite correction for unequal variances, followed with multiple comparisons of mean Induction Ratios with p-values adjusted via simulation to keep an overall alpha of $\geq 0.05$.

3. Results

Large-scale oil spills increase the overall concentration of toxic chemicals in affected environments. Some of these substances, such as volatile organic compounds (VOCs) and semi-volatile polycyclic aromatic hydrocarbons (PAHs), can be released from water and sediments affecting the quality of air in areas heavily contaminated with the natural crude oil. To address the potential health risk associated with chronic exposure to natural crude oil, we collected air samples from coastal areas polluted by the 2010 DWH oil spill in the Gulf of Mexico. Fig. 1 shows the locations of three collection sites, which are superimposed on the map of relative oiling of the Louisiana coastline as of January 23, 2011 (Jan 23 2011 Environmental Response Management Application (ERM) derived as part of the Shoreline Cleanup Assessment Technique (SCAT) Program (ERMA, 2011)). The samples were collected from Grand Isle (A), Port Fourchon (B), and Elmers’ Island (C) in spring, summer and fall of 2011, 2012, 2013 and 2014 using portable PM samplers (SKC Biosampler Impinger). The collected samples were stored in −80 °C, and were subsequently tested for the ability to induce a genotoxic response in genetically engineered bacterial reporter system (umu-ChromoTest from EBPI). PM samples collected from a non-contaminated area (Sea Rim State Park, TX) served as a control for background genotoxicity. The results in Fig. 2 demonstrate that samples collected from Grand Isle in March, July and October of 2011 demonstrated 443%, 570%, and 315% increases in genotoxic response (expressed as Induction Ratio) in comparison to the corresponding control samples. Significant increases in genotoxicity were also detected in samples from Elmer’s Island and Port Fourchon, LA. Genotoxicity analysis of samples from Elmer’s Island in 2011 demonstrated a 356% increase in March, 440% increase in July, and 264% increase in October. Analysis of samples collected at Port Fourchon demonstrated a 198% increase in March; 397% increase in July; and 223% increase in October (all statistically significant).

Additionally, we also observed significantly higher genotoxicity values in July 2011 in comparison to March and October of the same year in all three locations. The results in Fig. 3A demonstrate that combined genotoxicity levels (from all three Louisiana locations) were 24% higher in July in comparison to March ($p = 0.048$);
and 37% higher in comparison to October ($p=0.001$). This seasonal trend of high summertime genotoxicity was also observed in 2013 and in 2014 (Fig. 3A). In contrast, this trend was not observed in 2012, when the highest genotoxicity values were observed in October. July 2012 samples exhibited significant increase in genotoxicity, both in comparison to controls (Fig. 2, last column) and in comparison to experimental samples collected in March of the same year (Fig. 3A). However, October samples from 2012 were collected 2 weeks after Hurricane Isaac made a landfall in late August of 2012. It is probable that this extreme weather condition increased the content of genotoxic substances in the airborne PM samples collected from the coastal area, which is still heavily loaded with natural crude oil and dispersants, and could be responsible for this effect.

Another key finding of this study is the persistence of air genotoxicity observed in all three locations over last four years (Fig. 2). In Grand Isle for example, March samples demonstrate average genotoxicity induction ratios of $4.6+/-0.14; 3.7+/-0.03; 3.6+/-0.1$, and $3.1+/-0.05$ in 2011, 2012, 2013 and 2014, respectively. In July, genotoxic values in this area were $5.7+/-0.9; 4.54+/-0.05; 4.24+/-0.1$; and $4.4+/-0.35$ in 2011, 2012, 2013 and 2014, respectively. In October, genotoxicity values were $3.74+/-0.03; 5.34+/-0.1; 3.6+/-0.08;$ and $3.2+/-0.16$ in 2011, 2012, 2013 and 2014, respectively. Results illustrated in Fig. 3B demonstrate a direct comparison of air genotoxicity between air samples collected in 2011 and 2014. In this comparison average data from all three locations have been combined together and grouped as average induction ratios for March, July and October 2011, and were compared with induction ratios obtained from March, July and October 2014. This direct comparison demonstrated only marginal (not significant) changes in PM genotoxicity between 2011 and 2014. These findings indicate the persistent presence of genotoxic PM in the air from these selected coastal areas of southern Louisiana.

Meteorological data presented in Fig. 4 were utilized to compare genotoxicity of the air samples with weather parameters registered during sampling campaigns. These weather parameters include temperature (°F), humidity (%), wind direction, wind speed (mph), heat index (°F), dew point (°F) and wind chill (°F). These data were utilized to evaluate seasonal trend of maximum genotoxicity observed in July of 2011, 2013, and 2014 (Fig. 3A), and to address possible correlation between genotoxicity and the extreme meteorological conditions associated with hurricane Isaac (Fig. 3A, histogram corresponding to year 2012). The results in Fig. 5A demonstrate a highly significant correlation of elevated genotoxicity (Induction Ratio) with temperature. In this calculation, all temperature records, expressed in Fahrenheit (°F), have been divided into four arbitrary temperature brackets: 30–45, 46–60, 61–75 and over 75 °F. Statistically significant increments of genotoxicity induction ratios were detected between almost all temperature brackets when lower temperature brackets were compared to higher temperature brackets (five comparisons). One exception to this trend was observed when 46–60 °F temperature bracket was compared to the 61–75 °F temperature bracket.

We also observed elevated correlation between genotoxicity and humidity (Fig. 5B). In this comparison statistical significance was reached only when 50–59% humidity bracket was compared
either with 60–69% humidity brackets ($p=0.0001$), or with 70–79% humidity bracket ($p=0.0001$). Of note, increases in humidity from 60–69% to 70–79% did not significantly affect genotoxicity induction ratios. These analyses demonstrated positive correlations between DWH oil spill related PM genotoxicity, with temperature and humidity, which were evaluated here using temperature and humidity groups relevant to the daily and seasonal changes in this region. These correlations between genotoxicity and meteorological data may also explain why genotoxicity peaks most commonly in summers, when both temperature and humidity are the highest.

In conclusion, these findings indicate that potential health risks associated with persistent airborne PM genotoxicity exist in locations along coastal Louisiana heavily oiled by the DWH oil spill. As such, this research advocates for continued monitoring of air quality in this region.
4. Discussion

The results of this study demonstrate elevated levels of airborne PM genotoxicity in the monitored areas of coastal Louisiana over the first four years post DWH oil spill. In particular, air genotoxicity peaks during summers, which correlates with high temperatures and high humidity levels. Analyses also indicate that this seasonal trend was disrupted by the hurricane Isaac landfall in September 2012, which further supports the conceptual model of a continuous release of the oil likely generated from the oil deposits that remain in the Gulf post DWH oil spill.

In terms of a direct environmental impact, research has provided new insights into oil spills that occur at unprecedented depths, release enormous amounts of oil that for the most part is retained at deep sea levels, emulsifies and disperses due to deep pressures, and affects marine ecosystems for long periods of time (Kleindienst et al., 2015; Lubchenco et al., 2012). These deep water spills behave differently from the more common contamination caused by broken pipelines and ruptured tankers; wherein oil floats to the surface, and can be dispersed chemically or by natural weathering. The use of unprecedented amounts of dispersants at the origin of the DWH oil spill is controversial because the natural behavior of deep oil spills is to remain deep below the surface, however, dispersants have already disrupted this natural process (Kleindienst et al., 2015; Lubchenco et al., 2012). Recent studies also indicate that the oil from DWH spill continues to resurface (Warnock et al., 2015). These oil deposits are suspected to be released continuously to the surface due to chemical modifications.
Weather Monitoring equipment

<table>
<thead>
<tr>
<th>Season</th>
<th>Temp. F</th>
<th>Humidity %</th>
<th>Wind Direction</th>
<th>Wind Speed mph</th>
<th>Heat Index F</th>
<th>Dew Point F</th>
<th>Wind Chill F</th>
</tr>
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<tbody>
<tr>
<td>2011</td>
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<tr>
<td>March</td>
<td>50-57</td>
<td>54-56</td>
<td>S-S/E</td>
<td>6-10</td>
<td>50-56</td>
<td>43-48</td>
<td>48-55</td>
</tr>
<tr>
<td>July</td>
<td>90-93</td>
<td>65-67</td>
<td>S/E</td>
<td>7-10</td>
<td>91-98</td>
<td>63-70</td>
<td>86-88</td>
</tr>
<tr>
<td>October</td>
<td>64-68</td>
<td>52-54</td>
<td>S</td>
<td>5-7</td>
<td>62-67</td>
<td>54-57</td>
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<td>2012</td>
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<tr>
<td>March</td>
<td>55-58</td>
<td>55-57</td>
<td>S/E</td>
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<td>44-46</td>
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<tr>
<td>July</td>
<td>86-94</td>
<td>67-71</td>
<td>S</td>
<td>5-10</td>
<td>88-94</td>
<td>63-72</td>
<td>83-88</td>
</tr>
<tr>
<td>October</td>
<td>64-68</td>
<td>71-76</td>
<td>S/E</td>
<td>4-11</td>
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<td>2013</td>
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<tr>
<td>March</td>
<td>45-47</td>
<td>52-58</td>
<td>S/E</td>
<td>5-8</td>
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<tr>
<td>July</td>
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<td>October</td>
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<tr>
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<td>65-67</td>
<td>S/E</td>
<td>5-12</td>
<td>87-93</td>
<td>71-74</td>
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<tr>
<td>October</td>
<td>63-67</td>
<td>50-55</td>
<td>S/E</td>
<td>5-10</td>
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<td>62-69</td>
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Fig. 4. Weather parameters including temperature (F), humidity (%) wind direction, wind speed (mph), heat index (F), dew point (F) and wind chill (F) were collected using Weather monitoring equipment (ACU > RITE Professional Weather Center-5-in-1 wireless sensor). These weather parameters were registered at the time of the collection of air samples from Grand Isle, Port Fourchon and Elmer’s Island, Louisiana, in spring, summer, and fall of 2011, 2012, 2013 and 2014.

(weathering) of the oil/dispersant complexes and/or by high pressure-induced emulsification of the oil (Gong et al., 2014; Lubchenco et al., 2012). Our data illustrate that both seasonal (temperature and humidity) and extreme (hurricane landfall) weather conditions have the potential to continuously increase air genotoxicity near the oil spill impacted shorelines.

As a part of the global health response, the National Institute for Environmental Health Sciences (NIEHS) has initiated large-scale epidemiological study to investigate health of the cleanup workers (GuLF Study) (Sandler et al., 2012). Briefly, it involves a cohort of approximately 55,000 workers who are administered a questionnaire to obtain information on the subjects’ overall health status and the cleanup jobs in which they were engaged. In addition, two sub cohorts: Active Follow-up Sub-cohort and Biomedical Surveillance Sub-cohort have been established for blood, urine, hair, fingernails, household dust collection; and for more detailed neurological and physiological assessment. These efforts have been employed to determine the overall health status and possible health effects associated with the persistence of contamination in these populations, and will be useful for evaluation in conjunction with ongoing studies on the Gulf environment.

Multiple chemicals associated with the oil spill can potentially cause both acute and chronic health problems. These also include volatile organic compounds (VOCs) such as benzene, toluene, xylene, and ethyl benzene, and semi-volatile compounds such as polycyclic aromatic hydrocarbons (PAHs). Although chronic effects of low doses of these substances on human health are not well defined, several PAHs are classified as potent carcinogens, which form covalent DNA adducts and cause oxidative DNA damage (Ji et al., 2010; Liu et al., 2010; Seike et al., 2003; Xue and Warshawsky, 2005). In respect to cancer risk, these PAH-induced primary DNA lesions may result in the accumulation of random mutations when cellular mechanisms responsible for DNA repair fidelity are compromised by inherited mutations of genes involved in DNA repair (Shields, 1993), excessive DNA damage, or by oncogenic viruses (Morales-Sanchez and Fuentes-Panana, 2014; White et al., 2005; Wilk et al., 2013). Further epidemiological
values adjusted via simulation to keep an overall alpha.

variances, followed with multiple comparisons of mean Induction Ratio with

evaluation was based on one-way ANOVA with Satterthwaite correction for unequal

duction Ratio when 60

midity (70

statistically signi

cant increases in the Induction Ratio with the increasing temperature between

all four groups. The results in Panel B demonstrate a similar correlation, which

have been calculated between the Induction Ratio data and humidity data arbitrary

divided in to three groups (50–59%; 60–69% and 70–79%). The results demonstrate

statistically significant increases in the Induction Ratio when the lowest humidity

(50–59%) was compared either to intermediate humidity (60–69%) or high hu

midity (70–79%). We did not observed statistically significant changes in the In

duction Ratio when 60–69% and 70–79% groups were compared. Statistical evalua

tion was based on one-way ANOVA with Satterthwaite correction for unequal variances, followed with multiple comparisons of mean Induction Ratios with p

values adjusted via simulation to keep an overall alpha—0.05.

studies are required to verify if indeed PM-induced DNA damage can contribute to the increased incidence of cancer especially in individuals whom are already predisposed to cancer by inherited mutations or oncogenic viruses.

5. Conclusions

The impact of the DWH oil spill extended beyond the damage of natural resources and called for comprehensive remediation, a conclusion that has prompted revision of current approaches that are mostly focused on calculating the damage and replacing value of individual resources. Possible health effects of the oil spill, both acute and chronic, are often neglected in these calculations, especially long-term health effects, which are still difficult to estimate. In this respect our current study provides a simple and low-cost approach of monitoring airborne PM genotoxicity as a possible surrogate marker in the assessment of health risks associated with oil spills.

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Human subjects

N/A.

Animal research

N/A.

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