4.3 Toxicity

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Executive Summary

The Trustees designed and implemented a comprehensive program to evaluate the toxic effects of *Deepwater Horizon* (DWH) oil on natural resources of the northern Gulf of Mexico. This program entailed performing a series of controlled laboratory studies that were designed to support the Trustees' resource and habitat-specific injury assessments. Through this comprehensive toxicity testing program, the Trustees created a body of information that greatly expands on the scientific literature available prior to the spill and provides an unprecedentedly large, coherent dataset from which conclusions about injury could be drawn.

Overall, the Trustees found that exposure to DWH oil causes a wide range of toxic effects to natural resources, including death, impaired reproduction, disease, and other physiological malfunctions that reduce the ability of organisms to survive and thrive. Measured and modeled concentrations of DWH oil in surface water and sediments in the Gulf of Mexico at a number of locations and times during and following the spill exceeded concentrations at which the Trustees documented toxicological effects from oil exposure in the laboratory.

As part of the evaluation of toxicity to **water column resources**, the Trustees exposed fish and invertebrates (both offshore and nearshore species) to DWH oil mixed into water and in surface sheens. The results of Trustee studies demonstrated that the embryos and larvae (i.e., early life stage) of fish (ichthyoplankton) and various stages of pelagic invertebrates (zooplankton) are particularly susceptible to the toxic effects of DWH oil. Measured and modeled concentrations of DWH oil in the Gulf of Mexico

exceeded lethal levels in a number of locations and times during and following the spill. Thin, rainbow sheens of surface slick oil were also found to be lethal to fish embryos and invertebrates. Oil is known to be more toxic in the presence of natural sunlight. The Trustees determined that DWH oil was roughly 10 to 100 times more toxic to semi-transparent invertebrates and early life-stage fish when exposed to ultraviolet (UV) light, an effect that is of particular concern for fish embryos and larvae at or near the surface of the water column. In addition to lethality, exposure to DWH oil also caused developmental abnormalities, growth inhibition, immunosuppression, and decreased swim performance. The lethal effect of DWH oil on the embryos and larvae of fish and several life stages of invertebrates has important ecological implications. In addition to sustaining fish and invertebrate populations, these small, planktonic organisms are an important base of the marine food web.

The evaluation of toxicity to **benthic resources** involved tests in which bottom-dwelling invertebrates were exposed to sediments contaminated with DWH oil. Testing demonstrated that exposure of amphipods to contaminated sediments resulted in mortality at concentrations observed in nearshore and deep-sea sediments following the spill.

The **nearshore resources** toxicity testing program involved studies on species selected to represent injury to the marsh faunal community and to serve as an overall indicator of adverse effects on nearshore marsh habitats. In addition to evaluating toxicity to water column resources described above, the Trustees evaluated: nearshore fish and invertebrate species exposed to contaminated sediment, nearshore species exposed to combinations of water and suspended sediment, and nearshore species exposed to contaminated marsh soil and vegetation.

Exposure of marsh organisms to sediments contaminated with DWH oil resulted in a series of adverse effects, including death, reduced growth, and reduced reproductive success. Higher concentrations of total polycyclic aromatic hydrocarbons (TPAH50; the sum of 50 individual PAHs measured) in sediments resulted in more severe adverse effects in more test species. Adverse effects were observed at concentrations as low as approximately 1 milligram of TPAH50 per kilogram of sediment (mg/kg). Toxicological responses to DWH oil observed in Trustee studies included damage to gill and liver tissues, reduced growth rates, and mortality in flounder; growth inhibition in juvenile red drum and Pacific white shrimp; reduced reproduction and survival in Gulf killifish; mortality to fiddler crab offspring exposed to relatively low concentrations of oil in or on sediments, when followed by exposure to sunlight; and increased mortality and an impaired ability to move away from oil in marsh periwinkles. Exposure to DWH oil caused adverse effects in all oyster life stages tested, at varying concentrations.

The Trustees performed studies on the effects of ingesting DWH oil to freshwater turtle species that could serve as surrogates for **sea turtles**. Test animals that ingested DWH oil exhibited alterations in multiple toxicity endpoints, such as oxidative damage, and DNA damage. The Trustees also observed evidence of dehydration, decreased digestive function, and poor absorption of nutrients.

In studies with cell lines and an experimental surrogate organism for **marine mammals**, exposure to DWH oil also was found to cause problems with the regulation of stress hormone secretion from adrenal cells and kidney cells (Gulf toadfish). Impacts such as these on the endocrine system will affect an animal's ability to regulate body functions, respond appropriately to stressful situations, and lead to reduced fitness.

There was a considerable degree of consistency among the types of toxic responses observed across the different organisms tested in the Trustees' toxicity testing program. For example, effects observed by the Trustees in laboratory studies using DWH oil included cardiac effects in both fish and birds; disruption of blood cells and function in fish and birds; evidence of oxidative damage in fish, birds, and turtles; and impairment of immune system function in fish and birds. Evidence of impairment to stress responses and adrenal function also was observed in fish, birds, and mammalian cells tested in the laboratory. Evidence of similar physiological impairments were observed in a number of different types of organisms in the wild that were exposed to DWH oil.

4.3.1 Introduction

Oil is known to be toxic to organisms. However, the toxicity of different oils can vary, and both the toxic effects and the concentrations of oil at which those effects occur can differ across species and types of exposures. To understand the toxic effects of *Deepwater Horizon* (DWH) oil exposure on the natural resources of the northern Gulf of Mexico, the Trustees conducted a series of controlled laboratory studies. These studies contributed to the Trustees' understanding of the range of natural resource injuries that occurred as a result of the spill, and informed the injury determination and injury quantification findings that are presented in the remainder of Chapter 4.

Section 4.3 describes the rationale (Section 4.3.1), design (Section 4.3.2), and results (Section 4.3.3) of the Trustees' toxicity testing program.

4.3.1.1 What Is Toxicity?

Toxicity refers to the nature and degree to which a chemical substance is poisonous to organisms. The toxicity of oil has been studied extensively, through both laboratory investigations and in response to oil spills (e.g., Douben 2003; Kingston 2002; Leighton 1993; Peterson 2001; Reynaud & Deschaux 2006; Teal & Howarth 1984). In many hundreds of published reports, oil has been shown to be toxic to fish (e.g., de Soysa et al. 2012; Hemmer et al. 2010), invertebrates (e.g., Baussant et al. 2011; Hannam et al. 2010), birds (e.g., Balseiro et al. 2005; Stubblefield et al.), mammals (e.g., Bowyer et al. 2003; Duffy et al. 1994), reptiles (e.g., Fritts & McGehee 1989; Lutcavage et al. 1995), plants (e.g., DeLaune et al. 2003; Ibemesim & Bamidele 2008), plankton (Bender et al. 1977; Gardiner et al. 2013), and bacteria (Fuller et al. 2004; Hodson et al. ; Suarez-Suarez et al.). Some of the toxic effects of oil include death (Aurand & Coelho 2005; Perkins et al. 2005); reduced growth rates (Barron et al. 1999; Cajaraville et al. 1992; Scarlett et al. 2007); impacts on tissues, such as lesions in the liver, skin, or elsewhere (e.g., Khan 2013; Lipscomb et al. 1993); developmental abnormalities and cardiac damage (e.g., de Soysa et al. 2012; Hatlen et al. 2010); reproductive impairment (Baussant et al. 2011; Truscott et al. 1992); immune effects, which can increase susceptibility to disease (Hannam et al. 2010; Payne & Fancey 1989); and cancer (Hawkins et al. ; Suchanek 1993).

Many of the original studies of oil toxicity focused on lethal effects to older organisms. More recently, scientists have found that oil causes a much wider variety of toxic effects, and that the early life stages of many animals (e.g., embryos and larvae for fish; eggs for birds and reptiles) are particularly sensitive to the toxic effects of oil (e.g., Carls et al. 1999; Colavecchia et al. ; Couillard & Leighton 1991; Finch et al. 2011). In aquatic habitats, this can have an especially significant effect on the ecosystem, since, in

addition to sustaining populations of fish and invertebrates, the food web depends upon early life-stage fish and other plankton (see Chapter 3).

When describing toxicity, scientists often refer to "lethal" and "sublethal" effects (Rand 1995). Lethal toxicity occurs when exposure to a chemical results in observable mortality (death) to an exposed animal. Sublethal toxicity refers to effects that do not result directly in observable death. However, sublethal toxic effects can shorten the life expectancy of organisms by reducing their overall health or "fitness." For example, animals whose fitness is compromised by sublethal toxic effects may 1) have more difficulty finding prey or avoiding predators, 2) exhibit greater susceptibility to disease, 3) demonstrate a reduced ability to tolerate natural stresses (such as elevated temperatures or reduced dissolved oxygen in water), or 4) have more difficulties reproducing. In the wild, organisms whose fitness is compromised are more likely to die (Rice 2014).

In addition to its chemical toxic effects, oil can also harm organisms through physical fouling (Fowler et al. 1995; Hurst et al. 1991; Pezeshki et al. 2000). Fouling refers to the physical coating of oil on an organism and is not strictly the same as "toxicity" because it does not involve the chemical interaction of oil compounds with physiological processes. Nonetheless, fouling can be lethal to organisms and can cause a range of effects such as smothering (lack of oxygen), clogging tissues (e.g., eyes, nasal cavities), loss of insulation from feathers (which can result in death from hypothermia), or impaired movement (inefficient flight or swimming). Fouling resulted in mortality to birds and turtles and other taxa (see Section 4.7, Birds, and Section 4.8, Sea Turtles).

4.3.1.2 What Factors Influence the Toxic Effects of Oil?

A number of different factors can influence the toxicity of oil. Animals can be affected in different ways and at different oil concentrations, depending on their physiology, behavior, and life history. For example, the effects of oil on fish, which "breathe" through the passage of water over their gills, can differ from the effects of oil on dolphins, which, like humans, have lungs and must surface to breathe air. Some of the factors that influence the toxic effects of oil and were considered by the Trustees are described below. Section 4.3.2, Approach to the Assessment, discusses how these different factors were addressed in the Trustees' toxicity testing program.

4.3.1.2.1 Species and Life Stage

The toxic effects of oil and the concentrations at which those effects occur can vary considerably between species. These variations can be caused by differences in anatomy and physiology, metabolism, and how an organism is exposed to a chemical [for example, gill exposure, inhalation, or ingestion (Rand 1995)]. The Trustees evaluated toxicity across a suite of representative organisms that utilize the different habitats of the northern Gulf of Mexico, including animals that live in the open ocean and in nearshore waters, animals that live in and on bottom sediments, and animals that rely on important shoreline habitats such as intertidal marshes. In addition, the Trustees considered the life stage and life history of organisms that were exposed in these habitats by including evaluations of the toxicity of oil to embryonic, juvenile, and adult life stages.

4.3.1.2.2 Chemical Composition of Oil

As described in Section 4.2 (Exposure), oil is a complex mixture of chemicals, and this chemical mixture, or composition, can change in the environment through the process of weathering (Morris et al. 2015c). Although the toxicity of oil results from exposure to this complex chemical mixture, many scientists evaluate oil toxicity based on the concentration of polycyclic aromatic hydrocarbons (PAHs), a group of chemical compounds that are known to be among the most toxic components of oil (Box 1). The Trustees generally have employed this convention in evaluating laboratory and field data, but recognize that oil toxicity is caused by a more complex mixture of chemicals.

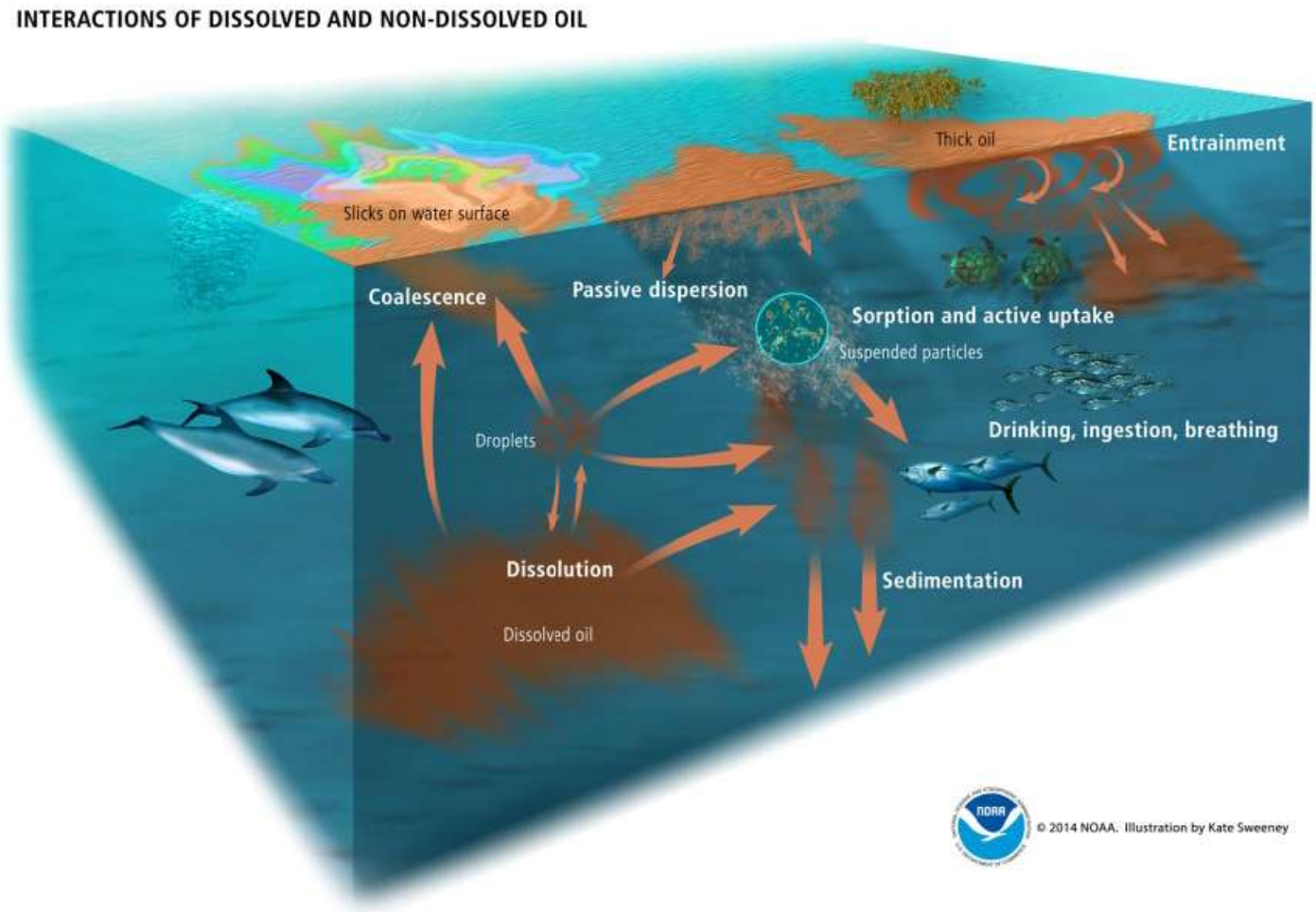
Because it is such a complex mixture of chemicals, the toxicity of different oils can vary, and the toxicity of a single type of oil (such as DWH oil) can change based on weathering and other environmental factors (NRC 2003). The Trustees evaluated the toxicity of DWH oils that were collected from the environment after the spill, including evaluating the relative toxicity of different field-collected oils that covered a range of weathering conditions (Morris et al. 2015c).

4.3.1.2.3 Oil Mixing, Dispersion, and Partitioning

Aquatic organisms in the Gulf of Mexico were exposed to DWH oil that was present as slicks floating on the surface of the water, oil that was mixed with and dissolved into water, and oil that was associated with sediments and organic material (Figure 4.3-1). The toxic effects of oil in water can be influenced by how the oil mixes and dissolves into water, and how it partitions between small droplets, water, and sediment (Payne et al. 2003). Oil-water dispersions can be natural, such as the formation of droplets of oil through physical action [e.g., wave action or the violent release of DWH oil from the well (Delvigne & Sweeney 1988)], or can be created by application of dispersant chemicals (NRC 2005).

Box 1. Why Are Polycyclic Aromatic Hydrocarbons (PAHs) Important for Toxicity?

Oil is composed of thousands of chemical compounds and the composition of the oil can affect its toxicity. Polycyclic aromatic hydrocarbons (PAHs) are some of the most toxic components of oil and have been the subject of extensive study over the past several decades (Adams et al. 2014; Billiard et al. 2008; Douben 2003; NRC 2003). Toxicologists often represent the toxicity of different oils in terms of the summed concentrations of many individual PAHs (e.g., EPA 2003; Incardona et al. 2014; NOAA 1991). Although this measure should not imply that PAHs are the only toxic components of oil, the Trustees largely have employed this convention in their chemical analyses of samples from the field and laboratory, particularly when evaluating potential toxicity. The specific measurement adopted in the Natural Resource Damage Assessment (NRDA) describes concentrations in terms of the sum of 50 individual PAH compounds (Forth et al. 2015). This value is referred to as total PAH50 (referring to the 50 individual PAHs measured), or TPAH50.



Source: *Kate Sweeney for NOAA.*

Figure 4.3-1. Natural resources of the Gulf of Mexico were exposed to oil floating on the surface of the water, mixed into the water through dispersion and natural mixing/dissolution processes, and suspended and bottom sediments.

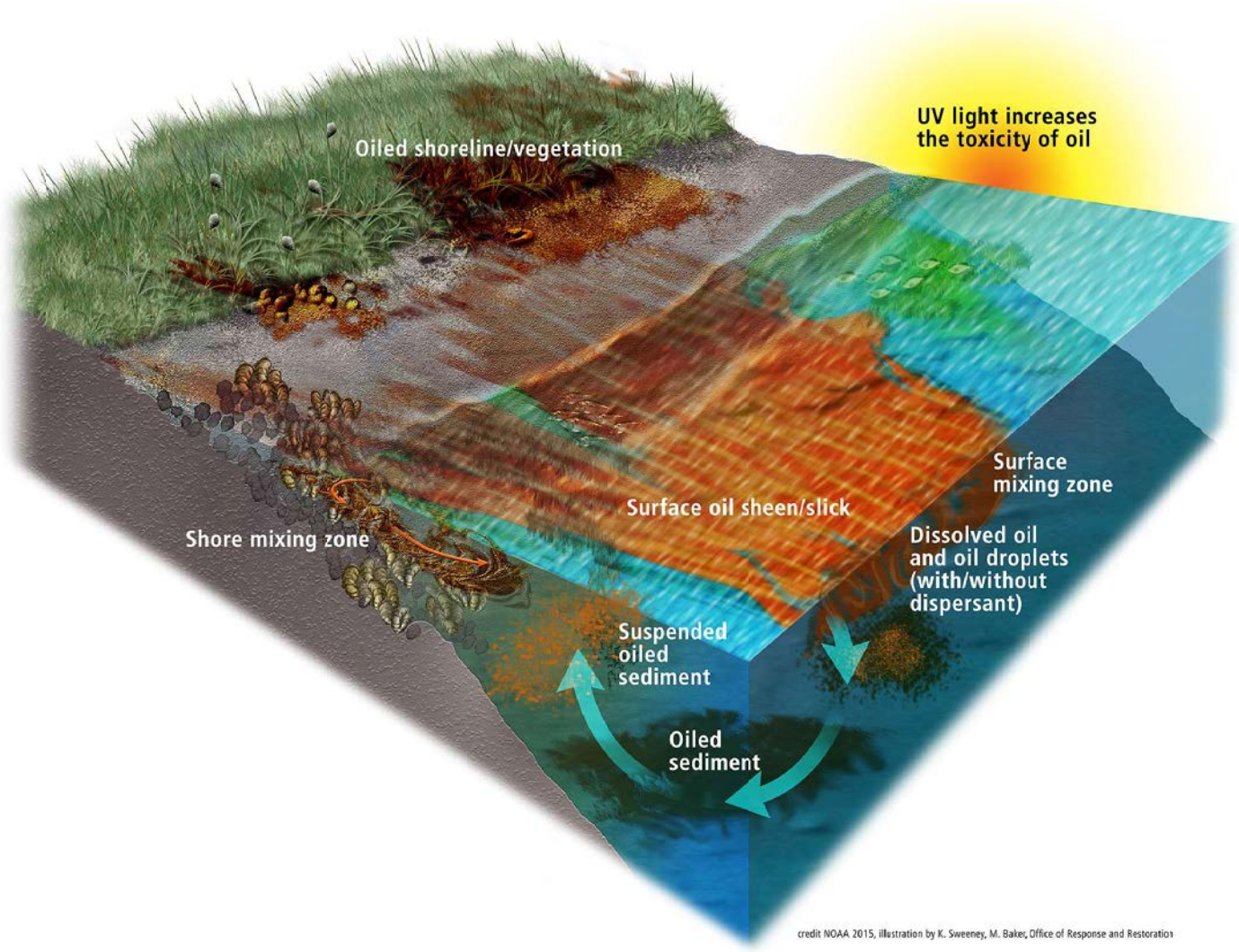
Chemical oil dispersants are mixtures of solvents (like nail polish remover), surfactants (like soaps), and other additives that are applied to oil slicks in order to break up the slick and mix the oil into the water column (Section 4.2, Natural Resource Exposure). Although dispersants are used to reduce the amount of thick, floating oil that can reach sensitive shoreline habitats, adding dispersants can increase the toxicity of oil to aquatic organisms by mixing more of it into the water column (NRC 2005). The Trustees' toxicity evaluation considered these different types of exposures to aquatic resources by evaluating the toxicity of different oil-water mixtures, as well as the toxicity of oil with added chemical dispersants (H.P. Forth et al. 2015; Morris et al. 2015b). Dispersants were used during the spill at depth near the wellhead and in offshore waters (see Section 4.2, Exposure).

4.3.1.2.4 Sunlight (Photo-Induced Toxicity)

When organisms are exposed to oil, the toxicity of the oil can increase substantially in the presence of natural sunlight (Oris & Giesy 1985; Sellin Jeffries et al. 2013). Known as photo-induced toxicity, this occurs because some PAH compounds in oil absorb ultraviolet (UV) light from the sun and produce toxic by-products that can damage DNA, cell membranes, and other tissues (Arfsten et al. 1996). This reaction occurs in transparent tissues, such as gills, and in transparent organisms (Arfsten et al. 1996; Oris & Giesy 1985) such as the embryos and larvae of many fish and shellfish. The Trustees evaluated the toxicity of DWH oil with and without UV light to represent the range of conditions that occur in the environment.

4.3.1.2.5 Exposure Pathways

The natural resources of the northern Gulf of Mexico were exposed to oil across a wide variety of habitats, and through a number of different exposure pathways (Section 4.2). Plants and animals were exposed to oil floating on the ocean surface, mixed in the water column, mixed in sediments and marsh soils, and on plants (Figure 4.3-2). As described in greater detail in the following sections of Chapter 4, animals were exposed to oil through breathing (including exposure through gills and lungs); inhalation and aspiration of oil into lungs; drinking and incidental intake of water; ingestion of sediment and food; and through physical contact (Figure 4.3-3). The Trustees designed studies to consider these different pathways and types of exposure.



Source: *Kate Sweeney for NOAA.*

Figure 4.3-2. The Trustees' toxicity testing evaluated the effects of DWH oil across a variety of habitats.

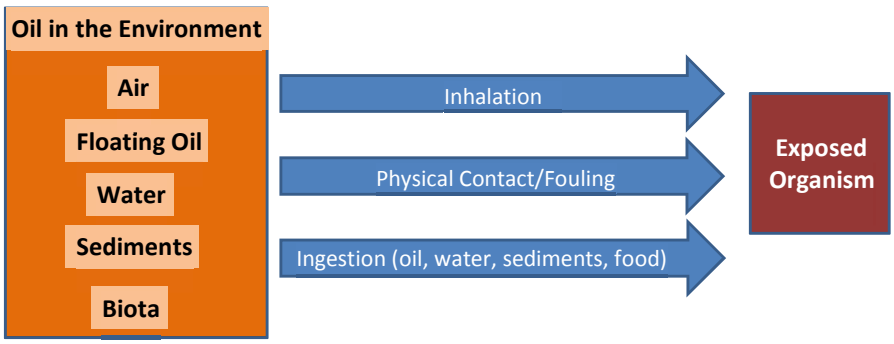


Figure 4.3-3. Potential pathways of exposure of organisms to DWH oil. Animals may be exposed through one or more of these pathways.

4.3.2 Approach to the Assessment

The Trustees' assessment approach involved performing a series of controlled laboratory studies that were designed to support the Trustees' resource and habitat-specific injury determination and quantification. As illustrated in Figure 4.3-4, the Trustees' toxicity testing program comprised studies designed to evaluate the toxicity of DWH oil for the following resource categories and exposure pathways in the injury assessment:

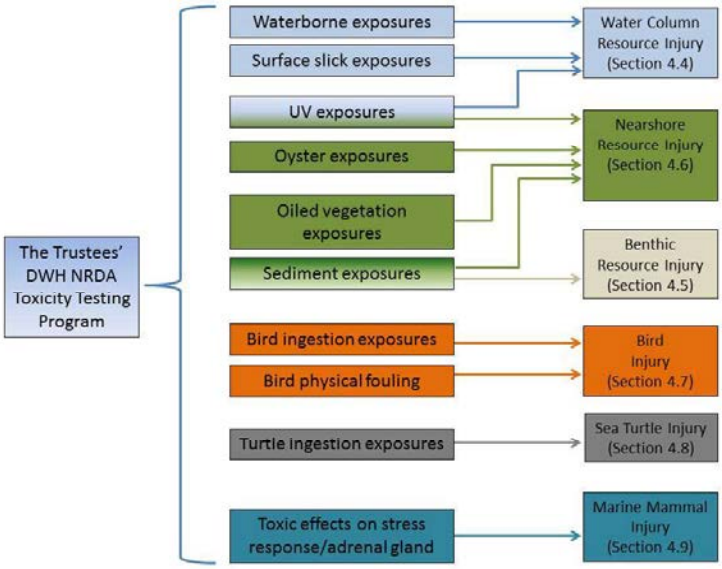


Figure 4.3-4. Overview of the Trustees' toxicity testing program showing the relationship between laboratory testing and resource-specific injury assessment.

- **Water column resources (Section 4.4)**, including fish and invertebrates found in offshore and nearshore areas. As part of the evaluation of toxicity to water column resources, the Trustees exposed fish and invertebrates to DWH oil mixed into water and in surface slicks.
- **Benthic resources (Section 4.5 and 4.6)**, specifically benthic invertebrates. The Trustees exposed bottom-dwelling invertebrates to sediments contaminated with DWH oil. The Trustees also evaluated the toxicity of oil to other bottom-dwelling organisms, including fish, oysters, and crustaceans as part of the nearshore resource toxicity testing work.
- **Nearshore resources (Section 4.6)**, including fish, crustaceans and other invertebrates, oysters, and snails. The Trustees exposed resources that live in nearshore habitats to DWH oil in sediments, and on marsh vegetation.

- **Birds (Section 4.7)**. The Trustees performed focused studies to evaluate the toxicity of DWH oil to birds when ingested and when externally exposed on feathers.
- **Sea turtles (Section 4.8)**. The Trustees performed studies on the effects of ingesting DWH oil to turtle species that could serve as surrogates for sea turtles.
- **Marine mammals (Section 4.9)**. To support evaluation of field data collected from bottlenose dolphins, the Trustees performed limited and focused laboratory studies using a mammalian cell line culture to evaluate the effects of DWH oil on stress response and adrenal gland function.

The Trustees designed their toxicity testing program to investigate the nature and extent of different types of adverse impacts to a variety of organisms based on observed, measured, and modeled exposure to oil and dispersant. The testing program was designed to address different types of exposures to DWH oil (e.g., exposure to weathered oil, to dispersed oil, to oil-water mixtures, to surface slicks, and to sediments), different environmental variables that can influence toxicity (primarily UV light), different test species, different life stages, and a series of different lethal and sublethal effects endpoints (Figure 4.3-5). To address the role of weathering on toxicity, a range of weathered DWH oils were used in laboratory tests (Box 2). Through this comprehensive toxicity testing program, the Trustees created a body of information that greatly expands on the scientific literature available prior to the spill and provides an unprecedentedly large, coherent dataset from which conclusions about injury could be drawn.

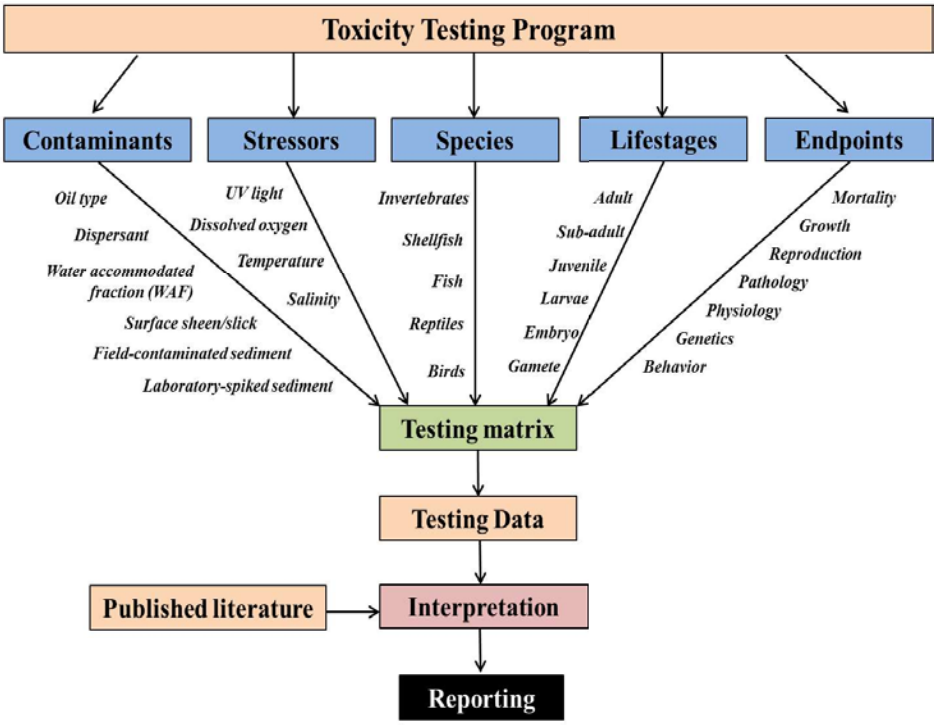


Figure 4.3-5. The Trustees' toxicity testing program was designed to evaluate different types of exposure to DWH oil, different environmental variables, a variety of Gulf of Mexico species and life stages, and a series of lethal and sublethal toxicological effects endpoints.

Box 2: What Types of Oil Were Used in the Toxicity Tests?

To better understand the influence of weathering on toxicity, four DWH oil samples at varying degrees of weathering were included in the toxicity testing program (H.P. Forth et al. 2015). The percent depletion of TPAH relative to a stable marker compound, hopane, is used as an indicator of the relative degree of weathering, and is generally presented as a percent (Morris et al. 2015c). The four oils tested were:

- "Source oil": *Deepwater Horizon* (BP's Macondo well) riser oil collected on July 26, 2010. This oil was almost entirely unweathered (8 percent weathered).
- "Artificially weathered oil": Source oil heated in a laboratory, removing the lightest components of the oil (e.g., volatile components like BTEX), in order to represent a slightly weathered oil (27 percent weathered).
- "Slick A": Surface slick oil collected on July 29, 2010, from the hold of a barge that was receiving oil from various skimmer vessels responding to the spill and therefore represents a natural degree of weathering that occurred in the Gulf environment (68 percent weathered).
- "Slick B": Surface slick oil collected on July 19, 2010, by a skimmer vessel near the Mississippi River Delta. This oil represents a higher degree of weathering that occurred naturally in the environment (85 percent weathered).

The Trustees' toxicity testing program evaluated a variety of species and included a series of laboratory tests (or "bioassays").

- **Test species.** Because it is impractical to test every species of organism that was exposed to DWH oil, the Trustees' testing program focused on using representative Gulf of Mexico species. The Trustees selected species for testing that are native to the northern Gulf of Mexico, could serve as example species from which generalizable inferences can be drawn (i.e., species whose physiology and life histories are representative of many species, or that occur in multiple habitats), could be tested in a laboratory setting, and play important or unique roles in the Gulf of Mexico ecosystem and/or economy. In some cases, tests were conducted with organisms closely related to Gulf of Mexico species. The Trustees conducted these tests using surrogate species to support evaluation of the toxicity of DWH oil to animals that cannot be tested in the laboratory (e.g., endangered sea turtles), or to gain a more mechanistic understanding of the toxic effects of DWH oil to facilitate broader inferences across different types of organisms. As shown in Table 4.3-1, the Trustees' toxicity testing program included 21 different species of fish and 12 species of invertebrates, as well as phytoplankton, freshwater turtles (as surrogates for sea turtles), and four different species of birds. The table also identifies the type(s) of exposures used in evaluating toxicity for each test species.
- **Toxicity testing procedures: bioassays.** Scientists typically evaluate the toxicity of environmental samples or chemicals by exposing test organisms to a range of concentrations under controlled conditions (Rand 1995). Such tests are often referred to as bioassays. Data generated from these tests are used by scientists to determine the types of adverse effects that

occur at different oil concentrations, under specific exposure conditions. The way that scientists often evaluate these adverse effects concentrations is through the determination of "dose-response relationships," which show the relationship between the concentration of a toxicant and the degree of adverse effects (Box 3). To simplify comparative analysis across bioassays, scientists refer to the toxic concentrations of a contaminant that cause a specific amount of some adverse effect, such as the concentration of oil that causes mortality to 50 percent of the test organisms exposed in a given study. These effects levels are known as "lethal concentration" (LC), or more broadly, "effects concentration" (EC) values. Using a standardized effects level such as an LC50 or an LC20 (that is, the concentration of a chemical that causes mortality to 50 percent or 20 percent of the test organisms, respectively) enables easier comparison between studies. For example, the relative sensitivity of two different species may be evaluated by comparing their respective LC50s or LC20s. It should be emphasized, however, that these effects concentrations do not represent "thresholds" for the onset of toxicity.

Table 4.3-1. Species and exposures included in the Trustees' toxicity testing program.

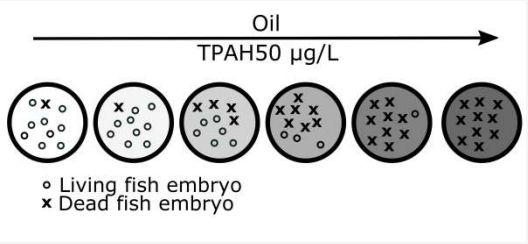
Species	Scientific Name	Exposure				
		WAF[a]/ Surface Slick	Sediment/ Oiled Substrate	Dietary	Dermal	UV[d]
Fish						
Atlantic croaker	*Micropogonias undulates*	X				
Bay anchovy	*Anchoa mitchilli*	X				X
Cobia	*Rachycentron canadum*	X				
Gulf killifish	*Fundulus grandis*	X	X			X
Gulf menhaden	*Brevoortia patronus*	X				
Gulf toadfish	*Opsanus beta*	X				
Inland silverside	*Menidia beryllina*	X				
Mahi-mahi	*Coryphaena hippurus*	X				X
Pacific bluefin tuna[b]	*Thunnus orientalis*	X				
Pacific mackerel[b]	*Scomber japonicus*	X				
Red drum	*Sciaenops ocellatus*	X	X			X
Red snapper	*Lutjanus campechanus*	X				X
Sand seatrout	*Cynoscion arenarius*	X				
Sheepshead minnow	*Cyprinidon variegatus*	X				X
Shovelnose sturgeon[b]	*Scaphirhynchus platorynchus*	X				
Southern bluefin tuna[b]	*Thunnus maccoyii*	X				
Southern flounder	*Paralichthys lethostigma*	X	X			
Speckled sea trout[c]	*Cynoscion nebulosus*	X				X
Yellowfin tuna	*Thunnus albacares*	X				
Yellowtail amberjack[b]	*Seriola lalandi*	X				
Zebrafish[b]	*Danio rerio*	X				

Species	Scientific Name	Exposure				
		WAF[a]/ Surface Slick	Sediment/ Oiled Substrate	Dietary	Dermal	UV[d]
Invertebrates						
Amphipod	*Leptocheirus plumulosus*		X			
Blue crab	*Callinectes sapidus*	X	X			X
Brown shrimp	*Farfantepenaeus aztecus*		X			
Copepod	*Acartia tonsa*	X				X
Eastern oyster	*Crassostrea virginica*	X	X	X		X
Fiddler crab	*Uca longisignalis*	X	X			X
Fiddler crab	*Uca minax*		X			
Grass shrimp	*Palaemonetes pugio*	X	X			X
Marsh periwinkle	*Littoraria irrorata*		X			
Mysid shrimp	*Americamysis bahia*	X				X
White shrimp	*Litopenaeus setiferus*	X	X			
Pacific white shrimp[b]	*Litopenaeus vannamei*	X	X			X
Phytoplankton						
Diatom	*Skeletonema costatum*	X				
Reptile						
Common snapping turtle[b]	*Chelydra serpentina*			X		
Red-eared slider[b]	*Trachemys scripta elegans*			X		
Birds						
Double-crested cormorant	*Phalacocorax auritus*			X	X	
Homing pigeon[b]	*Columba livia*			X	X	
Laughing gull	*Leucophaeus atricilla*			X		
Western sandpiper	*Calidris mauri*			X	X	

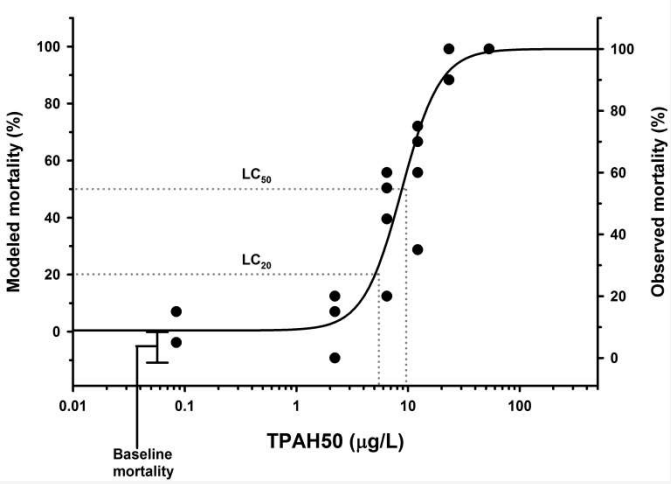
[a] *Water accommodated fraction.*
[b] *Surrogate species.*
[c] *Many common names for this species, including spotted seatrout.*
[d] *Ultraviolet light exposure in addition to oil exposure.*

Box 3: What Is a Toxicity Test and How Is Toxicity Measured?

A toxicity test, or bioassay, is a controlled study in which the lethal or sublethal effects of a chemical are evaluated in test organisms (Rand 1995). One common approach to toxicity testing is to place a similar number of organisms into replicate exposure chambers (such as tanks or beakers) that have different concentrations of oil. An unoiled "control" treatment is used to quantify the measured effect under the conditions of the test, but without oil. After exposing the organisms to oil for a given period of time, scientists determine the frequency or degree of adverse effects, such as the number of living and dead organisms in each exposure chamber.

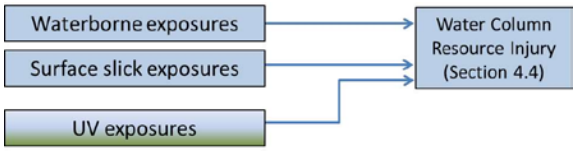


Data from a toxicity test then are compiled to evaluate the dose-response relationship between the exposure concentration, such as the concentration of TPAH50 in water, and the degree of effect, such as the percent of test organisms killed by the exposure.



The figure to the left shows an example of a dose-response curve derived from one of the Trustees' toxicity tests (Morris et al. 2015b). For this toxicity test, mahi-mahi embryos were exposed to increasing concentrations of oil, shown in terms of the TPAH50 concentration in micrograms of oil per liter of water (µg/L). The dose-response curve shows the relationship between TPAH50 and mortality based on the experimental data, illustrating that greater concentrations of TPAH caused higher mortality rates. LC50 and LC20 values are shown as the points on the horizontal axis that are associated with 50 percent and 20 percent mortality.

4.3.2.1 Water Column Resource Toxicity Testing



The water column resource toxicity testing program focused on evaluating the toxicity of DWH oil–with and without added dispersant–to fish, invertebrates, and diatoms, a kind of phytoplankton (see Table 4.3-1). Test organisms were exposed to oil mixed in water and as surface slicks. Because of the important role that natural sunlight plays in enhancing the toxicity of PAH, extensive testing was performed to evaluate the degree of this photo-induced toxicity.

Toxicity tests included studies to determine the concentrations of oil that kill organisms, as well as studies to determine concentrations of oil that cause adverse effects on the health or viability of

organisms. In addition to mortality, the Trustees' toxicity tests documented the following adverse effects on health and viability:

- Impaired early life-stage growth and development (Brown-Peterson et al. 2015; Incardona et al. 2014; Incardona et al. 2013; Lay et al. 2015b; Morris et al. 2015b)
- Impaired reproductive success (Morris et al. 2015b; Vignier et al. [In Press])
- Impaired cardiac development and function (Bursian et al. 2015b; Bursian et al. 2015c; Dorr et al. 2015; Incardona et al. 2014; Incardona et al. 2013; Morris et al. 2015b)
- Reduced immune system function and increased susceptibility to disease (Morris et al. 2015b; Ortell et al. 2015)
- Biochemical, cellular, and genetic alterations, and adverse changes to organ tissue (Brown-Peterson et al. 2015; Bursian et al. 2015b; Bursian et al. 2015c; Dorr et al. 2015; Morris et al. 2015b; Takeshita et al. 2015)

For purposes of the summary provided in this draft PDARP/PEIS, the Trustees' focus is primarily on the results of tests incorporated directly in the resource-specific injury determination and quantification presented in the following sections of this chapter. More detailed information is provided in related technical appendices and publications contained in the References section.

Prior to initiating the testing program, the Trustees developed a standardized set of methods and procedures to ensure consistency across the testing laboratories, and to ensure that quality assurance and quality control (QA/QC) was maintained. These efforts included using the same oil samples for testing, creating exposure solutions using the same methods, reviewing testing plans and procedures prior to conducting each test, collecting data in a standardized format, and implementing a detailed data validation and verification process (Morris et al. 2015b).

Toxicity tests with water column organisms included bioassays performed with oil-water mixtures and tests in which organisms were exposed to surface slicks. For oil-water mixtures, the Trustees developed a suite of methods to achieve a range of chemical concentrations and compositions similar to the range of conditions that organisms would have encountered in the ocean, but in a standardized manner that could be easily replicated by different laboratories conducting toxicity studies (Box 4). By using four different oils (Box 2) and three different mixing methods, the Trustees were able to develop test solutions that captured variability in conditions encountered in the environment and bolstered our understanding of oil toxicity across that range of conditions (H.P. Forth et al. 2015; Incardona et al. 2013).

Box 4: Producing Oil-Water Mixtures for Toxicity Testing

During the *Deepwater Horizon* spill, oil mixed with water in the environment in a variety of ways, including high energy mixing near the wellhead, through wave action at the ocean surface, and through application of chemical dispersants. While toxicity testing laboratory procedures do not need to mimic how oil mixed into water in the environment, toxicologists do want to test solutions with similar chemical compositions and concentrations to those found in the environment. Three methods were used to create oil-water mixtures, known as water accommodated fractions or WAFs: a low-energy mixing procedure (LEWAF); a high-energy mixing procedure (HEWAF); and a medium-energy, chemically enhanced mixing procedure (CEWAF) (H.P. Forth et al. 2015; Morris et al. 2015b). Researchers have used LEWAFs and CEWAFs for many years (Aurand & Coelho 2005; H.P. Forth et al. 2015; Singer et al. 2000), and these mixing methods produce solutions with chemical compositions similar to those measured in the field (H.P. Forth et al. 2015). Although HEWAFs have also been used in the field of petroleum toxicology for many years (Aurand & Coelho 2005; Echols et al. 2015; Girling 1989; Incardona et al. 2014), the Trustees developed a standardized blender-mixing method to achieve better consistency across test solutions and laboratories (H.P. Forth et al. 2015; Incardona et al. 2014; Morris et al. 2015b). Although DWH oil did not mix into the ocean like a blender, the method was used to prepare reproducible oil-water mixtures with chemical compositions and concentrations that were also similar to those measured in the environment (H.P. Forth et al. 2015).

In addition to tests with oil mixed into water, the Trustees also conducted a series of toxicity tests on different fish and invertebrate species using very thin surface slicks or sheens similar to the surface slicks that covered large expanses of the Gulf of Mexico during and following the spill (Figure 4.3-6).

4.3.2.1.1 Photo-Induced Toxicity Testing

As described previously, photo-induced toxicity can increase the toxicity of oil (Alloy et al. 2015; Oris & Giesy 1985; Sellin Jeffries et al. 2013). This reaction is important because, in the Gulf of Mexico, many developing fish and invertebrates live in the upper water column near the surface of the ocean where they are exposed to UV light (see Section 4.4).

The Trustees evaluated the UV photo-induced toxicity of DWH oil in a number of controlled tests using both natural sunlight and artificial lights that produced UV exposures similar to those encountered in nature (Box 5).

Source: *Abt Associates (top), NOAA (bottom).*

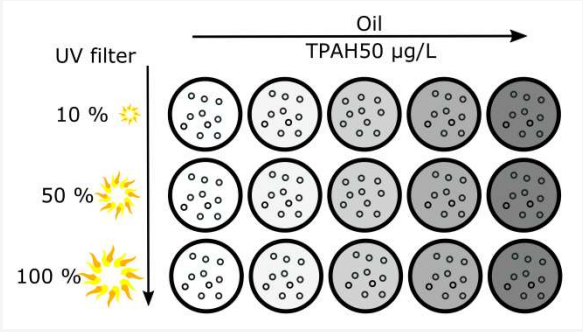
Figure 4.3-6. *Top*: Thin oil sheen [about 1 micron (µm) in thickness – see Forth et al. 2015 TR.09] generated in a beaker using DWH oil (Slick A). This oil, visible as a slightly reflective sheen on the water surface, was used to conduct bioassays with fish and invertebrates. *Bottom*: DWH oil sheen in the northern Gulf of Mexico, photographed from an airplane.

Box 5: What Are Photo-Induced Toxicity Tests?

The upper 15 to 30 meters of the water column in the northern Gulf of Mexico are clear enough that UV light penetrates at intensities sufficient to cause photo-induced toxicity (Lay et al. 2015a). When UV light is absorbed by PAHs in the tissues of semi-transparent organisms, damaging by-products are produced that can destroy tissues and cells. Thus, during the *Deepwater Horizon* oil spill, semi-transparent organisms that came into contact with oil and UV in both offshore and nearshore environments would be susceptible to photo-induced toxicity. The Trustees carried out a series of toxicity tests to determine how much more toxic DWH oil became in the presence of UV light.

A typical bioassay included 2 to 8 hours of exposure to waterborne (WAF) or surface slick oil followed by or in conjunction with 6 to 9 hours of exposure to outdoor sunlight or UV light generated with special light bulbs in a laboratory. Filters were used to vary the amount of UV exposure to test organisms.

The amount of sunshine can vary from day to day depending on weather and time of day during summers in the northern Gulf of Mexico. Therefore, researchers measured the amount of UV light exposure experienced by organisms during each test (Lay et al. 2015b) for comparison to field measurements to enable quantification of photo-induced toxic effects in the environment.

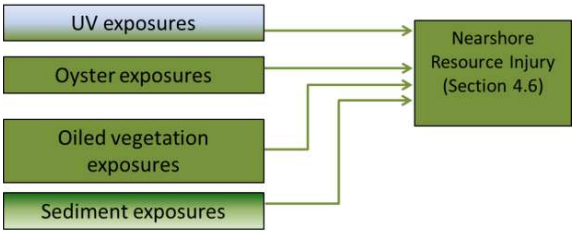


4.3.2.2 Benthic Resource Toxicity Testing

The benthic resource toxicity testing focused on evaluating the toxicity of sediments contaminated with DWH oil to bottom-dwelling invertebrates (Table 4.3-1). Extensive areas of bottom sediment were contaminated with DWH oil in nearshore and deep-sea habitats (see Section 4.2, Exposure, and Section 4.5, Benthic Resources). Therefore, bioassays used contaminated sediment collected from coastal areas of the northern Gulf of Mexico that experienced heavy oiling, as well as adding varying amounts of DWH oil to clean sediments collected from the field (Krasnec et al. 2015a). Additionally, BP conducted bioassays using contaminated sediment collected from deep-sea habitats, which are discussed in Section 4.3.3.2 (Krasnec et al. 2015b). In addition to being used for the benthic resource injury assessment, sediment toxicity testing also was used in the nearshore resource assessment, as discussed below.

4.3.2.3 Nearshore Resource Toxicity Testing

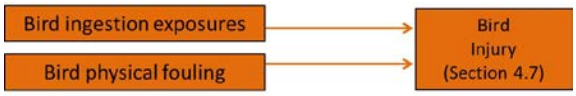
As part of the nearshore resource injury assessment (Section 4.6), the Trustees studied the effects of DWH oiling on marsh species (Table 4.3-1) that were selected to represent injury to the marsh faunal community more broadly, and as an overall indicator of adverse effects on nearshore marsh habitats.



In addition to evaluating toxicity to water column resources (Section 4.3.2.1), the Trustees evaluated nearshore species exposed to contaminated sediment, combinations of water and suspended sediment, and contaminated marsh soil and vegetation. The nearshore studies included evaluating the toxicity of DWH oil to Gulf sturgeon because this species migrates through nearshore areas when they move from salt water into coastal rivers to spawn. Nearshore studies also included tests to evaluate UV effects.

4.3.2.4 Bird Toxicity Testing

The Trustees conducted a series of focused laboratory toxicity studies to evaluate the toxicity of ingested oil, and to evaluate the implications of physical fouling of feathers.



4.3.2.4.1 Evaluating Effects of Ingested Oil

Deepwater Horizon response workers documented the presence of thousands of dead birds during and after the spill (see Section 4.7, Birds). Field surveys also documented the occurrence of thousands of oiled birds that were not sufficiently impaired to allow their capture and cleaning by oil spill responders (Section 4.7). When birds preen to clean oil off their feathers, they inevitably swallow oil. In order to assess the toxic effects of ingested oil on Gulf of Mexico bird species, the Trustees conducted a series of tests using representative and surrogate bird species, such as double-crested cormorants, spotted sandpipers, laughing gulls, and homing rock pigeons. Oil ingestion studies for gulls and cormorants were performed by injecting dead fish with DWH oil and feeding the contaminated fish to birds. As described later in this section, when birds ingested oil-contaminated fish, they suffered from a variety of adverse health effects, including anemia, liver dysfunction, kidney damage, hypothermia, weight loss, lethargy, abnormal feces, feather damage, heart abnormalities, moribundity (near death), and death (Bursian et al. 2015b; Bursian et al. 2015c; Dorr et al. 2015).

4.3.2.4.2 Evaluating Physical Fouling Effects of Oil

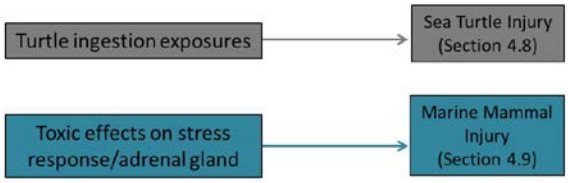
In addition to its chemical toxicity, the viscous, sticky nature of oil can adversely affect the ability of birds to take off, fly, and follow efficient flight paths. A wind tunnel was used to measure how oil on the body (not the wings) affects flight energetics and flight ability for western sandpipers (Maggini et al. 2015). The Trustees also employed high-speed video to determine how trace levels of oil on wing and tail feathers affects the speed and angle of a bird's takeoff movements, and how that affected flight energy costs.

To further investigate how DWH oil affects a bird's ability to fly in a natural environment, the Trustees assessed the effects of externally applied oil on the field-based flight performance of homing pigeons.

The Trustees compared the flight performance of individual homing pigeons before and after oiling over trips of 50, 85, and 100 miles (from the release site to their home loft). Studies also were designed to observe adverse health effects in oiled animals, including effects on body mass and effects on organ function (Pritsos et al. 2015).

4.3.2.5 Sea Turtles and Marine Mammals Toxicity Testing

The Trustees' injury assessment of sea turtles and marine mammals included very limited toxicity testing because these organisms are federally protected.



4.3.2.5.1 Sea Turtles

During the spill, response workers collected many oiled sea turtles from the Gulf of Mexico. Sea turtles had DWH oil covering their bodies and coating their esophagi, and this physical fouling with heavy oil was associated with mortality (Section 4.8, Sea Turtles). To help investigators evaluate the toxicity of ingested oil on sea turtles, the Trustees conducted a limited series of tests with surrogate turtle species (red-eared sliders and snapping turtles), in which animals were exposed to DWH oil to emulate ingestion exposures. Testing focused on the evaluation of sublethal effects on health and fitness (Mitchelmore & Rowe 2015).

4.3.2.5.2 Marine Mammals

As described in Section 4.9 (Marine Mammals), field studies demonstrated that coastal bottlenose dolphins suffered from adrenal gland disease and dysfunction as a result of the *Deepwater Horizon* spill. As additional support in evaluating the physiological mechanisms associated with this injury, a limited series of laboratory studies was performed with mammalian adrenal cell lines to study cellular dysfunction (Takeshita et al. 2015). In addition, studies were performed with the Gulf toadfish to further evaluate the effects of DWH oil on the release of stress response hormones which are related to adrenal function (Morris et al. 2015b).

4.3.3 Toxic Effects of DWH Oil

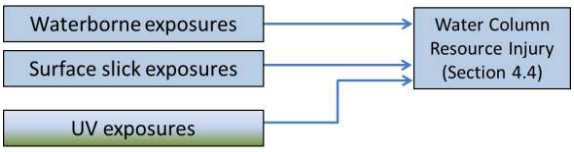
This section summarizes important findings from the Trustees' toxicity testing program. This summary emphasizes toxicity testing data used directly in the resource-specific sections of this chapter. A considerable amount of other supporting toxicity data was developed through the comprehensive testing program (see technical appendices and published manuscripts included in the References section). Although not all these data were included directly in the resource-specific injury determinations or quantifications, the data and findings informed the Trustees' decision-making regarding the nature and scope of injuries, and will help inform future restoration planning efforts. Finally, the data developed through this program contribute substantially to the Trustees' understanding of the toxicological effects of oil and the susceptibility of Gulf of Mexico species to oil pollution.

4.3.3.1 Toxicity of DWH Oil to Water Column Resources

Key Findings

- Fish embryos and larvae and invertebrates are particularly susceptible to the toxic effects of DWH oil, both when mixed with water and when present in the form of a surface slick. Measured and modeled concentrations of DWH oil in the Gulf of Mexico exceeded lethal levels in a number of locations and times during and following the spill.
- Thin, rainbow sheens of surface slick were lethal to developing fish and invertebrates.
- DWH oil is roughly 10 to 100 times more toxic to invertebrates and developing fish in the presence of natural sunlight.
- In addition to lethality, exposure to DWH oil causes developmental abnormalities, including heart and spinal defects. Many of these developmental abnormalities are severe enough to kill early life-stage fish.
- Older fish (juveniles or adults) are less susceptible than embryos and larvae to lethal effects of DWH oil exposure. At most of the oil concentrations that occurred after the spill, the toxic effects of oil on older fish are more likely to have manifested as sublethal injuries, including growth inhibition, immunosuppression, decreased swim performance, and an abnormal stress response.
- The lethal effect of DWH oil on fish embryos and larvae and invertebrates has important ecological implications. In addition to sustaining fish and invertebrate populations, these small, planktonic organisms are an important base of the marine food web.

4.3.3.1.1 Lethality



Oil causes a range of adverse effects to organisms. Short-term lethality, sometimes referred to as "acute lethality" is the most severe of those effects. The Trustees conducted extensive laboratory testing to evaluate the concentrations of oil that cause mortality. Lethal responses were used for purposes of quantifying water column resource injuries (Section 4.4). However, sublethal toxicity occurs at lower oil concentrations than mortality and the sublethal responses observed in the laboratory can result in reduced survival and reproduction in the wild. Consequently, lethality-based injury quantification underestimates the full scope of injuries.

Toxicity of Oil-Water Mixtures (WAF Tests)

The Trustees evaluated the lethal toxicity of DWH oil-water mixtures (WAF bioassays) to a large number of species of Gulf fish (or close surrogates) included as part of the water column resources assessment. Tested species included offshore, pelagic fish such as mahi-mahi, tunas, and cobia; as well as fish that live along the continental shelf or in more nearshore waters such as sea trout, red drum, menhaden, and bay anchovy (see Table 4.3-1). The Trustees also evaluated the toxicity of WAF exposure to shrimp, and to several invertebrates (blue crabs, oysters, and fiddler crabs), the larvae of which occupy the water

column during part of their developmental cycle. Testing was also performed with two species that make up part of the marine food web: a diatom phytoplankton and a copepod zooplankton.

As illustrated in Figure 4.3-7, which provides examples of WAF tests performed with embryos of mahi-mahi, red drum, and bay anchovy, the studies demonstrated a clear dose-response relationship between exposure to DWH oil and increased mortality rates. Table 4.3-2 summarizes LC20 values—concentrations that cause mortality in 20 percent of the test organisms—determined for water column fish species from these dose-response relationships. As shown in Table 4.3-2, LC20 values were as low as approximately 1 µg/L TPAH50, which can also be expressed as 1 part per billion (ppb) (Box 6). As discussed in greater detail below and in Section 4.4 (Water Column), many of these lethal effects concentrations for early life-stage fish were exceeded in the environment during and following the spill in some locations and times.

Box 6: What Is a Part Per Billion?

Toxicologists typically report the results from tests involving oil-water mixtures in units of micrograms of oil or TPAH per liter of water (µg/L). 1 µg/L is the same as 1 part per billion (ppb). 1 ppb is roughly equal to thoroughly mixing *one drop* of ink (about 0.0017 liquid ounces) into a large tanker truck full of water (about 11,600 gallons).

For a given species and life stage, the toxicity of DWH oil to fish was generally similar across WAF preparation methods when toxicity is expressed in terms of the concentration of TPAH50 (Morris et al. 2015b). In our tests with oils at four different weathering states, the toxicity of the WAF, expressed in TPAH50, generally increased with increasing degree of weathering (Morris et al. 2015c).

Table 4.3-3 presents the results of toxicity tests with invertebrates in terms of LC20 values. These effects values were somewhat higher than were observed in the more sensitive early life-stage fish tests and any exceedances of these higher invertebrate toxicity values in the environment would have been less frequent.

As with fish, the toxicity of weathered DWH oil exceeded that of less weathered oils (Morris et al. 2015c). In invertebrates, the toxicity of CEWAFs (containing dispersant) often was greater (i.e., lower LC values) than in LEWAFs/HEWAFs (without dispersant; Table 4.3-3). In toxicity tests with dispersant alone, invertebrates tended to be somewhat more sensitive than fish (Box 7). Therefore, the dispersant itself may have contributed to the toxicity of CEWAFs in some invertebrate tests (Morris et al. 2015b). However, the cumulative surface area of the ocean over which dispersant was applied (305 square-mile [mi^2] days (Houma 2010)[1] was only 0.06 percent of the cumulative area of surface oiling (475,000 mi^2 days; Section 4.4, Water Column).

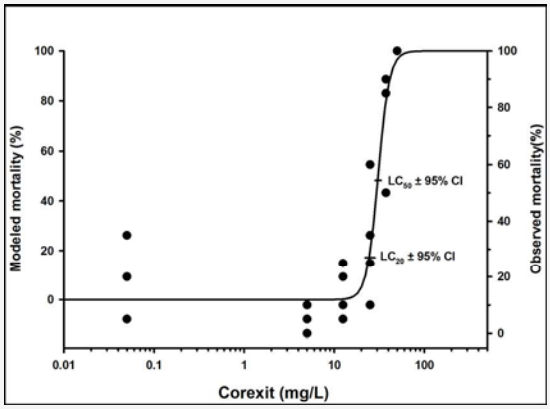
[1] A mi^2 day is a compound unit that means one square mile for one day, in any combination of area and time. For example, 100,000 mi^2 days could mean 1,000 mi^2 for 100 days, 10,000 mi^2 for 10 days, or 100,000 mi^2 for 1 day.

Box 7: Dispersant Toxicity

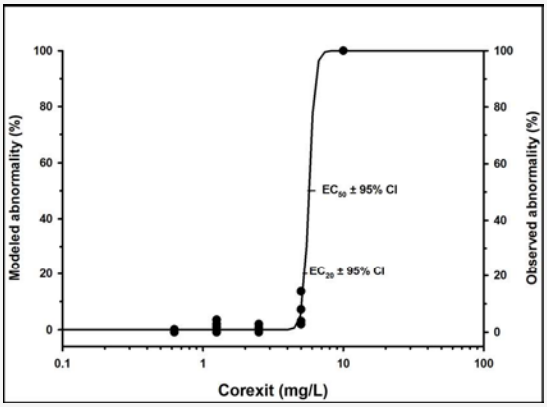
In addition to evaluating the toxicity of dispersant (Corexit 9500) in combination with oil in CEWAF tests, the Trustees performed bioassays with dispersant alone. Lethal effects concentrations for dispersant generally occurred in the parts-per-million (ppm, or mg/L) range of Corexit in water, whereas effects concentrations for TPAH50 were in the parts-per-billion (ppb, or µg/L) range (about 1,000 times lower).

For example, the left panel below shows the results of a test using larval mahi-mahi. In this test, the LC20 value was 25 ppm and the LC50 value for this test was 31 ppm. The right panel in the figure below shows the results of a bioassay in which abnormal development in oyster larvae was measured. In this test, the EC20 and EC50 concentrations were 5.3 and 5.7 ppm, respectively (Morris et al. 2015b).

It is possible that dispersant may have contributed to the observed toxicity in the Trustees' toxicity testing program CEWAF exposures to invertebrates (Morris et al. 2015b). However, the cumulative surface area of the ocean over which dispersant was applied was only 0.06 percent of the cumulative area of surface oiling (Section 4.4, Water Column). Consequently, any potential contribution from dispersant to total toxic effects would have been minimal relative to the injury caused by oil.



Effects of dispersant on survival of larval mahi-mahi.



Effects of dispersant on abnormal development of oyster larvae.

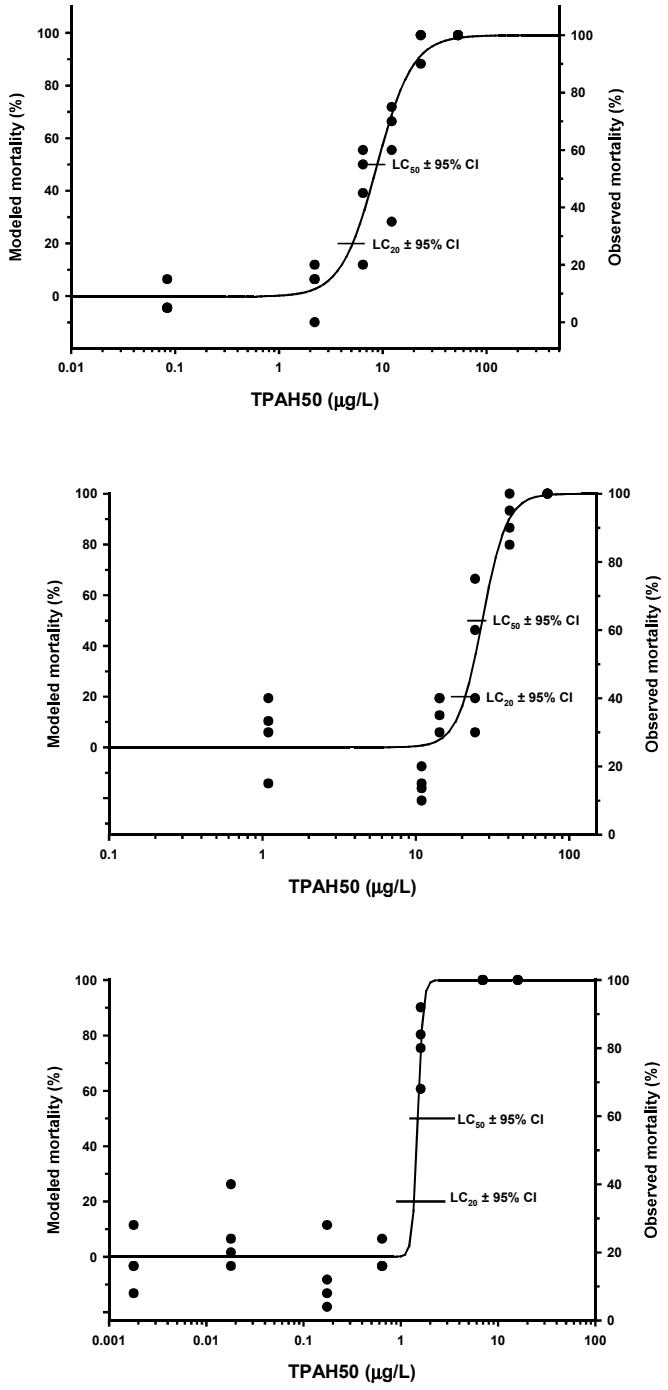


Figure 4.3-7. Results of HEWAF toxicity testing showing the relationship between the exposure concentration of TPAH50 and percent mortality (Morris et al. 2015b)**Top panel:** Mahi-mahi embryo/larvae exposed to Slick A oil. The LC20 for this test after 96 hours of exposure was 5.1 (95% confidence interval [CI] 3.7–6.6) µg/L TPAH50 **Middle panel:** Red drum embryo/larvae exposed to Slick A oil. The LC20 for this test after 72 hours was 20.5 (95% CI 17.8–23.7) µg/L TPAH50. **Bottom panel:** Bay anchovy embryo/larvae exposed to Slick B oil. The LC20 for this test after 48 hours was 1.3 (95% CI 0.8–NS) µg/L TPAH50.

When evaluating the data presented in Table 4.3-2 and Table 4.3-3, it should be emphasized that the toxicity of DWH oil increased considerably in the presence of UV light, for both fish and invertebrates. Consequently, these LC20 values would be overestimated for organisms exposed to UV light at or near the surface of the water. Additional information about UV phototoxicity and the relationship to environmental conditions is presented below.

Table 4.3-2. Ranges of LC20 values observed in toxicity tests with early life-stage fish exposed to different DWH oils using different WAF preparation methods (Morris et al. 2015b).

Species	Test Duration (hours)	LC20 (µg/L TPAH50)
Bay anchovy	48	1.3–3.4
Cobia	96	17.3–27.5
Mahi-mahi	96	5.1–40.2
Red drum	60–72	10.9–21.7
Speckled sea trout	72	6.2–25.6
Yellowfin tuna	24	0.7

Table 4.3-3. Ranges of LC20 values observed in toxicity tests with invertebrates exposed to oil-water mixtures (e.g., Morris et al. 2015b). Invertebrates tended to be less sensitive to DWH oil than many of the early life-stage fish tested. However, invertebrates generally appeared to have greater sensitivity to dispersants than the early life-stage fish. Invertebrates typically were more sensitive to DWH oil and dispersant (CEWAF) than oil alone (HEWAF).

Species	Life Stage	Duration (hours)	LC20 µg/L TPAH50
CEWAF			
Blue crab	Zoea	96	12.7–12.9
White shrimp	Juvenile	96	50.9
Grass shrimp	Adult	96	73
HEWAF			
Blue crab	Zoea	48	56.8–105.1
Copepod	Adult	96	33.5
White shrimp	Juvenile	96	80.4

Section 4.4 (Water Column) describes how these dose-response relationships were used to quantify lethality injuries to fish and invertebrates.

Toxicity of Surface Slicks

DWH oil covered an extremely large area of the ocean during the summer of 2010. The embryos and larvae of many fish and many life stages of invertebrates live in the upper water column near the ocean surface where they could come into contact with floating oil. The Trustees performed toxicity tests in which early life stages of different fish species and mysid shrimp were held in water covered with very thin sheens of DWH oil (see Figure 4.3-6).

The results of these studies demonstrated that exposure to even thin sheens of oil were extremely toxic to developing fish. The longer organisms spent in contact with the oil sheen, the more likely they were to die. For example, red drum embryos exposed to sheens made with weathered oil (Slick A) for 24, 48, or 60 hours experienced an average of 34, 68, or 74 percent mortality in excess of controls, respectively (Figure 4.3-8). Similarly, bay anchovy exposed to sheens made with weathered oil (Slick A or Slick B) for 48 hours suffered 94 and 88 percent mortality, respectively (Morris et al. 2015a). These results

demonstrate that direct exposure to the surface sheen and/or the water in close proximity to the sheen is highly toxic to early life-stage fish and invertebrates.

Photo-Induced Toxicity of DWH Oil

The Trustees evaluated the UV photo-induced toxicity of DWH oil in a number of controlled tests with fish and invertebrates that live in surface waters and are exposed to natural sunlight. Following a short exposure period (less than 8 hours) to relatively low concentrations of oil, semi-transparent organisms appear normal, but then die when they are exposed to UV light for short durations (less than 8 hours). Each of the species tested was susceptible to photo-induced toxicity when exposed to oil and UV light (Lay et al. 2015b). Toxicity in the presence of UV light was generally on the order of 10 to more than 100 times greater than toxicity without UV light. For example, the toxicity of waterborne DWH oil to speckled sea trout embryos/larvae was nearly 200 times greater in the presence of UV light than without UV (Figure 4.3-9).

As with photo-induced toxicity in WAF exposures, exposure to UV light also increased the toxicity of oil in thin surface sheens. Exposure to surface sheens and UV light resulted in very high mortality in tests with speckled sea trout embryos (70 percent mortality), juvenile mysid shrimp (97 percent mortality), and bay anchovy embryos (92 percent mortality).

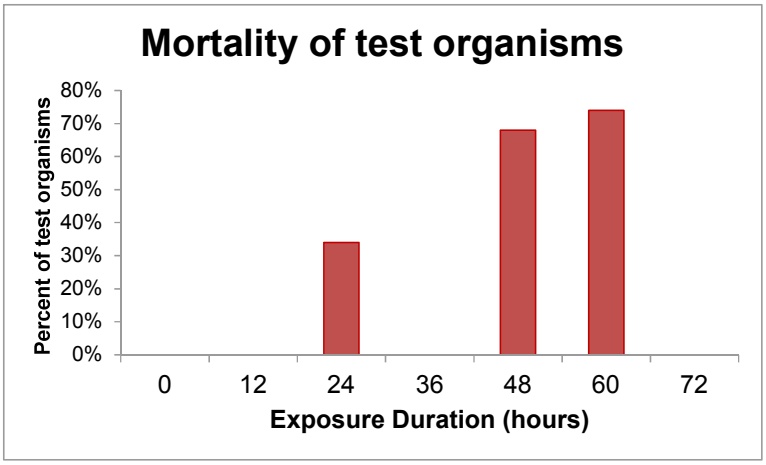


Figure 4.3-8. Percent mortality observed in red drum embryos following exposure in water with a thin sheen of floating DWH oil (Morris et al. 2015b).

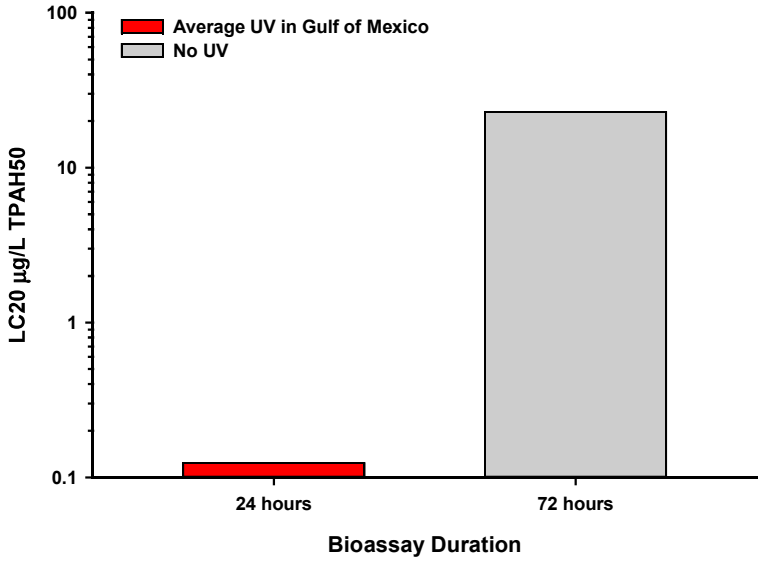


Figure 4.3-9. Comparison of lethal concentration (LC20) of DWH oil to early life-stage speckled sea trout in the presence of UV (red bar) at average UV levels recorded during the spill (Lay et al. 2015a) with bioassays conducted with embryos/larvae exposed to oil but no UV (gray bar) (Morris et al. 2015b). LC20 values for 72 hour exposures generally are lower than for 24 hour exposures (i.e., more toxicity). However, the toxicity to sea trout larvae for a 24 hour exposure in the presence of UV light was nearly 200 times greater than the toxicity for a 72 hour exposure without UV light.

The degree of photo-induced toxicity was found to be a function of the amount of incident UV light exposure (Lay et al. 2015b; Morris et al. 2015a).

Data from the Trustees' UV/oil bioassays were used to develop a method to adjust the dose-response curves for fish and invertebrates to account for photo-induced toxicity (Lay et al. 2015b). UV-adjusted toxicity values were used to quantify mortalities in water column resources near the ocean surface (Table 4.3-4; Section 4.4).

Table 4.3-4. LC50 values for fish and invertebrates showing adjustment for phototoxicity. Toxicity increased (i.e., lower LC50s) with exposure to UV (Lay et al. 2015b).

Species	Oil	Duration (hours)	LC50 µg/L TPAH50 No UV	LC50 µg/L TPAH50 UV-Adjusted
Ichthyoplankton				
Bay anchovy	B	48	1.4	0.1
Speckled sea trout	B	72	24.7	0.2
Red drum	A	72	27.1	0.2
Bay anchovy	A	48	3.9	0.2
Speckled sea trout	A	72	30.3	0.2
Red drum	B	60	30.9	0.2
Mahi-mahi	A	96	8.8	0.6
Zooplankton				
Copepod	A	96	64.4	2.4
Blue crab	B	48	79.0	2.9

Comparing Lethal Effects Concentrations Determined from Laboratory Testing to Conditions in the Environment During and Following the Spill

The laboratory toxicity tests described in this section are important for establishing quantitative relationships between oil concentrations and toxic effects (i.e., dose-response relationships). As illustrated in Figure 4.3-10, many water samples collected from the Gulf during the spill exceeded concentrations that would cause mortality to water column resources.

Analysis of the degree of toxicity that occurred in the environment is presented in Section 4.4 (Water Column).

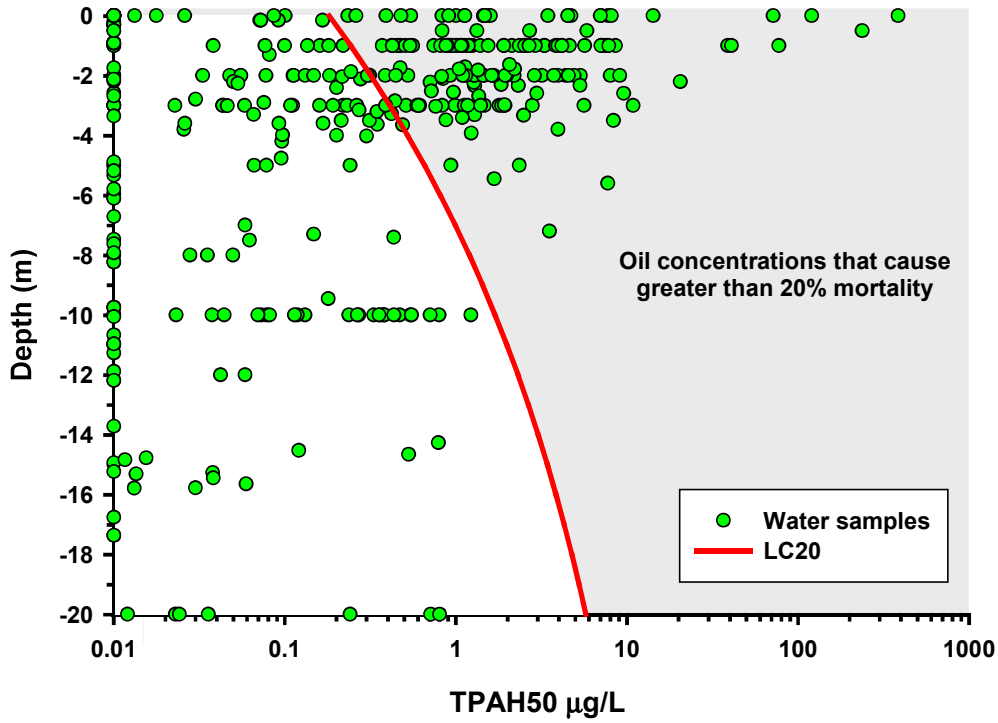


Figure 4.3-10. Many water samples collected during the spill had concentrations of TPAH that exceeded lethal levels determined from the Trustees toxicity test program (see Section 4.4, Water Column). TPAH50 concentrations in water samples collected at different depths during the spill (green dots) are plotted against the LC20 values for early life-stage speckled sea trout adjusted for photo-induced toxicity (red line). The LC20 value (red line) increases (i.e., less toxicity) with depth because ambient UV light is attenuated in the water. Samples falling in the gray-shaded area represent conditions in which mortality to ichthyoplankton would be expected to exceed 20 percent (see Section 4.4, Water Column; (see Section 4.4, Water Column; Lay et al. 2015b)).

4.3.3.1.2 Other Toxic Effects of DWH Oil on Water Column Resources

In addition to lethality, the Trustees evaluated other toxic effects of DWH oil. Toxic responses documented in water column resources included cardiac (heart) toxicity and other developmental effects, reductions in growth rates, impaired immune function, reduced swimming performance, and other adverse physiological responses. Although not incorporated explicitly in the Trustees' quantification of injuries to water column resources, these other injuries—which can impair the health, fitness, and long-term survival of animals—provide additional context regarding the effects of the spill on the environment.

Cardiac Toxicity and Developmental Abnormalities

One of the more severe types of petroleum toxicity, only discovered relatively recently, is the adverse effect of low concentrations of PAH on heart development and function in fish embryos and larvae. Cardiac impacts on developing fish that are severe enough to impair survival occur at TPAH concentrations lower than those associated with lethality (Carls et al. 1999; de Soysa et al. 2012; Heintz et al. 2000; Incardona et al. 2014; Incardona et al. 2013).

The Trustees evaluated cardiotoxicity in a number of different water column species, including yellowtail amberjack, bluefin and yellowfin tuna, mahi-mahi, and red drum. Exposed fish demonstrated heart-related abnormalities including decreased heart rates; abnormal heartbeat rhythms; abnormal heart development (e.g., tube heart); and edema (abnormal accumulation of fluid) near the heart, yolk sac and abdominal areas (Incardona et al. 2014; Incardona & Scholz 2015; Incardona et al. 2013; Morris et al. 2015b; Morris et al. 2015d) (Figure 4.3-11).

Source: *NOAA.*

Figure 4.3-11. Cardiotoxic effects of oil on developing fish. Two images are shown of early life-stage red drum from Trustee studies (Morris et al. 2015b; Morris et al. 2015d). The top picture is a control fish that was not exposed to oil. The bottom fish was exposed to DWH oil (Slick A LEWAF) for 36 hours. This fish developed edema (excess fluid) around the heart (arrow). Other developmental deformities observed included curved spine and reduced growth (Incardona & Scholz 2015). Young fish in the wild with these types of effects are considerably less likely to survive.

Fish developed edema and decreased heart rates at very low oil concentrations, with some EC20 values less than 1 µg/L TPAH50 over 36 to 48 hours (Table 4.3-5; (Incardona et al. 2014; Incardona & Scholz 2015; Morris et al. 2015b; Morris et al. 2015d).

Table 4.3-5. Concentrations of DWH oil (as TPAH50) that resulted in 20 percent effects concentrations (EC20) for cardiotoxicity (e.g., Incardona et al. 2014; Incardona & Scholz 2015; Incardona et al. 2013; Morris et al. 2015b; Morris et al. 2015d).

Species	Life Stage	Duration (hours)	EC20 µg/L TPAH50
Mahi-mahi	Embryo	48	1.3–8.7
Red drum	Embryo	48	1.0–15.7
Southern bluefin tuna	Embryo	36	0.6–3.3
Yellowfin tuna	Embryo	48	0.5–4.1
Yellowtail amberjack	Embryo	48	2.8–8.3

Reduced Growth

Depending on the situation, smaller fish may be at a competitive disadvantage, as larger animals are less likely to be eaten by predators, are better able to catch prey, and have greater reproductive potential. Juvenile red drum and red snapper exposed to waterborne DWH oil for 1 to 2 weeks were smaller (shorter lengths and reduced weights) compared to control animals (Figure 4.3-12, (Ortell et al. 2015). These results are consistent with tests in fish and invertebrates exposed to DWH oil contaminated

sediment (see Sections 4.3.3.2 and 4.3.3.3, below), and field experiments in which shrimp were placed near heavily oiled marsh areas (Rozas et al. 2014).

Reduced Immune Function

Oil can compromise the immune system's ability to protect oil-exposed animals from disease (Kennedy & Farrell 2008; Khan 1990). The ocean is rich in pathogenic organisms, such as parasites, viruses, and bacteria. While healthy animals are typically able to fight off infections, or at least quickly recover from them, organisms that are exposed to oil would be more susceptible to infections and their negative repercussions.

To determine the potential adverse effects of exposure to DWH oil on the fish immune system, the Trustees conducted tests on red snapper, Atlantic croaker, and red drum in which fish were exposed to both DWH oil and an endemic bacterium from the Gulf of Mexico, *Vibrio anguillarum (V. anguillarum)*. For example, juvenile red drum were exposed to waterborne oil for 4 days, followed by a 1-hour bacterial challenge. Survival and immune system effects of these fish were then compared to animals exposed to waterborne oil only, animals exposed only to bacteria (and were never exposed to oil), and animals that were not exposed to oil or bacteria.

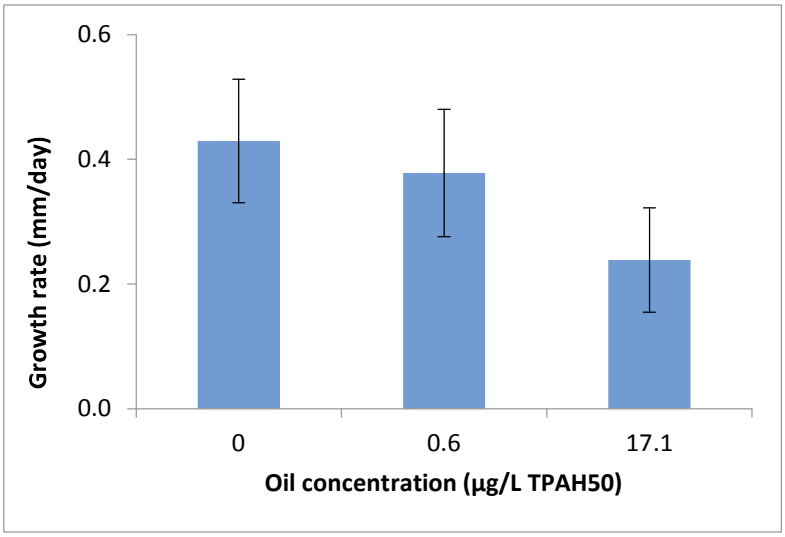


Figure 4.3-12. Effects of DWH oil (Slick A HEWAF) on growth rates of juvenile red snapper (75 days post-hatch) exposed to oil for 17 days (Morris et al. 2015b). Increasing concentrations of TPAH50 were associated with decreased growth rates. Bars show average growth rate over the 17 day period in millimeters per day. The error bars are one standard deviation of the mean.

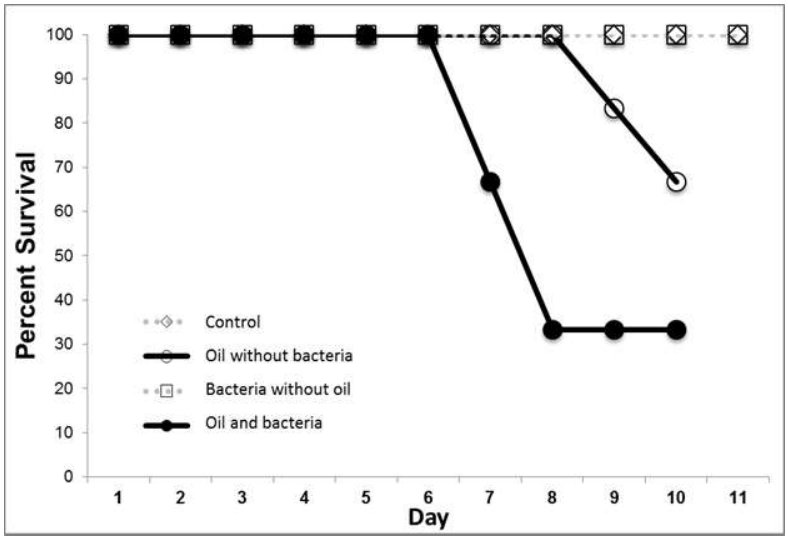


Figure 4.3-13. Percent survival of juvenile red drum exposed to one of four treatments: 1) neither oil nor bacteria (*Vibrio anguillarum*); 2) DWH oil without bacteria; 3) bacteria without oil; 4) DWH oil and bacteria. Exposure to oil and bacteria caused considerably more mortality than in the other treatments (Ortell et al. 2015).

Fish survival results are summarized below and in Figure 4.3-13 (Ortell et al. 2015):

- Greater than 60 percent of the fish exposed to oil and bacteria died.
- Thirty five percent of fish exposed to just DWH oil (at the same concentration) died.
- All fish exposed to just bacteria without oil or those exposed to no bacteria or oil survived ((Ortell et al. 2015); Figure 4.3-13).

Tests on immune function also demonstrated that exposure to DWH oil resulted in reduced red blood cell counts and a negative effect on genes that are associated with the production of antibodies that help fight off infection.

Based on these data, the Trustees have concluded that exposure to DWH oil causes immunosuppression, which can lead to increased vulnerability to infectious diseases and the ability to recover from infections. Immunosuppressed animals will be at a severe disadvantage compared to unaffected animals.

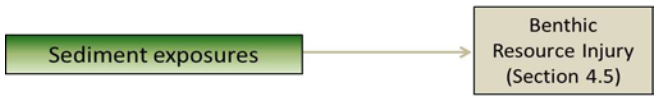
Reduced Swim Performance

The ability of fish to survive and thrive is dependent upon their ability to swim, whether to catch prey or escape from predators. To evaluate potential impacts on swim performance, the Trustees conducted laboratory studies with different life stages of mahi-mahi. The studies showed that the swim performance and associated metabolic and physiological status of mahi-mahi were adversely affected following short-term exposure to DWH oil, whether animals were exposed as embryos, juveniles, or adults. Embryos exposed to as little as 1.2 µg/L TPAH50 (Slick A HEWAF; based on average exposure concentrations) for 48 hours shortly after fertilization and then held and raised in clean water for 30 days experienced significantly reduced swim performance (Mager et al. 2014). Juvenile (30-day old) and adult mahi-mahi exposed to 30 µg/L or 8.4 µg/L TPAH50 (Slick A HEWAF), respectively, for 24 hours also experienced significantly reduced swim performance compared to controls (Mager et al. 2014; Morris et al. 2015b). Although this injury was not directly quantified, the Trustees concluded that fish exposed to DWH oil may have suffered from swim performance injuries that could have reduced their ability to escape predators or capture prey.

4.3.3.2 Toxicity of DWH Oil to Benthic Resources

Key Findings

- DWH oil was toxic to benthic resources in Gulf of Mexico sediments contaminated in the field during the spill and sediment spiked with DWH oil in the laboratory.
- Bottom-dwelling invertebrates experienced reduced survival, growth, and reproduction when exposed to DWH oil in Gulf of Mexico sediments from benthic nearshore and deep-sea environments.



The benthic resources toxicity testing focused on evaluating the toxicity of sediments contaminated with DWH oil to bottom-dwelling invertebrates (Table 4.3-1). The Trustees conducted bioassays using contaminated sediment collected from coastal areas of the northern Gulf of Mexico that experienced heavy oiling, or by adding varying amounts of DWH oil to clean sediments collected from the field (Krasnec et al. 2015a). The Trustees also evaluated the results of toxicity tests conducted with contaminated deep-sea sediments and amphipods performed by BP (Krasnec et al. 2015b).

In addition to being used for the benthic resources injury assessment (Section 4.5), sediment toxicity testing was also used to support the nearshore resources assessment. Those tests are discussed below in Section 4.3.3.3.

Benthic invertebrates, such as amphipods, are highly abundant, burrow into the sea floor, and are an important food source for many fish and invertebrate species. *Leptocheirus plumulosus (L. plumulosus),* a burrowing amphipod, were exposed to sediments contaminated with DWH oil to investigate effects on survival, reproduction, and growth. The amphipods were exposed for either 10 or 28 days to contaminated sediments collected from the northern Gulf of Mexico or sediments spiked in the laboratory using Slick A or B oils.

Exposure of the amphipods to oil-contaminated sediments resulted in mortality, with a calculated LC20 value of 7.16 mg/kg TPAH50 in 10-day tests over a range of sediment types (Figure 4.3-14; (Morris et al. 2015b)). Adverse effects of contaminated sediments on growth and reproduction of the amphipods were also observed. This information is important in assessing injury in deep-sea sediments, in sediment adjacent to marsh and beach environments, and in marsh soils that have been contaminated by oil.

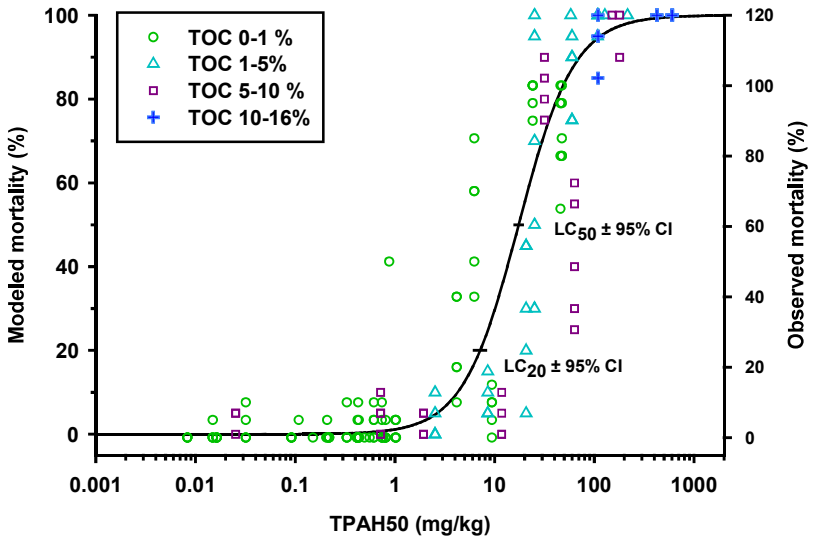
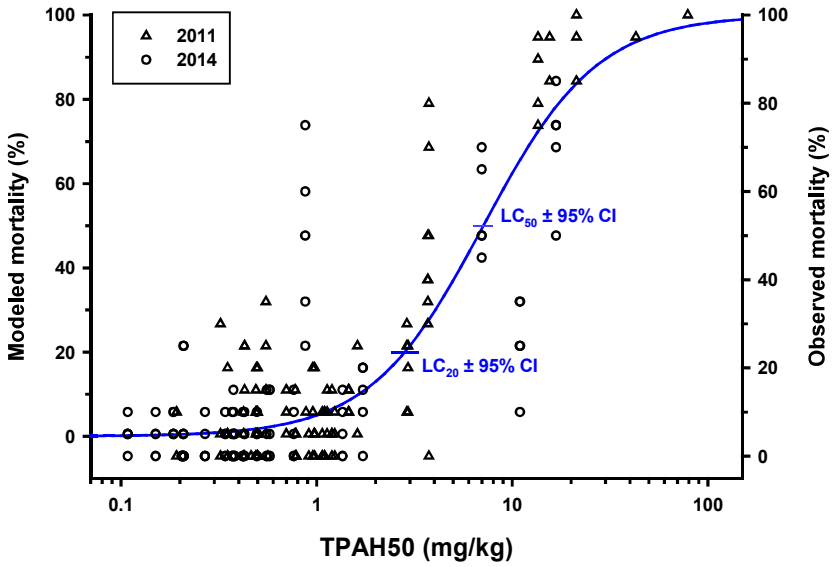


Figure 4.3-14. Juvenile amphipod (*L. plumulosus*) mortality after a 10-day exposure to contaminated sediments collected from the northern Gulf of Mexico or sediments spiked in the laboratory with DWH oil. The LC20 and LC50 values (95% confidence interval) are 7.2 (6.3, 8.2) and 17.4 (16.0, 19.0) mg/kg TPAH50, respectively (Morris et al. 2015b). Data are binned according to total organic carbon (TOC) concentrations.

BP also performed sediment toxicity tests with amphipods (*L. plumulosus*) exposed to sediments collected at various times and locations in the deep sea, both near the wellhead and further afield. The results of these tests were shared with the Trustees. The Trustees' analysis of the BP data determined that the combined LC20

across the different assays was 2.8 mg/kg TPAH50, a value similar to the results of the Trustees' tests, particularly when considering the low organic carbon content (1.2 percent) of the deep-sea sediments (Figure 4.3-15).

Section 4.5 presents information on injuries to benthic resources, including a comparison of effects concentrations to concentrations in sediments collected from the environment following the spill. Additional data collected by the Trustees on the toxicity of sediments to nearshore resources are described below in Section 4.3.3.3.



Source: *Krasnec et al. 2015*

Figure 4.3-15. Lethal toxicity (percent [%] mortality) of sediments collected from benthic deep-sea environments to amphipods. Data were generated by BP and shared with the Trustees who completed independent data analyses (Krasnec et al. 2015b). The average total organic carbon (TOC) content in these sediments was 1.2 percent (±0.3).

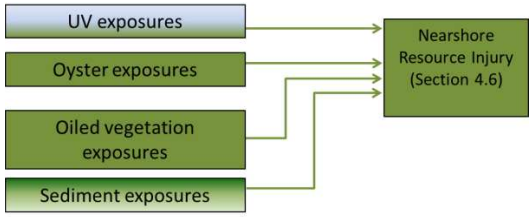
4.3.3.3 Toxicity of DWH Oil to Nearshore Resources

Key Findings

- The results of the Trustees' laboratory toxicity tests demonstrated that exposure of marsh organisms to sediments contaminated with DWH oil resulted in a series of adverse effects, including death, reduced growth, and reduced reproductive success.
- Higher concentrations of TPAH50 in sediments resulted in more adverse effects in more test species. Adverse effects were observed at concentrations as low as approximately 1 mg/kg TPAH in sediment.
- Southern flounder were adversely affected by exposure to oil-contaminated sediment. Toxic effects included damage to gill and liver tissues, reduced growth rates, and mortality.
- Exposure to oil-contaminated sediments caused growth inhibition in juvenile red drum and Pacific white shrimp.
- Gulf killifish embryos exposed to oil-contaminated suspended sediment were less likely to hatch or to survive after hatching.

- There was substantial mortality to fiddler crab offspring exposed to relatively low concentrations of oil in or on sediments as embryos, when followed by exposure to sunlight in clean water as hatched larvae.
- When marsh periwinkles were exposed to DWH oil on plants, they exhibited increased mortality and an impaired ability to move away from oil.
- Exposure to DWH oil caused adverse effects in all oyster life stages tested, at varying effects concentrations.

4.3.3.3.1 Southern Flounder (*Paralichthys lethostigma*)



Flatfish, such as flounder, are particularly vulnerable to the toxic effects of contaminated sediments because they spend most of their lives partially covered by sediments on the sea floor. Southern flounder, a flatfish that lives in the northern Gulf of Mexico, were exposed to sediments spiked with weathered DWH oil (Slick B).

The tests showed a suite of toxic effects that ranged from tissue damage to lethality, depending on the concentration of TPAH50 in the sediment. Southern flounder exposed to contaminated sediment showed evidence of damage to gill tissues at lower concentrations (EC20 values from 0.3 mg/kg to 1.3 mg/kg TPAH50). Damage to gill tissue can have a detrimental effect on an animal's respiratory system (their ability to extract oxygen from water). Reduced growth rates were observed at higher concentrations (Figure 4.3-16; EC20 = 12.8 mg/kg TPAH50). Both of these impacts could have a negative impact on fish in the wild. Exposure to contaminated sediments also resulted in direct mortality, with an LC20 value of 36.3 mg/kg TPAH50 (Brown-Peterson et al. 2015; Morris et al. 2015b).

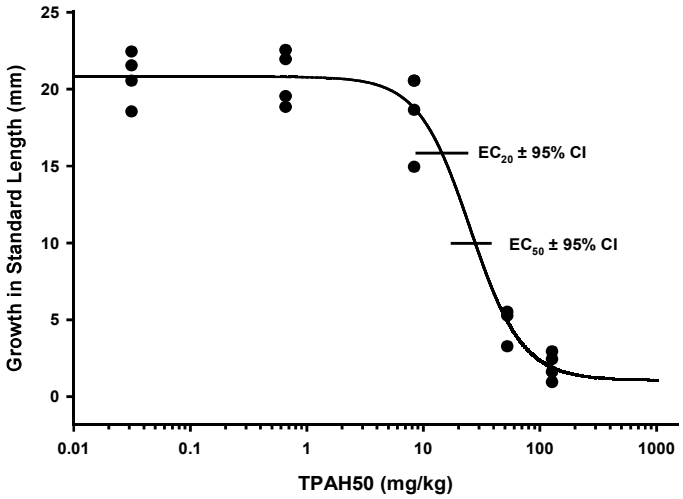


Figure 4.3-16. Growth in juvenile southern flounder after a 32-day exposure to sediments spiked with Slick B oil. The EC20 and EC50 values (with the 95% confidence intervals in parentheses) are 12.8 (8.8-23.7) and 26.7 (17.6-37.4) mg/kg TPAH50, respectively (Brown-Peterson et al. 2015; Morris et al. 2015b).

As discussed earlier, oil exposure can cause immunotoxicity, putting animals at a higher risk of infection and reducing their ability to survive infections (Kennedy & Farrell 2008; Khan 1990). Trustee testing involved exposing Southern flounder to sediment spiked with DWH oil (58 mg/kg TPAH50) for 7 days, followed by a 1-hour exposure to seawater containing *V. anguillarum* for 1 hour. Two days after the exposure to bacteria, 94 percent of the fish

died, whereas there was no mortality at the same time period (7 days) when fish were exposed to oil alone (Morris et al. 2015b; Ortell et al. 2015). This large increase in oil toxicity that occurred following a short exposure to bacteria that is widespread in the Gulf of Mexico illustrates that the potential effects of oil in the environment—where animals are subject to natural stresses—may be greater than in carefully controlled laboratory conditions.

4.3.3.3.2 Red Drum *(Sciaenops ocellatus)*

Juvenile red drum forage in sediments and inundated marsh soils. The Trustees found that exposure to oil-contaminated sediments for 13 days caused growth inhibition in juvenile red drum (Figure 4.3-17). The growth of the exposed fish was reduced, with an EC20 value of 37 mg/kg TPAH50 (Morris et al. 2015b).

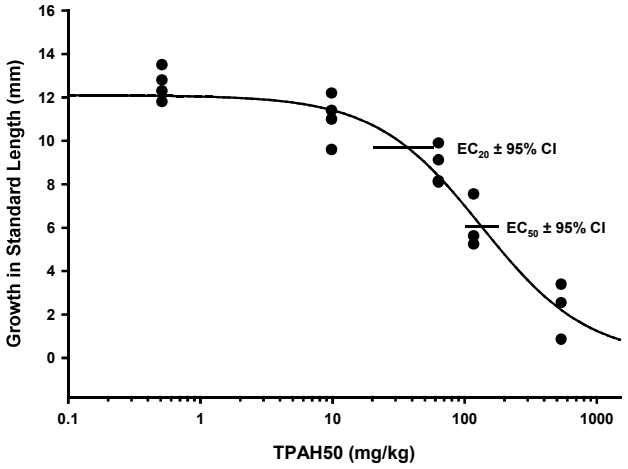


Figure 4.3-17. Growth in juvenile red drum after a 13-day exposure to sediments spiked with Slick B oil. The EC20 and EC50 values (with the 95% confidence intervals in parentheses) are 37.1 (20.9-56.8) and 134 (103-178) mg/kg TPAH50, respectively (Morris et al. 2015b).

4.3.3.3.3 Gulf Killifish *(Fundulus grandis)*

In addition to direct contact with sediment on the seafloor, organisms in some nearshore environments were exposed to suspended sediments contaminated with oil. To investigate the toxic effects of contaminated suspended sediment, the Trustees exposed Gulf killifish embryos to fine-grained sediments, spiked with Slick B oil. The sediments were suspended in the exposure water by gently moving the test chambers on an orbital shaker table. Exposure to contaminated suspended sediments impaired Gulf killifish embryo development and resulted in decreased hatch rates and increased mortality. These effects were combined into an "unviable embryo" endpoint. The LC20 for this unviable embryo response was 15.5 mg/kg TPAH50 in the underlying sediment that was suspended (Figure 4.3-18; (Morris et al. 2015b)). These data demonstrate that exposure to oil-contaminated suspended sediments can cause toxic effects on reproduction and survival.

4.3.3.3.4 Shrimp

The Trustees relied on field evaluations to determine injury to white and brown shrimp. To augment field studies, a laboratory toxicity test was performed. Pacific white shrimp were used as a surrogate species for the brown and white shrimp which occur in the Gulf of Mexico. Young shrimp (about 10-day old post-larvae) demonstrated reduced growth after only 6 days of exposure to sediment mixed with DWH oil, with an EC20 of 4.3 mg/kg TPAH50 (Morris et al. 2015b).

4.3.3.3.5 Fiddler Crabs *(Uca longisignalis)*

Fiddler crabs live in the intertidal zone of marshes, mudflats, and beaches. The burrows that they dig in the sediment are often inundated by seawater during high tides. In order to evaluate the UV photo-induced toxicity of sediment contaminated with DWH oil on early life-stage fiddler crabs, the Trustees designed an experiment to expose female adult crabs and their external egg masses to oiled sediment and UV light.

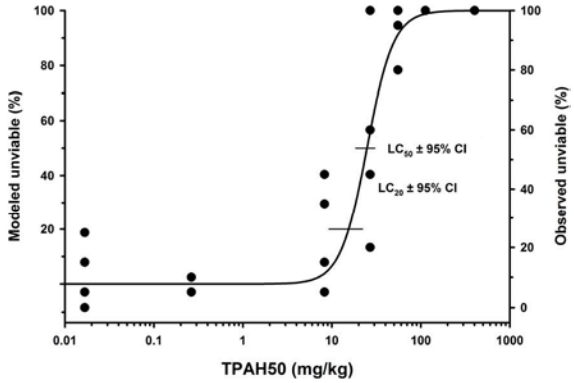


Figure 4.3-18. Relationship between TPAH50 (mg/kg) and viability of Gulf killifish embryos following a 20-day exposure to suspended sediments spiked with Slick B oil. The LC20 and LC50 values (with 95% confidence intervals in parentheses) are 15.5 (9.3-22.1) mg/kg and 24.8 (18.5-30.4) mg/kg TPAH50, respectively. These values were calculated using the concentrations of TPAH50 in the sediment which was suspended during the bioassay (Morris et al. 2015b).

In this study, adult fiddler crabs were placed on sediments coated with DWH oil for 10 days. The TPAH50 concentrations in the upper 2 centimeters (cm) of these sediments ranged from 0.07 (clean reference sediment) to 26 mg/kg. During the exposure period, several female crabs became gravid (i.e., produced fertilized egg masses that remain attached to the female; also known as "sponge crab"). The Trustees removed the gravid females from the oiled sediment exposures after 10 days and placed them in clean water for another 2 to 4 days until the embryos in their external egg masses hatched. After hatch, the zoea (i.e., larvae) were collected and either held indoors in clean water or exposed to varying levels of ambient sunlight in clean water for approximately 7 hours. Zoea from females exposed to contaminated sediment as embryos *and* subsequently exposed to sunlight in clean water experienced substantial mortality, with a calculated LC20 value of 0.62 mg/kg TPAH50 in the upper 2 cm of sediment (Figure 4.3-19; (Morris et al. 2015b)) This study demonstrates substantial toxicity to fiddler crab offspring at relatively low concentrations of oil in or on sediments, even though the oiled sediments did not affect adult survival, fecundity, or behavior.

4.3.3.3.6 Marsh Periwinkle Snails *(Littoraria irrorata)*

As oil was deposited on shorelines, it covered sediment, soil, and vegetation that provides habitat for invertebrates like snails and insects. The Trustees' assessment team determined that marsh periwinkles were injured by oiling: the densities of marsh periwinkles in oiled coastal habitats (e.g., marsh shoreline edge and marsh interior) were dramatically reduced compared with unoiled marsh (Section 4.6, Nearshore). To augment the field-based injury evaluation, the Trustees conducted a series of tests in which periwinkles were exposed to oiled marsh grass. These studies were designed to be similar to conditions on heavily oiled marsh platform sites where vegetation along the exposed marsh edge was most heavily oiled and often was lying flat on the marsh surface (Section 4.6). The tests were designed to assess whether exposure to oiled marsh platforms would affect periwinkle survival or their ability to move away from the oil towards cleaner upright plants further back in the marsh.

In the first series of tests, periwinkles were placed on *Spartina* plant shoots coated with DWH oil at a thickness similar to that measured at heavily oiled marsh sites (about 1 cm; (Zengel et al. 2015)). The amount of time it took for the periwinkles to move off of the oiled vegetation to non-oiled standing *Spartina* shoots about 23 cm away was recorded (Garner et al. 2015). Control animals in trays without oil reached the standing vegetation more quickly than the oil-exposed periwinkles. In the control group, 70 percent of the animals reached the standing vegetation in about 1 hour, and 85 percent of the periwinkles reached standing vegetation in about 2.5 hours. In contrast, only 18 percent of the oiled group reached the standing vegetation in about 4 hours, and only 22 percent reached the vegetation over the entire 72-hour duration of the test. Periwinkles in the oiled treatment that did not reach the standing vegetation in this test experienced very high mortality (77 percent). The results of this laboratory test suggest that periwinkles living in areas with heavily oiled marsh vegetation would likely have died.

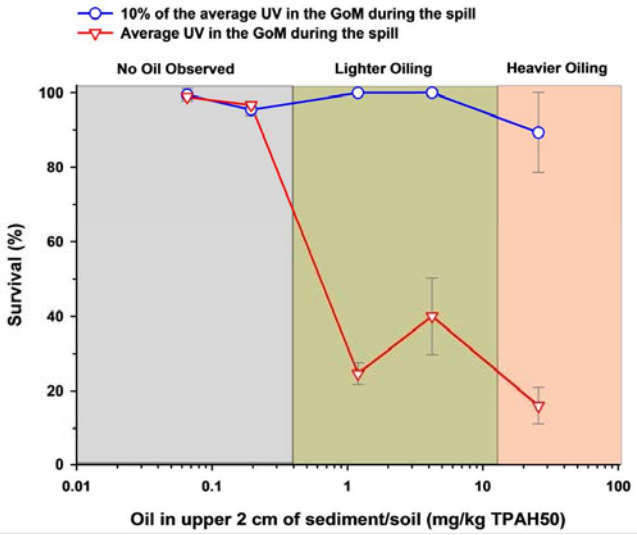


Figure 4.3-19. Survival of fiddler crab larvae exposed to contaminated sediments as embryos for up to 10 days and exposed in clean water for 1 day to natural UV light. Larvae exposed to full sunlight (100 percent of average incident UV in the northern Gulf of Mexico) suffered high mortality (triangles with red line). Little mortality was observed when crabs were only exposed to 10 percent of average incident UV light (circles with blue line). The colored bands represent the range of TPAH50 measured in different marsh shoreline oiling categories discussed in Section 4.6 (Morris et al. 2015b).

A follow-up test showed that periwinkle mortality was clearly related to the duration of oil exposure. After 16 hours, 35 percent of the periwinkles exposed to oil died. Mortality was 68 percent and 98 percent after 32 and 72 hours of oil exposure, respectively (Garner et al. 2015). Control periwinkles not exposed to oil had 0 percent mortality. Overall, the Trustees' laboratory test data support the findings and conclusions of the field studies in which heavily oiled sites had greatly reduced periwinkle abundance (see Section 4.6, Nearshore, for additional information).

4.3.3.3.7 Eastern Oysters *(Crassostrea virginica)*

The Trustees relied on field studies to evaluate injury to nearshore and subtidal eastern oysters. To augment these studies, a series of laboratory toxicity tests were performed with Eastern oysters (native to the northern Gulf of Mexico). The DWH oil spill occurred during the peak of the oyster spawning season and contaminated oyster habitats that support spawning adults, developing embryos, and larvae (Section 4.6, Nearshore).

The Trustees tested oysters at all stages of development including gametes (sperm and unfertilized eggs), embryos, planktonic veligers (i.e., larvae), spat (i.e., larvae attached to substrate), juveniles, and adults (Section 4.6 presents more information on the oyster life cycle). Oysters at various life stages were exposed to DWH oil in the water column and in sediment (both settled and suspended) to address different routes of exposure.

When oyster gametes (eggs and sperm) were exposed in the laboratory to DWH oil in water or suspended sediments, fertilization rates decreased. For example, when oyster gametes were exposed to oil (Slick A HEWAF) and UV light, EC50 values for fertilization ranged from 13 µg/L to 116 µg/L TPAH50 depending on the UV dose (Morris et al. 2015b). When gametes were exposed to suspended sediments that were collected during the response effort (Krasnec et al. 2015a), fertilization rates also decreased (EC20 = 40.6 µg/L TPAH50; Figure 4.3-20; (Morris et al. 2015b)).

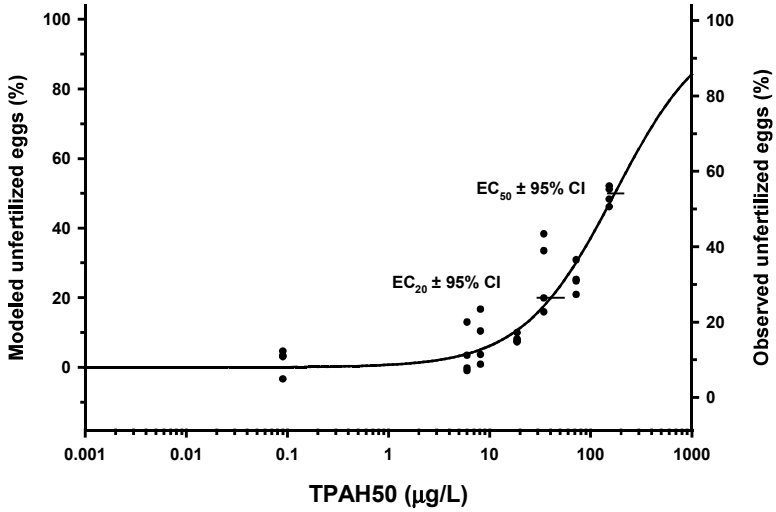


Figure 4.3-20. Effects of exposure to suspended fine-grain sediment contaminated with DWH oil on oyster fertilization. Gametes were exposed shortly after spawning and sampled after 1 hour of exposure to assess fertilization success. The EC20 on unfertilized eggs after 1 hour of exposure was 40.6 (95% CI 29.8-54.1) µg/L TPAH50 (Morris et al. 2015b).

Early life-stage oysters (embryos, veligers, pediveligers, and early spat) were also adversely affected by exposure to DWH oil. Waterborne weathered oil (Slick A HEWAFs) caused lethal and sublethal effects to early life -stage oysters [LC20 and EC20 (abnormality) values ranging from 33 µg/L to 645 µg/L TPAH50]. Interestingly, the addition of dispersant (Slick A CEWAFs) resulted in lethal and sublethal effects at lower concentrations of oil [higher toxicity; LC20 and EC20 (abnormality) values ranging from 12 µg/L to 133 µg/L TPAH50; (Morris et al. 2015b)].

In addition to WAF exposures, early life-stage oysters were also exposed to contaminated suspended sediments that were collected during the response effort (Krasnec et al. 2015a). Regardless of the exposure duration or the life stage tested, the veligers from those exposures were more likely to have developmental abnormalities than unexposed animals (Vignier et al. [In Press]). Veligers that were raised from contaminated sediment-exposed gametes were the most sensitive (abnormality EC20 = 1.1 µg/L TPAH50 for 24 hours of exposure), compared to veligers that were raised from exposed embryos (abnormality EC20 = 77.7 µg/L TPAH50 for 24 hours of exposure) or exposed as veligers themselves (abnormality EC20 = 95.9 µg/L TPAH50 for 48 hours of exposure; (Morris et al. 2015b)). Pediveligers that were exposed to sediment spiked with Slick B oil had decreased settlement rates (EC20 = 6.5 mg/kg TPAH50).

4.3.3.3.8 Summary of Sediment Toxicity Testing Results

The Trustees' sediment toxicity tests revealed a series of different adverse effects over a range of sediment exposure concentrations. Overall, the higher the concentration of TPAH50 in the sediments, more effects were observed in more test species, and at a greater degree of severity (Figure 4.3-21). As described in Section 4.6 (Nearshore), these adverse effects concentrations were exceeded along extensive lengths of marsh shorelines that were oiled as a consequence of the *Deepwater Horizon* spill.

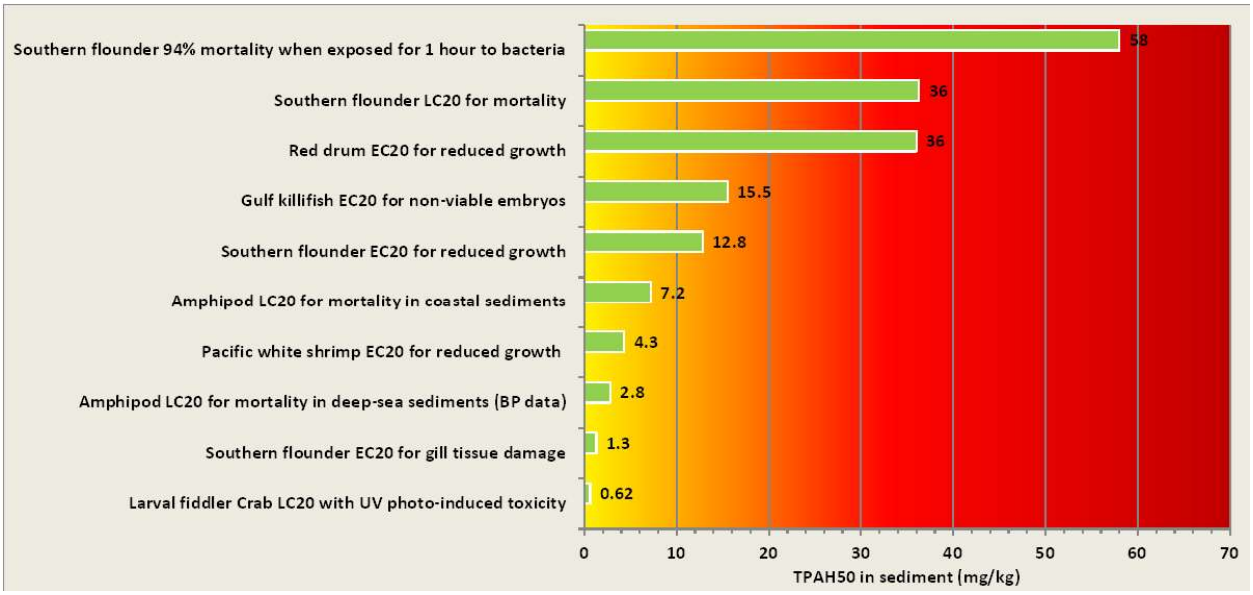


Figure 4.3-21. Toxicological effects concentrations, shown in mg TPAH50 per kg of sediment, for organisms exposed to sediment contaminated with DWH oil. Higher sediment concentrations of TPAH50 resulted in more severe effects to more test species (Morris et al. 2015b).

4.3.3.3.9 Gulf Sturgeon

The Gulf sturgeon (*Acipenser oxyrinchus desotoi*) is an anadromous fish, which migrates from salt water into large coastal rivers to spawn. Data collected from the field by the Trustees indicated that these protected fish were potentially exposed to DWH oil.

The Trustees performed controlled exposures of shovelnose sturgeon as a surrogate for the Gulf sturgeon. Juvenile shovelnose sturgeon were exposed to HEWAFs at a concentration range of 5 µg/L to 10 µg/L TPAH33 for 7 or 28 days (FWS 2015). Investigators identified significant changes in cell process pathways related to immune system function (including neutrophils and T and B cell processes), wound healing, and DNA replication. Genetic analysis identified changes in cell processes related to DNA damage and repair, as compared to control fish (FWS 2015).

The results of this laboratory study suggest that Gulf sturgeon were likely experiencing differential gene expression alterations after both short-term and longer-term oil exposure. Overall, the laboratory study provided evidence of adverse health outcomes of DNA damage at the molecular and biochemical levels, and immune injury at the molecular, biochemical, cellular, and organ levels (FWS 2015).

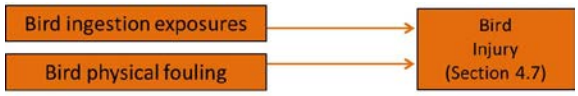
Section 4.6.7 presents additional information on injury determination for Gulf sturgeon.

4.3.3.4 Toxicity of DWH Oil to Birds

Key Findings

- When birds ingested food contaminated with DWH oil, they suffered from a variety of adverse health effects, including hemolytic anemia, liver dysfunction, kidney damage, hypothermia, weight loss, lethargy, abnormal feces, feather damage, moribundity (near death), and death.
- Ingestion of DWH oil caused several types of organ damage and dysfunction, including to liver, kidney, gastrointestinal tract, and cardiovascular systems. Ingestion of DWH oil disrupted digestive tract function, resulting in direct damage to tissues and poor absorption of fluids and nutrients.
- The Trustees' studies found previously undescribed alterations in heart function following oil ingestion, including heart tissue abnormalities, changes to heart function, and decreased blood pressure. Overall, disruption of organ physiology and function would have considerable negative consequences for a bird's fitness and survival.
- External oiling caused feather damage and reduced flight performance. Oiled birds demonstrated more erratic and less-efficient flying, slower take-off speeds, shorter flight times, and higher energy costs.

4.3.3.4.1 Effects of Ingested Oil on Birds



In Trustee laboratory tests, when birds ingested oil-contaminated fish, they suffered from a variety of adverse health effects, including hemolytic anemia, liver dysfunction, kidney damage, hypothermia, weight loss, lethargy, abnormal feces, feather damage, moribundity (near death), and death.

Double-crested cormorants and laughing gulls were orally dosed daily with 0, 5, or 10 milliliters of oil per kg body weight for up to 21 days (cormorants) and 28 days (gulls) (Bursian et al. 2015b; Bursian et al. 2015c). The results of the oral toxicity studies indicate that oral dosing of Gulf-relevant species with DWH oil resulted in clinical signs and changes in a number of hematological, biochemical, and tissue endpoints consistent with oil exposures in previous field and laboratory studies (Ziccardi 2015), including:

- Clinical signs of anemia consistent with oxidative damage to red blood cells included decreased packed cell volume (PCV), increased incidence of red blood cells containing Heinz bodies (an indicator of impacts to hemoglobin), and changes in plasma clinical chemistries.
- Changes in oxidative stress endpoints that provide further evidence of systemic oxidative damage.

- Increased liver weights and decreased plasma cholesterol, glucose, and total protein concentrations, and concentrations of protein fractions found in dosed birds were indicative of liver dysfunction.
- Increased plasma urea and uric acid, along with pathological changes in the kidney were suggestive of kidney damage.
- During blood sample collections, many of the dosed birds were found to have diminished blood clotting abilities. Also during necropsies, some of the dosed birds were found to have hearts that were enlarged and flaccid. Both of these observations were new findings in birds exposed to oil.

Birds that ingested oil during preening of contaminated feathers also suffered adverse health effects, including lethargy, abnormal feces (i.e., watery feces with evidence of blood and tissue), feather damage, feather plucking, moribundity, signs of anemia, and heart defects (Dorr et al. 2015). Approximately 13 grams of oil were applied to the breast and back of the double crested cormorants every 3 days over a 2-week period (total of six applications). The level of oiling on the birds' plumage in these studies was consistent with a "moderate" degree of oiling (21 to 40 percent coverage of body), as described in Section 4.7 (Birds).

Exposed animals demonstrated a significant decrease in packed cell volume and significant increases in Heinz bodies and reticulocyte counts. Similar effects were found in birds collected from oil contaminated areas during the summer and fall of 2010 (Fallon et al. 2014). Like in the study with contaminated prey, many of the dosed birds had diminished blood clotting abilities. Liver, kidney, and gastrointestinal tract weight increased in response to oil application; and some exposed birds had cardiac abnormalities (as diagnosed by echocardiograms). In a similar study, birds that preened oil from their feathers had reduced body temperatures and a greater loss of body mass/body fat (Maggini et al. 2015).

A major consequence of oil ingestion, supported by Trustee laboratory studies, Trustee field studies (Fallon et al. 2014), and previous published work, is significant alterations to red blood cell presence and function. Oil exposure leads to the denaturation of hemoglobin, formation of Heinz Bodies within cells, and reduction in the oxygen carrying capacity. This can have significant effects on bird performance, limit their ability to fly, swim, and forage, with subsequent increased risk of death. Ingestion of DWH oil also decreased white blood cell counts, with related adverse effects on immune function. Immune impairment can reduce a bird's ability to combat bacterial, fungal, viral, or parasitic infections, increasing the risk of death.

Ingestion of DWH oil caused several types of organ damage and dysfunction, including liver, kidney, gastrointestinal tract, and cardiovascular systems. Ingestion of DWH oil disrupted digestive tract function, resulting in direct damage to tissues and poor absorption of fluids and nutrients. Finally, the Trustee studies found previously undescribed alterations in cardiovascular function following oil ingestion, including heart tissue abnormalities (e.g., flaccid heart musculature), changes to heart function (e.g., increased ejection velocities and volumes), and decreased blood pressure. Overall, disruption of organ physiology and function would have considerable negative consequences for an animal's fitness and survival (Ziccardi 2015).

4.3.3.4.2 Physical Effects of External Oil

In addition to the toxic effects of oil, the viscous, sticky nature of oil negatively impacts birds' abilities to take off, fly, and follow efficient flight paths.

Using a wind tunnel, the Trustees measured how oil on the body (not the wings) affected flight energetics and flight ability for western sandpipers (Maggini et al. 2015). Trace oiling (less than 5 percent of body surface) and moderate oiling (21 to 40 percent) caused increases in the average energy cost of flight relative to baseline, with moderately oiled birds being the most affected. External oiling also caused more erratic flying (birds were more likely to run into the wall of the wind tunnel) and a preference for shorter flight times (Maggini et al. 2015). Moderately oiled birds had faster wingbeat frequencies and larger wing movements, leading to higher energy costs.

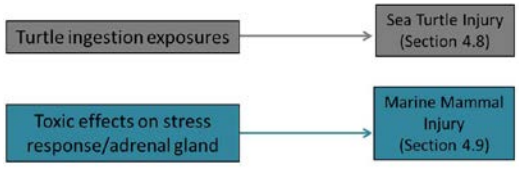
The flight performance of birds with oil on their wings and tail feathers was similarly affected. The Trustees used high-speed video to determine how trace levels of oil on wing and tail feathers affected the speed and angle of a bird's takeoff movements, and how that affected flight energy costs. Takeoff speed was slower in oiled birds, and they had to work harder to achieve flight when compared to unoiled birds (Maggini et al. 2015).

To investigate how oil affects a bird's ability to fly in a natural environment, the Trustees assessed the effects of externally applied DWH oil on the field-based flight performance of homing pigeons (Pritsos et al. 2015). The Trustees compared the flight performance of individual homing pigeons before and after oiling over trips of 50, 85, and 100 miles (from the release site to their home loft). After birds were exposed to oil, even in the light oiling category, they flew less efficiently (e.g., greater fluctuation in flight altitude over the course of the flight and altered flight paths) and took longer to return to their home loft. The studies also examined adverse health effects in oiled animals. Compared to their pre-oil flights, oiled animals weighed less after they returned to their home loft and took longer to recover the weight that is lost normally during the flight. Oiled birds also had indications of liver dysfunction and increased mortality.

The results of these flight studies indicate that even a trace amount of oil can cause a substantial increase in energy costs and can ultimately affect flight energetics and migratory performance. Mechanical effects (e.g., increased drag due to external oil) were partly responsible for increased energy demands for flight, implying that these results could be applied to all flying birds. In migratory birds, the added energetic costs of flight when oiled would result in additional time spent feeding, resting and preening, which in turn would slow down their migration affecting both their breeding performance and survival probability. These results imply that oiled birds will be less fit than non-oiled birds, leading to increased vulnerability to predation, reduced energy stores, and delayed arrival at breeding grounds (Ziccardi 2015).

4.3.3.5 Toxicity of DWH Oil to Sea Turtles and Marine Mammals

The Trustees' injury assessment of sea turtles and marine mammals included very limited toxicity testing because these organisms are federally protected.



4.3.3.5.1 Sea Turtles

During the spill, response workers collected many oiled sea turtles from the Gulf of Mexico. Sea turtles had DWH oil covering bodies and coating their esophagi (Section 4.8). As a component of the sea turtle injury assessment, the Trustees conducted limited laboratory testing with two surrogate turtle species, red-eared sliders (*Trachemys scripta elegans*) and snapping turtles (*Chelydra serpentina*). Exposures with surrogate species allowed us to obtain information on the toxicity of ingested oil that could not be measured directly in federally protected sea turtles.

Two oil exposure regimens were used (in addition to a control group) to approximate exposures estimated for minimally, lightly, and moderately oiled sea turtles observed in the field (see Section 4.8, Sea Turtles). Turtles were dosed daily for 14 days. Findings from the surrogate study, presented in Mitchelmore et al. (2015); Mitchelmore and Rowe (2015), included the following:

- Most of the turtles (greater than 96 percent) exposed in the laboratory continued to voluntarily feed despite the oil exposure.
- Dose-dependent increases in the levels of PAH were measured, demonstrating uptake and metabolism of oil at levels similar to those measured in the limited number of field-collected sea turtles (Ylitalo et al. 2014).
- Oiled turtles exhibited alterations in multiple toxicity endpoints, including oxidative and DNA damage.
- Observed physiological abnormalities in oil-exposed turtles included evidence of dehydration, decreased digestive function, and decreased assimilation of nutrients.

4.3.3.5.2 Marine Mammals

Bottlenose dolphins living in habitats contaminated with DWH oil showed signs of adrenal dysfunction, and dead, stranded dolphins from areas contaminated with DWH oil had smaller adrenal glands (Schwacke et al. 2014; Venn-Watson et al. 2015). Endocrine systems, including the adrenal gland in mammals (and the kidney in fish), enable vertebrates to respond to changes in their environment. In response to disturbances or stressful situations, chemical signals from the brain trigger a cascade of hormone releases into the bloodstream.

To further investigate the effect of DWH oil on an exposed organism's ability to respond to stress, the Trustees conducted laboratory tests with the Gulf toadfish (*Opsanus beta*) and laboratory-cultured human adrenal cells. Preliminary studies demonstrate that kidney cells from fish exposed to DWH oil exhibit an inhibition in their ability to secrete important stress hormones in response to a stimulant. Similarly, DWH oil caused dysregulation of stress hormone production in preliminary studies with human adrenal cells (the H295R cell line) (Takeshita et al. 2015).

4.3.4 Conclusions

The Trustees conducted a comprehensive program to evaluate the toxic effects of DWH oil on natural resources. The testing program consisted of studies designed to evaluate toxicity for the following resource categories and exposure pathways in the injury assessment.

Overall, the Trustees found that exposure to DWH oil causes a wide range of toxic effects, including death, impaired reproduction, disease, and other physiological malfunctions that reduce the ability of organisms to survive and thrive. Measured and modeled concentrations of DWH oil in surface water and sediments in the Gulf of Mexico at a number of locations and times during and following the spill were greater than the range of concentrations shown to cause these toxic effects in the Trustees' laboratory studies.

Specific findings from the Trustees' toxicity testing program are presented below.

4.3.4.1 Water Column Resources

- The embryos and larvae (i.e., early life-stage) of fish (ichthyoplankton) and various life stages of pelagic invertebrates (zooplankton) are particularly susceptible to the toxic effects of DWH oil, both when mixed with water and when present in the form of a surface slick. Measured and modeled concentrations of DWH oil in the Gulf of Mexico exceeded lethal levels in a number of locations and times during and following the spill.
- Thin, rainbow sheens of surface slick oil were extremely lethal to fish embryos and invertebrates.
- DWH oil is roughly 10 to 100 times more toxic to semi-transparent invertebrates and early life-stage fish in the presence of natural sunlight.
- In addition to lethality, exposure to DWH oil causes developmental abnormalities, including heart and spinal defects. Many of these developmental abnormalities are severe enough to kill early life-stage fish.
- Older fish (juveniles or adults) are less susceptible than embryos and larvae to the short-term lethal effects of DWH oil exposure. At most of the oil concentrations that occurred after the spill, the toxic effects of oil on older fish are more likely to have manifested as sublethal injuries, including growth inhibition, immunosuppression, decreased swim performance, or an abnormal stress response.
- The lethal effect of DWH oil on fish embryo and larvae and invertebrates has important ecological implications. In addition to sustaining fish and invertebrate populations, these small, planktonic organisms are an important base of the marine food web.

4.3.4.2 Benthic Resources

- Exposure of amphipods, a bottom-dwelling invertebrate, to sediments contaminated with DWH oil resulted in mortality at concentrations observed in deep-sea sediments and nearshore sediment and/or marsh soils following the spill.

4.3.4.3 Nearshore Resources

- Exposure of marsh organisms to sediments contaminated with DWH oil resulted in a series of adverse effects, including death, reduced growth, and reduced reproductive success.

- Higher concentrations of TPAH50 in sediments resulted in more adverse effects in more test species. Adverse effects were observed at concentrations as low as approximately 1 mg/kg TPAH50.
- Southern flounder were adversely affected by exposure to oil-contaminated sediment. Toxic effects included damage to gill and liver tissues, reduced growth rates, and mortality.
- Exposure to oil-contaminated sediments caused growth inhibition in juvenile red drum and Pacific white shrimp.
- Gulf killifish embryos exposed to oil-contaminated suspended sediments were less likely to hatch or to survive after hatching.
- There was substantial mortality to fiddler crab offspring exposed to relatively low concentrations of oil in or on sediments, when followed by exposure to sunlight.
- When marsh periwinkles were exposed to DWH oil on plants, they exhibited increased mortality and an impaired ability to move away from oil.
- Exposure to DWH oil caused adverse effects in all oyster life stages tested, at varying effects concentrations.

4.3.4.4 Birds

- When birds ingested food contaminated with DWH oil, they suffered from a variety of adverse health effects, including hemolytic anemia, liver dysfunction, kidney damage, hypothermia, weight loss, lethargy, abnormal feces, feather damage, moribundity (near death), and death.
- Ingestion of DWH oil caused several types of organ damage and dysfunction, including to liver, kidney, gastrointestinal tract, and cardiovascular systems. Ingestion of DWH oil disrupted digestive tract function, resulting in direct damage to tissues and poor absorption of fluids and nutrients.
- The Trustee studies found previously undescribed alterations in heart function following oil ingestion, including heart tissue abnormalities, changes to heart function, and decreased blood pressure. Overall, disruption of organ physiology and function would have considerable negative consequences for a bird's fitness and survival.
- External oiling caused feather damage and reduced flight performance. Oiled birds demonstrated more erratic and less-efficient flying, shorter flight times, and higher energetic costs.

4.3.4.5 Sea Turtles and Marine Mammals

- Surrogate species of freshwater turtles that ingested DWH oil turtles exhibited statistically significant alterations in multiple toxicity endpoints, oxidative damage, and DNA damage.
- Observed physiological abnormalities in oil-exposed turtles included evidence of dehydration, decreased digestive function and assimilation of nutrients.

- Exposure to DWH oil causes dysregulation of stress hormone secretion from adrenal cells (human cell line) and kidney cells (Gulf toadfish). Impacts on the endocrine system will affect an animal's ability to maintain homeostasis, respond appropriately to stressful situations, and lead to reduced fitness.

4.3.4.6 Common Observations Across the DWH Toxicity Testing Program

When organisms are exposed to chemical contaminants, the resulting toxic effects can manifest in a variety of manners depending on the species, individual life history, and the nature and concentration of the exposure. Despite this variation, the Trustees found a considerable degree of consistency among the types of toxic responses observed across the different organisms tested. For example, the Trustees observed cardiac effects in both fish and birds (Dorr et al. 2015; Incardona et al. 2014; Incardona et al. 2013; Morris et al. 2015b). Disruption of blood cells and function also was observed in fish and birds, both in the laboratory and in the field (Bursian et al. 2015b; Bursian et al. 2015c; Dorr et al. 2015; Morris et al. 2015b). Evidence of oxidative damage following exposure to oil was observed in fish, birds, and turtles (Bursian et al. 2015a; Mitchelmore et al. 2015; Morris et al. 2015b). Impairment of immune system function following exposure to oil was also observed in fish and birds, and was observed in field studies of dolphin health (Bursian et al. 2015b; Bursian et al. 2015c; Dorr et al. 2015; Morris et al. 2015b; Ortell et al. 2015; Venn-Watson et al. 2015). Evidence of impairment to stress responses and adrenal function was observed in fish, birds, and dolphins (Morris et al. 2015b; Takeshita et al. 2015; Venn-Watson et al. 2015). Evidence of impaired swim performance was observed in fish (Mager et al. 2014) and impaired flight performance in birds (Maggini et al. 2015; Pritsos et al. 2015).

Figure 4.3-22 illustrates the range of potential toxicological effects associated with exposure to DWH oil. Not every organism exposed to oil will experience all of the adverse health effects presented, and there is a very wide range in sensitivities between species and between individuals of the same species. However, all of the organisms that were exposed to elevated concentrations of DWH oil were forced to use energy to deal with the toxic insult. Many of those organisms would eventually have recovered fully, but others would have suffered from irreversible physiological effects that resulted in death, reduced life expectancy, or reduced reproduction.

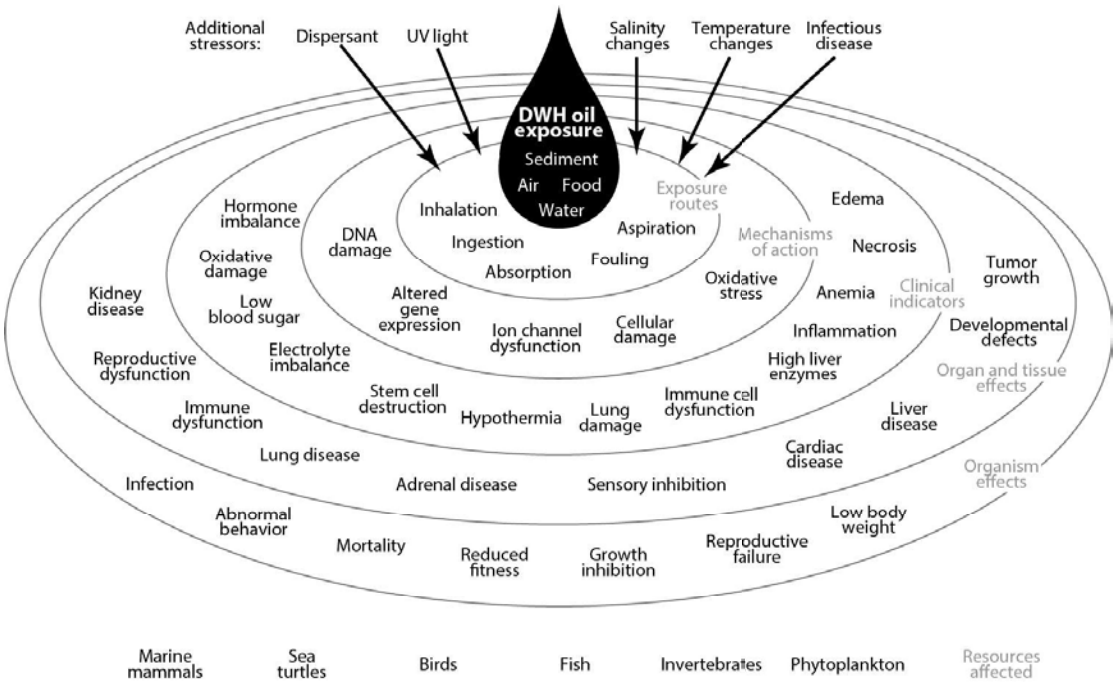


Figure 4.3-22. Conceptual illustration of the constellation of relationships between oil exposure and toxicological effects in organisms that were exposed to DWH oil during and after the spill. All of the exposure scenarios and resulting effects shown are supported by information in the literature and/or data generated through the Trustees' toxicity testing program.

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