Attachment C

Isaac Wirgin Affidavit

Riverkeeper, Inc. and Scenic Hudson Comments on Docket USCG-2014-0602

Review and Update of the New York/New Jersey Area Contingency Plan

October 10, 2014

SUPREME COURT OF THE STATE OF NEW YORK
COUNTY OF ALBANY

----------x

In the Matter of the Application of
RIVERKEEPER, INC., SIERRA CLUB ATLANTIC CHAPTER, WATERKEEPER ALLIANCE, INC., CENTER FOR BIOLOGICAL DIVERSITY, and EZRA PRENTICE TENANTS' ASSOCIATION

Index No. _____

Petitioners/Plaintiffs,

for a judgment pursuant to Article 78 and Section 3001 of the Civil Practice Law and Rules,

AFFIDAVIT OF ISAAC WIRGIN, Ph.D.

-against-

NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION, JOE MARTENS, as Commissioner of the New York State Department of Environmental Conservation, and GLOBAL COMPANIES, LLC,

Respondents/Defendants.

----------x

STATE OF NEW YORK)
: ss.:
COUNTY OF NEW YORK)

ISAAC WIRGIN, Ph.D., being duly sworn deposes and says:

1. I am an Associate Professor in the Department of Environmental Medicine of the New York University ("NYU") School of Medicine. I have been a faculty member in the Department since 1987 and my research is focused in two primary areas, the toxic effects of contaminants on ecosystem and human health and the population genetics of anadromous and marine fishes. I teach two courses in the Environmental Health Science program of NYU including one entitled, "Ecotoxicology-A Hudson River Case Study," that is offered annually and a second "Aquatic Toxicology," course that is offered on demand and more infrequently. I

am also a regular lecturer in two other NYU courses, "Toxicology" and "Genetic Susceptibility to Toxicant Induced Disease."

2. I am currently an Associate Editor of three related peer-reviewed journals including *Marine Environmental Research*, *North American Journal of Fisheries Management*, and *Marine and Coastal Fisheries* and in the past I have served on the Editorial Board of *Transactions of the American Fisheries Society*. To date, I have published 116 papers in peer-reviewed journals on the toxic effects of pollutants on ecosystem and human health, the population genetics of marine and anadromous fishes, and genetic susceptibility of human populations to several different cancers. I am a noted authority on the toxic effects of Hudson River-borne pollutants on its ecosystem and have frequently served as an invited speaker on various aspects of this topic locally, nationally, and internationally.

3. My research has been supported by a variety of funding agencies over the past 25 years. I have been the Principal Investigator on a variety of grants from the National Institute of Environmental Health Sciences (NIEHS), the Superfund Basic Research Program, the Hudson River Foundation, New York Sea Grant, the Electric Power Research Institute, the Gulf States Marine Fisheries Commission, National Oceanic and Atmospheric Administration's ("NOAA") Marfin Program, NOAA's Saltonstall Kennedy Program, and NOAA's Northeast Protected Resources Division. Currently, my research on sturgeons is supported by the Hudson River Foundation and NOAA's Office of Response and Restoration for studies on the toxic effects of polychlorinated biphenyls ("PCBs") congeners, PCB mixtures and dioxins, singly, and in binary combination, with global warming on early life-stages of Atlantic sturgeon and shortnose sturgeon.

4. My ground-breaking research on the development of resistance in early life-stages of Atlantic tomcod from the Hudson River to the toxic effects of PCBs and dioxin and its mechanistic basis was published in the prestigious journal *Science* and gained local, national, and international recognition. Coverage of this article was featured in the *New York Times Science Times*, Associated Press, *National Geographic News*, *Discovery News*, *Chemical Research in Toxicology*, the *Economist*, *Science News* and web sites of sponsor agencies at the Superfund Research Program and NIEHS. International coverage was provided by the *London Daily Mail* and featured in a Danish newspaper. Coverage of the story was also featured in broadcasts from National Public Radio nationally and in New York, the British Broadcasting Company, and the Canadian Broadcasting Company. Finally, resistance in Hudson River tomcod was portrayed in a soon to be released documentary produced by the TeraMatter/Skyvision entitled "Unnatural Selection."

5. I prepared this affidavit for Riverkeeper, Inc., and the Center for Biological Diversity, Inc., to inform their analysis of the potential impacts on sturgeons and other species from rail and marine vessel transport of heavy, sinking crude oils in and around the Hudson River Estuary, and spills of heavy, sinking crude oil, in the context of the application by Global Companies, LP to the New York State Department of Environmental Conservation (NYSDEC) to amend its Title V Air Permit to allow the heating, handling and transfer of heavy crude oil at its Terminal in Albany, New York.

6. There are 25 species of sturgeons worldwide and many or most populations in all species are threatened with extirpation or severe decline. The threats most often cited for demise of sturgeon populations include habitat alterations, compromised water quality, overharvest, and chemical pollution. Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*, and shortnose sturgeon,

Acipenser brevirostrum are two species of concern in the Hudson River and coastwide. Both species are afforded protection under the U.S. Endangered Species Act. Shortnose sturgeon is a charter member under this designation having been listed as "endangered" in 1973 and all populations of Atlantic sturgeon were added to the list in February 2012.

7. While shortnose sturgeon is listed as a single species coastwide, Atlantic sturgeon is listed as five distinct population segments ("DPS") coastwide, with the New York Bight DPS, which includes the Hudson River and Delaware River populations, listed as "endangered." Shortnose sturgeon is also listed under Appendix I of CITES (Convention on International Trade in Endangered Species) and both species are on the IUCN (International Union of the Conservation of Nature) red list. Thus, the status of both Hudson River sturgeons is of national and international concern.

8. Atlantic sturgeon is considered one of the three major glamour Hudson River resource species (the other two are striped bass and American shad). Its importance to life in the Hudson River valley is exemplified by its logo as the signature species on road signs on major roadways traversing the Hudson River and its major tributaries. That recognition may be in part due to its longevity (up to sixty years) and its large size (historically up to 800 lbs.). During the 1880s and 1890s, the Hudson River hosted large riverine fisheries for large spawning Atlantic sturgeon, but these in the Hudson and other rivers coastwide collapsed by 1900 to less than 10% of their historic highs and remained at low levels throughout the 20th century. The failure of these fisheries to rebound, and the development in the late 1980s of new coastal fisheries in the New York Bight targeting migrating subadult Atlantic sturgeon from the Hudson and elsewhere resulted in the imposition in 1998 of a 40-year coastwide moratorium in the U.S. on the harvest of Atlantic sturgeon. Continued perception of the failure of most populations to rebound over

the next 15 years resulted in the federal listing under ESA of Atlantic sturgeon in 2012. One of the major threats to the stabilization and rebuilding of Atlantic sturgeon populations listed in the U.S. federal listing document was chemical pollutants.

9. Contemporary coastwide distributions of both sturgeon species extends from Atlantic Canada (St. Lawrence River, Quebec-Atlantic sturgeon; Saint John River, New Brunswick-shortnose sturgeon) to rivers in northern Florida and southern Georgia. Atlantic sturgeon is anadromous (spawn in freshwater and spend the majority of the remainder of their lives in marine waters) and shortnose sturgeon is amphidromous (spend their entire lives within estuaries but move upstream to freshwater portions to spawn). Historically, Atlantic sturgeon were believed to spawn in 35 rivers coastwide, but today spawning persists in a maximum of 20 rivers, of which the Hudson is one. Both species are seasonally distributed for almost the entire length of the tidal Hudson River extending from just below the Federal Dam in Troy, New York, to New York City and thus both are likely to be exposed to a wide range of contaminants that are present to varying degrees throughout the entire 150 mile Estuary. Atlantic sturgeon subadults exit from the Hudson River at 2 to 6 years of age into coastal waters and non-natal estuaries and adults only return to spawn at 11 to 19 years of age. Shortnose sturgeon are usually resident throughout their life histories in natal rivers such as the Hudson, although they do make seasonal movements between upriver and downriver sites.

10. In Atlantic sturgeon, spawning is intermittent with females in the Hudson River spawning at three to five-year intervals and males at shorter intervals, a characteristic combined with spawning at advanced ages, which makes the rebuilding of depleted populations more problematic. In shortnose sturgeon, Hudson River females spawn every third year and males may spawn annually.

11. In the Hudson River, shortnose sturgeon spawn in April-May whereas Atlantic sturgeon spawn later in mid-June-early July. Shortnose sturgeon spawn from the mid-Hudson to Coxsackie, New York, whereas Atlantic sturgeon spawn above the salt front in an area centered around Hyde Park, New York. Recent evidence also suggests additional spawning elsewhere in the Hudson River including the Catskill and Albany areas.

12. Atlantic sturgeon in the Hudson River spawn over rubble bottom, although it is not clear what habitat shortnose sturgeon prefer. The success of young life stages determines the recruitment success of individual year classes to the overall adult populations of both sturgeon species, and these same young life stages of both Hudson River sturgeon species would be particularly vulnerable to the toxic effects of heavy, sinking oil spilled into the river, as I describe below.

13. Early life-stages of fishes are sensitive, logistically tractable, and statistically robust models to evaluate the toxic effects of many pollutants on ecosystem health. In controlled laboratory experiments, young life-stages (embryos and larvae) of many fish species are hypersensitive to the toxic effects of a variety of contaminants—much more so than older juvenile and adult life stages. Non-fatal perturbations in young life-stages may persist and adversely impact adult performance. Contaminants which are known to induce toxic responses in embryos and larvae of fishes include polycyclic aromatic hydrocarbons ("PAHs") found in crude oils, dioxins, furan, coplanar PCBs, and some metals. However, several studies have demonstrated that the sensitivities of fish species differ dramatically, over several orders of magnitude, to the toxic effects of PCBs and dioxin. For example, lake trout, brook trout and rainbow trout embryos are extremely sensitive to the toxic effects of dioxin ("TCDD") and PCBs, whereas northern pike, white suckers, and zebra fish are much less so[1]. Our recent studies

with young life-stages of Atlantic sturgeon and shortnose sturgeon suggest that they are among the most sensitive of fish species to toxicities from these contaminants [2,3].

14. Several studies have demonstrated that the toxic effects of exposure of fish embryos and larvae to coplanar PCBs, dioxin, and larger PAHs (but not smaller PAHs) in crude oil result from a single molecular mechanism-activation of the aryl hydrocarbon receptor ("AHR") pathway The AHR pathway and its functions are conserved (shared) among all vertebrate species from fishes to humans. One difference between fishes and higher vertebrates is that fishes have two forms of AHR; AHR1 and AHR2, whereas mammals have only a single form, AHR1. The AHR is a transcription factor that resides in the cytoplasm of all cells and binds lipophilic contaminant molecules such coplanar PCBs, dioxin, and many PAHs when they diffuse into the cell. This binding of contaminants to AHR triggers a cascade of downstream events that eventually results in the translocation of the AHR-contaminant complex to the nucleus where it binds specific short DNA domains in promoter regions upstream of the coding region of a battery of genes in the AHR battery that are critical to the detoxification and sometimes activation of these contaminants. This binding action of AHR activates the transcription of these genes and through unknown mechanisms mediates their toxicity. Knockout or knockdown of the AHR has been shown to eliminate many of the toxic effects of exposure to these chemicals. One gene in the AHR battery whose expression is highly and dose-responsively increased (induced) by exposure to all of these contaminants at the transcriptional and translational levels is cytochrome P4501A (CYP1A). This induced expression of CYP1A is particularly pronounced in livers and hearts of fishes. Thus, level of CYP1A expression is widely used in fishes to measure their exposure to aromatic hydrocarbon contaminants. It is also

employed as an early indicator of activation of the AHR2 pathway in fishes and likely ensuing downstream organismic toxic events.

15. Studies specifically in fishes have demonstrated that "knockdown" of AHR2 protein expression ameliorates the toxic actions of these chemicals. Further studies in fishes, birds, and mammals have demonstrated that there are genetic polymorphisms in AHR1 and AHR2 among populations within species and genetic variation among species that dramatically impact their sensitivities to the toxic effects of these chemicals. For example, my studies found that a genetic polymorphism in AHR2 in Atlantic tomcod from the Hudson River in combination with intense selective pressure for the variant AHR2 protein (less functional AHR2 protein) resulted in the dramatic resistance to PCBs and dioxin observed in the Hudson River population[4]. Similarly, studies with Atlantic killifish embryos from PCBs contaminated New Bedford Harbor, MA, suggest that several genetic polymorphisms in AHR2 serve as the mechanistic basis to PCB resistance in that population. Clearly, genetic variation in the AHR pathway among species of fishes can serve as the mechanistic basis to their relative sensitivities to contaminant toxicities. Recent studies suggest that the forms of AHR in sturgeons may make them particularly vulnerable to toxicities of some PAHs, PCBs, and dioxin.[5] Recent studies with white sturgeon suggest indicate that unlike in other fishes, both forms of their AHR; AHR1 and AHR2, are functionally active which may make them among the most sensitive species to the effects of these contaminants[6].

16. Toxicities that are often observed in young life-stages of fishes include reduced embryonic survivorship, altered duration to hatch, reduced hatching success, impaired eye development, smaller overall body size, and a variety of morphometric alterations including craniofacial malformations and abnormal spinal curvature. Hallmark toxicities that are always

seen in all fish models also include pericardial and yolk sac edemas. All of these alterations are incompatible with successful development of these life-stages in natural environments because of the reduced ability to avoid predators or capture prey.

17. Most, if not all, of these toxicant-induced aberrations result from alterations in cardiac morphology and function[7,8]. The hearts of toxicant-exposed embryos and larvae are usually smaller, contain fewer myocardial cells, and assume abnormal three-dimensional structures by altering the normal folding of the heart's two chambers. These alterations typically impair cardiovascular function through irregular atrial arrhythmia and bradycardia, a slowing of the heart beat rate. Toxic effects of these atypical cardiac morphology result in reduced blood flow to peripheral blood vessels.

18. When examining the toxic effects of crude oils on early life-stages of fishes, it is important to remember that all crude oils are different, but nonetheless each is comprised of complex mixtures of different families of PAHs that contain 3 to 7 fused benzene rings. Toxicities of the various PAHs in oils differ as does the mechanistic basis of their actions[9,10]. Also, the composition of crude oils released into the environment changes during the weathering process because of volatilization of the smaller PAHs and resulting increasing concentration of higher molecular weight PAHs.

19. Concern over the effects of crude oils on fishes and our knowledge of the subject emanate primarily from two events, the Exxon Valdez and Deepwater Horizon disasters. From the Exxon Valdez event, it was learned that early life-stage of fishes, such as Pacific herring and Pink salmon, are extremely sensitive to oil-induced early life-stage toxicities and that effects of exposure can persist to the adult life-stage and significantly affect recruitment into populations. Furthermore, studies demonstrated that these toxic effects of oils in embryos and larvae occur

primarily through structural and functional impairment of the developing heart[7,8]. Studies with low molecular weight PAHs found in weathered Exxon Valdez crude oil demonstrated that exposed fish embryos developed similar toxic syndromes to those described previously for coplanar PCBs and dioxin, including pericardial and yolk sac edemas, craniofacial alterations and body axis defects—all teratogenic defects resulting from cardiovascular dysfunction. PAHs in this oil directly disrupted cardiac function by interfering with the interdependent processes of heart chamber formation and blood circulation. Most interestingly, these studies demonstrated that the low molecular weight tricyclic PAHs that incurred this cardiovascular dysfunction did so through an AHR-independent mechanism. In contrast, crude oil with 4-5 and greater ringed PAHs often, although not always, induced their early life-stage toxicity through an AHR2-dependent mechanism. Even within the group of 5-ringed PAHs, it was found that both AHR2-dependent and AHR2-independent mechanisms were operative to induce tissue-specific toxicities[9]. Thus, it can be seen how different crude oils, and with different PAHs constituents and different patterns of weathering induce different toxicities through more than one molecular mechanism.

20. A recent study reported in the *Proceedings of the National Academy of Sciences of the U.S.* on controlled laboratory investigations on the effects of the Deepwater Horizon wellhead blowout on early life-stages of pelagic fishes such as southern bluefin tuna, yellowfin tuna, and amberjack, confirmed that a very different crude oil, Mississippi Canyon252, was toxic to embryonic fish hearts[11]. The study revealed essentially the same manifestations of cardiovascular toxicity with Deepwater Horizon crude as seen previously for Exxon Valdez oil, namely bradycardia, and irregular arrhythmia followed by edemas. In this case, toxic perturbations of oil exposure also included finfold defects and reduced fin growth. Furthermore,

results of a companion study with field-collected Deepwater Horizon oil reported in *Science* demonstrated that oil-exposed cardiomyocytes isolated from bluefin tuna and yellowfin tuna exhibited impaired cardiac excitation-contraction by disrupting repolarization by blocking the K+ channel and sarcolemmal and sarcoplasmic reticulum Ca2+ cycling[12]. These mechanistic findings provided a direct link between crude oil exposure, disruption of cellular excitability, altered cellular contractability, and the morphological and functional cardiovascular toxicities described previously in the hearts of fish embryos and larvae. Because the mechanistic basis of cardiomyocyte contractibility is highly conserved across all vertebrates, it is likely that this cascade of injuries from crude oil will similarly affect all fishes, including Hudson River sturgeons. Both sturgeons are bottom-dwelling, including their embryos and larvae which adhere to benthic substrates. The heavy, viscous nature of certain lipophilic contaminants, such as heavy crude oils, may cause the contaminants to sink and persist in the benthic environment, potentially increasing duration of exposure for sturgeons and preventing or hindering development during critical early life-stages.

21. Because of their spawning and distribution of young life-stages in upper portions of the tidal Hudson River estuary, sturgeons potentially can be highly exposed to spilled oil products. Similarly, their benthic eggs and larvae put them at risk to toxicity from lipophilic contaminants such as heavy crude oil, which will sink to the bottom and directly impact benthic organisms and habitat, unlike lighter forms of crude which float at or near the water's surface. Moreover, recent empirical studies suggest that North American sturgeons may be among the most sensitive of freshwater and anadromous fishes to the early life-stage toxic effects of these chemicals.

22. In collaboration with Dr. R.C. Chambers of the Northeast Fisheries Center of NOAA at Sandy Hook, N.J., my group evaluated under controlled laboratory conditions the sensitivities of Atlantic sturgeon and shortnose sturgeon embryos and larvae to toxicities of graded doses of a single coplanar PCB congener ("PCB126") and dioxin (TCDD). In these studies, embryos of both sturgeons were exposed for 24 hr. to PCB126 and TCDD, singly, and a variety of toxic endpoints were evaluated including survival to hatch, time to hatch, hatching rate success, 10 morphometric measurements in larvae reflective of normal patterns of growth, an eye development index, and survival success of unfed larvae. These responses in chemically exposed young life-stages of both species were compared to those in similarly aged specimens that were untreated or treated with solvent vehicle.

23. In summary, we found that survival to hatching of shortnose sturgeon decreased with increasing dose of PCB 126 and dioxin, while the duration of the embryonic period showed little evidence of chemical alteration for either sturgeon species[2]. A variety of morphometric features of hatchling larvae of both sturgeon species was affected by dose, most notably a shortening of the body, reduction in head size, increased inflation (edema) around the yolk sac, a reduction in the quantity (length) of yolk reserves, and a reduction in eye size. Eye development in both species decreased inversely with dose of each chemical. Finally, the persistence of unfed larvae (median larval lifespan) of both species decreased inversely with dose with both chemicals with sharp declines in lifespan occurring at PCB126 and dioxin nominal doses of > 1 part per billion (ppb) and > 0.1 ppb, respectively. The anadromous Atlantic sturgeon appeared more sensitive than the amphidromous shortnose sturgeon and observed dose-responsive early life-stage toxicities of both species are among the more sensitive found in fishes to date. Our study was among the first to demonstrate that sturgeon species are sensitive to the hallmark early

life-stage toxicities induced by AHR activating toxins such as coplanar PCBs and dioxin. Most importantly, the toxic responses for many of these endpoints in these sturgeons were induced at levels of PCBs or dioxin typically found in the Hudson River. Because the toxicities of PAHs, PCBs, and dioxin often occur by an identical AHR activated mechanism, it is likely that Hudson River sturgeons will also be unusually sensitive to the toxic effects of PAHs in crude oil.

24. In related laboratory studies, my group explored the sensitivity of Atlantic sturgeon and shortnose sturgeon to PCB126 and dioxin induction of levels of CYP1A expression, an indicator of AHR2 pathway activation and a predictor of likely downstream organismic toxicities. After partially cloning the CYP1A gene in both Hudson River sturgeon species, we showed that CYP1A expression (and presumably AHR2 activation) in embryos of both sturgeons was dose responsive and significantly induced at environmentally relevant doses of these toxicants as low as 10 parts per trillion for PCB126 and 1 part per trillion for TCDD[3].

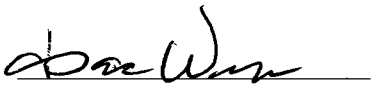
25. Recent studies in white sturgeon provide a mechanistic basis for the possible hypersensitivity of sturgeons to these contaminants. Studies by researchers at the University of Saskatchewan on white sturgeon demonstrated their tissue-specific sensitivity to beta-naphthoflavone (a PAH analogue) induction of CYP1A expression occurred at levels that were similar to the previously demonstrated hypersensitive trout models[5]. Further studies by the Canadian group on AHRs in white sturgeon indicated that unlike in other fishes, both AHR1 and AHR2 are likely functional, highly inducible by beta-naphthoflavone and widely expressed across many tissues suggesting that sturgeon species may be more sensitive to aromatic hydrocarbon contaminants such as PAHs than other fishes because of the potential cooperative activities of the two AHRs[6]. In summary, both early life-stage toxicity studies and sensitivity of CYP1A to induction by PAHs, PCBs, and dioxin indicate that sturgeons are unusually sensitive,

more so than most fishes, to the AHR-mediated toxic effects of exposure to PCB126 and PAHs and a mechanistic basis for this extreme vulnerability is suggested.

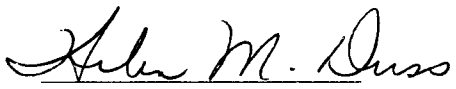
26. In summary, spillage of heavy crude oils into the tidal Hudson River environment will almost certainly adversely impact its ecosystem which is already burdened with unusually high levels of other damaging sediment-borne contaminants. It is likely that these heavy crude oils will be highly persistent in the benthic environment and will be acutely toxic to adult life stages of its fish community. More importantly, semi-soluble PAH constituents of these crude oils will be bioavailable for years to early life-stages of fishes in the Hudson River community. These spills are likely to occur near or upstream of spawning and nursery grounds of two listed sturgeon species of local, federal, and international concern. Many studies have demonstrated that early life-stages of fishes are particularly sensitive to the toxic effects of PAHs in crude oils through a proven highly conserved mechanism, cardiovascular dysfunction. Furthermore, exposure of embryonic fish can permanently reduce cardiac performance that can potentially lead to delayed mortalities and decreased performance in juveniles and adults. Moreover, recent investigations with aromatic hydrocarbon contaminants such as PAHs and PCBs have indicated that sturgeons are among the most sensitive of fishes to early life-stage toxicities induced by these contaminants. Because the developing heart in fishes, and perhaps particularly sturgeons, is an exceptionally sensitive and consistent indicator of crude oil impacts, the Hudson River population of these two protected species will almost certainly be challenged and damaged by the spillage of heavy crude oil in the environment.

27. I declare, under penalty of perjury, that the foregoing is true and correct.

Isaac Wirgin, Ph.D.



Sworn before me this
6th day of June, 2014



Notary Public

HELEN M DUSS
NOTARY PUBLIC-STATE OF NEW YORK
No. 01DU6286225
Qualified in Orange County
My Commission Expires July 22, 2017