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Letter to the Editor

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Letter to the Editor

The authors' reply:

Page et al. [1] appear to have missed our point that the teratogenic effects of oil on fish derive from embryonic exposure to environmentally persistent 3- and 4-ring polycyclic aromatic hydrocarbons (PAHs) [2]. They regard our conclusions as incorrect because we failed to demonstrate causality or a clear dose-response relationship. As evidence for lack of causality, they indicate that our data were not replicated. However, our report was a companion paper to a similar one demonstrating PAH-induced teratogenesis in herring embryos [3], also in the low ppb. Current literature corroborates our data and careful consideration of our conclusion demonstrates that their inclusion of low molecular weight PAHs in their dose-response relationship is counter to their thesis that dose measures should only include toxic compounds.

When we published this work 12 years ago, the concept that PAHs with high octanol-partition coefficient (K_{OW}) were teratogenic at concentrations below their solubility was considered novel. Since then, the sensitivity of developing fish embryos to ppb concentrations of PAHs dissolved in water has been confirmed for fish embryos exposed to oiled sediments [3], dissolved mixtures of PAHs derived from oiled sediments, and specific high molecular weight PAHs dissolved in water; additional references will be found in the Supplemental Data. More recently, experiments involving specific PAHs with partition-controlled delivery systems also have demonstrated toxic effects at levels below aqueous solubility limits. At least eleven reports replicate our findings in seven different fish species and support our conclusion that accumulation of PAHs by embryos depends on the kinetics of the transfer of PAHs from oil to egg rather than PAH solubility.

As with any pioneering research, our study 12 years ago lacked corroboration in the literature initially, but since then, our conclusions have inspired research elucidating mechanisms of PAH embryotoxicity in much greater detail. At the time we published our paper, the narcosis model of PAH toxicity was presumed to be the most important source of injury following oil spills. At least two more toxicity mechanisms have been described since that time. Induction and effects of CYP1A similar to those of dioxin-like compounds have been demonstrated for high K_{OW} compounds such as some C2 phenanthrenes. More recently, Incardona et al. [4] demonstrated that compounds such as unsubstituted phenanthrene and dibenzothiophene disrupt signal conduction in developing hearts, causing decreased aerobic capacity in survivors [5].

Despite this decade of effort, Page et al. [1] suggest that our effects may have resulted from metabolic intermediates produced during microbial digestion of oil in our incubators. However, the compounds and mechanisms by which microbial degradation of oil results in teratogenesis have not been described, nor has microbial degradation of oil been shown to increase the toxicity of oil-contaminated effluents.

Page et al.'s [1] misunderstanding of the relationship between weathering and PAH toxicity is apparent when they indicate that we believe that ''oil becomes more toxic as it weathers.'' We have noted, as described above, that the most toxic components of oil are also the most environmentally persistent and thus become more concentrated in the oil as weathering proceeds because other, less toxic oil components are lost more quickly. Although this means a unit mass of very weathered oil is more toxic than a unit mass of less weathered oil, it does not follow that a unit mass of oil will increase in toxicity as it weathers.

The compositional differences in the oil used in our study revealed the most embryotoxic components to be the PAH with highest molecular weights. Our weathered oil (WO) and very weathered oil (VWO) were both derived from Alaska North Slope crude oil, the cargo oil of the *Exxon Valdez,* but had different compositions caused by the weathering differences. For example, the dibenzothiophenes, phenanthrenes, and chrysenes accounted for more than 80% of the total PAH (TPAH) in the oil phase of our VWO but contributed to less than half the TPAH of our WO. However, the absolute concentrations of these heavy compounds were similar in the WO and VWO oils because of the effects of loss rate kinetics. Comparison of the effects associated with exposure to WO and VWO amounted to an examination of the potency of compounds present in WO but not in VWO. It was from these observations that we concluded that ''...the smaller PAHs contribute relatively little to observed toxicity'' [2] in our embryotoxicity tests.

As a result of misunderstanding these observations, Page et al. [1] offer a dose-response curve that includes compounds with little toxic effect such as naphthalenes. Including these lower molecular weight PAHs in their dose measures causes Page et al. [1] to inflate their doses relative to our VWO dose; hence, plots using these inflated values create the misleading impression that our VWO response does not fit a dose-response relationship. If embryotoxicity were dependent on a narcosis mechanism of toxicity, then the lighter compounds such as napthalenes would be much more relevant, but that is not the case. Consider the relationship between embryo mortality and the most environmentally persistent PAHs shown in Figure 1. In this figure we present the geometric mean concentration of these compounds in water during the first 63 d of exposure. Instead of concluding that no dose response exists, we conclude that the lowest effective concentration of these compounds is near 0.1 ppb.

Page et al.'s misapprehension [1] extends to their analysis of the dose-response relationships in Barron et al. [6], where they claim that the VWO failed to fit Barron et al's. toxic-unit based dose-response relationships. Barron et al. compared the explanatory value of different toxicity models based on data derived from our experiments [2] and those of Carls et al. [3]. The point made by Barron et al. was that narcosis models of toxicity did not predict effects as well as models based on more environmentally persistent PAHs such as C2 to C4 phenanthrenes. This

All Supplemental Data may be found in the online version of this article.
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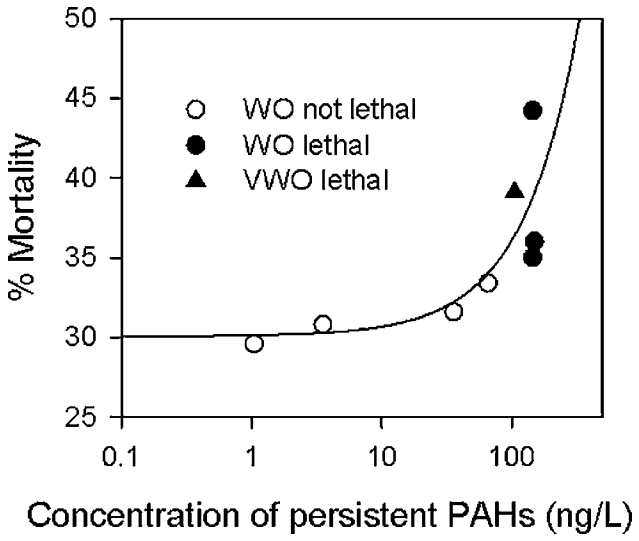

Fig. 1. Mortality of developing pink salmon embryos following exposure to the seven most environmentally persistent polycyclic aromatic hydrocarbons (PAHs) in *Exxon Valdez* crude oil. Concentrations are the geometric mean of PAHs with loss-rate constants less than 0.1 [7] in incubator effluents averaged over the first 63 d exposure [2].

is in agreement with our conclusions. What is more important is that these different modes of toxicity are not mutually exclusive and that toxicity proceeds by multiple pathways. The importance of these different pathways will vary with the composition of toxicant mixtures, as well as by species and life stage. Consequently, adherence to a single view of toxicity can lead to misinterpretation of results.

In summary, Page et al. [1] do not follow their own admonition when they describe our findings as unsupported. They initially argue that the appropriate dose-response relationship should include only those components that cause toxicity, but then evaluated our dose measures with a metric they identified as inappropriate. They claim our work lacks corroboration or confirmation, ignoring the substantial body of literature over the last twelve years demonstrating otherwise. We therefore dismiss the concerns raised by Page et al. [1] as entirely without merit, and stand by our conclusions.

SUPPLEMENTAL DATA

Supplemental References. (29.7 KB DOC).

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