2008

Letter to the Editor: COMMENT ON "TOXICITY OF WEATHERED *EXXON VALDEZ* CRUDE OIL TO PINK SALMON EMBRYOS"

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SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 27, No. 7, pp. 1475–1476, 2008

Printed in the USA
0730-7268/08 $12.00 + .00

Letter to the Editor

COMMENT ON "TOXICITY OF WEATHERED *EXXON VALDEZ* CRUDE OIL TO PINK SALMON EMBRYOS"

To the Editor:

We take issue with an article by Brannon et al. [1] in *Environmental Toxicology and Chemistry* questioning the validity of our work on the toxicity of petroleum-derived polycyclic aromatic hydrocarbons (PAHs) to the early life stages of fish. Beginning in 1997, we published a series of articles demonstrating adverse effects in response to PAH exposure at concentrations in the low parts per billion [2–6]. Based on their attempt to reproduce our results, Brannon et al. [1] argue that the effects we described were an artifact caused by contact with PAH-laden oil microdroplets instead of dissolved PAHs and, therefore, that our results likely are not applicable to most field situations. The arguments advanced by Brannon et al. [1] are flawed for a number of reasons, and we stand by our published work.

This letter focuses only on our fundamental concerns about the Brannon et al. article [1]. We have posted a detailed review at http://www.afsc.noaa.gov/ABL/Habitat/pdfs/review-3.pdf.

Any scientist should welcome independent efforts at confirmation of his or her work, if only as an indication of its potential significance, and indeed we do. But equally, any scientist who attempts to confirm the results of another has an obligation to faithfully duplicate the important aspects of the experimental conditions employed. Failure to do so may produce results at odds with those of the original work.

We designed our dosing apparatus to mimic PAH desorption from oil-coated rock substrate into interstitial water of beaches, where the PAH-contaminated water might be transported to developing salmon embryos [4,7,8], using oil from the same source (Alaska North Slope, USA) as that released during the 1989 *Exxon Valdez* spill. We suspected this exposure pathway may have been important during the years immediately following the 1989 *Exxon Valdez* oil spill, during which time elevated salmon embryo mortality was measured in streams contaminated with *Exxon Valdez* oil [9,10].

When we conducted our experiments, we were keenly aware that introduction of oil microdroplets by the dosing apparatus might seriously confound interpretation of the results. We described in detail the steps taken to prevent microdroplet formation, and we performed chemical measurements to evaluate the efficacy of these steps [2–6,11]. To suppress production of oil microdroplets, we sprayed an oil aerosol onto continuously tumbling rock for at least 90 s to minimize oil film thickness and, hence, promote adhesion. Because oil losses to the mixing container walls were obvious, we reported the oil loadings on the basis of direct measurement of oil adhered to the rock, which was only approximately 40% of the oil sprayed during application. To assess contributions from oil microdroplets during dosing, we used the large difference in the oil–water partitioning behavior of phytane (a branched aliphatic hydrocarbon) compared to that of PAHs. An absence of phytane in water samples indicates an absence of bulk-phase oil in the dosing water [12], and the ratio of PAH to phytane in the dosing water indicated that PAH contributions from bulk-phase oil were negligible.

By their own admission, Brannon et al. [1] took none of these precautions. Instead of spraying the oil onto their rock as an aerosol, they simply added the oil as a single aliquot, minimizing adhesion and making their preparation more prone to droplet formation. Instead of measuring the oil that actually adhered to the rock at the end of their mixing process, they simply assumed complete adhesion, explaining away the interpretive discrepancies this causes with what we feel are incorrect statements. Instead of actually measuring PAH contributions from oil microdroplets in their dosing water, they provide a rationale supported by a single visual observation in their highest dose—the one most prone to droplet formation—despite clear chemical evidence in their data that contributions from oil droplets cannot have been substantial (http://www.afsc.noaa.gov/ABL/Habitat/pdfs/review-3.pdf).

We accept the observation by Brannon et al. [1] of visible oil droplets in their highest exposure dose at face value, but the ensuing speculation is unwarranted. If oil microdroplets were present in their highest dose, perhaps they played some part in the toxic effects observed for the embryos exposed to that dose, although based on their chemical data, we doubt it. In any case, this does not necessarily imply that microdroplets were present in their lower doses, at which toxic effects also were observed. Furthermore, because the dosing method used by Brannon et al. [1] was more prone to droplet formation than our own, their observation does not imply the presence of microdroplets in any of our doses; we have strong chemical evidence that microdroplets were not present [2–6]. Brannon et al. [1] simply have no basis for their claim that microdroplets affected our experiments beyond their speculative extrapolation from their less carefully executed experiment.

Despite these experimental inconsistencies, we note that Brannon et al. [1] nonetheless have confirmed our basic findings. This may not be apparent, because they characterize their doses mainly in terms of nominal oil loadings (i.e., the amount of oil added per unit mass of rock) and compare these with our measured PAH doses in water. However, when viewed in terms of PAH concentrations measured in their dosing water (their Tables 1 and 2), the toxicity end points they monitored appear at aqueous PAH concentrations that are comparable with the results of our studies and those of others [2–6,13–15]. So, at the very least, perhaps we can agree that in every study that has subjected fish embryos to aqueous PAH concentrations in the low parts-per-billion range, adverse effects have been detected, provided that the embryos are monitored for their delayed manifestation.

The experimental finding that would cast doubt on the toxicity role that we have ascribed to PAHs would demonstrate an absence of toxic effects following exposure to dissolved

PAHs only. Neither Brannon et al. [1], nor anyone else to our knowledge, have reported such a result. Until this finding is persuasively presented in the scientific literature, we will stand by our published interpretations regarding the embryotoxicity of dissolved PAHs in the low parts-per-billion range [2–6].

In conclusion, although the procedures used by Brannon et al. [1] were more prone to droplet formation than our own, their chemical and biological evidence demonstrates the toxicity of oil droplets was negligible, corroborating our multiple studies [2–6]. Aqueous PAH concentrations, including hypothetical oil droplets, were damaging at less than 8 μg/L (ascites) in the Brannon et al. [1] study, which also is consistent with our results. What distinguishes the Brannon et al. [1] report from our own is that they base their interpretations on dose added (nominal oiling) instead of on dose measured (aqueous TPAH concentration).

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REFERENCES

1. Brannon EL, Collins KM, Brown JS, Neff JM, Parker KR, Stubblefield WA. 2006. Toxicity of weathered *Exxon Valdez* crude oil to pink salmon embryos. *Environ Toxicol Chem* 25:962–972.
2. Carls MG, Rice SD, Hose JE. 1999. Sensitivity of fish embryos to weathered crude oil: Part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). *Environ Toxicol Chem* 18:481–493.
3. Carls MG, Heintz RA, Marty GD, Rice SD. 2005. Cytochrome P4501A induction in oil-exposed pink salmon (*Oncorhynchus gorbuscha*) embryos predicts reduced survival potential. *Mar Ecol Prog Ser* 301:253–265.
4. Heintz RA, Short JW, Rice SD. 1999. Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered *Exxon Valdez* crude oil. *Environ Toxicol Chem* 18:494–503.
5. Heintz RA, Rice SD, Wertheimer AC, Bradshaw RF, Thrower FP, Joyce JE, Short JW. 2000. Delayed effects on growth and marine survival of pink salmon (*Oncorhynchus gorbuscha*) after exposure to crude oil during embryonic development. *Mar Ecol Prog Ser* 208:205–216.
6. Marty GD, Short JW, Dambach DM, Willits NH, Heintz RA, Rice SD, Stegeman JJ, Hinton DE. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 75:989–1007.
7. Carls MG, Thomas RE, Lilly MR, Rice SD. 2003. Mechanism for transport of oil-contaminated groundwater into pink salmon redds. *Mar Ecol Prog Ser* 248:245–255.
8. Carls MG, Rice SD, Marty GD, Naydan DK. 2004. Pink salmon spawning habitat is recovering a decade after the *Exxon Valdez* oil spill. *Trans Am Fish Soc* 133:834–844.
9. Bue BG, Sharr S, Moffitt SD, Craig AK. 1996. Effects of the *Exxon Valdez* oil spill on pink salmon embryos and preemergent fry. *American Fisheries Society Symposium* 18:619–627.
10. Bue BG, Sharr S, Seeb JE. 1998. Evidence of damage to pink salmon populations inhabiting Prince William Sound, Alaska, two generations after the *Exxon Valdez* oil spill. *Trans Am Fish Soc* 127:35–43.
11. Carls MG, Marty GD, Meyers TR, Thomas RE, Rice SD. 1998. Expression of viral hemorrhagic septicemia virus in prespawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil. *Can J Fish Aquat Sci* 55:2300–2309.
12. Payne JR, Driskell WB, Short JW. 2004. Prince William Sound RCAC long-term environmental monitoring program, 2003-2004 LTEMP monitoring report. Prince William Sound Regional Citizens' Advisory Council, Anchorage, AK, USA.
13. Kiparissis Y, Akhtar P, Hodson PV, Brown RS. 2003. Partition-controlled delivery of toxicants: A novel in vivo approach for embryo toxicity testing. *Environ Sci Technol* 37:2262–2266.
14. Rhodes S, Farwell A, Hewitt LM, MacKinnon M, Dixon DG. 2005. The effects of dimethylated and alkylated polycyclic aromatic hydrocarbons on the embryonic development of the Japanese medaka. *Ecotoxicol Environ Saf* 60:247–258.
15. Farwell A, Nero V, Croft M, Bal P, Dixon DG. 2006. Modified Japanese medaka embryo-larval bioassay for rapid determination of developmental abnormalities. *Arch Environ Contam Toxicol* 51:600–607.

The authors' reply:

We respond to the Carls et al. [1] critique of Brannon et al. [2], in which we questioned the validity of previous experimental investigations regarding the toxicity of petroleum-derived polycyclic aromatic hydrocarbons (PAHs) to pink salmon embryos conducted at Auke Bay Laboratory (ABL), Alaska, USA [3–5]. Carls et al. [1] defend their studies based on differences in methods for preparing oiled gravel, superior statistics, and greater replication compared to Brannon et al [2]. We believe the problem with the ABL experiments was an inherently flawed experimental design and misinterpretation of results.

Our investigation [2] was not meant to duplicate the ABL studies but, rather, to independently assess the toxicity of weathered *Exxon Valdez* crude oil, which we believed was needed because of inconsistencies between the results of laboratory studies performed by ABL [3–5] and those of field studies performed in oiled streams within Prince William Sound (PWS; AK, USA) shortly after the *Exxon Valdez* spill [6,7]. Our study [2] concentrated on measuring the toxicity of weathered crude oil collected from a beach in PWS and of crude oil artificially weathered in the laboratory. We prepared doses by applying oil directly to gravel tumbling in a mixer for 5 min to simulate oiled gravel on the shores of PWS. We also evaluated the ABL method of applying the oil mixed with *n*-pentane to the gravel to test for effects on toxicity, but we found no statistically significant differences. Thus, we reported the results that were most applicable to the field situation. After contaminating the gravel and flushing the mixture for 4 d with clean water before beginning the exposure, we periodically monitored exposure doses of total PAH (TPAH) concentration in gravel, water, and embryo tissues from postfertilization to the fry stage, enabling us to make comparisons among test doses as well as with other studies. Weathered oil from the beach was not toxic at the oiled gravel concentrations tested, but for this discussion, we will concentrate on results for the artificially weathered oil similar to that used in the ABL studies.

Embryo mortality significantly greater than that among controls was observed at TPAH concentrations in exposure water as low as 1 ppb in the ABL studies [3]. However, the dosing apparatus used in their experiments did not exclude oil droplets [2]. Carls et al. [1] offer the absence of phytane, a branched-chain aliphatic hydrocarbon with very low solubility in water, as proof for the absence of oil microdroplets in the dose water. Therefore, by their criterion, its presence in water would confirm the presence of an oil droplet phase. To evaluate the Carls et al. [1] claim, we downloaded the pertinent water chemistry data from the *Exxon Valdez* Trustees Hydrocarbon Database [8] (http://www.afsc.noaa.gov/ABL/Habitat/ablhab_exxonvaldez_