It's the Dose, Not the Hypothesis: Reply to Heintz *et al.* (2014)

Dear Editor,

The basis for ecotoxicology is the relationship between dose and response. Hypothesis testing can be indicative of difference, but should never be relied on without considering whether there is a true dose-response relationship. Hypothesis testing cannot inform the researcher regarding causation or uncertainty associated with an observed response, particularly in the case of complex exposures.

We (Page *et al.* 2012a) evaluated experiments conducted by Heintz *et al.* (1999) and Carls *et al.* (1999) that assessed the toxicity of petroleum to developing pink salmon and herring embryos. The results of these experiments, based on hypothesis testing, were reported as evidence of toxicological effects from exposure to 1 $\mu\text{g}/\text{L}$ or lower of dissolved total polycyclic aromatic hydrocarbons (TPAH), and as evidence that oil toxicity increased with weathering. Our evaluation was not, as Heintz *et al.* (2014) incorrectly stated, focused on “supposed failure to adequately control exposure levels” in the experiments. Although we found a number of issues with the work as reported that led us to question the certitude of the conclusions reached, our main focus was on the lack of a dose-response relationship in those experiments rather than on the hypothesis testing relied on by Heintz *et al.* (1999) and Carls *et al.* (1999). We found that these two articles and the articles that followed, which relied on these initial studies, did not provide technically defensible causal evidence for TPAH toxicity at 1 $\mu\text{g}/\text{L}$ or lower (the toxicity benchmark was at least an order of magnitude higher), nor that oil toxicity increased with weathering.

Below we provide responses to all of the concerns raised by Heintz *et al.* (2014) regarding our evaluations of their earlier work (Page *et al.* 2012a,b,c; Landrum *et al.* 2012, 2013; Neff *et al.* 2013). We (Landrum *et al.* 2012) previously congratulated Heintz, Carls, and co-workers “for providing more data and information for analysis than other papers that evaluated toxicological responses to time-varying concentrations of individual compounds or complex mixtures.” We again congratulate them for their pioneering work, but respectfully request that they fully consider the evidence we present, which is not “speculative misinterpretation.” Their interpretation of their findings, based on hypothesis testing, was not unreasonable as a preliminary analysis. However, with further consideration, particularly of the lack of dose-response relationships, and of the continuing failure to establish cause and effect, we find their interpretation is not technically defensible without corroborative testing. Such corroborative testing has not been reported (see below); in fact,

Carls *et al.* in 1999 were unable to replicate the “very weathered oil” findings of Heintz *et al.* (1999) as described by Page *et al.* (2012a)

Heintz *et al.* (2014) state “others (Turcotte *et al.* 2011) have performed the corroborating studies.” This is not the case. Turcotte *et al.* (2011) showed that alkylated PAH (not high molecular weight polycyclic aromatic compounds [PAC]) are toxic at relatively high concentrations under constant exposure conditions—not the same exposure conditions as Heintz *et al.* (1999) and Carls *et al.* (1999). Further, toxicity occurred at concentrations in the 40 to 100 $\mu\text{g/L}$ range with the exception of retene, which was around 10 $\mu\text{g/L}$. TPAH toxicity at 1 $\mu\text{g/L}$ was not demonstrated. Thus, Turcotte *et al.* (2011) have not in fact “performed the corroborating studies.” Similarly, Barron *et al.* (2003) focused on photo-induced toxicity for herring but not pink salmon, with semi-static exposure using daily renewal (*i.e.*, they did not conduct similar testing to Carls *et al.* [1999]); photo-induced toxicity was not the focus of Heintz *et al.* (1999). Barron *et al.* (2004) found that the “very weathered oil” exposures of Heintz *et al.* (1999) did not fit their alkyl phenanthrene toxicity model, based on the high toxicity of retene, for pink salmon where the very weathered oil had lower toxic units than exposures not causing toxicity. Retene (a C4-phenanthrene: 1-methyl-isopropyl-phenanthrene) is rare in crude oils, usually representing 2–20% of total C4-phenanthrenes in oil (Stout *et al.* 2001), and has an atypical toxicity profile (Scott *et al.* 2009). It is not representative of the alkyl-phenanthrenes in North Slope crude oil; thus, these papers were not corroborative but in fact support our concern regarding lack of a monotonic dose-response. Incardona *et al.* (2005) exposed fish embryos to saturated solutions of 4 non alkylated PAH, so didn’t confirm that fish embryos are sensitive to 1 $\mu\text{g/L}$ and lower concentrations of TPAH. They did, however, provide new evidence of the mechanisms of toxicity to fish embryos at high concentrations of different non-alkylated PAH. Kiparissis *et al.* (2005) showed that exposure depends on first order loss-rate kinetics but, again, this work did not corroborate Heintz *et al.* (1999) or Carls *et al.* (1999).

Heintz *et al.* (2014) accuse us of “selective reading of the literature” and specifically cite eight papers that they accuse us of not considering. In fact, we have been as broad in our reading of the literature as possible; we did consider all the articles we are accused of not considering, and more. None of these articles, or others we have recently reviewed and considered (Jung *et al.* 2013), provide evidence that TPAH toxicity occurs at 1 $\mu\text{g/L}$ or lower, nor that oil toxicity increases with weathering. Many of these papers rely on Heintz *et al.* (1999) and Carls *et al.* (1999) without questioning their conclusions (Carls and Meador 2010; Heintz *et al.* 2012).

We have repeatedly and in different forums pointed out to Heintz and co-workers that they have not considered key evidence. We do not believe this evidence was purposefully withheld. However, we are concerned that it has been consistently dismissed as irrelevant to their hypothesis. For example, Heintz *et al.* (2014) have not “addressed most of . . . [our] . . . criticisms . . . in a series of letters exchanged in *Environmental Toxicology and Chemistry* in 2012 (Heintz *et al.* 2012).” Our criticisms (Page *et al.* 2012b,c) were dismissed, but the dismissal was not based on strong scientific proof. We hope that Heintz and co-workers are not relying on constant repetition to sustain their hypothesis, even though the evidence against it, based on their own data, is compelling. One cannot have exposures at lower concentrations

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that produce effects when exposures at higher concentrations do not; all data must be considered and explained with a consistent conceptual model.

Heintz *et al.* (2014) provide a great deal of text criticizing us for raising the issue of possible confounding effects of oil microdroplets and bacterial/fungal growth. We do not in fact “feel entitled to presume . . . environmentally relevant information” from these possibilities. But we definitely feel entitled to raise the issue of confounding factors that could explain the curious, non-monotonic dose-response relationships reported by these workers. The data clearly demonstrate (Page *et al.* 2012a) that microbial degradation occurred and may have contributed to the observed toxic responses. Microdroplets also remain a possible confounding factor, as does binding to dissolved natural organic carbon. Redman *et al.* (2012) showed that the less weathered and more weathered rock column effluents of Carls *et al.* (1999) and the artificially weathered effluents of Heintz *et al.* (1999) contained microdroplets and colloids enriched in higher molecular weight PAHs, the same PAHs to which Carls *et al.* (1999) and Heintz *et al.* (1999) attributed the high embryo-toxicity of weathered crude oil. We agree that there is no proof that these potential confounding effects were causative nor do we imply the extent of contribution of these factors to the response, but there are sufficient data to further question the hypothesis set forth by Heintz *et al.* (1999) and Carls *et al.* (1999), independent of the major issue of no monotonic dose-response.

Heintz *et al.* (2014) conclude their letter by stating that, “Were Page *et al.* willing to consider this literature, they would find the details that address their concerns.” We have considered all the literature they cite and more. The problem is that Heintz and co-workers are favoring a hypothesis that is not only inconsistent with their own data, but also with the key technical arguments we have put forward, which include: (1) the unexplained higher toxicity at lower PAH concentrations compared to higher PAH concentrations without toxicity, (2) the lack of a monotonic dose-response relationship, and (3) potential confounding factors.

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REFERENCES

- Barron MG, Carls MG, Short JW, *et al.* 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. *Environ Toxicol Chem* 22:650–60

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- Barron MG, Carls MG, Heintz R, *et al.* 2004. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. *Toxicol Sci* 78:60–7
- Carls MG, Rice SD, and 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 pallasii*). *Environ Toxicol Chem* 18:481–93
- Carls MG, Meador JP. 2010. A perspective on the toxicity of petrogenic PAHs to developing fish embryos related to environmental chemistry. *Hum Ecol Risk Assess* 15:1084–98
- Heintz RA, Short JW, and 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
- Heintz RA, Rice SD, Carls MG, *et al.* 2012. Authors reply. *Environ Toxicol Chem* 31:472–3
- Heintz RA, Short JW, Carls MG, *et al.* 2014. Letter to the Editor. *Hum Ecol Risk Assess* (in press)
- Incardona JP, Carls MG, Day HL, *et al.* 2009. Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasii*) exposed to crude oil during weathering. *Environ Sci Technol* 43:201–7
- Jung J-H, Hicken CE, Boyd D, *et al.* 2013. Geologically distinct crude oils cause a common cardiotoxicity syndrome in developing zebrafish. *Chemosphere* 91:1146–55
- Kiparissis Y, Akhtar P, Hodson PV, *et al.* 2003. Partition-controlled delivery of toxicants: A novel in vivo approach for embryo toxicity testing. *Environ Sci Technol* 37:2262–66
- Landrum PF, Chapman PM, Neff J, *et al.* 2012. Evaluating the aquatic toxicity of complex organic chemical mixtures: Lessons learned from polycyclic aromatic hydrocarbon and petroleum hydrocarbon case studies. *Integr Environ Assess Manage* 8:217–30
- Landrum PF, Chapman PM, Neff J, *et al.* 2013. Influence of exposure and toxicokinetics on measures of aquatic toxicity for organic contaminants: A case study review. *Integr Environ Assess Manage* 9:196–210
- Neff JM, Page DS, Landrum PD, *et al.* 2013. The importance of both potency and mechanism in dose-response analysis: An example from exposure of Pacific herring (*Clupea pallasii*) embryos to low concentrations of weathered oil. *Mar Pollut Bull* 67:7–15
- Page DS, Chapman PM, Landrum PD, *et al.* 2012a. A perspective on the toxicity of low concentrations of petroleum-derived polycyclic aromatic hydrocarbons to early life stages of herring and salmon. *Hum Ecol Risk Assess* 18:229–60
- Page DS, Landrum PF, Neff J, *et al.* 2012b. Sensitivity of pink salmon (*Oncorhynchus gorbuscha*) embryos to weathered crude oil. *Environ Toxicol Chem* 31:469–71
- Page DS, Landrum PF, Neff J, *et al.* 2012c. Author's reply to Heintz *et al.* [1]. *Environ Toxicol Chem* 31:473–5
- Redman AD, McGrath JA, Stubblefield WA, *et al.* 2012. Quantifying the concentration of crude oil microdroplets in oil-water preparations. *Environ Toxicol Chem* 31:1814–22
- Scott JA, Ross M, Lemire BC, *et al.* 2009. Embryotoxicity of retene in cotreatment with 2-aminoanthracene, a cytochrome P4501A inhibitor, in rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 28:1304–10
- Stout SA, Uhler AD, and McCarthy KJ. 2001. A strategy and methodology for defensibly correlating spilled oil to source candidates. *Environ Forensics* 2:87–98
- Turcotte D, Akhtar P, Bowerman M, *et al.* 2011. Measuring the toxicity of alkyl-phenanthrenes to early life stages of medaka (*Oryzias latipes*) using partition-controlled delivery. *Environ Toxicol Chem* 30:487–95