ACUTE AND CHRONIC EFFECTS OF CRUDE AND DISPERSED OIL ON PRE-SMOLT STAGE CHINOOK SALMON

A Research Preproposal for the Oiled Wildlife Care Network – April 2005

Principal Investigators
Ronald S. Tjeerdema, Department of Environmental Toxicology, University of California, Davis, CA, 95616-8588; 530-754-5192; 530-752-3394 (FAX); rstjeerdema@ucdavis.edu

Ching Yu Lin, Department of Environmental Toxicology, University of California, Davis, CA, 95616-8588; 530-752-2534; 530-752-3394 (FAX); clin@ucdavis.edu

Hypothesis and Specific Aims
This project will compare the toxic impacts of the water-accommodated fraction (WAF) and the chemically enhanced WAF (CEWAF) of Prudhoe Bay Crude Oil (PBCO) on the pre-smolt stage of Chinook salmon (*Oncorhynchus tshawytscha*). The null hypothesis to be tested is: *The toxic impacts of crude oil on Chinook salmon pre smolts are not increased by application of oil dispersants.*

The specific aims are:
1. To conduct field-modeled laboratory exposures of pre-smolt salmon to declining concentrations of the WAF or CEWAF of PBCO, with the goal of determining the concentrations that induce metabolic stress, narcosis, and mortality; stress will be assessed using NMR-based metabolomics.
2. To determine the long-term growth and metabolic viability of the acutely exposed fish by culturing them in clean (oil- and dispersant-free) freshwater.

Experimental Plan
Currently, over 40% of Pacific salmon stocks are in serious decline. While they are struggling to recover from over fishing, habitat decline and pollution, there is serious concern that coastal oil spills (and response activities) near rivers of spawning importance could cause deleterious impacts to pre-smolts entering the ocean [1]. Our laboratory is currently comparing the impacts of the WAF and CEWAF (in seawater) of PBCO on salmon smolts. It is being conducted to provide resource agencies with toxicity information to be applied to spill response situations in the near-shore environment.

Of equal concern to the Department of Fish and Game's Office of Spill Prevention and Response (OSPR) is the relative toxicity of dispersed and non-dispersed oil in near-shore freshwater habitats. For example, there is significant potential for crude oil spills in San Francisco Bay, where a large amount of transport occurs. The northern estuary is largely a freshwater habitat, and there is concern that use of dispersants, such as Corexit 9500, would enhance crude oil toxicity by increasing its dispersion, thus bioavailability, to the freshwater stages of migrating salmon [2]. Therefore, we propose to use the same approach developed in our current OWCN project to investigate the relative toxicity of dispersed and non-dispersed PBCO to the pre-smolt stage of Chinook salmon, via freshwater exposures. Our exposure system is a large-volume version of the system previously developed for near-microscopic larvae [3-5]. The declining exposure protocol has been used extensively to determine the toxicity of petroleum and dispersants to a variety of marine organisms [6-12], and this approach is currently the standard in the field, having been adopted by the Chemical Response of Oil Spill Effects Research Forum (CROSERF) [13, 14].

Pre-smolts (~3 cm FL) will be obtained from the DFG Feather River Hatchery (available in early summer 2006), and exposed for 96 h to five water concentrations (in declining fashion) of either the WAF or CEWAF of PBCO (in freshwater) using methods developed for our current OWCN project [15-16]. The WAF and CEWAF will be fully characterized for hydrocarbons by gas chromatography-flame ionization detection (GC-FID). Declining concentrations will better simulate actual spill conditions, where both dilution and dispersion occur, and will also be monitored using GC-FID. Acutely exposed animals will be evaluated for metabolic stress (see below), narcosis, and mortality. All experiments will be repeated a minimum of three times for statistical validity.

Surviving pre-smolts will be placed in clean freshwater and routinely weighed and measured for 12 months to determine any chronic effects. A few will be regularly sacrificed for assessment of metabolic status using ^{1}H-NMR-based metabolomics. This approach combines the metabolic profiling capabilities of NMR with powerful pattern recognition (chemometric) methods [17, 18]. It will enable comprehensive assessment of the metabolic effects of WAF and CEWAF by profiling the hundreds of low-molecular weight endogenous metabolic compounds. It also is an ideal tool for identifying subtle metabolic changes that could impair growth and reproduction. We have previously conducted NMR-based metabolomic experiments with salmon and medaka embryos and adult abalone, and have established protocols and a demonstrated ability to conduct the proposed studies [19–23].

Significance to Oiled Wildlife Health
The National Marine Fisheries Service and the OSPR need experimental data to assess the impacts of spill remediation on endangered anadromous fish species (M. Sowby, pers. Comm., 2005). Our current OWCN project will provide information on the risk to saltwater-acclimated smolts of treating oil with dispersants. However, there is little information on the effects of oil treatment to the sensitive freshwater acclimated pre-smolt stage of salmon. Thus, this project represents a logical addition to our current project, as it provides additional information to assess risk to coastal salmon during key phases of their migration. It is designed to provide resource managers with important information on the toxicity of oil spills to salmon migrating in toward estuaries, and to provide support for decisions regarding the advisability of applying dispersants under spill conditions where the pre-smolt stages of migrating salmon are present, such as in San Francisco Bay.

This project also addresses the desire of the OWCN to investigate the direct and indirect effects of oil on wildlife. It will address applied questions regarding the risk of oil to endangered species. Experiments on the biochemical responses of pre-smolts to oil will also address basic questions concerning the mechanisms of oil toxicity in early life stages using a state-of-the-art approach. An oil spill would seriously impact salmon populations if it occurred during key periods of their migration, particularly when pre-smolts are moving from the Sacramento River into San Francisco Bay. This study will provide data on the toxicity of the bioavailable fractions of oil on salmon pre-smolts. It will also provide complementary information to allow comparison of the relative toxic actions of WAF and CEWAF of PBCO to the freshwater- and seawater-adapted stages of juvenile Chinook salmon.

Project Duration
The duration of the proposed project would be two years. During Year 1 declining acute salmon exposures using WAF and CEWAF will be completed, and the chronic growth experiments will be initiated. During Year 2 the chronic effects on growth, and metabolomic analysis from the acute tests, will be performed and completed.

Estimated Budget

	Year 1	Year 2	Totals
Personnel (Postdoctoral Researcher; salary and benefits)	34,287.00 (75%)	36,002.00 (75%)	70,289.00
Equipment	0.00	0.00	0.00
Supplies	4,000.00	4,000.00	8,000.00
Travel (Between Davis and Granite Canyon)	500.00	500.00	1,000.00
Other Expenses (NMR recharge)	2,000.00	2,000.00	4,000.00
Indirect costs	0.00	0.00	0.00
Totals	40,787.00	42,502.00	83,289.00

Budget Justification
The postdoctoral researcher would be primarily responsible for performing the metabolomic analyses. Supplies include glassware and chemicals for the exposures. Travel funds would support travel between UCD (where the metabolomic analyses would be conducted) and the DFG Granite Canyon Marine Pollution Studies Laboratory (near Monterey, CA), where the exposures will be conducted.

Supplemental Funds
A matching award application (two years, $75,000/year) to the OSPR will be submitted in the fall of 2005. Sponsored by Michael Sowby of OSPR, it would provide support for the Marine Pollution Studies Laboratory staff to conduct the pre-smolt exposures, and for the necessary chemical analyses.

Literature Cited

1. Sowby, M. L., R. S. Tjeerdema and M. F. Wolfe, 1999. *Environmental Fate of Petroleum Hydrocarbons and Dispersing Agents.* CDFG-UCSC Cooperative Oil Spill Research Program, Final Report (1998-1999), 13 pp.
2. Tjeerdema, R. S., M. M. Singer, M. F. Wolfe, G. J. Blondina and M. L. Sowby, 1999. Deriving fate and effects information to assess petroleum risk. In: *Dispersant Application in Alaska* (K. Trudel, ed.), Prince William Sound Oil Spill Recovery Institute, Cordova, AK, pp. 249–258 (invited).
3. Singer, M. M., D. L. Smalheer and R. S. Tjeerdema, 1990. A simple continuous-flow toxicity test system for microscopic life stages of aquatic organisms. *Wat. Res.* 24, 899–903.
4. Tjeerdema, R. S. and M. M. Singer, 1991. Closed flow-through aquatic toxicity testing and microscopic organisms: Not necessarily incompatible. *Mar. Pollut. Bull.* 22, 59–61.

5. Tjeerdema, R. S., M. M. Singer and D. L. Smalheer, 1991. Continuous-flow toxicity tests using the microscopic life stages of various marine organisms. *Can. Tech. Rep. Fish. Aquat. Sci.* 1774, 348–354.
6. Singer, M. M., D. L. Smalheer, R. S. Tjeerdema and M. Martin, 1990. Toxicity of an oil dispersant to the early life stages of four California marine species. *Environ. Toxicol. Chem.* 9, 1387–1395.
7. Singer, M. M., S. George, D. Benner, S. Jacobson, R. S. Tjeerdema and M. L. Sowby, 1993. Comparative toxicity of two oil dispersants to the early life stages of two marine species. *Environ. Toxicol. Chem.* 12, 1855–1863.
8. Singer, M. M., S. George, S. Jacobson, I. Lee, R. S. Tjeerdema and M. L. Sowby, 1994. Comparative toxicity of Corexit 7664 to the early life stages of four marine species. *Arch. Environ. Contam. Toxicol.* 27, 130–136.
9. Singer, M. M., S. George, S. Jacobson, I. Lee, R. S. Tjeerdema and M. L. Sowby, 1994. Comparative effects of oil dispersants to the early life stages of topsmelt (*Atherinops affinis*) and kelp (*Macrocystis pyrifera*). *Environ. Toxicol. Chem.* 13, 649–655.
10. Singer, M. M., S. George, S. Jacobson, I. Lee, L. L. Weetman, R. S. Tjeerdema and M. L. Sowby, 1995. Acute toxicity of the oil dispersant Corexit 9554 to marine organisms. *Ecotoxicol. Environ. Saf.* 32, 81–86.
11. Singer, M. M., S. George, S. Jacobson, I. Lee, L. L. Weetman, R. S. Tjeerdema and M. L. Sowby, 1996. Comparison of acute aquatic effects of the oil dispersant Corexit 9500 with those of other Corexit series dispersants. *Ecotoxicol. Environ. Saf.* 35, 183–189.
12. Singer, M. M., S. George, S. Jacobson, I. Lee, L. L. Weetman, G. J. Blondina, R. S. Tjeerdema, D. Aurand and M. L. Sowby, 1998. Effects of dispersant treatment on the acute aquatic toxicity of petroleum hydrocarbons. *Arch. Environ. Contam. Toxicol.* 34, 177–187.
13. Singer, M. M., D. Aurand, J.Clark, G. Sergy, M. L. Sowby and R. S. Tjeerdema, 1995. CROSERF: Toward a standardization of oil spill cleanup agent ecological effects research. In: *Eighteenth Arctic Marine Oilspill Program.* Environment Canada, Ottawa, Ontario, pp. 1263–1270.
14. Singer, M. M., D. Aurand, G. E. Bragin, J. R. Clark, G. M. Coehlo, M. L. Sowby and R. S. Tjeerdema, 2000. Standardization of preparation and quantitation of water-accommodated fractions of oil and their use in aquatic toxicity testing. *Mar. Pollut. Bull.* 40, 1007–1016.
15. Singer, M. M., D. L. Smalheer, R. S. Tjeerdema and M. Martin, 1991. Effects of spiked exposure to an oil dispersant on the early life stages of four marine species. *Environ. Toxicol. Chem.* 10, 1367–1374.
16. Singer, M. M., R. S. Tjeerdema and D. L. Smalheer, 1992. Evaluation of the toxicological effects of oil dispersants by modeled-exposure toxicity testing. *Can. Tech. Rep. Fish. Aquat. Sci.* 1863, 175–182.
17. Nicholson, J. K., J. Connelly, J. C. Lindon and E. Holmes, 2002. Metabonomics: a platform for studying drug toxicity and gene function. *Nat. Rev. Drug. Discov.* 1, 153–161.
18. Lindon, J. C., J. K. Nicholson, E. Holmes and J. R. Everett, 2000. Metabonomics: Metabolic processes studied by NMR spectroscopy of biofluids. *Concepts Magn. Reson.* 12, 289-320.
19. Viant, M. R., E. S. Rosenblum and R. S. Tjeerdema, 2003. NMR-based metabolomics: A powerful approach for characterizing the effects of environmental stressors on organism health. *Environmental Science and Technology* 37, 4982-4989.
20. Viant, M. R., I. Werner, E. S. Rosenblum, A. S. Gantner, R. S. Tjeerdema and M. L. Johnson, 2003. Correlation between heat-shock protein induction and reduced metabolic condition in juvenile steelhead trout (*Oncorhynchus mykiss*) chronically exposed to elevated temperature. *Fish Physiology and Biochemistry.* 29, 159-171.

21. Pincetich, C. A., M. R. Viant, D. E. Hinton and R. S. Tjeerdema, 2005. Metabolic changes in Japanese medaka (*Oryzias latipes*) during embryogenesis and hypoxia determined by in vivo ^{31}P NMR. *Comp. Biochem. Physiol.* 140, 103–113.
22. Pincetich, C. A., M. R. Viant, D. E. Hinton and R. S. Tjeerdema. Metabolic effects of dinoseb in Japanese medaka (*Oryzias latipes*) embryos as measured by *in vivo* ^{31}P NMR. *Aquat. Toxicol.* (submitted for publication)
23. Pincetich, C. A., M. R. Viant and R. S. Tjeerdema. Metabolic effects of dinoseb, diazinon, and esfenvalerate in eyed eggs and alevins of Chinook salmon (*Oncorhynchus tshawytscha*) as determined by ^{1}H NMR metabolomics. *Aquat. Toxicol.* (submitted for publication)