GEM PROPOSAL SIGNATURE FORM

THIS FORM MUST BE SIGNED BY THE PROPOSED PRINCIPAL INVESTIGATOR AND SUBMITTED ALONG WITH THE PROPOSAL. If the proposal has more than one investigator, this form must be signed by at least one of the investigators, and that investigator will ensure that Trustee Council requirements are followed. Proposals will not be reviewed until this signed form is received by the Trustee Council Office.

By submission of this proposal, I agree to abide by the Trustee Council's data policy (*Trustee Council/GEM Data Policy**, adopted July 9, 2002) and reporting requirements *(Procedures for the Preparation and Distribution of Reports***, adopted July 9, 2002).

PROJECT TITLE: Quantifying Temporal Variation in Harlequin Duck Exposure To Exxon Valdez Oil

Printed Name of PI: Dan Esler

Signature of PI: _____ Date 5 Jan 2005

Printed Name of co-PI: _____

Signature of co-PI: _____ Date _____

Printed Name of co-PI: _____

Signature of co-PI: _____ Date _____

* Available at http://www.oilspill.state.ak.us/pdf/admin/datapolicy.pdf

** Available at http://www.oilspill.state.ak.us/pdf/admin/reportguidelines.pdf

Trustee Council Use Only
Project No: _____
Date Received: _____

GEM PROPOSAL SUMMARY PAGE

(To be filled in by proposer)

Project Title: **Quantifying Temporal Variation in Harlequin Duck Exposure to Exxon Valdez Oil**

Project Period: FY 05

Proposer(s): Dr. Dan Esler

Study Location: Lab analysis and data analysis of samples collected in Prince William Sound.

Abstract: Measurements of cytochrome P4501A (P450) have proven to be extremely useful for quantifying the degree of exposure to hydrocarbons following the EVOS for a number of vertebrates, including harlequin ducks. However, the ability to document interannual changes in exposure for harlequin ducks is eroded by dramatic differences in average P450 values between years, both for oiled and unoiled areas. There is no reasonable biological explanation for these widely differing values among years and we speculate that these are the result of differences within the laboratory. Because the P450 data are so critical for documenting changes in oil exposure over time, as well as for linking individual survival with oil exposure, we propose to concurrently reanalyze all archived HADU samples. We propose to conduct these analyses at the same time samples from March 2005 are being analyzed (this is already funded by EVOSTC). This approach will result in a database in which all samples can be compared both within and between years, allowing for confident interpretation of the level of exposure in oiled areas and changes in that exposure over time.

Funding:

EVOS Funding Requested:	FY 04	$	
	FY 05	$ 39,000	
	FY 06	$	TOTAL: $39,000
Non-EVOS Funds to be Used:	FY 04	$	
	FY 05	$	
	FY 06	$	TOTAL:

Date: 5 January 2005

(NOT TO EXCEED ONE PAGE)

GEM RESEARCH PLAN

I. NEED FOR THE PROJECT

A. Statement of Problem

Harlequin ducks (*Histrionicus histrionicus*) have been the subject of a broad array of investigations following the 1989 Exxon Valdez oil spill. The process of population recovery of this species has been monitored and studied intensively, and it is one of the few species (along with sea otters) for which information is available that quantifies the demographic factors leading to population change. The Nearshore Vertebrate Predator project (NVP), funded by the EVOSTC, was initiated in 1995, and consisted of a suite of studies addressing constraints to population recovery for 4 vertebrates, including harlequin ducks. Results of the NVP included the surprising finding that several nearshore vertebrates (Jewett et al. 2002, Bodkin et al. 2002), including harlequin ducks (Trust et al. 2000), continued to be exposed to residual oil through at least 1998. This duration of exposure was much longer than expected, based on conventional assumptions about persistence of spilled oil (Peterson et al. 2003). NVP studies also documented demographic problems that corresponded with continued exposure to lingering oil, including reduced survival of sea otters (Monson et al. 2000) and harlequin ducks (Esler et al. 2000). In fact, exposure to lingering oil was considered to be a primary factor constraining population recovery of sea otters (Bodkin et al. 2002) and harlequin ducks (Esler et al. 2002).

Based on NVP findings, the EVOSTC funded a new set of studies (//423), which included consideration of the relationship between continuing oil exposure and population demography of harlequin ducks, at both individual and population levels. This work led to the conclusion that differences in harlequin duck survival between oiled and unoiled areas diminished over time, and were equivalent by 2002 (Bodkin et al. 2003). This was accompanied by suggestions of corresponding diminishment of oil exposure over time (see below). These are important findings because they: (1) document the full timeframe over which oil exposure persisted and (2) corroborate suggestions from NVP studies that there was a cause-effect relationship between oil exposure and population demographic processes.

Clearly, quantification of oil exposure is an important component of the conclusions described above. For harlequin ducks, along with other nearshore vertebrates, inferences about oil exposure have been drawn through quantification of cytochrome P4501A (P450). P450 has proven to be a sensitive and specific biochemical measurement for assessing exposure to PAHs. Certain PAHs induce P450 responses, therefore measuring resultant enzyme production or activity can indirectly indicate exposure to oil constituents. In the case of harlequin ducks, liver samples were collected to assess P450 induction by measuring 7-ethoxyresorufin-O-deethylase (EROD) activity. EROD, which is the catalytic function of hydrocarbon-inducible CYP 1A, activity is a widely used and recognized method for quantifying P450.

However, the interpretation of P450 data for harlequin ducks has been hampered by dramatic interannual differences in EROD activity results. As described in Fig. 1, average EROD activity reported for oiled areas ranged from 40.2 to 1981.8 pmol/min/mg across years and, for unoiled

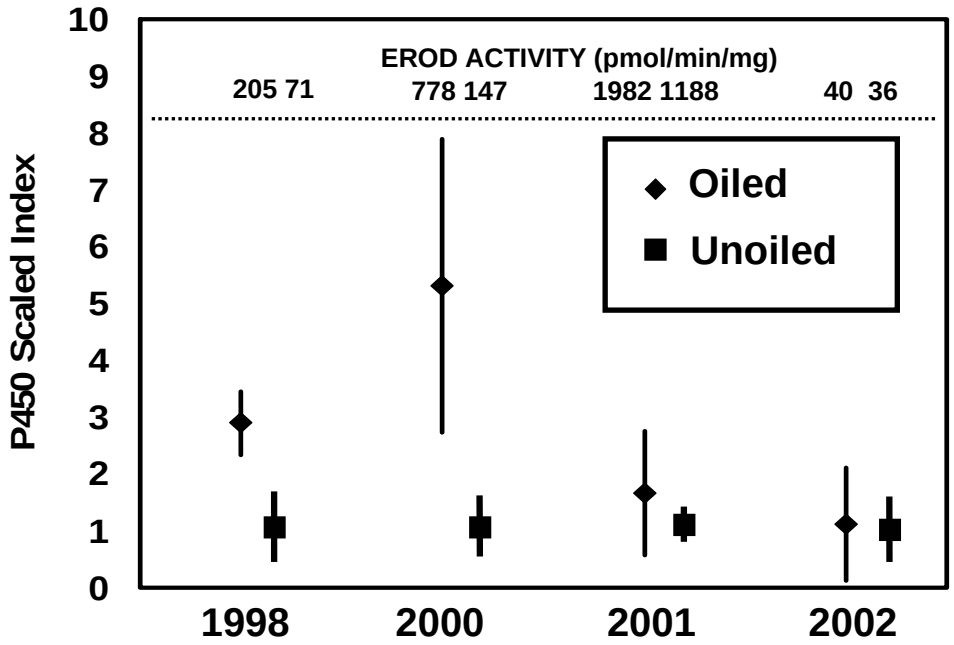

Figure 1. Cytochrome P450 (as measured by EROD activity) in harlequin ducks from oiled and unoiled areas of Prince William Sound, Alaska. The values above the dotted line are the average EROD activity reported from the lab. The figure scales the EROD data, setting the unoiled area at 1 for each year, to illustrate the change in the ratio of oiled:unoiled over time.

areas, the range was from 36.0 to 1187.9 pmol/min/mg across years. These almost certainly do not reflect real differences in exposure over time. Not only is the magnitude of differences among years in oiled areas far beyond what one would expect, one should predict that average exposure in oiled areas would decline over time with diminishing availability of oil, as has been described for other species. Also, there is no biological explanation for the dramatic differences across years in unoiled areas. One would expect that average EROD activity should remain stable over time in the unoiled areas. Also, interannual differences are fairly consistent between areas when considered across years; e.g., for both areas results are more than 30 times higher in 2001 than 2002. We are left to conclude that dramatic interannual differences are the result of variation in the laboratory processing.

Results from studies of captive harlequin ducks at the Alaska SeaLife Center corroborate the hypothesis of lab-induced interannual differences. During 2 winters (2000 and 2001) female harlequin ducks were captured from an unoiled area and held from September to March in captivity. In each winter, ducks ingested oil in controlled amounts and their P450 response was measured at season's end. Despite similar, controlled handling and dosing of ducks, as well as sample handling, between years, dramatically different results were reported in the 2 years. EROD activity of oiled birds was 634.6 and 2239.4 pmol/min/mg, respectively, in 2000 and 2001. More surprisingly, EROD activity of control birds was 86.7 and 235.3 pmol/min/mg in 2000 and 2001, respectively. The ratio of EROD activity for oiled:control birds was similar between years (7.3:1 and 9.5:1 in 2000 and 2001, respectively), suggesting that the magnitude of the differences was valid, but that values could not be directly compared across years.

To compare EROD activity across years for harlequin ducks captured in oiled and unoiled areas of Prince William Sound, we created an index for each year, scaling the results from unoiled areas to 1 and corresponding oiled area data by the same factor. This approach assumes that EROD activity would be similar in unoiled areas across years, which is reasonable under the assumption that residual Exxon Valdez oil is the primary inducer of P450. Based on this analysis (Figure 1), the difference in EROD activity diminishes over time and areas are statistically similar in 2001 and 2002. This pattern is concordant with those described for other nearshore species. However, the confidence in this conclusion, and its important implications for harlequin duck population recovery, would be enhanced by addressing the unexplained interannual variation.

Because the P450 data are so critical for documenting changes in oil exposure over time, as well as for linking individual survival with oil exposure, we propose to concurrently reanalyze all archived HADU samples. We propose to conduct these analyses at the same time samples from March 2005 are being analyzed (this is already funded by EVOSTC). This approach will result in a database in which all samples can be compared both within and between years, allowing for confident interpretation of the level of exposure in oiled areas and changes in that exposure over time.

Finally, as another check on data quality, we propose to have samples collected in March 2005 analyzed by 2 labs, 1 at Woods Hole Oceanographic Institute (where all of the historical data were generated) and 1 at University of California Davis with a history of doing the same method of EROD activity analyses.

B. Relevance to GEM Program Goals and Scientific Priorities

Lingering oil issues continue to be important for the EVOSTC and GEM. Recovery of the Prince William Sound ecosystem from the *Exxon Valdez* oil spill may not be considered complete until individuals are no longer exposed to spilled oil. Clear quantification of changes in exposure over time is central to that measure of recovery. Further, the proposed work will allow clearer interpretation of demographic processes related to changes in oil exposure, which in turn lead to conclusions about appropriate restoration.

II. PROJECT DESIGN

A. Objectives

This proposal consists of a single, simple objective:

1. Concurrently analyze all contemporary and archived harlequin duck liver samples using EROD activity to provide P450 data that can be confidently compared within and between years.

B. Procedural and Scientific Methods

Frozen liver samples collected in March 2005 will be shipped to the laboratory of Dr. John Stegeman at the Woods Hole Oceanographic Institute for preparation and analysis. Individual

liver pieces will be homogenized in 7 ml final volume homogenizing buffer (0.05 M Tris, 0.15 M KCl, pH 7.4), and microsomes sedimented by differential centrifugation as described previously (Stegeman et al., 1979). All other samples (i.e., those from previous studies of wild and captive harlequin ducks) are archived at Woods Hole as prepared microsomes. Microsomes will be resuspended in approximately 2 ml per g tissue with resuspension buffer (0.05 M Tris, 0.1 mM EDTA, 1 mM DTT, 20% v/v glycerol, pH 7.4). Protein will be determined in a 96 well plate using the micro- procedure of Smith et al. (1985). 7-Ethoxyresorufin-O-deethylase (EROD), the catalytic function of hydrocarbon-inducible CYP1A, will be measured using a kinetic modification of the plate-based assay of Kennedy et al. (1993). EROD activity will be determined in duplicate in a 48 well plate at 20° C using a Cytofluor® fluorescent plate reader (Millipore, Bedford, MA). Each well will contain 200 µl consisting of 1µl of microsomes (4-15 µg protein), 2 µM 7-ethoxy resorufin in 50 mM Tris buffer, 0.1 M NaCl, pH = 7.8. Catalytic activity will be initiated by the addition of NADPH in buffer to a final 1.67 mM concentration. Fluorescence will be determined at 1 min intervals over 6 min, and the linear slope (fluorescence per minute) will be divided by the slope of the resorufin product standard curve (fluorescence per pmol) determined under the same conditions to yield pmol per minute per mg protein catalytic rates.

C. Data Analysis and Statistical Methods

Using the new data derived from concurrent analysis of March 2005 samples and reanalysis of all archived samples, we will use a General Linear Model to evaluate variation in EROD activity in relation to area (oiled vs. unoiled), year (categorical variable with levels for 1998, 2000, 2001, 2002, and 2005), and the interaction of area by year. We will use an information-theoretic approach to model selection (Burnham and Anderson 2002), finding the most parsimonious grouping of cells within the area by year matrix.

D. Description of Study Area

The samples used in these analyses have been, or will be, collected from sites described in project 040774. In brief, these include areas within Prince William Sound that were oiled during the Exxon Valdez spill (Green Island, Bay of Isles, Lower Passage, Herring Bay, Crafton Island, Main Bay, and Foul Bay) and nearby unoiled sites on northwestern Montague Island. These are the same sites that have been used since the initiation of NVP studies in 1995.

E. Coordination and Collaboration with Other Efforts

This proposal builds on previously funded EVOSTC projects, including NVP and //423 studies. This is essentially an amendment to EVOSTC project 040774, which was designed to sample P450 across an array of species, including harlequin ducks, for comparison to previously-collected samples.

III. SCHEDULE

A. Project Milestones and Measurable Project Tasks

Objective 1. Arrange lab analysis contracts and schedules – Feb 2005.

Collect new samples (project 040774) – March 2005.

Ship new samples to labs – April 2005.

Data delivered – June 2005.

Data analyzed and provided to EVOSTC in brief – July 2005.

Final report – April 2006.

IV. RESPONSIVENESS TO KEY TRUSTEE COUNCIL STRATEGIES

A. Community Involvement and Traditional Ecological Knowledge (TEK)

This proposal does not include a field component, so community involvement in field activities does not apply. However, over the years of data acquisition, we have consistently chartered boats and aircraft support from local operators.

B. Resource Management Applications

The data generated under this proposal will provide clear answers to questions about effects of lingering Exxon Valdez oil on migratory bird populations, including the duration of exposure and subsequent chronic effects. These are useful not only for understanding effects of the Exxon Valdez spill, but also in the context of risk assessment for other catastrophic events. Finally, these data will contribute to the understanding of effects of other sources of chronic contamination on wildlife populations. These kinds of data are already being used by the U.S. Fish and Wildlife Service and the Canadian Wildlife Service.

V. PUBLICATIONS AND REPORTS

No funds are requested in this proposal for publications. A final report will be submitted by April 30, 2006.

VI. PROFESSIONAL CONFERENCES

No funds are requested in this proposal for attending meetings.

LITERATURE CITED

Bodkin, J.L., B. Ballachey, T.A. Dean, F.K. Fukuyama, S.C. Jewett, L.L. McDonald, D.H. Monson, C.E. O'Clair, and G.R. Van Blaricom. 2002. Sea otter population status and the process of recovery following the 1989 *Exxon Valdez* oil spill. Marine Ecology Progress Series 241:237-253.

Bodkin, J. L., B. E. Ballachey, D. Esler, and T. Dean. 2003. Patterns and processes of population change in selected nearshore vertebrate predators. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project //423).

Esler, D., J.A. Schmutz, R.L. Jarvis, and D.M. Mulcahy. 2000a. Winter survival of adult female harlequin ducks in relation to history of contamination by the Exxon Valdez oil spill. Journal of Wildlife Management 64:839-847.

Esler, D., T.D. Bowman, K.A. Trust, B.E. Ballachey, T.A. Dean, S.C. Jewett, C.E. O'Clair. 2002. Harlequin duck population recovery following the *Exxon Valdez* oil spill: Progress, process, and constraints. Marine Ecology Progress Series 241: 271-286

Jewett S.C., T.A. Dean, B. R. Woodin, M. K. Hoberg, and J. J. Stegeman. 2002. Exposure to hydrocarbons ten years after the Exxon Valdez oil spill: evidence from cytochrome P4501A expression and biliary FACs in nearshore demersal fishes. Marine Environmental Research 54(1):21-48.

Monson, D.H., D.F. Doak, B.E. Ballachey, A. Johnson, and J.L. Bodkin. 2000. Long-term impacts of the *Exxon Valdez* oil spill on sea otters, assessed through age-dependent mortality patterns. Proc. Nat'l. Acad. Sciences, USA 97(12):6562-6567.

Peterson, C.H, S.D. Rice, J.W. Short, D. Esler, J. L. Bodkin, B.E. Ballachey, D.B. Irons. 2003. Long-term ecosystem responses to the Exxon Valdez oil spill. Science 302:2082-2086.

Short, J. W., M. R. Lindeberg, P. M. Harris, J. M. Maselko, J. J. Pella, and S. D. Rice. 2004. Estimate of oil persisting on beaches of Prince William Sound, 12 after the Exxon Valdez oil spill. Environmental Science and Technology 38(1): 19 25.

Trust, K. A., D. Esler, B. R. Woodin, and J. J. Stegeman. 2000. Cytochrome P450 1A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. Marine Pollution Bulletin 40:397-403.

CURRENT AND PENDING SUPPORT FORM

The following information must be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: Dr. Dan Esler	Other agencies to which this proposal has been/will be submitted:

Support: XCurrent ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: EVOSTC project #040774

Collection of the final round of samples during March 2005 is supported by this project.

Source of Support: EVOSTC

Total Award Amount: $65,000 Total Award Period Covered: FY05

Location of Project: Prince William Sound

Months of Your Time Committed to the Project: FY04 1 FY 05 FY 06 Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Months of Your Time Committed to the Project: FY 04 FY 05 FY 06 Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Months of Your Time Committed to the Project: FY04 FY 05 FY 06 Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Months of Your Time Committed to the Project: FY 04 FY 05 FY 06 Sumr:

*If this project has previously been funded by another entity, please list and furnish information for immediately preceding funding period.

(USE ADDITIONAL SHEETS AS NECESSARY)

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07

Budget Category:	Proposed FY 05	Proposed FY 06	Proposed FY 07		TOTAL PROPOSED	
Personnel	$6.8	$0.0	$0.0		$6.8	
Travel	$0.0	$0.0	$0.0		$0.0	
Contractual	$29.0	$0.0	$0.0		$29.0	
Commodities	$0.0	$0.0	$0.0		$0.0	
Equipment	$0.0	$0.0	$0.0		$0.0	
Subtotal	$35.8	$0.0	$0.0		$35.8	
General Administration (9% of subtotal)	$3.2	$0.0	$0.0		$3.2	
Project Total	$39.0	$0.0	$0.0		$39.0	

Cost-share Funds:
In this box, identify non-EVOS funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

FY 05-07

Date Prepared:

Project Number:
Project Title: Harlequin Duck P450 Reanalysis
Agency: USGS

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07

Personnel Costs:		GS/Range/	Months	Monthly		Personnel
Name	Description	Step	Budgeted	Costs	Overtime	Sum
PI - Esler	salary and benefits		1.0	6.8		6.8
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
	Subtotal		1.0	6.8	0.0	
					Personnel Total	$6.8

Travel Costs:	Ticket	Round	Total	Daily	Travel
Description	Price	Trips	Days	Per Diem	Sum
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
				Travel Total	$0.0

FY 05

Project Number:
Project Title: Harlequin Duck P450 Reanalysis
Agency: USGS

EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 05 - FY 07

Contractual Costs:	Contractual Sum
Description	
Reanalysis of archived field samples (166@ $120 each) (1998 = 37 samples; 2000 = 33; 2001 = 54; 2002 = 42; TOTAL = 166)	19.9
Reanalysis of archived samples from the ASLC (36 @ $120 each) (2000 = 17; 2001 = 19; TOTAL = 36)	4.3
Duplicate analysis of samples to be collected March 2005 (40 @ $120 each)	4.8
If a component of the project will be performed under contract, the 4A and 4B forms are required. **Contractual Total**	$29.0

Commodities Costs:	Commodities Sum
Description	
Commodities Total	$0.0

FY 05

Project Number:
Project Title: Harlequin Duck P450 Reanalysis
Agency: USGS

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07

New Equipment Purchases:	Number	Unit	Equipment
Description	of Units	Price	Sum
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
		New Equipment Total	$0.0

Existing Equipment Usage:	Number	Inventory
Description	of Units	Agency

FY 05

Project Number:
Project Title: Harlequin Duck P450 Reanalysis
Agency: USGS

FY 06

Project Number:
Project Title: Harlequin Duck P450 Reanalysis
Agency: USGS

FY 06

Project Number:
Project Title: Harlequin Duck P450 Reanalysis
Agency: USGS

FY 06

Project Number:
Project Title:
Agency:

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07

FY 07

Project Number:
Project Title:
Agency:

FY 07

Project Number:
Project Title:
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FY 07

Project Number:
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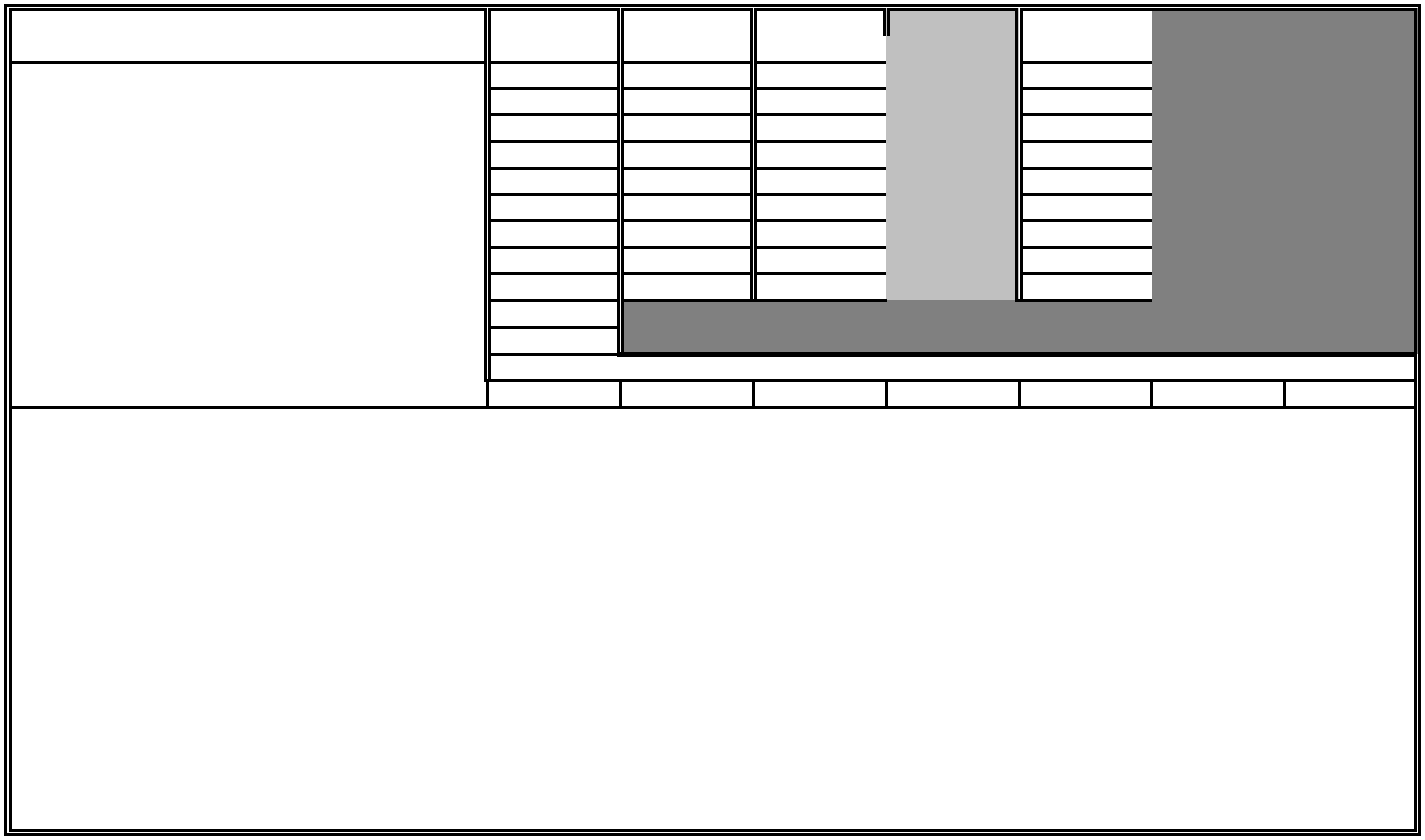
Agency:

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07



FY 05-07

Project Number:
Project Title:
Name of Contractor:

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07

FY 05

Project Number:
Project Title:
Name of Contractor:

FY 05

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***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07

FY 06

Project Number:
Project Title:
Name of Contractor:

FY 06

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Name of Contractor:

FY 06

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Name of Contractor:

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07

FY 07

Project Number:
Project Title:
Name of Contractor:

FY 07

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Project Title:
Name of Contractor:

***EXXON VALDEZ* OIL SPILL TRUSTEE COUNCIL**
DETAILED BUDGET FORM FY 05 - FY 07

FY 07

Project Number:
Project Title:
Name of Contractor: