Exxon Valdez Oil Spill State/Federal
Natural Resource Damage Assessment
Final Report

Hematology and Clinical Chemistry of Sea Otters Captured in Prince William Sound, Alaska Following the *Exxon Valdez* Oil Spill

Marine Mammal Study 6-17
Final Report

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Study History: Marine Mammal Study 6 (MM6), titled *Assessment of the Magnitude, Extent and Duration of Oil Spill Impacts on Sea Otter Populations in Alaska*, was initiated in 1989 as part of the Natural Resource Damage Assessment (NRDA). The study had a broad scope, involving more than 20 scientists over a three year period. Final results are presented in a series of 19 reports that address the various project components. Earlier versions of components of this report were included in the NRDA Draft Preliminary Status Reports for MM6 (January 1990--"Clinical Evaluation: Blood Pathology"; November 1990--"Section 7 - Bioindicators"; December 1990--MM6 Report submitted by Prince William Sound Science Center; November 1991--"Section 7 - Hematology and Chemistry").

Abstract: Hematologic and serum chemical analyses were performed on sea otter blood samples collected from 31 adult males, 63 adult females, and 42 pups captured in western Prince William Sound (oiled area), and 12 adult males, 40 adult females, and 15 pups captured in eastern Prince William Sound (unoiled area) in 1989 and 1990. Hematologic differences between eastern and western adult males were minimal. Both hematocrits and hemoglobins were higher in western than eastern otters but the biological significance of this is equivocal. Western males had higher absolute eosinophil counts, suggesting possible systemic hypersensitivity reactions. Western males had higher serum protein and serum globulin levels than eastern males, suggesting greater antigenic stimulation (more inflammatory and/or infectious conditions). There were no differences in hematologic parameters between eastern and western female otters. Some chemistry changes were present, but the degree of difference was small. Total protein and serum globulin levels were slightly higher in western females, a finding also seen in adult males. Mean levels of liver enzymes for western females were somewhat higher than for the eastern otters, suggesting the possibility of subclinical liver disease. As a group, western pup hematocrits, hemoglobins, and red cell counts were significantly lower than those of eastern pups. From a biological perspective, these reductions were minimal but supported by individual animal data. The red cell data suggest a mild anemia in western pups; however, the degree of anemia was minimal, so that biological significance was equivocal. Other hematologic and clinical chemical differences between eastern and western pups were not striking and were also of equivocal biological significance.

Key Words: carcasses, *Enhydra lutris*, *Exxon Valdez*, mortality, oil spill, sea otter.

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INTRODUCTION

After the March 1989 oil spill from the T/V *Exxon Valdez* in Prince William Sound (PWS), Alaska, efforts to assess damages to wildlife in the path of the oil were initiated. The sea otter population in western Prince William Sound suffered heavy losses, with several thousand otters estimated to have died acutely (i.e., within a few months of the spill). Additional injury to the sea otter population may have occurred through sub-lethal initial exposure of otters or chronic exposure to petroleum hydrocarbons persisting in the environment. Chronic exposure may occur directly, when sea otters groom oil from their fur, or indirectly through ingestion of oil-contaminated prey items.

One approach to search for potential sublethal effects in sea otters residing in oil-affected habitat is to examine various hematologic and serum chemical parameters. Comparing the values of these parameters from sea otters inhabiting oiled areas to values from otters in areas uncontaminated by the oil spill may provide information on the general health and possible changes associated with exposure to petroleum hydrocarbons.

The objective of this study was to compare blood and serum samples collected from sea otters in unoiled and oiled areas of PWS as a means of evaluating long-term injury to the sea otter population following the *Exxon Valdez* oil spill.

METHODS

Sample collection and analysis

Hematologic and serum chemical analyses were performed on blood samples collected from 203 sea otters captured in PWS in 1989 and 1990 for telemetry studies. Samples were collected from 43 adult males, 103 adult females, and 57 pups. Twelve of the males, 40 of the females, and 15 of the pups were from eastern PWS (unoiled) while the remaining animals were from oiled areas of western PWS.

Approximately 30 cc of blood was collected by jugular venipuncture from each otter. A subsample was transferred to a sterile vacutainer coated with EDTA for hematologic analyses. Another subsample was processed to obtain blood serum. Both subsamples were kept chilled and air-expressed to Smith-Kline Laboratories in Van Nuys, California, for analyses of hematologic parameters and serum chemistry.

Statistical treatment

Data from the eastern otters were used to establish reference ranges (mean +/- 2 standard deviations) for each of the analytes measured. Population data as well as individual animal data on western otters were compared to these reference ranges. Additionally, blood values from eastern otters were compared to values from western otters using the t-test procedure from SAS statistical software (SAS Institute, Cary, North Carolina). Although a number of the variables were not normally distributed, by convention most clinical laboratories do not transform data; therefore transformations were not done.

RESULTS AND DISCUSSION

Male Adult Sea Otters

Hematologic differences between eastern and western adult males were minimal (Table 1). Both hematocrits and hemoglobins were significantly higher in western otters than in eastern otters but the biological significance of this is equivocal, especially since the western means fall within the eastern reference range. From a biological perspective, the difference in oxygen carrying capacity of the blood at the values recorded is minimal. Western males had significantly higher absolute eosinophil counts, suggesting the possibility of systemic hypersensitivity reactions in western otters.

The important serum chemical differences between eastern and western males were found in protein and electrolyte levels. Western males had higher serum protein and serum globulin levels than eastern males. These findings suggest greater antigenic stimulation (more inflammatory and/or infectious conditions) in western than in eastern otters. This finding was supported by the eosinophilia in western males as well as the trend (although not statistically significant) toward higher circulating neutrophil numbers in western males.

Serum sodium and serum chloride were significantly higher and serum potassium was significantly lower in western males than in their eastern counterparts. The mean western serum potassium was still within the eastern reference range. This pattern of electrolyte change was unusual; the possibility of stress-induced hyperadrenalism should be ruled out by measurement of serum cortisol levels.

Other differences between western and eastern males included lower lactate dehydrogenase (LDH), phosphorus, and glucose, and higher cholesterol and carbon dioxide in western otters. LDH differences were disregarded because of the nonspecific nature of the enzyme and the wide eastern reference range. Glucose differences were not of biological importance because the relationship of sample collection to eating could not be ascertained and was undoubtedly not constant. Phosphorus differences were not important when considered in light of the large reference range. Cholesterol differences were not specific, difficult to interpret, and probably biologically insignificant. Differences in carbon dioxide levels were biologically insignificant especially in light of the large eastern reference range.

Female Adult Sea Otters

There were no differences in hematologic parameters between eastern and western female otters (Table 2). Some chemistry changes were present, but the degree of difference was small so that data must be interpreted cautiously.

Although statistically different than the eastern mean, mean serum protein and globulin levels for western female otters were within the eastern reference range. Total protein and serum globulin levels were slightly higher in western females, a noteworthy finding since it mirrors the same trend seen in western adult males in 1989 and 1990. As mentioned previously, a trend to higher globulin levels suggests possible antigenic stimulation.

Liver enzyme levels (alanine aminotransferase (ALT), alkaline phosphatase) for western females also fell within the eastern reference range but again mean values were somewhat higher (and statistically significant) for the western females than for the eastern

otters. This suggests the possibility of subclinical liver disease and merits careful monitoring in the coming months and years. This is particularly important in light of elevated liver enzymes seen in otters brought to rehabilitation centers shortly after the oil spill. Aspartate aminotransferase (AST) and LDH levels followed the same patterns as ALT and alkaline phosphatase; however, these enzymes are nonspecific and are influenced by factors such as hemolysis. Consequently, even less interpretive significance was given to these values.

Electrolyte levels in western females also generally fell within eastern reference ranges. Once again, however, a similar pattern to that seen in adult males was present; that is sodium and chloride mean values were somewhat higher (and statistically significant) and potassium mean values were somewhat lower (and statistically significant) in western females than in those from the east. Whether this subtle difference was biologically significant is still uncertain. However, as with males, a possible stress-induced hyperadrenalism should be ruled out by measurement of serum cortisol levels.

Sea Otter Pups

As a group, western pup hematocrits, hemoglobins, and red cell counts were significantly lower than those of eastern pups (Table 3). From a biological perspective, these reductions were minimal but supported by individual animal data. Mean corpuscular hemoglobin concentration (MCHC) was also significantly lower in western pups, but the degree of this decrease was not regarded as biologically significant.

When considered collectively, red cell data suggest a mild anemia in western pups. Although reticulocyte counts were not available, the normal red cell indices suggested that this anemia was most likely nonregenerative. The degree of anemia was minimal so that biological significance was equivocal.

Leukocyte group data for eastern versus western pups showed minimal differences. The only difference of interest was a higher mean lymphocyte count in western pups. When the individual animal data was examined, this difference truly reflected a higher absolute lymphocyte count in a number of western pups. Lymphocytosis may be seen in animals with chronic antigenic stimulation; however, this change is usually accompanied by hypergammaglobulinemia and therefore elevated serum globulin levels. Globulin levels in western pups were normal, so it was difficult to suggest chronic antigenic stimulation in the present case. In some animals, most notably cats, lymphocytosis is seen when the animal is excited at the time of sample collection. While this may also be the case in sea otters, it is difficult to rationalize a relatively greater effect in western than in eastern pups. It is noteworthy that the eosinophilia seen in western adult males was not a prominent finding in the pups.

The only other interesting hematologic observation was the presence of a significantly elevated mean platelet count in western pups. On an individual animal basis, this elevation was seen in a relatively high proportion of individuals (10 of 24 measured). While the elevation appeared to be real, the degree of elevation from a biological perspective was not extreme and no particular interpretation could be attached to this finding.

Chemistry data were relatively unremarkable. Statistically significant differences in blood urea nitrogen, LDH, glucose, and triglycerides were not regarded as biologically significant. Both sodium and chloride levels were statistically lower in western pups than in those from the east. The degree of reduction was so slight as to be of no biological

consequence; however, the observation is noteworthy in that it is in direct contrast to findings described earlier for adults where sodium and chloride were increased while potassium was reduced.

In summary, hematologic and clinical chemical differences between eastern and western pups were not striking and at best were of equivocal biological significance. Perhaps the most significant observation was that trends seen in adults were not present in the pups observed to date.

ACKNOWLEDGEMENTS

Blood samples from adult female sea otters and sea otter pups, and a portion of the samples from adult male sea otters, were collected by Dr. Lisa Rotterman of the Prince William Sound Science Center during the course of related NRDA sea otter studies.

Table 1. Results of hematologic and chemical analyses for male adult sea otters in Prince William Sound[1].

Blood variable	Units	West mean	West S.D.	East mean	East range (mean ± 2S.D.)
White blood cells	#/μl	9014	2865	8127	5089-11201
Hemoglobin	g/dl	19	1	18	16-20
Red blood cells[2]	#/μl	4.89	0.30	4.78	3.96-5.60
Hematocrit	%	58	3	54	48-60
Mean corpuscular volume	fl	130	52	113	99-126
Mean corpuscular hemoglobin	pg	39	2	38	34-42
Mean corp. hemoglobin conc.	g/dl	33	1	34	32-35
Platelets[3]	#/μl	329	76	340	240-440
Neutrophils	#/μl	4999	1314	4448	2962-5935
Lymphocytes	#/μl	3125	1343	3501	999-6004
Monocytes	#/μl	251	268	161	20-302
Eosinophils	#/μl	594	969	17	0-92
Basophils	#/μl	45	51	0	-----
Glucose	mg/dl	164	33	191	87-296
Total protein	g/dl	6.4	0.5	5.9	5.3-6.4
Creatinine	mg/dl	0.6	0.1	0.6	0.4-0.8
Cholesterol	mg/dl	126	26	111	98-124
Triglycerides	mg/dl	65	26	51	19-84
Alkaline phosphatase	IU/l	112	43	121	74-168
Aspartate aminotransferase	IU/l	292	142	290	128-453
Alanine amino transferase	IU/l	261	102	235	122-348
Lactic dehydrogenase	IU/l	348	135	548	243-852
Total bilirubins	mg/dl	0.1	0.1	0.1	0.0-0.2
Sodium	mEq/l	159	7	154	149-158
Potassium	mEq/l	3.8	0.4	4.3	3.4-5.2
Chloride	mEq/l	122	4	116	113-120
Calcium	mg/dl	9.3	0.5	9.2	7.8-10.6
Phosphorus	mg/dl	4.4	1.9	6.2	3.2-9.2
Albumin	g/dl	2.6	0.3	2.8	2.4-3.1
Globulin	g/dl	3.8	0.5	3.1	2.5-3.8
Blood urea nitrogen	mg/dl	51	14	51	27-76
Creatine kinase	IU/l	1419	1356	2423	0-7300
Carbon dioxide	mEq/l	28.33	2.32	26.18	20.90-31.46

[1] Hematology: west N = 28, east N = 11. Chemistry: west N = 31, east N = 12.
[2] x 10^6
[3] x 10^3

Table 2. Results of hematologic and chemical analyses for female adult sea otters in Prince William Sound[1].

Blood variable	Units	West mean	West S.D.	East mean	East range (mean ± 2S.D.)
White blood cells	#/μl	9082	2203	8233	2890-13576
Hemoglobin	g/dl	19	1	19	16-20
Red blood cells[2]	#/μl	4.73	0.38	4.67	4.0-5.4
Hematocrit	%	58	5	58	47-69
Mean corpuscular volume	fl	122	7	124	110-138
Mean corpuscular hemoglobin	pg	41	2	40	37-44
Mean corp. hemoglobin conc.	g/dl	33	2	33	29-37
Platelets[3]	#/μl	370	113	397	237-557
Neutrophils	#/μl	5890	1658	4974	1744-8204
Lymphocytes	#/μl	2570	981	2936	378-5494
Monocytes	#/μl	249	169	203	0-509
Eosinophils	#/μl	331	1513	62	0-392
Basophils	#/μl	42	111	36	0-1545
Glucose	mg/dl	247	72	194	124-264
Total protein	g/dl	6.5	0.6	6.2	5.2-7.3
Creatinine	mg/dl	0.5	0.1	0.5	0.3-0.6
Cholesterol	mg/dl	140	27	147	99-195
Triglycerides	mg/dl	76	74	64	18-110
Alkaline phosphatase	IU/l	151	97	109	22-196
Aspartate aminotransferase	IU/l	531	360	340	0-857
Alanine aminotransferase	IU/l	358	165	266	30-503
Lactic dehydrogenase	IU/l	675	645	400	68-732
Total bilirubins	mg/dl	0.2	0.1	0.3	0.0-0.8
Sodium	mEq/l	160	7	157	150-164
Potassium	mEq/l	4	0.4	4.8	4.0-5.6
Chloride	mEq/l	120	6	117	112-123
Calcium	mg/dl	9.4	0.6	8.2	6.8-9.7
Phosphorus	mg/dl	6.7	2.1	5.4	1.7-9.2
Albumin	g/dl	2.7	0.3	2.8	2.3-3.2
Globulin	g/dl	3.9	0.6	3.5	2.6-4.4
Blood urea nitrogen	mg/dl	44	10	46	15-77
Creatine kinase	IU/l	8572	15980	1913	0-5992
Carbon dioxide	mEq/l	30.69	2.94	-----	-----

[1] Hematology: west N = 39, east N = 15. Chemistry: west N = 52, east N = 36.
[2] x 10^6
[3] x 10^3

Table 3. Results of hematologic and chemical analyses for sea otters pups in Prince William Sound[1].

Blood variable	Units	West mean	West S.D.	East mean	East range (mean ± 2S.D.)
White blood cells	#/μl	9180	3925	8000	4671-11329
Hemoglobin	g/dl	17	1	20	18-22
Red blood cells[2]	#/μl	4.47	0.36	4.93	4.4-5.5
Hematocrit	%	51	3	56	49-63
Mean corpuscular volume	fl	114	5	113	100-126
Mean corpuscular hemoglobin	pg	39	2	39	36-43
Mean corp. hemoglobin conc.	g/dl	34	1	35	33-27
Platelets[3]	#/μl	377	71	289	182-396
Neutrophils	#/μl	5148	2424	5439	2645-8233
Lymphocytes	#/μl	3751	1817	2357	976-3737
Monocytes	#/μl	206	210	122	0-296
Eosinophils	#/μl	8	23	42	0-161
Basophils	#/μl	67	63	40	0-127
Glucose	mg/dl	184	33	235	126-344
Total protein	g/dl	5.2	0.5	5.5	4.8-6.1
Creatinine	mg/dl	0.3	0.1	0.4	0.1-0.7
Cholesterol	mg/dl	147	22	158	118.3-197.5
Triglycerides	mg/dl	80	34	67	47-88
Alkaline phosphatase	IU/l	307	156	297	200-394
Aspartate aminotransferase	IU/l	207	142	232	57-406
Alanine aminotransferase	IU/l	311	215	256	118-393
Lactic dehydrogenase	IU/l	279	86	345	141-550
Total bilirubins	mg/dl	0.2	0.1	0.2	0.1-0.3
Sodium	mEq/l	155	2	158	148-168
Potassium	mEq/l	4.5	0.4	4.4	3.4-5.4
Chloride	mEq/l	114	3	115	110-120
Calcium	mg/dl	10.0	0.5	10.0	8.9-11.0
Phosphorus	mg/dl	7.5	1.4	7.8	6.1-9.4
Albumin	g/dl	2.8	0.3	3.1	2.7-3.4
Globulin	g/dl	2.4	0.5	2.4	1.9-2.9
Blood urea nitrogen	mg/dl	37	11	27	18-35
Creatine kinase	IU/l	-----	-----	-----	-----
Carbon dioxide	mEq/l	-----	-----	-----	-----

[1] Hematology: west N = 25, east N = 12. Chemistry: west N = 40, east N = 15.
[2] x 10^6
[3] x 10^3