Evaluation of changes in hematologic and clinical biochemical values after exposure to petroleum products in mink (*Mustela vison*) as a model for assessment of sea otters (*Enhydra lutris*)

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Objective—To determine the effects of petroleum exposure on hematologic and clinical biochemical results of mink and to identify variables that may be useful for making management decisions involving sea otters (*Enhydra lutris*) that have been exposed to oil in their environment.

Animals—122 American mink (*Mustela vison*).

Procedures—Mink were exposed once to a slick of oil (Alaskan North Slope crude oil or bunker C fuel oil) on seawater or via low-level contamination of their daily rations.

Results—In the acute phase of exposure, petroleum directly affected RBC, WBC, neutrophil, and lymphocyte counts, fibrinogen, sodium, calcium, creatinine, total protein, and cholesterol concentrations, and alanine transaminase, creatine kinase, alkaline phosphatase, and γ–glutamyltransferase activities. Aspartate transaminase, alkaline phosphatase, γ–glutamyltransferase, and lactate dehydrogenase activities and cholesterol concentration also varied as a result of chronic low-level contamination of feed.

Conclusions and Clinical Relevance—Our results are in agreement with reports that attribute increased alanine transaminase and alkaline phosphatase activities and decreased total protein concentration to petroleum exposure in sea otters during an oil spill. Sodium, calcium, creatinine, cholesterol, and lactate dehydrogenase may be valuable variables to assess for guidance during initial treatment of sea otters exposed to oil spills as well as for predicting which petroleum-exposed sea otters will reproduce following an oil spill. Measurement of these variables should aid wildlife professionals in making decisions regarding treatment of sea otters after oil spills. (*Am J Vet Res* 2000;61: 1197–1203)

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Effects on wildlife of exposure to petroleum products often are difficult to assess during an oil spill. It has been almost impossible to definitively attribute changes in hematologic and clinical biochemical values to petroleum exposure because of the lack of control groups and a paucity of baseline information on species affected by exposure to oil in their environment. These issues, which involve causal influence as well as medical treatment and follow-up care of wildlife subjected to oil, become increasingly complex when threatened or endangered species such as the southern sea otter (*Enhydra lutris nereis*) are affected. Measurement of hematologic and clinical biochemical variables is used commonly by veterinarians to assess the health and well-being of animals. Therefore, these values should be used at crucial points during the response to an oil spill when evaluating sea otters that need medical treatment, assessing the quality and progression of rehabilitation, and evaluating the likelihood for a rehabilitated animal to be able to reproduce after release.

We designed a field trial that used mink (*Mustela vison*) as an animal model for sea otters to identify hematologic variables likely to change and the extent of that change after exposure to petroleum products. We chose mink, because they were used by Natural Resource Damage Assessment researchers investigating the effects of an oil spill on the reproductive success of oil-exposed populations.[a] Characteristics of mink that validate their use as a model for sea otters include the fact that, similar to sea otters, they are members of the mustelid family, have a high metabolic rate, intense grooming behavior, and semiaquatic nature, make use of a diverse group of prey items, and have an exquisite susceptibility to environmental contaminants.[1] The objectives of the study reported here were to evaluate the effects of exposure to petroleum products on hematologic and clinical biochemical values of mink that were exposed acutely to oil in their environment or that received low-level contamination of their food for an extended period and to determine whether the hematologic and clinical biochemical variables were predictors of exposure and subsequent reproductive performance. We hypothesized that exposure of mink through their food would simulate the recolonization by sea otters not directly exposed to petroleum products in environments previously contaminated with oil and would facilitate the investigation of potential chronic effects of petroleum contamination on multiple organ systems.

Materials and Methods

The randomized field study reported here was part of a large-scale study in a commercial breeding colony of standard dark ranch mink. The study investigated the potential effects of exposure to petroleum products on multiple organ systems of sea otters. All procedures were approved by a university animal care and use committee.

Animals—We randomly selected yearling female mink from a commercial breeding colony and allocated them to 1 of 5 exposure groups. To ensure consistency, physical examinations were performed and blood samples evaluated for all selected mink prior to allocation to treatment groups and initiation of the study.

Procedure—We selected **Alaskan North Slope (ANS)** crude oil and bunker C fuel oil as exposure products, because they are commonly transported or used as fuel in the Pacific and, therefore, are more likely to be spilled in the habitat of sea otters than other petroleum products. Groups initially consisted of 24 mink exposed to a 1.5-cm-thick slick of ANS crude oil on seawater for 1 minute (external: crude oil), 24 mink exposed to a 1.5-cm-thick slick of bunker C fuel oil on seawater for 1 minute (external: bunker C), 24 mink fed diets containing 500 µg of ANS crude oil/g of feed (internal: crude oil), 24 mink fed diets containing 500 µg of bunker C fuel oil/g of feed (internal: bunker C), and 26 control mink. Because assessment of the effects of petroleum exposure on reproductive performance was key to the objective of the large-scale study, selection of sample size was based on detecting, with 95% confidence and 80% power,[b] a difference of 45% between mean litter size of affected and unaffected groups.

The dose of 500 µg/g of feed for the internal trial was selected to simulate exposure to a petroleum product that would result from consumption of contaminated invertebrates by sea otters after an oil spill. Estimated total oil concentrations in mussels (*Mytilus trossulus*) from some areas in the path of the oil spill from the *Exxon Valdez* exceeded 1,250 µg/g in 1989 and remained close to that concentration for more than a year after the incident.[2] The concentration of 500 µg/g of feed corresponded to a total daily dose of approximately 0.065 g/kg of body weight for the mink in our study, which was a much lower daily dose of oil than in other studies in which investigators evaluated the effects of petroleum products on mammals.[2,3] Because we fed mink this diet for < 6 months, we believe the selected petroleum concentration and duration of exposure were appropriate. Our choice of dose also was supported by findings of persistent petroleum residues in contaminated mussel beds 4 years after the oil spill from the *Exxon Valdez*, which would have resulted in daily consumption of sea otters of weathered oil at a rate of 0.019 g/kg.[4]

Petroleum exposure was initiated 8 weeks prior to breeding, and animals were exposed in accordance with protocols established by the United States Environmental Protection Agency[5]; in the internal portion of the study, exposure continued until kits were weaned. Blood samples were collected from all mink 1 week after initial exposure to enable us to evaluate acute effects for the 2 exposure routes. Blood also was obtained after weaning of the kits (from the reproductive phase of the project) to enable us to assess the chronic effects of petroleum exposure. Females were not handled between breeding and weaning of kits, because we hypothesized that the stress of disturbance could affect reproductive outcomes.

Collection and analysis of samples—Mink were medicated with atropine (0.02 mg/kg, IM) and then immobilized with ketamine hydrochloride (25 mg/kg, IM) prior to collection of blood samples. Samples (2.5 ml) were obtained via cardiac puncture, using a 3-ml syringe and 22-gauge needle. One milliliter of each sample was mixed with EDTA to prevent coagulation and refrigerated until a CBC, using an automated technique, and differential WBC counts, using examination of blood smears stained with Wright stain, were performed at the **Veterinary Medical Teaching Hospital (VMTH)**, University of California, Davis. Hematologic analysis was performed within 24 hours of collection. The remaining 1.5 ml of blood was placed in a serum separator tube[c] and centrifuged for 5 minutes. Serum was removed and stored at –20 C until thawed and analyzed at the VMTH. Hematologic variables included RBC, nucleated RBC, platelet, WBC, and differential WBC (based on examination of 200 cells) counts, reticulocyte percentage, and fibrinogen concentration.

Serum biochemical analysis included concentrations of sodium, potassium, chloride, total carbon dioxide, phosphorous, calcium, BUN, creatinine, glucose, total protein, cholesterol, triglycerides, and total bilirubin as well as activities of **alanine transaminase (ALT)**, **aspartate transaminase (AST)**, **creatine kinase (CK)**, **alkaline phosphatase (AP)**, **γ–glutamyltransferase (GGT)**, and **lactate dehydrogenase (LDH)**. Blood collection was not successful on all animals at every sample collection. Some samples were of insufficient volume for all analyses. Fewer mink were available at the postweaning sample collection because of attrition from the toxic effects of exposure and the multiple organ system study.

Statistical analysis—A 1-way ANOVA and the Tukey method for multiple comparisons[d] were used to test for differences among means. Nucleated RBC, metamyelocyte, band neutrophil, monocyte, eosinophil, and basophil counts were not included in these analyses, because these cells were rarely identified during CBC counts and had skewed distributions.[6] Transformations of specific variables were made when appropriate. Kruskal-Wallis 1-way ANOVA and pairwise comparison procedures[d] were used to analyze postweaning data, because sample sizes were lower.

Sea otters have dark pelts with a natural sheen that makes it difficult to determine whether an otter in the vicinity of an oil spill has been exposed. Additionally, capture, restraint, and rehabilitation of potentially exposed wildlife are costly and stressful to the animals.[7] Therefore, hematologic and clinical biochemical values were used to classify mink on the basis of their exposure status to allow us to determine, using a multivariate logistic regression model, those variables that may be predictive of exposure. Only hematologic and clinical biochemical variables that were perceived to have clinical relevance and were found to be altered by petroleum exposure (on the basis of results of 1-way ANOVA or Kruskal-Wallis 1-way ANOVA) were included in the stepwise technique[d] for each of the sample collection times.

Because only those sea otters that reproduce after treatment and rehabilitation for the effects of an oil spill and subsequent release to the wild truly contribute to the free-ranging population, the hematologic and clinical biochemical variables also were used to classify mink on the basis of their reproductive success, using multivariate logistic regression. Sea otters in the vicinity of an oil spill may be captured and rehabilitated even when they have not been directly exposed to petroleum; thus, exposure status also was included in this portion of the analysis as a potential predictor of reproductive success. Reproductive success was defined as giving birth to ≥ 1 live kit. Appropriate interaction terms were included for explanatory variables with significant results, and transformation of specific variables was made when appropriate. A forward stepwise algorithm was used.[d] The likelihood ratio test (logarithm of the ratios of the maximized likelihood functions for 2 models, 1 with and 1 without the variable in question) was used to test for the entry or removal of variables in the model at each step ($P < 0.10$ to enter and $P > 0.15$ to remove). Improvement in the log likelihood at each step was assessed, using a χ^2 test. Overall fit of the final model

was assessed, using a **Hosmer-Lemeshow (H-L)** goodness-of-fit χ^2 test. Odds ratios were determined for each model by calculating biologically meaningful values of the explanatory variables for a high-risk animal and comparing those results with relevant values of the explanatory variables for a lower-risk animal.

Results

Results of the hematologic analysis performed on samples obtained 1 week after exposure were summarized (**Table 1**). Hematologic variables that differed significantly among exposure groups included RBC count ($P = 0.003$), WBC count ($P < 0.001$), segmented neutrophil and lymphocyte values (absolute counts as well as relative frequencies used for statistical purposes; $P < 0.001$), and fibrinogen concentration ($P < 0.001$). In general, mink exposed externally to bunker C fuel oil were most severely affected, with mean values differing from means of the control group for all 5 variables. Mean values for mink exposed externally to crude oil also varied from control means for all variables other than WBC count. Similar to externally exposed mink, mink exposed to bunker C fuel oil through their diet had higher mean RBC counts and fibrinogen concentration than control mink.

Differences among hematologic values after weaning were summarized (**Table 2**). Although differences among exposure groups were detected, all mean values reported were within reference ranges established for healthy mink.[8,9] For this sample, hematologic values that differed significantly ($P \leq 0.001$) among exposure groups included RBC, reticulocyte, and segmented neutrophil counts and lymphocyte values (absolute counts as well as relative frequencies used for statistical purposes). Mink exposed to bunker C fuel oil through their diet were more severely affected, with values differing significantly for all 4 variables. Similar to the group exposed to bunker C fuel oil in their diet, mink exposed to crude oil through their diet had lower mean reticulocyte and lower segmented neutrophil counts with a concomitant increase in the number of lymphocytes. Mean reticulocyte counts also were lower in the externally exposed mink than in control mink.

Results of clinical biochemical analysis for samples obtained 1 week after exposure were summarized (**Table 3**). Clinical biochemical variables that differed significantly ($P < 0.001$) among exposure groups were sodium, chloride, calcium, creatinine, and total protein concentrations and ALT, CK, AP, and GGT activities. For this acute sample, only mink exposed externally to petroleum products had altered clinical biochemical values, compared with values for the control mink. Mink exposed externally to crude oil had the most extensive effects, with altered values for all 9 variables. Effects on mink externally exposed to bunker C fuel oil also were severe, with all values differing for all variables except chloride and creatinine.

Mean cholesterol and bilirubin concentrations were within reference ranges for healthy mustelids[10] (data not shown); however, mean cholesterol concentrations were significantly ($P < 0.001$) lower for all exposed groups than for the control group, and the mean total bilirubin concentration was significantly ($P = 0.003$) higher for the group exposed externally to crude oil.

Table 1—Mean (± SEM) hematologic values of American mink (*Mustela vison*) that differed among exposure groups 1 week after initial exposure to petroleum products

Exposure group	No. of samples	RBC count ($\times 10^6$ cells/L)	WBC count (cells/μl)	Neutrophils (cells/μl [%])*	Lymphocytes (cells/μl [%])*	Fibrinogen (mg/dl)
Control	21	7.89 ± 0.33[a]	9,548 ± 640[a]	6,630 ± 594 67 ± 3.2[a]	2,081 ± 148 23 ± 1.8[a]	181 ± 19[a]
Internal ANS crude oil	19	8.38 ± 0.14[a,b]	8,453 ± 485[a]	6,060 ± 377 72 ± 1.8[a]	1,718 ± 187 21 ± 1.9[a]	184 ± 21[a]
Bunker C	22	8.82 ± 0.18[b]	8,682 ± 316[a]	5,944 ± 236 69 ± 1.6[a]	1,926 ± 162 22 ± 1.6[a]	291 ± 26[b]
External ANS crude oil	18	8.94 ± 0.24[b]	9,844 ± 509[a]	8,104 ± 496 82 ± 1.6[b]	1,114 ± 141 12 ± 1.6[b]	378 ± 33[b]
Bunker C	19	9.01 ± 0.21[b]	16,000 ± 1,268[b]	13,017 ± 1,081 84 ± 1.1[b]	1,774 ± 208 11 ± 1.1[b]	342 ± 29[b]

*Results determined on the basis of percentages to maintain statistical independence from the overall WBC counts. [a,b]Within a column, means with different superscript letters differ significantly ($P < 0.05$). ANS = Alaskan North Slope.

Table 2—Mean (± SEM) hematologic values of American mink that differed among exposure groups 6 months after initial exposure to petroleum products (ie, after weaning of kits)

Exposure group	No. of samples	RBC count ($\times 10^6$ cells/L)	Reticulocyte count ($\times 10^6$ cells/L [%])*	Neutrophils (cells/μl [%])*	Lymphocytes (cells/μl [%])*
Control	22	8.86 ± 0.16[a]	0.13 ± 0.01 1.5 ± 0.2[a]	4,356 ± 339 67 ± 2.4[a]	1,496 ±172 24 ± 2.5[a]
Internal ANS crude oil	21	8.02 ± 0.22[a]	0.04 ± 0.02 0.5 ± 0.2[b]	2,829 ± 244 54 ± 3.0[b]	2,065 ± 205 39 ± 3.1[b]
Bunker C	21	7.31 ± 13[b]	0.03 ± 0.02 0.5 ± 0.2[b]	3,198 ± 365 52 ± 3.4[b]	2,316 ± 247 38 ± 3.0[b]
External ANS crude oil	7	8.65 ± 0.20[a]	0.02 ± 0.02 0.3 ± 0.3[b]	3,709 ± 342 73 ± 2.7[a]	975 ± 136 19 ± 1.5[a]
Bunker C	13	8.55 ± 0.19[a]	0.02 ± 0.01 0.2 ± 0.2[b]	3,536 ± 304 66 ± 2.5[a,b]	1,356 ± 119 26 ± 2.0[a,b]

*Analyses determined on the basis of percentages to maintain statistical independence from the overall RBC and WBC counts, respectively. [a,b]Within a column, values with different superscript letters differ significantly ($P < 0.05$).

Table 3—Mean (± SEM) clinical biochemical values of American mink that differed among exposure groups 1 week after initial exposure to petroleum products

Exposure group	No. of samples	Sodium (mmol/L)	Chloride (mmol/L)	Calcium (mg/dl)	Creat (mg/dl)	TP (g/dl)	ALT* (U/L)	CK* (U/L)	AP* (U/L)	GGT* (U/L)
Control	19	153.9[a] (0.7)	109.2[a] (0.7)	9.9[a] (0.1)	0.63[a] (0.04)	6.19[a] (0.10)	115.3[a] (27.2)	585.6[a] (52.1)	66.8[a] (4.2)	0.1[a] (0.1)
Internal										
ANS crude oil	13	153.3[a,c] (0.7)	108.9[a] (0.8)	9.5[a] (0.1)	0.72[a] (0.03)	5.92[a] (0.13)	191.7[a,b] (104.3)	533.6[a,b] (64.9)	69.8[a] (6.1)	0.9[a] (0.8)
Bunker C	22†	152.2[a,c] (0.9)	108.1[a] (1.6)	9.6[a] (0.1)	0.62[a] (0.04)	5.78[a] (0.15)	99.8[a] (18.9)	640.7[a] (53.3)	73.8[a] (5.2)	0.6[a] (0.3)
External										
ANS crude oil	13‡	139.8[b] (2.3)	98.5[b] (1.3)	7.9[b] (0.3)	0.98[b] (0.07)	4.25[b] (0.17)	405.5[b] (86.1)	342.2[b] (63.9)	221.2[b] (47.3)	82.9[b] (22.2)
Bunker C	12	148.4[c] (2.0)	104.2[a,b] (1.3)	8.7[c] (0.2)	0.73[a] (0.03)	4.25[b] (0.12)	461.6[b] (109.6)	320.2[b] (31.8)	149.8[a] (28.9)	31.8[a,b] (10.9)

*Comparisons based on values determined by logarithm transformation. †n = 21 for sodium, chloride, and calcium concentrations. ‡n = 12 for sodium, chloride, and calcium concentrations. [a,b,c]Within a column, means with different superscript letters differ significantly ($P < 0.05$). Creat = Creatinine. TP = Total protein. ALT = Alanine transaminase. CK= Creatine kinase. AP = Alkaline phosphatase. GGT = γ-glutamyltransferase.

Table 4—Mean (± SEM) clinical biochemical values of American mink that differed among exposure groups 6 months after initial exposure to petroleum products (ie, after weaning of kits)

Exposure group	No. of samples	AST(U/L)	AP(U/L)	GGT(U/L)	LDH(U/L)	Chol(mg/dl)
Control	24	135.8 ± 12.1[a]	69.7 ± 3.9[a]	0.0 ± 0.0[a]	722 ± 89[a]	268.1 ± 9.8[a,b]
Internal						
ANS crude oil	19	152.0 ± 19.6[a,b]	94.4 ± 13.4[a,b]	0.3 ± 0.2[a]	1,236 ± 121[b]	286.0 ±11.3[a,c]
Bunker C	22	153.1 ± 12.5[a,b]	104.1 ± 26.9[a,b]	4.7 ± 1.2[b]	1,078 ± 92[a,b]	235.8 ± 6.4[b]
External						
ANS crude oil	8	156.9 ± 21.5[a,b]	88.6 ± 8.4[a,b]	0.0 ± 0.0[a]	1,518 ± 235[b]	346.4 ± 19.4[c]
Bunker C	16	200.4 ± 22.2[b]	96.7 ± 5.1[b]	0.3 ± 0.3[a]	1,650 ± 157[b]	346.4 ± 15.3[c]

AST = Aspartate transaminase. LDH = Lactate dehydrogenase. Chol = Cholesterol. *See* Table 3 for key.

Results of clinical biochemical analyses for samples obtained after weaning were summarized (**Table 4**). Clinical biochemical variables that differed significantly among exposure groups included AST ($P = 0.02$), AP ($P = 0.005$), GGT ($P < 0.001$), and LDH ($P < 0.001$) activities and cholesterol concentration ($P < 0.001$). Mean GGT activity was higher in mink exposed to bunker C fuel oil through their diet, and mean LDH activity was higher in mink exposed to crude oil through their diet, compared with values for control mink. Other clinical biochemical variables differed significantly ($P < 0.001$) among exposure groups, but mean values, including potassium, calcium, BUN, creatinine, total protein, and bilirubin concentrations (data not shown), were not outside reference ranges for healthy mink or other mustelids.[9,10]

The only variables that entered the stepwise regression model for classification of exposure status for samples obtained 1 week after exposure were cholesterol and creatinine concentrations. The resulting model for exposure was as follows:

$$\log_e \frac{P(X)}{1 - P(X)} = 14.93 + 6.634\,(\text{creatinine concentration}) - 0.078\,(\text{cholesterol concentration})$$

This model fit the data well (H-L χ^2; $P = 0.64$). Using this model, a mink with a serum creatinine concentration of 1.0 mg/dl and cholesterol concentration of 180 mg/dl was > 20,000 times more likely to have been exposed to a petroleum product than a mink with a creatinine concentration of 0.6 mg/dl and cholesterol concentration of 275 mg/dl.

For the sample collected after weaning, variables that entered the stepwise regression model for the classification of exposure status were sodium and calcium concentrations and LDH activity. The resulting model for exposure was as follows:

$$\log_e \frac{P(X)}{1 - P(X)} = 50.53 - 0.218(\text{sodium concentration}) - 1.943(\text{calcium concentration}) + 0.003\,(\text{LDH activity})$$

This model fit the observed data well (H-L χ^2; $P = 0.99$). Using this model, a mink with a serum sodium concentration of 150 mmol/L, serum calcium concentration of 9.0 mg/dl, and LDH activity of 1,500 U/L was 139 times more likely to have been exposed to crude oil than a mink with a serum sodium concentration of 154 mmol/L, serum calcium concentration of 10 mg/dl, and LDH activity of 725 U/L.

The only variables that entered the logistic model for the prediction of reproductive success for samples obtained 1 week after exposure were exposure group and creatinine concentration. Interaction terms between exposure and all other variables and between WBC count and the proportion of WBC that were segmented neutrophils were not significantly associated with reproductive success. All coefficients in the model selected were significantly different from zero, except those for exposure in the externally exposed groups. Resulting models for reproductive success in mink exposed externally to ANS crude oil and bunker C fuel oil, respectively, in their diet fit the data well, as determined on the basis of results of the H-L χ^2 ($P = 0.71$). The resulting models for reproductive success were as follows:

ANS crude oil,

$$\log_e \frac{P(X)}{1 - P(X)} = -0.596 - 6.587(\text{creatinine concentration})$$

and bunker C fuel oil,

$$\log_e \frac{P(X)}{1 - P(X)} = -0.762 - 6.587(\text{creatinine concentration})$$

Analysis of these models indicated that unexposed mink with serum creatinine concentrations of 0.6 mg/dl were 180 times more likely to produce live offspring than mink exposed acutely to bunker C fuel oil and that had a serum creatinine concentration of 1.0 mg/dl. Furthermore, mink exposed to ANS crude oil and that had a serum creatinine concentration of 1.0 were 1.2 times more likely to produce live offspring in the same year than mink exposed acutely to bunker C fuel oil and that had the same serum creatinine concentration.

For the sample obtained after weaning, variables that entered the logistic model for prediction of reproductive success were exposure and LDH activity. Again, interaction terms were not significantly associated with reproductive success, and coefficients for exposure in the externally exposed groups were not significantly different from zero. Resulting models for the reproductive success in mink exposed to ANS crude oil and bunker C fuel oil in their diets, respectively, fit the observed data well (H-L χ^2; $P = 0.94$). The models for these 2 groups were as follows:

ANS crude oil,

$$\log_e \frac{P(X)}{1 - P(X)} = -2.354 - 0.002(\text{LDH activity})$$

and bunker C fuel oil,

$$\log_e \frac{P(X)}{1 - P(X)} = -4.342 - 0.002(\text{LDH activity})$$

Therefore, unexposed mink that had a serum LDH activity of 725 U/L were 122 times more likely to produce live offspring than mink that had a serum LDH activity of 1,500 U/L and had been exposed to bunker C fuel oil through their diet. Furthermore, mink exposed to ANS crude oil through their diet and that had a serum LDH activity of 1,500 U/L were 7.3 times more likely to produce live offspring than mink exposed to bunker C fuel oil in their diet that had a similar LDH activity.

Discussion

Hematologic and clinical biochemical data can aid veterinarians, wildlife biologists, and rehabilitators when they make important decisions regarding treatment and medical care of animals after exposure to petroleum products. However, changes in these values attributable to environmental exposure to oil could not have been differentiated from those related to the stress of handling and rehabilitation prior to evaluation in a controlled study. In general, the study reported here provided evidence that sea otters acutely exposed to oil in the environment and then removed from the source should have changes in hematologic and clinical biochemical variables early in the rehabilitation process, whereas sea otters exposed to long-term oil contamination in the environment may have more profound changes later during the process.

Most hematologic changes 1 week after exposure were evident in acutely exposed mink. Mink exposed externally, as well as mink exposed to bunker C fuel oil in their diet, had slightly higher RBC counts.[9] Whereas the magnitude of increase was probably not of clinical importance, it may have been indicative of a stress response that caused splenic contraction and release of RBC.[11] Dehydration was less likely to be the cause of higher RBC counts, because there was not a concomitant increase in total protein concentration. Similarly, fibrinogen concentration also was higher in these 3 groups, compared with values for control mink; however, none of the exposure groups had fibrinogen concentrations outside reference ranges for domestic animals.[11] The slight increases in fibrinogen concentration may have been attributable to increased production in response to inflammation or trauma resulting from exposure. Plasma fibrinogen concentration is a more sensitive indicator of acute inflammation than WBC counts in some species, such as cattle.[11] Only mink exposed externally to bunker C fuel oil had an increased WBC count. Lack of leukocytosis in mink exposed externally to crude oil was probably indicative of a difference in toxicity between the 2 products. Leukocytosis without a neutrophilic left shift may be caused by corticosteroids, mild inflammation, or direct toxic effects.[12] Therefore, the physiologic stress associated with exposure to bunker C fuel oil may be greater than that for exposure to ANS crude oil; there may have been pain associated with exposure, more secondary stress associated with thermoregulatory problems, more associated inflammatory processes, or more severe toxic effects attributable to composition of the product. Physiologically induced stress associated with acute exposure was confirmed by the increase in number of segmented neutrophils and relative decrease in number of lymphocytes[11] evident in both groups of mink exposed externally.

Hematologic values after weaning were all within reference ranges for mink and other healthy mustelids and domestic animals.[8-10] However, significant differences among exposure groups were detected. It was not surprising that the values varied within a narrow range, because the mink selected for use in this study were homogenous females of a single breed and age class, in contrast to the heterogeneous groups of putatively healthy animals usually used to establish reference ranges. As expected, the greatest effects were found in the mink exposed internally at the later sample collection periods. Especially interesting was the reestablishment of the appropriate neutrophil-to-lymphocyte ratio in mink exposed externally and the decreased number of neutrophils relative to number of lymphocytes in mink exposed internally. This shift may have been attributable to a reduction in the production of neutrophils,[12] possibly resulting from toxic effects on hematopoietic stem cells.

Again, variation from reference ranges was not evident in the clinical biochemical values measured 1 week

after exposure in the mink exposed internally. However, many significant changes were identified in mink acutely (externally) exposed to petroleum products. Whereas sodium, chloride, and calcium concentrations were less than the reference range for mink,[9] they probably were not clinically relevant.[13] The decreased sodium and chloride concentrations in mink exposed externally may have been directly attributable to toxic effects of petroleum from the ingestion of oil through grooming,[13] and all 3 measurements may have been affected by gastrointestinal tract disturbances associated with exposure, including anorexia and diarrhea.

Although reference values for mink or other mustelids were not available for CK activity, all groups of mink, including control mink, had increased CK activity, compared with values for domestic animals. The relatively decreased values in mink exposed externally were most likely attributable to behavioral results of exposure. Healthy mink are extremely active and aggressive during capture and restraint. The greater activity of control mink and mink exposed internally prior to anesthesia and collection of blood samples most likely resulted in increases in CK activity. The relative lethargy and inactivity in mink exposed externally resulted in less resistance to capture and, subsequently, lower CK values.

These behavioral changes also support the diagnosis of hepatic insufficiency that resulted in CNS abnormalities.[14] Mink exposed externally often were stuporous, laterally or dorsally recumbent, and blind during the initial period (5 to 15 days) after exposure. The increased ALT, AP, and GGT values and decreased total protein concentration were all indicative of hepatic toxicosis.[15,16] These variations in clinical biochemical values and the increased creatinine concentration indicated direct toxic effects on the liver and kidneys resulting in hepatocellular damage, cholestasis, and potentially decreased glomerular filtration. This set of clinical biochemical values as well as increased ALT and AP activity and decreased total protein concentration were found in sea otters that died within 10 days after examination at rehabilitation centers following the oil spill from the *Exxon Valdez* in March 1989.[17] Behavioral abnormalities similar to those seen in mink also have been reported in harbor seals[18] and sea lions exposed to petroleum products.

Although we expected to find alterations from typical clinical biochemical values in the values determined for samples collected after weaning from mink exposed internally, we were surprised to detect alterations in values persisting in apparently recovered, externally exposed mink; interestingly, increases in AST, AP, and LDH activities and cholesterol concentration were apparent 6 months after exposure in mink exposed externally. The increased values for AST, AP, cholesterol (externally exposed groups only), GGT (internally exposed bunker C fuel oil group only), and LDH were indicative of mild liver damage, which may have included hepatocellular damage and cholestasis.[16] It is also interesting that AST activity was increased in this sample without a simultaneous increase in ALT activity. Activity of ALT is considered to be more specific for hepatic damage than AST activity. The increase of AST activity alone may be attributable to the chronic nature of damage. The validity of AST activity as a useful measure in the assessment of chronic exposure is supported by the identification of increased values in river otters 2 years after the incident involving the *Exxon Valdez*.[19]

In an attempt to identify useful variables for the classification of exposure status for each animal, we fit a logistic regression model, using hematologic and clinical biochemical values obtained 1 week after external exposure. Although many variables were highly correlated ($P < 0.001$) with exposure, creatinine and cholesterol concentrations had the best fit. Our analysis also revealed that sodium and calcium concentrations and LDH activity may be useful for identifying exposed animals 6 months after a single acute exposure or initiation of chronic exposure via diet.

Only those sea otters that reproduce after exposure to oil spills contribute to the free-ranging population. If hematologic and clinical biochemical values that can be measured after capture but prior to release of sea otters from rehabilitation centers are predictive of future reproductive success, they may be useful when clinicians make triage, treatment, and release decisions. We have described elsewhere[e] the reproductive effects of exposure to petroleum products on mink as a model for sea otters. Briefly, the mean number of liveborn kits per breeding female was dramatically reduced for mink exposed to ANS crude oil (57% reduction) and bunker C fuel oil (87% reduction) through their diets, compared with values for control mink (mean, 5.3 liveborn kits/litter). However, the mean number of liveborn kits per breeding female for mink exposed externally was not different from that of the control mink.

Analysis of the logistic model indicated that reproductive performance can be explained by oil-exposure status and creatinine concentrations measured during the acute phase of exposure. This information may be useful if wildlife personnel are faced with another environmental catastrophe, such as the incident involving the *Exxon Valdez*. When confronted with more injured wildlife than can be cared for immediately, it may be scientifically justifiable to euthanatize those animals that have severe clinical signs and high creatinine concentrations in their serum, especially on the basis that creatinine concentration is associated with both acute exposure and reproductive performance. However, the study reported here does not provide evidence that animals with high creatinine concentrations may not recover and produce offspring in future years. Therefore, caution must be used when basing decisions for euthanatizing animals solely on the evaluation of clinical biochemical data.

Exposure status and LDH activity were predictive of reproductive success 6 months after a single acute exposure to petroleum products or initiation of chronic low-level dietary exposure. The selection of LDH values by the stepwise procedure was not surprising given that LDH was the only clinical biochemical variable altered in both externally and diet-exposed mink for the sample collected after weaning. Therefore, exposure status and LDH activity may be useful in predicting which sea otters will reproduce in the year following an oil spill and should be considered when

evaluating an animal for release from an oil spill rehabilitation facility; an animal that has truly recovered would be expected to have serum LDH activity and values for other hepatic enzymes within reference ranges at the time of release.

[a]Blake J, White R, Sousa M, et al. Influence of oil hydrocarbons on the reproduction of mink (abstr), in *Proceedings*. 42nd Annu Conf Wildl Dis Assoc 1993;45.

[b]VetStat, BioMedware, Davis, Calif.

[c]Vacutainer, Becton Dickinson, Rutherford, NJ.

[d]BMDP/Dynamic Release 7.0, BMDP Statistical Software, Los Angeles, Calif.

[e]Mazet JA, Jessup D, Gardner IA, et al. Reproductive effects of petroleum product exposure on American mink (*Mustela vison*) as a laboratory model for sea otters (*Enhydra lutris*)(abstr), in *Proceedings*. Joint Conf Am Assoc Zoo Vet Wildl Dis Assoc Am Assoc Wildl Vet 1995;41.

References

1. Wren CD. Cause-effect linkages between chemicals and populations of mink (*Mustela vison*) and otter (*Lutra canadensis*) in the Great Lakes Basin. *J Toxicol Environ Health* 1991;33:549–585.

2. Hartung R. Assessment of the potential for long-term toxicological effects of the *Exxon Valdez* oil spill on birds and mammals. In: Wells PG, Butler JN, Hughes JS, eds. *Exxon Valdez oil spill: fate and effects in Alaskan sea waters. ASTM STP 219*. Philadelphia: American Society for Testing and Materials, 1995;693–725.

3. Leighton FA. The systemic toxicity of Prudhoe Bay crude and other petroleum oils to CD-1 mice. *Arch Environ Contam Toxicol* 1990;19:257–262.

4. Boehm PD, Mankiewicz PJ, Hartung R, et al. Characterization of mussel beds with residual oil and the risk to foraging wildlife 4 years after the *Exxon Valdez* oil spill. *Environ Toxicol Chem* 1996;15:1289–1303.

5. Ringer RK, Hornshaw TC, Aulerich RJ. Mammalian wildlife (mink and ferret) reproduction tests. In: *Mammalian wildlife (mink and ferret) toxicity tests protocols*. Corvallis, Ore: US Environmental Protection Agency–National Oceanic and Atmospheric Administration, 1992;23–49.

6. Bennett PM, Gascoyne SC, Hart MG, et al. Development of LYNX: a computer application for disease diagnosis and health monitoring in wild mammals, birds, and reptiles. *Vet Rec* 1991;128: 496–499.

7. Estes JA. Catastrophes and conservation: lessons from sea otters and the *Exxon Valdez*. *Science* 1991;254:1596.

8. Jain NC. Normal values in blood of laboratory, fur-bearing, and miscellaneous zoo, domestic, and wild animals. *Schalm's veterinary hematology*. 4th ed. Philadelphia: Lea & Febiger, 1986;315–318.

9. Weiss DJ, Wustenberg W, Bucci TJ, et al. Hematologic and serum chemistry reference values for adult brown mink. *J Wildl Dis* 1994;30:599–602.

10. Ryland LM, Bernard SL, Gorham JR. A clinical guide to the pet ferret. *Compend Contin Educ Pract Vet* 1983;5:25–32.

11. Jain NC. Comparative hematology of common domestic animals. *Essentials of veterinary hematology*. Philadelphia: Lea & Febiger, 1993;36–37, 42–44.

12. Duncan JR, Prasse KW, Mahaffey EA. Leukocytes. *Veterinary laboratory medicine: clinical pathology*. 3rd ed. Ames, Iowa: Iowa State University Press, 1994;37–62.

13. Willard MD. Electrolyte and acid-base abnormalities. In: Willard MD, Tvedten H, Turnwald GH, eds. *Small animal clinical diagnosis by laboratory methods*. Philadelphia: WB Saunders Co, 1989;103–120.

14. Greene CE, Braund KG. Diseases of the brain. In: Ettinger SJ, ed. *Textbook of veterinary internal medicine*. 3rd ed. Philadelphia: WB Saunders Co, 1989;618.

15. Duncan JR, Prasse KW, Mahaffey EA. Liver. In: *Veterinary laboratory medicine: clinical pathology*. 3rd ed. Ames, Iowa: Iowa State University Press, 1994;130–151.

16. Willard MD. Gastrointestinal, pancreatic, and hepatic disorders. In: Willard MD, Tvedten H, Turnwald GH, eds. *Small animal clinical diagnosis by laboratory methods*. Philadelphia: WB Saunders Co, 1989;211–221.

17. Rebar AH, Lipscomb TP, Harris RK, et al. Clinical and clinical laboratory correlates in sea otters dying unexpectedly in rehabilitation centers following the *Exxon Valdez* oil spill. *Vet Pathol* 1995;32:346–350.

18. Frost KJ. Marine mammals study number 5: assessment of injury to harbor seals in Prince William Sound, Alaska and adjacent areas. *State-federal natural resource damage assessment for April – December 1989*. Fairbanks, Alaska: Alaska Department of Fish and Game, 1990;1–22.

19. Duffy LK, Bowyer RT, Testa JW, et al. Chronic effects of the *Exxon Valdez* oil spill on blood and enzyme chemistry of river otters. *Environ Toxic Chem* 1994;13:643–647.