CLINICAL FINDINGS IN KIT FOXES AND DEER MICE FROM AN OIL FIELD ENVIRONMENT

KRISTIN G. CHARLTON,[1] School of Veterinary Medicine, University of California, Davis, California 95616

DAVID W. HIRD, School of Veterinary Medicine, University of California, Davis, California 95616

STEVEN M. GRIFFEY, School of Veterinary Medicine, University of California, Davis, California 95616

JOSEPH G. ZINKL, School of Veterinary Medicine, University of California, Davis, California 95616

LINDA K. SPIEGEL, California Energy Commission, 1516 Ninth Street, MS-40, Sacramento, CA 95614

ABSTRACT: Oil fields in the southern San Joaquin Valley of California have been identified as important habitat for the federally-designated endangered San Joaquin kit fox (*Vulpes macrotis mutica*). However, the potential effects of oil field-related contaminants on kit foxes have not been evaluated. From 1992 to 1993 we collected blood samples from kit foxes inhabiting the Midway-Sunset oil field and from kit foxes inhabiting a reference site, the undeveloped Lokern Natural Area (Kern County, California), for hematology and serum chemistry analysis. We also collected tissue samples from deer mice (*Peromyscus maniculatus*), one of their prey species, for histologic analysis. A significantly higher proportion of kit foxes from the oil field had circulating immature red blood cells than foxes from the undeveloped reference area. A significantly higher proportion of deer mice from the oil field exhibited extramedullary hematopoiesis and adrenocortical vacuolation than deer mice inhabiting the undeveloped reference area. We conclude that kit foxes and deer mice inhabiting oil fields may be exposed to conditions that lead to potentially pathologic changes, although other factors unrelated to the oil field environment must also be considered. The potential detrimental effects of oil field exposure on terrestrial wildlife should be considered in the recovery planning process given the importance of oil fields as habitat for the endangered San Joaquin kit fox.

Key words: San Joaquin kit fox, *Vulpes macrotis mutica*, deer mice, *Peromyscus maniculatus*, oil fields, endangered species, histology, hematology, serum chemistry

2001 TRANSACTIONS OF THE WESTERN SECTION OF THE WILDLIFE SOCIETY 37:61-65

The San Joaquin kit fox (*Vulpes macrotis mutica*) has been federally designated as an endangered species since 1966 (Federal Register 32:4001). It is found primarily in the southern San Joaquin Valley of California where loss of habitat to agricultural, urban, and industrial development has contributed to its endangered status. However, unlike intensive agricultural or urban developments, petroleum development often retains enough suitable habitat to support kit fox populations (California Energy Commission 1996). Several petroleum production areas have been identified as important kit fox habitat, and evaluating the effects of petroleum-related contaminants in kit foxes has been identified as a research goal of San Joaquin kit fox recovery (United States Fish and Wildlife Service 1989).

Crude oil contains many organic and inorganic components that are known to be toxic to animals. In addition, several toxic compounds are used in the petroleum extraction process (Peakall et al. 1982, Seiler et al. 1988, Edwards 1991). Potential routes of exposure to oil field contaminants include inhalation of volatile substances or soil particulates, consumption of wastewater, incidental soil ingestion, ingestion of plants, prey, or debris that may contain toxic substances, and dermal contact. The fact that both kit foxes and deer mice are burrowing animals with intimate contact with soil may make them more susceptible to oil field contaminants.

Changes in serum chemistry and hematology values have been reported in several wildlife species after acute or chronic exposure to crude oil (Engelhardt 1981, Leighton et al. 1983, Williams 1990). Exposure to crude oil has also been associated with the histologic lesions in wildlife (Peakall et al. 1980, Engelhardt 1983, Fry and Lowenstine 1985, Lipscomb et al. 1993). However, most reports are of marine mammals or birds that were either victims of an oil spill or were otherwise acutely exposed to crude oil. Hazards of oil field wastes to domestic livestock have been described (Edwards 1989), but little information is available on the effects of low-level chronic exposure of terrestrial wildlife to oil field pollutants (McBee and Bickman 1988).

The purpose of this study was to identify potential pathologic changes in kit foxes and one of their prey species, deer mice (*Peromyscus maniculatus*), which may

[1]Present address: 8352 McCourtney Rd., Lincoln, CA 95648 USA, email: kgcharlton@aol.com

be related to inhabiting an oil field environment. The specific objectives were to determine whether differences in kit fox hematology, kit fox serum chemistry, and deer mouse histology could be detected between populations inhabiting an oil field and those inhabiting an undeveloped reference area.

STUDY AREA

The Midway-Sunset oil field is located approximately 9 km north of Taft, California (Kern County; 35°08'N, 119°27'W). Surface disturbance of the approximately 20-km² study area was >70%. Disturbance consisted of oil pump platforms, power generation facilities, pipe and steam lines, graded areas and cut banks, and other oil-related structures and roads. There was an average of 243 oil pumps per km². Kit fox density was estimated to be 0.6 foxes/km². The undeveloped reference area was approximately 32 km² and located within the Lokern Natural Area, approximately 5 km east of Buttonwillow, California (Kern County; 35°24'N, 119°28'W). Surface disturbance was limited to unpaved, infrequently used roads and the California Aqueduct along the northern border. Kit fox density was estimated to be 1.2 foxes/km². Vegetation at both sites consisted mainly of saltbush scrub (*Atriplex* spp.) and non-native annual grasses (*Bromus* spp.) (California Energy Commission 1996).

METHODS

Blood was collected from unanesthetized adult foxes that were live-trapped in box traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) for radiotelemetry studies from May 1992 to January 1993. Animals were removed from traps and sampled within 1-2 hours of sunrise at both sites. Samples were collected from 10 foxes (5 males, 5 females) inhabiting the oil field and from 11 foxes (6 males, 5 females) inhabiting the reference area. Approximately 10 ml of blood were obtained from each fox via jugular venipuncture and mailed overnight to a commercial veterinary diagnostic laboratory (Consolidated Veterinary Diagnostics, Sacramento, California, USA) for hematology and serum chemistry analysis.

Hematological variables measured were red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, and white blood cell differential count. RBC morphology was also recorded. Serum chemistry variables measured were alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatinine kinase, total protein, albumin, globulin, total bilirubin, indirect bilirubin, blood urea nitrogen, creatinine, cholesterol, glucose, calcium, phosphorous, bicarbonate, chloride, potassium, and sodium. Laboratory personnel performing the hematology and serum chemistry analyses did not know whether foxes were from the oil field or the reference area. Kit fox hematology and serum chemistry results were analyzed using the Mann-Whitney test and Fisher's exact test (Zar 1984). A P-value of ≤ 0.05 was considered significant for both tests.

Twelve adult deer mice (4 males, 8 females) from the oil field and 8 adult deer mice (2 males, 6 females) from the reference area were live-trapped (H. B. Sherman Traps, Tallahassee, Florida, USA) in September 1992. Captured deer mice were transported to nearby laboratory facilities where they were euthanized via cervical dislocation within 24 hours of capture. Livers, spleens, kidneys, adrenal glands, hearts, lungs, and brains were collected at the time of death and fixed in 10% phosphate-buffered formalin. Fixed tissues were routinely processed, embedded in paraffin, cut into 5μm thick sections, and stained with Mayer's hematoxylin and eosin. Tissues were examined by a veterinary pathologist who was unaware of where the animals were collected. Prevalence of histologic lesions in the 2 populations were compared using the 2-tailed Fisher's exact test (Zar 1984) with a P-value of ≤ 0.05 considered significant. Prevalence was defined as the number of individuals with a specific lesion or abnormality divided by the total number of those individuals examined. Blood samples were not collected from deer mice.

RESULTS

Five of 10 (3 male, 2 female) kit foxes from the oil field had polychromatic RBCs and an additional kit fox had circulating nucleated RBCs. A single kit fox (1/11) from the reference area was reported to have polychromatic RBCs. Significantly more foxes from the oil field had polychromatic or nucleated circulating RBCs ($P = 0.021$). No significant differences were detected between the two populations for any remaining hematological or serum chemistry values.

No gross lesions were noted in any of the deer mice at necropsy. Extramedullary hematopoiesis (EMH) was found in 6/12 (2 male, 4 female) deer mice from the oil field and in 0/8 deer mice from the reference area. Of the 6 deer mice in which EMH was present, 2 exhibited EMH in the liver only, 1 in the spleen only, and 3 in the liver, spleen, and mesenteric fat. The degree of EMH was slight to moderate when present. The prevalence of EMH was significantly higher in deer mice inhabiting the oil field ($P = 0.024$).

Cortical vacuolation of the adrenal gland was seen in 5/9 (2 male, 3 female) mice from the oil field and in 0/6 mice from the natural area. Adrenocortical vacuolation involved only the *zona fasciculata* in 3/5 deer mice and both the *zona fasciculata* and the *zona reticularis* in 2/5

deer mice. The prevalence of adrenocortical vacuolation was significantly higher in deer mice inhabiting the oil field ($P = 0.042$).

Several additional incidental histologic findings were seen in mice from both populations. Mild to severe centrilobular to generalized vacuolation of hepatocytes was present in 11/12 deer mice from the oil field and 6/8 deer mice from the natural area. These vacuoles were characterized by irregular borders and fine cytoplasmic septae. Pulmonary emphysema was noted in 9/11 deer mice from the oil field and 5/8 deer mice from the natural area. Lymphoid hyperplasia of the spleen was noted in 10/11 deer mice from the oil field and 5/8 deer mice from the natural area. None of these differences were statistically significant ($P > 0.05$).

DISCUSSION

Polychromatic or nucleated RBCs were present in >50% of the foxes from the oil field and <10% of the foxes from the reference area. Polychromasia is usually characteristic of immature RBCs released prematurely from the bone marrow into the peripheral circulation in response to tissue hypoxia. In extreme cases of tissue hypoxia, nucleated RBCs may also be released. This occurs as a result of decreased production or increased destruction or loss of RBCs (Meyer and Harvey 1998).

Extramedullary hematopoiesis (EMH) was present in a significantly higher proportion of deer mice inhabiting the oil field. While 50% of deer mice from the oil field exhibited EMH, no deer mice from the natural area exhibited EMH. In most mammalian species, EHM occurs when normal bone marrow hematopoiesis cannot produce enough RBCs to meet the animal's oxygen transport demands (Cotran et al. 1994). Impaired hemoglobin or enzyme function can also result in failed oxygen transport and subsequent hypoxia (Cotran et al. 1994). In response to tissue hypoxia, EMH produces more RBCs and thus provides adequate oxygen delivery to tissues.

In laboratory mice (*Mus musculus*) and to a lesser degree in laboratory rats (*Rattus rattus*), splenic hematopoiesis is considered normal (Percy and Barthold 1993). It is not known whether EMH is normal in deer mice. However, given the significantly higher proportion of oil field-exposed deer mice that exhibited EMH in this study, these findings suggest that the EMH may be related to the oil field environment.

Possible explanations for the apparent relationship between inhabiting the oil field and development of evidence of tissue hypoxia include Heinz body hemolytic anemia, exposure to heavy metals, or as yet unidentified factors. Acute exposure to crude oil is known to cause oxidation and denaturation of hemoglobin in the RBCs of marine birds (Leighton et al. 1983). The denatured hemoglobin forms Heinz bodies on the RBCs and anemia results when these damaged cells are removed from circulation by the spleen (Cotran et al. 1994). Birds suffering from Heinz body anemia exhibited increased hematopoiesis in their bone marrow, however, no EMH was reported (Leighton 1986). Anemia has been reported in sea otters and polar bears acutely exposed to crude oil, but no evidence of EMH was reported in these studies (Williams 1990, Engelhardt 1981).

Since hematology was not performed on deer mice, and histology was not performed on kit foxes, it was not possible to determine whether there may have been a link between the observed abnormal RBC morphology in kit foxes and the EMH in deer mice in the present study. However, the statistically significant findings of immature RBCs in kit foxes and EMH in deer mice from the oil field suggest the possibility that these animals may be exposed to conditions that may cause or induce tissue hypoxia.

Certain trace metals (zinc, cadmium, copper, lead) are known to inhibit RBC production (Thompson et al. 1977, Davis and Avram 1978). Tissue metal concentrations were not determined for deer mice in this study. However, they were determined for kit foxes inhabiting the same area and were not found to be elevated (Charlton et al. 2001).

Adrenocortical changes resembling those observed histologically in deer mice from the oil field have been chemically induced in laboratory rodents. The mechanism of action in experimental animals is believed to be via inhibition of a hydroxylation step in the conversion of cholesterol to cortisol and other steroid hormones. When hydroxylation of cholesterol is inhibited, it accumulates in the cells of the adrenal cortex resulting in cortical vacuolar degeneration (Yarrington 1983). We did not attempt to characterize the staining characteristics or ultrastructural features of the adrenocortical vacuoles in this study, so where unable to identify the contents.

Of the 6 oil field-exposed deer mice in which EMH was present, and of the 5 in which adrenocortical vacuolation was present, 4 deer mice exhibited both of these lesions. This suggests that exposure to a common agent may be responsible for the development of these 2 findings.

It is not clear whether these observations represent markers of exposure, an adaptive response to the environment, markers of an adverse effect with secondary consequences, or incidental findings. Likewise, the significance of these findings to the animals, either on an individual or population basis, is not known. Outcome variables such as survival and reproductive success did not appear to differ between the two kit fox populations during this study period. While overall abundance of

both kit foxes and deer mice was less in the oil-field exposed animals, this was attributed to lower carrying capacity as a result of habitat reduction and fragmentation caused by oilfield-related construction and maintenance activities (California Energy Commission 1996).

The proposed links between the pathological findings in the 2 species reported here are tenuous. Before firm conclusions can be made regarding the relationship between inhabiting the oil field and the development of clinical changes, the limitations of this study need to be fully acknowledged. Many unknown variables other than the exposure to the oil field environment, such as genetic, hormonal, nutritional, and infectious disease factors, could differ between the 2 groups of animals. The mechanisms responsible for our observations are likely multifactorial and, without a more controlled study, cannot be fully elucidated. While the exact mechanisms may not be clearly defined, we found that kit foxes and deer mice inhabiting the oil field in this study exhibited potential pathologic changes not seen in animals from an undeveloped reference area. These preliminary findings warrant further investigation, especially considering the importance of oil fields as habitat for the endangered San Joaquin kit fox.

ACKNOWLEDGEMENTS

This research was funded by the California Energy Commission. Trapping, handling, and sampling of deer mice were conducted in accordance with California Department of Fish and Game Scientific Collector's Permit number 7082 and the Protocol for Animal Use and Care at the University of California, Davis. Kit foxes were trapped, handled, and sampled in accordance with United States Fish and Wildlife permit number SPIELK-4. We thank Tony Dao, Michelle Disney, Michael Small, and Judy Thom for trapping and sampling foxes. Bob Stafford assisted with collecting samples from deer mice. Dr. Harvey Olander, Dr. Dan Anderson, and Dr. Walter Boyce provided encouragement and advice during the early stages of this study.

LITERATURE CITED

California Energy Commission. 1996. Studies of the San Joaquin kit fox in undeveloped and oil-developed areas. Sacramento, California.

Charlton, K. G., D.W. Hird, and L. K. Spiegel. 2001. Trace metal concentrations in kit foxes from the southern San Joaquin Valley of California. California Fish and Game. 87:45-90..

Cotran, R. S., V. Kumar, and S. L. Robbins. 1994. Robbins Pathologic Basis of Disease. Fifth edition. W.B. Saunders Company, Philadelphia, Pennsylvania.

Davis, J. R. and M. J. Avram. 1978. A comparison of the stimulatory effects of cadmium and zinc on normal and lead-inhibited human erythrocytic aminolevulinc acid dehydratase activity in vitro. Toxicology and Applied Pharmacology 44:181-190.

Edwards, W. C. 1989. Toxicology of oilfield wastes: hazards to livestock associated with the petroleum industry. Pages 363-374 *in* The Veterinary Clinics of North America: Clinical Toxicology. Vol. 5, No. 2. W.B. Saunders Company, Philadelphia, Pennsylvania.

Edwards, W. C. and D. G. Gregory. 1991. Livestock poisoning from oil field drilling fluids, muds, and additives. Veterinary and Human Toxicology 33:502-504

Engelhardt, F. R. 1981. Oil pollution in polar bears: exposure and clinical effects. Pages 139-179 *in* Proceedings of the Fourth Annual Marine Oil Spill Program Technical Seminar, Edmonton, Alberta.

Engelhardt, F. R. 1983. Petroleum effects on marine mammals. Aquatic Toxicology 4:199-217.

Fry, D. M. and L. J. Lowenstine. 1985. Pathology of common murres and Cassin's auklets exposed to oil. Archives of Environmental Contamination and Toxicology 14:725-737.

Leighton, F. A., D. B. Peakall, and R. G. Butler. 1983. Heinz-body hemolytic anemia from the ingestion of crude oil: a primary toxic effect in marine birds. Science 220:871-873.

Leighton, F. A. 1986. Morphological lesions in RBCs from herring gulls and Atlantic puffins ingesting Prudhoe Bay crude oil. Veterinary Pathology 22:393-402.

Lipscomb, T. P., R. K. Harris, R. B. Moeller, J. M. Pletcher, R. J. Haebler, and B. E. Ballachey. 1993. Histopathologic lesions in sea otters exposed to crude oil. Veterinary Pathology 30:1-11.

McBee, K. and J. W. Bickham. 1988. Petrochemical-related DNA damage in wild rodents detected by flow cytometry. Bulletin of Environmental Contamination and Toxicology 40:343-349.

Meyer, D. J. and J. W. Harvey. 1998. Veterinary laboratory medicine. Second edition. W. B. Saunders Company, Philadelphia, Pennsylvania.

Peakall, D. B., D. J. Hallet, D. S. Miller, R. G. Butler, and W. B. Kinter. 1980. Effects of ingested crude oil on black guillemots: a combined field and laboratory study. Ambio 9:28-30.

Peakall, D. B., D. J. Hallet, J. R. Bend, G. L. Foureman, and D. S. Miller. 1982. Toxicity of Prudhoe Bay crude oil and its aromatic fractions to nestling herring gulls. Environmental Research 27:206-215.

Percy, D. H. and S. W. Barthold. 1993. Pathology of laboratory rodents and rabbits. Iowa State University Press, Ames, Iowa.

Seiler, H. G., H. Sigel, and A. Sigel. 1988. Handbook on toxicity of inorganic compounds. Marcel Dekkar, Inc., New York, New York.

Thompson, J., D. Jones, and W. H. Beasley. 1977. The effect of metal ions on the activity of aminolevulinc acid dehydratase. British Journal of Industrial Medicine 34:32-36.

United States Fish and Wildlife Service. 1989. San Joaquin kit fox recovery plan. Portland, Oregon.

Williams, T. M. 1990. Evaluating the long term effects of crude oil exposure in sea otters: laboratory and field observations. Wildlife Journal 13:42-48.

Yarrington, J. T. 1983. Chemically induced adrenocortical lesions. Pages 69-74 *in* T.C. Jones, U. Mohr, and R.D. Hunt, editors. Endocrine system. Springer-Verlag, New York, New York.

Zar, J. H. 1984. Biostatistical analysis. Second Edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.