This is to certify that the

thesis entitled

The Impact of Naturally Weathered North
Slope Crude Oil on Reproduction and Survival
in Mallards

presented by

Gregg Allan Hancock

has been accepted towards fulfillment
of the requirements for
Natural Science MS degree in Fisheries & Wildlife

Harold H. Prince
Major professor

Date July 20, 1992

THE IMPACT OF NATURALLY WEATHERED NORTH SLOPE CRUDE OIL ON REPRODUCTION AND SURVIVAL IN MALLARDS

By

Gregg Allan Hancock

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1991

ABSTRACT

THE IMPACT OF NATURALLY WEATHERED NORTH SLOPE CRUDE OIL ON REPRODUCTION AND SURVIVAL IN MALLARDS

By

Gregg Allan Hancock

A series of toxicological tests were conducted to assess the impact of ingestion of naturally weathered North Slope crude oil (WNSCO,) from the Exxon Valdez accident, on survival and reproduction in mallards. The studies performed were a LD_{50}, a LC_{50}, a food avoidance, a 14-day dietary feeding study, a 22-week one-generation reproduction study and an eggshell application study. No outward signs of toxicity were noted in any of the birds in any study. The LD_{50} was > 5,000 mg WNSCO/kg body weight, the LC_{50} was > 50,000 ppm in the diet and the median food avoidance concentration (FAC_{50}) was > 20,000 ppm in the diet. The only effects noted in birds fed up to 100,000 ppm WNSCO in the diet in the 14-day dietary feeding study were significantly ($P \leq 0.05$) increased livers in males when normalized on the basis of body and brain weights. Hens in the high dose (20,000 ppm) group in the reproduction study laid eggs with significantly reduced ($P \leq 0.05$) shell thickness and strength. Application of WNSCO to the eggshell showed no toxicity related affects on developing embryos. WNSCO does not appear to be a highly toxic compound and outward physical contact should be of more concern than ingestion.

ACKNOWLEDGEMENTS

I would like to thank Dr. Harold Prince, Dr. Robert Ringer and Dr. Scott Winterstein for serving on my committee. Thanks especially to Dr. Prince for serving as my major professor and giving me guidance and advise and to Dr. Ringer for all the extra time he took to help me on the project.

I would like to especially thank Cathy Cook for all her fantastic help on my project as well as in my classes. Her encouragement and guidance were above and beyond the call of duty and I am deeply in her debt.

Thanks also go to Sara Thompson, whose hard work and dedication was greatly appreciated and will not be forgotten.

Finally, I would like to thank my wife Tammi for putting up with me over the last 3 years. I know it has not been easy but I hope it has been worth it. Thank you from the bottom of my heart and remember that I love you more than anything.

TABLE OF CONTENTS

LIST OF TABLES v

INTRODUCTION 1

MATERIALS AND METHODS 2

Acute Oral LD50 2

Subacute Dietary LC50 3

Food Avoidance 4

14-day Dietary Feeding 5

Reproduction Study 6

Eggshell Application Study 9

RESULTS AND DISCUSSION 10

Acute Oral LD50 10

Subacute Dietary LC50 12

Food Avoidance 14

14-day Dietary Feeding 14

Reproduction Study 17

Eggshell Application Study 31

CONCLUSION 35

APPENDIX 39

LIST OF REFERENCES 79

LIST OF TABLES

Table 1. Survival, feed consumption and body weight ($\bar{x}$ ± S.E.) in male and female mallards orally dosed with WNSCO. 11

Table 2. Survival, feed consumption and growth rate ($\bar{x}$ ± S.E.) in mallard ducklings fed WNSCO in the diet for 5 days. 13

Table 3. Mean daily feed consumption and growth rate ($\bar{x}$ ± S.E.) of mallard ducklings given a choice of food treated with WNSCO and untreated feed in the diet for 5 days. 15

Table 4. Mean daily feed consumption, body weight and organ parameters ($\bar{x}$ ± S.E.) in adult mallards fed WNSCO in the diet for 14 days. 16

Table 5. Mean daily feed consumption and body weights ($\bar{x}$ ± S.E.) in mallards fed WNSCO in the diet for 22 weeks. 18

Table 6. Egg production, percent fertility, percent hatchability and percent survival of ducklings from eggs laid by female mallards fed WNSCO in the diet for 22 weeks. 23

Table 7. Mean eggshell strength and thickness ($\bar{x}$ ± S.E.) in eggs laid by hen mallards fed WNSCO in the diet for 22 weeks. 25

Table 8. Mean initial body weight and growth rate ($\bar{x}$ ± S.E.) of hatchlings of hen mallards fed WNSCO in the diet for 22 weeks. 26

Table 9. Mean organ weights (g) in mallards fed WNSCO in the diet for 22 weeks in a reproduction study ($\bar{x}$ ± S.E.). 28

Table 10. Mean clinical blood parameters ($\bar{x}$ ± S.E.) in adult mallards fed WNSCO in the diet for 22 weeks. 29

Table 11. Number hatched, number 14-day survivors and hatchling growth rate (g/bird/day) from mallard eggs externally treated with various amounts of WNSCO. 32

Table 12. Mortalities observed in adult mallards orally dosed with weathered North Slope crude oil. 39

Table 13. Body weights (g) of adult mallards orally dosed with weathered North Slope crude oil (mean ± S.E.). 40

Table 14. Food consumption of adult mallards orally dosed with weathered North Slope crude oil (mean ± S.E.). 41

Table 15. Mortalities observed in mallard ducklings fed weathered North Slope crude oil in the diet for 5 days. 42

Table 16. Food consumption of mallard ducklings fed weathered North Slope crude oil in the diet for 5 days (mean ± S.E.). 43

Table 17. Body weights (g) of mallard ducklings fed weathered North Slope crude oil in the diet for 5 days (mean ± S.E.). 44

Table 18. Mortalities observed in mallard ducklings given a choice of food treated with weathered North Slope crude oil and untreated food in the diet for 5 days. 45

Table 19. Feed consumption (g) in mallard ducklings given a choice between feed treated with weathered North Slope crude oil and untreated feed in the diet for 5 days (mean ± S.E.). 46

Table 20. Body weights (g) of mallard ducklings given the choice of feed treated with weathered North Slope crude oil or untreated feed in the diet for 5 days (mean S.E.). 47

Table 21. Mortalities observed in adult mallards fed weathered North Slope crude oil in the diet for 14 days. 48

Table 22. Mean daily feed consumption (g) in adult mallards fed weathered North Slope crude oil in the diet for 14 days (mean ± S.E.). 49

Table 23. Body weights (g) of adult mallards fed weathered North Slope crude oil in the diet for 14 days (mean ± S.E.). 50

Table 24. Hematology results of adult mallards fed weathered North Slope crude oil in the diet for 14 days (mean ± S.E.). 51

Table 25. Clinical chemistry results of adult mallards fed weathered North Slope crude oil in the diet for 14 days (mean ± S.E.). 53

Table 26. Mortalities observed in adult mallards fed weathered North Slope crude oil in the diet for 22 weeks. 55

Table 27. Mean feed consumption (g/b/d*) by adult mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 56

Table 28. Body weights (g) by sex of adult mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 57

Table 29. Organ:brain weight ratios of adult female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 58

Table 30. Organ:brain weight ratios of adult male mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 59

Table 31. Organ:body weight ratios of adult female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 60

Table 32. Organ:body weight ratios of adult male mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 61

Table 33. Clinical chemistry results of adult female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 62

Table 34. Clinical chemistry results of adult male mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 65

Table 35. Hematology results of adult female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 68

Table 36. Hematology results of adult male mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 70

Table 37. Reproductive data from mallard hens fed weathered North Slope crude oil in the diet for 22 weeks. 72

Table 38. Reproductive data from mallard hens fed weathered North Slope crude oil in the diet for 22 weeks - eggs set. 73

Table 39. Reproductive data from female mallards fed weathered North Slope crude oil in the diet for 22 weeks - number hatched. 74

Table 40. Mean eggshell strength, in Newtons (N), of eggs laid by female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 75

Table 41. Mean eggshell thickness (mm) of eggs laid by female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 76

Table 42. Mean initial and 14-day survivor body weights of mallard hatchlings of hen fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.). 77

Table 43. Mortalities observed in hatchlings of mallard hens fed weathered North Slope crude oil in the diet for 22 weeks. 78

INTRODUCTION

Although seepage of petroleum through parts of the ocean floor has occurred for thousands of years, an ever increasing consumption of petroleum products worldwide since World War II has resulted in discharge of large quantities of petroleum into the environment (Bourne 1968, Prince 1983). Oil pollution is the inevitable consequence of dependence on a largely oil-based technology. Approximately 10^{12} g of oil is released into the oceans each year from shipping alone. Petroleum pollution has been implicated as a significant factor in the demise of some seabird populations during the 20th century (Holmes 1984).

Oil spills pose a threat to many forms of aquatic wildlife, especially birds. Bourne (1968) states that oil pollution affects birds sooner and more lethally than any other form of wildlife. Direct physical contact with oil can soil the feathers resulting in loss of insulation and buoyancy, thereby causing mortality. Ingestion of contaminated food and water, as well as from preening oil covered feathers has been shown to affect metabolic rates, reproductive success, and survivability under stress. Incubating adults that have been exposed to oil may transfer it to the egg shells which can result in reduced hatchability from embryo mortality due to toxicity or from suffocation due to blockage of pores.

Shortly after midnight on 24 March 1989 the oil tanker Exxon Valdez, carrying 53 million gallons of crude oil, ran aground on Bligh Reef in Prince William Sound in the Gulf of Alaska. Within 8 hours of the accident an estimated 10 million gallons of Alaskan North Slope crude oil had spilled into the Sound and the slick measured 1000 feet wide by 4 miles long. Within 24 hours of the accident efforts were underway to pump the remaining 43 million gallons of crude oil off the Valdez. Equipment shortages, logistic problems and weather conditions confounded containment and clean-up. The spill was not contained, quickly spreading and soiling hundreds of miles of shore in and around the Sound. The latitude and currents in the semi-enclosed Prince William Sound accentuated the severity, resulting in reduced rates of weathering and biodegradation. Spring migration and large numbers of waterbirds and shorebirds would potentially be exposed to the spilled oil since the spill preceded spring migration. The initial impact of the oil on wildlife was directly observable on waterbirds and sea otters (*Enhydra lutris*). Concern was expressed over the effects of the spill on the fisheries of the area, so the herring and roe fishery was closed that year.

Approximately three months (1 July 1989) after the spill occurred, weathered oil lost from the Exxon Valdez was recovered by a skimmer for a series of tests on its relative toxicity.

This project was undertaken to evaluate the potential of impact of naturally weathered North Slope crude oil (WNSCO) on survival and reproduction of waterbirds using the mallard (*Anas platyrhyncos*) as the test subject. All studies were conducted under Good Laboratory Practices guidelines.

MATERIALS AND METHODS

Acute Oral LD50

The acute toxicity test (LD_{50}) is designed to determine the single oral dose level which kills fifty percent of the treatment population. Prior to test initiation, food was withheld from all test groups for 17 hours. Weathered North Slope crude oil was administered as a single oral gavage in gelatin capsules. Preliminary range-finding studies indicated no mortalities through 5000 mg/kg body weight dosages. Treatments included a male and female treatment group (5000 mg/kg body weight) and a male and female control group (0 mg/kg body wt.). Doses were administered in 5 capsules per bird and control birds received empty capsules.

Adult mallards were purchased from a commercial source (Whistling Wings, Hanover, IL). Birds were housed in 70 x 68 x 28 cm (L x W x H) galvanized steel cages (2 males or 2 females per cage) and photoperiod was set at 8:16 hr light:dark. Test subjects were randomly assigned to treatments. Birds were dosed following a 2 week acclimation period. Food (duck maintenance) and water were provided *ad libitum*. Food consumption, mortality and clinical signs of toxicity were recorded daily throughout a 14-day post-treatment observation period. Body weights were taken at initiation, day 7 and termination of the study. Gross necropsies were performed on all birds at study termination.

Body weights and food consumption were analyzed by t-test using TOXSTAT Version 3.2 software (Gulley et al. 1990).

Subacute Dietary LC50

The dietary concentration of weathered crude oil at which fifty percent of the treatment population dies (LC_{50}) was evaluated. Preliminary studies indicated no mortalities when ducklings were fed up to 20,000 ppm WNSCO mixed in the diet. Treatments included a control group given untreated food and a high dose group exposed to food containing 20,000 ppm WNSCO in the diet, consisting of 20 and 10 birds, respectively. The control group in this study was fed a diet with 2% mineral oil. A supplemental test was also conducted consisting of a new control group given untreated feed and one dose group given feed containing 50,000 ppm WNSCO, each containing 12 birds.

One-day old ducklings were purchased from a commercial source (Whistling Wings, Hanover, IL). Ducklings were housed in an animal room with a 16:8 hr light:dark photoperiod in 91 X 71 x 23 (L x W x H) cm galvanized steel brooders with a temperature gradient from approximately 36°C at the rear to room temperature (26 to 29°C) at the front of the brooder. Ducklings were acclimated to duck starter ration and cages for three days prior to test initiation and were 5 days old at test initiation. The appropriate dietary concentrations were presented for 5 days after acclimation. A test material-free diet was presented for 3 additional days after the 5 day test, prior to sacrifice. Body weights were recorded at test initiation, day 5 and at termination (day 8). Food consumption, mortality and clinical signs of toxicity were recorded daily throughout the 8 day test period. Food and water were provided *ad libitum*.

Body weights, mean growth and food consumption were analyzed by t-test using TOXSTAT Version 3.2 software (Gulley et al. 1990).

Food Avoidance

The purpose of this experiment was to determine if mallard ducklings avoided feed containing various concentrations of WNSCO. Treatment levels were set at 0, 1,250, 2,500, 5,000, 10,000 and 20,000 ppm WNSCO in the diet. Mineral oil was used as a carrier in the treatments. Each test group was given the choice of untreated food and food treated with WNSCO. Feed hoppers were rotated daily to adjust for position effect Kononen et al (1986). Food and water was provided *ad libitum*.

One-day old ducklings were purchased from a commercial source (Whistling Wings, Hanover, IL). Ducklings were housed in an animal room with a 16:8 hr. light:dark photoperiod in 91 X 71 x 23 (L x W x H) cm galvanized steel brooders with a temperature gradient from approximately 36°C at the rear to room temperature (26 to 29°C) at the front of the brooder. Ducklings were acclimated to duck starter ration and cages for three days prior to test initiation and were 5 days old at test initiation. The appropriate dietary concentrations were presented for 5 days after acclimation. A material-free diet was presented for 3 additional days after the 5 day test, prior to sacrifice. Body weights were recorded at test initiation, day 5 and at termination (day 8). Food consumption, mortality and clinical signs of toxicity were recorded daily throughout the 8 day test period.

Body weight, mean growth and food consumption were analyzed by one-way ANOVA followed by Dunnett's test or the Bonferroni t-test (for an unequal number of replicates) using TOXSTAT Version 3.2 software (Gulley et al. 1990).

14-day Dietary Feeding

This experiment evaluated the toxicity of weathered crude oil to adult mallards during a 14-day exposure. These data were helpful in the determination of experimental concentrations of WNSCO in the food given to mallards in an experiment designed to evaluate the effect of WNSCO on egg production and survival of mallard ducklings. Food (duck breeder) treated with 0, 10,000, 30,000 and 100,000 ppm WNSCO was fed to 4 groups of 10 birds each (5 per sex per group). Twenty birds (5 per treatment level) were randomly chosen for evaluation of clinical blood parameters at study initiation and termination. Parameters evaluated include: red cell count, white cell count, differential count, total hemoglobin, mean cell volume, packed cell volume, mean cell hemoglobin, mean cell hemoglobin content, reticulocyte count, AST (aspartate aminotransferase), uric acid, total protein, albumin, CPK, ALT (alanine aminotransferase), alkaline phosphatase, calcium, glucose, potassium, and sodium. Upon termination of the study all surviving birds were euthanized by CO_2 asphyxiation and post mortem examinations were performed. Wet weights were taken for liver, kidney, adrenal, spleen and brain. In addition to these tissues, lung, bone marrow, eye, skin, salt gland, heart, skeletal muscle, gastrointestinal tract, reproductive organs and all gross lesions were taken for histopathological evaluation.

Adult mallards were purchased from a commercial source (Whistling Wings, Hanover, IL) and housed in an animal room with an 8:16 hour light:dark cycle in 70 x 68 x 28 cm (L x W x H) galvanized steel cages containing 1 male and 1 female. There was a 2 week acclimation period prior to test administration. Food and water were provided *ad libitum*. Food consumption was recorded every other day and body weights were recorded at initiation, day 7 and termination of the study. Mortality and clinical signs were recorded daily during the 14-day exposure period. Photoperiod was set at 8:16 hours light:dark.

Body weight, organ weight, organ:brain weight ratios, organ:body weight ratios, food consumption, and blood chemistry parameters and hematology were analyzed by one-way analysis of variance followed by Dunnett's test or a Bonferroni t-test. Data that did not meet assumptions of homogeneity of variance or normality were transformed and analyzed as above. Data that did not meet these assumptions through transformation were analyzed by the Kruskal-Wallis test followed by Dunn's multiple comparison. All analyses were done using TOXSTAT Version 3.2 software (Gulley et al. 1990).

Reproduction Study

This study assessed the impact of an extended dietary exposure of mallards on egg production, fertility, oviposition, hatchability, eggshell thickness and strength, and survivability of ducklings. Clinical blood parameters of adult birds were evaluated as indicators of any physiological changes resulting from the oil consumption.

One-hundred twenty mallards at least 16-weeks old were obtained from a commercial source (Whistling Wings, Hanover, IL) and housed in 70 x 68 x 28 cm (L x W x H) galvanized steel cages. There was a 2 week acclimation period prior to test initiation. Photoperiod was set at 7:17 hr. light:dark for the first eight weeks of the study and shifted to 17:7 hr light:dark for the remainder of the study to sexually stimulate the birds. Treatment levels of WNSCO in duck breeder-layer were set at 0, 200, 2,000 and 20,000 ppm based on the results from the 14-day dietary feeding study. As up to 100,000 ppm was readily consumed in the 14 day study without apparent adverse affects the upper limit for this study was set at 20,000 ppm as any higher concentration might have resulted in caloric imbalances. Food and water were provided *ad libitum*. Body weights were recorded at weeks 0, 2, 4, 6, 8 (prior to egg production) and at termination of the study. Forty birds (10 per treatment level, 5 per sex) were randomly chosen for evaluation of clinical blood parameters at study initiation, weeks 5 and 10 (prior to egg production), and at study termination (at the end of the ten week egg production period). Blood was drawn from the area of the brachial vein. Parameters evaluated included: red cell count, white cell count, differential count, total hemoglobin, mean cell volume, packed cell volume, mean cell hemoglobin, mean cell hemoglobin content, reticulocyte count AST (aspartate aminotransferase), uric acid, total protein, albumin, CPK, ALT (alanine aminotransferase), alkaline phosphatase, calcium, glucose, potassium, sodium and total bilirubin.

When at least 50% of the hens in the control group (0 ppm) had begun to lay eggs, the 10 week period of egg production was begun. Eggs were collected daily and stored at 50°F until set. All eggs were inspected for cracks prior to incubation. Eggs were set in a Petersime incubator at 37.5°C and 60 to 70% humidity. Eggs were misted with distilled water daily and candled on days 14 and 21 of incubation. Eggs were transferred from the incubator to a Petersime hatcher, set at 37.5°C and 70 to 80% humidity, on day 24 of incubation.

Eggshell thickness and strength measurements were made on eggs laid the first day of weeks 1, 3, 5, 7 and 9 of the experiment. Eggshell strength was measured on an Instron Testing Machine (Model No. 4202). Force was applied to the egg until the shell fractured. Egg shell thickness (including the shell membrane) was then measured with a micrometer at 4 points around the circumference of the shell after the contents of the shell had been rinsed out with tap water and the shell dried for at least 24 hours at room temperature.

Ducklings were housed in 91 x 71 x 23 (L x W x H) cm galvanized steel brooders (Petersime Brood Unit, Model No. 2.S.D.). with a temperature gradient from approximately 36 to 22°C for 14 days. Photoperiod was set on a 14:10 hour light:dark cycle. Food (duck starter) and water were provided *ad libitum* and health checks were made daily. At the end of the two week observation period all surviving ducklings were euthanized by CO_2 asphyxiation.

All surviving adult birds were euthanized by CO_2 asphyxiation upon termination of the study and post mortem examinations were performed. Wet weights were taken for liver, kidney, adrenal, spleen and brain. In addition to these tissues lung, bone marrow, eye, skin, salt gland, heart, skeletal muscle, gastrointestinal tract, reproductive organs and all gross lesions were taken and stored in 10% buffered formalin for histopathological evaluation.

Body weight, organ weight, organ:brain/body weight ratios, food consumption, egg shell strength and thickness, and blood clinical chemistry and hematology parameters and reproductive parameters were analyzed using the one-way analysis of variance procedure followed by a Dunnett's test or a Bonferroni t-test to test for significant differences between treatments and control. Data that did not meet assumptions of homogeneity of variance or normality were transformed and analyzed as above. Data that did not meet these assumptions through transformation were analyzed by the Kruskal-Wallis test followed by Dunn's multiple comparison. All analyses were done using TOXSTAT Version 3.2 software (Gulley et al. 1990). Analysis of percentage of hens laying eggs was conducted using the chi-square test.

Eggshell Application Study

WNSCO was applied externally to mallard egg shells and its effects on embryo mortality and duckling survival were assessed. Freshly laid eggs were ordered from a commercial supplier (Whistling Wings, Hanover, IL) and inspected for cracks upon receipt. Each egg was divided into six approximately equal application zones by using a template and marking with a lead pencil (the air sac was not included) and set in a Petersime incubator at 37.5° C and 70-80% humidity. Treatment with petrolatum or WNSCO occurred on day 7 of incubation. All dead and infertile embryos, as determined by candling, were removed at this time. Petrolatum was used as a positive control to test for gas exchange effect. Preliminary experiments using petrolatum as a pore blocker demonstrated that covering more than 1/3 of the egg resulted in almost total mortality of embryos. Based on these results no more than 1/3 of the egg was covered with weathered crude oil in this experiment. There were 4 treatment levels of petrolatum or WNSCO: coverage of the top 1/6 of the egg; the top 1/3; the middle 1/6; and the middle 1/3. The crude oil was a thick, brown mousse in consistency and appearance so it was applied with a watercolor paintbrush by covering the appropriate zones. Eggs were weighed prior to and after treatment to determine dose amount. Eggs were not candled during the course of the incubation as the oil made the eggs slippery and the possibility of spreading the oil to larger portions of the egg.

Ducklings were housed in 91 x 71 x 23 (L x W x H) cm galvanized steel brooders (Petersime Brood Unit, Model No. 2.S.D.). with a temperature gradient from approximately 36 to 22°C for 14 days to evaluate survivability. Food (duck starter) and water were provided *ad libitum*.

Initial duckling body weight and 14-day growth rate were analyzed by one-way ANOVA followed by Dunnett's test or the Bonferonni t-test (Gulley et al. 1990). Analysis of hatching success was conducted using the chi-square test.

RESULTS AND DISCUSSION

Acute Oral LD50

No mortalities or signs of toxicity were observed in any of the test groups (Table 1). Boersma et al. (1988) found no effect on gross morphology or immediate survival in fork-tailed storm-petrels (*Oceanodroma furcata*) orally dosed with artificially weathered Prudhoe Bay crude oil (PBCO). A single dose of artificially weathered South Louisiana crude oil (SLCO) resulted in reduced survival in herring gull (*Larus argentatus*) chicks (Butler and Lukasiewicz 1979). No oil related mortalities were reported in adult sandhill cranes (*Grus canadensis*) repeatedly dosed with raw PBCO, however the high dose group was observed to be weaker and more lethargic as well as maintaining a different standing and walking posture (Fleming et al. 1982). Using single oral doses of crude oil on both young herring gulls and adult black guillemots (*Cepphus grylle*), other researchers report no significant mortalities (Peakall et al. 1980, Peakall et al. 1982, Peakall et al. 1983, Peakall et al. 1985). Peakall et al. (1980) used weathered SLCO. Even multiple doses of up to 20 mg crude oil/kg bodyweight/day for as long as to 6 days does not significantly increase mortality in nestling herring gulls and Atlantic puffins (Leighton et al. 1983, Lee et al. 1985, Leighton 1985, Leighton et al. 1985).

Male mallards consumed an average of 34 g more food than females which is consistent with the 13% differential in weight between sexes (Table 1). A significant reduction of 18 g (17%) ($P \leq 0.05$) was noted for males consuming treated food. This pattern was not observed in mean daily food consumption of females. Engel et al. (1978) noted reduced food intake on day 1 following dosing in Japanese quail orally dosed with PBCO, but consumption returned to normal the following day.

Table 1. Survival, feed consumption and body weight ($\bar{x}$ ± S.E.) in male and female mallards orally dosed with WNSCO.

	Treatments (mg/kg)			
	Male		Female	
Parameter	0	5000	0	5000
Survival				
Lived	6	6	6	6
Died	0	0	0	0
Feed Consumption (g/b/d)[*]	127 ± 4.0	109[a] ± 5.6	93 ± 3.5	94 ± 2.3
Body weight (g)				
Initial	1123 ± 44	1166 ± 30	994 ± 22	1008 ± 34
Terminal	1166 ± 30	1224 ± 33	1059 ± 40	1069 ± 26

[a]Significantly different from male control ($P \leq 0.05$)
[*]g/b/d = grams per bird per day

Postmortem examination of all ducks at study termination showed no compound-related lesions. The single dose acute LD_{50} of mallard ducks orally dosed with WNSCO is greater than 5,000 mg/kg.

Subacute Dietary LC50

Consumption of duck starter treated with 20,000 and 50,000 ppm WNSCO in the diet resulted in no compound-related mortalities or grossly observable signs of toxicity in ducklings during the course of the study (Table 2). Some previous studies report no significant treatment related mortalities in young birds fed crude oil in the diet (Szaro et al. 1978b, Eastin and Murray 1981, Eastin and Rattner 1982). Rattner and Eastin (1981) reported 2 mortalities in mallard ducklings fed 1.5% PBCO, but this was not statistically significant. Pullet chicks fed 2, 4, and 6% artificially weathered Nigerian light crude oil in the diet for 42 days exhibited significant treatment related mortality (Nwokolo and Ohale 1986). Up to 5% weathered crude oil in our tests showed no observable effects on the ducklings.

Ducklings between the ages of 5 to 10 days ate 21.0 to 23.9 g of food per day. Mean food consumption between treatment and control groups was not significant ($P > 0.05$). The mean daily growth rates ranged from 15.1 grams per bird in the 50,000 ppm treatment group to 16.3 grams per bird in the control group. Postmortem examination of all ducklings at study termination showed no compound-related effects. Eastin and Murray (1981) fed mallard ducklings 2.5% Prudhoe Bay crude oil and found no significant difference in mean body weights or growth. Mallard ducklings fed SLCO up to 5% from hatching to 8 weeks showed a significantly depressed growth rate compared to controls (Szaro et al. 1978b).

Table 2. Survival, feed consumption and growth rate ($\bar{x} \pm SE$) in mallard ducklings fed WNSCO in the diet for 5 days.

	Treatment (ppm)			
Parameter	0	20,000	0	50,000
Survival				
Lived	20	10	12	12
Died	0	0	0	0
Mean Feed Consumption[a] (g/bird/day)	21.8 ± 0.9	23.9 ± 1.5	22.7 ± 1.0	21.0 ± 0.8
Growth[b] rate (g/bird/day)	16.3 ± 0.8	16.0 ± 1.2	15.8 ± 1.3	15.1 ± 1.1

[a]Day 0 to day 5
[b]Day 0 to day 8

Food Avoidance

Ducklings that were given a free choice of food treated with up to 20,000 ppm WNSCO versus non-treated food did not display a preference (Table 3). Food consumption averaged from 24.1 to 27.3 grams per bird per day. Postmortem examination of all ducklings at study termination showed no compound-related effects. Nwokala and Ohale (1986) noted a significant decrease in food consumption for pullet chicks fed 2, 3 and 6% artificially weathered crude oil in the diet. They found that the contaminated feed was initially rejected and feel that the degree of rejection was related to the level of contamination, but by the end of the second week their consumption was back to normal. Eastin and Murray (1981) noted an initial drop in weight of mallard ducklings fed 2.5% PBCO but thereafter weight gain was similar to controls. They felt this probably reflected food avoidance.

The average mean daily growth rate of 16.9 grams per duckling in this experiment was similar to the growth rates of 15.8 grams per duckling found in the LC_{50} experiment where there was no choice of food and treatment concentrations were as high or higher. No significant differences ($P > 0.05$) in growth were noted between any of the dietary exposure groups.

14-day Dietary Feeding

Mallards consumed an average of 100 grams of food per bird per day treated with as much as 10,000 ppm WNSCO (Table 4). Mean food consumption of test group and control mallards was not significantly different ($P > 0.05$). Consumption of WNSCO treated food resulted in no significant changes ($P > 0.05$) in body weight compared with controls.

Consumption of food treated with WNSCO resulted in no significant change in organ weight parameters compared with controls ($P > 0.05$) with the exception of high

Table 3. Mean daily feed consumption and growth rate ($\bar{x} \pm$ S.E.) of mallard ducklings given a choice of food treated with WNSCO and untreated food in the diet for 5 days.

	Treatment (ppm)					
Parameter	0	1,250	2,500	5,000	10,000	20,000
Feed Consumption (g)						
Treated[a]	11.9 ± 1.3	13.1 ± 1.7	12.2 ± 1.8	15.2 ± 2.0	13.7 ± 2.0	11.2 ± 1.2
Untreated[b]	12.9 ± 1.4	11.8 ± 1.4	12.0 ± 2.1	12.1 ± 1.9	13.8 ± 2.2	12.9 ± 1.7
Total	24.1 ± 1.4	24.9 ± 2.0	24.2 ± 1.8	27.3 ± 2.4	27.5 ± 2.3	24.1 ± 1.9
Growth rate (g/b/d)[c]	16.9 ± 0.7	16.8 ± 1.2	16.1 ± 1.9	17.9 ± 1.2	17.7 ± 0.9	16.4 ± 1.1

[a]Hopper with WNSCO treated feed (for 0 ppm, this contained untreated feed)
[b]Hopper with clean feed
[c]From 0 to 8 days

Table 4. Mean daily feed consumption, body weight and organ parameters ($\bar{x}$ ± S.E.) in adult mallards fed WNSCO in the diet for 14 days.

	Treatments (ppm)			
Parameter	0	10,000	30,000	100,000
Feed Consumption (g/bird/day)	100.3 ± 5.9	103.2 ± 6.1	98.8 ± 5.7	97.5 ± 5.6
Body Weight (g)				
Male: initial	1227 ± 45	1208 ± 61	1265 ± 65	1210 ± 61
terminal	1247 ± 69	1219 ± 70	1278 ± 73	1204 ± 67
Female: initial	1101 ± 51	1091 ± 23	1107 ± 67	1096 ± 52
terminal	1120 ± 51	1105 ± 35	1105 ± 70	1096 ± 57
Organ Weights (g)				
Liver				
male	27.0 ± 2.5	30.3 ± 2.2	30.4 ± 2.8	34.7 ± 1.7
female	27.4 ± 2.7	28.5 ± 0.7	30.5 ± 2.6	31.9 ± 0.9
Kidney				
male	9.5 ± 1.1	9.4 ± 0.6	9.6 ± 1.0	9.6 ± 0.7
female	8.5 ± 0.8	9.1 ± 0.2	9.0 ± 0.7	9.1 ± 0.9
Spleen				
male	0.64 ± 0.07	0.75 ± 0.13	0.67 ± 0.08	0.55 ± 0.07
female	0.88 ± 0.17	0.86 ± 0.07	0.73 ± 0.12	0.58 ± 0.03
Adrenal				
male	0.17 ± 0.02	0.20 ± 0.02	0.19 ± 0.05	0.21 ± 0.02
female	0.14 ± 0.02	0.11 ± 0.02	0.21 ± 0.01	0.17 ± 0.03
Brain				
male	5.1 ± 0.2	5.1 ± 0.1	4.8 ± 0.2	4.8 ± 0.1
female	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.5 ± 0.2
Liver:Brain Weight (male)	5.4 ± 0.6	5.9 ± 0.5	6.3 ± 0.4	7.3[a] ± 0.4
Liver:Body Weight (male)	0.022 ± 0.001	0.025 ± 0.002	0.024 ± 0.002	0.029[a] ± 0.002

[a]Significantly different from control ($P \leq 0.05$)

dose male liver weights normalized on the basis of either brain or body weights. Male liver weights normalized on brain weight and body weight were 35 and 32% larger, respectively, in the high dose group when compared with control birds.

Liver enlargement can be an indicator of increased function associated with detoxification (Szaro 1977). Consumption of crude oil has been shown to result in an increase in liver size (Miller et al. 1977, Patton and Dieter 1980, Gorsline et al. 1981). The ability of birds to withstand oil pollution is thought to lie in the ability of the liver to metabolize the hydrocarbons that are ingested. The mixed function oxidase (MFO) system is the primary means by which the liver metabolizes these hydrocarbons (Gorsline et al. 1981). Gorsline et al. (1981) found an increase in specific and total liver microsomal enzyme activity in mallards fed five different crude oils. Gorsline and Holmes (1982a) attempted to determine if crude oil could in turn induce the MFO system in the ducklings of hens consuming crude oil. They found more than a two-fold increase in specific liver activity of ducklings hatched from hens consuming 1 and 3% SLCO and 3% PBCO.

Control and high dose tissues were examined histopathologically. Due to the lack of consistent and significant difference in the appearance of these tissues the treatments in between were not examined. The only differences noted were that spleens showed a small increase in the amount of white pulp in the birds from the high dose group. The high dose livers had lower numbers of focal accumulations of lymphocytes.

Reproduction Study

The 22 week experimental period included a 12 week prelaying and a 10 week laying period. Prior to the breeding period, food consumption averaged about 97 grams per bird per day (Table 5). During the period of egg production, food consumption

Table 5. Mean daily feed consumption and body weights ($\bar{x}$ ± S.E.) in mallards fed WNSCO in the diet for 22 weeks.

	Treatment (ppm)			
Parameter	**0**	**200**	**2,000**	**20,000**
Feed Consumption (g/b/d)				
Pre-breeding	99.3 ± 2.6	101.4 ± 2.4	95.2 ± 2.1	93.2 ± 2.0
Breeding	177.3 ± 11.5	153.2 ± 7.0	169.7 ± 9.6	166.9 ± 12.3
Adult Body Weight (g)				
Male :initial	1150 ± 25	1177 ± 26	1188 ± 32	1203 ± 26
:terminal	1228 ± 28	1276 ± 23	1233 ± 24	1304 ± 33
Female :initial	1007 ± 24	1006 ± 22	1046 ± 22	1033 ± 26
:terminal	1250 ± 21	1254 ± 45	1251 ± 46	1200 ± 17

increased markedly in all groups, averaging about 167 grams per bird per day. No significant differences (P > 0.05) were noted between treatment groups and controls, either before or during the breeding period.

The effect of crude oil on food consumption is a variable parameter in the literature. Compared to control birds receiving no crude oil, there have been increases, decreases and no difference in feed consumption reported. No significant differences in food consumption were noted in mallards consuming SLCO contaminated food up to 5% of the diet (Szaro et al. 1978b, Gorsline and Holmes 1982b, Gorsline and Holmes 1982c, Vangilder and Peterle 1982). Adult mallards fed 1.5% PBCO for 7 days showed decreased food consumption the first day but not the remainder of the time period (Rattner 1981). Domestic mallards fed North Sea crude oil suffered reduced intake for the first 2 weeks but increased to control levels by the third week (Harvey et al. 1982). This reduction was accompanied by a fall in body weight which was not regained until the ninth week of oil ingestion. Most studies note an increase in food consumption in controls after photoperiod was increased but not in treatment birds. All treatment groups in our study showed increased food consumption after photoperiod was increased.

Hyperphagia, increased food consumption normally in response to nutritional deficit, appears to be somewhat common in birds fed crude oil contaminated feed (Holmes et al. 1978b, Holmes et al 1979, Gorsline and Holmes 1981, Gorsline et al. 1981). Mallards fed 1% and 3% SLCO in the diet for 50 days exhibited no significant difference in food consumption during the first week, but by the end of the 50 day test period food consumption was significantly higher in the treatment groups (Gorsline and Holmes 1981). Harvey et al. (1982) feel that differences in the amount of food eaten by ducks fed different types of crude oil appear to reflect differences in their chemical composition. Of the studies discussed here, SLCO is the predominant crude oil used and all studies where no significant change in feed consumption occurred used this oil. However, in all three studies with significantly increased food consumption, SLCO was used. PBCO resulted in reduced feed consumption in one study and increased in another. These results may very well reflect the differences in composition in crude oils. Crude oils from different locations are known to differ in chemical composition, but Peakall et al. (1983) have shown that oils from the same location collected at separate times can also differ. The oils used were South Louisiana crude oils collected in 1976 and 1978. The batch from 1976 caused significant effects in parameters they were evaluating while the 1978 batch exhibited no such effects.

There is a sexual dimorphism in body weight between males and female mallards. This is apparent in the initial body weight data where average male body weight is 1180 grams while average female body weight is 1023 (Table 5). However, during the breeding period the body weights of males and females are similar. Male body weights have increased about 7% over initial body weight while females have increased over 20% of initial body weight. Most of this is due to increased weight of organs involved with reproduction. Females put more into the reproductive effort, therefore they exhibit a greater increase in weight.

A number of dietary feeding studies with adult mallards fed up to 3% SLCO, KCO or PBCO in the diet report no significant difference in body weights for oil treatment groups (Holmes et al. 1978a, Coon and Dieter 1981, Gorsline and Holmes 1981, Rattner 1981, Gorsline and Holmes 1982a, Gorsline and Holmes 1982b, Gorsline and Holmes 1982c, Vangilder and Peterle 1982). Mallards fed SLCO in the diet for 26 weeks showed no significant reduction in body weight (Coon and Dieter 1981). Pattee and Franson (1982) fed naturally weathered Ixtoc I crude oil to American kestrels and noted that birds fed 3% crude in the diet lost significantly more weight than the controls. In separate experiments, Vangilder (1981) noted a significant decrease in body weight in mallard hens fed 2% SLCO compared to controls, but a significant increase in hens fed 0.5% SLCO. When Gorsline et al. (1981) examined the effect of 50 days exposure of 5 different crude oils in mallards they found significant losses in body weight in some groups, even in those that consumed significantly more food than controls. Both 1 and 3% PBCO resulted in a significant loss in body weight by the termination of the experiment when compared to initial body weight, while controls did not.

The four experimental groups of mallards in this study laid nearly 3000 eggs during the 70 day test period (Table 6). The rate of oviposition was similar in all groups ($P > 0.05$). Fertility and hatchability averaged 97.4% and 83.8%, respectively, and was similar in all groups ($P > 0.05$). Survivability of 14 day ducklings was very similar in all treatment groups ($P > 0.05$).

Numerous investigators have reported various effects on these reproductive parameters in birds ingesting crude oil. Ainley et al. (1981) force fed wild Cassin's auklets a single oral dose of 1000 mg PBCO in gelatin capsules and reported a marked decrease in percentage of eggs laid from controls, but the eggs hatched normally. Mallards fed SLCO at 0.25 and 2.5% in the diet for 26 weeks showed a significant reduction in egg production, but fertility and hatchability were not affected (Coon and Dieter 1981). Egg production in Japanese quail fed single oral doses of up to 800 mg PBCO was significantly reduced on day 2 following dosing, but by day 4 was back to normal (Engel et al. 1978). Hartung (1965) reported that 3 ducks fed 2g/kg crude oil by oral intubation stopped laying immediately and didn't resume for 2 weeks while sham-dosed birds continued laying throughout that period. Vangilder (1981) found that SLCO in the diet significantly reduces reproductive performance in mallards, lowering egg production by 36.6%, but did not affect fertility. Egg production was significantly reduced in mallards fed SLCO (Vangilder and Peterle 1982). Vangilder and Peterle (1983) noted that egg production, onset of laying and egg fertility were unaffected by dietary ingestion of 0.5% SLCO, however, hatchability of SLCO hens was significantly reduced compared to controls. Cavanaugh et al. (1983) determined that mallards ingesting 3% SLCO took significantly longer to complete the reproductive cycle than control birds, 71 days as opposed to 56 days for controls. Cavanaugh and Holmes (1982, 1987) found that ovarian maturation was delayed significantly in mallards ingesting 3% SLCO in the diet. In both instances, mean plasma estradiol concentrations were significantly lower in contaminated birds after increase in photoperiod. They believe that the crude oil in some way interferes with the transmission of information from the photoreceptor to hormonally-regulated events. Domestic mallards fed 5% North Sea crude oil in the diet exhibited a significantly reduced oviposition rate and the onset of laying was delayed by at least 4 weeks (Harvey et. al 1981, Harvey et al. 1982). Holmes et al. (1978a) found that mallards consuming 1% SLCO or Kuwait crude oil (KCO) in the diet had no effect on oviposition but at 3% SLCO caused significant inhibition of oviposition while 3% KCO caused complete inhibition.

Survivorship to two weeks is a key measure of the number of young that will be recruited into the population (Vangilder and Peterle 1982). Survivorship of hatchlings from treated birds in our study was not significantly different ($P > 0.05$) from hatchlings of control birds (Table 6). Survival data in the literature deals mainly with hatchling survival under stress conditions. Vangilder (1981) observed that hatchlings of mallards fed 0.5% SLCO in the diet had a significantly reduced survival time compared to controls when one-hour old hatchlings were placed at 20^{0}C but the same did not occur in a separate experiment with hatchlings of mallards fed 2% SLCO in the diet. Survival in SLCO ducklings was significantly less under stress conditions (Vangilder and Peterle 1982).

Table 6. Egg production, percent fertility, percent hatchability and percent survival of ducklings from eggs laid by female mallards fed WNSCO in the diet for 22 weeks.

	Treatment (ppm)			
Parameters	0[a]	200[b]	2,000[a]	20,000[b]
Eggs laid (N)	730	711	778	616
Rate (eggs/hen/day)	.695	.703	.741	.625
Fertility (%)	97.4	99.4	94.4	98.4
Hatchability (%)	84.5	82.2	84.6	84.0
14-Day Survivability (%)	98.7	97.9	98.0	98.6

[a] Based on 15 hens
[b] Based on 14 hens

Eggshell thickness and strength are important measures of the effects of environmental contaminants on reproductive performance in birds. The eggshell provides a physical barrier that protects the embryo from desiccation and infection, and this protection is compromised in the event of eggshell thinning. Both total eggshell thickness and egg shell strength in the 20,000 ppm WNSCO treatment group were significantly reduced ($P \leq 0.05$) by approximately 8% when compared to the control group (Table 7). Some investigators have recorded similar reductions in eggshell thickness in birds exposed to crude oil while others have noted no effect. Harvey et al. (1981) found significant eggshell thinning in eggs from domestic mallard hens fed 5% North Slope crude oil in the diet. Refeeding uncontaminated diet to these hens increased egg shell thickness, but not to control levels. Eggs from mallards ingesting 2% SLCO had shells that were significantly thinner than control eggs (Vangilder 1981). Holmes et al. (1978a) reported that ingestion of SLCO caused significant thinning of eggshells at 1 and 3% while KCO did not at either concentration. When these birds were put back on uncontaminated feed, eggshell thickness became similar to the control group. Vangilder and Peterle (1982) also found significant eggshell thinning in mallards ingesting SLCO. Coon and Dieter (1981), on the other hand, fed mallards up to 2.5% SLCO for 26 weeks and found no reduction in eggshell thickness in treatment birds. A single oral dose of PBCO in Japanese quail resulted in significantly reduced eggshell thickness on day 1 following dosing, but was back to normal the next day (Engel et al. 1978).

Initial body weights for ducklings of hens consuming 200 and 2,000 ppm WNSCO were significantly higher ($P \leq 0.05$) than controls over the 10 week period (Table 8). However, ducklings of hens consuming 20,000 ppm WNSCO in the diet were not significantly different ($P > 0.05$) than controls. On the contrary, the mean daily growth rate in the 20,000 ppm ducklings was significantly higher ($P \leq 0.05$) than

Table 7. Mean eggshell strength and thickness ($\bar{x}$ ± S.E.) of eggs laid by hen mallards fed WNSCO in the diet for 22 weeks.

	Treatment (ppm)			
Parameter	0	200	2,000	20,000
Egg shell thickness (mm)	0.417 ± 0.004	0.417 ± 0.004	0.405 ± 0.004	0.386[a] ± 0.006
Egg shell strength (Newtons)	32.3 ± 0.5	31.6 ± 0.7	32.1 ± 0.6	29.5[a] ± 0.8

[a]Significantly different from control ($P \leq 0.05$)

Table 8. Mean initial body weight and growth rate ($\bar{x}$ ± S.E.) of hatchlings of hen mallards fed WNSCO in the diet for 22 weeks.

	Treatment (ppm)			
Parameter	0	200	2,000	20,000
Initial Body Weight (g)	35.3 ± 0.2	36.2[a] ± 0.2	36.3[a] ± 0.2	35.8 ± 0.2
Growth rate (g/b/d)[b]	9.7 ± 0.1	10.0 ± 0.1	9.6 ± 0.1	10.5[a] ± 0.1

[a]Significantly different from control ($P \leq 0.05$)
[b]For 14 days

controls while the 200 and 2,000 ppm ducklings were not different from the control.

No significant differences ($P > 0.05$) were found between treatment and control mallards by sex for mean organ weights (Table 9) or organ weights normalized on the basis of body weight or brain weight. Liver weights of males were 24 g less than females and similar to liver weights of non-reproductive males in the 14-day study. The large liver weights of females in this study can be associated with it being a storage site for fat going to yolk deposition.

The females consuming 20,000 ppm WNSCO in the diet suffered a 26% reduction in serum albumin and a 17% decrease in serum total protein (Table 10). Calcium levels were reduced by over half (54%) in the 20,000 ppm females compared to controls. Ingestion of crude oil did not significantly impact other clinical blood parameters.

The decrease in plasma calcium and protein in the high dose group appears to be related to the reduced eggshell thickness and strength of eggs observed for this group. Calcium and protein are important components in shell structure and if a hen suffers from reduced levels, this could compromise shell thickness and strength. The lower calcium level in the high dose birds may be the result of reduced absorption in the intestinal mucosa as the amount of food consumed by the high dose group is not significantly less than the controls. Harvey et al. (1982) suggest that contamination of the diet with crude oil may irritate the gastrointestinal tract and it is possible therefore that petroleum may have a direct effect on the absorptive properties of the mucosa cells in the small intestine which might indirectly lead to a reduction in egg production and egg quality. They found that domestic mallards fed North Sea crude oil exhibited a significantly lower plasma calcium level compared to control. This reduction remained even after oil had been removed from the diet and food consumption increased, and they feel this provides evidence that petroleum ingestion

Table 9. Mean organ weights (g) in mallards fed WNSCO in the diet for 22 weeks in a reproduction study ($\bar{x}$ ± S.E.).

	Treatment (ppm)			
Parameter	0	200	2,000	20,000
Liver				
male	27.8 ± 1.2	27.5 ± 0.9	27.3 ± 1.2	30.5 ± 1.1
female	49.1 ± 4.0	53.2 ± 5.1	53.8 ± 4.8	51.6 ± 4.5
Kidney				
male	8.5 ± 0.3	8.8 ± 0.3	8.5 ± 0.3	8.9 ± 0.4
female	10.7 ± 0.4	10.5 ± 0.4	10.4 ± 0.5	10.6 ± 0.3
Spleen				
male	0.74 ± 0.05	0.69 ± 0.04	0.69 ± 0.05	0.57 ± 0.03
female	0.91 ± 0.11	0.79 ± 0.06	1.52 ± 0.79	0.62 ± 0.06
Adrenal				
male	0.28 ± 0.02	0.37 ± 0.03	0.29 ± 0.02	0.29 ± 0.03
female	0.42 ± 0.04	0.33 ± 0.03	0.30 ± 0.03	0.31 ± 0.03
Brain				
male	5.0 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
female	4.6 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.1

Table 10. Mean clinical blood parameters ($\bar{x}$ ± S.E.) in adult mallards fed WNSCO in the diet for 22 weeks.

	Treatment (ppm)			
Parameter	0	200	2,000	20,000
Female				
Albumin (g/dl)	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	1.7[a] ± 0.2
Total Protein (g/dl)	5.7 ± 0.2	5.9 ± 0.5	5.8 ± 0.1	4.4[a] ± 0.6
Calcium (mg/dl)	33.4 ± 3.6	32.7 ± 2.5	27.4 ± 1.3	15.5[a] ± 4.2
Basophils (%)	4.3 ± 0.6	4.3 ± 1.3	2.0[a] ± 0.4	1.8[a] ± 0.5
Male				
Albumin (g/dl)	1.9 ± 0.1	1.5[a] ± 0.1	1.8 ± 0.1	1.9 ± 0.1
RBC (10^6 cell/mm^3)	3.1 ± 0.21	3.0 ± 0.03	2.7[a] ± 0.04	2.7[a] ± 0.07
Packed Cell Volume (%)	48.2 ± 0.4	48.6 ± 0.9	44.2[a] ± 1.1	47.4 ± 1.0

[a]Significantly different from control ($P \leq 0.05$)

inhibits calcium uptake.

Two adult females died during the 10-week egg production period. An adult female in the high dose treatment group died during the first week of egg production and an adult female in the 200 ppm treatment group was found dead 4 weeks after egg production began. Upon autopsy, neither death was determined to be treatment related. No birds exhibited signs of gross toxicity. Our mortality results are consistent with other studies of adult mallards fed crude oils in the diet at concentrations up to 5% have found no treatment related mortality even after 30 weeks of continuous ingestion (Holmes et al. 1978a, Holmes et al. 1978b, Holmes et al. 1979, Coon and Dieter 1981, Gorsline and Holmes 1981, Gorsline et al. 1981, Rattner 1981, Cavanaugh and Holmes 1982, Gorsline and Holmes 1982c, Harvey et al. 1982, Cavanaugh and Holmes 1983, Cavanaugh et al. 1983, Vangilder and Peterle 1983). Vangilder (1981) reported 5 mortalities in an experiment where mallards were fed SLCO but these were not treatment related.

No treatment related gross abnormalities or lesions were observed at necropsy in this study. Histopathological examination revealed no consistent differences in tissues of high dose birds compared to control birds, by sex, except in the number of circumscribed nodules of lymphoid tissue in the spleens of females. There was a higher average number of circumscribed nodules in the spleens of high dose females than in controls. Coon and Dieter (1981) reported no histopathological differences between birds fed 2.5% SLCO in the diet and control birds. Pathological responses of birds from oil spill sites or of birds given single oral doses of oil as noted by Leighton et al. (1983) have consistently included hepatic fatty changes, lipid pneumonia, gastrointestinal irritation and renal tubular nephrosis.

Eggshell Application Study

Hatching success was significantly reduced ($P \leq 0.05$) in only the WNSCO treatment that covered the middle 1/3 of the egg (Table 11). On the contrary, hatching success was significantly reduced ($P \leq 0.05$) from the control group in all petrolatum treatment groups (Table 11). As petrolatum is not known to be toxic, this is most likely an effect of pore blockage resulting in inefficient gas and water exchange. Embryos from unhatched eggs in the petrolatum application group were examined and most showed signs of being hydrocephalic, a sign of insufficient water exchange. Many embryos from unhatched eggs in the WNSCO treatment groups also showed signs of being hydrocephalic. The apparent differences between treatments is most likely due to the efficiency of the treatment in blocking pores. The petrolatum, being smooth and applying evenly, proved an efficient pore blocker. The WNSCO was of a firmer consistency and did not spread as evenly and was probably not an efficient pore blocker, so more WNSCO was needed to significantly reduce hatchability. This suggests that the weathered crude is not toxic to the embryos at the levels applied and they would be impacted by pore blockage before toxicity.

Crude oil toxicity to developing embryos has been demonstrated in the literature and is a viable concern. Typically, only microliter quantities of crude oils ($\leq$ 50 ul) applied directly to the egg shell of bird eggs are needed to result in significant embryo mortality (Albers 1978, Hoffman 1978, Szaro et al. 1978a, Hoffman 1979a, Hoffman 1979b, Macko and King 1980, Lambert et al. 1982, Couillard and Leighton 1989, Couillard and Leighton 1990). Hartung (1965) demonstrated that oil can be transferred from feathers to eggs. Albers (1980) has taken this one step further and shown that an incubating bird will enter oiled water, become oiled, and subsequently transfer the oil to the eggs. He also showed that at 100 ml of PBCO/m^2 of water surface there was a significant reduction in hatchability in mallard eggs. Hallet et al.

Table 11. Number hatched, number 14-day survivors, and hatchling growth rate (g/b/d) from mallard eggs externally treated with various amounts of WNSCO.

Treatment	Number hatched	Number 14-day survivors	Hatchling growth rate ($\bar{x}$ ± S.E.)
Control*	43	42	11.2 ± 0.4
Petrolatum			
Top 1/6	32[a]	31	12.4 ± 0.6
Top 1/3	3[a]	3	13.4 ± 2.8
Middle 1/6	34[a]	32	12.6 ± 0.6
Middle 1/3	4[a]	4	9.6 ± 2.5
WNSCO			
Top 1/6	42	42	11.3 ± 0.4
Top 1/3	38	38	11.2 ± 0.4
Middle 1/6	39	39	11.4 ± 0.4
Middle 1/3	37[a]	37	11.1 ± 0.4

*All test groups N=45

[a]Significantly different from control ($P \leq 0.05$)

(1983) have demonstrated that aromatic hydrocarbons from petroleum pass through the shell and membrane and into the developing embryo.

The age of the developing embryo at which exposure to oil takes place is an important factor as well. Albers (1978) treated mallard eggs with 5 ul of SLCO on days 2, 6, 10, 14, 18, and 22 of a 26 day incubation period and found that hatchability of eggs decreased as the age of the embryo decreased. His results show that after day 10 the impact on the embryo is reduced to a large extent. Hoffman (1979b) demonstrated two periods within the first ten days of incubation in which mallard embryos were most susceptible to petroleum toxicity. Mallard eggs were treated with crude oil on day one of development, and three and seven days after treatment there were significant embryo mortalities while there was little mortality after 13 days. Mallard eggs on day 3 of development exhibited a significant decline in embryo mortality by day 6 and then a rapid decline on days 8 and 9 (Hoffman 1978). Using chicken embryos, Hoffman (1978) found little mortality within four days after treatment on day 3 but on the seventh day there was a sudden increase in embryo mortality.

Two studies have looked at the impact of weathered crude oils on bird eggs. Szaro et al. (1980) treated mallard eggs with 0 to 50 ul of raw PBCO or PBCO that had been artificially weathered for 10 days, either indoors or outdoors. All treatment groups caused significant embryo mortality and weathering had no effect on toxicity of the PBCO. They then weathered it outdoors for 4 weeks. PBCO did not show a decrease in toxicity until the third week, and even at 4 weeks as little as 20 ul caused significant mortality. The weathering process that our PBCO underwent occurred under natural conditions and had weathered for over 3 months and the physical state it was in probably did not allow for it to leach into the egg in large quantities. Ten microliters of naturally weathered Libyan crude oil collected after a pipeline rupture caused significant embryo mortality in heron eggs but surprisingly the raw Libyan crude oil did not (Macko and King 1980). This naturally weathered crude was collected from bottom sediments which may account for the difference in toxicity. The crude oil in bottom sediments may contain concentrated aromatic hydrocarbons as these can enter the water column through dissolution and may salt out into the bottom sediments.

Ellenton (1982) has tried to elucidate the fraction of crude oil which is responsible for the embryotoxicity. Vangilder and Peterle (1983) also looked at PBCO fractions and their effects on chicken embryos. They found that the aromatic fraction of PBCO was the most toxic fraction of the oil. Ellenton (1982) split PBCO into aliphatic, 2 or 3 ring (F3) aromatic, and 4 or 5 ring (F4)aromatic fractions, and applied these to chicken embryos. The aliphatic portion caused no decrease in survival or increase in developmental abnormalities, but did result in significant decrease in weight, crown-rump length and head length. The F3 and F4 fractions had no effect on weight or length and only the F3 fraction caused a significant increase in developmental abnormalities. Their results led them to the conclusion that the embryotoxic activities reside in the F3 fraction.

Survivability of the hatchlings from any of the treatment groups was not different ($P > 0.05$) from the control group (Table 10). Albers (1980) found no significant difference in mallard hatchling survival up to one week, nor did they find any gross behavioral or physical abnormalities, in hatchlings of hens exposed to PBCO. Under similar conditions, Albers and Gay (1982) found no significant difference in one week survivability of one week old hatchlings. Growth rate of the hatchlings did not differ significantly ($P > 0.05$) for any treatment level for either treatment when compared to the controls (Table 11).

CONCLUSION

The results of this series of studies seem to be in agreement with the majority of the literature, which shows that crude oils, in general, appear to be of low toxicity. At levels of up to 5000 mg/kg body weight in a single oral dose there was no mortality or loss of body weight, but the male birds did eat significantly less food than controls over the two week observation period. No mortalities, adverse behavioral reactions, or effects on growth or food consumption were noted in mallard ducklings fed up to 50,000 ppm weathered crude oil in the diet for 5 days. No food avoidance behavior was noted in mallard ducklings given a choice of clean feed and contaminated feed up to 20,000 ppm in the diet. So we can assume that feed contaminated with weathered crude oil is not a deterrent to ingestion up to at least 2% of the diet. The only pathological changes noted in the organs was in the significant increase in liver weights of birds consuming 100,000 ppm in the 14-day dietary feeding study. No difference in food consumption or body weights was found in the 14-day dietary feeding or reproduction studies. No significant differences were noted in the reproductive parameters except for a decrease in eggshell thickness and strength of hens consuming 20,000 ppm in the diet. Reduced concentrations of plasma calcium, total protein and albumin may have led to this condition. Hens from this group also laid fewer eggs and in a more erratic fashion than control hens, but this was not statistically significant. The results from the eggshell application experiment show that the main concern of transfer of crude oil, at this stage of weathering, from the hen to the eggs would be physical covering, which blocks the pores and results in reduced gas and water exchange.

Weathering is a complicated process involving a complex interaction of environmental variables that is not likely to be accurately duplicated in a laboratory situation. Results from artificial weathering experiments have been variable at best and show that artificial weathering does not always reduce the toxicity of crude oil (McEwan and Whitehead 1978, Butler and Lukasiewicz 1979, Peakall et al. 1980, Szaro et al. 1980, Peakall et al. 1983, Leighton 1985, Fry et al. 1986, Nwokolo and Ohale 1986, Boersma et al. 1988). McEwan and Whitehead (1978) and Szaro et al. (1980) have shown slight decreases in toxicity of crude oil after weathering, and in the case of Szaro et al. it still only took microliter quantities to cause significant embryo mortality. In the other studies the effects of weathered crudes were similar to raw crudes. There have been only two studies that I am aware of that have looked at naturally weathered crude oil (Pattee and Franson 1982, Macko and King 1980). Ixtoc I crude oil from the Ixtoc well blowout in the Gulf of Mexico had little effect on American kestrels ingesting it up to 3% in the diet (Pattee and Franson 1982). Macko and King (1980) found naturally weathered Libyan crude oil collected from bottom sediments at the spill site to be highly toxic to Louisiana heron embryos while raw Libyan crude was not. In this case, weathering actually enhanced the toxicity of the oil. This oil had only weathered for 4 weeks and might be different in composition to weathered oil skimmed from the surface. There are many opportunities giving access to naturally weathered crude oils and other petroleum products resulting from accidental spills, and these need to be taken advantage of and studied.

Most of the chronic tests have been done in the laboratory under 'ideal' conditions where all environmental variables remain relatively constant. Studies have shown that when birds ingesting oil are removed from the 'ideal' laboratory conditions and put under some type of environmental stressor they are unable to adapt to the new conditions as well as the control birds, and suffer greater mortality (Holmes et al. 1978b, Holmes et. al 1979). The physiological response of oiled birds to adaption to sea water, as well as the effect of temperature stress, was examined. This would be a major concern in the area at the Valdez spill. Holmes et al. (1978b) adapted domestic mallards to artificial seawater and then exposed them to SLCO or KCO in the diet while maintaining them at 27°C. They found that after exposing them to mild cold stress at 3°C, birds that consumed crude oil died earlier and in greater numbers than seawater- or fresh-water maintained control birds. Holmes et al (1979), in a similar study, looked at the effects of five different crude oils. The results obtained were similar, and they also felt that PBCO to be the most toxic oil tested, as the birds ingesting PBCO consumed the least oil and suffered the highest mortality under cold stress.

Crocker et al. (1974) suggest that ingested oil might hinder intestinal absorption in birds and be an important factor in seabird mortality. When exposed to hypertonic saline solution, equivalent to ocean water, there was an increase in exchange rates of water and sodium in the mucosal lining of the intestine. After giving mallard ducklings a single oral dose of crude oil, Crocker et al. (1974) found that birds given oil at the start of maintenance on hypertonic seawater never developed high mucosal rate transfers, and in birds adapted to hypertonic saline solution prior to dosing the high mucosal rate transfer that had been established was lost within 24 hours of treatment. In a follow up experiment, Crocker et al. (1975) looked at the effects of different crude oils on mucosal transfer rate. They found that of the three crudes tested, PBCO had the least effect while SLCO and KCO had the greatest effect. They also artificially weathered crude oil and found enhanced suppression of mucosal rate transfer. Eastin and Murray (1981) looked at the effects of dietary ingestion of PBCO on intestinal absorption on sodium, chloride, potassium and water in freshwater-adapted mallards. They found no effect on intestinal absorption and suggest that the effect of crude oil on transport might be related to mechanisms stimulated by saltwater-adaption. From these results, and as suggested by Harvey et al. (1982), it could also be postulated that the nutrient absorptive qualities of the intestine may be compromised by crude oil ingestion, which may explain reduced reproductive potential, i.e. depressed egg production, slowed ovarian maturation, delayed oviposition, thinned egg shells, reduced hatchability, etc. This becomes critically important, especially in an arctic or subarctic environment in which the breeding season is short and the timing of the reproductive cycle is critical. If young are not fledged and flying on their own within this critical time period then recruitment into that population could be severely reduced.

From the data presented in this series of experiments, it appears that naturally weathered crude oil is not highly toxic when fed to birds under controlled laboratory conditions. There is a great need to look at the effects of naturally weathered crude oil as there is a severe lack in the literature. We need to be able to determine whether this oil poses a threat to seabird populations after going through the weathering process, including looking at the effects on birds ingesting oil and then put under environmental stresses. It is hard to extrapolate results from controlled laboratory experiments to wild populations and there needs to be an effort to assess its impact from this angle. At this point, it seems that the greatest threat to seabird populations is from physical covering with oil resulting in loss of buoyancy and insulation, and inhalation of toxic fumes in the first hours of a spill, both resulting in death.

APPENDIX

Table 12. Mortalities observed in adult mallards orally dosed with weathered North Slope crude oil.

Diet Concentration (mg/kg)	#Dead/#Exposed/#Exhibiting Toxic Signs
0	0/12/0
5,000	0/12/0

Table 13. Body weights (g) of adult mallards orally dosed with weathered North Slope crude oil (mean ± SE).

Diet Concentration (mg/kg)	Sex	Mean Body Weight (g) ± SE		
		Initiation	Day 7	Termination
0	M	1123 ± 44	1189 ± 36	1191 ± 36
0	F	994 ± 22	1042 ± 33	1059 ± 40
5,000	M	1166 ± 30	1217 ± 35	1224 ± 32
5,000	F	1008 ± 34	1055 ± 33	1069 ± 26

Table 14. Food consumption (g) of adult mallards orally dosed with weathered North Slope crude oil (mean ± SE).

Diet Concentration (mg/kg)	Sex	Mean Food Consumption (g) ± SE		
		Initiation - Day 7	Day 7 - Termination	Initiation - Termination
0	M	135 ± 5.8	118 ± 4.9	127 ± 4.0
0	F	99 ± 4.7	86 ± 4.9	93 ± 3.5
5,000	M	122 ± 9.5	96 ± 4.4	109[a] ± 5.6
5,000	F	102 ± 2.8	87 ± 2.9	94 ± 2.3

[a]Significantly different from control males ($P \leq 0.05$)

Table 15. Mortalities observed in mallard ducklings fed weathered North Slope crude oil in the diet for 5 Days.

Diet Concentration (ppm)	#Dead/#Exposed/#Exhibiting Toxic Signs
0	0/20/0
20,000	0/10/0
0	0/12/0
50,000	0/12/0

Table 16. Food consumption (g) of mallards ducklings fed weathered North Slope crude oil in the diet for 5 Days (mean ± SE).

Diet Concentration (ppm)	Study Days	
	Initiation - Day 5	Day 5 - Termination
0	21.8 ± 0.9	33.9 ± 1.4
20,000	23.9 ± 1.5	35.7 ± 1.8
0	22.7 ± 1.0	34.5 ± 2.4
50,000	21.0 ± 0.8	31.6 ± 1.7

Table 17. Body weights (g) of mallard ducklings fed weathered North Slope crude oil in the diet for 5 days (mean ± SE).

Diet Concentration (ppm)	N	Study Day: Initiation	5	Termination
0	20	66.5 ± 1.9	137.4 ± 5.7	197.3 ± 7.5
20,000	10	68.3 ± 2.1	140.8 ± 9.4	196.0 ± 13.5
0	12	61.9 ± 4.0	130.5 ± 9.6	188.3 ± 13.2
50,000	12	65.4 ± 4.4	130.1 ± 9.0	185.9 ± 12.5

Table 18. Mortalities observed in mallard ducklings given a choice of food treated with weathered North Slope crude oil and untreated food in the diet for 5 days.

Diet Concentration (ppm)	#Dead/#Exposed/#Exhibiting Toxic Signs
0	0/20/0
1.250	0/10/0
2,500	0/10/0
5,000	1/10/0
10,000	0/10/0
20,000	0/10/0

Table 19. Feed consumption (g) in mallard ducklings given a choice between feed treated with weathered North Slope crude oil and untreated feed in the diet for 5 days (mean ± SE).

Diet Concentration (ppm)	N	Feed Consumption (g) ± SE		
		Treated Feed	Control Feed	Total
0	20	11.9 ± 1.3	12.9 ± 1.4	24.3 ± 1.5
1,250	10	13.1 ± 1.7	11.8 ± 1.4	24.9 ± 2.0
2,500	10	12.2 ± 1.8	12.0 ± 2.1	24.2 ± 1.8
5,000	10	15.2 ± 2.0	12.1 ± 1.9	27.3 ± 2.4
10,000	10	13.7 ± 2.0	13.8 ± 2.2	27.5 ± 2.3
20,000	10	13.2 ± 2.0	12.3 ± 1.5	25.4 ± 2.6

Table 20. Body weights (g) of mallard ducklings given the choice of feed treated with weathered North Slope crude oil or untreated feed in the diet for 5 days (mean ± SE).

Diet Concentration (ppm)	N	Study Day Initiation	Day 5	Termination
0	20	67.6 ± 2.2	141.0 ± 5.2	202.7 ± 7.4
1,250	10	67.9 ± 3.7	141.8 ± 9.1	202.1 ± 12.9
2,500	10	64.5 ± 4.1	135.1 ± 12.2	193.6 ± 18.5
5,000	10	64.9 ± 4.9	144.9 ± 9.0	212.2 ± 12.2
10,000	10	67.9 ± 3.9	146.6 ± 8.4	209.6 ± 10.3
20,000	10	65.9 ± 3.0	139.3 ± 8.6	196.7 ± 11.3

Table 21. Mortalities observed in adult mallards fed weathered North Slope crude oil in the diet for 14 Days.

Diet Concentration (ppm)	#Dead/#Exposed/#Exhibiting Toxic Signs
0	0/10/0
10,000	0/10/0
30,000	0/10/0
100,000	0/10/0

Table 22. Mean daily feed consumption (g) in adult mallards fed weathered North Slope crude oil in the diet for 14 days (mean ± SE).

Diet Concentration (ppm)	N	Feed Consumption (g) ± SE		
		Day 0-8	Day 8-14	Day 0-14
0	10	108.9 ± 7.9	88.8 ± 8.0	100.3 ± 5.9
1,250	10	103.5 ± 7.8	102.9 ± 10.0	103.2 ± 6.1
2,500	10	92.1 ± 7.7	107.6 ± 8.2	98.8 ± 5.7
5,000	10	94.1 ± 8.2	102.1 ± 7.3	97.5 ± 5.6

Table 23. Body weights (g) of adult mallards fed weathered North Slope crude oil in the diet for 14 days (mean ± SE).

		Study Day		
Diet Concentration (ppm)	Sex	Initiation	Day 7	Termination
0	M	1227 ± 45	1259 ± 61	1247 ± 69
10,000	M	1208 ± 61	1237 ± 70	1219 ± 70
30,000	M	1265 ± 65	1266 ± 76	1278 ± 73
100,000	M	1210 ± 61	1225 ± 71	1204 ± 67
0	F	1101 ± 51	1146 ± 53	1120 ± 51
10,000	F	1091 ± 23	1119 ± 27	1105 ± 35
30,000	F	1107 ± 67	1096 ± 59	1105 ± 70
100,000	F	1096 ± 52	1116 ± 52	1096 ± 57

Table 24. Hematology results of adult mallards fed weathered North Slope crude oil in the diet for 14 days (mean ± SE).

		Exposure Concentration (ppm)			
Parameter	Day	0	10,000	30,000	100,000
Red Blood	0	2.5 ± 0.13	2.6 ± 0.09	2.5 ± 0.08	2.4 ± 0.06
Count	14	2.7 ± 0.11	2.6 ± 0.09	2.6 ± 0.10	2.5 ± 0.07
(10^6cell/mm^3)					
White Blood	0	9467 ± 865	12500 ± 2191	9505 ± 1603	8521 ± 1210
Count	14	10145 ± 1434	8747 ± 699	8322 ± 1793	8873 ± 2093
(cells/mm^3)					
Lymphocytes	0	25.4 ± 5.4	36.2 ± 6.4	25.2 ± 5.0	25.2 ± 7.5
(%)	14	28.2 ± 4.3	34.4 ± 8.8	34.8 ± 3.6	45.4 ± 6.1
Monocytes	0	6.8 ± 1.9	7.0 ± 2.4	12.4 ± 3.1	3.8 ± 1.4
(%)	14	9.2 ± 3.2	11.6 ± 5.9	5.4 ± 1.6	3.8 ± 1.0
Heterophils	0	63.0 ± 4.4	55.4 ± 5.0	59.8 ± 6.6	68.8 ± 7.0
+ Eosinophils	14	58.2 ± 4.2	51.2 ± 10.9	56.0 ±4.2	48.2 ± 5.9
(%)					
Basophils	0	4.8 ± 0.8	1.4 ± 0.7	2.6 ± 0.9	2.2 ± 1.0
(%)	14	4.4 ± 1.3	2.8 ± 1.4	3.8 ± 1.0	2.6 ± 1.3
HGB	0	15.6 ± 0.3	15.8 ± 0.4	15.1 ± 0.4	14.8 ± 0.3
(g/dl)	14	16.0 ± 0.6	15.9 ± 0.4	14.6 ± 0.5	14.5 ± 0.2
Mean Cell	0	186.8 ± 6.1	181.3 ± 2.9	190.4 ± 3.6	192.0 ± 3.6
Volume	14	170.8 ± 5.8	177.8 ± 5.1	171.4 ± 3.8	176.8 ± 4.5
(fL)					

Table 24 (cont'd).

Parameter	Day	0	10,000	30,000	100,000
Mean Cell	0	62.0 ± 2.5	62.0 ± 0.6	59.9 ± 1.4	62.0 ± 1.4
Hemoglobin	14	59.0 ± 2.1	61.0 ± 2.0	56.6 ± 1.8	57.5 ± 1.3
(uug)					
Mean Cell	0	33.2 ± 0.5	33.4 ± 0.7	31.4 ± 0.3	32.2 ± 0.3
Hemoglobin	14	34.4 ± 0.4	34.2 ± 0.3	33.0 ± 0.5	32.7 ± 0.3
Concentration					
(%)					
Packed Cell	0	47.2 ± 1.5	47.4 ± 1.5	48.2 ± 1.6	45.8 ± 0.5
Volume	14	46.4 ± 1.7	46.2 ± 1.1	44.4 ± 1.5	44.4 ± 0.5
(%)					
Reticulocyte	0	3.8 ± 0.7	3.8 ± 0.6	4.3 ± 0.3	4.3 ± 0.6
Count	14	3.7 ± 0.6	2.6 ± 0.3	2.9 ± 0.3	2.9 ± 0.2

Table 25. Clinical chemistry results of adult mallards fed weathered North Slope crude oil in the diet for 14 days (mean ± SE).

		Exposure Concentration (ppm)			
Parameter	Day	0	10,000	30,000	100,000
Na	0	144.2 ± 0.5	146.2 ± 0.8	145.4 ± 0.5	144.2 ± 0.7
(mEq/L)	14	144.4 ± 1.1	144.0 ± 1.4	145.2 ± 0.7	143.2 ± 0.9
K	0	2.7 ± 0.2	2.7 ± 0.3	2.4 ± 0.2	2.3 ± 0.1
(mEq/L)	14	2.6 ± 0.1	2.6 ± 0.2	2.7 ± 0.2	2.7 ± 0.2
Alk Phos	0	226.0 ± 29.8	210.4 ± 31.3	150.0 ± 16.2	230.8 ± 33.3
(U/L)	14	156.8 ± 20.8	166.2 ± 15.9	130.8 ± 18.1	152.6 ± 22.7
CPK	0	317.4 ± 107.0	349.2 ± 53.4	285.6 ± 39.8	305.8 ± 46.0
(U/L)	14	293.0 ± 107.6	841.4 ± 467.3	282.4 ± 59.3	212.2 ± 33.3
Glucose	0	188.2 ± 6.4	191.4 ± 8.7	181.4 ± 7.2	181.6 ± 7.3
(m/dl)	14	186.8 ± 2.9	193.4 ± 4.2	194.6 ± 7.8	179.2 ± 5.9
ALT	0	23.8 ± 1.9	29.2 ± 3.1	19.0 ± 2.3	24.4 ± 3.2
(SGPT)	14	22.2 ± 3.2	33.8 ± 3.1	28.4 ± 0.9	32.6 ± 2.5
(U/L)					
AST	0	7.6 ± 2.3	9.8 ± 2.6	8.8 ± 1.2	8.8 ± 1.5
(SGOT)	14	9.2 ± 2.1	25.2 ± 11.3	10.6 ± 2.0	10.0 ± 1.6
(U/L)					
Total	0	3.7 ± 0.2	3.7 ± 0.1	3.9 ± 0.2	3.8 ± 0.16
Protein	14	4.0 ± 0.3	3.8 ± 0.2	4.3 ± 0.2	4.4 ± 0.0
(g/dl)					

Table 25 (cont'd).

Parameter	Day	0	10,000	30,000	100,000
Albumin	0	1.6 ± 0.1	1.5 ± 0.0	1.6 ± 0.1	1.7 ± 0.1
(g/dl)	14	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.0
Globulin	0	2.1 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.1 ± 0.1
(g/dl)	14	2.3 ± 0.2	2.0 ± 0.1	2.5 ± 0.2	2.5 ± 0.1
Uric Acid	0	8.7 ± 1.5	7.6 ± 0.8	7.4 ± 1.5	8.1 ± 1.6
(mg/dl)	14	4.6 ± 0.6	4.0 ± 0.5	6.2 ± 1.2	4.4 ± 0.2
Ca	0	10.0 ± 0.3	10.1 ± 0.2	10.4 ± 0.3	10.4 ± 0.2
(mg/dl)	14	10.3 ± 0.3	10.3 ± 0.2	11.0 ± 0.4	11.1 ± 0.4

Table 26. Mortalities observed in adult mallards fed weathered North Slope crude oil in the diet for 22 weeks.

Diet Concentration (ppm)	#Dead/#Exposed/#Exhibiting Toxic Signs
0	0/30/0
200	1/30/0
2,000	0/30/0
20,000	1/30/0

Table 27. Mean feed consumption (g/b/d*) by adult mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.).

	Diet Concentration (ppm)			
Week	0	200	2,000	20,000
1 & 2	105.6 ± 4.4	113 ± 4.9	107.6 ± 3.9	100.2 ± 5.1
3 & 4	96.8 ± 4.3	94.4 ± 4.4	92.2 ± 3.5	91.4 ± 3.4
5 & 6	97.1 ± 5.3	106.2 ± 4.4	92.9 ± 4.1	91.2 ± 3.7
7 & 8	97.9 ± 6.6	91.3 ± 3.6	88.3 ± 3.4	90.2 ± 3.4
9 & 10	97.6 ± 6.8	96.8 ± 5.9	93.5 ± 4.9	87.9 ± 4.3
11 & 12	141.4 ± 10.3	150.2 ± 10.4	127.3 ± 7.1	129.7 ± 11.9
13 & 14	146.0 ± 8.3	142.7 ± 8.3	125.2 ± 4.7	122.3 ± 9.4
15 & 16	126.4 ± 9.7	151.3 ± 13.0	121.1 ± 9.7	138.4 ± 7.6
17 & 18	146.4 ± 12.1	140.3 ± 7.0	130.7 ± 8.3	147.0 ± 7.4
19 & 20	144.4 ± 15.0	142.0 ± 7.5	144.5 ± 9.3	143.6 ± 12.1
21 & 22	157.8 ± 11.3	174.0 ± 11.0	173.1 ± 8.5	186.1 ± 18.0

* g/b/d = grams per bird per day

Table 28. Body weights (g) by sex of adult mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

Diet Concentration (ppm)	Sex	Mean Body Weight (g) ± SE					
		Week 0	Week 2	Week 4	Week 6	Week 8	Termination
0	F	1007 ± 24	1025 ± 22	1029 ± 20	1049 ± 24	1043 ± 28	1250 ± 21
200	F	1006 ± 22	1030 ± 23	1034 ± 22	1053 ± 24	1027 ± 27	1254 ± 45
2,000	F	1046 ± 22	1067 ± 26	1076 ± 28	1082 ± 25	1062 ± 25	1251 ± 46
20,000	F	1033 ± 26	1043 ± 26	1052 ± 24	1060 ± 24	1032 ± 24	1200 ± 17
0	M	1150 ± 25	1211 ± 28	1220 ± 40	1222 ± 33	1192 ± 34	1228 ± 28
200	M	1177 ± 26	1218 ± 30	1215 ± 32	1225 ± 29	1181 ± 25	1276 ± 23
2,000	M	1188 ± 32	1248 ± 32	1252 ± 33	1256 ± 33	1221 ± 34	1233 ± 24
20,000	M	1203 ± 26	1237 ± 33	1251 ± 33	1263 ± 36	1225 ± 35	1304 ± 33

Table 29. Organ:brain weight ratios of adult female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

Concentration (ppm)	Liver	Kidney	Spleen	Adrenal
0	10.7 ± 1.0	2.3 ± 0.1	0.20 ± 0.02	0.091 ± 0.009
200	11.8 ± 1.1	2.3 ± 0.1	0.17 ± 0.01	0.073 ± 0.005
2,000	11.8 ± 1.2	2.3 ± 0.1	0.34 ± 0.18	0.066 ± 0.006
20,000	11.6 ± 1.1	2.4 ± 0.1	0.14 ± 0.01	0.069 ± 0.007

Table 30. Organ:brain weight ratios of adult male mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

Concentration (ppm)	Liver	Kidney	Spleen	Adrenal
0	5.6 ± 0.2	1.7 ± 0.1	0.15 ± 0.01	0.058 ± 0.004
200	5.5 ± 0.2	1.8 ± 0.1	0.14 ± 0.01	0.073 ± 0.006
2,000	5.5 ± 0.2	1.7 ± 0.1	0.14 ± 0.01	0.058 ± 0.005
20,000	6.2 ± 0.3	1.8 ± 0.1	0.12 ± 0.01	0.059 ± 0.006

Table 31. Organ:body weight ratios of adult female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

Concentration (ppm)	Kidney	Liver	Spleen	Adrenal	Brain
0	0.0085 ± 0.0002	0.039 ± 0.003	0.00073 ± 0.00009	0.00034 ± 0.00004	0.0037 ± 0.00010
200	0.0084 ± 0.0002	0.042 ± 0.003	0.00063 ± 0.00004	0.00027 ± 0.00002	0.0037 ± 0.00013
2000	0.0084 ± 0.0006	0.043 ± 0.003	0.00151 ± 0.00093	0.00025 ± 0.00003	0.0038 ± 0.00015
20,000	0.0088 ± 0.0003	0.043 ± 0.004	0.00052 ± 0.00005	0.00026 ± 0.00002	0.0038 ± 0.00006

Table 32. Organ:body weight ratios of adult male mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

Concentration (ppm)	Kidney	Liver	Spleen	Adrenal	Brain
0	0.0069 ± 0.0002	0.023 ± 0.001	0.00061 ± 0.00005	0.00023 ± 0.00002	0.0041 ± 0.0001
200	0.0069 ± 0.0002	0.021 ± 0.001	0.00054 ± 0.00003	0.00029 ± 0.00002	0.0040 ± 0.0001
2000	0.0069 ± 0.0002	0.022 ± 0.001	0.00055 ± 0.00093	0.00023 ± 0.00002	0.0041 ± 0.0001
20,000	0.0069 ± 0.0003	0.024 ± 0.001	0.00044 ± 0.00003	0.00023 ± 0.00002	0.0038 ± 0.0001

Table 33. Clinical chemistry results of adult female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

		Diet Concentration (ppm)			
Parameter	Week	0	200	2,000	20,000
K	0	2.7 ± 0.46	2.8 ± 0.18	3.0 ± 0.23	2.8 ± 0.31
(mEq/L)	5	2.6 ± 0.14	2.8 ± 0.19	2.9 ± 0.29	2.3 ± 0.06
	10	2.9 ± 0.74	2.2 ± 0.19	2.7 ± 0.45	2.4 ± 0.24
	Term.	2.4 ± 0.29	2.8 ± 0.30	2.8 ± 0.11	2.2 ± 0.20
Na	0	147.8 ± 1.9	148.2 ± 1.4	147.0 ± 1.2	147.0 ± 1.0
(mEq/L)	5	145.8 ± 1.1	145.6 ± 0.4	143.6 ± 0.8	144.0 ± 0.9
	10	145.4 ± 1.5	144.4 ± 1.8	146.0 ± 1.5	148.0 ± 1.6
	Term.	134.6 ± 2.2	135.8 ± 1.7	136.4 ± 1.2	136.2 ± 1.2
Alk Phos	0	302.8 ± 67.6	313.8 ± 39.3	215.2 ± 30.1	188.6 ± 47.1
(U/L)	5	133.4 ± 25.2	199.8 ± 32.6	110.6 ± 13.0	101.6 ± 18.7
	10	422.2 ± 98.4	456.6 ± 112.0	195.6 ± 71.7	246.2 ± 86.3
	Term.	927.6 ± 531.1	207.2 ± 62.3	103.0 ± 8.3	81.4 ± 11.6
CPK	0	401.2 ± 197.3	332.8 ± 40.0	407.6 ± 144.0	305.6 ± 39.1
(U/L)	5	273.4 ± 71.0	277.2 ± 43.5	247.2 ± 58.2	313.6 ± 69.9
	10	715.2 ± 100.3	637.6 ± 120.4	599.0 ± 124.4	370.2 ± 85.3
	Term.	302.6 ± 70.3	356.0 ± 64.7	275.4 ± 45.9	292.6 ± 29.3
Glucose	0	212.6 ± 6.1	240.6 ± 10.5	240.0 ± 11.1	232.7 ± 6.8
(m/dl)	5	207.4 ± 10.4	202.6 ± 11.4	201.8 ± 9.5	216.6 ± 15.8
	10	187.2 ± 10.3	186.6 ± 6.2	204.4 ± 4.5	200.0 ± 6.8
	Term.	206.9 ± 7.1	215.8 ± 9.2	218.0 ± 8.3	211.6 ± 8.0

Table 33. (cont'd).

Parameter	Week	0	200	2,000	20,000
ALT	0	18.4 ± 1.4	21.6 ± 7.3	26.4 ± 6.1	21.6 ± 2.0
(SGPT)	5	23.2 ± 3.6	22.2 ± 1.9	23.2 ± 2.1	27.2 ± 3.0
(U/L)	10	22.6 ± 6.3	26.2 ± 2.9	31.6 ± 2.6	36.6 ± 2.1
	Term.	26.2 ± 3.9	25.2 ± 4.2	31.2 ± 1.9	42.8 ± 11.4
AST	0	11.2 ± 2.5	13.4 ± 1.6	15.0 ± 3.9	11.2 ± 1.7
(SGOT)	5	11.2 ± 1.3	11.8 ± 1.4	10.4 ± 2.1	10.0 ± 3.8
(U/L)	10	15.2 ± 3.0	21.6 ± 7.7	15.0 ± 3.1	10.6 ± 1.8
	Term.	3.6 ± 1.7	6.4 ± 2.1	8.4 ± 2.2	13.2 ± 7.4
Total	0	4.1 ± 0.40	3.8 ± 0.13	3.5 ± 0.17	3.8 ± 0.15
Protein	5	3.6 ± 0.21	3.6 ± 0.10	3.9 ± 0.16	3.9 ± 0.11
(g/dl)	10	5.2 ± 0.36	5.4 ± 0.70	4.7 ± 0.32	4.5 ± 0.28
	Term.	5.7 ± 0.23	5.9 ± 0.49	5.8 ± 0.10	4.4[a] ± 0.55
Albumin	0	1.8 ± 0.04	1.9 ± 0.09	1.7 ± 0.13	1.9 ± 0.04
(g/dl)	5	1.6 ± 0.08	1.6 ± 0.06	1.7 ± 0.10	1.7 ± 0.08
	10	2.2 ± 0.10	2.2 ± 0.20	2.0 ± 0.12	2.0 ± 0.10
	Term.	2.3 ± 0.12	2.3 ± 0.14	2.2 ± 0.14	1.7[a] ± 0.17
Globulin	0	2.3 ± 0.40	1.7 ± 0.22	1.8 ± 0.13	1.9 ± 0.12
(g/dl)	5	2.0 ± 0.19	2.0 ± 0.11	2.3 ± 0.19	2.2 ± 0.10
	10	2.9 ± 0.26	3.2 ± 0.52	2.7 ± 0.25	2.5 ± 0.19
	Term.	3.4 ± 0.23	3.6 ± 0.40	3.6 ± 0.08	2.6 ± 0.40
Uric Acid	0	6.2 ± 0.7	4.5 ± 0.8	4.7 ± 1.0	6.4 ± 0.9
(mg/dl)	5	4.8 ± 0.9	3.9 ± 0.3	4.5 ± 0.7	5.1 ± 1.0
	10	6.0 ± 0.5	4.5 ± 0.8	5.9 ± 0.9	5.1 ± 0.6
	Term.	6.0 ± 0.5	5.3 ± 0.9	6.5 ± 0.9	5.2 ± 1.1

Table 33 (cont'd).

Parameter	Week	0	200	2,000	20,000
Ca	0	11.0 ± 0.4	10.9 ± 0.3	10.6 ± 0.3	10.6 ± 0.3
(mg/dl)	5	10.4 ± 0.2	10.0 ± 0.4	10.8 ± 0.2	10.5 ± 0.3
	10	28.4 ± 6.5	36.6 ± 18.9	16.0 ± 4.2	16.1 ± 4.3
	Term.	33.4 ± 3.6	32.7 ± 2.5	27.4 ± 1.3	15.5[a] ± 4.2
Total	0	0.24 ± 0.08	0.12 ± 0.02	0.24 ± 0.08	0.22 ± 0.05
Bilirubin	5	0.34 ± 0.04	0.32 ± 0.02	0.36 ± 0.04	0.32 ± 0.04
(mg/dl)	10	0.42 ± 0.15	0.66 ± 0.27	0.60 ± 0.45	0.24 ± 0.10
	Term.	0.40 ± 0.10	0.38 ± 0.07	0.34 ± 0.04	0.16 ± 0.02

[a]Significantly different from control ($P \leq 0.05$)

Table 34. Clinical chemistry results of adult male mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

		Diet Concentration (ppm)			
Parameter	Week	0	200	2,000	20,000
K	0	2.3 ± 0.20	2.6 ± 0.31	2.8 ± 0.18	2.8 ± 0.43
(mEq/L)	5	2.2 ± 0.20	2.1 ± 0.07	2.9 ± 0.25	3.4 ± 1.16
	10	2.3 ± 0.22	2.2 ± 0.11	2.9 ± 0.19	4.4 ± 1.66
	Term.	2.5 ± 0.39	2.3 ± 0.19	2.6 ± 0.15	3.9 ± 1.28
Na	0	146.4 ± 1.3	146.6 ± 1.5	146.0 ± 0.5	146.4 ± 1.3
(mEq/L)	5	145.2 ± 1.5	144.4 ± 1.4	145.4 ± 0.5	145.0 ± 0.8
	10	148.4 ± 0.9	148.2 ± 1.2	146.2 ± 0.9	149.0 ± 2.3
	Term.	137.8 ± 1.8	136.6 ± 0.7	139.4 ± 1.2	140.2 ± 0.7
Alk Phos	0	131.8 ± 30.0	198.6 ± 19.1	218.4 ± 44.7	177.8 ± 22.4
(U/L)	5	89.8 ± 27.4	109.6 ± 17.9	102.4 ± 11.1	98.8 ± 11.9
	10	63.6 ± 11.6	66.0 ± 7.1	56.2 ± 4.0	84.8 ± 15.8
	Term.	50.8 ± 9.6	57.2 ± 7.0	45.8 ± 2.4	67.0 ± 10.6
CPK	0	466.0 ± 197.6	281.4 ± 38.6	384.0 ± 37.6	279.8 ± 77.2
(U/L)	5	551.0 ± 95.9	255.4 ± 49.2	482.0 ± 152.5	393.4 ± 76.9
	10	641.4 ± 181.7	605.2 ± 78.4	550.2 ± 115.8	432.4 ± 161.4
	Term.	418.4 ± 22.8	341.0 ± 83.2	375.2 ± 83.7	413.6 ± 93.8
Glucose	0	217.0 ± 4.3	245.0 ± 9.1	216.9 ± 4.5	221.4 ± 6.2
(m/dl)	5	200.8 ± 13.0	208.4 ± 12.5	225.6 ± 10.5	213.0 ± 11.0
	10	188.8 ± 7.5	198.2 ± 6.7	193.2 ± 5.3	183.4 ± 3.5
	Term.	196.8 ± 8.3	199.6 ± 6.6	201.6 ± 6.5	205.2 ± 5.2

Table 34. (cont'd).

Parameter	Week	0	200	2,000	20,000
ALT	0	24.8 ± 2.0	21.6 ± 3.3	22.2 ± 6.7	23.8 ± 1.2
(SGPT)	5	24.0 ± 4.3	16.4 ± 4.1	26.8 ± 5.2	29.8 ± 2.7
(U/L)	10	31.2 ± 3.6	26.6 ± 3.2	31.6 ± 4.8	33.8 ± 4.2
	Term.	53.8 ± 27.1	28.4 ± 5.0	32.2 ± 5.4	43.2 ± 3.9
AST	0	10.0 ± 3.5	9.0 ± 1.8	9.0 ± 1.3	10.0 ± 1.4
(SGOT)	5	14.2 ± 2.4	10.0 ± 1.5	12.6 ± 5.1	13.2 ± 3.2
(U/L)	10	18.6 ± 5.9	12.4 ± 1.4	13.4 ± 2.8	14.0 ± 3.1
	Term.	10.6 ± 1.7	7.8 ± 0.4	8.8 ± 2.6	9.8 ± 0.9
Total	0	3.8 ± 0.20	3.6 ± 0.11	3.9 ± 0.14	3.8 ± 0.16
Protein	5	3.7 ± 0.17	3.6 ± 0.14	3.7 ± 0.05	3.6 ± 0.16
(g/dl)	10	4.2 ± 0.23	3.7 ± 0.15	4.0 ± 0.15	4.2 ± 0.07
	Term.	4.1 ± 0.26	3.8 ± 0.25	4.2 ± 0.13	4.0 ± 0.11
Albumin	0	1.8 ± 0.10	1.7 ± 0.07	1.8 ± 0.09	1.8 ± 0.07
(g/dl)	5	1.5 ± 0.13	1.5 ± 0.02	1.6 ± 0.07	1.6 ± 0.11
	10	1.9 ± 0.11	1.5[a] ± 0.08	1.8 ± 0.08	1.9 ± 0.06
	Term.	1.6 ± 0.06	1.6 ± 0.08	1.8 ± 0.11	1.7 ± 0.09
Globulin	0	2.0 ± 0.12	1.9 ± 0.11	2.1 ± 0.12	2.0 ± 0.11
(g/dl)	5	2.2 ± 0.12	2.1 ± 0.15	2.0 ± 0.06	2.0 ± 0.06
	10	2.2 ± 0.21	2.2 ± 0.15	2.2 ± 0.09	2.3 ± 0.06
	Term.	2.5 ± 0.22	2.2 ± 0.26	2.4 ± 0.12	2.3 ± 0.11
Uric Acid	0	5.1 ± 0.8	6.2 ± 1.0	4.6 ± 0.3	6.0 ± 0.6
(mg/dl)	5	4.6 ± 0.6	4.0 ± 0.5	6.2 ± 1.2	4.4 ± 0.2
	10	6.7 ± 2.0	5.8 ± 0.3	6.1 ± 1.4	5.6 ± 0.4
	Term.	7.4 ± 1.4	7.8 ± 0.7	6.7 ± 0.9	5.1 ± 0.9

Table 34 (cont'd).

Parameter	Week	0	200	2,000	20,000
Ca	0	10.4 ± 0.2	10.2 ± 0.4	10.3 ± 0.2	10.0 ± 0.1
(mg/dl)	5	10.1 ± 0.4	10.0 ± 0.2	9.8 ± 0.3	9.8 ± 0.2
	10	10.1 ± 0.3	10.7 ± 0.2	10.0 ± 0.2	10.4 ± 0.3
	Term.	10.6 ± 0.3	10.3 ± 0.3	10.9 ± 0.3	11.0 ± 0.2
Total	0	0.18 ± 0.04	0.14 ± 0.04	0.14 ± 0.04	0.22 ± 0.06
Bilirubin	5	0.28 ± 0.05	0.22 ± 0.02	0.20 ± 0.05	0.20 ± 0.03
(mg/dl)	10	0.26 ± 0.05	0.22 ± 0.05	0.20 ± 0.06	0.26 ± 0.09
	Term.	0.16 ± 0.02	0.26 ± 0.02	0.22 ± 0.06	0.20 ± 0.06

[a]Significantly different from control ($P \leq 0.05$)

Table 35. Hematology results of adult female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

		Diet Concentration (ppm)			
Parameter	Week	0	200	2,000	20,000
Red Blood	0	2.6 ± 0.17	2.7 ± 0.14	2.7 ± 0.15	2.7 ± 0.13
Count	5	3.0 ± 0.23	2.8 ± 0.10	3.0 ± 0.11	2.8 ± 0.07
(10^6cell/mm^3)	10	2.7 ± 0.04	2.6 ± 0.15	2.8 ± 0.15	2.8 ± 0.09
	Term.	2.3 ± 0.14	2.4 ± 0.09	2.3 ± 0.13	2.5 ± 0.17
White Blood	0	13740 ± 5110	17400 ± 6164	10060 ± 2470	10600 ± 2844
Count	5	9520 ± 1715	14880 ± 4269	10740 ± 2922	12780 ± 3127
(cells/mm^3)	10	8475 ± 844	10350 ± 601	10620 ± 1075	10556 ± 3593
	Term.	16340 ± 3225	19020 ± 4835	19700 ± 2710	10720 ± 2042
Heterophils	0	57.6 ± 14.1	40.4 ± 10.8	65.2 ± 8.2	71.8 ± 5.8
+ Eosinophils	5	56.8 ± 6.1	52.2 ± 6.8	48.2 ± 5.1	53.6 ± 6.7
(%)	10	62.8 ± 6.6	63.5 ± 11.5	58.2 ± 11.4	60.0 ± 6.1
	Term.	75.6 ± 6.1	79.6 ± 3.7	81.8 ± 4.4	66.8 ± 8.7
Lymphocytes	0	28.0 ± 12.7	39.6 ± 6.5	26.2 ± 6.3	15.0 ± 5.2
(%)	5	27.8 ± 5.2	30.6 ± 6.0	32.0 ± 5.3	30.2 ± 5.1
	10	20.0 ± 6.2	21.5 ± 8.9	27.0 ± 8.5	30.0 ± 7.0
	Term.	16.0 ± 4.6	12.2 ± 1.4	12.8 ± 4.5	26.8 ± 9.1
Monocytes	0	11.8 ± 2.6	16.0 ± 4.6	5.4 ± 1.6	11.4 ± 1.5
(%)	5	12.4 ± 3.6	13.0 ± 2.4	16.8 ± 2.9	12.4 ± 2.2
	10	13.0 ± 3.3	10.8 ± 4.5	12.8 ± 3.7	8.2 ± 1.9
	Term.	6.8 ± 1.7	4.0 ± 1.4	3.0 ± 0.6	3.6 ± 2.7
Basophils	0	2.6 ± 1.2	4.0 ± 0.9	3.2 ± 1.4	1.8 ± 0.6
(%)	5	3.0 ± 1.3	4.2 ± 1.1	3.0 ± 0.7	3.8 ± 1.1
	10	4.3 ± 0.6	4.3 ± 1.3	2.0[a] ± 0.4	1.8[a] ± 0.5
	Term.	1.6 ± 0.9	4.2 ± 2.6	2.4 ± 0.7	2.8 ± 0.9

Table 35 (cont'd).

Parameter	Week	0	200	2,000	20,000
HGB	0	14.2 ± 0.9	14.8 ± 0.8	14.3 ± 0.8	14.4 ± 0.6
(g/dl)	5	14.6 ± 0.4	14.3 ± 0.4	15.0 ± 0.2	14.8 ± 0.3
	10	15.9 ± 0.6	14.7 ± 0.8	14.8 ± 0.8	14.4 ± 0.5
	Term.	13.2 ± 0.9	13.0 ± 0.5	12.1 ± 0.9	13.7 ± 1.3
Mean Cell	0	166.6 ± 2.8	170.6 ± 2.2	164.2 ± 3.0	161.8 ± 3.2
Volume	5	152.8 ± 10.0	162.0 ± 4.1	159.8 ± 5.4	163.8 ± 2.5
(fL)	10	164.3 ± 3.1	166.0 ± 3.7	163.6 ± 3.2	168.5 ± 5.1
	Term.	150.6 ± 5.2	147.2 ± 9.6	139.4 ± 6.4	152.2 ± 4.5
Mean Cell	0	54.1 ± 1.4	55.4 ± 1.5	52.7 ± 0.9	52.6 ± 2.1
Hemoglobin	5	49.6 ± 3.4	51.6 ± 1.3	50.9 ± 1.4	52.8 ± 1.2
(uug)	10	59.5 ± 3.8	55.5 ± 1.4	53.8 ± 0.9	53.3 ± 1.4
	Term.	57.0 ± 1.6	55.1 ± 2.0	52.7 ± 2.4	53.9 ± 3.2
Mean Cell	0	32.6 ± 0.8	32.5 ± 0.5	32.2 ± 0.9	32.5 ± 0.4
Hemoglobin	5	32.4 ± 0.4	31.9 ± 0.3	31.8 ± 0.5	32.2 ± 0.4
Concentration	10	36.3 ± 2.2	33.3 ± 1.2	32.9 ± 0.6	31.6 ± 1.0
(%)	Term.	38.0 ± 1.6	38.1 ± 2.9	38.0 ± 2.0	36.2 ± 1.8
Packed Cell	0	43.6 ± 2.6	45.6 ± 2.7	44.6 ± 3.1	44.2 ± 1.7
Volume	5	45.0 ± 1.1	45.0 ± 1.6	47.0 ± 0.5	46.0 ± 0.5
(%)	10	44.4 ± 0.9	42.0 ± 2.7	45.0 ± 1.7	46.0 ± 0.9
	Term.	35.0 ± 2.7	34.4 ± 1.3	32.2 ± 3.0	38.8 ± 3.4
Reticulocyte	0	1.5 ± 0.1	1.6 ± 0.3	1.4 ± 0.3	1.2 ± 0.2
Count	5	1.7 ± 0.3	1.2 ± 0.2	1.3 ± 0.4	1.6 ± 0.2
(%)	10	2.5 ± 0.4	2.0 ± 0.4	1.9 ± 0.6	2.8 ± 1.0
	Term.	4.0 ± 0.5	2.7 ± 0.5	4.3 ± 0.7	3.9 ± 1.8

[a]Significantly different from control ($P \leq 0.05$)

Table 36. Hematology results of adult male mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± SE).

		Diet Concentration (ppm)			
Parameter	Week	0	200	2,000	20,000
Red Blood	0	2.9 ± 0.09	2.9 ± 0.05	2.8 ± 0.05	2.9 ± 0.09
Count	5	3.1 ± 0.21	3.0 ± 0.03	2.7[a] ± 0.04	2.7[a] ± 0.07
(10^6cell/mm^3)	10	2.9 ± 0.04	2.9 ± 0.05	2.8 ± 0.07	2.8 ± 0.13
	Term.	2.9 ± 0.11	2.7 ± 0.10	2.7 ± 0.07	2.6 ± 0.12
White Blood	0	10520 ± 1519	8940 ± 1622	10706 ± 1231	9760 ± 2109
Count	5	14340 ± 4557	10100 ± 1621	7280 ± 702	13700 ± 2729
(cells/mm^3)	10	11020 ± 1332	11400 ± 3000	7940 ± 1399	10520 ± 2166
	Term.	16340 ± 3225	19020 ± 4835	19700 ± 2710	10720 ± 2042
Heterophils	0	47.8 ± 5.3	52.2 ± 6.2	53.0 ± 5.7	44.8 ± 5.3
+ Eosinophils	5	55.2 ± 7.3	70.6 ± 1.5	52.8 ± 7.5	52.6 ± 11.7
(%)	10	70.8 ± 4.5	62.8 ± 3.6	67.2 ± 7.6	53.4 ± 3.7
	Term.	62.2 ± 5.3	58.2 ± 8.3	58.6 ± 4.2	68.0 ± 6.5
Lymphocytes	0	35.8 ± 8.2	29.2 ± 5.2	30.4 ± 5.5	38.8 ± 6.8
(%)	5	34.6 ± 7.7	19.0 ± 1.6	31.0 ± 5.9	31.8 ± 10.4
	10	15.8 ± 4.5	22.4 ± 4.1	18.2 ± 8.1	34.6 ± 2.2
	Term.	29.8 ± 4.6	33.8 ± 7.3	35.4 ± 4.7	23.8 ± 7.2
Monocytes	0	15.0 ± 3.4	13.8 ± 4.0	8.8 ± 2.0	13.2 ± 3.1
(%)	5	7.2 ± 1.6	6.6 ± 1.6	12.8 ± 2.7	11.8 ± 2.7
	10	11.4 ± 2.0	11.0 ± 0.7	9.2 ± 2.3	8.8 ± 2.7
	Term.	5.4 ± 1.6	4.8 ± 1.2	4.2 ± 2.0	3.8 ± 1.8
Basophils	0	1.4 ± 0.7	4.8 ± 2.1	7.6 ± 3.4	3.2 ± 0.5
(%)	5	3.0 ± 0.7	3.8 ± 1.0	3.4 ± 0.7	3.8 ± 1.2
	10	2.0 ± 1.3	3.8 ± 1.0	5.4 ± 1.6	3.2 ± 1.2
	Term.	2.6 ± 0.9	3.2 ± 1.4	1.8 ± 0.9	4.4 ± 0.9

Table 36 (cont'd).

Parameter	Week	0	200	2,000	20,000
HGB	0	15.7 ± 0.2	15.4 ± 0.4	14.7 ± 0.3	15.3 ± 0.3
(g/dl)	5	15.0 ± 0.5	14.9 ± 0.1	14.2 ± 0.2	14.0 ± 0.2
	10	15.0 ± 0.6	15.4 ± 0.3	15.0 ± 0.4	14.6 ± 0.6
	Term.	15.5 ± 0.5	15.2 ± 0.8	15.6 ± 0.4	14.2 ± 0.6
Mean Cell	0	167.8 ± 4.3	166.6 ± 0.9	160.2 ± 3.2	161.2 ± 3.2
Volume	5	157.2 ± 10.0	158.4 ± 2.0	169.4 ± 1.9	167.6 ± 4.4
(fL)	10	168.6 ± 3.0	162.8 ± 1.7	165.2 ± 1.3	164.2 ± 1.5
	Term.	170.2 ± 3.2	169.4 ± 2.9	171.8 ± 3.8	172.5 ± 3.9
Mean Cell	0	54.5 ± 1.2	53.8 ± 0.6	53.0 ± 0.9	52.0 ± 1.0
Hemoglobin	5	49.2 ± 3.2	50.1 ± 0.4	53.2 ± 0.6	52.4 ± 0.6
(uug)	10	52.4 ± 2.2	53.6 ± 1.0	54.4 ± 0.5	52.8 ± 0.7
	Term.	54.0 ± 3.1	55.3 ± 1.9	58.2 ± 2.0	54.7 ± 1.8
Mean Cell	0	32.6 ± 0.2	32.2 ± 0.3	33.2 ± 0.3	32.3 ± 0.3
Hemoglobin	5	31.3 ± 0.2	31.7 ± 0.4	31.5 ± 0.3	31.3 ± 0.6
Concentration	10	31.2 ± 1.1	32.9 ± 0.3	32.9 ± 0.4	32.2 ± 0.4
(%)	Term.	31.7 ± 1.7	32.6 ± 0.8	33.9 ± 1.3	31.8 ± 1.2
Packed Cell	0	48.2 ± 0.4	47.6 ± 0.9	44.2[a] ± 1.1	47.4 ± 1.0
Volume	5	48.0 ± 1.9	47.2 ± 0.9	45.0 ± 0.4	44.6 ± 1.0
(%)	10	48.2 ± 0.4	46.8 ± 0.8	45.6 ± 0.9	45.4 ± 1.9
	Term.	49.0 ± 1.9	46.4 ± 1.4	46.2 ± 1.2	44.6 ± 1.6
Reticulocyte	0	1.4 ± 0.2	1.2 ± 0.1	1.5 ± 0.4	1.2 ± 0.2
Count	5	2.0 ± 0.3	1.7 ± 0.3	2.2 ± 0.5	1.6 ± 0.2
(%)	10	2.3 ± 0.5	1.6 ± 0.4	1.9 ± 0.3	2.3 ± 0.5

[a]Significantly different from control ($P \leq 0.05$)

Table 37. Reproductive data from mallard hens fed weathered North Slope crude oil in the diet for 22 weeks.

	Diet Concentration (ppm)			
Parameters	0	200	2,000	20,000
Eggs Laid	730	711	778	616
Eggs Laid/Max Laid*(%)	69.5	72.6	74.1	62.9
Eggs Cracked/Eggs Laid(%)	4.1	1.8	4.0	3.4
Fertile Eggs/Eggs Set(%)	97.4	99.4	94.4	98.4
Viable 21-day Embryos/ Fertile Eggs(%)	94.4	94.2	92.4	93.5
Ducklings/Fertile Eggs(%)	84.5	82.2	84.6	84.0
14-Day Survivors/Hatch(%)	98.7	97.9	98.0	98.6

* Max Laid = the maximum # of egg laying days (70)

Table 38. Reproductive data from mallard hens fed weathered North Slope crude oil in the diet for 22 weeks - eggs set.

	Diet Concentration (ppm)			
Parameters	0	200	2,000	20,000
Eggs Laid	730	711	778	616
Shell-less Eggs Laid	5	0	0	56
Eggs Removed for Thickness/Strength	51	46	52	37
Eggs Cracked	30	13	31	19
Eggs Cracked During Incubation	9	1	3	4
Eggs Set	644	654	695	504

Table 39. Reproductive data from female mallards fed weathered North Slope crude oil for 22 weeks - number hatched.

	Diet Concentration (ppm)			
Parameters	0	200	2,000	20,000
Eggs Laid	730	711	778	616
Eggs Cracked	30	13	31	19
Eggs Set	644	654	695	504
Fertile Eggs	627	650	656	496
Viable 21-day Embryos	592	612	608	464
Ducklings	530	534	555	417
14-day Old Survivors	523	523	544	411

Table 40. Mean egg shell strength, in Newtons (N), of eggs laid by female mallards fed weathered North Slope crude oil in the diet for 22 weeks (Mean ± SE).

Diet Concentration	Mean Egg Shell Strength (N)					
(ppm)	Week 1	Week 3	Week 5	Week 7	Week 9	Total
0	(7)[*] 31.1 ± 1.4	(13) 32.3 ± 0.9	(9) 31.8 ± 1.3	(9) 30.2 ± 1.5	(13) 34.6 ± 0.9	(51) 32.3 ± 0.5
200	(7) 30.0 ± 1.0	(10) 31.4 ± 1.4	(9) 31.5 ± 1.9	(11) 31.1 ± 1.5	(8) 34.3 ± 1.9	(45) 31.6 ± 0.7
2,000	(4) 30.3 ± 1.9	(13) 32.5 ± 1.0	(13) 28.6 ± 1.6	(12) 33.0 ± 0.5	(9) 36.1 ± 0.9	(51) 32.1 ± 0.6
20,000	(4) 29.1 ± 4.2	(10) 29.5 ± 1.5	(9) 29.1 ± 0.9	(6) 26.7 ± 1.8	(5) 33.7 ± 1.7	(34) 29.5[a] ± 0.8

[*]Numbers in parenthesis equal sample size
[a]Significantly different from control ($P \leq 0.05$)

Table 41. Mean egg shell thickness (mm) of eggs laid by female mallards fed weathered North Slope crude oil in the diet for 22 weeks (mean ± S.E.).

Diet Concentration	Mean Egg Shell Thickness (mm)					
(ppm)	Week 1	Week 3	Week 5	Week 7	Week 9	Total
0	(7)* 0.439 ± 0.010	(13) 0.422 ± 0.007	(9) 0.407 ± 0.008	(9) 0.420 ± 0.009	(13) 0.403 ± 0.008	(13) 0.417 ± 0.004
200	(8) 0.430 ± 0.008	(10) 0.424 ± 0.006	(9) 0.419 ± 0.013	(11) 0.410 ± 0.009	(8) 0.404 ± 0.010	(8) 0.417 ± 0.004
2,000	(5) 0.402 ± 0.016	(13) 0.412 ± 0.007	(13) 0.388 ± 0.011	(12) 0.412 ± 0.007	(9) 0.412 ± 0.005	(9) 0.405 ± 0.004
20,000	(4) 0.395 ± 0.026	(10) 0.398 ± 0.011	(9) 0.384 ± 0.011	(7) 0.373[a] ± 0.017	(7) 0.380 ± 0.010	(7) 0.386[a] ± 0.006

*Numbers in parenthesis equal sample size
[a]Significantly different from control ($P \leq 0.05$)

Table 42. Mean initial and 14-day survivor body weights (g) of mallard hatchlings of hens fed weathered North Slope Crude Oil in the diet for 22 weeks (mean ± SE).

	Treatment (ppm)							
	0		200		2000		20000	
Week	Initial	14-Day	Initial	14-Day	Initial	14-Day	Initial	14-Day
1	35.7 ± 0.6	143.0 ± 7.0	36.9 ± 0.7	157.8 ± 5.0	36.1 ± 0.7	147.6 ± 8.0	34.7 ± 0.6	135.3 ± 11.9
2	36.3 ± 0.4	138.1 ± 5.0	37.7 ± 0.5	148.0 ± 5.4	37.2 ± 0.5	160.9[a] ± 7.5	35.2 ± 0.5	172.1[a] ± 7.8
3	34.2 ± 0.6	161.4 ± 4.5	36.1 ± 0.5	189.1[a] ± 4.3	35.7 ± 0.5	180.6[a] ± 4.7	35.1 ± 0.6	186.4[a] ± 3.6
4	33.1 ± 0.5	224.0 ± 5.6	35.9[a] ± 0.5	209.0 ± 4.7	35.2[a] ± 0.5	168.5[a] ± 3.7	35.6[a] ± 0.5	210.4 ± 5.8
5	35.7 ± 0.5	196.1 ± 3.9	39.0[a] ± 0.5	183.7 ± 3.9	35.9 ± 0.8	187.6 ± 4.4	35.9 ± 0.6	186.7 ± 5.1
6	34.6 ± 0.5	179.6 ± 3.7	35.9 ± 0.5	164.5[a] ± 3.2	36.8[a] ± 0.6	191.9[a] ± 3.0	34.5 ± 0.6	178.4 ± 4.1
7	33.3 ± 0.6	187.3 ± 6.2	34.9 ± 0.6	187.9 ± 4.6	36.3[a] ± 0.6	183.8 ± 4.9	36.4[a] ± 0.6	191.6 ± 5.6
8	37.3 ± 0.5	148.7 ± 5.0	36.4 ± 0.7	183.4[a] ± 4.0	37.0 ± 0.7	156.8 ± 5.5	36.9 ± 0.7	193.6[a] ± 6.8
9	35.6 ± 0.7	173.3 ± 5.7	34.0 ± 0.6	170.5 ± 4.7	35.7 ± 0.5	157.6 ± 4.0	37.0 ± 0.5	187.4 ± 6.9
10	35.9 ± 0.5	174.3 ± 4.8	34.8 ± 0.5	170.9 ± 5.3	37.2 ± 0.5	165.4 ± 4.9	36.9 ± 0.5	163.9 ± 6.7

[a] Significantly different from control ($P \leq 0.05$)

Table 43. Mortalities observed in hatchlings of mallard hens fed weathered North Slope crude oil in the diet for 22 weeks.

Diet Concentration (ppm)	#Dead/#Exposed/#Exhibiting Toxic Sign
0	6/529/0
200	10/546/0
2,000	9/557/0
20,000	7/416/0

LIST OF REFERENCES

LIST OF REFERENCES

Ainley, D.G., C.R. Grau, T.E. Roudybush, S.H. Morrell, and J.M. Utts. 1981. Petroleum ingestion reduces reproduction in Cassin's auklets. Marine Poll. Bull. 12:314-317.

Albers, P.H. 1978. The effects of petroleum of different stages of incubation bird eggs. Bull. Environ. Contam. 19:624-630.

------. 1980. Transfer of crude oil from contaminated water to bird eggs. Environ. Research 22:307-314.

------, and M.L. Gay. 1982. Effects of a chemical dispersant and crude oil on breeding ducks. Bull. Environ. Contam. Toxicol. 29:404-411.

Boersma, P.D., E.M. Davies, and W.V. Reid. 1988. Weathered crude oil effects on chicks of fork-tailed storm-petrels (*Oceanodroma furcata*). Arch. Environ. Contam. Toxicol. 17:527-531.

Bourne, W.R.P. 1968. Oil pollution and bird populations. Pages 99-121 in J. D. Carthy and D.R. Arthur, eds. The biological effects of oil pollution on litteral communities. Field Studies Council, London.

Butler, R.G., and P. Lukasiewicz. 1979. A field study of the effect of crude oil on herring gull (*Larus argentatus*) chick growth. Auk 96:809-812.

Cavanaugh, K.P., and W.N. Holmes. 1982. Effects of ingested petroleum on plasma levels of ovarian steroid hormones in photostimulated mallard ducks. Arch. Environ. Contam. Toxicol. 11:503-508.

------, and W.N. Holmes. 1987. Effects of ingested petroleum on the development of ovarian endocrine function in photostimulated mallard ducks (*Anas platyrhynchos*). Arch. Environ. Contam. Toxicol. 16:247-253.

------, A.R. Goldsmith, W.N. Holmes, and B.K. Follet. 1983. Effects of ingested petroleum on the plasma prolactin levels during incubation and on the breeding success of paired mallard ducks. Arch. Environ. Contam. Toxicol. 12:335-341.

Coon, N.C., and M.P. Dieter. 1981. Responses of adult mallard ducks to ingested South Louisiana crude oil. Environ. Research. 24:309-314.

Couillard, C.M., and F.A. Leighton. 1989. Comparative pathology of Prudhoe Bay crude oil and inert shell sealants in chicken embryos. Fundam. Applied Toxicol. 13:165-173.

------, and F.A. Leighton. 1990. The toxicopathology of Prudhoe Bay crude oil in chicken embryos. Fund. Applied Toxicol. 14:30-39.

Crocker, A.D., J. Cronshaw, and W.N. Holmes. 1974. The effects of a crude oil on intestinal absorption in ducklings (*Anas platyrhynchos*). Environ. Pollution. 7:165-177.

------, J. Cronshaw, and W.N. Holmes. 1975. The effects of several crude oils and some petroleum distillation fraction on intestinal absorption in ducklings (*Anas platyrhynchos*). Environ. Physiol. Biochem. 5:92-106.

Eastin, W.C. Jr., and G.C. Murray. 1981. Effects of crude oil on avian intestinal function. Can. J. Physiol. Pharmacol. 59:1063-1068.

------, and B.A. Rattner. 1982. Effects of dispersant and crude oil ingestion on mallard ducklings (*Anas platyrhynchos*). Bull. Environ. Contam. Toxicol. 29:273-278.

Ellenton, J.A. 1982. Teratogenic activity of aliphatic and aromatic fractions of Prudhoe Bay crude and fuel oil no. 2 in the chicken embryo. Toxicol. Appl. Pharmacol. 63:209-215.

Engel, S.E., T.E. Roudybush, J.C. Dobbs, and C.R. Grau. 1978. Depressed food intake and reduced reproduction in Japanese quail after a single dose of Prudhoe Bay crude oil. In Developmental Toxicology of energy related pollutants. Proceedings of the 17th annual Hanford Biology Symposium at Richland, Washington. Technical Information Center. U.S. Department of Energy. eds. D.D. Mahlum, M.R. Sikov, P.L. Hackett and F.D. Andrew.

Fleming, W.J., L. Sileo, and J.C. Franson. 1982. Toxicity of Prudhoe Bay crude oil to sandhill cranes. J. Wildl. Manage. 46:474-478.

Fry, D.M., J. Swenson, L.A. Addiego, C.R. Grau, and A. Kang. 1986. Reduced reproduction of wedge-tailed shearwaters exposed to weathered Santa Barbara crude oil. Arch. Environ. Contam. Toxicol. 15:453-463.

Gorsline, J., and W.N. Holmes. 1981. Effects of petroleum on adrenocortical activity and on hepatic naphthalene-metabolizing activity in mallard ducks. Arch. Environ. Contam. Toxicol. 10:765-777.

------, W.N. Holmes, and J. Cronshaw. 1981. The effects of ingested petroleum on the naphthalene-metabolizing properties of liver tissue in seawater-adapted mallard ducks (*Anas platyrhynchos*). Environ. Research. 24:377-390.

------, and W.N. Holmes. 1982a. Ingestion of petroleum by breeding mallard ducks: some effects on neonatal progeny. Arch. Environ. Contam. Toxicol. 11:147-153.

------, and W.N. Holmes. 1982b. Variations with age in the adrenocortical responses of mallard ducks (*Anas platyrhynchos*) consuming petroleum-contaminated food. Bull. Environ. Contam. Toxicol. 29:146-152.

------, and W.N. Holmes. 1982c. Suppression of adrenocortical activity in mallard ducks exposed to petroleum-contaminated food. Arch. Environ. Contam. Toxicol. 11:497-502.

Gulley, D.D., A.M. Boelter, and H.L. Bergman. 1990. TOXSTAT version 3.2. Fish Physiology and Toxicology Laboratory. Department of Zoology and Physiology. University of Wyoming. Laramie, WY.

Hallet, D.J., F.I. Onuska, and M.E. Comba. 1983. Aliphatic and polyaromatic components of weathered and unweathered Southern Louisiana crude oil. Marine Environ. Research. 8:73-85.

Hartung, R. 1965. Some effects of oiling on reproduction of ducks. J. Wildl. Manage. 29:872-874.

Harvey, S., H. Klandorf, and J.G. Philips. 1981. Reproductive performance and endocrine responses to ingested petroleum in domestic ducks (*Anas platyrhynchos*). Gen. Compar. Endocrinol. 45:372-380.

------, P.J. Sharp, and J.G. Philips. 1982. Influences of ingested petroleum on the reproductive performance and pituitary-gonadal axis of domestic ducks (*Anas platyrhynchos*). Compar. Biochem. Physiol. 72C:82-89.

Hoffman, D.J. 1978. Embryotoxic effects of crude oil in mallard ducks and chicks. Toxicol. Appl. Pharmacol. 46:183-190.

------. 1979a. Embryotoxic effects of crude oil containing nickel and vanadium in mallards. Bull. Environ. Contam. Toxicol. 23:203-206.

------. 1979b. Embryotoxic and teratogenic effects of crude oil on mallard embryos on day one of development. Bull. Environ. Contam. Toxicol. 22:632-637.

Holmes, W.N. 1984. Petroleum pollutants in the marine environment and their possible effects on seabirds. Reviews in Environ. Toxicol. 1:251-317.

------, K.P. Cavanaugh, and J. Cronshaw. 1978a. The effects of ingested petroleum on oviposition and some aspects of reproduction in experimental colonies of mallard ducks (*Anas platyrhynchos*). J. Repro. Fert. 54:335-347.

------, J. Cronshaw, and J. Gorsline. 1978b. Some effects of ingested petroleum on seawater adapted ducks (*Anas platyrhynchos*). Environ. Res. 17:177-190.

------, J. Gorsline, and J. Cronshaw. 1979. Effects of mild cold stress on the survival of seawater-adapted mallard ducks (*Anas platyrhynchos*) maintained on food contaminated with petroleum. Environ. Res. 20:425-444.

Kononen, D.W., J.R. Hochstein and R.K. Ringer. 1986. A quantitative method for evaluating avian food avoidance behavior. Environ. Toxicol. Chem. 5:823-830.

Lambert, G., D.B. Peakall, B.J.R. Philogene and F.R. Engelhardt. 1982. Effect of oil and oil dispersant mixtures on the basal metabolic rates of ducks. Bull. Environ. Contam. Toxicol. 29:520-524.

Lee, Y.-Z., F.A. Leighton, D.B. Peakall, R.J. Norstrom, P.J. O'Brien, J.F. Payne and A.D. Rahimtula. 1985. Effects of ingestion of Hibernia and Prudhoe Bay crude oils on hepatic and renal mixed function oxidase in nestling herring gulls (*Larus argentatus*). Environ. Res. 36:248-255.

Leighton, F.A. 1985. Morphological lesions in red blood cells from herring gulls and Atlantic puffins ingesting Prudhoe Bay crude oil. Vet. Path. 22:393-402.

------, 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.

------, Y.-Z. Lee, A.D. Rahimtula, P.J. O'Brien, and D.B. Peakall. 1985. Biochemical and functional disturbances in red blood cells of herring gulls ingesting Prudhoe Bay crude oil. Toxicol. Appl. Pharmacol. 81:25-31.

Macko, S.A., and S.M. King. 1980. Weathered oil: Effect on hatchability of heron and gull eggs. Bull. Environ. Contam. Toxicol. 25:316-320.

McEwan, E.H., and P.M. Whitehead. 1978. Influence of weathered crude oil on liver enzyme metabolism of testosterone in gulls. Can. J. Zool. 56:1922-1924.

Miller, D.S., D.B. Peakall, and W.B. Kinter. 1977. Crude oil ingestion impairs osmoregulatory and nutrient transport in herring gulls. Fed. Proceed. 36:1008.

Nwokolo, E., and L.O.C. Ohale. 1986. Growth and anatomical characteristics of pullet chicks fed diets contaminated with crude petroleum. Bull. Environ. Contam. Toxicol. 37:441-447.

Pattee, O.H., and J.C. Franson. 1982. Short-term effects of oil ingestion on American kestrels (*Falco sparverius*). J. Wildl. Diseases. 18:235-241.

Patton, J.F., and M.F. Dieter. 1980. Effects of petroleum hydrocarbons on hepatic function in the duck. Compar. Biochem. Physiol. 65:33-36.

Peakall, D.B., D. Hallett, 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.

------, 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. Environ. Res. 27:206-215.

------, D.S. Miller, and W.B. Kinter. 1983. Toxicity of crude oils and their fractions to nestling herring gulls - 1. Physiological and biochemical effects. Marine Environ. Res. 8:63-71.

------, D.A. Jeffry, and D.S. Miller. 1985. Weight loss of herring gulls exposed to oil and oil emulsion. Ambio 14:108-110.

Prince, H.H. 1983. Effects of petroleum on wildlife. Presented at USA/Chechoslovakian Seminar on Toxic Substances and Wildlife, Oct. 3-4, 1983, Strbske Pleso, Czechoslovakia. Mich. Ag. Exp. Station J. Article No. 11140.

Rattner, B.A. 1981. Tolerance of adult mallards to subacute ingestion of crude petroleum oil. Toxicol. Letters 8:337-342.

------, and W.C. Eastin Jr. 1981. Plasma corticosterone and thyroxine concentrations during chronic ingestion of crude oil in mallard Ducks (*Anas platyrhynchos*). Compar. Biochem. Physiol. 68:103-107.

Szaro, R.C. 1977. Effects of petroleum on birds. Transactions of the North American Wildlife and Natural Resources conference. 42:374-381.

------, P.H. Albers, and N.C. Coon. 1978a. Petroleum: Effects on mallard egg hatchability. J. Wildl. Manage. 42:404-406.

------, M.P. Dieter, G.H. Heinz, and J.F. Ferrell. 1978b. Effects of chronic ingestion of South Louisiana crude oil on mallard ducklings. Environ. Res. 17:426-436.

------, N.C. Coon, and W. Stout. 1980. Weathered petroleum: Effects on mallard egg hatchability. J. Wildl. Manage. 44:709-713.

Vangilder, L.D. 1981. Relationships among egg quality, survivorship of newly hatched young, and pollutants in the mallard. Ph.D Thesis, The Ohio State Univ., Columbus. 124pp.

------, and T.J. Peterle. 1982. Impairment of mallard reproduction by dietary crude oil or DDE. XVth International Congress of Game Biologists 17-24 May 1981. Madrid, Spain.

------, and T.J. Peterle. 1983. Mallard egg quality: Enhancement by low levels of petroleum and chlorinated hydrocarbons. Bull. Environ. Contam. Toxicol. 30:17-23.