Exxon Valdez Oil Spill Trustee Council

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STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
AND RESTORATION STRATEGY PLAN

I. COVER PAGE

TITLE: INFLUENCE OF OIL HYDROCARBONS ON REPRODUCTION OF MINK (*Mustela vison*)

STUDY ID NUMBER: TM #6

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Abstract

Influence of Oil Hydrocarbons on Reproduction of Mink (*Mustela vison*)

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The impact of consuming low concentrations of weathered Prudhoe Bay crude oil (WPBCO) on nutrition and reproduction in mink (*Mustela vison*) was investigated. The study was initiated following the Exxon Valdez oil spill for use as a potential model for carnivores living in oil contaminated areas. Commercial mink food contaminated with WPBCO at 1,10,100 and 1000 ppm was of high palatability and no significant decline in food digestibility was noted over a 6 day feeding trial. However, a linear dose dependent decline in total mean retention time of the water-protein and lipid-oil phase of digesta was noted. In addition, a linear dose dependent decline was noted in alimentary fill. These results have negative implications for the absorption of nutrients of low availability, particularly on natural diets where oil contamination exceeds 1000 ppm. Oil hydrocarbon levels in liver and bile increased in a dose dependent fashion and were significantly higher than controls at and above 100 ppm. Short-term (7 day) ingestion of WPBCO at 100 ppm did not affect reproductive performance when contaminated food was ingested during any of four reproductive stages tested: pre-estrus, diapause, pregnancy, and lactation. Similarly, reproduction was not affected in a group of mink fed 100 ppm WPBCO for 120 days

beginning at pre-estrus. Mink remained healthy throughout the trial and few differences were observed in blood analyses between the treatment and control groups. Mink did not discriminate between control food and oiled food, contaminated at 100 ppm.

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PROJECT OBJECTIVE

The ultimate objective of this study was to assess the effect of ingesting feed contaminated with trace levels of weathered Prudhoe Bay Crude Oil (WPBCO) on reproduction in a carnivore (mink) with a known sensitivity to environmental toxicants.

SPECIFIC OBJECTIVES

PALATABILITY/TOXICITY TRIAL

This portion of the study was undertaken to identify the minimum concentration (ppm WPBCO) in feed that produces detectable hydrocarbons in liver and bile without producing clinical illness or significantly ($P<0.05$) reduced palatability.

DIGESTIBILITY AND RATE OF PASSAGE TRIALS

These studies examined the behavior of trace amounts of dietary WPBCO on digestive functions, specifically dry matter digestibility and the rate of food passage through the alimentary tract.

7-DAY INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL

The hypothesis that short-term (7-day) ingestion of a low level of WPBCO during pre-estrus, diapause, pregnancy or lactation does not significantly ($P<0.05$) affect reproduction in female mink was tested.

120-DAY INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL

The hypothesis that continuous, low-level ingestion of WPBCO beginning in pre-estrus and extending through weaning of kits does not significantly ($P<0.05$) affect reproduction in female mink was tested.

FEED DISCRIMINATION TRIAL

This study was conducted to determine whether mink would discriminate against feed contaminated with trace levels of WPBCO.

INTRODUCTION

Mink are carnivorous mammals inhabiting the margins of streams, lakes, marshes, and marine islands throughout most of North America. In northern, temperate regions mating occurs in March and kits (average 5 per litter) are born in late April or early May following an average 51 day gestation (Enders, 1952; Sundqvist et al., 1989). Gestation length is affected by a variable period of delayed implantation (Enders, 1952; Sundqvist et al., 1989).

Mink are at the top of the food chain and thus exposed to a wide variety of environmental contaminants. Studies with ranched mink have documented a marked sensitivity to many chemical and biological compounds (Sundqvist et al., 1989). Some of those known to interfere with reproduction include heavy metals, halogenated hydrocarbon pesticides and other aromatic, halogenated hydrocarbons (Ringer, 1981; Sundqvist et al., 1989). In the mid-1960s, a decline in reproductive performance in ranched mink (Hartsough, 1965) was eventually traced to high polychlorinated biphenyl (PCB) content of Great Lakes fish used in commercial mink diets (Aulerich et al., 1971; Aulerich et al., 1973). Ranched mink fed PCBs (2-5 ppm), polybrominated biphenyls (1-2.5 ppm), or hexachlorobenzene (5-25 ppm) suffered complete reproductive failure, significantly reduced litter size and/or excessive kit mortality (Ringer et al., 1972; Aulerich and Ringer, 1977; Aulerich and Ringer, 1979; Bleavins et al., 1980; Ringer, 1981; Hornshaw et al., 1983; Rush et al., 1983;

Bleavins et al., 1984; Aulerich et al., 1985; Wren et al., 1987). Particularly dangerous are compounds like PCBs that accumulate in the subcutaneous fat (Hornshaw et al., 1983).

Mink inhabiting the coastal area of Prince William Sound, Alaska, feed on fish, small mammals, frogs, aquatic insects, and occasionally birds. It is highly probable that this population will be exposed to petroleum hydrocarbons originating from the Exxon Valdez oil spill. Crude oil released into the environment is immediately subjected to a variety of weathering processes (Payne and McNabb, 1984). Within a few weeks of an oil spill the majority of the more toxic, lower molecular weight compounds are eliminated primarily through evaporation. However, heavier distillate products not subject to significant evaporative loss persist in the environment and are likely to enter the food chain in significant quantities (Payne and McNabb, 1984). Because of the sensitivity of mink to environmental toxicants, they will be used as a model species to study the impact of consuming low concentrations of weathered Prudhoe Bay crude oil on reproduction in carnivores.

STUDY METHODOLOGY

WEATHERED PRUDHOE BAY CRUDE OIL (WPBCO)

Prudhoe Bay crude oil, taken from the Exxon Valdez, was subjected to laboratory evaporation at room temperature using gentle agitation for a 7 day period (Appendix A). Gas chromatography on a sample of the laboratory weathered oil was performed to ensure that there were no hydrocarbons lighter than n-C_{10} (Appendix A). This is similar to the hydrocarbon composition of oil following 2 weeks of natural weathering processes (Payne and McNabb, 1984).

PALATABILITY/TOXICITY TRIAL

Twenty-five yearling, female mink (Source: Oregon State University Fur Farm, Corvallis, OR) were randomly assigned to one of five treatment groups (n=5 per group). Treated groups were fed either 1, 10, 100, or 1000 ppm WPBCO, diluted in salmon oil and mixed in commercial mink ration (Northwest Fur Breeders Coop, Edmonds, WA). Control groups received an equivalent amount of salmon oil in their ration (10 ml/kg)(Appendix B). Samples of oiled food were frozen for hydrocarbon analysis as a quality control. Animals were weighed the day before the oil-feeding trial began and again at the end of the treatment period (6 days). Mink were fed 225 g of the prepared diet each morning. The following morning all remaining food was removed, including food spilled or cached in the nest box, and weighed to provide an estimate of daily food consumption. After 6 days, 2 randomly-

selected mink from each group were euthanized and necropsied (Appendix C). Detection of hydrocarbons in the tissues (bile and liver) was used to confirm oil ingestion by the animals and acted as a quality control. In addition, hematology, serum biochemical profiles and histopathology were used to evaluate potential physiological and morphological changes resulting from the oil treatment (Appendix D). These studies were conducted between Jan 8-18, 1990.

DIGESTIBILITY ESTIMATES

During the preliminary palatability/toxicity trial all feces were collected daily, weighed and stored frozen. Samples were thawed, mixed and sub-sampled to determine dry matter (DM) content. Likewise, daily food samples were stored frozen, then subsequently thawed, combined and sub-sampled for DM content.

Estimates of the cumulative fresh food eaten and fresh feces produced, over a 3 day period, were converted to a daily DM intake using the average DM contents of food and feces.

Dry matter digestibility was estimated as:

$$\text{DM digestibility (DMD, \%)} = \frac{\text{DM intake} - \text{DM output}}{\text{DM intake}} * 100$$

Thus, a single DMD estimate was obtained for each animal composing the control and treatment groups.

RATE OF PASSAGE

Rate of passage of the food water-protein and the lipid-oil phases were estimated using a single dose of ^{51}Cr-EDTA to determine the water-protein phase (Holleman and White 1989) and tritiated glycerol-triether (^{3}H-GTE) to measure the lipid-oil phase (Carlson and Bailey 1972; Roby et al., 1989).

A known quantity of marker was given to each mink in a small food sample following 4 hours without food. All mink consumed the entire offered dose. Mink were then given their usual meal, and feces were collected at 0.5h intervals for 10h, at 2h intervals for another 10h and at 6h intervals for an additional 52h, giving a total collection period of 72h. Fecal samples were weighed and representative sub-samples taken for assay of ^{51}Cr by gamma spectroscopy (Holleman and White 1989). The ^{3}H-GTE in feces was recovered by extraction with hexane and ^{3}H was assayed by liquid scintillation counting. To provide standards for radioisotope assay of ^{51}Cr, representative food samples were used to dilute ^{51}Cr-EDTA.

Total mean retention time (TMRT) of each marker was estimated as described by Holleman and White (1989). TMRT is given as the ratio of two integrals derived from the fecal specific radioactivity (SR, cpm/g fecal DM) excretion curves, viz;

$$TMRT = {}_{0}\int^{\infty} T.SRt.dt \; / \; {}_{0}\int^{\infty} SRt.dt$$

where the numerator is the area under the curve obtained by

multiplying each fecal SR at time T (SRt) by its respective time since dosing (T). The denominator is the area under the SR versus time curve for feces.

The theoretical nondigestible fill (Vn, g DM) of the alimentary tract was estimated from feces output (DM output, g/d) and TMRT (h) of the lipid-oil marker ^{3}H-GTE (Holleman and White 1989) viz.;

$$Vn = (\text{DM output} / 24) \cdot \text{TMRT}$$

When DM is absorbed linearly within the alimentary tract, then the total alimentary fill (F, g DM) is given by (Holleman and White 1989);

$$F = Vn + ((Vn \cdot DMD/100) / 2(1-DMD/100))$$

Dry matter absorbed in the alimentary tract was expressed on a daily basis (DMA g/d), i.e.;

$$DMA = \text{DM intake} - \text{DM output}$$

And during the mean time a meal resides in the gut (A, g) viz.;

$$A = ((\text{DM intake} - \text{DM output}) / 24) \cdot \text{TMRT}$$

7-DAY INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL

Before the start of the breeding season, yearling, female mink were randomly assigned to a control group (n=20) or a pre-estrus treatment group (n=19). These females and all others in the colony (110 total) were bred between March 3-26 using untreated, yearling males (n=30; Source: Oregon State University

Fur Farm, Corvallis, OR). The breeding schedule (Appendix E) was designed to maximize litter size by taking advantage of superfetation and reduce effects of natural male infertility by breeding the females to 2 or 3 different males. Unassigned, mated females (n=48) were randomly allocated to the remaining groups: diapause, pregnancy, and lactation (n=16 each).

Based on the results of the palatability/toxicity trial, animals in treatment groups were fed 100 ppm WPBCO (appendix B). Control animals were fed the commercial mink ration with 10 ml/kg salmon oil. The housing, care, feeding and maintenance of all animals are outlined in Appendix F.

Representative samples of treated and untreated diet were periodically frozen for hydrocarbon analysis as a quality control. The treatment diet was fed for a period of 7 days as follows: pre-estrus, Feb 26-Mar 4; diapause, Mar 26-Apr 1; pregnancy, Apr 13-Apr 19; and lactation, beginning the third day after delivery of kits. These treatment times were chosen to correspond with potentially sensitive periods during the female reproductive cycle. Daily food consumption was determined for control and treated groups during each 7 day trial.

All females were weighed prior to the start of the study (Feb 20, 1990) at the end of the breeding season (March 26, 1990), in mid-pregnancy (Apr 10, 1990), in late pregnancy (Apr 25, 1990) and at necropsy (throughout June and July).

Beginning April 28, nest boxes were checked each morning for the presence of kits. Litters found in the morning were counted

and weighed that afternoon, and again at 2, 4, and 6 weeks and at necropsy (7 weeks). Kits remained with their dams throughout the study and were weaned on to commercial mink ration.

Reproductive variables were mating success, determined from observations of successful breeding during March; gestation length, defined as the number of days from the first successful mating to parturition (this period encompasses a variable period of delayed implantation (diapause) as well as true pregnancy); whelping success, defined as the successful delivery of 1 or more live kits; the total number of kits per litter; the number of live kits per litter, recorded within the first 48 hours; the mean litter birth weight; the number of kits surviving to weaning; kit growth rate from birth to weaning.

At 7 weeks post-partum dams and kits were euthanized (Appendix C). Blood samples were taken via cardiac puncture with the animals under ketamine anesthesia prior to euthanasia by CO_2 administration. Complete necropsies were done on all females. Kits from treatment groups were euthanized and partial necropsies (physical exam, weight and collection of liver, kidney and gonads for histology) were conducted on a randomly selected male and female kit from each litter. Twenty remaining kits from the control group were not euthanized and were returned to individual cages.

120-DAY INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL

Twenty females were randomly allocated to a treatment group fed 100 ppm WPBCO daily from February 20 until they were euthanized in July. This study was run in parallel with the 7-day study and used the same control group. Breeding and care of the treated females in this study was identical to, and carried out in association with, the 7-day trial. Food consumption in the 120-day group was measured at pre-estrus, diapause, pregnancy and lactation in conjunction with the other treatment groups. Kits from the 120-day treated females were weaned on to the same treated mink food as their dams. At 7 weeks post-partum dams were euthanized and necropsied (Appendix C&D). In addition, one male and one female kit from each litter were randomly selected for euthanasia and partial necropsy as described above. Twenty kits were returned to individual cages and maintained on the treated diet.

FEED DISCRIMINATION TRIAL

Ten male mink were offered, daily for 10 days, 150 g of the control diet and 150 g of treatment diet containing 100 ppm WPBCO in 10ml/kg salmon oil. One of the diets contained food color to enable identification of treated and untreated food. The diet containing food color was alternated daily. Food consumption was estimated by subtracting the uneaten portion removed from the cage the following day. This study was conducted in July 1990.

DATA ANALYSIS

Data were analyzed using a statistical program for personal computers (SOLO version 2.0, BMDP statistical software and Sigma Stat version 2.0, Jandel Scientific) either by One-way ANOVA (normally distributed data) Kruskal-Wallis ANOVA (non-normally distributed data), t-test, or two sample proportion test (non-parametric) as appropriate.

STUDY RESULTS

PALATABILITY/TOXICITY TRIAL

The addition of 1-1000 ppm WPBCO to the diet of female mink did not affect daily food consumption ($P > 0.05$, Fig. 1), or the total amount of food consumed over a 6 day trial ($P > 0.05$, Fig. 2). There was no significant difference in weight loss (all groups lost weight) associated with the oiled diet over the trial period ($P > 0.05$, Fig. 3).

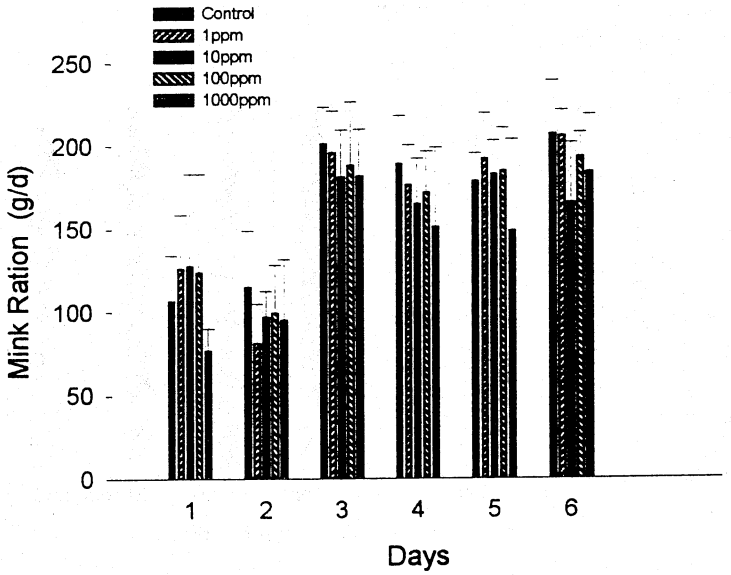

Fig. 1. Mean (± sd) daily food consumption for each treatment group. No differences (P>0.05) were found between treatment groups and controls

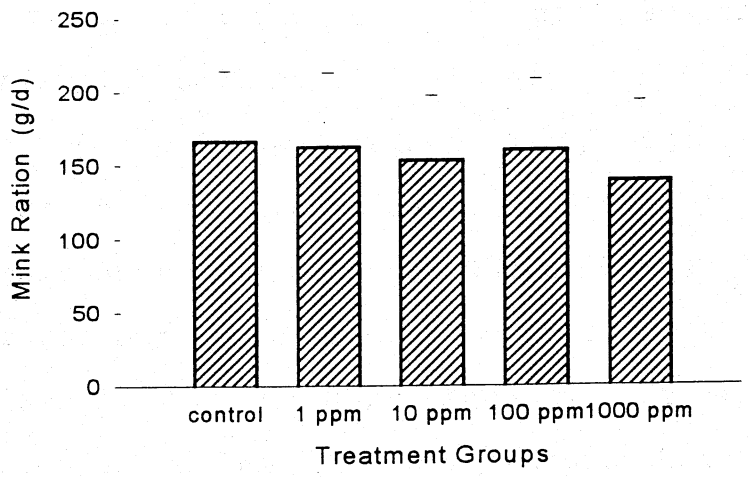

Fig. 2. Mean (± sd) total food consumption for each treatment group over the 6 day pretrial . No differences (P>0.05) were found between treatment groups and controls.

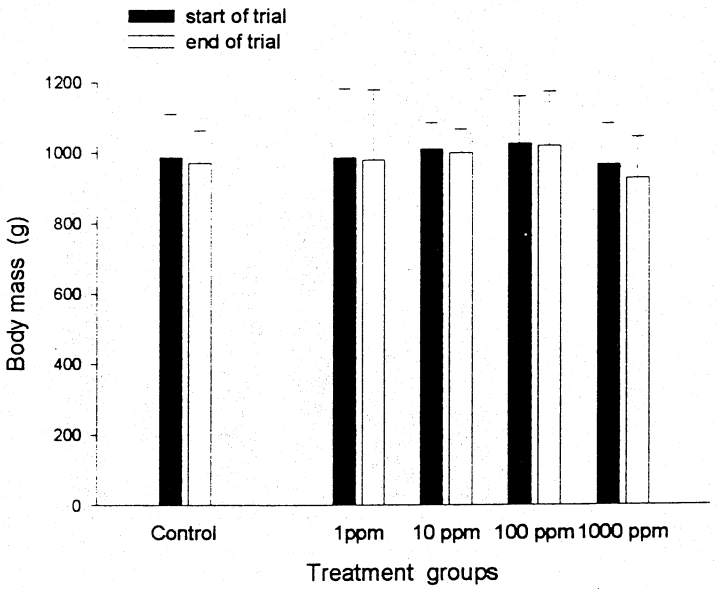

Fig. 3 Mean (± sd) body mass of female mink at the beginning and end of the 6 day pretrial trial. All groups lost body mass but the difference was not significant (P>0.05) between treatment and controls.

Significantly elevated levels of hydrocarbons could not be measured in feed samples containing 1 or 10 ppm WPBCO. Similarly these diets did not result in significant levels of hydrocarbons in liver or bile. Diets containing 100 or 1000 ppm WPBCO did result in significant levels of hydrocarbons in both bile and liver that reflected the level of food contamination (Table 1; Fig. 4&5). At no time during the study did any of the mink exhibit clinical symptoms indicative of oil toxicity. Hematology and serum chemistry profiles did not differ between treated and control groups and no pathological lesions were associated with any treatment level.

Table 1. Hydrocarbons were measured as a pristane to phytane ratio (PRIS/PHY) in feed samples and liver and as a carbon preference index (CPI) in liver only. Declining PRIS/PHY ratio and CPI are indicative of petroleum contamination. Increasing levels of Phenanthrene (PHN) and napthalene (NPH), measured in bile, indicate oil contamination. Liver PRIS/PHY and CPI data were log transformed for analysis (T-test comparisons between treatments and controls, BMDP - Solo), **P<0.05; ***P<0.001.

	FEED	LIVER		BILE	
treatment	PRIS/PHY (N=3) X ± SD	PRIS/PHY (N=5) X ± SD	CPI (N=5) X ± SD	PHN (N=5) Ng/ml X ± SD	NPH (N=5) Ng/ml X ± SD
Control	256.9 ± 10.9	232.9 ± 148.9	12.97 ± 10.28	1,100 ± 190	13,000 ± 5,500
1 ppm	242.9 ± 22.3	379.1 ± 558.5	10.8 ± 2.13	1,100 ± 220	8,000 ± 3,500
10 ppm	210.0 ± 49.3	85.5 ± 22.58	8.8 ± 4.0	1,500 ± 720	10,000 ± 4,000
100 ppm	54.7 ± 7.2***	41.0 ± 4.2**	5.6 ± 2.2	8,200 ± 1060***	34,000 ± 5,200***
1000 ppm	8.8 ± 1.6***	6.5 ± 0.9***	3.4 ± 2.3**	55,600 ± 20,020**	144,000 ± 55,500**

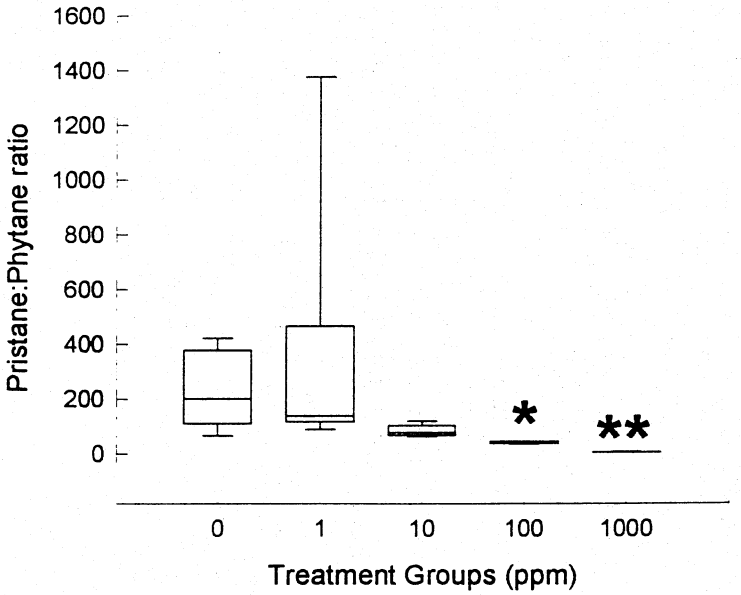

Fig. 4. The pristane to phytane ratio was used as a measure of liver hydrocarbons (n=2/group). A decreasing ratio is indicative of hydrocarbons. Significant differences were found at the 100 ($P<0.05$) and 1000 ($P<0.001$) ppm treatment levels.

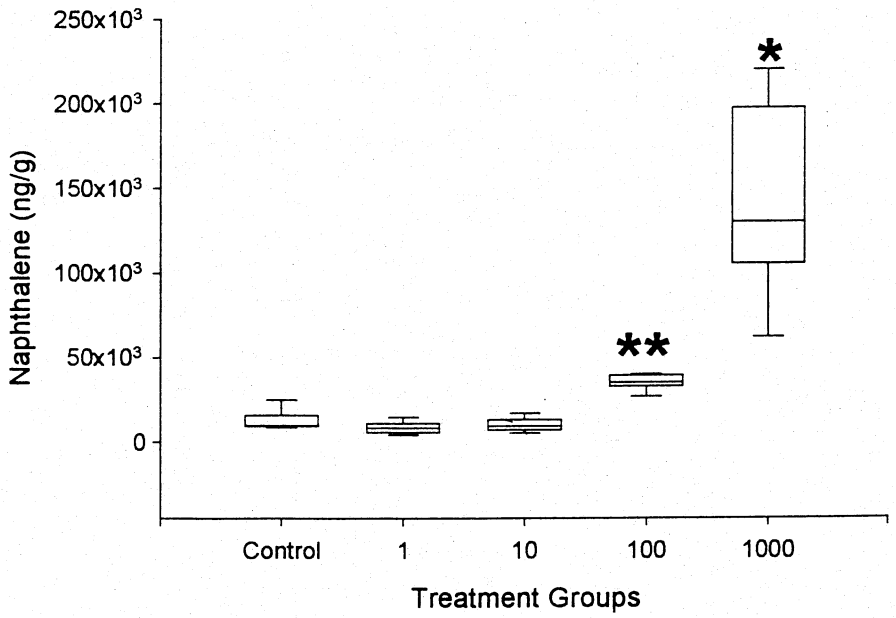

Fig. 5. Increasing napthalene in mink bile (n=2/group) indicates the presence of hydrocarbons. Differences are significant at the 100 ($P<0.001$) and 1000 ($P<0.05$) ppm treatment levels.

DIGESTIBILITY AND RATE OF PASSAGE TRIAL

Food intake: Inclusion of weathered crude oil resulted in a voluntary food DM intake that was individually variable but was not significantly ($P>0.05$, AOV) affected by treatment (Table 2).

Apparent DMD: The treated mink ration (DMD = 76 $\pm$ 2.3%) lowered the DMD by 3 to 6 digestibility units (Table 2). Increasing levels of oil were not associated with a change in DMD, i.e. there was no dose effect. At the highest levels of crude oil intake (54 - 73 mg/d at 1000 ppm) the contribution of unabsorbed oil to feces output was negligible (0.31 - 0.41% of feces DM).

Table 2. Mean ($\pm$ sem) daily food intake (g DM) and apparent dry matter digestibility of food (%) by mink consuming either a control ration or a ration containing WPBCO (n=3 mink/group)

Treatment	Daily Dry Matter Intake	Apparent Dry Matter Digestibility
Control	83 $\pm$ 3.4	76 $\pm$ 2.3
1 ppm	74 $\pm$ 4.3	70 $\pm$ 1.7
10 ppm	67 $\pm$ 10.7	71 $\pm$ 3.1
100 ppm	83 $\pm$ 0.8	73 $\pm$ 3.6
1000 ppm	66 $\pm$ 5.9	72 $\pm$ 1.9

DM absorption: Inclusion of oil had a negligible effect on daily amount of DM absorbed, however a significant reduction in response to oil dose ($r= -0.609$, $n=12$, $P<0.05$) was noted when absorption of DM was corrected to the TMRT of the GTE marker.

Rate of passage: Both the aqueous and lipid-oil markers were excreted as a major event following a transit time in the

alimentary tract of approximately 5h. This major event was followed by an exponential decline of marker concentration in feces for a further 15h. In some animals a small secondary peak in marker concentration (<1/100 of primary peak) was noted after 15h.

a) Aqueous phase: TMRT of the aqueous phase increased from 4.2 $\pm$ 0.14 h to 5.1 $\pm$ 0.28 ($P>0.05$) at an oil dose of 1 ppm (Table 3). The dose response curve showed a linear decline in TMRT (Y, h) with increasing oil contamination from 1 to 1000 ppm (X, ppm) given by equation [1](Fig. 6).

[1] $Y = 4.8 - 0.191 \ln X$ $\quad r = 0.630$ $(n=12)$ $P<0.025$

b) Lipid-oil phase: TMRT of the lipid-oil phase was 6.1 $\pm$ 0.80 h (Table 3). Food contaminated with 1 ppm WPBCO resulted in no change in the TMRT of ^{3}H-GTE. The dose response curve showed a linear decline in TMRT (Y, h) with increasing oil contamination (X, ppm) given by equation [2] (Fig. 6). The lowest TMRT (3.5 $\pm$ 0.22 h) at 1000 ppm was significantly lower than controls ($P<0.05$).

[2] $y = 6.2 - 0.395 \ln X$ $\quad r = 0.826$ $(n=12)$ $P<0.001$

At 1000 ppm, TMRT of the aqueous and lipid-oil phases were identical indicating no differentiation in the way the two food components are handled within the alimentary tract. In the control ration, the lipid component was retained longer ($P<0.1$) than the aqueous-protein phase of the food. Thus inclusion of crude oil in the ration differentially changed the way protein

and lipid phases functioned in the alimentary tract, resulting in separate dose response curves (Fig. 6).

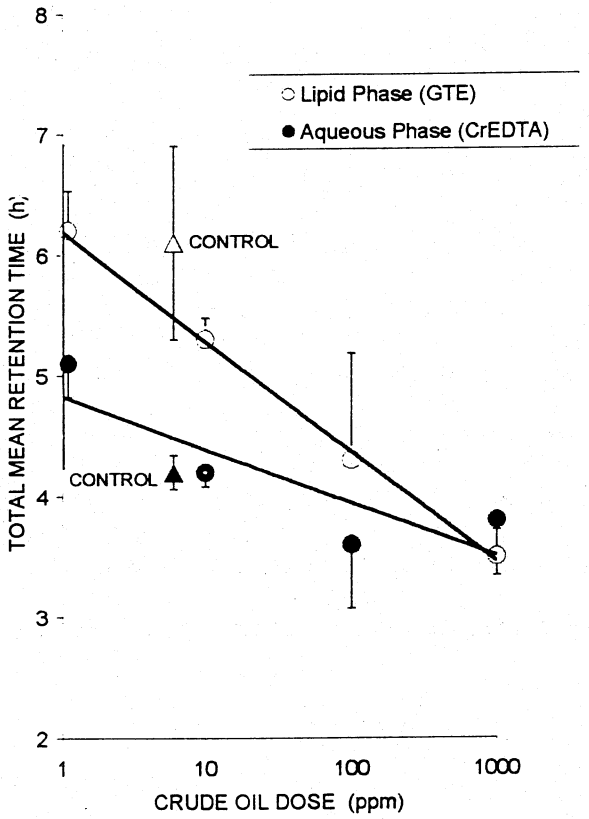

FIG. 6. Dose response curve for the effect of crude oil on digesta rate of passage (TMRT) in mink. Changes in total mean retention time of the lipid-oil and aqueous-protein phases of the ration are shown in relation to the dietary concentration of weathered crude oil. Triangles are means (±sem) for controls. Equation [1] and [2] in text give lines of best fit for the two phases. Values represent means (n=3; ± sem).

Table 3. Effect of contamination of food with WPBCO on rate of passage as a total mean retention time (TMRT (h), mean ± sem) of the aqueous and lipid-oil phases. Each mink was given a single meal containing ^{51}Cr-EDTA and ^{3}H-GTE to mark the aqueous and lipid-oil phases of the diet (n=3 mink/group).

Treatment	TMRT aqueous	TMRT lipid-oil
Control	4.2 ± 0.14	6.1 ± 0.80
1 ppm	5.1 ± 0.28	6.2 ± 0.33
10 ppm	4.2 ± 0.12	5.3 ± 0.18
100 ppm	3.6 ± 0.53	4.3 ± 0.88
1000 ppm	3.8 ± 0.46	3.5 ± 0.22

At a dose of 10 to 1000 ppm, mink in this study lowered theoretical fill of nondigestible DM. Nondigestible DM fill (Y, g) declined with intake of crude oil (X, ppm) as given by equation [3] (Fig. 7).

[3] $Y = 5.7 - 0.403 \ln X$ $r = 0.59$ $(n=12)$ $P<0.05$

Total alimentary fill also showed a dose response as given by equation [4] (Table 4, Fig. 7).

[4] $Y = 12.3 - 0.821 \ln X$ $r = 0.62$ $(n=12)$ $P<0.025$

where Y=total alimentary fill (gDM) and X=oil dose (ppm). Differences in slopes and elevations of the regression lines (Fig. 7) show that the increasing oil ingestion differentially reduced fill of the digestible over the indigestible component of gut fill.

Table 4. Effect of inclusion of dietary WPBCO on apparent dry matter absorption and alimentary fill (mean ± sem) in domestic mink (n=3 mink/group).

Treatment	Apparent Dry Matter Absorption		Alimentary fill
	g/d	(g/residence time)	(g)
Control	63 ± 4.4	11 ± 1.0	14 ± 0.3
1 ppm	52 ± 10.1	11 ± 2.6	12 ± 2.2
10 ppm	47 ± 6.3	8 ± 0.9	10 ± 1.9
100 ppm	60 ± 3.0	9 ± 1.3	10 ± 2.1
1000 ppm	48 ± 4.8	8 ± 1.5	6 ± 0.7

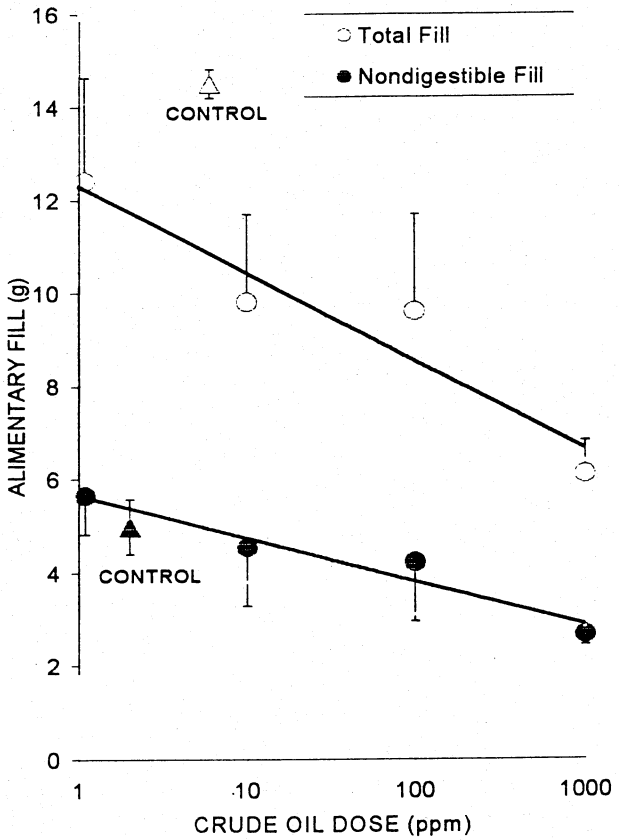

FIG. 7. Dose response curve for the effect of crude oil on theoretical alimentary fill in mink. Triangles are means (±sem) for control mink. Equation [3] and [4] in the text give lines of best fit for Vn and F versus dose of oil. Values represent means (n=3; ±sem).

7-DAY AND 120-DAY INGESTION OF WEATHERED PRUDHOE BAY CRUDE OIL

Because the 7-day and 120-day studies were conducted simultaneously using the same control group, data from the two studies have been combined.

Twenty-seven mink were removed from the experiment, 23 of these due to illness unrelated to the experimental protocol (Appendix G). Twenty-one mink were diagnosed with lactational anemia during their sixth week of nursing, a common condition

seen in ranched mink. One animal died prior to whelping with a very similar clinical appearance to lactational anemia and one other mink had an infection (22% bands) at the end of the experiment.

With the exception of the lactational anemia, which was independent of treatment, all animals remained clinically healthy. The total red blood cell count for the lactation group was significantly ($P<0.05$) lower than controls. Lactate dehydrogenase (LDH) for two treatment groups (pregnant and 120-day) was significantly higher ($P<0.05$) than the control. No significant difference ($P<0.05$) was seen in any of the other parameters measured in the complete blood count or the serum chemistry profile (Table 5 & 6). The blood parameters are presented individually to accommodate comparison of treatment groups with the control group.

There were no significant findings in any mink at post-mortem examination or during histopathological assessment of tissues.

Neither food consumption (Fig. 8) nor weight changes in the dams varied significantly with treatment. All dams typically lost weight during the suckling period (Fig. 9).

Table 5. Complete blood count (mean ± sd) for mink in the control group and each of the treatment groups.

Parameter	Treatment Groups (n) means ± sd					
	Control (14)	120-Day (14)	Pre-estrus (15)	Diapause (13)	Pregnancy (12)	Lactation (11)
Red Blood Cells[1] (10^6/ml^3)	8.61 ± 0.68	8.30 ± 0.48	8.63 ± 0.54	8.64 ± 0.72	8.30 ± 0.71	*7.83 ± 0.44
Hemoglobin[1] (g/dl)	16.99 ± 1.00	16.36 ± 1.06	17.18 ± 1.14	17.22 ± 1.28	16.41 ± 1.00	15.81 ± 0.78
Hematocrit[1] (vol%)	52.63 ± 3.77	50.99 ± 3.37	52.75 ± 3.18	53.55 ± 4.60	51.31 ± 3.77	49.50 ± 3.12
Mean Corpuscular Volume[1] (femtoliters)	61.31 ± 2.73	61.94 ± 2.57	60.76 ± 3.30	62.00 ± 2.37	62.04 ± 3.02	63.27 ± 3.02
Mean Corpuscular Hemoglobin[2] (pgs)	19.78 ± 0.88	19.79 ± 0.79	19.91 ± 0.53	19.85 ± 0.62	19.88 ± 0.84	20.14 ± 0.75
Mean Corpuscular Hemoglobin Conc[2] (g/dl)	32.34 ± 1.08	32.05 ± 0.88	32.60 ± 1.05	31.4 ± 2.42	32.03 ± 0.93	32.06± 1.17
Red Cell Distribution Width[2] (%)	16.39 ± 1.50	15.92 ± 1.03	16.09 ± 0.91	15.80 ± 0.62	16.30 ± 1.29	16.16 ± 0.92
Platelets[1] (10^3/ml^3)	660.86 ± 146.70	738.40 ± 81.10	639.07 ± 157.77	640.46 ± 165.41	715.42 ± 138.67	616.46 ± 259.43
Mean Platelet Volume[1] (femtoliters)	8.73 ± 1.09	8.89 ± 0.86	8.99 ± 1.14	9.05 ± 0.64	8.93 ± 0.78	8.60 ± 1.18
White blood Cells[2] (10^3/ml^3)	5.56 ± 1.65	5.13 ± 1.33	5.80 ± 3.88	5.81 ± 2.49	5.51 ± 2.01	6.22 ± 1.24
Neutrophils[2] (10^3/ml^3)	3.50 ± 1.80	3.23 ± 1.19	3.85 ± 2.98	3.75 ± 2.03	3.42 ± 1.45	3.95 ± 0.65
Lymphocytes[2] (10^3/ml^3)	1.80 ± 1.02	1.55 ± 0.82	1.77 ± 1.05	1.72 ± 0.87	1.72 ± 1.33	2.03 ± 0.86
Monocytes[2] (10^3/ml^3)	0.07 ± 0.05	0.11 ± 0.07	0.09 ± 0.08	0.11 ± 0.10	0.14 ± 0.14	0.10 ± 0.07
Eosinophils[2] (10^3/ml^3)	0.19 ± 0.18	0.23 ± 0.24	0.09 ± 0.14	0.23 ± 0.39	0.20 ± 0.28	0.16 ± 0.12

[1] Normal data with equal variance. Analysis by One Way Analysis of Variance. Paired treatment vs control group analyzed using Tukey Test.

[2] Normality test failed. Analysis by Kruskal-Wallis One Way Analysis of Variance. Paired treatment vs control group analyzed using Dunn's method.

* Significantly different from the control group $p<0.05$; power of the test = 0.707.

Basophils were not detected in any mink except 1 individual from the control group (relative count = 1%).

Bands were not detected in any mink except 1 individual from the pregnancy group (relative count = 1%).

Table 6. Serum biochemical profiling (mean ± sd) for mink in the control group and each of the treatment groups.

Parameter	Treatment Groups (n) means ± sd					
	Control (15)	120-Day (15)	Pre-estrus (15)	Diapause (14)	Pregnancy (12)	Lactation (11)
Sodium[1] (mEq/L)	143.33 ± 3.40	144.33 ± 3.48	143.00 ± 5.66	144.21 ± 3.96	143.42 ± 3.55	144.73 ± 2.15
Potassium[1] (mEq/L)	4.05 ± 0.35	4.24 ± 0.36	4.2 ± 0.34	4.1 ± 0.30	4.25 ± 0.25	4.28 ± 0.32
Chloride[1] (mEq/L)	108.87 ± 3.72	110.27 ± 3.60	109 ± 5.50	109.07 ± 3.32	108.75 ± 2.45	109.73 ± 2.57
Calcium[1] (mg/dl)	9.23 ± 0.55	9.25 ± 0.56	9.2 ± 0.36	9.34 ± 0.45	9.41 ± 0.37	9.27 ± 0.41
Phosphorus[1](mg/dl)	3.79 ± 1.12	4.16 ± 0.92	3.76 ± 0.91	3.87 ± 1.22	4.08 ± 0.90	5.52 ± 0.80
Blood Urea Nitrogen[1] (mg/dl)	31.6 ± 13.31	28.47 ± 7.62	32.4 ± 11.61	31.07 ± 10.16	27.83 ± 5.51	31.73 ± 13.34
Creatinine[2] (mg/dl)	0.33 ± 0.09	0.33 ± 0.10	0.34 ± 0.07	0.34 ± 0.10	0.34 ± 0.07	0.31 ± 0.07
Total Bilirubin[2] (mg/dl)	0.27 ± 0.1	0.34 ± 0.07	0.33 ± 0.19	0.35 ± 0.09	0.34 ± 0.11	0.39 ± 0.19
Direct Bilirubin[2] (mg/dl)	0.25 ± 0.09	0.33 ± 0.08	0.31 ± 0.2	0.33 ± 0.11	0.32 ± 0.13	0.36 ± 0.22
Total Protein[2] (g/dl)	6.11 ± 0.31	6.12 ± 0.22	6.173 ± 0.32	6.32 ± 0.34	6.42 ± 0.39	6.12 ± 0.22
Albumin[2] (g/dl)	3.17 ± 0.24	3.15 ± 0.13	3.12 ± 0.21	3.25 ± 0.16	3.26 ± 0.15	3.09 ± 0.16
Albumin:Globulin[2]	1.09 ± 0.15	1.07 ± 0.06	1.05 ± 0.08	1.04 ± 0.06	1.04 ± 0.08	1.01 ± 0.08
Cholesterol[2] (mg/dl)	273 ± 70	269 ± 35	273 ± 30	285 ± 43	304 ± 65	310 ± 41
Alkaline Phosphatase[2] (IU/L)	147.48 ± 65.03	146.80 ± 40.69	134.87 ± 31.40	123.21 ± 27.39	133.83 ± 41.49	128.46 ± 31.98
GGT[2] (IU/L)	4.67 ± 1.59	4.80 ± 2.24	5.33 ± 2.77	4.14 ± 0.36	4.67 ± 2.31	5.55 ± 2.91
Aspartate transaminae[2] (IU/L)	114.93 ± 131.34	99.87 ± 48.48	141.47 ± 136.68	219.64 ± 302.15	101.75 ± 33.27	107.36 ± 41.71
Alanine transaminase[2] (IU/L)	284.40 ± 489.40	122.07 ± 96.32	187.33 ± 145.86	205.86 ± 268.23	166.25 ± 164.14	183.46 ± 159.68
Lactate dehydrogenase[2] (IU/L)	624.33 ± 240.28	*1164.33 ± 396.57	1005.33 ± 599.55	1135.86 ± 726.58	*1289.42 ± 440.95	1044.00 ± 447.10
Creatine kinase[2] (IU/L)	309.47 ± 136.14	613.93 ± 451.79	443.40 ± 329.92	387.57 ± 259.31	500.58 ± 199.08	547.27 ± 335.12
Glucose[1] (IU/L)	95.67 ± 19.63	88.07 ± 23.62	92.00 ± 34.52	97.64 ± 21.79	97.75 ± 24.84	91.55 ± 22.55

[1] Normal data with equal variance. Analysis by One Way Analysis of Variance. Paired treatment vs control group analyzed using Tukey Test.

[2] Normality test failed. Analysis by Kruskal-Wallis One Way Analysis of Variance. Paired treatment vs control group analyzed using Dunn's method.

* Significantly different from the control group $p<0.05$.

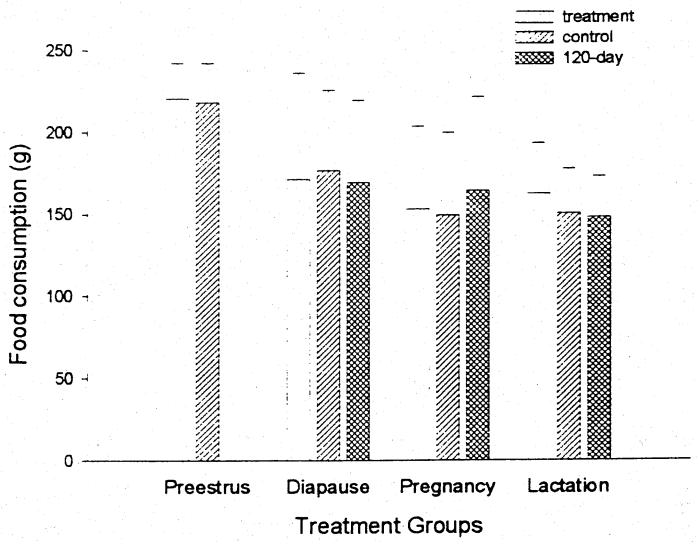

Fig. 8. Mean (± sd) daily food consumption over each 7 day trial representing critical stages during the reproductive cycle. There were no differences (P>0.05) between groups in the amount of food eaten at each trial period.

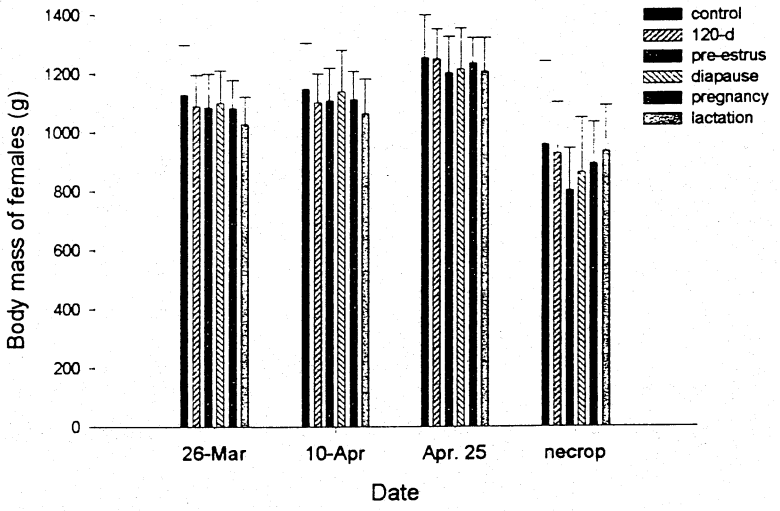

Fig. 9. Mean (±sd) body mass of dams in each treatment group starting with the end of the breeding season (Mar 26), early pregnancy (Apr 10), late pregnancy (Apr 25), and necropsy (7 weeks after whelping). There was no significant difference (P>0.05) between the treatment and controls at any stage.

There was no difference in the percentage of females successfully mated in the pre-estrus group (100%, n=19) or the 120-day group (95% n=19) compared to controls (95% n=20). The length of total gestation (diapause/pregnancy) between treatment and control groups did not vary (Table 7).

Whelping success was not affected by oil ingestion (P>0.05, Table 7). There was no difference in the number of kits per litter (P=0.47), the number of live kits per litter (P=0.67) or the survival of kits to weaning (P>0.05).

There was no significant difference in kit birth weight (P=0.24) and weight at 2 (P=0.35), 4 (P=0.63) and 6 weeks (P=0.30)(Fig. 10).

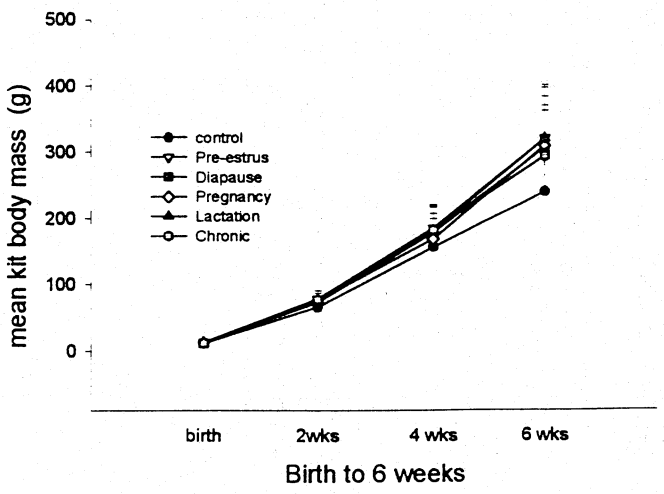

Fig. 10. Mean (± sd) kit body mass for each treatment group. Values were obtained by weighing each litter and dividing by litter size. Weights were taken at 2 week intervals. There was no significant difference (P>0.05) between treatment groups and controls.

Table 7. The number of females whelping, mean number of kits per litter, live kits per litter and total litter weight for all treatment groups.

Treatment	Mated Females Whelped % (n)	Gestation Length (mean ± sd)	Mean number of kits /litter	Mean number live kits/litter	Litter Weight (mean ± sd)
Control	89 (19)	54.8 ± 5.0	6.6	6.3	61.8 ± 25.3
Pre-estrus	79 (19)	54.7 ± 3.6	5.6	5.4	60.3 ± 15.4
Diapause	88 (16)	54.6 ± 4.3	5.5	5.3	58.0 ± 28.3
Pregnancy	81 (16)	55.7 ± 5.5	5.0	4.8	55.4 ± 30.4
Lactation	75 (16)	54.7 ± 4.1	6.5	6.3	63.9 ± 20.8
120-Day	83 (18)	54.7 ± 4.8	5.9	5.8	60.0 ± 18.1

FEED DISCRIMINATION TRAIL

There was no difference (P=0.14) in the total amount of oiled (941 g/mink) and unoiled (743 g/mink) food consumed in a ten-day period, and mink did not show a preference for food containing food color (p=0.30, 880 g red food/mink vs. 825 g uncolored food/mink). There was an apparent sampling of the two foods, with most mink consuming primarily oiled diet on some days and mainly unoiled diet on other days, rather than eating equal quantities of both diets daily (Fig. 11).

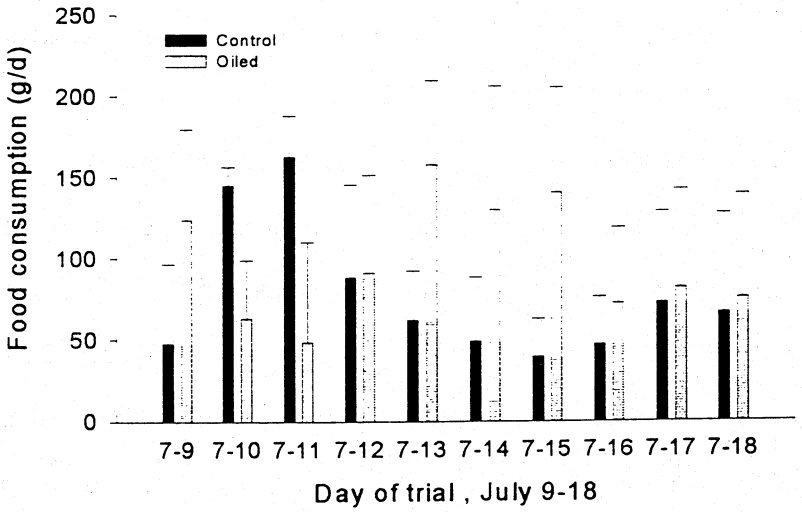

Fig. 11. Mean (±sd) daily food consumption of oiled and unoiled mink food offered simultaneously to 10 male mink for 6 days. The mink showed no consistent preference for one diet over the other.

DISCUSSION

Mink readily consumed feed contaminated with 1-1000 ppm WPBCO over a 6 day period. The dose-related levels of hydrocarbons detected in liver and bile at 100 and 1000 ppm confirmed oil consumption and absorption of hydrocarbons. Body weight did not vary significantly and the mink remained clinically healthy over the course of treatment. When offered 100 ppm oiled and unoiled food simultaneously, mink did not discriminate between the treated or untreated diet. Although these results are specific to the experimental conditions used here, it is highly probable that wild mink will similarly consume feed contaminated with trace amounts of weathered oil. It is also equally probable that ingestion of trace amounts of weathered oil, such as used in this study, will not cause any clinical symptoms in the short term.

The daily amount of food DM absorbed showed an 8 to 25% decrease at 1, 10 and 1000 ppm treatments. The decrease in absorption was attributed to a combination of responses. A slight decrease in DMD was combined with a significant increase in food passage rate. For the control animals, during the mean time that food resides in the alimentary tract, approximately 11g DM is absorbed. Although an oil intake of 0.052 mg (1 ppm in food) does not affect this absorption function, at intakes of 0.470 mg (10 ppm) to 48 mg (1000 ppm) absorptive function is reduced by 24%, i.e. to 8-9 g per mean residence time. The functional response of the mink to oil ingestion was to lower

alimentary fill. Presumably mink ate more frequently in order to maintain a normal daily DM intake.

Contamination of the ration with increasing amounts of crude oil has negative implications for absorption of nutrients despite no significant decline in DMD. The effect would be particularly important to those nutrients that may be limiting in the diet (e.g. carbohydrates and calcium in the wild) or those nutrients that are not absorbed by active transport (e.g. most macro-minerals).

For limiting nutrients, and those absorbed by passive diffusion, a reduced residence time would result in lower net absorption and there is no means of adapting by producing more transporter or carrier proteins in order to compensate for the low availability.

Lactational anemia was diagnosed in the control and all treatment groups and is considered a husbandry problem independent of oil ingestion. Except for individuals removed from the study due to this disease, all remaining mink were considered healthy based on the lack of gross and microscopic lesions and evaluation of clinical pathology. Interpretation of a clinical pathology panel requires evaluating the entire panel as a unit. Isolating parameters may lead to inappropriate conclusions (see Duncan et. al., 1994). The study was not designed to identify subtle effects of oil on mink clinical pathology. Due to the high inherent variability in hematology and serum biochemistry, subtle effects can only be identified with a large sample size. Although

statistically significant, the slightly lower mean red blood cell count for the lactation group and the slightly higher lactate dehydrogenase levels in the pregnancy and 120-day groups was not associated with any adverse health or reproductive effect and is not clinically meaningful.

The relative lack of clinical impact, and ready ingestion of trace amounts of WPBCO parallels the situation of hexoclorobenezene (HCB) and polyclorinated biphenyl (PCB) contamination of mink food in the Great Lakes Region (Rush et al., 1983; Aulerich et al., 1973). However, in these studies, trace amounts of HCBs and PCBs (1-5 ppm) had serious, negative effects on reproduction. This contrasts markedly with the results found in this study. Trace amounts of WPBCO ingested at critical times during the reproductive cycle (pre-estrus, diapause, pregnancy and lactation) had no measurable impact on any of the reproductive parameters investigated. From these data it appears that short term ingestion (7 days) of feed contaminated with WPBCO will not impair reproduction, regardless of the timing of that exposure. Ingestion of 100 ppm WPBCO continuously for 120 days similarly did not have a negative effect on reproduction.

No conclusions can be made regarding the fitness of animals consuming contaminated feed longer than 4 months. These data do not address the effects of volatile components in fresh crude oil on intake, digestion and reproduction. Problems arising from repeated exposure to weathered crude oil over a longer time frame were not investigated and remain a distinct possibility. Although

offspring from both the controls and the 120-day treated females were maintained on their respective diets, funding was terminated before studies on the F_1 generation could be undertaken.

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APPENDIX A

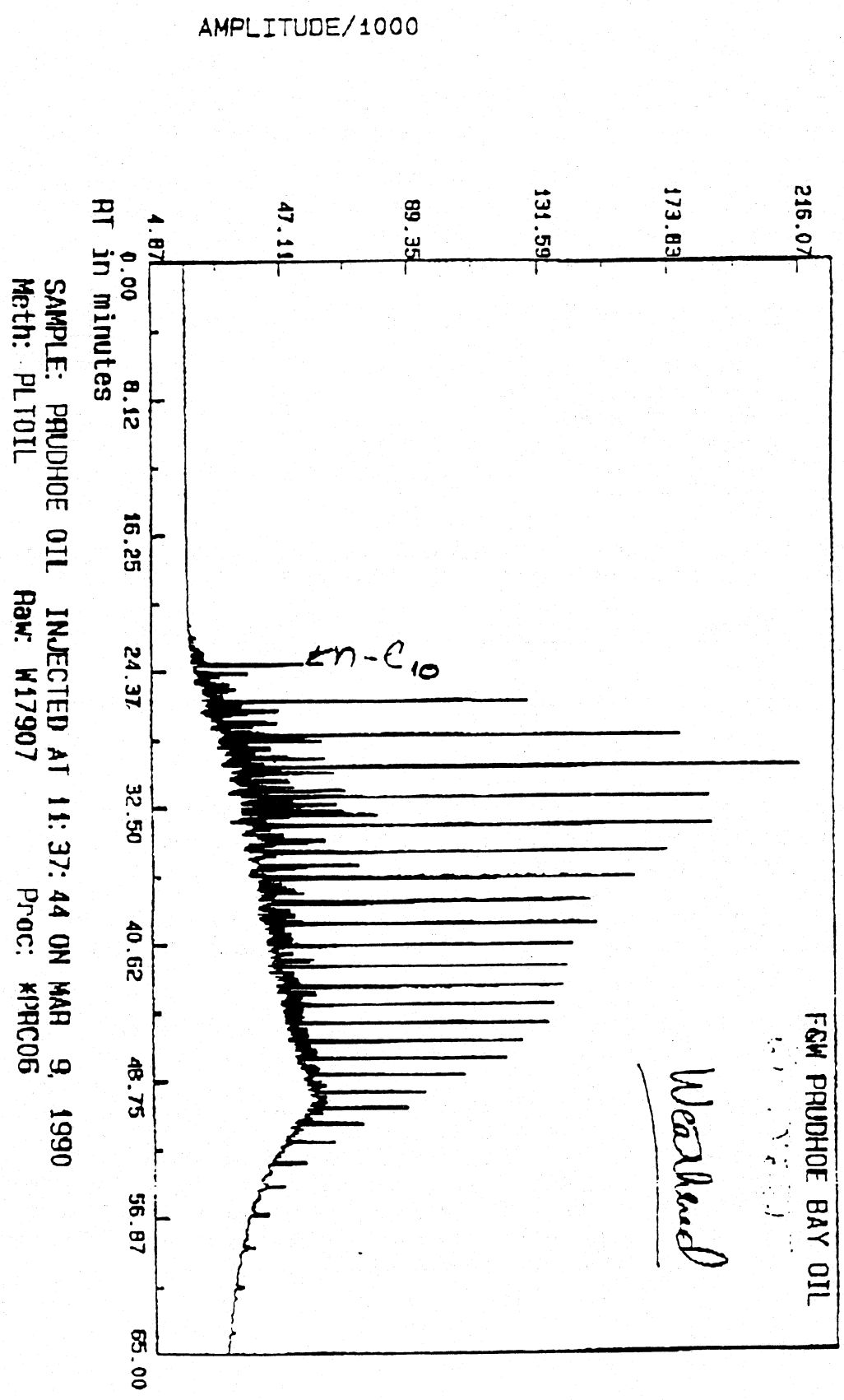
LABORATORY WEATHERING OF PRUDHOE BAY CRUDE OIL

SOURCE:

Prudhoe Bay Crude Oil from the Exxon Valdez will be obtained from Dr David Shaw, Institute of Marine Science, University of Alaska Fairbanks.

- 1 liter of Prudhoe Bay crude oil will be placed in a flat bottomed, solvent-rinsed vessel to a depth no greater than 2cm.
- The oil will be gently agitated in a fume hood for 7 days (168 hours) at room temperature (20C).
- To characterize the oil, gas chromatography will be performed at the end of 168 hours (Payne, and McNabb, 1984). The objective is to approximate 2 weeks of weathering, thus all hydrocarbons lighter than n-C_{10} must be removed from the oil (Payne, and McNabb, 1984)(Fig. A1).
- A sample of the final, laboratory weathered product will be submitted to the Analytical Chemistry Technical Team for characterization.

Laboratory weathered oil will be stored in sealed glass containers and stored in the dark at room temperature until used in the experiment.


AMPLITUDE/1000
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173.83
131.59
89.35
47.11
4.87
0.00
8.12
16.25
24.37
32.50
40.62
48.75
56.87
65.00
RT in minutes
F&M PRUDHOE BAY OIL
Weathered
n-C10
SAMPLE: PRUDHOE OIL INJECTED AT 11:37:44 ON MAR 9, 1990
Meth: PLTOIL
Raw: W17907
Proc: *PRC06

APPENDIX B

CONTAMINATION OF MINK FEED

1. PALATABILITY/TOXICITY TRIAL

The specific gravity of WPBCO is 1.0, based on the weight of 1 ml of WPBCO. Dilutions are calculated on a weight:weight basis.

a. A stock solution of 1:100 WPBCO will be prepared at the beginning of the trial. This solution will be used in the preparation of the 1 ppm, 10 ppm, and 100 ppm oil-contaminated feed needed for the 7 days of the study.

WPBCO 1:100 (1 ml WPBCO diluted to 100 mls with salmon oil):

1. add approximately 50 mls salmon oil to cleaned volumetric cylinder.
2. Add 1 ml WPBCO.
3. QS to 100 ml with salmon oil.
4. Place in waring blender and mix on medium speed for 1 minute.

b. Each day 1.3 kg oil-contaminated feed must be prepared for each treatment group following the table below.

Dose (ppm)	1:100 stock	salmon oil (ml)	mink feed(kg)
0	0	13	1.3
1	130 ul	12.8	1.3
10	1.3 ml	11.7	1.3
100	13 ml	0	1.3

For these dilutions, 5 ml salmon oil is added to a 25 ml graduated cylinder. Add WPBCO. QS to 13 ml. Mix thoroughly and add to feed.

For 1000 ppm, add 1.3 mls WPBCO (not the 1:100 diluted stock) to 11.7 ml salmon oil. Mix thoroughly and add to feed.

c. Mix oil and feed (thawed out the previous day) in Hobart mixer at medium speed for 5 minutes.

d. To prevent contamination of the various diets, the control feed should be mixed in separate glassware and Hobart dishes(bucket 1). The 1ppm followed by the 10ppm should be mixed in bucket 2, and the 100 ppm and 1000 ppm should be mixed in bucket 3. Diets are always mixed from low to high dose. Separate glassware should be used for each dilution to avoid cross-contamination.

e. Glassware and Hobart containers should be thoroughly washed with soap and water. Washing should be done from low dose to high dose.

2. 7-DAY AND 120-DAY INGESTION STUDIES

a. All animals will be fed diets containing 10 ml salmon oil/kg feed, except when they are being fed their specific oil-tainted diet. The salmon oil will be mixed with the feed (237 ml salmon oil/50 lb bag feed) using a paint-drill mixer.

b. Animals on oil-contaminated feed are to be fed mink ration containing 100 ppm WPBCO and 10 ml salmon oil/kg. A stock of contaminated solution (1:100 WPBCO:salmon oil)will be made by diluting 10 ml WPBCO in 1 liter salmon oil. The stock will be stored in the refrigerator. The appropriate amount of oil-contaminated feed will be mixed daily by adding 10 ml stock solution to 1 kg of feed. This will be mixed in a Hobart mixer on medium speed for 5 minutes.

APPENDIX C

EUTHANASIA, GROSS NECROPSY AND COLLECTION OF HISTOLOGICAL SPECIMENS

Euthanasia of mink will be done by exposure to carbon dioxide under the supervision of a licensed veterinarian. Procedures will follow the standards designated by the American Veterinary Medical Association.

A large number of animals will be euthanized starting June 30 (weaning). Euthanasia will be scheduled in accordance to the speed in which necropsies and sample collection can be done. This will enable the pathologist and prosectors to collect tissue and fluid samples from mink immediately after death.

GROSS NECROPSY:

Each dead mink will be assigned an Institute of Arctic Biology, Pathology Accession number. Standard necropsy protocol will be performed, or supervised, by a board eligible veterinary pathologist. All necropsies will be performed at the Institute of Arctic Biology, University of Alaska Fairbanks. Each anatomical system will be examined and any gross lesions will be recorded on the pathology record sheet. Between each necropsy the stainless steel necropsy surface, cutting board and all instruments will be cleaned and rinsed following the procedures outlined in the Hydrocarbon Analysis.

COLLECTION OF HISTOLOGICAL SPECIMENS:

Samples for histological examination will be collected using standard technique. Tissues will be cut to a maximum of $1cm^3$ and placed in 10% buffered formalin. Tissue samples for histopathology will be stored in leak-proof, screw-top containers and labeled:

OIL SPILL TM6
MINK ID:_____
PATHOLOGY #_____
DATE: _____

Containers will be double labeled: 1) outside labeling using stick-on labels with information recorded using an indelible marker,; and 2) a water-resistant card label inside the jar with data written in pencil.

The following samples will routinely be collected:

heart (3 sections)
lung (3 sections)
stomach
duodenum
jejunum
ileum
colon
pancreas
liver
spleen
mesenteric lymph node

retropharyngeal lymph node
adrenal gland
thyroid gland
pituitary gland
brain (left side)
psoas muscle
section from quadriceps
skin (face and thoracic)
bone marrow

Male:
testicles
accessory glands

Female:
uterus (2 sections)
ovaries

COLLECTION OF SAMPLES FOR HYDROCARBON ANALYSIS

Samples for hydrocarbon analyses will be collected during the necropsy.

A. Source of samples

1. All blood and tissue samples will be collected from animals just after euthanasia.
2. All fecal samples will be collected from solvent rinsed tin foil in catch trays below the pens of caged mink.

B. Materials for collecting and storing samples

1. Blood samples

New needles, syringes and vacutainers will be used for each blood sample collected. Vacutainers will contain heparin, EDTA or no additive as appropriate.

2. Tissue samples

All tissue samples will be collected from freshly killed animals. Instruments used will be solvent rinsed (rinsed with acetone followed by hexane) prior to use and between animals. Tissues will be wrapped in solvent-rinsed aluminum foil (as per State/federal damage assessment plan for analytical chemistry, quality assurance/quality control). All persons handling tissues will wear disposable gloves, to be changed between animals.

3. Fecal collection

Prior to collection, the catch trays beneath the animal pens will be lined with solvent rinsed tin foil. Fecal matter will be picked up with solvent rinsed instruments, and wrapped in solvent rinsed aluminum foil.

C. Storage of samples

1. Blood

All samples will be processed as soon after collection as possible, by centrifuging to collect serum or plasma. Serum and plasma will be stored frozen in fresh vacutainers without additives. CBCs will be analyzed soon after collection.

2. Tissue and fecal samples

All tissue and fecal samples will be stored frozen at the University of Alaska Institute of Arctic Biology until turned over to the appropriate agency.

D. Identification of samples

All samples will be clearly labeled on the outside of the solvent rinsed tin foil wrapper (time tape) in waterproof ink. Each tin foil wrapped sample will then be sealed inside a whirl-pak^{R} with a cardboard label. Scientific name of species, project and experiment number, animal number, date of collection, type of tissue, and initials of collecting individual will identify each sample.

HYDROCARBON ANALYSIS

Specimens will be archived at the University of Alaska Fairbanks and an inventory will be provided to the Analytical Chemistry Technical Team (Everett Robinson-Wilson, USF&W). Upon receipt of instructions, specified samples for hydrocarbon assay will be sent by courier to a laboratory designated by the Analytical Chemistry Technical Team(Trustee Council, 1989).

APPENDIX D

CLINICAL PATHOLOGY

Immediately after death, blood will be collected from each mink by cardiac puncture with a 12cc syringe and a 20g, ½inch needle. Blood will be placed directly into:

1. a 3ml lavender top Monoject^{R} blood collection vial (containing EDTA) for a complete blood count (CBC).
2. a 10ml red top Monoject^{R} blood collection vial for subsequent separation of serum for clinical chemistry.

** NOTE: Hemolysis and subsequent spuriously elevated serum potassium and phosphorus were encountered during the palatability/toxicity experiment. We therefore make the following modification to the blood collection procedure: Each animal will be anesthetized with ketamine hydrochloride (0.05-0.1mg/g) and blood will be collected by cardiac puncture as described above. The animal will then be placed in a gas anesthetic chamber for euthanasia by carbon dioxide (see Appendix C) and subsequent necropsy.

Lavender top tubes and red top tubes will immediately be submitted to the Fairbanks Memorial Hospital Laboratory for complete blood count and serum chemistry as described below.

Complete Blood Count (CBC):
Blood smears will be prepared and stained with Wright's-Giemsa for a differential count performed by a hospital laboratory technician. The remainder of the CBC will be performed on a Coulter S+4 counter. The CBC includes: total white blood cell count, total red blood cell count, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, total platelet count, mean platelet volume, and differential count. Red cell morphology is assessed during the differential count.

Serum Chemistry:
Serum is separated from the clot and the chemistry panel is run using a Kodak Ektachem 700. The panel includes: Sodium, Potassium, Chloride, Calcium, Phosphorus, Blood Urea Nitrogen (BUN), Creatinine, Cholesterol, Total and Direct Bilirubin, Total Protein, Albumin, Albumin:Globulin Ratio, Gamma-glutamyltransferase (GGT), Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate Dehydrogenase (LDH), and Creatinine Phosphokinase (CPK).

APPENDIX E

BREEDING SCHEDULE

PROCEDURE:

The mink will be bred using a 1+8+9 day breeding schedule recommended for yearling mink (Murphy, 1983; Friend and Crampton, 1960).

- Females will be put with the males for the first time on the 2-3 of March (Dr. B. Murphy will supervise initial breeding).
- Females in the control and pre-estrus groups will be randomly assigned prior to the breeding season. Only mated females will be assigned to the remaining 3 groups.

Breeding procedure: Males will be locked out of their nest boxes prior to breeding sessions and a female placed in the male's cage. The initial response of the animals will be watched and recorded. All efforts will be made to leave the animals undisturbed during this 30 minute period (a non-breeding female will be removed from the male's cage only if serious fighting occurs).

Following breeding, a vaginal smear will be taken to confirm mating and check the fertility of the males.

Eight days after her first breeding, the female will be mated with a second male and to a third male the following day (day 9).

Females mated for the first time after Mar 17 will be mated on a 1+1 schedule.

Each male will be used a maximum of 2 times on any given day (AM & PM). Males with high breeding success will be used preferentially.

Individual records will be kept for each female detailing the number of males with which she has been bred and mating success.

APPENDIX F

DAILY FEEDING AND CARE

Mink will be held in individual wire cages with attached wooden nest boxes containing bedding material (hay or straw). Cages will be stacked in 2 tiers with catch trays under each wire cage. The cages will be located outdoors in a fenced, roofed facility. Food will be offered in stainless steel bowls either in the nest box (when on special diets) or in the wire cage. The mink will have ad lib access to water via an automatic watering system.

Individual animal health will be checked daily. Animals, which appear dull or listless, have a matted coat, ocular or nasal discharge, signs of bleeding, reduced appetite, diarrhea, or constipation will be checked more thoroughly by the University veterinarian.

Fecal catch trays will be cleaned twice weekly, and nest boxes weekly (more often if needed). The facility will be swept and otherwise cleaned as needed. Lights will go on at 9:00 AM and off at 4:00 PM. Natural lighting will be allowed to exceed artificial light. Any sampling after dark will be done under red light. Animals will live at ambient temperatures to -30^{0}C, at which time auxiliary heaters will be used.

Animal Welfare

The University of Alaska Fairbanks (UAF) has on file with the Office for Protection Against Research Risks (OPRR), National Institutes of Health, an "Assurance of Compliance with Public Health Service Policy on Humane Care and Use of Laboratory Animals". This University's Animal Facilities are licensed by the United States Department of Agriculture and are subject to twice yearly, surprise inspections by USDA veterinarians to ensure compliance with the Animal Welfare Act.

UAF has a full-time, staff veterinarian who supervises the veterinary care program as outlined in the Regulations of the Animal Welfare Act. This University also has an Institutional Animal Care and Use Committee (IACUC) mandated by Public Health Service Policy and the Animal Welfare Act. This project was reviewed and approved, without modifications, by this committee.

APPENDIX G: ANIMALS DELETED FROM EACH TREATMENT FOR THE 7- AND 120-DAY OIL INGESTION EXPERIMENTS

GROUP	#	REASON	WT	Na	K	Phos	BUN	Cr
CONT	025	dead-LA*	764	---	---	---	---	---
	047	dead-LA	490	---	---	---	---	---
	049	sick-LA	506	107	9.4	10	152	0.3
	070	feeding error	---	---	---	---	---	---
	100	dead-LA	453	---	---	---	---	---
	108	sick-LA	642	114	7.4	15.1	215	1.3
120D	048	dead-LA	513	---	---	---	---	---
	075	sick-LA	634	108	8.3	31.5	641	4.5
	101	dead-LA	576	---	---	---	---	---
	102	died before whelping	---	185	6.2	14.4	191	1.8
	103	dead-LA	762	---	---	---	---	---
PREE	012	feeding error	---	---	---	---	---	---
	034	dead-LA	738	---	---	---	---	---
	038	sick-LA	546	113	7.8	12.3	269	1.6
	039	sick-LA	531	120	6.7	8.2	159	0.8
	107	ate all 14 kits	1013	147	3.7	4.2	19	0.3
DIAP	002	sick-22% bands	785	148	4.3	4.4	34	0.3
	062	sick-LA	527	116	6.3	4.4	75	0.7
PREG	028	sick-LA	594	113	6.7	7.8	229	0.3
	069	sick-LA	458	---	---	---	---	---
	072	sick-LA	530	126	4.5	7.8	187	1.2
	074	sick-LA	675	103	9.4	22.5	473	5.6
LACT	017	sick-LA	641	120	8.3	31.2	579	7.3
	032	serum hemolyzed	1050	140	8.6	8.5	29	0.3
	044	sick-LA	583	111	7.1	9.3	143	1.1
	090	sick-LA	488	113	6.2	10.4	268	1.7
	109	sick-LA	525	102	9.3	16.6	380	2.0

*LA = LACTATIONAL ANEMIA