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for

Office of Biological Services

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Abstract

Mallard ducks fed 0.25, 1.0, 2.5, 3.0, or 5.0 percent South Louisiana crude oil exhibited decreases in egg production, fertility, and hatchability in proportion to the concentration of oil. Extremely low amounts of petroleum hydrocarbons were also toxic to fertile, incubating mallard eggs. At 8 days of development the external application of 20 µl of oil products, including South Louisiana and Kuwait crudes, and No. 2 fuel oil, killed all embryos. The embryotoxicity was age dependent. Mortality from 5 µl South Louisiana crude oil varied from 32-92 percent when applied to mallard eggs with 2-10 day old embryos. In addition mallard ducklings were adversely affected by the ingestion of crude oil. When fed 2.5 or 5.0 percent South Louisiana crude oil from hatch to 8 weeks of age ducklings failed to develop normal flight feathers, and exhibited morphological and biochemical evidence of liver and kidney damage. Thus far adult mallard ducks fed 0.25 or 2.5 percent South Louisiana crude oil, or a chemically defined aromatic mixture representative of this oil, have not shown the severity of pathological changes evidenced in the ducklings.

Our data suggests that adult waterfowl may be able to adapt and tolerate high concentrations of ingested petroleum hydrocarbons, but that all phases of reproduction and early development are severely impaired by low concentrations of petroleum hydrocarbons.

Acute and Chronic Studies with Waterfowl Exposed to Petroleum Hydrocarbons

Our requirements for energy and economic independence will rest largely on petroleum hydrocarbons for several decades. This recognition has resulted in concerted efforts to find, obtain, process and deliver oil at an ever increasing rate. Coupled with this phenomena is our nation's awareness and dedication to the maintenance of ecological balance. Many government agencies have been assigned the task of insuring our wildlife heritage during the removal of non-living natural resources. We represent a small part of this effort -- an examination of the "Ecological and Physiological/Toxicological Effects of Oil on Birds." Our studies encompass numerous aspects of avian biology, and are proceeding on a broad front as fast as possible in an attempt to answer one basic question. Do sublethal concentrations of petroleum hydrocarbons pose a significant threat to bird populations? The effects of petroleum hydrocarbons on aquatic birds have been the subject of two recent symposia (Dieter, 1976, and Szaro, 1977). These reviews pointed out that the base of scientific information was scant and needed expansion. The available evidence was nevertheless alarming, and indicated the potential damage that petroleum hydrocarbons might inflict on susceptible avian populations.

The research program at Patuxent has been evaluating the biological effects of petroleum hydrocarbons on all stages of the waterfowl life cycle. These studies include i) the hatchability of eggs exposed to oils, ii) the development of ducklings fed oil from the day of hatch, and iii) reproduction in adult waterfowl fed oil. The third study is

complemented by another with adult waterfowl fed a chemically defined mixture representative of the concentration and type of aromatic compounds present in oil. In addition experiments have been initiated to evaluate biological responses to petroleum hydrocarbons incorporated via the food chain. Analytical development is proceeding in concert with all of these biological studies.

We have repeatedly demonstrated that microliter amounts of crude or refined petroleum hydrocarbons result in high mortality of developing embryos. Table I shows that 5 µl of South Louisiana crude oil, Kuwait crude oil, or No. 2 fuel oil, applied to fertile eggs at 8 days of incubation, caused 76-98% mortality. We are also able to conclude that i) mortality is a result of toxicity and not oxygen deprivation, and ii) that an alkane mixture tested was non-toxic, implicating the aromatic fraction or other constituents of crude oil as the toxic components.

The embryotoxicity of oils was not confined to the mallard. Eider eggs collected from the Maine seacoast were externally oiled with No. 2 fuel oil resulting in 31% mortality (Table II). Eider embryos were about 1/20 as sensitive as mallard embryos to external egg oiling. Some of the species difference can be ascribed to different dose-weight relationships, as the eider embryos are twice the size of mallard embryos. Age dependent sensitivity to petroleum hydrocarbons is another factor that was responsible (Table III). Embryos that were at earlier stages of development were much more sensitive to egg oiling than later stages. This was supported by another series of experiments shown in Table IV.

Mortality from petroleum hydrocarbon application was clearly age dependent; 5 µl applied on or before 10 days of development resulted in

92% or greater mortality from South Louisiana cr de oil and 32-87% mortality from No. 2 fuel oil (Table IV). Treat ent of eggs on or beyond day 14 did not result in a significant increase n mortality. This line of investigation will be continued with eggs frc different species and with other crude oil products, and extended by i conducting field studies with eggs oiled in marked nests, and ii) conduct ng outdoor pen studies with oiled waterponds and naturally incubating h ns.

We also report that hens from paired mallar s fed 2.5% South Louisiana crude oil laid fewer eggs than pairs f d 0.25%, a mixture of 1% paraffins, or untreated control pairs. Egg p oduction for 30 days dropped from an average of 25 eggs/hen in untrea ed to 11 eggs/hen in those fed 2.5% South Louisiana crude oil. Our c ntract investigator has reported similar results with mallards fed 1, 3 r 5% South Louisiana crude oil (Holmes, personal communication). He eported that hens fed 3% oil ceased laying, and that those fed 1% oil aid one-third as many eggs as controls. It is evident that during the reproductive season petroleum hydrocarbons could pose a threat to wa erfowl populations by interfering with the production and viability of eggs.

Duckling growth was impaired when diets cor aining 5% South Louisiana crude oil were ingested from hatching until 8 we ks of age (Table V). More importantly, in these ducks as well as thos fed 2.5% South Louisiana crude oil flight feathers failed to develop norm lly. Liver hypertrophy and splenic atrophy was evidence of the patholog cal effects of the oil; biochemical lesions that occurred included eleva ion of plasma alanine aminotransferase and ornithine carbamyl transfer se activity. These plasma enzymes appear in the circulation as a result of liver and kidney damage.

The same enzymes are being measured in studies with adult ducks fed South Louisiana crude oil or representative aromatic compounds of this crude oil. These variables have thus far failed to reveal dysfunction in adult ducks fed up to 2.5% South Louisiana crude oil, or in those fed the aromatic mixtures.

More sophisticated measurements of liver function were performed with the drakes fed the aromatic mixture. The ability of the liver to remove toxic substances from the circulation was followed by measuring the change in the clearance rate of injected indocyanine green dye, a compound that is metabolized entirely by the liver. The ingestion of the 4000 ppm aromatic mixture caused a compensatory response to the toxicants reflected by a significant elevation of plasma clearance rates above control levels (Table VI). The data suggests that adult waterfowl may be able to adapt and tolerate higher concentrations of petroleum hydrocarbons than ducklings.

Our present evidence thus indicates that all phases of the reproductive sequence, fertilization, laying, embryonic and duckling development, are highly sensitive to petroleum hydrocarbons. Hopefully extensive means could be undertaken to prevent oil exposure during this critical period.

In other studies waterfowl are fed various invertebrates that have been exposed to petroleum hydrocarbons to determine the nature and extent of the products accumulated in their tissues. Crayfish have been selected as food items that are readily accepted by mallard ducks. A radiolabelled aromatic compound is used as a marker to follow the oil through the food chain.

Along with the biological work, a concerted effort has been made to develop analytical capability for petroleum hydrocarbons. This poses a special challenge because of the overwhelming number of chemical compounds present in one sample, and the fact that this array is unique for different oils and even for different batches of the same oil.

The first problem addressed was to demonstrate that petroleum hydrocarbons accumulate in avian tissues. High resolution gas chromatography and mass spectrometry were used to identify the petroleum-derived, saturated hydrocarbons in tissues of oil-dosed ducks (Lawler, et al., In press, 1977). The distribution pattern of n-alkanes, the homologous series of petroleum hydrocarbons and the $\underline{n}$-C_{17}/pristane ratio were used to provide evidence that petroleum hydrocarbons had accumulated in the tissues. Selective uptake and/or metabolism had apparently occurred since tissue levels of individual saturated petroleum hydrocarbons differed from the relative amounts present in South Louisiana crude oil. No accurate quantitation of saturated petroleum hydrocarbons was possible, but relative accumulation by tissues was: skin > uropygial gland > breast muscle > heart muscle > liver > blood. No saturated petroleum hydrocarbons were detectable in the brain and only trace amounts in the blood.

The unsaturated petroleum hydrocarbons (aromatic compounds) are now being measured in the same tissue extracts. The tissue accumulation of aromatic hydrocarbons was similar to that of the alkanes, except that detectable quantities of aromatics were present in the brain.

Our analytical efforts favor the aromatic fraction because the biological evidence indicates the aromatic compound in petroleum hydrocarbons are toxic. We are streamlining present analytical methods and are

selecting marker compounds in petroleum hydrocarbons to serve as references for quantitation. Pending completion of these tasks, tissues from mallard ducklings fed South Louisiana crude oil are scheduled for petroleum hydrocarbon analysis. Other material that will soon become available for analysis includes tissues from adult mallards fed either South Louisiana crude oil or an aromatic mixture representative of this oil.

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Table I.[1]

Embryotoxicity from Egg Oiling[a] with Different Petroleum Products (N=50).

Treatment	Percent Mortality after Treatment with: South La. crude oil	Kuwait crude oil	No. 2 fuel oil
none	8	8	12
propylene glycol (50 µl)	6	-	-
alkane mixture (50 µl)[b]	4	-	-
oil (1 µl)	38[c]	28[c]	36[c]
oil (5 µl)	98[c]	76[c]	82[c]
oil (10 µl)	98[c]	84[c]	90[c]
oil (20 µl)	100[c]	94[c]	100[c]

[a]Fertile mallard eggs treated externally at day 8 of development.

[b]See Table VI for components of alkane mixture.

[c]Significantly different from controls, $P < 0.01$.

[1]Data derived from the studies of Szaro, Albers, and Coon (In preparation, 1977).

Table II.[1]

Embryotoxicity in Oiled Common Eider Eggs[a] (N=48).

Treatment	Percent Mortality
none	4
propylene glycol (50 µl)	4
oil (5 µl)	8
oil (20 µl)	31[b]

[a]Fertile eider eggs of various developmental stages treated externally with No. 2 fuel oil.

[b]Significantly different from controls, $P < 0.01$.

[1]Data derived from the studies of Szaro and Albers (In press, 1977).

Table III.[1]

Age Dependent Embryotoxicity[a] to Oil in Common Eider Eggs.

No. 2 Fuel oil (20 µl)	N	Age in Days (Mean ± S.E.)
Dead	15	4.3 ± 1.4
Survivors	33	16.1 ± 0.7

[a]Significantly different ages, $P < 0.05$.

[1]Data derived from the studies of Szaro and Albers (In press, 1977).

Table IV.[1]

Embryotoxicity from Egg Oiling[a] at Different Developmental Stages (N=50).

Age of Treated Embryo (days)	Percent Mortality after Treatment with: South Louisiana Crude Oil	No. 2 Fuel Oil
controls[b]	0	20
2	100[c]	87[c]
6	97[c]	67[c]
10	92[c]	32[c]
14	22	17
18	12	20
22	5	7

[a] Fertile mallard eggs treated externally with 5 µl of oil.

[b] Mortality of untreated control eggs checked by candling at each interval.

[c] Significantly different from controls, $P < 0.01$.

[1] Data derived from the study of Albers (In preparation, 1977).

Table V.[1]

Morphological and biochemical responses in mallard ducklings fed South Louisiana crude oil from hatch to 8 weeks of age. Means ± S.E., N=10.

MORPHOLOGY

Concentration of oil fed (ppm)	Body weight g	Liver weight g	Liver weight % BW	Spleen weight g	Spleen weight % BW
0	1119	34.5 ± 1.5	3.2	0.81 ± .06	.074
250	1127	33.0 ± 1.9	2.9	0.84 ± .12	.067
2500	1109	31.5 ± 1.7	1.9	0.64 ± .04	.058[a]
25,000	1069	51.5 ± 1.9	4.8[a]	0.39 ± .06	.037[a]
50,000	913	69.7 ± 4.7	7.4[a]	0.30 ± .03	.032[a]

BIOCHEMISTRY[b]

Plasma Enzyme Activity (Milliunits)

	Alanine aminotransferase (from liver)	Ornithine Carbamyl transferase (from kidney)	α-hydroxybutyric acid dehydrogenase (from heart)
0	11.6 ± 1.0	12.5 ± 0.6	96.4 ± 9.0
250	12.7 ± 2.1	23.1 ± 1.2[a]	112.3 ± 8.9
2500	23.5 ± 3.3[a]	19.0 ± 1.8[a]	84.6 ± 7.8
25,000	19.0 ± 1.6[a]	18.8 ± 1.1[a]	77.8 ± 7.7
50,000	16.3 ± 1.4[a]	20.7 ± 1.5[a]	86.3 ± 12.5

[a]Significantly different from controls, $P < 0.05$.

[b]Additional plasma enzymes measured but showing no significant change included aspartate aminotransferase, lactate dehydrogenase and cholinesterase.

[1]Data derived from the study of Szaro (Unpublished data, 1977).

Table VI.[1]

Liver function in mallard drakes fed aromatic mixtures[a] representative of those in South Louisiana crude oil. Means ± S.E., N=12.

	Months			
	Pretreatment	1	3	5
Treatment	Plasma Clearance Rate (ml /min/kg body weight)			
Control	13.7 ± 1.1	13.5 ± 0.8	13.5 ± 0.7	13.1 ± 0.7
Alkane mix, 10,000 ppm	14.0 ± 1.1	14.7 ± 0.7	14.9 ± 0.7	13.7 ± 0.7
Aromatic mix, 400 ppm	13.4 ± 1.0	15.5 ± 0.8	15.2 ± 0.7	14.7 ± 0.5
Aromatic mix, 4000 ppm	14.1 ± 1.0	17.0 ± 1.4[c]	18.5 ± 1.0[c]	18.5 ± 1.2[c]

[a] The aromatic mixture consists of an equimolar ratio of 10 compounds (ethyl benzene, 1,2,3,4-tetrahydronaphthalene, dimethylnaphthalene, 2,3-trimethylindolenine, acenaphthylene, acenaphthene, phenanthrene, 2-methylbenzothiazole, dibenzothiophene, and 2,6-dimethylquinoline) dissolved in 1% alkanes (equimolar mixture of tridecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4,6,6-pentamethyl heptane, 2,2,4,4,6,8,8-heptamethylnonane, 2,6,10,14-tetramethylpentadecane, and decahydronaphthalene) in the feed.

[b] The removal of injected indocyanine green dye from the circulation is a specific measure of liver function.

[c] Significantly different from controls, $P < 0.05$.

[1] Data derived from the study of Patton (Unpublished data, 1977).