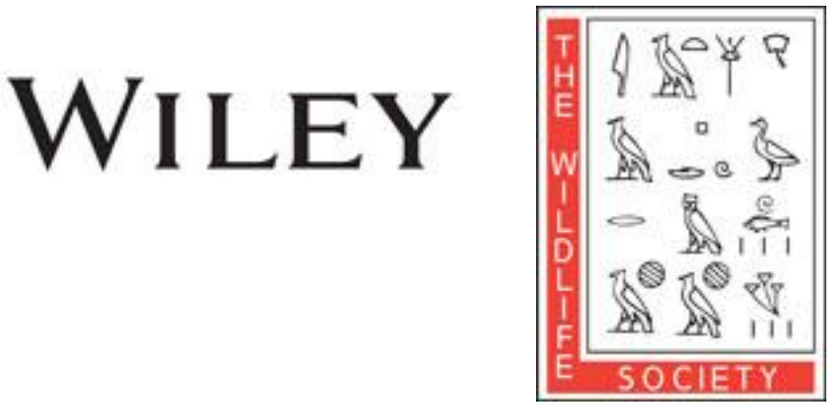

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TOXICITY OF SOME OILS TO WATERFOWL[1]

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Abstract: A number of industrial oils were tested for their toxic effects on waterfowl. All oils were able to cause lipid pneumonia, gastrointestinal irritation, fatty livers, and adrenal cortical hyperplasia when fed to ducks in single doses by stomach tube. Feeding of a cutting oil and a diesel oil also resulted in acinar atrophy of the pancreas. The diesel oil and a fuel oil produced toxic nephrosis in a number of animals. Feeding the cutting oil produced a definite inhibition of cholinesterase activity while the diesel oil depressed cholinesterase activity only slightly. Approximate LD_{50} values were determined for a number of oils under different environmental conditions. Gross examination of a series of 41 ducks which had been killed by oil pollution in the wild showed, at autopsy, changes similar to those encountered in the experimentally fed ducks. It was concluded that the toxicity of polluting oils is a definite factor in the observed mortalities due to oil pollution.

Oil pollution has become a serious hazard to waterfowl during the last 30 years. The extent of waterfowl mortalities due to oil pollution has been reviewed by Hawkes (1961) and Erickson (1963). These reviews indicate that many thousands of birds die annually as a result of oil pollution. Two major factors appear to be involved in these deaths: the external oiling of the ducks and the actual ingestion of the oil. Richardson (1956) and Giles and Livingston (1960:299) have suggested that oil may be ingested by waterfowl during preening and may have toxic effects. Hartung (1963) demonstrated experimentally, using isotope-labeled oils, that oiled ducks ingest significant quantities of oil in preening their plumage. The present study was undertaken to investigate the toxic effects of oils on ducks.

METHODS AND MATERIALS

The pathological and physiological effects of ingested oils were evaluated by feeding oils to wild-trapped and domesticated ducks. Oils were fed undiluted as single doses by stomach tube. The oils utilized in these experimental feedings were: a light fuel oil (No. 1) containing less than 1 percent phenolic compounds as anti-oxidants; a diesel oil containing less than 1 percent organically bound phosphorus, phenols, and other unidentified additives; a simple, sulfuretted, low additive SAE 10W motor lubricating oil; a sulferetted SAE 10-W-30 motor lubricating oil with high detergent content; a high pressure cutting oil additive containing 30 percent chlorine and 10 percent phosphorus in organically bound form; and a cutting oil formulated with 10 percent of this additive, 10 percent triglycerides, and 80 percent mineral oil.

Tissues for histological examination were collected immediately after sacrifice by decapitation, and fixed in buffered 10 percent formalin. In those cases where early histological examinations indicated specific organ damage, appropriate biochemical tests were made in subsequent experiments to try to determine the extent of alteration in function of these organs.

Blood for analytical procedures was obtained by heart puncture or from the metatarsal vein, depending upon the amount of blood required. Hemoglobin was analyzed spectrophotometrically as oxyhemo-

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globin using the method described by Sunderman et al. (1953:559). Plasma lipase levels were determined according to Johnson and Bockus (1940) as an indicator of the existence of pancreatic injury. Liver damage was evaluated by determining plasma glutamic-oxalacetic transaminase levels by the method of Karmen (1955), and by conducting Bromsulphalein liver function tests using the method described by Gaebler (1945). Non-protein nitrogen levels in blood as an indicator of kidney function were determined by the procedure of Folin and Wu (1919) adapted as a micro-method. Some of the oils under investigation contained organic phosphates which might inhibit acetylcholinesterase, which has an essential function in the proper transmission of nervous impulses across many types of nerve synapses and the neuromuscular junction. The status of acetylcholinesterase in these ducks was monitored indirectly by determining plasma cholinesterase levels by the electrometric method of Michel (1949). The method was slightly modified by the addition of a calibration procedure which permitted translating the change in pH into the corresponding amount of acetylcholine hydrolyzed by the enzyme. The presence of occult blood in the intestines was determined by the benzidine test according to Todd et al. (1953:101).

A sample of 41 wild oil-killed ducks was autopsied and examined to determine whether there was any correlation between experimentally induced changes due to oil ingestion, and those encountered in waterfowl mortalities due to oil pollution in the wild. This sample represented a fraction of the waterfowl killed by a number of small oil pollution episodes on the lower Detroit River between 1960 and 1964. Presumably a number of different oils were involved in these episodes.

The statistical significance of the data was evaluated using a one-sided *t*-test without assuming equality of variances (Snedecor 1956).

RESULTS

Respiratory System

Twenty-four percent of all the experimentally oil-fed ducks developed lipid pneumonia. All oils tested were able to produce lipid pneumonia, sometimes at dose levels as low as 1 ml/kg of oil, but more commonly at dose levels above 2 ml/kg. Among pollution-killed ducks the incidence of pneumonia reached 61 percent. After microscopic examination of frozen tissue sections stained with oil red O of a subsample of five pneumonic lungs from the pollution-killed ducks, four were diagnosed as lipid pneumonia.

Freiman et al. (1940) and Schneider (1949) indicated that the ingestion of oils by healthy human adults could result in lipid pneumonia because some of the oil was either inhaled or leaked past the glottis during swallowing. In ducks, the glottis is located on the surface of the base of the tongue and the inhalation of oils, or leakage of oils past the glottis, may occur more readily than in mammals. Ducks fed doses of more than 3 ml of some oils attempted to regurgitate it. Regurgitation seemed to be difficult and was accompanied by vigorous shaking of the head. Ducks could inhale some oil during this process.

The lungs of ducks suffering from lipid pneumonia were markedly hyperemic. Usually less than half the total mass of the lungs was involved. The walls of the air-capillaries in the parabronchi were greatly thickened because of an intense inflammation which was characterized by the presence of many lymphocytes and proliferation of connective tissue. The air-capillaries were frequently occluded by this thicken-

Table 1. Blood values for ducks before and after ingestion of fuel oil.

Species and Treatment	Number of Ducks	RBC × 10^6 Mean ± se		Hematocrit Percent ± se		Hemoglobin (g/100 ml) Mean ± se	
Mallard normal	28	2.59 ± 0.056		42.6 ± 1.02		13.7 ± 0.206	
Mallard 8 days after ingestion of 2 g/kg of fuel oil	9	2.11	0.102‡	41.3	1.79*	11.9	0.887†
Pekin normal	8	2.65	0.084	45.6	2.96	14.2	0.299
Pekin 8 days after ingestion of 2 g/kg of fuel oil	7	2.23	0.104‡	37.1	1.48†	11.7	0.808†

* Not significant.
† Significant ($p < 0.05$).
‡ Significant ($p < 0.01$).

ing. Foreign body giant cells occurred only rarely. Near areas where oil droplets were lodged in the parabronchi, the inflammation was hemorrhagic. Frequently the outline of the oil droplets was visible in paraffin tissue sections because of the outline left by the erythrocytes, lymphocytes, and neutrophils which were incorporated into the droplet. The area surrounding the droplet was usually devoid of parabronchial tissue and was filled with a hemorrhagic, serous exudate. Frequently the area was partially walled off by connective tissue.

Digestive System

Most of the oils tested irritated the gastrointestinal tract to some extent. The degree of irritation was judged by the relative level of hyperemia of the intestinal wall, and by the relative amount of occult blood found in the intestines by the benzidine test. By these criteria the fuel oil and the diesel oil produced severe irritation in all ducks when fed at a level of 1 ml/kg or higher. The lubricating oils were judged to be only slightly irritating, and in comparison medicinal mineral oil was found to be nonirritating. Even though the amount of occult blood found in the intestines of ducks which had been fed fuel or diesel oil was high, microscopic examination of tissue sections from these ducks did not show any specific areas where hemorrhage into the lumen was ocurring. Apparently the blood was lost by diapedesis.

Ducks which were fed oils developed diarrhea within 6 hours. This diarrhea persisted up to several days in ducks fed fuel or diesel oil. The feces were bright green, probably due to bile pigments. Large quantities of uric acid were also frequently present. The feces of ducks fed the diesel oil or the cutting oil usually contained quantities of mucus.

Hematological studies indicated that ducks fed 2 g/kg of fuel oil were slightly anemic 2 days after they were fed the oil (Table 1). This anemia may have resulted from the blood loss into the intestine.

On the average, the gastrointestinal tracts of oil-killed ducks from the wild exhibited a greater degree of irritation than was found in ducks fed up to 3 ml of fuel oil or diesel oil. The gastrointestinal tracts of all oil-killed wild ducks were hyperemic and contained occult blood as determined by the benzidine test.

Table 2. Liver function of Pekin ducks before and after ingestion of oils.

TREATMENT	NUMBER OF DUCKS	PLASMA GOT UNITS MEAN ± SE	NUMBER OF DUCKS	BSP DYE RETENTION 5 MIN (%) ± SE
Normal	13	29.2 ± 2.70	7	10.4 ± 0.51
2 ml/kg 10-W-30 lube oil	7	28.3 2.89*	3	10.3 1.30*
3 ml/kg diesel oil	3	39.0 1.53†	3	10.8 3.33*
6 ml/kg diesel oil	3	48.3 2.04†	3	11.7 1.76*
12 ml/kg diesel oil	3	68.0 10.2†	3	19.0 1.90†
24 ml/kg diesel oil	3	153.7 25.2†	3	25.1 3.72†

* Not significant.
† Significant ($p < 0.05$).

The livers of all experimental ducks and also a few control ducks showed at least some fatty infiltration and degeneration.

Plasma glutamic-oxalacetic transaminase (GOT) levels were increased significantly 24 hours after the ingestion of 3 ml/kg of the diesel oil. At this time, Bromsulphalein (BSP) liver function tests showed significantly increased retention of the dye only at dose levels above 12 ml/kg of the diesel oil (Table 2). The 10-W-30 lubricating oil fed at 2 ml/kg had no significant effect on plasma GOT or BSP retention.

Microscopic examination of the pancreas showed a reduction in zymogen granules in many animals sacrificed 48 hours after the ingestion of any oil. The reduction of the number of these granules is indicative of a reduction in the quantity of stored enzyme precursors in the pancreas. In the most severe cases of pancreatic damage, which occurred in ducks fed 6 ml/kg of the diesel oil, the zymogen granules were completely absent. The parenchyma of the pancreas exhibited cloudy degeneration and appeared to be in the early stages of acinar atrophy. In spite of the histological changes found in the pancreas, the plasma lipase levels in experimental ducks were only slightly elevated above those of the controls.

Table 3. Non-protein nitrogen concentrations (NPN) of mallard blood before and 48 hours after ingestion of oils.

TREATMENT	NUMBER OF DUCKS	(MG/100 ML) ± SE
Normal	12	52.5 ± 7.05
2 ml/kg 10-W-30 lubricating oil	6	48.2 4.08*
2 ml/kg diesel oil	11	89.2 8.80†
2 ml/kg fuel oil	7	76.7 7.96†

* Not significant.
† Significant ($p < 0.05$).

Excretory System

The kidneys of ducks which had been fed the lubricating oils, or the cutting oil, developed minor changes limited to cloudy swelling of the proximal convoluted tubules and the occurrence of small amounts of cellular debris in Bowman's capsule. These conditions were not found among normal controls. Changes in the kidneys were more conspicuous in ducks which had been fed large doses of fuel oil or diesel oil. In these cases, the distal convoluted tubules frequently contained albuminoid casts combined with some tubular hydropic degeneration pointing to the existence of a toxic nephrosis. The changes in non-protein nitrogen (NPN) concentrations in the blood reflected the changes observed during histologic examinations. The NPN levels (Table 3) were elevated after the ingestion of the fuel oil or the diesel oil

Table 4. Cholinesterase activities of the plasma of control and experimental ducks.

Species and Treatment	Number of Ducks	Av. mg ACh Hydrolyzed per ml Blood Plasma per Hour ± se
Black duck normal	15	7.22 ± 0.595
Black duck 1 ml/kg cutting oil additive	3	1.80 0.494*
Black duck 2 ml/kg cutting oil additive	4	0.92 0.225*
Mallard normal	16	7.30 0.503
Mallard 5 ml/kg cutting oil	5	1.96 0.296*
Mallard 5 ml/kg diesel oil	8	4.11 0.488*
Pekin normal	7	3.41 0.559
Pekin 250 mg/kg Malathion	3	0.07 0.032*
Pekin 1 ml/kg cutting oil additive	3	0.92 0.225*

* Significant ($p < 0.01$).

but not after the ingestion of a comparable amount of lubricating oil.

Adrenal Glands

The adrenal glands of many experimental ducks which had been fed more than 1 ml/kg of any oil were greatly enlarged. Microscopic examination showed that most of this enlargement was due to hyperplasia of cortical-type tissue. Adrenal tissue of the medullary type was affected only minimally, while cortical-type tissue often increased twofold. Gross examination of the autopsied wild oil-killed ducks presented a similar picture where the adrenal glands were definitely enlarged in 75 percent of the cases. These changes observed in the adrenal gland appeared to be similar to those described by Selye (1946) as a general adaptation syndrome in response to stress.

Nervous System

Ducks which were fed high doses of the cutting oil or the diesel oil exhibited incoordination, ataxia, tremors, and constricted pupils. Since these oils contained organic phosphates, inhibition of acetylcholinesterase activity was suspected. Inhibition of this enzyme would drastically alter the functioning of many segments of the nervous system. Plasma-cholinesterase is also prone to inhibition by organic phosphates. Plasma levels were monitored to indicate the status of the acetylcholinesterase activity in the nervous system. The measured plasma-cholinesterase levels were significantly depressed after ingestion of the diesel oil or the cutting oil (Table 4). These measurements therefore strongly suggest that the observed changes in motor activity were caused by the inhibition of acetylcholinesterase activity.

Lethal Doses

The oils fed were relatively nontoxic to ducks kept under optimal conditions. Under these conditions only the cutting oil additive caused mortalities with an approximate LD_{50} of 3 ml/kg. All animals tested under optimal conditions survived doses of up to 7 ml/kg of the cutting oil, 24 ml/kg of the diesel oil, and up to 20 ml/kg of the two types of lubricating oil. However, in a stressed group of ducks, kept outdoors under crowded conditions at temperatures ranging from 0 to –10 C, the toxicity of these oils was greatly increased. Under these conditions, the cutting oil additive was found to have an approximate LD_{50} of 1 ml/kg, the cutting oil an LD_{50} of 3 ml/kg, and the diesel oil an LD_{50} of 4 ml/kg. The lubricating oils were not tested under these latter conditions.

DISCUSSION

Some of the toxic effects noted among ducks after oil ingestion appear to be common to all industrial oils tested. These effects are lipid pneumonia, gastrointestinal irritation, fatty changes in the liver, and adrenal cortical hyperplasia. Other effects appeared to be more specifically related to the kind of oil involved. Thus the diesel oil and the cutting oil produced some acinar atrophy in the pancreas, and the fuel oil and the diesel oil produced some toxic nephrosis in the kidney. The cholinesterase levels were affected only by the cutting oil, and to a slight extent by the diesel oil.

The dose levels utilized in these studies may appear to be unreasonably high. However, previous studies, using isotope-labeled oils, had indicated that ducks will preen approximately 50 percent of any polluting oil from their feathers within the first 8 days after exposure, and will ingest most of this oil in the process (Hartung 1963:51). Later studies have also indicated that ducks can readily acquire an average of 7 g of polluting oils on their plumage under natural conditions (Hartung 1964). The ingestion of the oil under natural conditions admittedly is spread over an extended time. However, the greatest amount of oil is ingested during the first day after oiling. After that the amounts ingested decrease in a logarithmic fashion. Thus an average oiled duck with 7 g of oil on its feathers should ingest approximately 1.5 g of oil during the first day. The ducks utilized in the present study weighed approximately 0.70 kg, and thus oral dosages of 2 to 3 g/kg appear to be realistic levels which could be readily encountered in the wild. It is nevertheless realized that the single doses of oil given experimentally do not fully correspond to natural conditions.

At the dose levels used in this study the tested oils produce undesirable effects in ducks kept under optimal conditions. Prestressed ducks succumbed to dosages of 3 to 4 g/kg of diesel oil and cutting oil. Therefore, it appears that the toxicity of ingested polluting oils plays a definite role in the observed waterfowl mortalities due to oil pollution. The magnitude of this role will depend upon the type of oil involved and on the magnitude of additional stresses such as food shortages, parasitism, and cold.

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SOME INFECTIOUS DISEASES OF WATERFOWL IN THE MISSISSIPPI FLYWAY

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Abstract: To establish the presence of selected infectious diseases in a "healthy" wild waterfowl population, a serologic and parasitologic study was initiated. Sera from 123 Canada geese (primarily *Branta canadensis interior*) and 179 mallards (*Anas platyrhynchos*) were screened for antibodies against six arboviruses. There was no neutralizing antibody detected against western viral encephalitis, eastern viral encephalitis, St. Louis viral encephalitis, Venezuelan viral encephalitis, California viral encephalitis, or vesicular stomatitis. Sera from 55 ducks and 12 geese were negative for agglutinating antibodies to *Salmonella pullorum, Salmonella typhimurium,* and *Mycoplasma gallisepticum.* Fifty-five duck sera and 11 goose sera were negative for complement-fixing antibodies of ornithosis. The most significant serologic finding was the presence of Newcastle disease antibody. Forty (17 percent) of 236 Canada geese were positive for Newcastle disease antibody. Thirty-seven (14 percent) of 267 mallard ducks had antibody titers for Newcastle disease virus. A limited study of parasitism revealed a low prevalence of *Haemoproteus, Leucocytozoon,* and microfilariae in both geese and mallards as well as *Plasmodium* in wild geese. The significance of the serological and parasitological findings is discussed.

The occurrence of infectious disease in waterfowl has been well documented (Halloran 1955:332–348, Quortrup et al. 1957, Sciple 1953, Rosen and Bischoff 1950, O'Roke 1931). Most reports of disease in waterfowl have been concerned with spectacular epizootics, individual mortality, an extension of host or geographic range, or a survey of an easily sampled species as part of a domestic animal or human disease problem. While there can be no doubt of the value of these types of reports, they lack continuity and contribute little to understanding the significance of disease in wild populations.

This is a report on the initial phase of an infectious disease investigation of selected waterfowl populations in the Mississippi flyway. Its aim is to establish the presence, prevalence, and significance of infectious disease in this apparently "healthy" waterfowl population. Previous experience of the birds with disease was determined by the presence of antibodies against a select

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