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METHODS USED AT PATUXENT WILDLIFE RESEARCH CENTER TO STUDY THE EFFECTS OF OIL ON BIRDS

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INTRODUCTION

The obvious damage to the flora and fauna following an oil spill receives a considerable amount of attention. News media accounts are usually replete with photographic evidence of the disastrous effects of these catastrophes. Although the numbers of dead and oiled animals found at the scene of a spill are somtimes large, the effect on wildlife from the low levels of oil remaining after cleanup procedures or from continuous low-level contamination in other areas may be equally damaging. However, these low-level exposures are difficult to detect. An understanding of their ecological impact requires scientific attention. Chronic low-level effects of oil on the environment are being investigated by a number of research groups. The Patuxent Wildlife Research Center has focused its oil contamination research on birds. Members of the research group include wildlife biologists, physiologists, and chemists. The purpose of this paper is to present the experimental approaches used by researchers at the Patuxent Center to resolve some of the questions concerning the effects of chronic low levels of oil on birds.

METHODS AND RESULTS

Oil can be ingested by birds directly while drinking or feeding, ingested indirectly via the food chain, or applied to incubating eggs by the adult. The effects of oil on avian species through these routes of exposure were determined in three types of related experiments: 1) indoor pen studies, 2) incubator studies, and 3) outdoor field studies. To assess the effects of oil ingestion on birds, investigators monitored several parameters, including mortality, growth (weight gain and wing measurements), plasma enzymes, and liver function. Several routine clinical biochemical techniques for assessing damage to mammalian systems were adapted for birds. Thus, measurements were made on plasma enzymes that reflect damage to specific organs such as kidney, liver, and heart. In addition, a technique established for mammals to directly indicate a change in hepatic function was found acceptable for avian species (Patton 1978). Organ damage measured biochemically was confirmed at both the macroscopic and microscopic level by a pathologist

(Szaro et al. 1978; Patton and Dieter, in press; Coon and Dieter, in preparation; Szaro, in preparation). In several studies, tissues from dosed and undosed ducks were chemically analyzed for oil components.

Indoor Pen Studies

Studies in indoor pens are advantageous because light, temperature, water, food intake, and other external elements can be precisely controlled. Under constant conditions, the effects on test animals of varying one or more factors at a time can be examined.

Feeding studies were conducted to determine the effects on birds of ingesting different doses of crude oil in their food. In initial studies with adult mallard ducks *(Anas platyrhynchos)*, various amounts of crude oil were included in commercial duck feed. Briefly, pairs of mallards were placed in separate cages and fed either clean feed or one of the several diets containing crude oil at environmentally realistic values ranging from 0.25 to 2.5%, for 26 weeks. Those receiving oil in their diet laid fewer eggs than did controls (Coon and Dieter, in preparation). In another experiment, adult mallards compensated for oil ingestion with an increased liver clearance capacity (Patton and Dieter, in press).

Subsequent experiments have been conducted, in which ducklings were fed test diets containing 0.025 to 5% oil from the day of hatching (Szaro 1978; Szaro, in preparation). After studying the effects of several crude oils, an experiment was done to examine the effects of various components of these crude oils (Patton and Dieter, in press). Crude oils, ingested with food, have retarded growth, increased liver size, increased plasma enzyme activities of alanine aminotransferase and ornithine carbamyl transferase (indicative of liver and kidney damage), and decreased spleen size and hematocrit in ducklings (Szaro et al. 1978; Szaro, in preparation).

Chemical analyses of these ducks showed that ingested oil components had been absorbed and could be found accumulated to a greater extent in fat than in liver, kidney, and several other tissues (Lawler et al. 1978; Lawler, Loong, and Laseter, 1978a, 1978b).

Indirect ingestion of oil may occur through the food chain. This hypothesis was tested using a radioactive isotope. Previous studies had strongly implicated the aromatic hydrocarbon fraction of crude and No. 2 fuel (diesel fuel) oils as being most toxic. When crude oil is mixed with water, these aromatic fractions are found in the water-soluble fraction (WSF). A volume of No. 2 fuel oil was poured onto water (5% oil by volume) to simulate an oil spill. This mixture was stirred for 20 hours. No. 2 fuel oil contains approximately 16% napthalenes and about 40% total aromatics. The aqueous phase containing the WSF was decanted, and a known activity of ^{14}C-naphthalene was added. Crayfish were placed in the WSF for 3 hours and then fed to adult ducks. The WSF, the crayfish, and the duck tissues were measured for radioactivity. Knowledge of the activity of ^{14}C-naphthalene that was added and the amount of aromatics or naphthalene in the WSF allowed calculation of the

amount of contaminant in each phase of the experiment (Tarshis, in preparation). The results from these experiments have supported the hypothesis that transfer of aromatics through a simple aquatic food chain is another route for oil contamination to reach waterfowl.

Taken together, the results from the studies described suggest that, to a degree, adult ducks ingesting oil with their food may be able to make physiological compensations. There may be damage to organ systems, however, that directly affects the ability of the bird (especially the young) to survive a second environmental challenge (Holmes, Gorsline, and Cronshaw 1979). There is also some evidence that oil ingestion by adults affects egglaying (Holmes, Cavanaugh, and Cronshaw 1978; Coon and Dieter, in preparation). Studies are underway at the Patuxent Center to further define this reproductive effect.

Incubator Studies

Incubators were used as an aid to study the effects of microliter (μl) quantities of crude and No. 2 fuel oils on developing avian embryos. A technique was developed in which oil was externally applied in precise amounts (ranging from 1 to 50 μl) to discrete areas of bird eggs (Szaro and Albers 1977, Albers 1977, 1978). The eggs were subsequently placed in an incubator maintained at optimum temperature and humidity. Eggs were routinely candled to monitor mortality and embryo development. Surviving embryos were examined at 18 days of age or permitted to hatch. External applications of oil were found to increase mortality and incidence of defects and stunting in mallard embryos (Eastin and Hoffman 1978; Hoffman 1978a, 1978b, Hoffman 1979a). In addition, a dose:mortality relationship was shown. Eggs from other species were collected from coastal areas and brought back to the laboratory (Szaro and Albers 1977, Coon et al. 1979, White et al. 1979). When these eggs were dosed with oil, as in the previous studies, the mortality was not as great. One major difference between the two studies was that the mallard eggs were synchronous in development, whereas field-collected eggs were at various stages of embryo development. Laboratory studies were performed to investigate the possibility that there is a differential sensitivity to external oiling during development. The results were clearcut and indicated high mortality at two distinct periods early in the development, but that egg oiling after this critical time had little effect.

It also was decided to examine in detail certain suspect trace metals and those components chemically identified in the aromatic hydrocarbon fraction of crude oil to try to further understand the toxicity to embryos. The porphyrin forms of vanadium and nickel found in crude oil are much less toxic to embryos than are some of the salts previously tested on birds and mammals. These porphyrin forms did, however, produce some defects in embryos (Hoffman 1979b). And mercury, if present in sufficient quantity, was toxic (Hoffman and Moore 1979a, 1979b). Of the aromatic fraction components, the polycyclic aromatics including chrysene, benzo(a)-pyrene, and dimethylbenzanthracene produced high mortality in embryos (Hoffman 1979a, Hoffman and Gay, in preparation). The incubation experiments just described

have helped elucidate the effects that minute amounts of oil would have during the reproductive season.

Outdoor and Field Studies

The indoor pen studies have been invaluable, because under optimally controlled environmental conditions they provided indicators of the subtle types of chronic effects oil may have in natural bird populations. It is highly essential, however, to show correlative effects of crude oil contamination occurring under normal field conditions. Therefore, studies were conducted to confirm effects of oil on birds under more realistic environmental conditions. Outdoor pens were used to determine whether oil applied to small pools of water used by mallards for drinking and swimming would be carried on the ducks' breast feathers to the nest. Eggs of these ducks became oiled, and mortality to the embryos was comparable to those incubated artifically (Albers, in preparation). Additional oil-related studies have been done in the field with a variety of birds. Most of this work was and continues to be done by Fish and Wildlife Service personnel and to a lesser extent under cooperative agreements with State universities. The effects of No. 2 fuel oil on hatchability of marine and estuarine bird eggs naturally incubated in the field were compared with its effects on eggs artifically incubated. Eggs of three species of birds from breeding colonies on the Texas coast were randomly selected, 20 μl of No. 2 fuel oil was applied to the eggshell surface, and the eggs were returned to the nest. After 5 days of natural incubation, embryo mortality was determined. No. 2 fuel oil produced 56% or greater mortality in this study, only slightly less than that in eggs artificially incubated (White, King, and Coon 1979). In other field studies, nesting marine birds were trapped, oil was applied to their breast feathers, and the birds were released. After some preening and swimming, these birds returned to their nests and continued incubating their eggs. Again, embryo mortality was similar to that in the incubator studies (King and Lefever, in preparation). These studies under natural conditions indicated that birds with oiled breast feathers return to their nests and contaminate the eggs they incubate. Literature reports and personal communications with biologists responding to oil spills include accounts of birds foraging in oil-contaminated areas and collecting oil on their breast feathers. Although not as well documented, bird nests containing oiled eggs also have been observed after oil spills occurred near breeding areas (Alexander et al. 1978). These kinds of observations need further documentation to assess the frequency of their occurrence.

SUMMARY

The results from the laboratory studies done at the Patuxent Center have shown the developmental stages to be extremely susceptible to the effects of low-level oil contamination. Although adult ducks may be able to physiologically compensate for oil in the diet, evidence suggests a lowered tolerance to a second environmental challenge. There is also some evidence that chronic ingestion of small amounts of oil by adult female ducks affects

reproduction. These secondary effects are not clear and require more study before their importance to the survival of avian populations can be fully understood. In addition to the experimental results, several reliable indicators have been established to assess the sublethal effects of crude and No. 2 fuel oils on all stages of the avian life cycle. More importantly, insight has been gained into the detection and measurement of the effects of crude oil on natural bird populations.

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