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# Embryotoxic Effects of Crude Oil Containing Nickel and Vanadium in Mallards

David J. Hoffman

*U. S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Md. 20811*

Recent studies (ALBERS 1977, SZARO & ALBERS 1977) have shown that only microliter amounts of crude oil applied to the surface of fertile eggs of several avian species including mallards (Anas platyrhynchos) result in considerable reduction in hatching. Other studies have reported the teratogenic and embryotoxic potential of South Louisiana crude oil, an API reference oil (HOFFMAN 1978a,b), and have shown that certain aromatic hydrocarbons are important contributing factors for embryotoxicity (EASTIN & HOFFMAN 1978). In addition to high aromatic hydrocarbon content certain crude oils contain high concentrations of nickel and vanadium; concentrations of vanadium reaching as high as 1400 ppm in Venezuelan crude oil (COMMITTEE ON BIOLOGICAL EFFECTS OF ATMOSPHERIC POLLUTANTS 1974). Both of these metals result in high embryonic mortality when injected into the yolk sac or chorioallantoic membrane of fertile chicken eggs (RIDGWAY & KARNOFSKY 1952). Egg production has been suppressed in laying hens fed vanadium (HAFEZ & KRATZER 1976) and lipid metabolism altered in laying mallard hens (WHITE and DIETER 1978). The present study was designed to compare the effects of crude oil with and without added vanadium and nickel on embryonic development in mallards.

## METHODS

Fertile mallard duck eggs were obtained within several days of collection from a commercial hatchery and on arrival at the Patuxent Center were placed in a Petersime incubator maintained at 37.5°C and 70% relative humidity. All eggs were candled before treatment and infertile ones were discarded. Eggs were randomly divided into four treatment groups of 70 and treated at 72 h of development as follows: (1) untreated controls (2) treated with 1 µL of South Louisiana crude oil[1], (3) treated with 1 µL of South Louisiana crude oil containing 700 ppm V, or (4) treated with 1 µL of South Louisiana crude oil containing 700 ppm Ni. Vanadyl meso-tetraphenylporphine or nickel mesotetraphenylporphine (Strem Chemicals Inc.) was added to South Louisiana crude oil to produce a V or Ni concentration of approximately 700 ppm. The content of these metals in South Louisiana crude oil is generally low and analysis of our sample revealed that concentration was only several ppm. This low level concentration provided an opportunity to compare the toxicity of the crude oil with and without these metals and in a molecular form (porphyrin) occurring naturally in crude

[1] Obtained from American Petroleum Institute

0007-4861/79/0023-0203 $01.00

oil (YEN 1975). The crude oil was applied by microliter pipet to the shell surface just below the air space of upright eggs and permitted to spread freely (ALBERS 1977).

All eggs were candled daily to determine mortality and dead embryos were removed and examined. All remaining eggs were opened on the 18th day of development. When the embryos were removed from the eggs, embryonic weights, crown-rump lengths, and bill lengths were recorded and all embryos were examined for external defects. One half of the embryos were examined for soft tissue defects by the sectioning technique of WILSON (1965) and by dissection. The other half were cleaned and stained with alizarin red S according to the method described by KARNOFSKY (1965) for skeletal examination.

Embryonic weights, crown-rump lengths, and bill lengths were compared by one way analysis of variance and Duncan's multiple range test. Survival data and number of embryos with one or more defects were compared by Chi square analysis.

## RESULTS

Treatment with crude oil or crude oil containing 700 ppm vanadium or nickel resulted in considerable and significant embryonic mortality compared with untreated controls (Table 1).

TABLE 1

Effects of Adding Vanadium or Nickel to South Louisiana Crude Oil on Mallard Duck Embryos (N=70).

| | Control | 1 µL Crude Oil | 1 µL Crude Oil w/Vanadium (700 ppm) | 1 µL Crude Oil w/Nickel (700 ppm) |
|---|---|---|---|---|
| Survival (%) | 97 | 54[a] | 47[a] | 44[a] |
| Weight (g) | 14.4±1.12[b] | 14.3 ±1.96 | 12.9[c]±2.52 | 13.0[c]±1.74 |
| Crown-rump (mm) | 85.6±3.57 | 81.7[c]±5.93 | 79.7[c]±5.77 | 80.3[c]±3.38 |
| Bill (mm) | 13.3±0.80 | 12.5[c]±1.02 | 11.6[c]±1.23 | 11.8[c]±1.33 |
| Abnormal survivors (%) | 1.5 | 10.7[a] | 36.0[a] | 44.0[a] |

a Significantly different from control group by chi-square, P<.01.
b Mean ± S.D.
c Significantly different by one way analysis of variance (P<.01) and Duncan's multiple range test (P<.05).

Survival of the treatment groups was reduced to 54%, 47%, and 44% in the crude oil, crude oil containing V, and crude oil containing Ni, respectively, compared with 97% survival in the control group. Survival rates among crude oil, crude oil containing V and crude crude oil containing Ni did not differ significantly.
Embryonic growth was generally more stunted when Ni or V was included in the treatment. The presence of Ni or V resulted in a significantly lower mean embryonic weight at 18 days of development

compared with treatment with crude oil alone or to untreated controls. Treatment with crude oil alone at this dose level did not result in a significantly lower mean embryonic weight compared to untreated controls. The presence of Ni or V resulted in a mean embryonic bill length that was significantly shorter than treatment with crude oil alone or untreated controls. All three treatments resulted in mean embryonic bill lengths that were significantly shorter than untreated controls. The mean embryonic crown-rump lengths were significantly shorter in all three treatment groups compared with untreated controls but did not differ significantly from each other. When either V or Ni was present, the percentage of surviving embryos that were abnormal was significantly greater than treatment with crude oil alone. All three treatments resulted in a significant incidence of abnormal survivors compared with untreated controls. Abnormal embryos included ones with bill and eye defects, hydrocephaly, and highly stunted embryos with reduced feather formation.

## DISCUSSION

The addition of relatively high concentrations of vanadium or nickel porphyrin compounds to a crude oil that only contained trace amounts of vanadium and nickel did not significantly increase embryonic mortality above the level caused by the crude oil alone. However, embryonic mortality appeared slightly greater in the presence of vanadium or nickel and it is possible that this effect might be amplified with a lower dose of oil. The temporal pattern of embryonic mortality did not differ in the presence of added vanadium or nickel and there were two major peaks of mortality as reported previously after treatment with crude oil (HOFFMAN 1978a,b). The first of these occurred on days 4 through 6 of development and the second on days 8 through 10 of development, probably representing uptake of oil and metal via the blood vessels of the yolk sac for the first peak and via the chorioallantoic membrane for the second peak. Other metals were more highly toxic when applied in this manner. Methyl mercury chloride increased embryonic mortality on days 4 through 6 of development when applied to the surface of mallard eggs (EASTIN & HOFFMAN 1978). Other forms of nickel and vanadium including the chloride and acetate salts cause high embryonic mortality when injected directly into the yolk sac of 4 day old chick embryos or onto the chorioallantoic membrane of 8 day old embryos (RIDGWAY & KARNOFSKY 1952). Although external applications of crude oil containing high concentrations of vanadium or nickel in the porphyrin form had about the same effect on embryonic survival as crude oil alone, embryonic growth was reduced and the percentage of abnormal survivors was significantly greater in the presence of either metal.

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