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Embryotoxic and Teratogenic Effects of Crude Oil on Mallard Embryos on Day One of Development

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Recent studies (ALBERS 1977, SZARO & ALBERS 1977) have shown that only 1-5 µL of South Louisiana crude or No. 2 fuel oil applied to the surface of fertile eggs of several species during the first two-thirds of the incubation period results in considerable reduction in hatching. The toxic dose of 1-5 µL of these oils covers only 5% of the egg surface at most and equivalent amounts of an aliphatic hydrocarbon mixture are not toxic (ALBERS 1977). Therefore, the embryotoxic effects of certain petroleum products applied to the exterior surface of incubating eggs may be produced by a mechanism that is not related to oxygen deprivation by blockage of the shell pores.

Previous studies (HOFFMAN 1978a,b) have examined the teratogenic and embryotoxic potential of microliter quantities of South Louisiana crude oil, an API reference oil, on mallard eggs of 3 days incubation. The present study examined the effects of equivalent exposures at day one of development.

MATERIALS AND METHODS

Fertile eggs of mallard ducks (Anas platyrhynchos) were obtained within several days of collection from a commercial hatchery (Whistling Wings, Hanover, Illinois) and on arrival at this laboratory were placed in a Petersime incubator maintained at 37.5°C and 70% relative humidity.

All eggs were candled before treatment and infertile ones discarded. Eggs were randomly divided into five groups of 70 and treated at 24 h of development as follows: untreated controls, 10 µL of a mixture of 9 aliphatic hydrocarbons[1], and 1 µL, 5 µL, or 10 µL of South Louisiana crude oil (obtained from the American Petroleum Institute). The aliphatic hydrocarbon mixture served as a control component of crude oil which coated the egg shell in about the same manner as crude oil (ALBERS 1977). Crude oil or the aliphatic hydrocarbon mixture was applied by microliter pipet

[1] These included the following mixed in equal proportions: pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4,6,6-pentamethylheptane, 2,2,4,4,6,8,8-heptamethylnonane, 2,6,10,14-tetramethylpentadecane, and decahydronaphthalene.

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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, sex, embryonic weights, crown-rump lengths, and bill lengths were recorded and all embryos were examined for external defects. One half of the embryos were then defeathered and placed in Bouin's solution for fixation and subsequent examination of soft tissues by the sectioning technique of WILSON (1965) and by dissection. The other half were cleared 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, sex distributions, and numbers of embryos with one or more defects were compared by Chi square.

RESULTS

External applications of crude oil on eggs resulted in a major decline in embryonic survival 3 days after treatment (Figure 1). By 6 days of incubation over half of all mortality for each dose level had occurred. A second major decline in survival occurred after day 7 and through day 10 of development and there was little further mortality after 13 days of development. By day 18 of development the percentage of survivors in the crude oil-treated groups ranged from 1.4 to 57% (Table 1). Treatment with aliphatic hydrocarbons (paraffin) had no effect on survival. Mean embryonic weights were significantly lower in the crude oil-treated group (sexes combined) and in the females of the 5 µL crude oil-treated group compared with controls and those treated with aliphatic hydrocarbons. The mean crown-rump length was significantly shorter in the 5 µL crude oil-treated group (sexes combined) and in the females of this group compared with controls and those treated with aliphatic hydrocarbons. Mean bill lengths were significantly shorter in the crude oil-treated groups (sexes combined) and in the females of these groups compared with controls and those treated with aliphatic hydrocarbons. Applications of crude oil produced a number of abnormal survivors in both the 1 µL and 5 µL crude oil-treated groups. In the 10 µL group there was only one survivor which was stunted. Abnormalities included deformed bills, incomplete ossification of the phalanges, reduction in the size of liver lobes, and general stunting with incomplete feather formation. Single instances of reduction in the number of ribs, abnormal cervical vertebrae, and spina bifida occurred. Treatment with aliphatic hydrocarbons did not result in any of these effects.

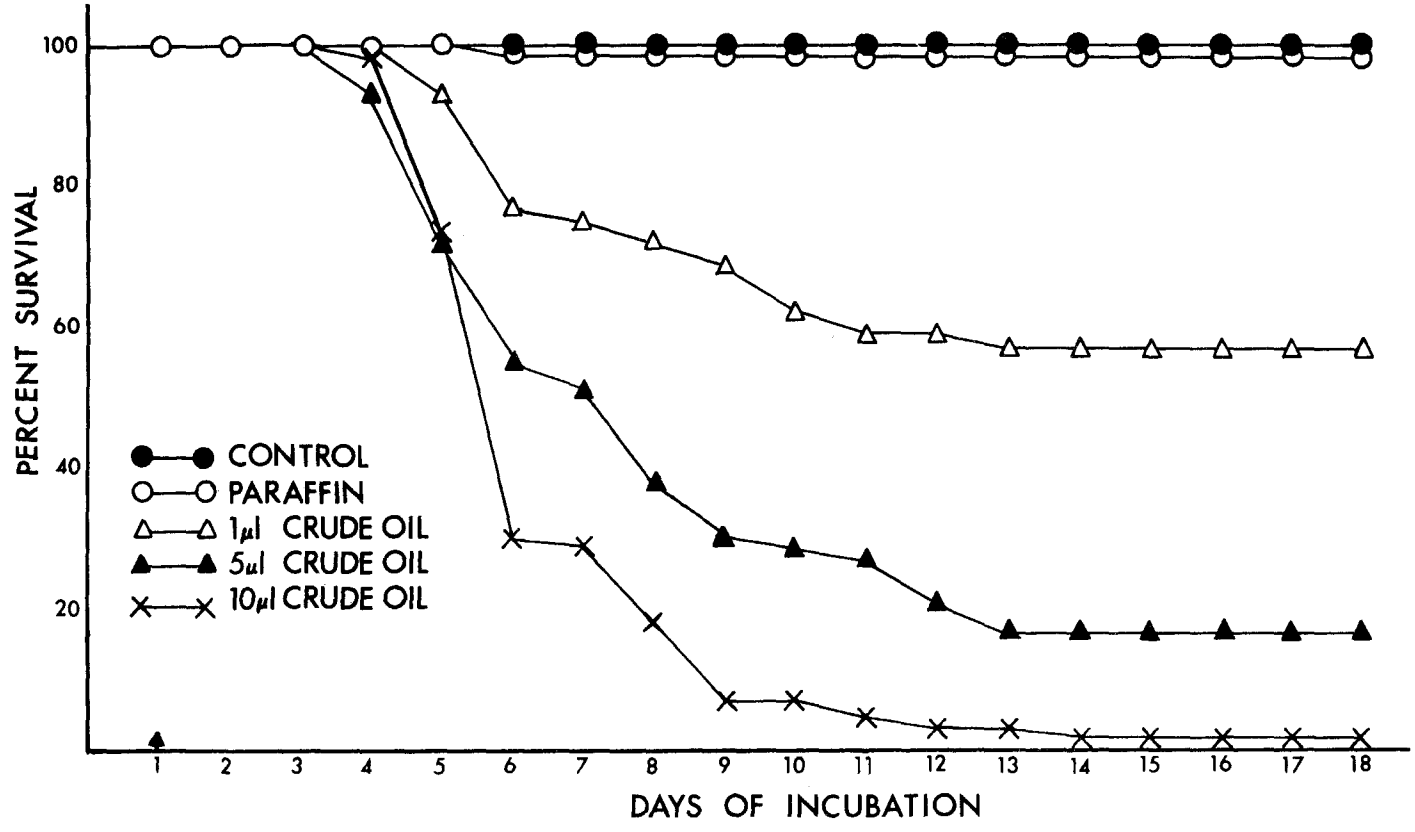

Figure 1. Effects of South Louisiana crude oil on survival of mallard embryos. (The oil was applied on day 1 of development).

TABLE 1

Effects of South Louisiana Crude Oil on Mallard Eggs on Day 1 of Development. (N = 70).

	Control	Aliphatic hydrocarbons	Crude Oil[a] 1 µl	Crude Oil[a] 5 µl
Survival (%)	98.5	100	57[b]	17[b]
Sex ratio, M:F	57:43	55:45	44:56	40:60
Weight (g)				
male	16.8±1.99[c]	17.1±2.22	16.2 ±2.04	16.2 ±2.04
female	16.3±1.22	16.4±2.28	14.9 ±2.93	12.1[d]±2.93
combined	16.6±1.73	16.8±2.28	15.4[d]±2.62	13.7[d]±2.62
Crown-rump (mm)				
male	87.7±3.50	88.7±3.36	87.2 ±3.88	86.9 ±1.11
female	85.9±2.96	86.9±4.28	83.9 ±5.20	79.4[d]±7.03
combined	87.0±3.38	87.9±3.93	85.4 ±4.90	82.4[d]±6.54
Bill (mm)				
male	13.8±0.50	13.6±0.43	13.3 ±0.75	13.4 ±0.48
female	13.6±0.56	13.6±0.45	12.9[d]±0.71	12.0[d]±1.58
combined	13.7±0.54	13.6±0.45	13.1[d]±0.74	12.6[d]±1.04
Abnormal[e] survivors (%)	3	1.5	29.4[b]	63.6[b]

[a] There was only one survivor in the 10 µl treatment group (male, 7.7 g; crown-rump, 69.5 mm; bill, 9.5 mm).

[b] Significantly different from control and aliphatic hydrocarbon-treated groups, $P < 0.01$, chi-square.

[c] Mean ± S.D.

[d] Significantly different from control and aliphatic hydrocarbon-treated groups, by one-way analysis of variance ($P < .01$) and Duncan's multiple range test ($P < .05$).

[e] These included controls - stunted growth (1), incomplete ossification (1); aliphatic hydrocarbons - stunted growth (1); 1 µl crude oil - spina bifida (1), visceral defects (4), incomplete ossification (3), stunted growth and feather formation (4); 5 µl crude oil - brain and eye defects (2), bill defects (2), visceral defects (2), incomplete ossification (2), stunted growth and feather formation (2).

DISCUSSION

In the present study external applications of crude oil on mallard eggs on day 1 of development resulted in a similar pattern of embryonic death when compared with treatment on day 3 of development (HOFFMAN 1978a,b). There were two major declines in survival with treatment on either day 1 or 3. The first period of embryonic death was somewhat more pronounced with treatment on day 1. The second major period of embryonic death occurred during the time of rapid outgrowth of the chorioallantoic membrane over the surface of the inner shell membrane, suggesting potential for further uptake of the oil by this highly vascular membrane. The extent of total mortality and dose-related mortality by 18 days of development was similar with treatment on either day 1 or 3 of development. Decreased growth as reflected by weight, crown-rump length, and bill length was significant in both the 1 and 5 µL oil-treated groups in the present study. With treatment on day 3 (HOFFMAN 1978a,b) these changes were less evident in the 1 µL oil-treated group. Treatment on day 1 resulted in a significant incidence of abnormal survivors in the 1 and 5 µL oil-treated groups which was only apparent in the 5 µL oil-treated group with treatment on day 3. These findings indicate that the effects of crude oil applications in mallards on days 1 and 3 result in equivalent embryonic death but the overall toxicity is greater when treatment is on day 1.

Treatment on day 1 with aliphatic hydrocarbons that occur in crude oil had virtually no effect on survival, embryonic growth, or number of defects, further demonstrating that the toxicity of crude oil was not due to blockage of shell pores and subsequent hypoxia. Other studies have identified individual aromatic hydrocarbons and combinations of aromatic hydrocarbons that occur in South Louisiana crude oil and are toxic when applied to mallard eggs (EASTIN and HOFFMAN 1978).

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REFERENCES

ALBERS, P. H.: In: Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms. D. A. Wolfe, ed., pp. 158-163. New York: Pergamon Press 1977.

EASTIN, W. C., and D. J. HOFFMAN: In: AIBS Conference on Assessment of Ecological Impacts of Oil Spills. (In press) 1978.

HOFFMAN, D. J.: Teratology 17(2):40A (1978a).

HOFFMAN, D. J.: Toxicol. Appl. Pharmacol. (In press) (1978b).

KARNOFSKY, D. A.: In: Teratology: Principles and Techniques. J. G. Wilson and J. K. Warkany, eds., pp. 194-213. Chicago and London: University of Chicago Press 1965.

SZARO, R. C., and P. H. ALBERS: In: Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms. D. A. Wolfe, ed., pp. 164-167. New York: Pergamon Press 1977.

WILSON, J. G.: In: Teratology: Principles and Techniques. J. G. Wilson and J. K. Warkany, eds., pp. 262-277. Chicago and London: University of Chicago Press 1965.