

## Effects of Corexit 9527 on the Hatchability of Mallard Eggs

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Oil dispersants are commonly used to treat oil spills on most water areas of the world. In the United States, dispersants can be used only if deemed necessary according to conditions set forth in the National Oil and Hazardous Substances Pollution Contingency Plan (COUNCIL ON ENVIRONMENTAL QUALITY 1975) and if the dispersant has been approved by the U. S. Environmental Protection Agency. Field testing of chemical dispersants in the territorial waters of the United States began in September 1978 off the coast of southern California. Sponsored by the American Petroleum Institute (API) and the Southern California - Petroleum Contingency Organization (SC-PCO) (SMITH and HOLLIDAY 1979), the tests were designed to evaluate dispersant effectiveness, application procedures, and effects of chemically dispersed oil on marine organisms. Two dispersants were used in these tests, Corexit 9527, a water soluble, "self-mixing", concentrated dispersant and BP 1100 WD, a water soluble, concentrated dispersant requiring mechanical mixing.

Toxicity testing of chemical oil dispersants increased rapidly following the biologically destructive use of dispersants at the wreck of the *Torrey Canyon* in 1968 (SMITH 1968). The newer dispersants proved to be much less toxic to fish, fish larvae, bivalves, and crustaceans than the older types (SWEDMARK et al. 1973, WILSON 1977). Corexit 9527 alone retarded fertilization and larval development of sea urchins and fish (LONNING and HAGSTROM 1976, HAGSTROM and LONNING 1977), was moderately toxic to 2 species of marine green algae (HELDAL et al. 1978), and was less toxic to fingerling rainbow trout than when combined with No. 2 fuel oil (WELLS and DOE 1976). Research on the effects of dispersants and chemically dispersed oil has not included birds, mammals, or reptiles.

Crude and refined oils are very toxic to the embryos of aquatic birds (ALBERS 1977, 1978; SZARO and ALBERS 1977, ALBERS and SZARO 1978, SZARO et al. 1978, HOFFMAN 1978, COON et al. 1979, WHITE et al. 1979, MCGILL and RICHMOND [unpublished]). Experiments by HARTUNG 1965, ALBERS (in preparation), and KING and LEFEVER (in preparation) have shown that oiled birds will resume incubation (unless heavily oiled) and transfer oil to the eggs. RITTINGHAUS 1956 and BIRKHEAD et al. 1973 reported the natural occurrence of adult-to-egg oil transfer and the

subsequent hatching failure for sandwich terns (*Sterna sandvicensis*) and great black-backed gulls (*Larus marinus*). Chemical dispersion of a surface layer of oil into small droplets that disperse rapidly into the water column may change the effects of the oil on living organisms. The dispersant also may be toxic and its effects must be considered when evaluating the impact of chemically dispersed oil. The present paper reports the results of a study of the effects of Corexit 9527 dispersant, crude oil, and crude oil/Corexit 9527 mixtures on mallard duck (*Anas platyrhynchos*) embryos.

## METHODS

After 6 days of incubation in a commercial incubator, viable mallard eggs were randomly divided into 13 groups of 50 eggs each. Twelve groups of eggs were then treated with either 1, 5, or 20  $\mu$ l of Prudhoe Bay (Alaska) crude oil, Corexit 9527, a 30:1 oil/Corexit mixture, or a 5:1 oil/Corexit 9527 mixture. The thirteenth group was an untreated control. All substances were applied to the surface of the egg near the air cell end by microliter syringe (ALBERS 1977). The 20  $\mu$ l treatment consisted of 4 5- $\mu$ l applications.

All eggs were candled on day 12 of incubation. Eggs with dead embryos were opened and the stage of development was recorded. The embryonic stage of development was not necessarily identical to the chronological age of the embryo (ALBERS 1977). Ducklings were weighed and examined for gross malformations within 24 hr of hatching. Eggs that failed to hatch after 29 days of incubation were opened and the stage of development of the embryo was recorded.

## RESULTS

The addition of Corexit 9527 to crude oil did not seem to alter the viscosity or appearance of the oil in the 30:1 mixture but the 5:1 mixture was less viscous and less homogeneous in appearance than the oil as it ran over the surface of the eggs. Corexit 9527 alone was less viscous than the crude oil.

Mallard eggs treated with 20  $\mu$ l of crude oil, Corexit 9527, 30:1 oil/Corexit 9527, 5:1 oil/Corexit 9527, and 5  $\mu$ l Corexit 9527 and 5:1 oil/Corexit 9527 had significantly ( $P < 0.01$ ) lower hatching success than the untreated control eggs (Table 1). The comparisons between treated groups and the control and among treated groups were used to create a general toxicity ranking; (Corexit 9527 = 5:1 oil/Corexit 9527) > Prudhoe Bay crude oil > 30:1 oil/Corexit 9527.

TABLE 1

Hatchability of mallard duck eggs treated with Prudhoe Bay crude oil, Corexit 9527 dispersant, a 30:1 oil/dispersant mixture, or a 5:1 oil/dispersant mixture after 6 days of incubation. N = 50 for each treatment group

Treatment	Dead embryos	Percent hatching success	Group comparisons
1. Control	6	88	2 x 5 2 x 8**
2. 20 $\mu$ l oil	34	32*	2 x 11
3. 5 $\mu$ l oil	9	82	2 x 6**
4. 1 $\mu$ l oil	9	82	3 x 9 3 x 12**
5. 20 $\mu$ l 9527	32	36*	4 x 7
6. 5 $\mu$ l 9527	17	66*	4 x 10
7. 1 $\mu$ l 9527	5	90	4 x 13
8. 20 $\mu$ l 30:1	22	56*	
9. 5 $\mu$ l 30:1	9	82	
10. 1 $\mu$ l 30:1	5	90	
11. 20 $\mu$ l 5:1	33	34*	
12. 5 $\mu$ l 5:1	26	48*	
13. 1 $\mu$ l 5:1	8	84	

\* Significantly different from the control, chi-square test,  $df = 1$ ,  $P \leq 0.01$

\*\* Hatching success of the 2 treatment groups is significantly different, chi-square test,  $df = 1$ ,  $P \leq 0.01$

The significant analysis of variance ( $P \leq 0.01$ ) and significant group comparisons ( $P \leq 0.10$ ) of the stage of embryonic development at death showed that 5  $\mu$ l of the 5:1 oil/Corexit 9527 mixture caused death earlier than 5  $\mu$ l of Corexit 9527 or 30:1 oil/Corexit 9527, and 20  $\mu$ l 5:1 oil/Corexit 9527 caused death earlier than 5  $\mu$ l 30:1 oil/Corexit 9527 (Table 2). The largest treatments (20  $\mu$ l) caused death earlier in embryonic development than the smallest treatments (1  $\mu$ l). This treatment-related effect was also reported in a previous study (ALBERS 1977). None of the treated groups were different from the control.

TABLE 2

The stage of development at death of mallard embryos dying during the experiment

Treatment	No. of dead embryos	Mean stage of development in days*	Group comparisons**
1. Control	6	18.2	6 x 12 9 x 11
2. 20 $\mu$ l oil	34	12.9	9 x 12
3. 5 $\mu$ l oil	9	14.8	2,5,8,11 x 4,7,10,13
4. 1 $\mu$ l oil	9	20.6	
5. 20 $\mu$ l 9527	32	13.5	
6. 5 $\mu$ l 9527	17	18.9	
7. 1 $\mu$ l 9527	5	19.8	
8. 20 $\mu$ l 30:1	22	13.2	
9. 5 $\mu$ l 30:1	9	21.7	
10. 1 $\mu$ l 30:1	5	22.4	
11. 20 $\mu$ l 5:1	33	10.8	
12. 5 $\mu$ l 5:1	26	10.3	
13. 1 $\mu$ l 5:1	8	17.4	

\*One-way analysis of variance, significant,  $P \leq 0.01$ .

\*\* Paired and complex comparisons, Scheffe's procedure, significant when  $P \leq 0.10$

Corexit 9527 appeared to penetrate egg shells and shell membranes as readily as crude oil. When a drop of the dispersant was unintentionally applied to the egg shell next to the embryo, the embryo became fused to the shell membrane and died within 24 hr. Neither crude oil, refined oil (ALBERS 1977), nor oil/Corexit 9527 mixtures caused this fusion of the embryo to the egg shell.

Hatching weights of mallard ducklings were not significantly different ( $P > 0.01$ ) and none of the paired comparisons between treated groups and the control or among treated groups were significantly different ( $P > 0.10$ ) (Table 3). The lowest mean group weights were those of the 5:1 oil/Corexit 9527 treatments.

No gross external malformations or behavioral abnormalities were observed.

TABLE 3

Hatching weights of mallard ducklings

Treatment	No. of ducklings	Mean hatching weight*	Group comparisons**
1. Control	44	42.6	
2. 20 $\mu$ l oil	16	43.4	
3. 5 $\mu$ l oil	41	42.1	
4. 1 $\mu$ l oil	41	42.0	
5. 20 $\mu$ l 9527	18	41.2	
6. 5 $\mu$ l 9527	33	42.1	
7. 1 $\mu$ l 9527	45	42.7	
8. 20 $\mu$ l 30:1	28	42.8	
9. 5 $\mu$ l 30:1	41	41.2	
10. 1 $\mu$ l 30:1	45	42.5	
11. 20 $\mu$ l 5:1	17	40.8	
12. 5 $\mu$ l 5:1	24	40.6	
13. 1 $\mu$ l 5:1	42	40.2	

\* One-way analysis of variance, not significant, ( $P > 0.01$ )

\*\* All possible paired comparisons were non-significant,  $P > 0.10$ , Scheffe's procedure

#### DISCUSSION

Wild birds probably would not encounter Corexit 9527 as a concentrate or as a diluted spray mixture (9 parts water to 1 part 9527) because of rapid dilution upon reaching the water surface (CANEVARI and LINDBLOM 1976). The toxicity of the concentrated dispersant must be determined, however, so that the effect of oil/dispersant mixtures can be properly interpreted.

Manufacturer's recommendations indicate that 1 part of Corexit 9527 usually is adequate to disperse 30-60 parts of oil, depending on the type of oil (EXXON CHEMICAL COMPANY 1978). In

field tests, Corexit 9527 used in a 30:1 ratio effectively dispersed 65% of spilled crude oil for 10 minutes (WELLS and DOE 1976). In another test it effectively dispersed crude oil when used at  $\leq 8.5:1$  (GILL 1977). The API/SC-PCO tests used oil and dispersants in a 5:1 ratio although the actual ratio was higher because some of the dispersant missed the floating oil (SMITH and HOLLIDAY 1979). Other reports also have shown that the amount of dispersant needed to effectively disperse oil is greater than the manufacturer's recommended ratios (SWEDMARK et al. 1973). The 30:1 and 5:1 oil/dispersant ratios used in this study therefore appear to be appropriate and realistic.

Prudhoe Bay crude oil was less toxic to mallard eggs than South Louisiana crude oil, Kuwait crude oil, or No. 2 fuel oil (ALBERS 1977; SZARO et al. 1978). Therefore, concentrated Corexit 9527 may be slightly more toxic to bird eggs than the least toxic crude or refined oil tested at the Patuxent Wildlife Research Center.

Corexit 9527 and 5:1 oil/Corexit 9527 appear to be slightly more toxic than Prudhoe Bay crude oil and 30:1 oil/Corexit 9527 because of the difference in toxicity at the 5  $\mu$ l level. The toxicity of Corexit 9527 relative to Prudhoe Bay crude oil may have been affected by an increase in egg surface coverage due to a lower viscosity for Corexit 9527 than for Prudhoe Bay crude oil (greater opportunity for penetration) or by differences in penetration capability. The Corexit 9527 in the 5:1 oil/Corexit 9527 mixture may have increased the toxicity of the crude oil by permitting greater penetration of the oil or by reducing the viscosity of the oil and increasing the surface area affected. The low toxicity of 30:1 oil/Corexit 9527 compared with the 5:1 mixture or with oil alone is difficult to explain. The small amount of dispersant in the mixture appeared to reduce the toxicity of crude oil at the 20  $\mu$ l level. Some interaction may have occurred, because the toxicity for 20  $\mu$ l 30:1 oil/Corexit 9527 was less and the toxicity of 5  $\mu$ l 5:1 oil/Corexit 9527 was more than would have been expected for the amounts of crude oil and dispersant present in the mixtures.

The 18.2-day mean for the stage of development of the 6 control embryos that died was lower than expected. In previous experiments, the stage of development for control mortality was 23-24 days even though hatchability was about the same as in the present study (ALBERS 1977; ALBERS [unpublished]). Therefore, results of the tests for significant differences between treated groups and controls probably are conservative. The early death produced by applications of 5:1 oil/Corexit 9527 suggests that Corexit 9527 may speed the lethal effect of the crude oil.

The low mean weights of ducklings hatched from eggs exposed to all 3 treatments of 5:1 oil/Corexit 9527 compared with hatching

weights of ducklings from eggs exposed to crude oil or Corexit 9527 alone suggests that certain mixtures of oil and Corexit might affect hatching weights. Although this suggestion is based on non-significant differences, I mention it because very few differences in hatching weights have been reported in similar studies (ALBERS 1977; SZARO and ALBERS 1977; ALBERS 1978; SZARO et al. 1978).

Because Corexit 9527 and oil/Corexit 9527 mixtures are toxic to bird eggs 2 important questions need to be addressed; (1) can chemically dispersed oil be transferred to eggs by breeding adults, and (2) can enough oil or dispersant be transferred to significantly affect egg hatchability or breeding bird behavior?

#### ACKNOWLEDGEMENTS

I thank Jeffrey Schnebelen for his assistance in the study and Lucille F. Stickel, William C. Eastin, and James Spann for their review of a preliminary draft of this paper.

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