Bull. Environm. Contam. Toxicol. 30, 17–23 (1983)

Mallard Egg Quality: Enhancement by Low Levels of Petroleum and Chlorinated Hydrocarbons

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Chlorinated and petroleum hydrocarbons are widespread and significant pollutants in global ecosystems (OHLENDORF et al. 1978, WHITE 1979, WOLFE 1977). When fed to mallards (Anas platyrhynchos), high levels of either cause a significant decline in egg quality (GRAU et al. 1977; HOLMES et al. 1978; VANGILDER and PETERLE 1980, 1981). We now report that petroleum hydrocarbons fed together with a chlorinated hydrocarbon enhance egg quality if fed to mallards at low, environmentally realistic levels. However, when low levels of either petroleum hydrocarbons or a chlorinated hydrocarbon are fed alone to mallards, egg quality is reduced. For the purposes of this paper, we defined egg quality in terms of egg size, the amount and relative proportions of the egg's components, the composition of certain components, egg hatchability, and duckling survivorship.

METHODS AND MATERIALS

Mallard hens (N = 80), 4 generations removed from wild stock, were maintained in 2- and 3-tiered poultry batteries at 10 to 25°C on a 6h light - 18h dark cycle commencing 30 January, 1980. Hens were given food and water ad lib. On 19 February 1980, 4 groups of 20 hens each were randomly assigned to receive a diet containing 0.5% (w/w) South Louisiana crude oil (SLCO), one containing 5 ppm DDE, one containing 0.5% (w/w) SLCO + 5 ppm DDE or a diet of untreated food (control) in order to examine single effects and interactions of the pollutants. Egg production was induced by increasing day length to 16h light on 5 April 1980. Each group of hens was released weekly into an enclosure with 10 untreated mallard drakes beginning 7 April 1980 to insure production of fertile eggs. Eggs were collected daily, identified by hen and laying date, and weighed (± 1 mg).

Alternate eggs laid by each hen were kept for egg quality analysis and the others were incubated. Those kept for egg quality analysis were hard-boiled and separated into 3 components: shell, yolk, and albumen. Each component was weighed (± 1 mg), identified by hen and date, and stored frozen in airtight jars. Egg quality was analyzed from a subsample of these eggs. This subsample consisted of the components of the first 5 unincubated eggs laid by each of 5 randomly selected hens in each group (N = 100 eggs, 25 per group). Yolks and albumens of these eggs were lyophilized to constant weight and analyzed for water, protein

0007-4861/83/0030-0017 $01.40

(Kjeldahl nitrogen determination), and gross energy content (bomb calorimetry).

Eggs assigned to be incubated were set weekly in Jamesway incubators to provide data on egg hatchability and to provide ducklings for the survival experiment. Four days before the expected hatching date, eggs were transferred to individual compartments in a hatching incubator. The incubator was continuously monitored so that time of hatching for each duckling could be determined.

The objective of the next phase of our experiment was to determine whether dietary exposure of mallards to oil and/or DDE would effect the ability of their ducklings to survive. Because starvation and chilling have been cited as major causes of mortality in young precocial birds (KEAR 1965, DZUBIN and GOLLOP 1972), we tried to simulate these conditions in the laboratory. One-hour old ducklings were weighed and placed into an environmental chamber in individual compartments at 20°C. No food or water was provided. The environmental chamber was continuously monitored so that survival time for each individual could be determined.

RESULTS

Reproductive Performance. The onset of laying, egg production, and egg fertility did not differ among the groups (Table 1; $P > 0.05$). Egg fertility was uniformly low among the groups because mallard drakes could not be maintained in good reproductive condition.

TABLE 1

Reproductive statistics for mallard hens fed a control, South Louisiana crude oil (SLCO) (0.5% w/w), DDE (5 ppm), or SLCO + DDE (0.5% w/w + 5 ppm) diet. Tabled values of egg production and fertility are means ± 1 standard error. Statistical analyses follow SNEDECOR and COCHRAN (1967:221, 515)

	CONTROL	SLCO	DDE	SLCO + DDE
Median date of first egg	19 April	18 April	20 April	20 April
Egg production (Eggs per 100 hen-days)	47.5±3.7	42.3±3.0	48.7±9.0	50.5±3.9
Fertility (% of incubated eggs fertile)	16.4±4.2	7.7±3.4	14.2±4.4	10.0±3.3

Egg Size. The mass ($\bar{x}$ = 58.07g) of eggs laid by hens fed SLCO, whether alone or in combination with DDE, was significantly greater than that of eggs laid by hens not fed SLCO ($\bar{x}$ = 54.07g) ($F_{1,16}$ = 6.55, P < 0.025). The mass ($\bar{x}$ = 57.09g) of eggs laid by hens fed DDE, whether alone or in combination with SLCO, was not different from that of hens not fed DDE ($\bar{x}$ = 55.06g) ($F_{1,16}$ = 1.68, P > 0.05) (2x2 nested factorial analysis of variance).

Egg Components and Composition. The increased mass of eggs laid by hens fed SLCO was accompanied by disproportionate changes in the weight of each component. Eggs laid by hens fed SLCO, whether alone or in combination with DDE, contained proportionately more shell ($F_{1,95}$ = 4.40, P = 0.0386) and yolk ($F_{1,95}$ = 6.46, P = 0.0127) and proportionately less albumen ($F_{1,95}$ = 9.60, P = 0.0026) than did eggs laid by hens not fed SLCO (Table 1). Eggs laid by hens fed diets containing DDE had proportionately less shell than did eggs laid by hens not fed DDE ($F_{1,95}$ = 30.33, P < 0.0001) (Table 2). Not only was the proportion of yolk greater in

TABLE 2

Adjusted marginal means from a factorial analysis of covariance (2x2) of shell, yolk, and albumen mass of eggs laid by hens fed South Louisiana crude oil (SLCO) (0.5% w/w) or DDE (5 ppm). To detect changes in the relative proportions of the eggs' components a 2x2 factorial analysis of covariance was conducted on shell, yolk, and albumen mass with egg mass as the covariate. Residual error variances and slopes did not differ among the groups and the SLCO x DDE interaction was not significant for the egg components (P > 0.05). Therefore, adjusted marginal means were used as estimates of the effects of SLCO and DDE. The standard error of the difference between SLCO marginal means is 0.0797, 0.2572, and 0.2649 g for shell, yolk, and albumen mass, respectively. The standard error of the differences between DDE marginal means is 0.0736, 0.2375, and 0.2447 g for shell, yolk, and albumen mass, respectively.

	SLCO (%)		DDE (ppm)	
	0.0	0.5	0.0	5
Shell mass (g)	5.76	5.93*	6.05	5.64**
Yolk mass (g)	20.26	20.91*	20.44	20.73
Albumen mass (g)	30.05	29.23**	29.58	29.70

* P < 0.05, **P < 0.01

eggs laid by hens fed SLCO, but the caloric density of yolks from these eggs was also greater. The caloric density of yolks from eggs laid by hens fed SLCO ($\bar{x}$ = 777.6 Kcal/100g dry yolk), whether alone or in combination with DDE, was 8.1 ± 0.3 Kcal/100 g dry

yolk greater than that of yolks from eggs laid by hens not fed SLCO ($\bar{x}$ = 769.5 Kcal/100g dry yolk) ($F_{1,16}$ = 9.25, P = 0.0078) (6). The caloric density of yolks from eggs laid by hens fed DDE ($\bar{x}$ = 773.5 Kcal/100g dry yolk), whether alone or in combination with SLCO, was not different than that from eggs laid by hens not fed DDE ($\bar{x}$ = 773.6 Kcal/100g dry yolk) ($F_{1,16}$ = 0.00, P = 0.9954) (2x2 nested factorial analysis of variance). Water and protein content of yolk were not affected (P > 0.05).

Egg Hatchability. There was a significant interaction between the two pollutants in their effect on egg hatchability. Although the size, proportion of yolk, and caloric density of yolk of eggs laid by hens fed SLCO was increased, hatchability of eggs [(number eggs hatching/number eggs fertile) x 100] laid by hens fed SLCO alone was significantly less than that of eggs laid by hens fed any other diet (P < 0.05). Hatchability was 29% (6/21), 69% (35/51), 52% (23/44), and 63% (19/30) for eggs laid by mallards fed the SLCO, control, DDE, and SLCO + DDE diets, respectively.

Duckling Survivorship. We hypothesized based on the results of the above egg quality analyses that because eggs laid by mallards fed SLCO contain more energy, by virtue of their larger size and proportion of yolk and higher caloric density of yolk, that ducklings hatching from these eggs would survive longer under our experimental conditions. The results of the survival experiment supported this hypothesis (Table 3). Eggs laid by mallards fed SLCO together with DDE were larger, produced larger ducklings, and the ducklings survived, on average, 12 hours longer than did those

TABLE 3

Survival time of mallard ducklings hatched from eggs laid by hens fed a control, South Louisiana crude oil (SLCO) (0.5% w/w), DDE (5 ppm), or SLCO + DDE (0.5% w/w + 5 ppm) diet.

	Control N=22		SLCO N=2		DDE N=18		SLCO + DDE N=9	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Egg mass (g)	54.22	0.88	61.10	-	55.82	1.10	60.16	1.53*
Duckling mass (g)	37.11	0.67	42.36	-	37.66	0.65	41.45	1.37*
Survival time (h)	58.74	2.42	67.92	-	61.29	2.56	70.47	3.10*

* P < 0.05, significantly different from control

of control hens. Unfortunately the sample size of eggs laid by hens fed SLCO alone was too small to make any meaningful comparisons. DDE had no effect on egg mass, duckling mass, or survival time.

DISCUSSION

Eggs laid by mallards fed SLCO together with DDE were of better quality than those laid by mallards fed any other diet (Table 4). The enhanced egg quality was probably the product of

TABLE 4

Summary of the effects of South Louisiana crude oil (SLCO) (0.5% w/w) DDE (5 ppm), and SLCO + DDE (0.5% w/w + 5 ppm) diets on reproductive performance and egg quality of mallards.

	SLCO alone	DDE alone	SLCO + DDE
Onset of laying	No effect	No effect	No effect
Egg production	No effect	No effect	No effect
Egg fertility	No effect	No effect	No effect
Egg size	Increased	No effect	Increased
Proportion of shell	Increased	Decreased	Increased
Proportion of yolk	Increased	No effect	Increased
Proportion of albumen	Decreased	No effect	Decreased
Amount of energy per gram of dry yolk	Increased	No effect	Increased
Egg hatchability	Decreased	No effect	No effect
Survival ability of young that hatch	?	No effect	Increased

1) the positive effects of SLCO on egg size and composition and 2) the antagonism of DDE to the embryotoxic effects of SLCO. Our results indicate that the physiological processes of mallard hens chronically exposed to low levels of SLCO, whether alone or in combination with DDE, were enhanced. Hormesis, the apparent enhancement of physiological processes, has also been found in crab zoeae exposed to low levels of petroleum hydrocarbons (LAUGHLIN et al 1981). PATTON and DIETER (1980) hypothesized that "short-term (less than one generation) adaptive changes may occur in response to the ingestion of petroleum hydorcarbons." Our data also show that ingestion of low levels of petroleum hydrocarbons alone by adult mallards may induce hormesis or adaptive changes in their reproductive physiology yet still be toxic to their offspring.

To our knowledge, this study is the first to demonstrate antagonism between petroleum and chlorinated hydrocarbons. WARE

(1980) attributed antagonism of 2 toxicants to the "inhibition by one toxicant of the enzymes involved in the activation of the other." Both chlorinated and petroleum hydrocarbons have been shown to increase the activity of hepatic microsomal enzymes (MILLER et al. 1978, PEAKALL 1967). Perhaps DDE competes with the aromatic hydrocarbon fraction of SLCO such that toxic products resulting from the induction of microsomal enzymes are not transferred to the embryo. Whatever the mechanism, the results indicate that interactions between pollutants can significantly alter reproductive characteristics of birds.

Higher levels of dietary SLCO (1-3%), cause eggshell thinning in eggs laid by mallards (HOLMES et al. 1978, VANGILDER and PETERLE 1980). Mallards fed a diet containing 2% SLCO laid eggs that were smaller and contained a smaller proportion of yolk than eggs laid by hens fed a control diet (VANGILDER and PETERLE 1981). The caloric density of the yolk, however, was not affected. Egg hatchability was also reduced. Japanese quail (Coturnix coturnix japonica), chickens (Gallus gallus domesticus), and Canada geese (Branta canadensis moffiti) fed single doses of petroleum produce eggs with abnormal yolk structure (GRAU et al. 1977). Production and hatchability of eggs laid by Japanese quail were also reduced. Mallard hens fed a diet containing 10 ppm DDE laid eggs that did not differ in size from eggs laid by control hens but that had a reduced proportion of yolk (VANGILDER and PETERLE 1981). Ducklings hatched from eggs laid by mallards fed either 2% SLCO or 10 ppm DDE exhibit reduced thermoregulatory ability and those that are able to thermoregulate show reduced survivorship (VANGILDER and PETERLE 1980).

The results of this study and our previous studies of SLCO and DDE at high levels (VANGILDER and PETERLE 1980, 1981) indicate that pollutant-induced effects may be positive or negative depending upon 1) the dose level 2) the life history stage of the organism and 3) interactions with other pollutants. This concept probably applies to other types of pollutants and suggests that current methods for assessing the biological impacts of chronic low level pollution are too simplistic.

ACKNOWLEDGEMENTS

This research was supported by the U. S. Fish and Wildlife Service, Patuxent Wildlife Research Center (USDI 14-16-0009-78-973) and the Ohio Cooperative Wildlife Research Unit. The senior author was supported during manuscript preparation by a grant from the U. S. Department of Energy (DE-AC09-76SR00819) to the University of Georgia. Scott Cameron and Tom Ranney provided technical assistance. I. L. Brisbin, J. D. Congdon, J. W. Gibbons, M. H. Smith, and L. J. Vitt critically reviewed the manuscript. Mallards were supplied by the Max McGraw Wildlife Foundation, Dundee, Ill. SLCO (American Petroleum Institute Reference Oil No. 2) was obtained from Dr. Jerry M. Neff, Texas A & M University.

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Accepted November 2, 1982