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Short Communication

DIFFERENCES IN MORTALITY AMONG BOBWHITE FED METHYLMERCURY CHLORIDE DISSOLVED IN VARIOUS CARRIERS

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Abstract—Twelve-day-old bobwhite chicks were fed a diet containing 0, 5.4 or 20 ppm methylmercury chloride. The methylmercury chloride was added to the diet either in a dry, pulverized form or dissolved in acetone, propylene glycol or corn oil. Mortality was measured for 6 weeks, and samples of liver were saved for mercury analysis. Mortality was significantly lower in birds fed 20 ppm methylmercury chloride when acetone was the solvent. The reduced mortality could not be explained by effects of acetone on dietary level of mercury or on uptake of mercury into the body.

Keywords—Bobwhite Methylmercury Carriers Solvents Toxicity Diluents

INTRODUCTION

Liquids such as acetone, corn oil and propylene glycol are often used as carriers (diluents or solvents) in dietary toxicity tests, yet little is known about their effects on the toxicity of test chemicals. Acetone at a level of 4% in the diet for a 5-d period had no adverse effects on Japanese quail (*Coturnix japonica*) and ring-necked pheasant (*Phasianus colchicus*) [1], nor did propylene glycol at a level of 8% in the diet have an effect on dogs in a 2-year study [2].

Hill and Camardese [3] suggested that a carrier should be used in all subacute (LC50) tests with birds because diets prepared with carriers appeared to yield better dose–response relationships than did diets in which milligram amounts of a test chemical were mixed directly into the feed. Turner [4] pointed out that dissolving a test chemical in corn oil may simulate the way that that chemical is ingested in some foods, such as animal matter, but not in others, such as sprayed vegetation.

The purpose of our experiment was to test whether acetone, corn oil and propylene glycol as carriers or the absence of a carrier affected the toxicity of methylmercury chloride in Northern bobwhite (*Colinus virginianus*).

METHODS

Ten 12-d-old bobwhite were randomized to each of 48 pens measuring 34 × 98 × 22 (high) cm. In each pen, a heated section was maintained at 35.2 to 38°C for the first week and then decreased about 2.8°C each week thereafter until 4 weeks, after which the temperature was maintained at 24 to 27°C. Outside the heated areas, the temperature followed a gradient down to a room temperature of about 20 to 24°C. Water and a commercial game bird starter mash (Zeigler Bros., Gardners, PA) were available ad libitum. A photoperiod of 14 h of light and 10 h of dark was used.

Sixteen randomly selected pens of 12-d-old bobwhites were assigned one of the following dietary concentrations of methylmercury chloride (approximately 100% pure; ICN Pharmaceuticals, Plainview, NY) for 6 weeks; 0, 5.4 or 20 ppm. Within each dietary concentration, the methylmercury chloride was added to the diet in one of four ways: (a) mixed dry into the diet, as a powder, (b) dissolved in a small amount of acetone and then into corn oil so that corn oil constituted 1% of the diet, (c) dissolved in a small amount of acetone and then into propylene glycol so that propylene glycol constituted 1% of the diet or (d) dissolved

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9430

in acetone so that acetone constituted 1% of the diet. The four control diets contained no solvent, 1% corn oil, 1% propylene glycol or 1% acetone. Within each dietary concentration of methylmercury chloride, there were four pens in each of the four carrier groups.

Table 1 lists the amount of mercury found in feed samples. We sampled some carrier groups more intensively than others to determine (a) whether the addition of pulverized methylmercury chloride to the diet resulted in a homogeneous mix and (b) whether the reduced mortality we observed with acetone as the carrier could have been caused by evaporation of methylmercury chloride from the feed.

Mortality was recorded for 6 weeks. At the end of the experiment, the amount of mercury was determined in the livers of 4 surviving control birds (1 from each carrier), 16 birds fed 5.4 and 16 birds fed 20 ppm methylmercury chloride (4 from each carrier).

Each sample for mercury analysis was homogenized with a Virtis blender and a 5-g subsample was taken for analysis. The subsample was digested using the method described by Monk [5]. Mercury was determined by cold-vapor atomic absorption spectrophotometry, according to the method of Hatch and Ott [6], with a Coleman model MAS50 mercury analyzer. Recoveries averaged 93% and the lower limit of detection was 0.02 ppm.

Final angular-transformed percentage mortalities among birds fed the same concentration of methylmercury chloride, but in different carriers, were compared by analysis of variance. Means were separated by Tukey's multiple comparison method [7].

RESULTS AND DISCUSSION

Birds fed a control diet or a diet containing 5.4 ppm methylmercury chloride experienced a low level of mortality (Fig. 1). Because there were no effects of solvents on mortality in controls or birds fed 5.4 ppm methylmercury chloride, only the average mortality is shown. There was heavy mortality in quail fed 20 ppm methylmercury chloride, and the mortality was influenced by the solvent used.

The highest mortality occurred either when methylmercury chloride was pulverized and added directly to the diet or when corn oil was used as the carrier. Propylene glycol appeared to lower mortality, but there was no statistically significant difference from the no-carrier and corn oil treatments. However, when acetone was used as the carrier, mortality was significantly less than when no carrier or corn oil was used. Mortality with acetone as carrier was not significantly lower than with propylene glycol as carrier.

We know of no other studies on the effects of solvents on the toxicity of methylmercury to birds. Gile et al. [8] found a suggestion of differences in the toxicities of carbofuran (2,2-dimethyl-2,3-dihydro-7-benzofuranyl-*N*-methylcarbamate), endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo,endo-1,4:5,8-dimethanonaphthalene) and dursban (*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate) in 5-d LC50 tests with bobwhite and mallards (*Anas platyrhynchos*) depending on the carrier used (corn

Table 1. Mercury measured in feed samples calculated to contain 0, 4.3 or 16 ppm mercury

Mercury calculated to have been added to the diet[a] (ppm)	Mercury measured in the diet (ppm) when solvent was:							
	No solvent		Acetone		Propylene glycol		Corn oil	
	Fresh mix[b]	From feeder[c]	Fresh mix[b]	From feeder[c]	Fresh mix[b]	From feeder[c]	Fresh mix[b]	From feeder[c]
0	0.03	—	0.14	—	0.05	—	0.04	—
4.3	5.3	4.3	4.7	4.2	4.1	4.0	4.1	3.3
16	15	15	15	18	13	17	18	14
	14		14	17				
	17							
	15							

[a]Diets mixed to contain 5.4 or 20 ppm methylmercury chloride should contain 4.3 and 16 ppm elemental mercury.
[b]Fresh mix: the feed sample was collected and frozen in a sealed jar immediately after the diet was mixed.
[c]From feeder: the feed sample was collected after it had remained in a feeder for 2 d.

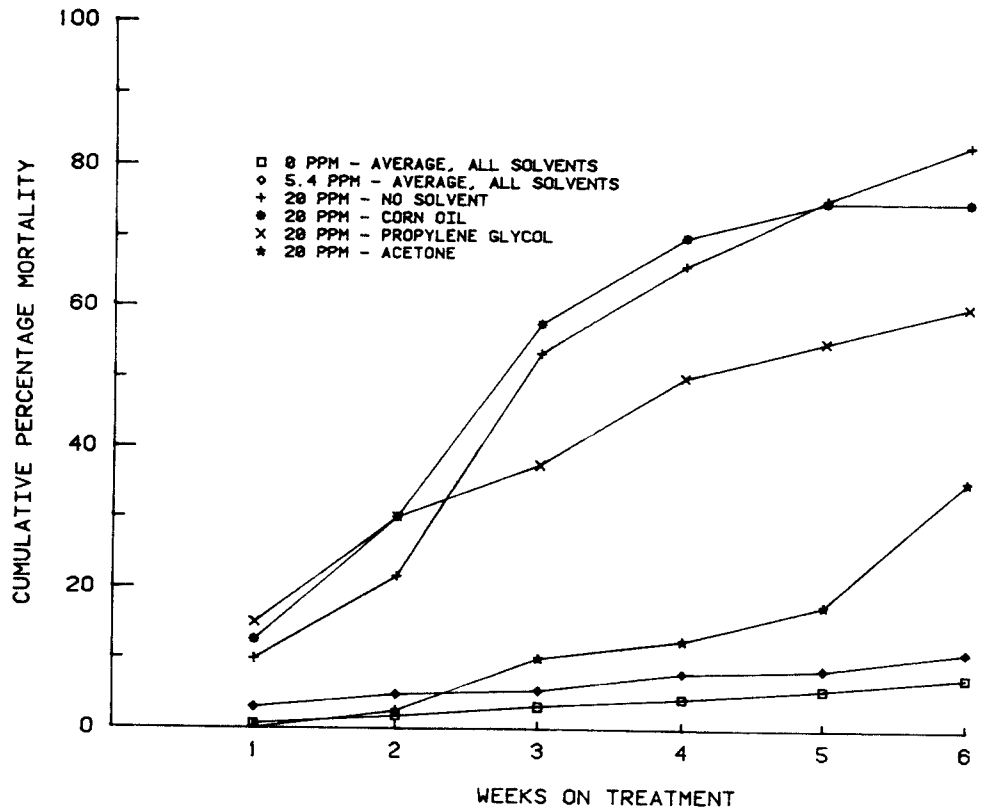

Fig. 1. Mortality of bobwhite fed 0 or 5.4 ppm methylmercury chloride (with results from all solvents combined) or 20 ppm methylmercury chloride (with each solvent group shown individually).

oil, propylene glycol, carboxymethylcellulose or distilled water), but these differences were not statistically significant. They also observed better dose-response relationships with some carriers than with others for some of the chemicals tested. In tests using excised rat skin, O'Brien and Dannelley [9] found that, in general, malathion (*O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate), dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo,exo-1,4:5,8-dimethanonaphthalene), carbaryl (1-naphthyl *N*-methylcarbamate) and famphur (*O,O*-dimethyl-*O,p*-(dimethylsulfamoyl)phenyl phosphorothioate) were absorbed from different solvents in the following (decreasing) order: acetone > benzene > corn oil.

Analyses of feed samples showed close agreement with calculated values (Table 1). We conclude from our feed analyses that (a) there were no mistakes in feed preparation that could account for the reduced toxicity of 20 ppm methylmercury chloride when acetone was the carrier, (b) all of the carrier conditions, including no carrier, resulted in homogeneous mixes and (c) there was no measurable loss of methylmercury chloride from the feed during the time it was in the feeder, regardless of carrier.

We do not know what caused the reduced mortality in birds fed 20 ppm methylmercury chloride dissolved in acetone. We could not attribute it to differences in available compound in the diet or to differences in uptake of mercury by the body. The livers of three controls contained no detectable mercury and a fourth contained only 0.03 ppm mercury (Table 2). There was slightly less mercury in the livers of birds fed 20 ppm methylmercury chloride in acetone and propylene glycol, but the differences were not statistically significant. Furthermore, the reverse was true at 5.4 ppm methylmercury chloride, so it does not appear that acetone affected the uptake of mercury, at least in birds surviving treatment.

Given the unexpected findings of our study, it

Table 2. Mercury in livers of bobwhite fed 0, 5.4 or 20 ppm methylmercury chloride dissolved in acetone, propylene glycol, corn oil or no solvent[a]

Methylmercury chloride added to diet (ppm)	n	Mercury (ppm, wet weight) in liver when the solvent was:			
		No solvent	Acetone	Propylene glycol	Corn oil
0	1	<0.02	<0.02	0.03	<0.02
5.4	4	13 ± 1.9 a	15 ± 1.5 a	16 ± 1.2 a	14 ± 0.9 a
20	4	42 ± 0.8 a	36 ± 3.3 a	36 ± 1.6 a	46 ± 10.6 a

[a]The detectable level of mercury was 0.02 ppm. Values are means ± SE. Values in the same row that share a letter in common were not significantly different at $\alpha = 0.05$ by analysis of variance.

would be wise to verify our results by conducting the same or a similar experiment. With large sample sizes, the mercury levels in the liver could be more carefully examined. Mercury levels in other tissues, including kidney and brain, should be examined. Mercury levels could be determined in birds that died, as well as in the survivors. One could experiment with different levels of acetone in the diet to see if mortality could be changed. Our findings suggests that acetone may have been absorbed along with the methylmercury chloride and acted as an antagonist to its toxicity; however, this would have to be verified in specially designed studies.

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