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The Effect of Chemical Carriers on Avian LC_{50} Toxicity Tests

Jay D. Gile,[1] Joann B. Beaver,[2] and Robert Fink[2]

[1]*Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR 97333, and* [2]*Wildlife International Ltd., St. Michaels, Maryland 21663*

The subacute dietary (LC_{50}) toxicity of a pesticide as prescribed by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) and of toxic substances as defined by the Toxic Substances Control Act (TSCA) is a routine data point for many chemicals. The methods under which the LC_{50} data are generated are quite specific and prescribed by law (e.g. FIFRA; Fed. Reg. Vol. 43, No. 132 Sect. 163.71-2). The dietary toxicity of a chemical is one of the fundamental parameters required by toxicologists in evaluating chemical hazard since dietary exposure is the most probable route of intoxication confronting wild species. The protocol is not designed to mimic a natural situation but to assess the impact on the most vulnerable individuals (juveniles) in a reproducible fashion.

In developing the test protocol it was recognized that additives may be necessary to insure uniform distribution of the test chemical in the feed. These additives, commonly known as "carriers", are presumed to be inert, i.e., they are not expected to alter chemical toxicity. The use of a carrier requires consideration of the amount and type to be used. According to the FIFRA protocol the amount of carrier can not exceed 2% of the total mass of feed. The selection of carrier type is left to the discretion of the investigator. The FIFRA protocol suggests corn oil or "other necessary vehicle." A list of generally accepted carriers would include corn oil, peanut oil, propylene glycol, carboxy methylcellulose and water.

This study is a pilot effort to address the question of effect of different carriers on dietary toxicity (LC_{50}) of a particular chemical. The carriers that were evaluated include table grade corn oil, propylene glycol, carboxy methylcellulose and distilled water. The test chemicals were carbofuran (2,3-Dihydro-2,2-dimethyl-7-benzofuranylmethylcarbamate), dursban (O, O-diethyl-O(3,5,7-trichloro-2-pyridyl)phosphorothioate, and endrin (1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a,-octahydro-endo-1,4-endo-5,8-dimethano-naphthalene) representing the general pesticide classes of carbamates, organophosphates and organochlorine insecticides, respectively.

MATERIALS AND METHODS

Test Species

Both bobwhite quail and mallard duck eggs were collected from Wildlife International Ltd.'s production flocks certified to be pullorum-typhoid free. Upon hatching, both ducklings and quail chicks were maintained in Beacon[1] battery brooders throughout both the acclimation and test periods. During the acclimation (through day 13) the quail received Headstart vitamin mix via their drinking water.

LC_{50} Test Protocol

All aspects of the LC_{50} testing was performed at the Wildlife International Ltd. facility in St. Michaels, Maryland. The test protocol used for LC_{50} evaluations was that specified by FIFRA (Fed. Reg. Vol. 43 No. 132 Sect. 163.71-2). At 14 days of age, test birds were randomly assigned to the following treatment groups: Control A (basal diet), Control B (basal diet + 2% carrier); Lab standard (dieldrin), Experimental (Test chemical + 2% carrier). Game bird starter ration (test medium) and water were available ad libitum throughout the study. The photoperiod throughout brooding and the 8-day study was 14 hours of light a day.

The birds were exposed to the appropriate dietary concentrations for 5 days, and then maintained on toxicant-free diet for an additional 3-day observation. One group of control birds received the basal diet throughout the study. An additional group of control birds received basal diet with 2% (by weight) distilled water added during the exposure phase of the study.

Body weights were recorded by pen at initiation and termination of the study. Feed consumption was recorded by pen during the 5-day exposure. Feed consumption was measured accurately, but is presented as an estimate due to the unavoidable wastage by the birds.

Symptoms of toxicity and mortality were recorded daily throughout the study. Mortality was analyzed statistically by probit analysis (FINNEY, 1971).

Test Diet Preparation

Carbofuran: The experimental material was weighed in a tared beaker and acetone (5-10 ml) was added to dissolve the material. The carrier was added in three portions with stirring after each addition. The total amount of carrier added was 64 ml for corn oil; 57 ml - propylene glycol; 59 ml - carboxy methylcellulose

[1] Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

and 60 ml of distilled water. The carbofuran appeared to form a homogenous solution with corn oil and propylene glycol but formed a precipitate with the first addition of both the carboxy methylcellulose and water. The beaker was emptied into the weighed starter ration and rinsed with 20 ml of acetone. The diet was then mixed for 10 min in a Hobart mixer.

Dursban: Dursban test diets were prepared by the same procedure as carbofuran with one exception. The dursban was liquified by warming the tared beaker in a hot water bath (65°C) rather then dissolving the material in acetone. Dursban formed in homogenous solution or appeared uniformly distributed with corn oil and propylene glycol. It formed discrete droplets with carboxy methylcellulose and a heavy precipitate in water.

Endrin: The endrin test diets were prepared using techniques identical to carbofuran diet preparation. Endrin behaved in an identical manner to carbofuran in terms of solubility in the four carriers.

For the purposes of diet preparation and residue analysis all three test chemicals were assumed to be 100% active ingredient.

Diet Analysis

To confirm nominal concentrations select samples of each test chemical with each carrier at low and high concentrations were subjected to residue analysis by Environmental Protection Agency staff at the Corvallis laboratory. The 10-g samples were extracted in 150 mls of solvent (carbofuran in acetonitrile, dursban and endrin in hexane) for 30 minutes. Fifty mls of the extract was then centrifuged at 1000 ppm for 20 min. A Hewlett Packard 5880 GC fitted with a 12m capillary column and an FID detector was used for analysis of the centrifuged extract.

RESULTS AND DISCUSSION

There are several ways in which a chemical carrier could affect the results of an LC_{50} test. First the carrier could affect the distribution of the test chemical in the food resulting in the test species not being exposed to the intended concentration of test material. Second, the chemical/carrier combination could alter the palatability of the test diet possibly resulting in avoidance by the test species. Third, if the carrier has some nutritional value of its own, amending the diet with this material could result in a reduction of the daily food intake required to maintain the test species. Finally, the carrier could alter the absorption through the intestinal tract thereby limiting the toxic response even though the animal is exposed to the chemical.

The selection of a particular carrier for a specific chemical will depend to a great extent on the solubility of the test chemical in water. The chemicals used in this study present a

wide range of solubilities (carbofuran - 700 ppm; dursban - 2 ppm, SPENCER 1982; endrin - 0.25 ppm, BIGGAR & RIGGS 1974). To examine the potential effects of the four carriers we conducted residue analysis of the food, and determined food consumption and growth in addition to estimating toxicity through an estimate of the LC_{50} and examination of the dose-response relationships.

A major problem with analyzing the results of this study is that no appropriate statistical test could be identified due to the nature of the data. Specifically, funding limitations did not permit replication of any of the tests. However several comments may be made on the potential effect of carriers on LC_{50} tests.

The dietary toxicity of carbofuran, dursban and endrin with each of the four carriers is presented in Table I. The order of toxicity for carbofuran with the four carriers in quail was propylene glycol > distilled water > corn oil > carboxy methylcellulose. For dursban in quail, toxicity varied as follows: carboxy methylcellulose > corn oil > distilled water > propylene glycol. For endrin the order of toxicity was distilled water > propylene glycol > corn oil > carboxy methylcellulose. For mallards the order of carbofuran toxicity was corn oil > propylene glycol > distilled water > carboxy methylcellulose. With dursban the toxicity ranked distilled water > carboxy methylcellulose > propylene glycol > corn oil. For endrin the order was corn oil > propylene glycol > distilled water > carboxy methylcellulose. There appear to be differences in the LC_{50} values associated with different chemical/carrier combinations, however, no pattern is evident for any of the carriers in either species.

If the dose-response relationships are examined (Table II) then clearly there is a most appropriate carrier for each test chemical based on mortality associated with concentration level. Propylene glycol resulted in the best dose-response relationship for carbofuran while corn oil appeared to be the best for dursban and endrin.

Food consumption for each test chemical/carrier combination as well as carrier controls is shown in Table III. With only three exceptions, those groups of birds presented with a corn oil carrier consumed less food than those presented with the other three carriers. Although those birds exposed to corn oil as a carrier generally consumed less food, the number of replicates is insufficient to demonstrate conclusively that birds will consume less of a diet amended with corn oil. It has been assumed that a 2% carrier amendment would not significantly alter the nutritional quality of the food; however if birds routinely consume less corn oil amended food, test results could be affected. Bird growth is presented in Table III as the average weight gained during the 8-day duration of the LC_{50} test. These data do not indicate any pattern in relation to the type of carrier used in spite of the reduced food consumption by those birds exposed to corn oil. Chemical analysis of the feed samples for each test chemical/carrier combination indicated no effect by any of the carriers on test chemical concentrations in the feed.

Table I. Comparative toxicity of test chemicals with different carriers.

Chemical	LC_{50} (95% confidence interval)[a] Carrier[b] CO	PG	CMC	H_2O
	Quail			
Carbofuran	509(389-662) 2.359[c]	354(254-507) 1.398	614 -[d] 1.338	481 - 1.718
Dursban	392(293-522) 1.937	421(332-535) 3.142	353(294-429) -[e]	397(318-498) 3.753
Endrin	7.3(4.9-9.6) 2.308	6.3 - 1.427	14.4 - 1.550	5.8(1.8-7.9) 1.915
Carbofuran	93(65-123) 1.930	117(86-148) 1.859	134(74-209) 0.932	118 - 1.369
Dursban	1080(707-2503) 0.954	900(764-1255) 1.796	757(478-1443) 0.892	671(322-2170) 0.615
Endrin	16(12-22) 1.932	19(14-26) 1.583	24(18-33) 1.823	21(16-27) 2.427

[a] LC_{50}'s (ppm) determined from 5 days on ad libitum toxic diet followed by 3 days on nontoxic feed. Five geometrically spaced concentrations (10 birds/concentration) were used for each LC_{50} determination.

[b] CO = corn oil; PG = propylene glycol; CMC = carboxy methylcellulose; H_2O = distilled water

[c] slope

[d] Confidence interval could not be calculated.

[e] slope not calculated due to lack of sufficient partial kills.

The LC_{50}'s for all chemical/carrier combinations compare favorably with those cited in the literature. In a study by HILL et al. (1975) the LC_{50} for carbofuran in Japanese quail was determined to be 438 ppm while the LC_{50} for mallards was estimated at 190 ppm. Both values are comparable to those observed in our study. In another study, HILL & CAMARDESE (1981) demonstrated the intrinsic variability between LC_{50} tests using carbofuran in Japanese quail. They observed LC_{50} values ranging from 477 to 1087 ppm over four replicate LC_{50} tests. A technical information sheet provided by the Dow Chemical Company lists LC_{50} values of 449 ppm for 1- to 5-day-old Bobwhite and 180 and 36 ppm for 1- to 7-day-old mallards. In a study funded by EPA the LC_{50} for Bobwhite (14-day-old) was determined to be 863 ppm (OSU 1981).

Table II. Response of test birds to graduated concentrations of carbofuran, dursban and endrin with different carriers.

Mortality %									
Quail					Mallard				
	Carrier[a]					Carrier[a]			
Conc. (ppm)	CO	PG	CMC	H_2O	Conc. (ppm)	CO	PG	CMC	H_2O
				Carbofuran					
100	0	10	0	0	56.2	20	10	20	0
178	0	30	40	10	100.0	50	40	40	80
316	10	40	0	0	178.0	90	70	60	50
562	70	60	30	90	316.0	100	100	80	90
1000	90	100	90	80	562.0	100	100	90	100
				Dursban					
178	10	10	0	0	178	0	0	0	20
316	30	20	30	20	316	10	0	30	40
562	70	80	100	90	562	40	30	60	50
1000	100	100	100	100	1000	50	50	40	30
1780	100	100	100	100	1780	60	90	80	90
				Endrin					
3.16	30	0	0	50	5.62	20	0	0	10
5.62	70	80	30	80	10.0	50	10	0	20
10.00	100	60	90	100	17.8	90	60	40	90
17.80	100	90	70	100	31.60	100	80	70	100
31.60	100	100	100	100	56.20	100	90	90	100

[a] CO - corn oil, PG - propylene glycol, CMC - carboxy methyl-cellulose, H_2O - distilled water.

In another EPA-funded study (PRODUCT SAFETY LABORATORY 1981) the LC_{50} for 14-day-old mallards was determined to be 1800 ppm. The source of variation between these studies could be bird age in the Dow study, strain, chemical purity, etc. No carrier was indicated for the Dow data; in the EPA study corn oil was used for the Bobwhite and propylene glycol for the mallards. Data from the literature for the LC_{50} of endrin indicate values of 15 ppm for Bobwhite and 21 ppm for mallard (HEATH & STICKEL 1965). While the quail value is approximately double that observed in this study, the LC_{50} for mallards falls within the range of our observations.

CONCLUSIONS

Although unsupported statistically, the data from this study especially the dose-response observations suggest that carriers do have an effect on LC_{50}. To provide conclusive evidence additional replication would be required. However, even if it could be proven statistically, the variation in LC_{50} estimates from the literature indicates a high degree of intrinsic variability in the LC_{50} test that may mask any effect of a carrier.

Table III. Food consumption (g/bird/day) and growth (g)

Test Chemical		Carrier[c] CO		PG		CMC		H_2O	
Carbofuran	(Q)[b]	7.0[c]	14.0[d]	8.2	13.5	8.4	10.8	7.2	12.0
	(D)	6.0	77.0	9.0	75.0	8.6	64.8	12.6	44.0
Dursban	(Q)	9.0	12.0	6.4	5.3	5.6	11.5	4.2	12.7
	(D)	9.5	26.8	10.4	78.0	13.6	43.4	11.2	66.8
Endrin	(Q)	4.2	10.5	8.8	7.3	6.0	11.7	4.8	11.0
	(D)	36.0	141.0	51.0	139.2	47.8	150.6	29.0	180.6
Control	(Q)	10.4	21.6	10.6	21.8	11.0	20.2	8.6	23.6
	(D)	74.6	197.6	82.8	219.6	80.8	207.4	81.8	203.6

[a] CO = corn oil; PG = propylene glycol; CMC = carboxy methylcellulose; H_2O = distilled water
[b] Q = quail
D = duck
[c] average food consumption based on all dose levels
[d] Average weight gain Day 1 - Day 8 of test.

References

BIGGAR, J. W., and I. R. RIGGS: Hilgardia 42:383 (1974).

DOW CHEMICAL COMPANY: Dursban insecticides technical information. Agric. Prod. Div. Midland, MI. undated.

FINNEY, D. J.: Statistical Methods in Biological Assay, second ed. London: Griffin Press (1971).

HEATH, R. G., and L. F. Stickel: Protocol for testing the acute and relative toxicity of pesticides to penned birds. p. 18-24 In The Effects of Pesticides on Fish and Wildlife. U.S. Fish and Wildl. Serv. Circ. 226 (1965).

HILL, E. F., R. G. HEATH, J. W. SPANN and J. D. WILLIAMS: Lethyl Dietary Toxicity of Environmental Pollutants to Birds. U.S. Fish and Wildl. Ser. Special Scientific Report 191 (1975).

HILL, E. F. and M. B. Camardese: Subacute toxicity testing with young birds: response in relation to age and intertest variability in LC_{50} estimates, pp. 41-65 In Avian and Mammalian and Wildlife Toxicology: Second Conference, ASTM STP 757, D. W. Lamb and E. E. Kenaga, Eds., American Society for Testing and Materials, (1981).

OREGON STATE UNIVERSITY: Avian environmental toxicology: pesticide effects. U.S. Envir. Prot. Ag. cooperative agreement #807472 (1981). (unpublished data).

PRODUCT SAFETY LABORATORIES: LC_{50} test with mallard ducks on sodium arsenite, strychnine, merphos, monitor and dursban. U.S. Envir. Prot. Ag. contract 1B1407NTSA (1981). (unpublished data).

SPENCER E. Y. Guide to Chemicals Used in Crop Protection. 7th ed. Agriculture Canada, Research Program Service, Ottawa, Ontario, publ. 1043 (1982).

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