Bull. Environ. Contam. Toxicol. (1988) 40:619–625

Bulletin of Environmental Contamination and Toxicology

Two Methods for Evaluating Avian Food Avoidance Data

Douglas W. Kononen

Department of Animal Science and Center for Environmental Toxicology, Michigan State University, East Lansing, Michigan 48824-1225

Recent years have seen the crystallization and implementation of a systematic framework for assessing the health risks associated with exposure to environmental toxicants (U.S. Environmental Protection Agency, 1986a-e). While these methods are oriented toward human public health risk assessment, they can be adapted for non-human (e.g. wildlife) environmental contaminant risk assessments. Broadly, risk assessment includes one or more of the following components: hazard identification, dose-response assessment, exposure assessment, and risk characterization (National Research Council, 1983). This paper discusses two regression methods for evaluating avian food avoidance data by determining an environmental toxicant discrimination threshold concentration (DTC). This information can then be incorporated into the exposure assessment component of environmental risk assessments.

A significant component of an animal's total exposure to a given environmental toxicant is via its diet. Exposure assessments can be enhanced by information obtained from studies of dietary avoidance behavior. Dietary avoidance studies, involving measurements of freely exposed animals' consumption of treated and untreated food or water at different contaminant concentrations, can indicate whether a given species is able to discriminate between contaminated and uncontaminated dietary sources. If (1) an animal can detect (e.g. by sight, smell, or taste) differences between contaminated and contaminant-free dietary sources, and (2) given such discriminating ability it can associate certain adverse effects (unpalatability, nausea, etc.) with consumption of "tainted" food, then (3) avoidance of the contaminated dietary source is possible (assuming availability of alternative untainted dietary sources). Estimates of the likely magnitude of dietary exposure to environmental toxicants would be improved over those derived from blanket assumptions of uniform dietary consumption rates if a risk assessor knew that a given species had the ability to detect and avoid food contaminated with a given toxicant.

Send reprint requests to Douglas W. Kononen, Operating Sciences Department, General Motors Research Laboratories, General Motors Technical Center, Warren, Michigan 48090-9055.

The following definition of discriminating behavior is proposed. Discriminating behavior (at a given dietary exposure level) occurs among a group of animals given free choice between equally available treated and untreated dietary sources if consumption of clean food exceeds consumption of treated food. The highest dietary contaminant concentration at which equal amounts of equally available clean and contaminated food are consumed is termed the discrimination threshold concentration (DTC). Dietary contaminant concentrations exceeding the DTC elicit avoidance behavior (the ratio of treated to total food consumption declines in a dose-related manner).

MATERIALS AND METHODS

Data were obtained from an experiment designed to evaluate northern bobwhite (Colinus virginianus) avoidance of carbofuran-contaminated food (Kononen, et al, 1987). For each of five treatment levels, two 10-day-old birds within each of five replications (pens) were freely exposed for five days to equal amounts of clean and carbofuran-contaminated food. Each pen's consumption of treated and clean food was measured daily. Five day average food consumption data (grams per bird-day) for each experimental unit is listed in Table 1. Table 2 summarizes Table 1 data by averaging (for each treatment level) food consumption across experimental units. In Table 3, Table 1 data are dichotomized to reflect, for each treatment level, the number of pens where the ratio of treated to total food consumption was less than 0.5. This dichotomization of Table 1 data was necessary to provide quantal response input (i.e., did or did not respond) to the probit analysis method discussed below. A log(dose)-log(response) and a log(dose)-probit(response) model were applied to each data set, respectively.

RESULTS AND DISCUSSION

The following simple linear regression model was applied to Table 1 and Table 2 data.

$$1. \quad \log_{10}(Tr/T) = \log_{10}(a) + b[\log_{10}(\text{dose})]$$

where Tr = grams per bird-day treated food consumption
T = grams per bird-day total food consumption
dose = dietary carbofuran concentration (ppm).

The results of Equation (1) applied to Table 1 data are,

$$2. \quad \log_{10}(Tr/T) = 0.2765 - 0.2615[\log_{10}(\text{dose})],$$

$$R^2 = 0.57, \; t_b = -5.52 \; (p < 0.001).$$

Setting (Tr/T) equal to 0.5 and solving for the DTC results in,

$$3. \quad DTC = \text{antilog}\{[\log_{10}(0.5) - 0.2765]/-0.2615\}$$

$$= 162 \text{ ppm}.$$

Using the linear calibration confidence interval estimation procedure outlined by Snedecor and Cochran (1967), a calculated ninety-five percent confidence interval for the above DTC (ppm) is (23,801).

Equation (1) applied to Table 2 data results in,

4. $$\log_{10}(Tr/T) = 0.2868 - 0.2642[\log_{10}(\text{dose})],$$
$$R^2 = 0.97,\ t_b = -9.64\ (p < 0.005).$$

5. $$DTC = \text{antilog}\{[\log_{10}(0.5) - 0.2868]/-0.2642\}$$
$$= 168 \text{ ppm}.$$

Ninety-five percent confidence limits (ppm) for this DTC estimate are (65,333).

Although it is always possible to solve for a DTC regardless of the level of statistical significance of the regression described by Equation (1), confidence interval estimation is not possible using the linear calibration method outlined in Snedecor and Cochran (1967) unless the slope of the regression is statistically significant at the 5% level. This fact is illustrated by fitting Equation (1) to Table 3 data, resulting in,

6. $$\log_{10}(R/N) = -1.0429 + 0.3498[\log_{10}(\text{dose})],$$
$$R^2 = 0.67,\ t_b = 2.48\ (p > 0.05).$$

7. $$DTC = \text{antilog}\{[\log_{10}(0.5) + 1.0429]/0.3498\}$$
$$= 132 \text{ ppm}.$$

Equation (1) applied to the summary food avoidance data listed in Tables 1-3 results in three comparable discrimination threshold concentration estimates (i.e., 162, 168, and 132 ppm, respectively). These results indicate that, under the conditions of the avoidance test, northern bobwhite do not begin to avoid carbofuran-contaminated food until concentrations are greater than 130 ppm.

Strictly speaking, the quantal response data required by probit analysis preclude application of this method to the type of data presented in Tables 1 and 2. An earlier paper (Kononen, et al, 1986) described an effort to determine a DTC (termed a food avoidance concentration 50 or FAC50) using a log(dose)-probit(response) method where the response variable was the proportion of total food consumption which was treated food. Although the non-quantal nature of this response measure violated the probit method assumptions, this application can yield acceptable point estimates of the discrimination threshold concentration. However, statistical confidence interval estimates calculated with this type of response information may be suspect.

Using the probit technique (SAS Institute, 1984) and the response variable described in Kononen, et al, 1986, we obtain the following model,

$$8. \quad \{\Phi^{-1}(y) + 5\} = A + B[\log_{10}(\text{dose})],$$

where $y = Tr/T$ and Φ^{-1} = inverse normal distribution.

Equation (8) applied to Table 2 data results in,

$$9. \quad \{\Phi^{-1}(y) + 5\} = 6.3369 - 0.6074[\log_{10}(\text{dose})],$$

Chi-square = 0.0261 with 3 d.f. ($p = 0.9989$),

FAC50 = DTC = 159 ppm.

With these data, no confidence interval estimates are calculable for the DTC. This illustrates the major difficulty of the FAC50 method. In Table 3, the data from Table 1 are dichotomized into a quantal all or none response. If the ratio of treated to total food consumption was less than 0.5 within a given dietary treatment replication (pen), then the birds in that pen were assumed to have responded to the treatment (by demonstrating a preference for clean over contaminated food). Application of the probit technique [Equation (8)] to these data results in,

$$10. \quad \{\Phi^{-1}(y) + 5\} = -6.2703 + 5.0120[\log_{10}(\text{dose})],$$

where $y = R/N$ (cf. Table 3),

Chi-square = 0.2286 with 3 d.f. ($p = 0.9895$).

The probit method yields a 177 ppm DTC estimate with approximate 95% confidence interval (ppm) given by (0,294). Comparing the point estimates of the DTC obtained with the probit method with those obtained with the simple log-log regression (Table 4), we see that both methods yield similar results. The advantage of the simple regression method is that it may be used with any of the levels of data aggregation summarized in Tables 1-3 while use of the probit technique requires the unabridged results (Table 1) which must be dichotomized to yield a quantal response variable.

Two simple regression methods are proposed which, when applied to the type of data commonly recorded during food avoidance experiments, can yield point estimates along with 95% confidence limits of the minimum concentration of dietary contaminant which will result in an avoidance response by exposed animals. This concentration, termed the discrimination threshold concentration (DTC), may prove useful in efforts to assess wildlife species' "real world" dietary toxicant exposures.

Acknowledgments. This research was supported by U.S. Environmental Protection Agency Cooperative Agreement CR-810421-01-0 but does not necessarily reflect the views of that agency. No official endorsement should be inferred.

Table 1. Northern bobwhite food avoidance data set I

Dietary Carbofuran Concentration (ppm)	Pen	S/N[1]	Treated Food Consumption[2] (g/b-d)	Total Food Consumption[3] (g/b-d)
154	1	2/2	3.3	5.6
	2	2/2	2.7	5.6
	3	2/2	3.2	6.0
	4	1/2	2.8	4.9
	5	2/2	2.1	5.6
278	1	2/2	2.8	5.4
	2	2/2	2.9	6.0
	3	2/2	2.1	7.1
	4	2/2	2.9	6.3
	5	2/2	2.6	5.5
500	1	2/2	1.9	6.5
	2	2/2	2.3	6.4
	3	2/2	2.3	5.5
	4	2/2	1.9	6.0
	5	2/2	3.2	7.5
900	1	1/2	3.3	7.9
	2	2/2	1.9	6.5
	3	2/2	2.0	5.8
	4	2/2	1.5	6.8
	5	2/2	2.4	4.9
1620	1	2/2	1.7	6.1
	2	2/2	1.5	5.6
	3	2/2	1.7	7.1
	4	2/2	1.9	6.4
	5	2/2	1.7	6.9

[1] S/N = No. birds surviving test period/No. birds tested.
[2] g/b-d = grams per bird-day.
[3] Total = treated plus clean food consumption.

Table 2. Northern bobwhite food avoidance data set II

Dietary Carbofuran Concentration (ppm)	Tr[1]	T[2]
154	2.8	5.5
278	2.7	6.1
500	2.3	6.4
900	2.2	6.4
1620	1.7	6.4

[1] Tr = Mean treated food consumption (g/b-d).
[2] T = Mean total (clean plus treated) food consumption (g/b-d).

Table 3. Northern bobwhite food avoidance data set III

Dietary Carbofuran Concentration (ppm)	R[1]	N[2]
154	2	5
278	4	5
500	5	5
900	5	5
1620	5	5

[1] R = No. pens where the ratio (treated/total consumption) was less than 0.5.
[2] N = No. pens per treatment level.

Table 4. Discrimination threshold concentration estimates (plus 95% confidence intervals) calculated with two separate methods

Method	Data Set	DTC (ppm)[1]	95% CI (ppm)[2]
Linear calibration	I	162	(23,801)
	II	168	(65,333)
	III	132	NC
Log-probit	I	NC	NC
	II	159	NC
	III	177	(0,294)

[1] DTC = Discrimination threshold concentration (ppm).
[2] Approximate 95% confidence interval for DTC.
NC = Not calculable with the method used.

REFERENCES

Kononen, DW, JR Hochstein, and RK Ringer (1986) A quantitative method for evaluating avian food avoidance behavior. Environ Toxicol Chem 5:823-830

Kononen, DW, JR Hochstein, and RK Ringer (1987) Avoidance behavior of mallards and northern bobwhite exposed to carbofuran-contaminated food and water. Environ Toxicol Chem 6:41-50

National Research Council (1983) Risk assessment in the Federal government: managing the process. National Academy Press, Washington, D.C.

SAS Institute (1984) SAS users guide: statistics. SAS Institute, Inc., Cary, NC

Snedecor, GW and WG Cochran (1967) Statistical methods, 6th edition. The Iowa State University Press, Ames, Iowa

U.S. Environmental Protection Agency (1986a) Guidelines for carcinogen risk assessment. Fed Reg 51(185):33992-34003

U.S. Environmental Protection Agency (1986b) Guidelines for mutagenicity risk assessment. Fed Reg 51(185):34006-34012

U.S. Environmental Protection Agency (1986c) Guidelines for the health risk assessment of chemical mixtures. Fed Reg 51(185): 34014-34025

U.S. Environmental Protection Agency (1986d) Guidelines for health risk assessment of suspect developmental toxicants. Fed Reg 51(185):34028-34040

U.S. Environmental Protection Agency (1986e) Guidelines for exposure assessment. Fed Reg 51(185):34042-34054

Received June 22, 1987; accepted November 12, 1987.