THE SIGNIFICANCE OF PREFERENCE IN LABORATORY BAIT ACCEPTANCE STUDIES

JAMES G. MILLER, Head, Department of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi

The palatability of rodenticide baits has recently been the subject of renewed interest in industry and regulatory agencies. The requirement that a bait, especially those with anticoagulant rodenticides, be consumed by the target species is a fundamental requirement for effectiveness. This has been overlooked at times, with the result that in some instances, rodenticides have been relatively ineffective due to a lack of acceptance.

The measurement of palatability and rodent acceptance is a controversial area. The procedures used have been subjected to criticism, often uninformed criticism, and the projection of the experimental data to use conditions has also been debated. The regulatory agencies have utilized a concept in that the rodenticide bait is in effect challenged by an attractive non-toxicant food source. The rationale is that under use conditions an existing food source is present to sustain the rodent population before the introduction of the rodenticide bait. The rodenticide bait should therefore be capable of being consumed in significant quantities in the presence of the food source. The problems arise in the interpretation of the data and in its projection to use conditions. Specifically, if bait A, is accepted at 33 percent and bait B is accepted at a level of 29 percent, how significant is the difference?

The experimental conditions have been relatively well standardized. Laboratory rats, at least 10 of each sex, are housed in individual cages. The rats are offered a highly palatable, easily consumed food source, and the candidate bait, over a period of 15 days. The acceptance of the candidate bait is computed as a percentage of the total food intake from all sources over the period of the test.

We have conducted a large number of the assays in the course of quality control, developmental, and compliance programs. The following data was developed within the program utilizing warfarin from a wide variety of sources as the toxicant and rats from the Charles River Breeding Laboratories as the test animal.

The data to be presented will deal with the role of sex, the variability of the acceptance data and the significance of differences between assays, and the utilization of a shortened experimental period.

Role of Sex of the Test Animal

A cursory examination of raw laboratory data indicates that male rats appear to discriminate against some anticoagulant baits to a greater degree than the female in the same trial.

To explore this observation the data from 40 experimental trials covering a range of acceptance from 10 percent to 50 percent was assembled. This range of acceptance represents severe rejection of the bait at 10 percent to a no choice at 50 percent.

The data, as summarized in Table 1, indicates that there is a greater rejection of anticoagulant bait by the male throughout the range of the tests. As would be expected the difference in acceptance between the male and female is greatest at the 10 percent level, where it is highly significant, gradually declining to a "not a significant difference" in baits with a composite acceptance of 50 percent.

Table 1. The role of sex of the test animal in bait acceptance.

	Acceptance Range			
Mean Acceptance	10-20%	20-30%	30-40%	40-50%
Male	12.5%	21.2%	32.0%	45.2%
Female	20.8%	28.6%	39.9%	50.6%
Significance	P<0.001	0.001 P<.01	0.01 P<.05	Not Significantly Different

This data indicates the necessity for the segregation of the data by sex, prior to statistical analysis. It would also be highly desirable to report acceptance data by sex as this difference between male and female could be altered with other circumstances or agents.

The necessity of using animals within a fairly narrow weight range is apparent. The utilization of heavy mature females, 250 grams and young males in the 125 gram range would materially bias the data.

The Significance of Difference Between Assays

The central question that faces every investigator and evaluator of data in this field is the significance of difference. This is determined within the limits that the investigator is willing to accept as to the possibility of error. The 5 percent level is frequently used, although in these experiments a good case could be made for operating at the 10 percent level. At the 5 percent level of significance, and employing a group size of 24 animals the significance of difference ranges from 6.7 to 10.0 percent at acceptance levels of 10 to 50 percent at the 20 to 30 percent range the significance is 8.7 percent. This places inhibitions on the absolute utilization of a single decision point of 33 percent. At the 50 percent or no detection of bait range, the 10.0 percent difference is operative. A group size of 12 rats is also included as this size group may be used for initial evaluation. It is apparent that the use of a 10 percent level of significance, which would be appropriate for screening purposes, requires differences of 7 to 12 percent to be significant.

The 36 animal group is also included to illustrate that the increase precision of the data is hardly worth the increased cost incurred and most decisions made at the 24 animal group size would not be altered by the 36 animal group (Table 2).

Table 2. Estimated least significance differences.

Rat/Group	Acceptance Range	Significance Levels			
		20%	10%	5%	1%
N = 12	10-20%	5.8%	7.6%	8.9%	11.8%
	20-30%	8.0%	10.3%	12.3%	16.6%
	30-50%	9.2%	11.8%	14.1%	18.8%
N = 24	10-20%	4.1%	5.2%	6.7%	8.3%
	20-30%	5.7%	7.3%	8.7%	11.6%
	30-50%	6.5%	8.3%	10.0%	13.3%
N = 36	10-20%	3.3%	4.3%	5.1%	6.8%
	20-30%	4.6%	5.9%	7.1%	9.5%
	30-50%	5.3%	6.8%	8.2%	10.9%

The Significant Number of Observations During an Assay

By far the largest number of assays conducted within a laboratory will be to provide the basis for decisions for alternate bait ingredients, packaging, shelf life studies, quality control, etc. Only a relatively few assays are conducted for regulatory purposes. Also because commercial rodenticide baits are formulated with such a large overdose of the toxicant, this type of assay is not well suited for the determination of toxicity. The toxicant is best monitored by chemical assays and other biological protocols.

It therefore appeared feasible to utilize a shortened form of the standard assay to generate decision making data in reference to palatability and acceptance characteristics. For all practical purposes, with warfarin baits, the onset of morbidity at the 5th day terminates significant bait consumption. With a bait acceptance of 15 percent or above, there will be little or no likelihood that bait consumption after the 5th day could alter the data obtained before this point. Baits with less than 15 percent consumption would have been rejected at this point regardless of subsequent bait consumption.

An analysis of the data as presented in Table 3 indicated that decisions regarding the acceptability of warfarin baits can be made with a high degree of confidence by the 3rd day, and there is no basis for prolongation of the studies beyond the 4th day.

This effects a considerable saving in the biological evaluation of rodenticide baits and coupled with screening groups of 12 animals materially increases the capability of a laboratory to examine a wider range of options and to monitor the full gamut of quality control from raw materials through production and inventory to the point of sale.

Table 3. Full scale EPA* tests in 5, 4, 3 day observation points.

Test Batch vs EPA	Rats - Mean Acceptance							
	Full Scale EPA Test		5 Day		4 Day		3 Day	
	Male N=12	Female N=12	Male N=12	Female N=12	Male N=12	Female N=12	Male N=12	Female N=12
1. EPA vs EPA (Blank)	50.2%	50.3%	50.2%	50.3%	50.2%	50.3%	50.2%	52.2%
2. Bait A	23.9%	38.1%	24.0%	38.0%	24.2%	38.5%	24.7%	39.5%
3. Bait B	24.6%	31.5%	24.5%	31.3%	24.7%	31.0%	25.9%	32.5%
4. Bait C	17.6%	31.0%	17.2%	30.8%	17.5%	31.3%	18.7%	32.2%
5. Bait D	18.1%	25.1%	18.1%	24.9%	18.1%	25.1%	18.5%	27.6%

* EPA = U.S. Environmental Protection Agency

FACTORS AFFECTING THE PALATABILITY OF BAITS

Toxicant

The toxicant may be a major source of palatability variation within baits. We have acquired extensive experience with warfarin from a wide range of sources. There are batches of warfarin that are rejected by the rodent when used at the 0.025 percent concentration. There are also other batches of warfarin that are sufficiently bland to permit complete masking of its presence and even preferential consumption, depending on the carrier. Impurities at certain levels may play a role in the palatability of warfarin, however, wide differences can exist within the range of relatively pure material. The control of these variables, and their monitoring has lead to the development of Wincon®, a highly refined warfarin. The taste factor that is involved is believed to be a persistent, intense bitter taste, almost an "after taste". It is perceived by humans after a few minutes and persists for several minutes. The ability of rats to detect bitter tastes has been previously documented.

Mixing

The choice of the carrier is critical in the manufactur of anticoagulant baits. However, the methods of mixing are as critical here as in any of the food and beverage industries. It is not sufficient to mix a bait by any means feasible to achieve the distribution of toxicant as required on the label claim. The bland character of the pretested toxicant, such as Wincon® can be readily lost if the mixing procedures are not developed to retain this bland quality.

Shelf Life

Manufactured baits required a prolonged shelf life - perhaps even exceeding those commonly employed in the edible cereal industry. The rodenticide baits have to be palatable at the point of sale. The qualities present at the time of formulation have to be extended over this period with the additional protection against odor transfer and insect infestation. One of the major factors in the loss of palatability is the development of rancidity. We have been able to use a human odor panel to monitor these changes. The trend of the percent age of volatile fatty acids, if monitored over the entire period, is also of value in the determination of shelf life characteristics.

A palatable anticoagulant bait used in adequate quantities and in an intelligent manner is capable of effective control of the vast majority of rodent infestations. In the public's mind the lack of control of a rodent problem and the presence of resistant rats is unfortunately often synonymous. It is of great importance that a well blended toxicant in a palatable carrier with an extended shelf life be utilized. In view of the minor incidence of the anticoagulant resistance rats it is a disservice to create unwarranted doubt in the minds of the consumer over the effectiveness of proven agents, effective against 99 percent or more of the rodent problems.

In our experience the use of laboratory rodents for the determination of the palatability of the baits is a useful, reproducible bioassay. The improvement in the quality of the rodenticide baits has been marked. However, we have major reservations over the production of this data to the final user situation. There are a great many factors which play a role

In the control or elimination of a rodent problem. Without adequate control of the habitat and alternate food sources the use of a rodenticide bait can at best be only temporarily pallative, at worst, ineffective. The preoccupation with the toxicant bait has been overdone. It is unlikely that a toxicant bait, whether it be of an established performance characteristic, or of a novel structure, will make a significant contribution to the effective control of the rodent problem without the use of the supporting measures. Certainly, if the toxicant bait was an overriding factor, we would have long since seen the effects of the overkill production of thousands of pounds of rodenticide.