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# TASTE-AVERSION LEARNING AND ITS IMPLICATIONS FOR RODENT CONTROL

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ABSTRACT: Although bait shyness has long been recognized as a problem to be overcome in the control of vertebrate pests, it has recently been suggested that the phenomenon might be turned to an advantage and used as an alternative, non-lethal form of control. Unfortunately, this technique has not proven to be as useful as hoped, as the work which has been done on coyotes is inconclusive at best and some recent work on rodents has cast serious doubts upon the method's potential. However, an extensive literature dealing with the formation of poison-based food aversions now exists, and insights gained from these studies can be used to increase the efficacy of traditional, lethal control techniques. For example, the efficacy of pre-baiting may be greatly increased if the pre-bait is treated with a non-toxic flavor which mimics the flavor of the subsequently used toxin, even if this non-toxic flavor decreases the acceptability of the pre-bait.

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## INTRODUCTION

For as long as man has tried to control rodents through poisoning, the animals' adeptness at avoiding toxic baits has been obvious. Recent findings suggest that the learning mechanisms involved in poison-based food-aversion learning may differ from those involved in other forms of learning. In particular, illness-based aversion learning is characterized by single-trial, long-delay acquisition, by an apparent specificity of cue to consequence, and by a strong resistance to extinction. That is, a single food/illness experience is sufficient to produce a profound and long-lasting aversion, specific to the taste of the food, even though the onset of the illness may not occur until several hours after the consumption of the food.

All of these attributes make this form of learning a serious problem to be overcome in vertebrate pest control. At the same time, the apparent specialization of this learning led some workers to suggest that, with the proper manipulation, illness-based aversion learning might be used to develop new and alternative forms of control in which the pests are "trained" to avoid foods of economic importance. Theoretically, this method offered a great deal of promise, since laboratory studies had demonstrated many times that a single flavor/illness pairing could cause animals to avoid that flavor for the rest of their lives. Therefore, it was reasoned, the simple development of techniques to bring this method to the field would result in new and effective means of pest control.

Although some early work suggested that this might be useful in the control of sheep predation by coyotes, more recent findings and a re-examination of some of the early reports (see review by Griffiths, Connolly, Burns, and Sterner 1978) now make this appear less likely. However, the size and intractability of coyotes render them difficult animals to study in a tightly controlled manner and thus one might still suggest that the observed difficulties may be due more to imperfections in the development of appropriate field techniques than to flaws in the original logic. However, some recent work on rodents [carried out in the laboratory, but under conditions more closely approximating field conditions than those previously employed (Robbins 1980)] indicates that there may indeed be flaws in the original logic.

Specifically, Robbins tested mice (*Peromyscus* and *Mus*) in groups, under ad lib fluid availability, to determine if animals faced with a three-bottle choice (LiCl & saccharin vs. NaCl & saccharin vs. water) would consume less of the "safe" NaCl/saccharin mixture than would control animals faced with a two-bottle choice (NaCl & saccharin vs. water). That is, he attempted to confer "protection" upon the safe NaCl mixture by simultaneously offering the animals a toxic LiCl solution. He found that with animals naive to the test flavors, only a temporary protection could be obtained, and that with animals that had had prior safe experience with the NaCl/saccharin mixture, no protection at all was observed. Furthermore, he found that if NaCl were not added to the saccharin to mimic the flavor of the LiCl, after 48 hrs no protection at all was found even with naive animals. The elimination of potential visual or position cues failed to make the system any more effective. Thus, this direct laboratory test of the efficacy of self-administered aversive conditioning in modifying rodent dietary selection seems to indicate that the technique offers more promise than performance. At a minimum, it suggests that a flavor with a less distinct taste than LiCl would have to be employed for the method to be successful.

Does this failure mean that a study of aversive conditioning has no applications in rodent control? I don't believe so. An extensive literature exists (see Riley & Clarke, 1977) which has

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examined many factors affecting poison-based aversion learning, including those which prevent its occurrence. An understanding of these findings might be used to increase the efficacy of standard, lethal control techniques through the reduction or elimination of bait shyness.

For example, pre-baiting has long been known to increase the effectiveness of rodent toxicants (Howard 1959; Peregrine 1973), while pre-exposure to the flavor later paired with illness has been shown to greatly reduce, or even eliminate, the occurrence of illness-induced flavor aversions (Robbins 1979). The parallel nature of these two findings immediately suggests the possibility of a similar underlying mechanism, and that in turn suggests that the pre-exposure effect in flavor-aversion learning might be used to devise a more effective pre-baiting technique. It is often assumed that pre-baiting increases the efficacy of toxicants by increasing their initial acceptability to the target animals through a reduction of the animals' innate wariness toward consuming novel foods or toward eating in novel environments. With this assumption in mind, it is usually recommended (a) that the pre-bait be made as desirable as possible to the animals, and (b) that the pre-baiting may be assumed to have achieved its maximum effect once the consumption of the pre-bait has begun to level off (Peregrine 1973, p. 526).

If pre-baiting acts by establishing some sort of behavioral "momentum to consume", then these recommendations are valid. However, if pre-baiting acts by decreasing the animals' ability to form learned aversions toward the toxic bait, then these recommendations should be replaced with (a) the pre-bait should be made to resemble the flavor of the bait plus toxicant as much as possible, even if this results in a decreased acceptance of the pre-bait, and (b) maximum acceptance of the pre-bait may not be presumed to indicate maximum effectiveness of the pre-bait -- several studies on the pre-exposure effect in taste-aversion learning have indicated that asymptotic consumption of the pre-exposure flavor does not correlate with the maximum inhibition of taste-aversion learning (cf. Robbins 1979, and papers cited therein). Thus, if it can be shown that pre-baiting acts, even partially, via the suppression of the animals' ability to form learned aversions, appropriate modifications of pre-baiting procedures should lead to a significant increase of the effectiveness of the toxic bait, especially when distinctly flavored acute toxicants are used.

Since evidence is available which suggests that even a very rapid initial rejection of a toxicant may actually reflect a quickly acquired learned aversion to that flavor, this reasoning seems promising. For example, Robbins (1978) compared the consumption of .2 M solutions of safe NaCl and of toxic LiCl by deer mice (Peromyscus maniculatus bairdi) which had had no prior experience with either flavor. At the end of a five-minute period there was a significant difference in the consumption of the two groups, with the animals drinking NaCl having consumed a mean of 1.9 mls and those drinking LiCl only .85 mls. Although this might have been taken to indicate that the two flavors had different initial acceptabilities to the animals, a closer examination of the consumption in ten-second intervals (see Figure 1) found that the two flavors were initially identically acceptable, but that the animals drinking the LiCl very quickly terminated their consumption, presumably due to the rapid onset of subtle toxic effects of the LiCl. Subsequent tests showed that the animals drinking LiCl had acquired a very strong learned aversion. Kusano (1975) has presented cumulative consumption curves for rats offered various acute toxicants. Since many of these curves are similar in shape to the cumulative LiCl curve in Figure 1, it is possible that rats' low initial acceptability of some toxicants may reflect the animals' rapid acquisition of learned aversions toward them. If this is true, it should be possible to design a modified pre-baiting procedure which would greatly increase the effectiveness of such toxicants. The remainder of this paper will be devoted to presenting and examining some experimental work intended to test this hypothesis.

#### EXPERIMENT 1

The reasoning offered above suggests that the effectiveness of a pre-bait should be increased by the addition of a non-toxic substance similar in flavor to the toxicant to be used later. This experiment will test that hypothesis by comparing the consumption of a sublethally toxic mixture of LiCl and saccharin by three groups of animals: one offered no pre-bait, another offered a pre-bait of saccharin alone, and the third offered a pre-bait of saccharin and NaCl. If the hypothesis is correct, the group given a pre-bait of saccharin and NaCl should show the greatest consumption of the subsequently offered toxic solution of saccharin and LiCl.

#### Methods

The 48 animals of this study were experimentally naive, adult (100-160 days of age) Peromyscus maniculatus gambelii. They were laboratory bred and reared from stocks maintained in the laboratory since their original capture near Mount Shasta, California, in 1967. Young mice were housed with their parents until weaning, then in same-sex groups until the experimental procedures were begun. During these studies the animals were housed in plastic laboratory cages (15 x 30 x 15 cm) equipped with wire lids and containing wood shavings as bedding. Lab chow was available ad lib.

At the beginning of the experiment, the animals were housed individually in cages as indicated and moved into an experimental room. Animals were assigned randomly, but balanced by sex, to one of three treatment groups ( $n = 16$  for each): Group 1 (no-pre) was scheduled to receive no pre-baiting, then be offered a choice between plain water and a mixture of saccharin and LiCl, a sublethal toxin. Group 2 (S-pre) was scheduled to receive pre-baiting with saccharin alone, then be offered the same choice of water versus saccharin and LiCl. Group 3 (S+N-pre) was scheduled to receive pre-baiting with a mixture of saccharin and NaCl, then also be offered the water versus saccharin and LiCl choice.

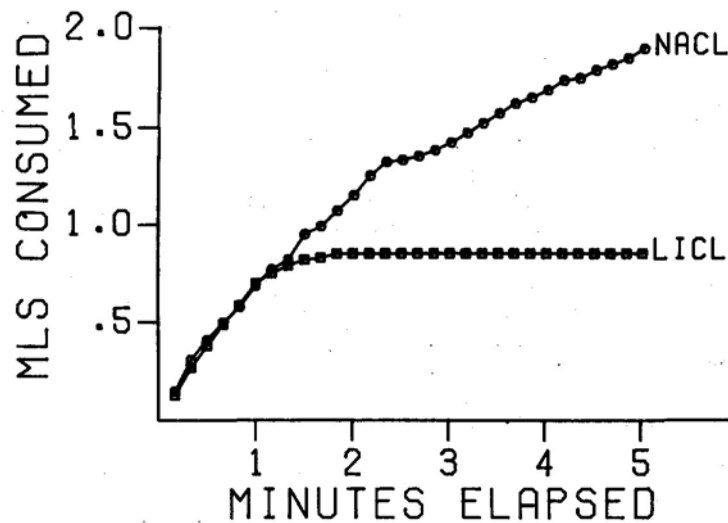


Fig. 1. Mean cumulative consumption of 0.2 M LiCl and NaCl solutions by naive animals. [Data from Robbins 1978]

For five days all animals received only plain water to drink, Then an eight-day pre-baiting was begun. Each cage had two tubes placed on it: one containing water and the other the assigned pre-baiting substance (a second tube of water for the no-pre group). The saccharin alone pre-bait was a 0.02 M solution of sodium saccharin. The S+N pre-bait was a 0.2 M solution of NaCl in 0.02 sodium saccharin.

Following the eight days of pre-baiting, the animals were given seven days of toxic baiting by offering saccharin plus LiCl in place of the pre-bait solution. The saccharin plus LiCl was a 0.2 M solution of LiCl in 0.02 M sodium saccharin.

All fluids were available 24 hours a day throughout the experiment. During this and the following experiments, fluid consumption was measured by offering the fluids in disposable plastic syringes that had been modified into calibrated drinking tubes (Robbins 1977a, 1977b).

### Results

The results for both the pre-baiting and the toxic-baiting periods are given as cumulative mean consumption curves in Figure 2. Cumulative consumption is used as the measured variable, since it is the variable of practical concern in rodent control.

Analysis of variance followed by planned contrasts found significant differences among the groups on each day of the pre-baiting schedule [ $F_{(2,45)} \geq 5.6341$ ,  $p \leq .05$ ], with the S-pre and S+N -pre groups drinking more than the controls [ $F_{(1,45)} \geq 4.4559$ ,  $p \leq .05$ ]. Although the S+N-pre group drank somewhat more than the S-pre group during the pre-baiting period, this difference was not significant on any day [ $F_{(1,45)} \leq 2.9287$ ].

A similar analysis on the data from the toxic baiting trials found significant differences among the groups on the first trial [ $F_{(2,45)} = 10.3806$ ,  $p \leq .001$ ]. On this trial, there was no difference between the no-pre and the S-pre groups [ $F_{(1,45)} = 0.0058$ ], while the S+N-pre group drank significantly more than either of the other two groups [ $F_{(1,45)} \geq 15.2681$ ,  $p \leq .001$ ]. Equivalent results were obtained with the cumulative data for the remaining six toxic-baiting trials.

### Discussion

These results show that the addition of a toxin-flavor mimic to the pre-bait does significantly increase the effectiveness of the pre-baiting procedure. Indeed, in this case, pre-baiting with the bait alone was completely ineffective; only pre-baiting with both the bait and the toxins-flavor mimic significantly increased the acceptance of the toxin/bait combination.

Although the results of the first trial did show a significant effect due to pre-baiting, it cannot be determined if this reflects an increased initial acceptability or a decreased learned aversion, since the data of the first trial actually represent the cumulative consumption over 24 hrs. A measurement with a finer temporal resolution would be required to distinguish between these two possibilities. This will be offered in the next experiment.

## EXPERIMENT 2

### Methods.

Forty-eight experimentally naive, male and female *Peromyscus maniculatus gambelii* were used to replicate the previous experiment exactly, except that a modified fluid-availability schedule was used

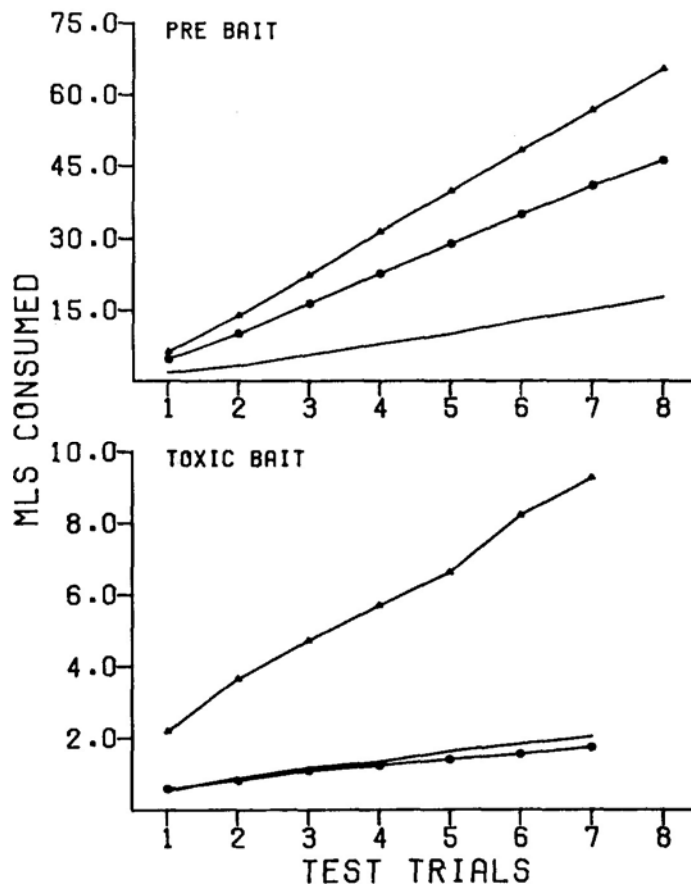


Fig. 2. Mean cumulative consumption by the animals of Experiment 1. The line connecting triangles indicates the consumption of the S+N-pre group; the line connecting circles that of the S-pre group; and the plain line gives that of the no-pre group. Curves on the upper axes represent consumption of the different pre-baits by the different groups, while the curves on the lower axes give the consumption of the same toxic bait by the different groups. Each trial represents consumption during a 24-hour period.

to permit a more precise measure of the toxic bait's acceptability on the first test trial. This was accomplished as follows: On Day 1, the animals were taken from their colony cages and placed into individual cages in the experimental room, with water tubes placed on the cages. On Day 2, the water tubes were removed, the data recorded, and a 24-hour fluid deprivation was begun. On Day 3, water tubes were placed on the cages for 20 minutes, then removed and the data recorded. Immediately following the recording of data, the tubes were refilled, replaced upon the cages, and left in position for 24 hours. This alternating fluid-availability/fluid-deprivation schedule was maintained throughout the experiment.

To ensure that the animals learned the schedule, they were offered water on this schedule for seven days. Then they were offered their assigned pre-bait flavors (as in Experiment 1) for eight days. Finally, they were tested with the toxic flavor for six days. The schedule was arranged so that the first toxic-baiting test would occur as a 20-minute drinking period. This allowed data to be taken which reflected the animals' acceptance of the toxic solution in this limited initial-contact period.

### Results

The results for both the pre-baiting and the toxic-baiting periods are given as cumulative mean consumption curves in Figure 3. Note that in this experiment the cumulative consumption curves have a "stair-step" appearance, since the odd-numbered trials represent consumption during a 20-minute period, while the even-numbered trials represent consumption during a 24-hour period.

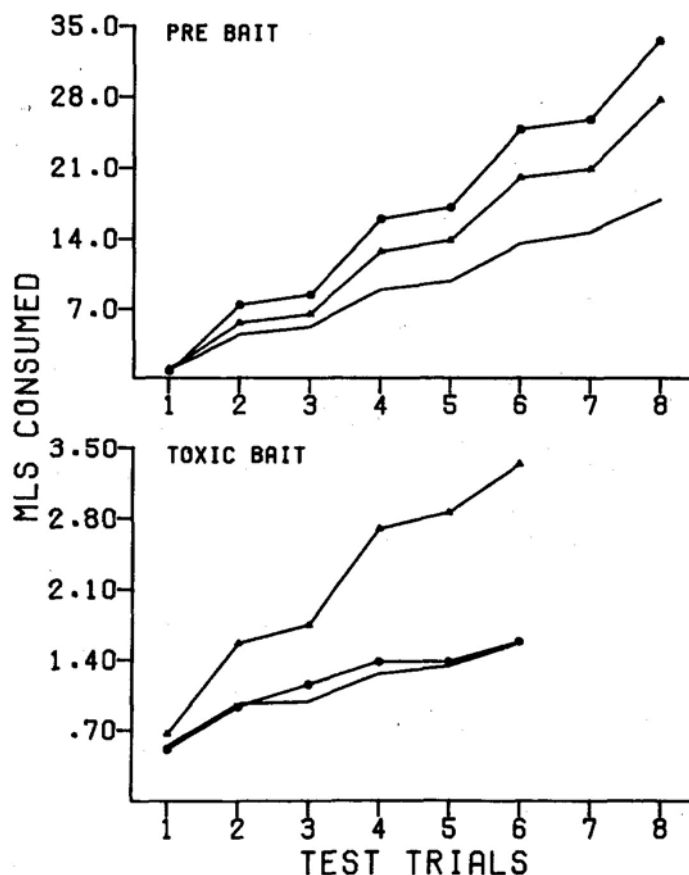
Analysis of variance, followed by planned contrasts, on the results of the pre-baiting trials found no differences among the groups on the first, 20-minute trial [ $F(2,45) = 0.9237$ ]. However, differences among the groups were detected on all subsequent pre-baiting trials [ $F(2,45) \geq 3.5695$ ,  $p \leq .05$ ]. The S-pre group drank more than the controls on pre-baiting trials 2-8 [ $F(1,45) \geq 7.0426$ ,  $p \leq .05$ ], but did not differ from the S+N-pre group on any pre-baiting trial [ $F(1,45) \leq 2.5560$ ]. The cumulative consumption of the S+N-pre group was significantly greater than that of the control group only on the sixth and eighth pre-baiting trial [ $F(1,45) \geq 4.6091$ ,  $p \leq .05$ ].

A similar analysis on the toxic-baiting trials found no differences among the groups on the first, 20-minute trial [ $F(2,45) = 0.6240$ ]. On the remaining trials, however, significant differences among the groups were obtained [ $F(2,45) \geq 6.7603$ ,  $P \leq .01$ ], with the S+N-pre group drinking more than either of the other two groups [ $F(1,45) \geq 10.1536$ ,  $p \leq .01$ ]. As in the previous experiment, no difference was observed between the no-pre controls and the S-pre groups on any of the toxic-baiting trials [ $F(1,45) \leq 0.6001$ ].

### Discussion

The results of this experiment have replicated the previous findings, in that again it has been found that pre-baiting increased the acceptance of the toxic bait only when the pre-bait included a non-toxic flavor similar to that of the subsequently used toxin. Additionally, since there were no differences among the groups on the first, 20-minute toxic-bait trial, it appears that in this case pre-baiting acted by inhibiting the occurrence of illness-induced rejection of the bait, rather than by increasing the bait's initial acceptability.

These results have confirmed the original hypothesis regarding the effectiveness of adding a toxin-flavor mimic to the pre-bait. If it is found that this is a general effect, not merely restricted to *Peromyscus* and to saccharin/LiCl mixtures, this work should provide the foundation upon which new pre-baiting procedures of greatly enhanced efficacy might be developed. Therefore, a test for the occurrence of a similar effect in animals of a different taxon will be provided in the next experiment.



**Fig. 3.** Mean cumulative consumption by the animals of Experiment 2. The line connecting triangles indicates the consumption of the S+N-pre group; the line connecting circles that of the S-pre group; and the plain line gives that of the no-pre group. Curves on the upper axes represent consumption of the different pre-baits by the different groups, while the curves on the lower axes give the consumption of the same toxic bait by the different groups. The odd-numbered trials represent consumption during 20-minute periods; even-numbered trials give consumption during 24-hour periods.

### EXPERIMENT 3

#### Methods

This experiment was designed to replicate Experiment 2, above exactly, except that adult, wild-type *Mus musculus* (derived from stocks originally captured near Salida, California, in 1972) were used as the test animals. Thirty-nine adult, laboratory-reared, male and female house mice were

selected from the colony and assigned to treatment groups, balanced by sex, as in the previous experiment. They were offered fluids on the same schedule as employed in Experiment 2, except that only four toxic-baiting trials were used.

### Results

The results for both the pre-baiting and the toxic-baiting periods are given as cumulative mean consumption curves in Figure 4. Note that again the cumulative consumption curves have a "stair-step" appearance due to the alternation of 20-minute and 24-hour trials.

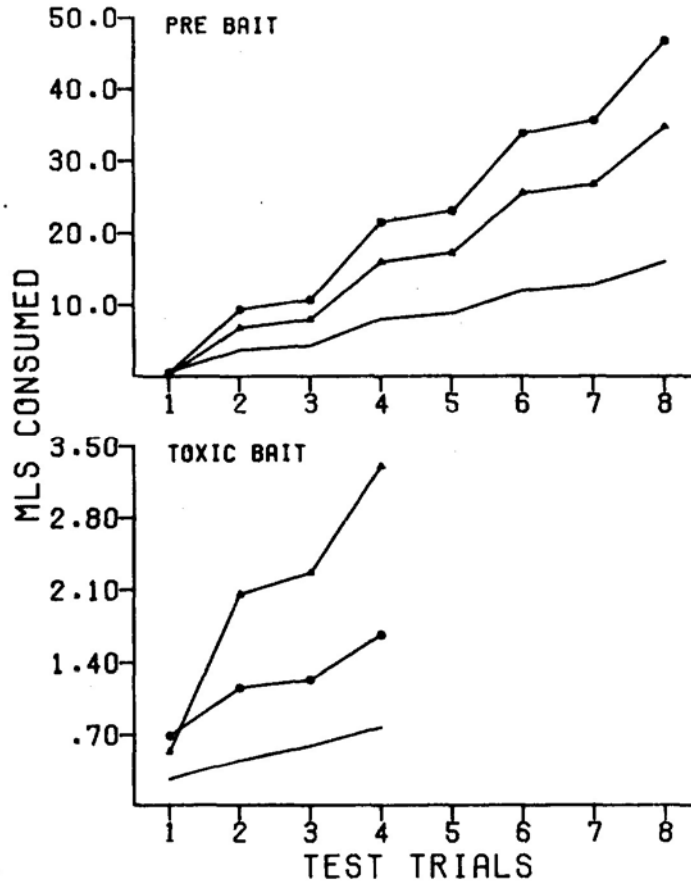


Fig. 4. Mean cumulative consumption by the animals of Experiment 3. The line connecting triangles indicates the consumption of the S+N-pre group; the line connecting circles that of the S-pre group; and the plain line gives that of the no-pre group. Curves on the upper axes represent consumption of the different pre-baits by the different groups, while the curves on the lower axes give the consumption of the same toxic bait by the different groups. The odd-numbered trials represent consumption during 20-minute periods; even-numbered trials give consumption during 24-hour periods.

Analysis of variance, followed by planned contrasts, on the results of the pre-baiting trials found no differences among the groups on the first, 20-minute trial [ $F(2,36) = 1.8251$ ]. However, differences among the groups were detected on all subsequent pre-baiting trials [ $F(2,36) \geq 17.5743$ ,  $p \leq 10^{-5}$ ]. The S-pre group drank more than the controls [ $F(1,36) \geq 34.7723$ ,  $p \leq 10^{-6}$ ] and more than the S+N-pre group [ $F(1,36) \geq 5.0879$ ,  $p \leq 0.05$ ] on pre-baiting trials 2-8. The cumulative consumption of the S+N-pre group was significantly greater than that of the control group on pre-baiting trials 2-8 [ $F(1,36) \geq 12.1075$ ,  $p \leq 0.01$ ].

A similar analysis on the toxic-baiting trials found differences among the groups on the first, 20-minute trial [ $F(2,36) = 8.9341$ ,  $p \leq .001$ ], with the S-pre and the S+N-pre groups each drinking more than the controls [ $F(1,36) \geq 7.3122$ ,  $p \leq 0.05$ ], but not differing from each other [ $F(1,36) = 2.1365$ ]. On the remaining trials, however, significant differences among the groups were again obtained [ $F(2,36) \geq 14.2005$ ,  $p < 10^{-4}$ ], but on these trials the S+N-pre group drank more than either of the other two groups [ $F(1,36) \geq 10.5458$ ,  $p \leq 0.01$ ], and the S-pre group drank more than the no-pre control group [ $F(1,36) \geq 4.1240$ ,  $p \leq 0.05$ ].

## Discussion

With house mice, the S-pre and the S+N-pre groups showed an equivalent increased acceptance of the toxic bait during the first 20-minute trial, suggesting that in this case pre-baiting, either with S+N or with S alone, acted to increase the initial acceptability of the toxic bait. However, the significantly increased consumption of the S+N-pre group on the subsequent trials shows that with *Mus*, as with *Peromyscus*, the inclusion of a toxin-flavor mimic in the pre-bait increases the effectiveness of a toxic bait by inhibiting the formation of illness-induced taste aversions. Additionally, since the S+N-pre group drank significantly less of the pre-bait but significantly more of the toxic bait, it appears that this technique can increase the acceptance of the toxic bait, even if it decreases the acceptance of the pre-bait.

This experiment has established the generality of the phenomenon across animals of different taxa, A test using actual rodenticides remains to be done.

### GENERAL DISCUSSION

Pre-baiting has long been known to increase the efficacy of acute rodenticides. However, trial and error has shown that the effectiveness of pre-baiting can vary from toxicant to toxicant and from species to species. The findings of the experiments reported above may shed some light upon the variability encountered in the use of pre-baiting.

The results above suggest that the effectiveness of pre-baiting should be related to the similarity between the flavor of the pre-bait and the flavor of the subsequently employed bait/toxicant combination. Therefore, the efficacy of pre-baiting should vary across toxicants since pre-baiting with the bait alone would be more effective, for toxicants that are relatively tasteless to the target species and much less effective for distinctly flavored toxicants.

Since even closely related organisms frequently show striking differences in their taste sensitivity to various compounds (Kare 1971), it is not surprising that the effectiveness of pre-baiting would vary from species to species. Indeed, the data from Experiments 2 and 3 above show a clear species-related difference in the efficacy of pre-baiting using the bait flavor alone: For *Mus* pre-baiting with saccharin alone significantly increased the subsequent acceptability of saccharin plus LiCl (cf. Figure 4), while for *Peromyscus* pre-baiting with saccharin alone did not increase the later acceptability of saccharin plus LiCl (cf. Figures 2 and 3). Therefore, if these experiments had been conducted using only traditional pre-baiting techniques, it would have been concluded that pre-baiting was effective with *Mus* but was not effective with *Peromyscus*. However, pre-baiting with the bait and a flavor mimic of the subsequently used toxicant was highly effective with both species. This suggests that the development of pre-bait techniques using flavor mimics of actual rodenticides should not only lead to much more effective baiting procedures, but should also decrease the variability in efficacy frequently encountered in pre-baiting.

Although these results with LiCl are reasonably persuasive, work with a real rodenticide remains to be done. Zinc phosphide would be a good subject for further investigation, since several studies (Cowan 1978, Cowan, Srihari & Sridhara 1979, Mukherjee & Jain 1979, Prakash & Jain 1971, Prakash & Ojha 1977, Ojha 1978, Sridhara & Srihari 1978, 1979) have found that this toxicant is especially prone to inducing shyness, despite the fact that its initial acceptability can be increased greatly with pre-baiting. This renders zinc phosphide effective primarily as a "one-shot" rodenticide. If the findings of the present experiments prove generalizable to the action of this toxicant, its efficacy could be greatly enhanced through the use of techniques similar to those presented here. Of course, this could also apply to other rodenticides known to be particularly prone to inducing shyness.

Additionally, as noted in the discussion of Figure 1, it is possible that low initial acceptability of a toxicant may actually reflect a rapid acquisition of learned aversions. If that is the case, this technique might also be employed to increase the acceptability of many toxicants, as well as to prevent the formation of bait shyness. In any event, the technique certainly seems to hold out promise and to warrant further study.

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