

## Liquid baits for rodent control: A comparison of wild Norway versus wild ricefield rat response to glucose plus saccharin solutions

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A series of two-bottle tests were conducted with wild Norway (*Rattus norvegicus*) and wild ricefield (*Rattus rattus mindanensis*) rats to assess liquid bait consumption levels. Glucose, a natural sugar, and saccharin, an artificial sweetening agent, were tested against a combination of the sweeteners in water solution with each rat species. Water baseline data indicated that ricefield rats drank relatively more water per day. No species differences, however, were found for consumption levels of 3.0% glucose or for 0.125% saccharin when fluid intake levels were adjusted for mean body weight differences between species using metabolic size data transformations to yield relative consumption levels. A species difference was shown for relative consumption of the mixture of 3.0% glucose plus 0.125% saccharin in water solution with either sweetener as the alternate choice. Ricefield rats showed a two-fold increase in relative consumption of the mixture compared with solutions of either sweetener alone. Norway rats, in contrast, showed a synergistic six-fold increase in relative consumption of the mixture compared with solutions of either component alone. A second series of two-bottle choice tests with new groups of rats showed that both saccharin and glucose solution consumption levels were similar in the two species. The lack of glucose plus saccharin synergism in the ricefield rat response was found to be related to less preference by ricefield rats for glucose when paired with saccharin solution compared to the Norway rat preference pattern. Implications on the potential application of these results to the control of the two species using liquid bait stations are discussed and summarized. Published by Elsevier Science Ltd

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Rats cause extensive damage to agricultural crops, are responsible for post-harvest losses of food, and can become vectors or intermediate hosts for a large number of disease pathogens (Fall, 1991; Pratt, Bjornson and Littig 1977; Rzoska, 1953). Grain baits are used in field situations to control rodent outbreaks throughout the world, and these are an effective media for rodenticide delivery. Taste additives can be used with rodent baits to improve palatability of grain bases (Shafi, Pervez, Ahmad and Ahmed, 1990; Shafi, Ahmed, Pervez and Ahmad, 1992).

In food warehouse and grain silo storage areas, however, solid food baits may not compete well with other freely available food sources that have become very familiar to the rodents. Added odor and taste agents may induce a few individuals to sample the solid bait material, and pre-baiting can sometimes be used to improve efficacy. However, a more advantageous tactic for rodent control in these situations would involve the use of liquid bait stations. Rats generally find and use

new sources of water in buildings without flooding problems or plumbing leaks. Most rats prefer sweet-tasting fluid that can be used as a vehicle for the delivery of rodenticides or other agents (e.g. contraceptive vaccines). A main objective of the current study was to evaluate the potential of two sweeteners alone and in combination as liquid bait media for controlling two species of wild rats.

Valenstein, Cox and Kakolewski (1967) reported that laboratory rats would consistently drink extremely large quantities of a solution containing a mixture of glucose, saccharin and water. When offered a mixture of 3.0% glucose and 0.125 or 0.250% saccharin in distilled water, albino rats drank amounts that were approximately equal to their individual body weights every 24 h. This excessive or polydipsic drinking was thought to result from a synergistic effect between the mixture components (i.e. a highly palatable sweet taste could be obtained without an associated high caloric content). At the same time, the slightly bitter taste of saccharin was thought to be partially masked by the sweet taste of glucose.

Lockard (1968) found that laboratory rats preferred foods with tastes preferred by humans. Wild Norway

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rats, however, have been found to be more calorically selective in their food habits (Maller and Kare, 1965) with palatability only playing a minor role in their food selection. In laboratory tests, wild Norway rats showed similar preference responses to albino laboratory strains (Shumake, Thompson and Caudill, 1971) for solid food treated with sucrose, citric acid, sodium chloride or quinine in various concentration levels.

In this study, the responses of wild Norway rats (*Rattus norvegicus*) and another species of wild rat native to the Philippines (*Rattus rattus mindanensis*) were evaluated to determine relative consumption for glucose (nutritive sweetener), saccharin (non-nutritive sweetener) and combinations of the two agents in deionized water. Subsequent tests were designed to isolate mixture components that could explain an observed species difference in relative consumption.

## Materials and methods

### Experiment 1. Replication of the glucose-saccharin synergistic effect in wild rat strains

**Animals.** Six male, wild Norway rats, live and trapped locally, weighing  $315.5 \pm 14.1$  g, and six male, wild ricefield rats, trapped in the Philippines, and weighing  $258.7 \pm 18.1$  g, were maintained throughout the tests on *ad libitum* Purina® Laboratory Rat Chow diet. They were maintained in a temperature ( $22^\circ \pm 2^\circ\text{C}$ ) controlled room with a forward 12:12 h light/dark cycle. All animals were individually housed and tested in standard wire mesh cages ( $24 \times 17.5 \times 18$  cm). Glass drinking bottles (450 ml) with rubber stoppers and stainless steel sipper tubes were attached to the front of the cages with spring clips.

**Chemicals.** All water, glucose (G), saccharin (S) and G + S solutions were premixed using deionized water and stored for 24 h under refrigeration before use in preference tests. Reagent grade granular anhydrous D-glucose (CAS No. 50-99-7; Mallinckrodt) and sodium saccharin (CAS No. 81-07-2; Merck) were used.

**Procedure.** Fluid consumption was measured by weighing the bottles to the nearest 0.5 g on a single beam balance before and after each 23 h exposure interval. Differences were taken to reflect consumption. Fluid spillage was found to be minimal, but it was not directly measured. As indicated by Mason and Clark (1994), spillage in two-choice tests presents problems of food particle or fluid type separation, and the degree of spillage is relatively proportional to consumption levels. First, water consumption was measured for each of the 12 animals on 4 successive days using single bottles attached to the front of each animal's cage (Test A1). Then, all animals were given 3.0% G and G + S (3.0% glucose and 0.125% saccharin) in separate bottles for 4 days (Test B1). Finally, the animals were offered a choice between 0.125% saccharin solution (S) and the G + S solution for 4 days of preference testing (Test C1). Positions of the test fluid bottles were alternated each day in the choice-preference tests.

Fluid consumption values were transformed into metabolic size measures (g fluid/kg body weight raised to the 0.75 power) as recommended by Schmidt-

Nielson (1984). A repeated measure of analysis of variance (ANOVA) was used (Winer, 1962). All analyses were performed on the transformed data values to control for differences in body weights between the two species. Test A1 was a 2 (species)  $\times$  4 (days) analysis, and B1 through C1 were 2 (species)  $\times$  2 (fluids)  $\times$  4 (days) analyses.

### Experiment II. Isolation of glucose and saccharin taste component effects

**Animals.** Six male, wild Norway rats with a mean body weight of  $312.4 \pm 10.1$  g and six male, ricefield rats with a mean body weight of  $232.9 \pm 15.5$  g were maintained throughout all tests on *ad libitum* Purina® Laboratory Rat Chow with the same room temperature and light cycle conditions as described for Experiment I. The same cage type, solution bottles, G and S sweeteners, and weigh-back balance as previously described were used. All test solutions were again stored under refrigeration for 24 h before offering them to the rats.

**Procedure.** First, the 12 rats were offered a choice between deionized water and 0.125% S solution for the first 4 test days and 23 h consumption levels were measured for each rat each day of the test (Test A2). Next, animals were offered a choice between deionized water and 3.0% G solution on each of 4 days (Test B2). Then, preference for 0.125% S versus 3.0% G solutions was tested for 4 days (Test C2). Because the results of these first three choice tests did not reveal clear species differences in preference, all rats were given a choice between water and 6.0% G plus 0.25% S solution for 4 days (Test D2). Finally, rats were offered a choice between deionized water versus 3.0% G plus 0.125% S over a 6-day interval (Test E2).

All metabolic size data sets for each of the preference tests (A2 through E2) were analyzed with the same ANOVA design (i.e. 2 species  $\times$  2 fluids  $\times$  4 or 6 days with repeated measures on days).

## Results

### Experiment I

*Figure 1* shows the mean + standard deviation fluid consumption levels for the two species based upon metabolic size measures. The ricefield rats weighed, on average, 18% less than the wild Norway rats. The ANOVA on the transformed water baseline data (Test A1) indicated that the ricefield rats drank significantly more water per day ( $F = 7.43$ ; 1,9 d.f.;  $P < 0.05$ ) even though they weighed consistently less than the Norway rats. Excessive G + S daily intake levels by the wild Norway rats occurred in tests B1 and C1.

The ANOVA on the G versus G + S choice preference test data (Test B1) indicated significant species ( $F = 48.59$ , 1,10 d.f.;  $P < 0.01$ ), test fluid ( $F = 124.22$ ; 1,10 d.f.;  $P < 0.01$ ), and species  $\times$  test fluid interaction ( $F = 68.56$ ; 1,10 d.f.;  $P < 0.01$ ) effects. There were also significant main effects for days ( $F = 2.95$ ; 3,30 d.f.;  $P < 0.05$ ) and a three-way interaction among species, test fluids, and days ( $F = 1.72$ ; 3,30 d.f.;  $P < 0.05$ ).

In the S versus G + S condition (Test C1), a separate

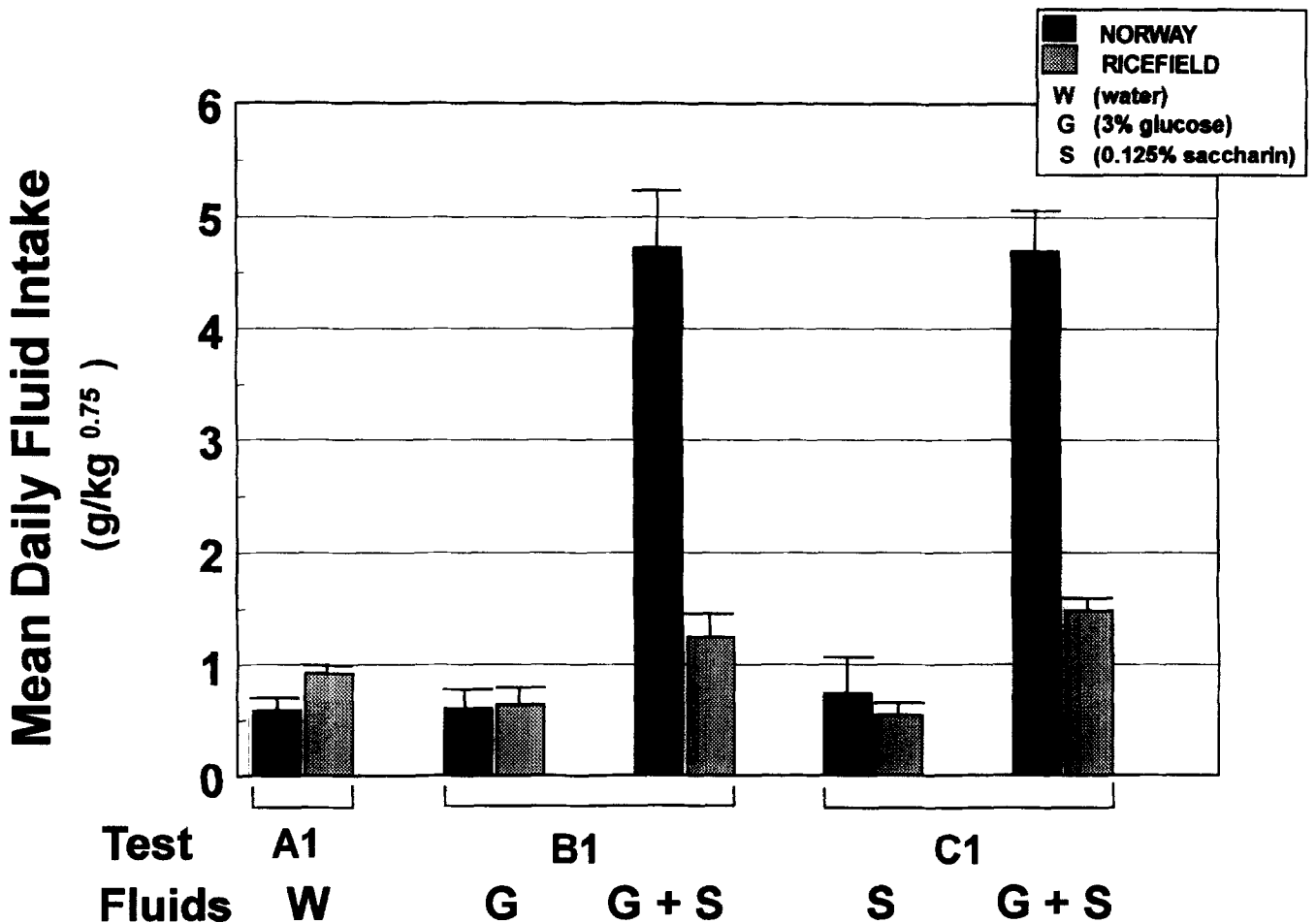


Figure 1. Mean (+ SD) consumption levels based upon metabolic size (grams of fluid intake per kilogram of body weight raised to the 0.75 power) for the two rat species in Experiment I. A1, B1, and C1 are successive 4-day preference tests for water, G versus G + S, and S versus G + S, respectively

repeated measures ANOVA was performed on metabolic size intake data. Significant species ( $F = 31.20$ ; 1,10 d.f.;  $P < 0.01$ ), test fluid ( $F = 94.68$ ; 1,10 d.f.;  $P < 0.01$ ), and species  $\times$  test fluid interaction ( $F = 35.93$ ; 1,10 d.f.;  $P < 0.01$ ) effects were found. The species effect was thus replicated on this second two-choice test. The ricefield rats drank mean daily amounts ( $92.9 \pm 3.7$  g) of the G + S fluid mixture representing less than half their individual body weights, whereas the Norway rats drank the same mixture in mean daily amounts ( $344.5 \pm 15.8$  g) often exceeding their body weights.

#### Experiment II

The wild ricefield rats had a mean body weight approximately 25% lower than the mean body weight of wild Norway rats. Figure 2 shows the results of the five preference tests (A2 through E2) in terms of mean + standard deviation fluid intake values based on the metabolic size measure. The high levels of G + S consumption in tests D2 and E2 are again quite apparent, particularly for wild Norway rats.

The transformed water versus S consumption data (Test A2) ANOVA indicated only a test fluid ( $F = 9.33$ ; 1,10 d.f.;  $P < 0.05$ ) effect with both species showing a preference for the S solution. There were no significant species, day, or interaction effects.

Water versus G preference data (B2) again yielded a

significant test fluid ( $F = 23.86$ ; 1,10 d.f.;  $P < 0.01$ ) effect with no species effect. However, there were species  $\times$  day ( $F = 5.14$ ; 1,10 d.f.;  $P < 0.01$ ) and species  $\times$  day  $\times$  fluid ( $F = 4.78$ ; 3,30 d.f.;  $P < 0.01$ ) interaction effects along with day ( $F = 3.45$ ; 3,30 d.f.;  $P < 0.05$ ) and fluid  $\times$  day ( $F = 3.18$ ; 3,30 d.f.;  $P < 0.05$ ) effects. The three-way interaction effect arose from the fact that ricefield rats and Norway rats showed equal preference for the G solution on the first test day, but then diverged in preference patterns with ricefield rats showing significantly less of a preference for G solution compared with wild Norway rats for the last 3 days of the test.

In the direct choice preference test for the two sweeteners (C2), there was no significant difference between species. However, there were significant fluid ( $F = 60.85$ ; 1,9 d.f.;  $P < 0.01$ ) and species  $\times$  fluid ( $F = 7.12$ ; 1,9 d.f.;  $P < 0.05$ ) effects. Essentially, both rat species preferred the G solution to the S solution, with both drinking close to equivalent levels of S solution based on metabolic size. However, the extent of the G preference was significantly more pronounced in the wild Norway rats resulting in the two-way interaction effect. The lack of day interactions demonstrated consistent preferences for the G solution by both species over the 4-day test.

The last two preference tests yielded similar statistical results. For the 4-day test (D2), there were significant species ( $F = 8.89$ ; 1,9 d.f.;  $P < 0.05$ ), fluid ( $F = 65.46$ ;

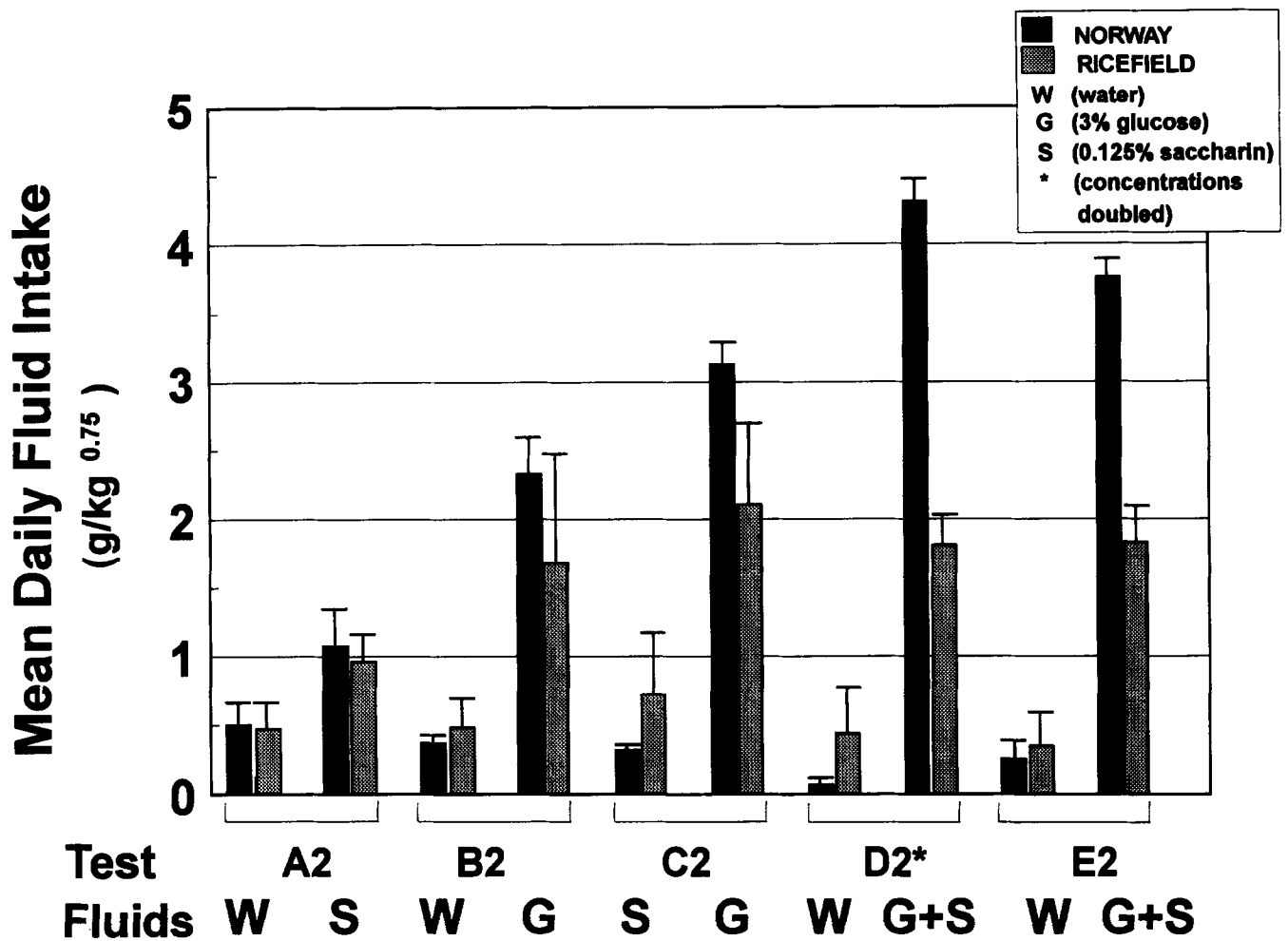


Figure 2. Mean (+ SD) consumption levels based upon metabolic size (grams of fluid intake per kilogram of body weight raised to the 0.75 power) for the two rat species in Experiment II. A2 through D2 are successive 4-day preference tests for water versus S, water versus G, S versus G, and water versus G + S, respectively. E2 is a 6-day preference test for water versus G + S

1,9 d.f.;  $P < 0.01$ ), and species  $\times$  test fluid ( $F = 17.18$ ; 1,9 d.f.;  $P < 0.01$ ) interaction effects. For the 6-day test (E2), there were significant species ( $F = 5.45$ ; 1,9 d.f.;  $P < 0.05$ ), fluid ( $F = 100.2$ ; 1,9 d.f.;  $P < 0.01$ ), and species  $\times$  fluid ( $F = 16.71$ ; 1,9 d.f.;  $P < 0.01$ ) interaction effects. All other main and interaction sources were non-significant in both ANOVAs. For each of these last two tests, the wild Norway rats showed significantly greater consumption of the G + S solution based on metabolic size intake measures ( $\bar{x} = 4.31 \pm 0.16$ , and  $3.76 \pm 0.13$ ) compared to G + S consumption levels of the ricefield rats ( $\bar{x} = 1.81 \pm 0.22$ , and  $1.83 \pm 0.26$ ), respectively. This factor accounted for the main part of the two-way species  $\times$  fluid interaction effects in both of these last two tests. The wild Norway rats, however, drank significantly reduced amounts of water when G + S was available as compared with the ricefield rats in Test D2, and this also contributed to the two-way interaction.

#### Discussion and management implications

In Experiment I, both wild rat species drank approximately equivalent mean amounts of the G and S solutions in both preference tests (B1 and C1). The Norway rats, however, drank at least three times more of the G + S

solution when compared to the relative consumption levels of ricefield rats. Wild Norway rats showed a level of polydipsia equal to that shown by albino (domesticated Norway) rats in previous studies. Valenstein *et al.* (1967) concluded in their tests that this G + S polydipsia was not due to excessive body fluid loss due to urination (polyuria) or to the inhibition of antidiuretic hormone levels in albino rats. Both G and S can produce increased insulin secretion thereby affecting caloric food intake regulation in the rat (Sclafani, 1973, Valenstein and Weber, 1965). Consumption of both S at low concentrations and a nutritive sweetener, sucrose, has been reported as reduced in hypothyroid rats (Gordon, Wong, Liu and Rivlin, 1992). However, the species difference in consumption of G + S solutions in the current study may not necessarily reflect direct differences in metabolic, caloric or osmoregulatory functions. On the sensory level, it is possible that the ricefield rats were more sensitive to the slightly bitter taste of saccharin or were less attracted to the glucose component of the mixture in terms of taste palatability. Genetic strain differences in laboratory mice for detection and preference of bitter-tasting substances (Harder, Whitney, Frey, Smith and Rashotte, 1984; Lush, 1984; Whitney and Harder, 1986; Whitney and Harder, 1994; Whitney, Harder and Gannon, 1986), as well as sweet-tasting substances,

(Capeless and Whitney, 1995; Iwaski, Kasahara and Sato, 1986) have been reported extensively.

Experiment II attempted to elucidate the question of relative palatability in these two species using different groups of wild ricefield and wild Norway rats. As indicated, a significant species by test fluid interaction occurred when 6.0% G and 0.25% S solutions were compared (Test D2). This species effect could be, at least partially, due to a lower preference for G and slightly higher preference for S by ricefield rats (Test C2). The G component for ricefield rats in the G + S solution may have been more blunted in terms of palatability compared with that shown by wild Norway rats. It is unclear from the data whether this lowered palatability for glucose stemmed from lesser taste sensitivity, different caloric regulatory systems, or other factors. However, on the basis of metabolic size, the ricefield rats drank relatively more water than did wild Norway rats pointing to a possible difference in osmoregulatory function between the species. The species  $\times$  fluid  $\times$  day triple interaction (Test B2) may have indicated a learning effect related to post-ingestive metabolic factors similar to those reported for albino (Norway) rats (Schalfani and Ackroff, 1994). Metabolic studies on G solutions and G + S solutions would be needed to further illuminate this aspect of the species difference.

On the basis of overall consumption by both species, however, the G + S solution would probably serve as an excellent base for water-soluble rodenticides, contraceptive drugs or other rodent control agents. The degree of preference for the G + S solution would serve to compete with other sources of water, particularly when wild Norway rats have become problems in structures. A sweetened-liquid bait (0.005% brodifacoum + 0.10% saccharin solution in water) has, in fact, been adopted for rodent control in grain storage structures in Taiwan (Lu, 1986) where *Rattus norvegicus*, as well as are two other species, *Rattus losea* and *Bandicota indica*, are major rodent pests. No published reports are available regarding potential G + S solution synergy in these latter two species.

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