

RODENTICIDE FLAVOR CHARACTERISTICS ASSESSED THROUGH GENERALIZATION OF CONDITIONED FLAVOR AVOIDANCE

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Abstract: Little information is available concerning the flavor qualities of toxicants or bait formulations. Using generalization of flavor avoidance learning (FAL) in Experiment 1, we investigated the flavor of alpha-chlorohydrin, alpha-naphthylthiourea, calciferol, scilliroside, and sodium (Na) warfarin as perceived by Sprague-Dawley rats (*Rattus norvegicus*). Scilliroside was apparently tasteless. For the other toxicants, FAL was observed. Alpha-chlorohydrin and alpha-naphthylthiourea avoidance generalized to "bitter" and "sour" flavors, calciferol avoidance generalized to "bitter" and "sweet" flavors, and Na warfarin FAL generalized to "bitter," "sweet," and "salty" flavors. In Experiment 2 we used generalization of FAL to examine the flavor of strychnine in agar or water. Strong FAL was observed, although avoidance generalization was narrower for strychnine in agar than in water. In Experiment 3 we examined FAL among alpha-chlorohydrin, alpha-naphthylthiourea, calciferol, strychnine, and Na warfarin. Flavor avoidance learning and generalization were obtained for some (but not all) rodenticides, and similarities among generalization profiles obtained in Experiments 1 and 2 did not predict generalization among the rodenticides, per se. We infer that toxicants can have complex flavors and are not only bitter or sour, as some have proposed. Applications of our findings may range from the empirical development of prebait formulations to the evaluation of bait enhancers and microencapsulation techniques.

J. WILDL. MANAGE. 55(1):188-198

The flavor characteristics of rodenticides are largely unknown. Such information could prove useful (Robbins 1980, Reidinger and Mason 1983) in developing rodenticide flavor mimics for use in prebait formulations. Flavored prebaits would promote habituation by rodents to the flavor of toxicant-containing bait, thereby increasing consumption and improving efficacy. In addition, knowledge about the flavor of a rodenticide might suggest flavor-masking agents to increase consumption of unpalatable but otherwise effective control substances (Reidinger and Mason 1983, Stewart et al. 1983, Mason et al. 1985).

Several methods have been used to assess the taste and/or flavor (taste, odor, texture) of foods and fluids to rodents (Erickson 1963, Morrison 1967, Kusano et al. 1975a). The most common method is response generalization following flavor avoidance learning (FAL) (Wiggins et al. 1989). Briefly, FAL occurs when ingestion of a distinctive flavor is paired with illness. After the pairing, animals avoid the distinctive flavor and generalize this avoidance to similar flavors. Nachman (1963, 1979) used this procedure to demonstrate that, for laboratory rats (*Rattus norvegicus*), the taste of sodium chloride resembled that of lithium chloride (LiCl) (i.e., learned

avoidance of one, readily generalized to the other). Conversely, compounds with less chemical similarity to LiCl (e.g., potassium chloride [KCl], potassium nitrate [KNO₃]) elicited less response. Nowlis and Frank (1977) also used generalization of FAL to demonstrate that laboratory rats and hamsters (*Mesocricetus aurata*) tend to categorize tastants into 4 groups ("sweet," "sour," "salty," and "bitter"), and DuVillard et al. (1980) used FAL to describe the effects of thaumatin, a sweetness enhancer, on the perceived sweetness of sucrose to rats.

Smith and Theodore (1984) used generalization of FAL to assess discrimination by rats of the flavor qualities in 2-component mixtures. The results indicated that rats can discriminate between mixture components; we have therefore used this method to assess the perception of rodenticides such as strychnine by rats (Stewart et al. 1983, Mason et al. 1985). Our present experiments represent an extension of this work—we sought to describe the flavor of an additional 4 rodenticides (Exp. 1), as well as to compare the flavor of strychnine in aqueous solution versus agar (Exp. 2). In Experiment 3, we examined generalization among rodenticides to assess whether the flavor profiles obtained in Experiments 1 and 2 could be used to predict

perceived similarities among the rodenticides themselves.

Our research was supported by U.S. Army Research Office Contract No. DAAG-29-82-K-0192 and U.S. Department of Agriculture Cooperative Agreement No. 14-16-0009-86-900. W. Bowles, S. Lewis, and C. A. Kornet provided technical advice and assistance. L. Clark, W. J. Jakubas, and G. K. Beauchamp critically reviewed earlier manuscript drafts.

MATERIALS AND METHODS

Experiment 1.—This experiment was designed to identify the principal flavor characteristics of technical grade alpha-chlorohydrin (Aldrich, Milwaukee, Wis.) (0.8 mg/mL), alpha-naphthylthiourea (K & K Fine Chemicals, Plainview, N.Y.) (0.75 mg/mL), calciferol (Rentokil Ltd., Sussex, England) (100 mg/mL), sciliroside (Sandoz Inc., Basel, Switzerland) (30 mg/mL), and sodium (Na) warfarin (Wisconsin Alumni Foundation, Madison, Wis.) (0.75 mg/mL). The selection of toxicants represented a wide variety of chemical structures (Thomson 1989), and presumably, a broad spectrum of flavor qualities. Because we were unable to obtain any evidence that rats could detect pure sciliroside, even when presented in 2-bottle drinking tests or at high concentrations in carboxymethylcellulose gel, data for that compound are not reported here. Contaminants in the production of red squill, the active ingredient of which is sciliroside (Thomson 1989:168), may be responsible for reports (P. Savarie, Denver Wildl. Res. Cent., pers. commun.) concerning the unpalatability of this toxicant to rats.

Rodenticides were prepared in aqueous solutions at either (1) $\frac{1}{2}$ of a single, acute, oral LD_{50} (mg/kg body mass), or (2) the limit of solubility in distilled water, if lower than (1). Preliminary experiments indicated that these concentrations would serve as reliable flavor stimuli for avoidance learning. Also, the concentrations were low enough to preclude obvious symptoms of poisoning. The objective was to provide little or no illness from the rodenticides, and then to elicit controlled malaise by injection of 0.15 M LiCl. The use of LiCl as the unconditioned stimulus standardized the degree of sickness experienced by the animals, as well as the interval between rodenticide ingestion and the onset of malaise.

Nine hundred and sixty adult (60 days old) male Sprague-Dawley Norway rats (272 ± 5 [x

\pm SD] g) were obtained in 4 cohorts ($n = 240$ /cohort), 1 cohort every 8 weeks for a period of 32 weeks. This large number of animals was actually the minimum number required to perform the generalization tests described below. The animals were individually caged (cage dimensions = $28 \times 20 \times 20$ cm) in a room with a 12:12 light:dark cycle and an ambient temperature of 23 ± 2 C. Free access to rodent chow (Wayne Lab Blox; Premier Laboratory Diets, Bartonville, Ill.) and water was provided, except as described below. At the conclusion of the experiments, rats either were euthanized by carbon dioxide inhalation or were donated to other research projects at the Monell Center.

For each rodenticide, a cohort of animals was adapted to a water deprivation regime that involved a 15-minute presentation of water during the first hour of light and a 30-minute presentation of water during the 10th hour of light (Mason et al. 1985). Water was presented in 30-mL syringes fitted with metal sipper tubes. After 2 weeks, the 240 animals were ranked according to mean water intake during the 15-minute periods, and then assigned to 48 groups ($n = 5$ /group) on the basis of water intake. The 48 groups were then paired (group 1 with group 2, group 3 with group 4, etc.) and within each pair, 1 group was randomly assigned to the experimental condition, and the other group was assigned as a matched control.

On the first day of conditioning, experimental groups were presented with an aqueous rodenticide solution (i.e., a conditioned stimulus) during the 15-minute morning period. Concurrently, control groups were presented with water. After at least 5 mL of fluid was consumed, or after 1 hour had passed, each animal was given an intraperitoneal (IP) injection of 0.15 M LiCl (100 mg/kg of body mass). On the day following conditioning, and during the light period on the next day, all animals were given free access to lab chow and water to facilitate recovery from conditioning. Water deprivation was begun again at dark onset of the second postconditioning day.

During the 15-minute drinking periods on each of the next 4 days, all animals were tested for generalization of FAL to 4 of 24 flavors (Table 1). These flavors and their concentrations were chosen on the basis of prior work (Nachman et al. 1979, Nowlis et al. 1980, Mason et al. 1985). Our hypotheses were (1) that similarities between a flavor and a rodenticide would

Table 1. Flavor stimuli used during generalization tests following flavor avoidance learning in Experiments 1 and 2. Chemical abbreviations below are used in the text.

Stimuli	Flavor ^a	Concentration
Ammonium chloride (NH ₄ Cl)	bitter-salty	0.3 M
Magnesium sulfate (MgSO ₄)	bitter	0.1 M
Acetic acid	sour	0.01 M
Sodium saccharin	sweet-bitter	0.04 M
Sodium saccharin	sweet-bitter	0.2 M
Urea	bitter	3.0 M
Citric acid	sour	0.003 M
Sodium nitrate (NaNO ₃)	salty-bitter	0.1 M
Fructose	sweet	0.3 M
Potassium chloride (KCl)	salty-bitter	0.3 M
Quinine hydrochloride (QHCl)	bitter	0.0001 M
Hydrochloric acid (HCl)	sour	0.01 M
Sucrose	sweet	0.1 M
Quassia bitters	bitter	0.2%
Gentain bitters	bitter	0.2%
Potassium nitrate (KNO ₃)	bitter	0.1 M
Sodium sulfate (Na ₂ SO ₄)	salty-bitter	0.2 M
Glucose	sweet	0.75 M
l-phenylalanine	bitter	0.15 M
Distilled water	weakly bitter	n/a
Ammonium carbonate (NH ₄) ₂ CO ₃	bitter-salty	0.1 M
Sucrose octaacetate (SOA)	bitter	0.001 M
Glycine	sweet	0.6 M
Sodium chloride (NaCl)	salty	0.1 M

^a Flavor attributes that humans provide. Where pairs of attributes are given, the stronger quality appears first.

be reflected by avoidance of the flavor, and (2) that the greater the avoidance response, the greater the perceived similarity. We based these assumptions on the results of an earlier experiment in which we used strychnine as the conditioned stimulus (Mason et al. 1985).

To encourage measurable consumption of all flavors, 1-bottle tests were used (Dragoin et al. 1971). To guard against extinction of FAL, the animals were given another pairing of rodenticide (experimental groups) or water (control groups) and LiCl on Day 5. After the 2-day rest period, animals were presented with another 4 of the 24 flavors. This cycle of conditioning and testing continued until each animal had been presented with all of the flavors. We used a Latin Square design to determine flavor presentations

among groups during the generalization tests. For each of 24 stimulus orders, 1 experimental and 1 control group were tested.

Drinking was restricted on conditioning days, but it appeared that experimental versus control group differences became larger over sessions. This possibility was assessed for each rodenticide in a 2-factor ANOVA with repeated measures over conditioning days (6 levels). We used Tukey Honestly Significant Difference (HSD) tests (Winer 1962:198) to identify significant differences among means ($P < 0.05$).

For each rodenticide, drinking during generalization tests was assessed in a 3-factor ANOVA with repeated measures over flavors (24 levels). The independent factors were (1) order of flavor presentation during generalization (24 levels), and (2) groups. Tukey HSD tests were used to identify significant differences among means ($P < 0.05$).

Experiment 2.—As in previous work (Mason et al. 1985), Experiment 1 was performed with rodenticides in aqueous solution. An unanswered question was whether similar results might be obtained if toxicants were presented in a solid matrix (i.e., 'food'). Experiment 2 was performed to investigate this issue.

Strychnine was selected as the conditioned stimulus (Stewart et al. 1983, Mason et al. 1985), and a cohort ($n = 120$) of 60-day-old male Sprague-Dawley rats (280 ± 6.0 g) served as subjects. Animals were randomly assigned to 2 subcohorts ($n = 60$ /subcohort) and were housed and maintained as previously described.

One subcohort was adapted to the water deprivation schedule described in Experiment 1. These animals were then assigned to 4 groups ($n = 15$ /group) on the basis of drinking during the 15-minute periods. The other subcohort was adapted to a 12-hour food deprivation schedule in which all food was removed from the cages at dark onset. During the hour following light onset, these animals were presented with 30 g of 5% agar (Sigma, St. Louis, Mo.), containing 1% powdered Wayne Lab Blox to encourage measurable consumption. Agar samples were presented in disposable plastic cups. After 1 hour, the cups were removed from the cages and weighed. Spillage collected on paper towels under the animals' cages was subtracted from the final mass of the agar in the cups. The remainder was taken as a measure of consumption, and this measure was used to assign animals to 4 groups

matched for agar consumption. After each session, the rats were then allowed free access to food (Lab Blox) and water until lights out.

On conditioning days, 1 experimental group was presented with strychnine (0.8 mg/g) in 5% powdered agar containing 1% powdered Wayne Lab Blox. The other experimental group was presented with strychnine (0.8 mg/mL) in aqueous solution. The 2 control groups were presented with 5% agar containing 1% powdered chow (1 group) or water (1 group) only.

After at least 5 mL of fluid or 2 g of agar were consumed, or after 1 hour had passed, all animals were given an IP injection of 0.15 M LiCl. On the day following conditioning and on the next day, all animals were given free access to food and water to facilitate recovery from conditioning. Food or water deprivation was reinstated at dark onset of the second postconditioning day.

During the first hour of light on each of the next 4 days, the animals were tested for generalization of strychnine avoidance to 4 of the 24 flavors presented in aqueous solution or in 5% agar in 1-bottle or 1-cup tests. The concentrations of each of the flavors in agar were identical to those used in fluid tests (Table 1). On the fifth postconditioning day, experimental animals were given another pairing of 0.0002 M strychnine (in agar or solution) and an IP injection of 0.15 M LiCl. Control animals received a pairing of plain agar or water and LiCl injection. After a 2-day rest period, the animals were presented with another 4 of the 24 flavors in their respective medium. These procedures were repeated until each animal had been presented with all 24 flavors.

We assessed conditioning and generalization test consumption in 2 ways. Within cohorts (i.e., within water or agar), we performed 2-factor ANOVA's with repeated measures over sessions (i.e., conditioning days or generalization tests). Between cohorts (i.e., between water vs. agar) we used 2-factor ANOVA's to assess preference ratios, calculated by dividing experimental consumption by total (experimental and control) consumption within each cohort. The use of preference ratios placed both feeding and drinking scores in the same metric, a series ranging from 0.0 (absolute rejection of a flavor by experimental animals relative to control animals) to 1.0 (absolute preference for a flavor by experimental animals relative to control animals).

Tukey HSD tests were used to identify significant differences among means ($P < 0.05$).

Experiment 3.—Generalization test results in Experiments 1 and 2 suggested flavor similarities among rodenticides. Experiment 3 was performed to assess whether these inferred similarities could be used to predict avoidance generalization from 1 rodenticide to the others. Alpha-chlorohydrin, alpha-naphthylthiourea, calciferol, strychnine, and Na warfarin were used at the concentrations presented to animals in Experiment 1.

Five hundred 60-day-old male Sprague-Dawley rats (271 ± 10 g) were housed and maintained as previously described. The animals were obtained in 5 cohorts ($n = 100$ /cohort) over a period of 15 weeks.

Five separate assessments were performed (each with a different cohort) to directly assess flavor similarities among rodenticides. For each assessment, a different rodenticide served as the conditioned stimulus. Each cohort was adapted to the water deprivation schedule and assigned to 10 groups ($n = 10$ /group, 5 experimental and 5 control rats) on the basis of consumption. On each of 2 successive conditioning days, we presented each of the 5 experimental groups with a rodenticide in aqueous solution. Control groups were presented with distilled water only. After drinking, all animals were given IP injections of 0.15 M LiCl. Two conditioning trials (rather than 1) were used to assure FAL. After 2 days free access to food and water, the animals were again deprived of water, and different groups were exposed to each of the 5 rodenticides (1 for each experimental and control group pair) in a single 1-bottle test.

We evaluated the results of each rodenticide assessment in a 2-way ANOVA with independent measures on both factors (groups, rodenticides). Tukey HSD tests were used to identify significant differences among means ($P < 0.05$).

RESULTS

Experiment 1

Conditioning.—For all rodenticides, the interaction between groups and sessions was significant (Figs. 1–4). Overall, experimental groups showed significant decreases in consumption among conditioning sessions and drank less than animals in the control groups.

Generalization.—For all rodenticides, there

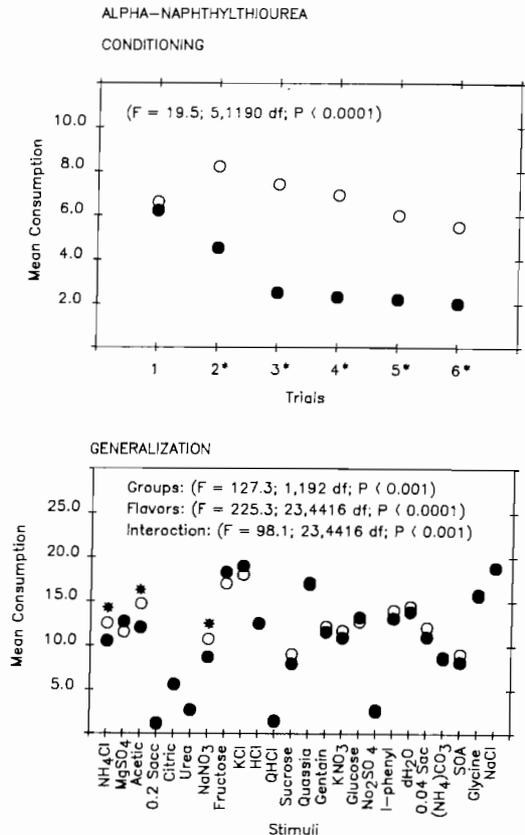
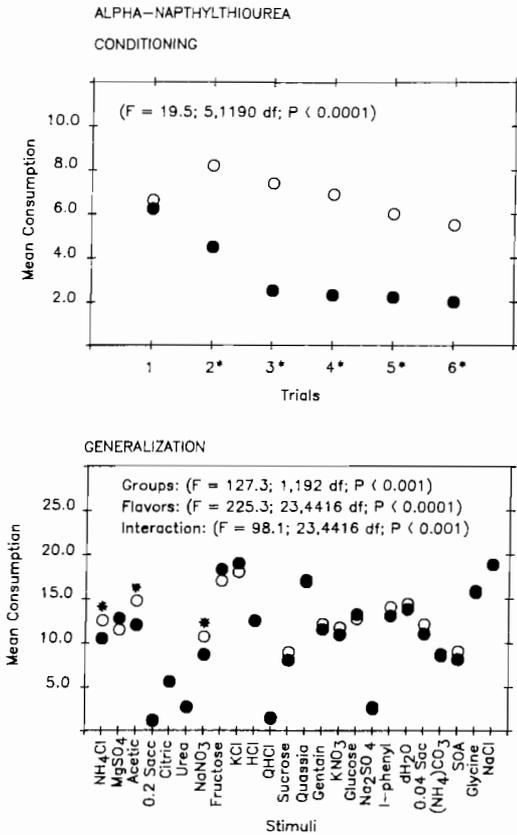


Fig. 1 (Top) Mean consumption (mL) of alpha-chlorohydrin (filled circles) or water (open circles) in conditioning trials (Exp. 1). The significant group × trial interaction term (indicating a difference between groups over trials) appears in the upper left corner of the panel. (Bottom) Mean consumption (mL) of flavor (filled circles) or water (open circles) in alpha-chlorohydrin generalization trials. Significant main effects for group and flavor, and significant group × flavor interactions, appear in the upper portion of the panel. Asterisks indicate significant group differences (P < 0.05).

Fig. 2. (Top) Mean consumption (mL) of alpha-naphthylthiourea (filled circles) or water (open circles) in conditioning trials (Exp. 1). The significant group × trial interaction term (indicating a difference between groups over trials) appears in the upper left corner of the panel. (Bottom) Mean consumption (mL) of flavor (filled circles) or water (open circles) in alpha-naphthylthiourea generalization trials. Significant main effects for group and flavor, and significant group × flavor interactions, appear in the upper portion of the panel. Asterisks indicate significant group differences (P < 0.05).

were significant differences among groups and flavors, and significant interactions between these terms. In each case, experimental animals drank less than control animals. Post hoc analyses of flavor effects and group by flavor interactions are provided below.

Alpha-chlorohydrin.—Sodium chloride (NaCl), sodium nitrate (NaNO₃), and fructose were consumed in larger quantities, and 0.2 M saccharin, urea, quinine hydrochloride (QHCl), and sodium sulfate (Na₂SO₄) in smaller amounts, than any of the other flavors. Post hoc examination of the interaction between groups and flavors revealed that, relative to control consumption, experimental groups avoided consumption of 0.3 M NH₄Cl, 0.3 M KCl, and 0.04

M saccharin (Fig. 1). Group differences were greatest for NH₄Cl, although in all cases, the differences, albeit significant, were small.

Alpha-naphthylthiourea.—NaCl, fructose, NaNO₃, and sucrose were consumed in larger quantities, and 0.2 M saccharin, urea, QHCl, and Na₂SO₄ in smaller quantities than any of the other flavors. Post hoc examination of the interaction between groups and flavors showed that, relative to control groups, experimental groups avoided 0.3 M NH₄Cl, 0.01 M acetic acid, and 0.1 M NaNO₃ (Fig. 2).

Calciferol.—NaCl, sucrose, fructose, and NaNO₃ were consumed in larger quantities, and 0.2 M saccharin, urea, QHCl, and Na₂SO₄ in smaller amounts than any of the other flavors.

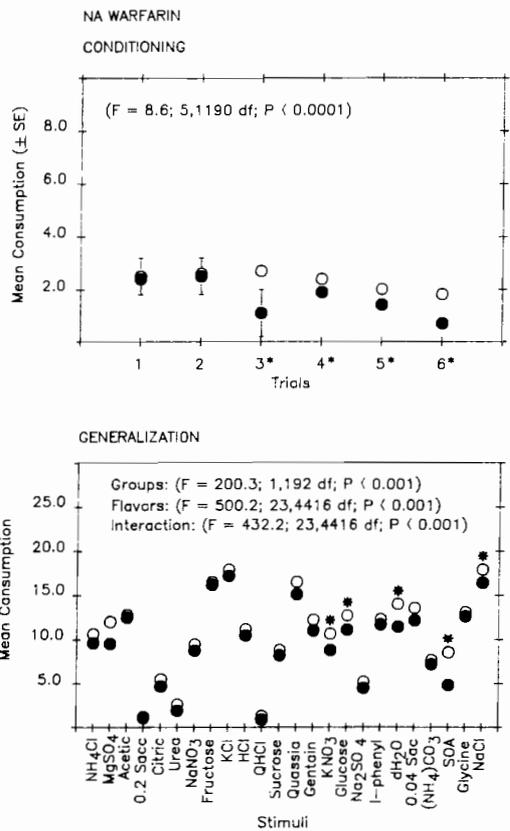
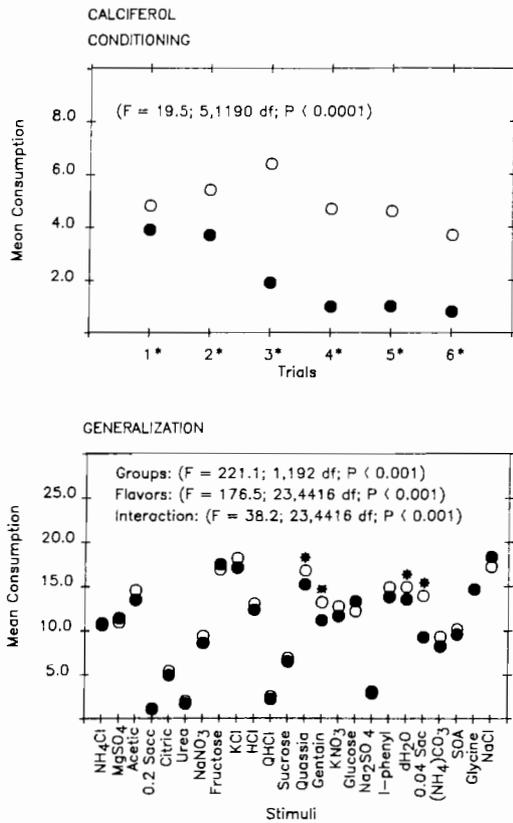


Fig. 3. (Top) Mean consumption (mL) of calciferol (filled circles) or water (open circles) in conditioning trials (Exp. 1). The significant group × trial interaction term (indicating a difference between groups over trials) appears in the upper left corner of the panel. (Bottom) Mean consumption (mL) of flavor (filled circles) or water (open circles) in calciferol generalization trials. Significant main effects for group and flavor, and significant group × flavor interactions, appear in the upper portion of the panel. Asterisks indicate significant group differences (P < 0.05).

Fig. 4. (Top) Mean consumption (mL) of Na warfarin (filled circles) or water (open circles) in conditioning trials (Exp. 1). The significant group × trial interaction term (indicating a difference between groups over trials) appears in the upper left corner of the panel. (Bottom) Mean consumption (mL) of flavor (filled circles) or water (open circles) in Na warfarin generalization trials. Significant main effects for group and flavor, and significant group × flavor interactions, appear in the upper portion of the panel. Asterisks indicate significant group differences (P < 0.05).

Post hoc examination of the interaction between groups and flavors showed that, relative to control groups, experimental groups avoided 0.2% quassia, 0.2% gentain, distilled water, and 0.04 M saccharin (Fig. 3). Of these stimuli, the strongest avoidance was toward 0.04 M saccharin, and the weakest, toward distilled water.

Na Warfarin.—Fructose, sucrose, NaCl, and NaNO₃ were consumed in larger quantities, and 0.2 M saccharin and QHCl in smaller amounts than any of the other flavors. Examination of the interaction between groups and flavors showed that, relative to control groups, experimental groups drank less 0.1 M MgSO₄, 0.1 M KNO₃, 0.75 M glucose, distilled water, 0.001 M SOA, and 0.1 M NaCl (Fig. 4). The greatest difference was exhibited toward SOA.

Experiment 2

Within Subcohorts: Conditioning Trials.—For the water subcohort, experimental animals drank less than control animals on all but the first conditioning day (Fig. 5). Whereas both experimental and control animals drank less on successive conditioning days, decreases exhibited by experimental animals were larger than decreases exhibited by control animals.

Conditioning trial results for the agar subcohort were similar to those described for the water subcohort. Although there were no overall differences between groups, experimental animals ate relatively less than control animals during sessions 3–6. Also, consumption by the experimental group decreased across sessions,

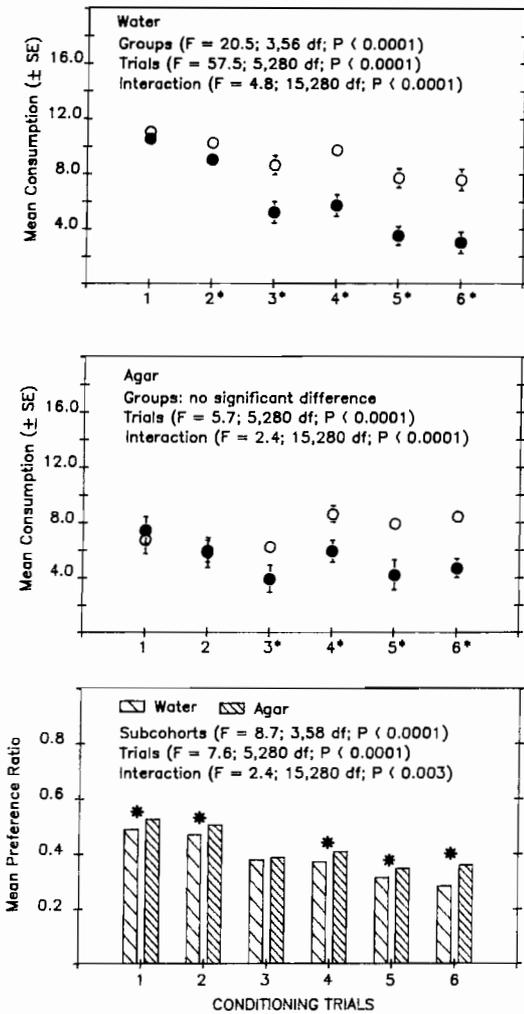


Fig. 5 (Top, middle) Mean consumption (mL, g) of strychnine (filled circles) or control substances (water or agar, open circles) in Experiment 2 conditioning trials. Significant main effects for group and trial, and significant group × trial interactions, appear in the upper portion of each panel. (Bottom) Preference ratios calculated by dividing mean experimental consumption by total (exp. and control) consumption on each conditioning day. Capped vertical bars represent standard errors of means; some of these are small and fall within data points.

whereas control consumption remained relatively constant.

Between Subcohorts: Conditioning Trials.— Preference ratios for animals in the water subcohort were significantly, albeit slightly, lower than those of animals in the agar subcohort.

Within Subcohorts: Generalization Trials.— Analysis of consumption by the water subcohort revealed a pattern of results similar to that reported previously for generalization of strychnine FAL (Mason et al. 1985). There were no overall differences in drinking between exper-

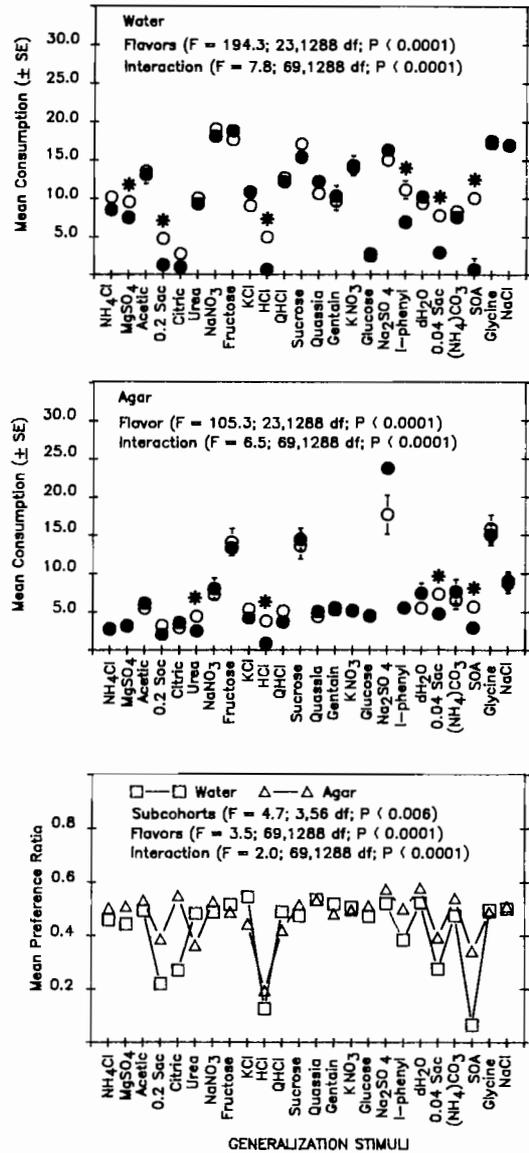


Fig. 6. (Top, middle) Mean consumption (mL, g) of flavors by animals given strychnine in water or agar (filled circles) or control substances (water or agar, open circles) in Experiment 2 generalization trials. Asterisks indicate significant group differences. (Bottom) Preference ratios calculated by dividing mean experimental consumption by total (exp. and control) consumption of each flavor, presented in water or agar. Significant main effects for group and flavor, and significant interactions between groups and flavors are given in the upper portion of each panel. Capped vertical bars represent standard errors of means; many of these are small and fall within data points.

imental and control groups. Among flavors, drinking of fructose, sucrose, glucose, NaCl, NaNO₃, and glycine was significantly higher than drinking of the other flavors. Examination of the significant interaction between groups and flavors revealed that experimental animals drank

less 0.1 M $MgSO_4$, 0.2 M or 0.04 M saccharin, 0.01 M HCl, 0.15 M l-phenylalanine, and 0.001 M SOA than did the control animals (Fig. 6).

Analysis of consumption by the agar subcohort revealed no overall differences between experimental and control groups. Among flavors, consumption of fructose, sucrose, glucose, and glycine was significantly higher than that of the other flavors. Examination of the significant interaction between groups and flavors revealed that the experimental animals ate significantly less agar flavored with 0.04 M saccharin, 0.01 M HCl, 3.0 M urea, or SOA than did the control animals.

Between Water and Agar Subcohorts: Generalization Trials.—Overall, the water subcohort had significantly lower preference scores (i.e., showed significantly stronger avoidance generalization) than the agar subcohort. Among flavors, preference ratios for 0.2 M and 0.04 M saccharin, QHCl, and SOA were significantly lower than those for the other flavors. Examination of the significant interaction term showed that although the water subcohort showed stronger avoidance of 0.2 and 0.04 M saccharin, urea, l-phenylalanine, and SOA than the agar subcohort, the agar subcohort showed relatively stronger avoidance of citric acid and HCl.

Experiment 3

Alpha-chlorohydrin.—Experimental animals drank significantly less than control animals, and among rodenticides, consumption of strychnine was significantly less than that of the other stimuli. Examination of the significant interaction between groups and rodenticides indicated that both experimental and control groups drank less when strychnine was presented; calciferol and alpha-chlorohydrin depressed drinking by the experimental group only (Fig. 7).

Alpha-naphthylthiourea.—Experimental animals drank less than control animals. Among rodenticides, consumption of strychnine was significantly less than consumption of the other stimuli. Examination of the significant interaction between groups and rodenticides showed that experimental animals drank less alpha-naphthylthiourea and calciferol than did control animals.

Calciferol.—There were no overall differences between groups. However, significantly less strychnine and calciferol were consumed than alpha-chlorohydrin, alpha-naphthylthiourea, and warfarin. Examination of the significant interaction between groups and rodenti-

cides showed that experimental animals drank less calciferol than did control animals.

Strychnine.—Experimental animals drank less of all 5 rodenticides than did control animals. Otherwise there were no significant differences.

Na Warfarin.—There were no significant differences between experimental and control groups.

DISCUSSION

The results of Experiment 1 are consistent with the notion that rats are capable of discriminating flavor components in alpha-chlorohydrin, alpha-naphthylthiourea, calciferol, and Na warfarin. In each instance, experimental animals exhibited avoidance toward rodenticide during conditioning, and avoidance generalization to a subset of the 24 flavors during testing.

Unexpectedly, FAL generalization was broadest for Na warfarin, even though conditioning was weak relative to the other rodenticides. If this generalization profile accurately reflects the perceived flavor of Na warfarin to rats, then this substance might be characterized anthropocentrically as having "bitter," "sweet," and "salty" qualities. Although additional experimentation is warranted to confirm this interpretation, the possibility that warfarin has a complex flavor contrasts with anecdotal and published (Thomson 1989:152) statements that warfarin is "tasteless." Perhaps, the assumption that warfarin lacks a distinctive flavor reflects a lack of FAL caused by the delay between ingestion of warfarin and onset of malaise (≥ 24 hr) under practical conditions. In classical FAL, long delays between flavor ingestion and malaise are usually associated with weak FAL (Andrews and Braveman 1975). In Experiment 1, ingestion of warfarin was immediately followed by LiCl-induced malaise, thus maximizing the likelihood of taste-sickness association.

Avoidance of calciferol generalized to quassia, gentain, distilled water, and saccharin. Because distilled water is characterized as weakly bitter by humans (Bartoshuk 1968), a possibility is that it may also be weakly bitter to rats. Accordingly, we infer that calciferol has both "bitter" (quassia, gentain, distilled water) and "sweet" (saccharin) qualities.

Avoidance of alpha-naphthylthiourea generalized to NH_4Cl , acetic acid, and $NaNO_3$. Thus, alpha-naphthylthiourea might be characterized as "salty-bitter" and "sour." This finding is consistent with reports that alpha-naphthylthiourea is avoided in 2-bottle tests (Kusano et al. 1975b).

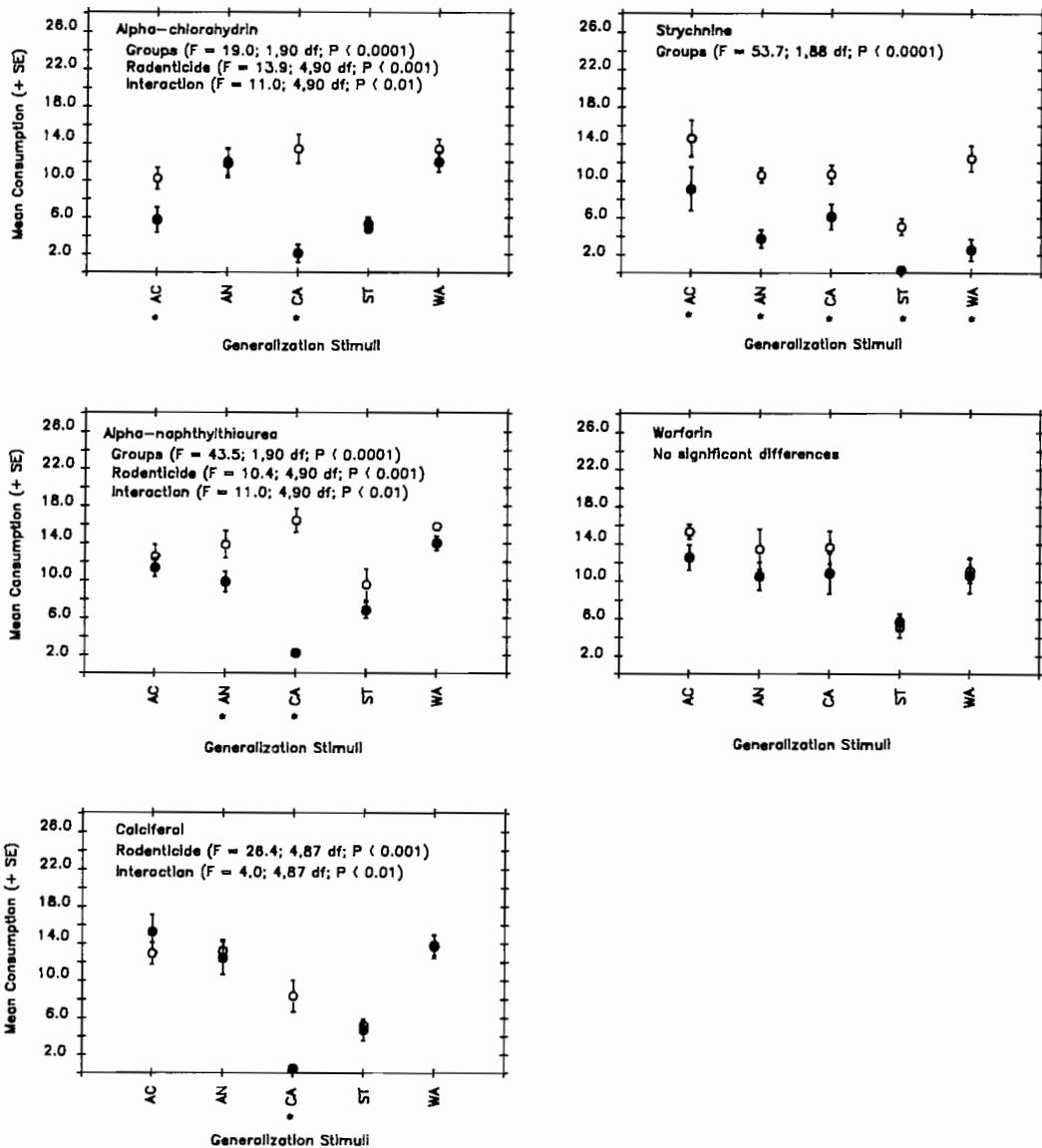


Fig. 7. Mean drinking of rodenticide (filled circles) or water (open circles) in Experiment 3 generalization tests. Significant main effects for group and rodenticide, and significant interactions between groups and rodenticides are given in the upper left portion of each panel. Capped vertical bars represent standard errors of means; a few of these are small and fall within data points. Asterisks indicate significant group differences ($P < 0.05$).

Although “sour” tastes, per se, are not particularly aversive to rats, adaptation to sour may cause plain water to taste sweet, as it does for humans (Bartoshuk 1968). Rats may not have rejected alpha-naphthylthiourea in 2-bottle tests because of its perceived flavor; rather, they may be showing preferences for sweet (i.e., plain) water. If such sweet-sour interactions occur for rats, then the present results clarify the findings of Kusano et al. (1975b), who reported that al-

pha-naphthylthiourea became more objectionable to rats when mixed with an 8.0% (mass/vol) aqueous sucrose solution. For humans, sour is enhanced by sucrose when tastants are mixed (Bartoshuk 1972).

Finally, avoidance of alpha-chlorohydrin generalized to NH_4Cl , KCl , and saccharin. Hence, the flavor might be characterized as having both “salty-bitter” and “sweet” components.

Overall, the flavor characteristics of the 4 ro-

denticides were primarily "bitter" and "salty," although calciferol, Na warfarin, and alpha-chlorohydrin apparently possessed "sweet" notes, whereas alpha-naphthylthiourea was at least partly "sour." Further, in all Experiment 1 generalization tests, relatively large amounts of KCl, NaCl, and sugar (e.g., sucrose, fructose) were consumed. Although high consumption of sweet solutions or NaCl solution is not surprising, the apparent preference for KCl was unexpected, as this substance is both bitter and salty to humans. These results can be taken as evidence against the simplistic assumption that humans and rats perceive flavors similarly, and thus that the unpalatable characteristics of toxicants (anthropocentrically described as bitter) can be masked by the addition of substances that decrease the bitterness of flavors for humans, such as sugar (e.g., Kusano et al. 1975b) or sodium bicarbonate (Stewart et al. 1983). Nevertheless, Experiment 1 only indicates the flavor qualities associated with each toxicant at the concentration tested. Flavor intensities remain unknown. Generalization was never to all of the flavors within a qualitative group. Thus, alpha-chlorohydrin FAL generalized to NH_4Cl , a "bitter" stimulus, but not to other bitter stimuli, such as urea, SOA, or QHCl. This selectivity could reflect differences in the quality of bitter stimuli but more likely, selectivity reflects differences in the perceived intensity of bitterness associated with the generalization flavors. In addition, because the strength of FAL depends on the concentration of the conditioned stimulus (Riley and Clarke 1977) and because flavor quality (for humans) changes with changing concentration, it is possible that different flavor profiles would be obtained if different rodenticide concentrations were used.

In Experiment 2, generalization of strychnine FAL in feeding was similar to, but not identical with, avoidance generalization expressed in drinking. Relative to animals in the agar cohort, animals in the water cohort showed earlier and more robust FAL during conditioning and slightly broader generalization during test sessions. We speculate that these dissimilarities could reflect weakened flavor intensities in the feeding context. Conditioned stimulus intensity affects the strength of the avoidance response (e.g., Bartoshuk 1972), and the perception of strychnine and/or of the generalization flavors might be obscured by the flavor of the 1% rat chow in the agar matrix. Flavors in a solid matrix might simply be less accessible to receptors

than are flavors in aqueous solution. Regardless, the principal flavor of strychnine in both contexts was "bitter," and the results are consistent with our view that FAL can be used to profile the flavor of rodenticides in food or fluid, although flavor profiles generated in 1 context may not apply to the other.

The results of Experiment 3 were not consistent with what might have been predicted on the basis of FAL generalization in Experiments 1 and 2. Indeed, the only relatively accurate prediction was for strychnine, in which generalization of FAL was observed toward all of the other rodenticides (all had "bitter" qualities), with the weakest generalization expressed toward alpha-chlorohydrin. As suggested in our discussion of Experiment 1, an explanation for these failures in prediction is that the predictions were based on flavor quality only, without consideration of perceived flavor intensities. Another explanation may be that weak FAL occurred in one or several of the assessments in Experiment 3, and that this, in turn, affected the strength of generalization among rodenticides (Barker 1976). Additional experiments, perhaps with >2 conditioning trials and/or test sessions, will be necessary to determine whether flavor profiles of individual rodenticides can be used to predict similarities among toxicants.

MANAGEMENT IMPLICATIONS

The flavor profiles generated in Experiment 1 might prove useful for the development of simple flavor mimics and masking agents. In a previous experiment (Mason et al. 1985), we reported that 0.0002 M strychnine (the concentration used in Exp. 2) was primarily "bitter" to rats, insofar as FAL generalized most readily to 0.001 M SOA, and about half as strongly to 1.5 M urea. When these substances were mixed in a 2:1 ratio, a successful flavor mimic of strychnine was obtained (i.e., learned avoidance of the nontoxic mixture generalized strongly to strychnine). Similar relationships may occur with the rodenticides tested in the present experiment, although the tastes of these compounds appeared to be relatively more complex than that of strychnine. Also, because each of the rodenticides in Experiment 1 had "bitter" components and because there is evidence to support the contention that NaCl reliably masks "bitter" perception (Mason et al. 1985), NaCl might effectively mask bitterness associated with some or all of these substances. Finally, Experiment 1 provided some evidence that NaNO_3 was at-

tractive. The possibility exists that this substance might enhance the palatability of bait.

Regarding Experiment 2, whereas FAL can be used to generate flavor profiles in both feeding and drinking contexts, it is clear that results collected in 1 context may not be transferable to the other. Nonetheless, we speculate that FAL set in a feeding context could be used to evaluate the flavor of rodenticides in solid bait formulations, as well as the flavor characteristics of commercial bait formulations (without incorporated rodenticides). The data also provide some evidence that glycine may enhance the palatability of bait, insofar as consumption of this flavor was high in both feeding and drinking contexts. Although consumption of glycine was not significantly higher than consumption of other flavors in Experiment 1, the mean consumption of glycine was fifth highest during calciferol and alpha-naphthylthiourea generalization tests, and was among those flavors eliciting the greatest consumption by both experimental and control animals in alpha-chlorohydrin and Na warfarin tests.

LITERATURE CITED

- ANDREWS, E. A., AND N. S. BRAVEMAN. 1975. The combined effects of dosage level and interstimulus interval on the formation of one-trial poison-based aversion in rats. *Anim. Learning Behav.* 3: 287-289.
- BARKER, L. M. 1976. CS duration, amount and concentration effects in conditioned taste aversions. *Learning Motivation* 7:265-273.
- BARTOSHUK, L. M. 1968. Water taste in man. *Perception Psychophysics* 3:184-186.
- . 1972. The chemical senses I: taste. Pages 169-190 in J. W. Kling and L. A. Riggs, eds. *Experimental psychology*. Holt, Rinehart and Winston, New York, N.Y.
- DRAGOIN, W., G. E. MCCLEARY, AND P. MCCLEARY. 1971. A comparison of two methods of measuring conditioned taste aversions. *Behav. Res. Methods and Instrumentation* 3:309-310.
- DUVILLARD, X., H. DUGAS, AND J. N. BROUWER. 1980. Enhancement of the perceived sweetness of sucrose in the rat by thaumatin. *Chem. Senses* 5:93-98.
- ERICKSON, R. P. 1963. Sensory neural patterns and gustation. Pages 205-213 in Y. Zotterman, ed. *Olfaction and taste I*. Pergamon Press, New York, N.Y.
- KUSANO, T., Y. KASAHARA, AND Y. KAWAMURA. 1975a. Gustatory effect of rodenticides and repellents in rats. *Food and Fert. Technol. Cent. Tech. Bull.* 47:1-25.
- , ———, AND ———. 1975b. Utilizing sugars to improve the taste-effectiveness of thiourea derivatives for use as rodenticides. *Appl. Entomol. Zool.* 10:19-29.
- MASON, J. R., R. F. REIDINGER, AND C. N. STEWART. 1985. Profiling, mimicking and masking the flavor of a selected rodenticide. *Physiol. Behav.* 35: 127-134.
- MORRISON, M. 1967. Behavioral response patterns to salt stimuli in the rat. *Can. J. Psychol.* 21:141-152.
- NACHMAN, M. 1963. Learned aversion to the taste of lithium chloride and generalization to other salts. *J. Comp. Physiol. Psychol.* 56:343-349.
- , J. RAUSCHENBERGER, AND J. H. ASHE. 1979. Stimulus characteristics in food aversion learning. Pages 105-131 in N. W. Milgram, L. Krames, and T. M. Alloway, eds. *Food aversion learning*. Plenum Press, New York, N.Y.
- NOWLIS, G., AND M. FRANK. 1977. Qualities in hamster taste: behavioral and neural evidence. Pages 241-248 in J. LeMagnen and P. MacLeod, eds. *Olfaction and taste VI*. Information Retrieval Inc., Washington, D.C.
- , ———, AND C. PFAFFMAN. 1980. Specificity of acquired aversions to taste qualities in hamsters and rats. *J. Comp. Physiol. Psychol.* 94: 932-942.
- REIDINGER, R. F., AND J. R. MASON. 1983. Exploitable characteristics of neophobia and food aversions for improvements in rodent and bird control. Pages 20-42 in D. E. Kaukiainen, ed. *Vertebrate pest control and management materials: 4th symposium*. Am. Soc. for Testing and Materials, Philadelphia, Pa.
- RILEY, A. L., AND C. M. CLARKE. 1977. Conditioned taste aversions: a bibliography. Pages 593-632 in L. M. Barker, M. R. Best, and M. Domjan, eds. *Learning mechanisms in food selection*. Baylor Univ. Press, Waco, Tex.
- ROBBINS, R. J. 1980. Taste aversion learning and its implications for rodent control. Pages 114-120 in *Proc. of the 9th vertebrate pest conference*. Univ. California Press, Davis.
- SMITH, D. V., AND R. M. THEODORE. 1984. Conditioned taste aversion: generalization to taste mixtures. *Physiol. Behav.* 32:983-989.
- STEWART, C. N., R. F. REIDINGER, AND J. R. MASON. 1983. Method for inferring taste qualities of rodenticides to rodents. Pages 155-165 in D. E. Kaukiainen, ed. *Vertebrate pest control and management materials: 4th symposium*. Am. Soc. for Testing and Materials, Philadelphia, Pa.
- THOMSON, W. T. 1989. *Agricultural chemicals book III: miscellaneous chemicals*. Thomson Publ., Fresno, Calif. 210pp.
- WIGGINS, L. L., R. A. FRANK, AND D. V. SMITH. 1989. Generalization of learned taste aversions in rabbits: similarities among gustatory stimuli. *Chem. Senses* 14:103-119.
- WINER, B. J. 1962. *Statistical principles in experimental design*. McGraw-Hill Book Co., New York, N.Y. 907pp.

Received 26 January 1990.

Accepted 6 September 1990.

Associate Editor: DeYoung.