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A Review of the Bird Repellent Effects of 117 Carbocyclic Compounds

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ABSTRACT

We evaluated 117 carbocyclic compounds for their bird repellent effects in no-choice (one-bottle) drinking tests and summarized the results in this paper. Compounds derivative of aromatic heterocycles, acetophenones and carbocyclic compounds containing sulfur are often strong repellents. Anthranilates, aromatic alcohols, aromatic aldehydes, and carbocyclic compounds containing nitrogen are moderately strong repellents. However, the potency of anthranilates is highly variable, depending upon the nature of the substitutions. Acetates and benzoates are weak repellents. Benzoic acids and amino acids are not repellent to starlings. In addition, discussions of the type of behavioral assay, units of measure, and dose-response characterizations include recommendations for future studies of repellents. Convergence on the types of information reported and the details of experiments will enhance our ability to compare the efficacy of repellents across studies.

KEY WORDS

bird repellents, trigeminal repellents, anthranilates, acetophenones, benzoic acid, capsaicin, aldehydes, acetates, starlings

INTRODUCTION

Identification of chemical repellents that are effective, economical, and environmentally safe is an area of active research in the areas of animal damage control and wildlife conservation (Mason and Clark 1992). However, investigators are often hampered in their evaluations of chemical repellents because studies on even single compounds frequently result in conflicting levels of reported efficacy. In part, the apparent contradictions are due to the different behavioral assays employed, the various measures of repellency used, and the different means of stimulus presentation, delivery, and verification of active ingredients.

Over the past several years, my laboratory has amassed a comprehensive database on the

efficacy of carbocyclic compounds as part of a larger research program in the study of structure-activity relationships of avian repellents. The first objective of this paper is to summarize the performance of carbocyclic compounds as bird repellents based on one-bottle drinking assays. The second objective of this paper is to emphasize the need for standardization in reporting the results of studies on repellents. To this end, the methods section is more reflective than normal, dealing in more detail on the rationale for reporting results according to specific minimum standards.

METHODS

Suggestions for Uniform Methods and Standards for Comparative Studies

Types of Laboratory Behavioral Assays

A standard means to evaluate repellency is to monitor water (or food) intake in a timed one- or two-bottle (or cup) test. Each type of test will yield slightly different results. For example, at equimolar concentrations, animals are more sensitive to repellents delivered in solution relative to delivery in solids (c.f., Clark and Shah 1991, Mason et al. 1991). This differential responsiveness primarily is attributable to molecules having greater access to receptors when in solution.

Animals are more sensitive to concentration effects in two-choice versus one-choice tests, especially under experimental conditions where intake of test material is maximized, i.e., conditions of mild food or water deprivation. However, the advantages of one-bottle (cup) tests are that they are simple to administer and they minimize side-bias effects within a cage. Moreover, one-bottle tests serve as a conservative index of a repellent's potency. During a one-bottle test, an animal is forced to drink fluid or undergo water deprivation. Because there is a tradeoff between an animal's thirst or hunger state and the threshold for palatability of a repellent, longer experiments will tend to yield even more conservative estimates of a repellent's potency. Generally, if a compound is repellent in a one-bottle test, it will also be repellent in a two-choice test situation and in the field. In contrast, while one bottle (cup) tests may overlook marginally effective repellents, two-bottle (cup) tests are likely to detect weak repellents. Minor levels of discomfort can be avoided because the animal has an alternative to satiate its hunger or thirst. Two-bottle (cup) tests may accurately gauge the intrinsic "unpalatability" of a compound while controlling for thirst or hunger levels, but are not good measures of a repellent's potency, i.e., its ability to suppress intake of treated material under the most challenging conditions (e.g., food or water deprivation, no-choice feeding or drinking situations).

The Dose-Response Curve

Irrespective of the behavioral assay used to evaluate repellency, it is important to accurately characterize the avoidance response. The dose-response relationship allows comparison of active agents across studies and should be incorporated into all studies for each new active ingredient,

regardless of the nature of the experiments.

Even when other factors are controlled, the repellent effect of a compound normally varies as a function of its concentration. This functional response is an interaction between the chemical properties of the active ingredient and the physiological and cognitive properties of the animal. Typically, the dose-response is a negative logistic function of compound concentration (Figures 1a-c). During pilot range-finding studies, it is important to find the concentration for which the active agent saturates the avoidance response. This is the range of concentrations where the avoidance response asymptotically converges on the maximum repellent effect (the asymptotic minimum). It is also important to find the concentrations of active agents that result in responses that are indistinguishable from the negative control. The negative control is the presentation of the carrier solution or matrix. Between these two concentrations, there should be a sufficient number of tests to adequately describe the doseresponse transition state. Generally, I have found that six test concentrations, at half-log intervals (including the concentrations encompassing the minimum and maximum responses), are sufficient to yield a robust description of the dose-response.

It is important for the mean response for each concentration, along with its standard error and sample size, to be reported in tabular form. In this way other investigators may extract the most information from a study. In the absence of tabular information, a description of the formula used to characterize the dose-response, along with the parameter values and their standard errors, should be reported.

Comparison Among Compounds: Operational Definitions of Potency for Repellents

Where the response varies as a function of the concentration of the active agent, I have found a nonlinear, four-parameter logistic equation to be a useful numerical characterization of the dose-response. The logistic equation takes the form, $R = (a-d) / [1 + (x/c)^b] + d$, where a is the asymptotic maximum consumption, b is the slope, c is the inflection, d is the asymptotic minimum consumption and x is the concentration. Solving for the parameter values is now routine with the use of most statistical packages for the personal computer. In solving for the parameter values, a constraint of $d > 0$ must be imposed, because negative consumption is not possible.

The different ways to estimate the potency of a repellent are apparent from the interpretation of the parameter values. The slope is a measure of sensitivity to changes in repellent concentration (Figure 1a). For example, at the extremes, large values for the slope indicate an "all or none" threshold response. That is to say, birds may be insensitive (tolerant?) to a repellent up to a critical threshold concentration, after which repellent intake is maximally suppressed. Small values for slope indicate a graded threshold response, i.e., the suppression of intake gradually changes as the concentration of the repellent changes. Repellents with steep slopes (step-functions) provide unambiguous signals for efficacy. An animal's intake of treated material is suppressed (or not) to criterion level. A second measure of sensitivity is the displacement of the dose-response curve along the concentration axis as estimated by the inflection point (Figure 1b). Small inflection values (leftward shift of the curve) indicate heightened responsiveness to chemical concentrations. Large inflection values (rightward shift of the curve) indicate diminished responsiveness to the chemical. A third measure of sensitivity is the maximum suppression of intake as estimated by the

minimum asymptotic consumption (Figure 1c). Suppression is self-explanatory and is usually the descriptor commonly associated with the term "repellency."

Relying on a single measure to categorize potency of a repellent gives only partial information about the effect of a repellent. The problem can be illustrated as follows: in toxicological studies, inflection is commonly used as a measure of activity. Reliance on this single measure of activity is possible because the asymptotes of the dose-response curve zero and one. However, this represents only the boundary conditions of repellent studies. In many cases, the minimum asymptote does not reach zero relative consumption.

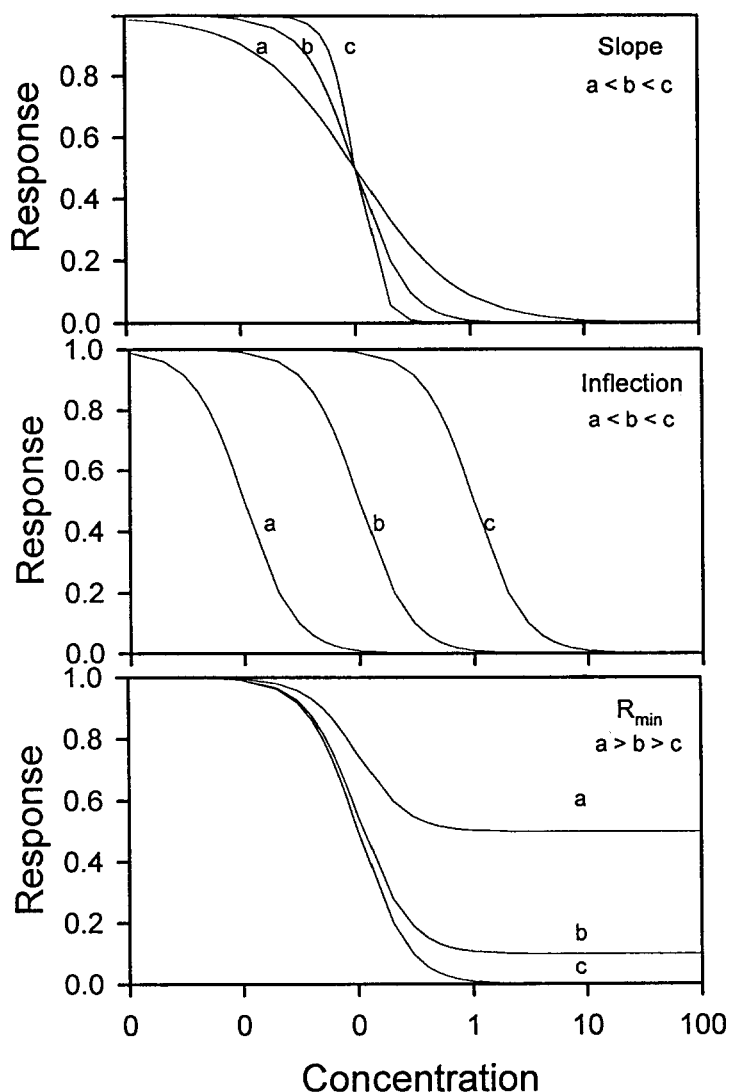


FIGURE 1.

[a] The effect of changing the slope on relative intake as a function of stimulus concentration for a four-parameter logistic dose-response curve. [b] The effect of changing the inflection of stimulus concentration for a four-parameter logistic dose-response curve. [c] The effect of changing the minimum asymptote intake as a function of stimulus concentration for a four-parameter logistic dose-response curve.

Therefore, reliance on inflection as a metric of repellency can prove misleading. For example, compounds illustrated in Figure 1c have identical inflection points. On this basis, one would conclude that the compounds have similar potency. However, inspection of the minimum asymptote shows the compounds to differ for maximum suppression effects. Similar trends are observed if slopes differ (Figure 1a). Thus, the question becomes: which of the indices is the most appropriate index of repellent potency? From a practical standpoint, this question is easily solved. The most potent repellent, in terms of performance of active agent alone, is the one that yields a critical performance level (avoidance response) at the lowest concentration. Thus, repellency should be described using two parameters, i.e., the inflection point and the maximum repellent effect. At least for carbocyclic compounds, slope is of less importance as a discriminating descriptor of performance (Clark and Shah 1994).

Units of Measure

Implicit in a comparative study of dose-response relationships is the selection of an appropriate measure for concentration. Measures commonly reported are percentage, ppm, or total mass or volume of material delivered to the animal relative to the mass or volume of vehicle, i.e., (g/g), (g/ml), or (ml/ml). Because the potential driving the avoidance response is the number of molecules accessing the receptors, the most accurate measure of concentration is molarity. At the very least, information necessary to calculate molarity of test substances should be provided, e.g., solubility, molecular weight, and the concentration metrics used. In addition, the Chemical Abstract Service (CAS) registry number and a statement on how concentration of test samples were verified should be reported. In the case of natural product extracts, reporting of concentrations as a percentage is the only viable option.

Experimental Design Used in One-Bottle Drinking Assays of Carbocyclic Repellents

After capture and adaptation to laboratory conditions starlings (*Sturnus vulgaris*) were tested in a standard one-bottle, 6-hr assay. On the first day (the pretest period), starlings were randomly assigned to groups and presented with tap water in graduated Richter (drinking) tubes. Fluid consumption was recorded every 2 hr for three recording periods. At the end of the 6 hr, the Richter tubes were replaced with a second set of tubes, and water was available ad libitum until the start of the test period the next day. As a precondition for further testing, similarity for group-average, 6-hr diurnal water consumption was verified using a two-way fixed-effects analysis of variance, where the main effects were bihourly consumption and group. Bihourly intake was recorded because it is a simple means to evaluate the behavioral mechanisms for repellency, i.e., avoidance of trigeminal irritants or formation of a conditioned taste, odor, or flavor aversion (Clark and Mason 1993, Clark 1996). On the second day, birds within groups were presented with one of the randomly assigned concentrations of the test substance. The protocol for the 6-hr drinking test was repeated. On the third day, birds were presented with tap water using the standard presentation protocol, and groups effects were tested to inspect for carry-over effects. Similarity of hour effects within concentrations on the test day and lack of group effects on the

post-test day indicate that the repellent is most likely a trigeminal irritant having no postingestional consequences, i.e., repellency is a transient sensory effect requiring intimate contact with the repellent (Clark 1996).

RESULTS AND DISCUSSION

A Comparison of Compounds for Bird Repellent Activity

Over the years, we have evaluated a number of compounds for bird repellent activity. Our primary goal was to develop a quantitative structure-activity model to be used in making predictions about repellent activity of yet-to-be-tested compounds. The development of such a predictive model would allow us to economically prospect the chemical databases for promising compounds that could then be evaluated empirically for actual activity under registration and manufacturing constraints imposed by the development process.

Several lists are provided. All are based on one-bottle, 6-hr drinking assays, and include, based on timed sampling, only those compounds we believe operate via trigeminally mediated avoidance. Table 1 (see tables at end of chapter) contains estimates of activity based upon complete doseresponse characterizations where the concentration of each of 61 compounds in solution was verified by spectrophotometric or high-performance liquid chromatography (HPLC) analysis. The activity of other compounds was examined for a single concentration, usually at the saturation limit in water (Table 2). For these compounds, no inflection point is available, though R_{min} is reported. A third list of activity is provided for those compounds where the test concentrations could not be verified by spectrographic techniques (Table 3). R_{min} is reported for the maximum concentration tested (0.5% w/w or w/vol). Table 4 depicts responses to amino acids.

Compounds were characterized into categories of potency based upon their R_{min} values (Figure 2). Strong repellents are defined for the condition when R_{min} is ≤ 0.20 . In this case, the fluid intake generally is not statistically different from zero consumption. Compounds are considered to be moderately aversive if, $0.2 < R_{min} < 0.4$. Compounds in this category generally are statistically different from zero consumption, but are often statistically similar to intake values characterizing strong repellents. Compounds are considered to be weakly aversive if, $0.4 < R_{min} < 0.6$. Such compounds generally are statistically different from strong repellents but not moderately aversive compounds. Compounds are not considered to be aversive if, $R_{min} > 0.6$. These compounds generally are not statistically different from controls (Clark and Shah 1991, 1994, Clark et al. 1991, Shah et al. 1991, Mason et al. 1991).

Previously, Clark and Shah (1994) summarized the qualitative chemical attributes of trigeminal bird repellents. In order of importance, the critical features affecting repellency are as follows: (1) the presence of a phenyl ring is critical for repellency; (2) basicity of the molecule, in general, enhances repellency. However, when an electron withdrawing group is present it must not contain an acidic function; and (3) good repellents possess a high degree of electronegativity. Steric effects and extreme delocalization of lone pairs of electrons, as might occur in meta isomers and aromatic structures with multiply substituted electron donating groups, tend to interfere with repellency.

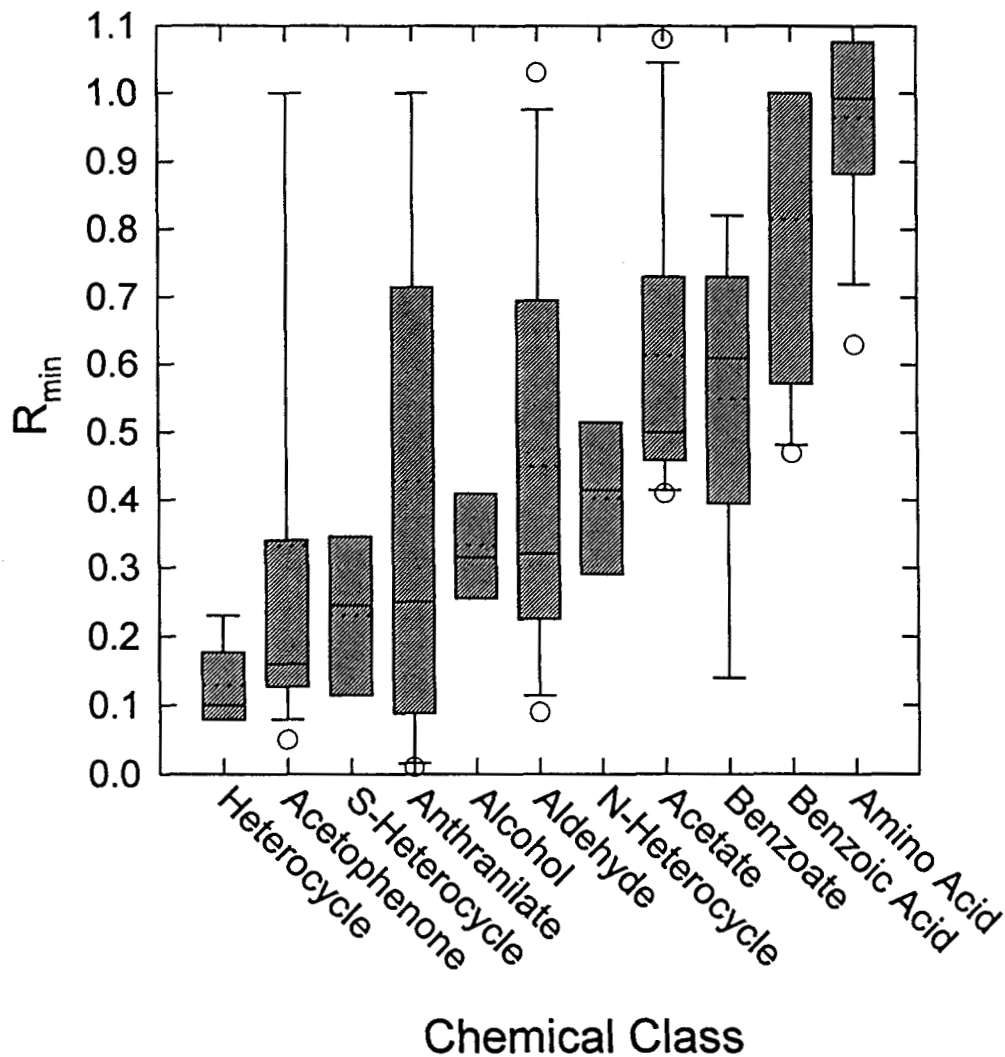


FIGURE 2. The relative reduction of fluid intake for repellents as a function of chemical class. Horizontal dashed lines depict the median R_{\min} . Horizontal solid lines depict the mean R_{\min} . The vertical shaded boxes depict the 75th percentile for R_{\min} . The vertical capped bars depict the 95th percentile for R_{\min} . The circles depict the maximum and minimum values for R_{\min} falling outside the 95th percentile range.

The core structures of many simple aromatic compounds show bird repellent activity (Figure 3). Repellency of the core structures is often enhanced by the substitution of an amino group, followed by methoxy, methyl, and hydroxy groups. Substitution at the ortho position generally leads to improved repellency. Enhanced repellency may also occur if substitution is at the para position. However, substitution at the meta position may increase or decrease repellent effects, depending upon the nature of the substitution. The strongest repellents are those aromatic structures with the fewest substitutions. Several other patterns emerge (Figure 2). The most

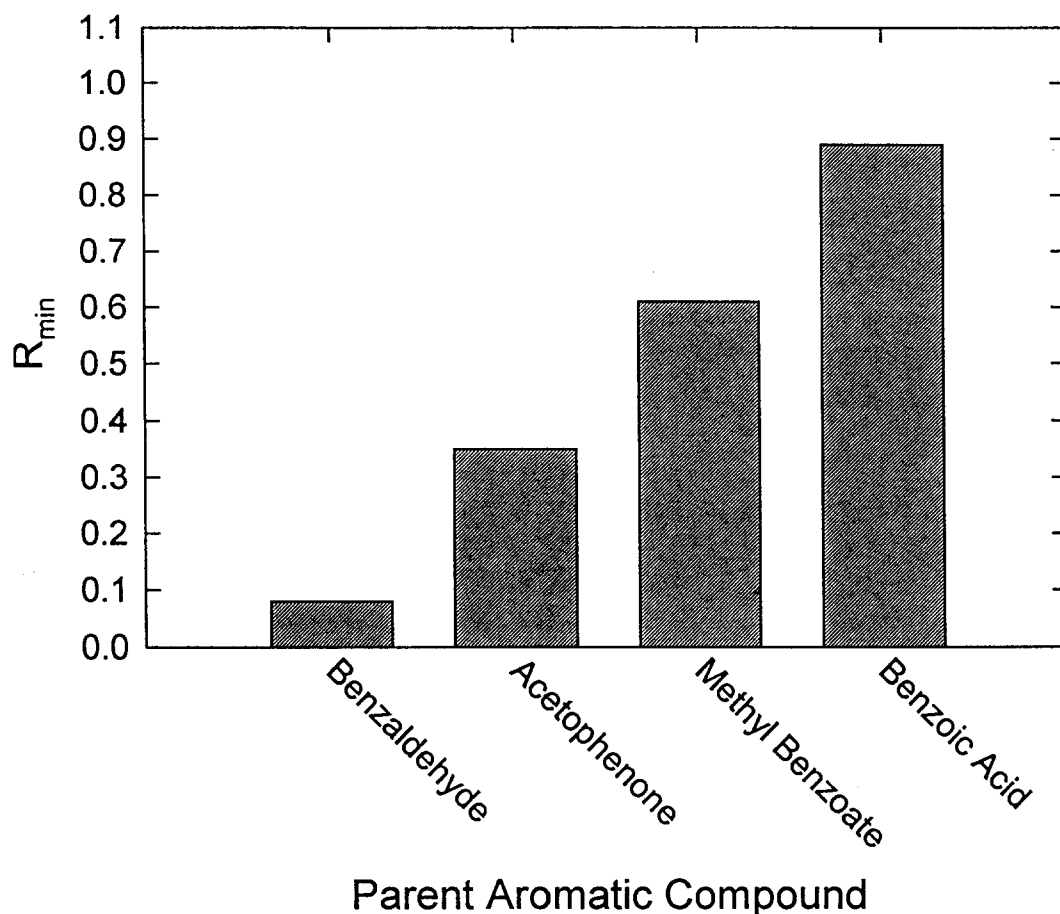


FIGURE 3.

The relative intake for the parent structures of aromatic aldehydes, acetophenones, benzoates, and benzoic acids. Substitutions on the carbonyl function that reduce the basicity of the molecule also detract from repellency. The effects of such substitutions can be compensated by addition of electron donating structures to the aromatic core structure.

potent repellents (where the median $R_{\min} \leq 0.2$) are structurally rigid and possess the resonance and electronegative properties outlined above (e.g., aromatic heterocycles containing nitrogens and simple acetophenone structures). Aromatic N-heterocycles are more uniformly strong repellents than are acetophenones. In the latter case, substitutions have a greater chance of delocalizing lone pairs of electrons. Compounds derived from S-heterocycles, anthranilates, aromatic alcohols, and aromatic aldehydes tend to be moderately good repellents ($0.2 < \text{median } R_{\min} \leq 0.4$). Birds consuming alcohols show signs of toxicosis, thus these compounds are not strictly trigeminal repellents. Anthranilates and aldehydes show a high degree of variability for activity. Compounds within these classes with little repellent activity tend to be nonplanar structures. Moderate and strong ($R_{\min} \leq 0.4$) repellents generally are derived from aromatic structures with high basicity, those characterized by lower basicity, e.g., benzoic acids are not, as a class, good repellents. Amino acids are not repellent.

MANAGEMENT IMPLICATIONS

Individual compounds listed above may form the basis for new active ingredients in formulated bird repellents. However, the appropriate formulation, delivery strategies for the formulated product in the field, and the cost-effectiveness of bringing any of the listed compounds to registration under the Federal Insecticide, Fungicide, and Rodenticide Act has yet to be determined for any compound except methyl anthranilate. Because the U.S. Environmental Protection Agency is relaxing standards for natural extracts, there may be other means to incorporate some of the described active ingredients into easily registerable bird repellent products. I showed the range of activities for classes of compounds. It may be possible to perform extractions for natural products by chemical class that would incorporate many of the same compounds characterized in this paper, thus reducing the costs of registering naturally derived bird repellents.

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Table 1. A summary of trigeminal repellent activity against European starlings for 61 carbocyclic compounds where the dose-response curves were characterized, and the concentrations were verified by spectrographic or HPLC analysis.

Compound Name	CAS ^a	C _{RT} ^b	C _{max} ^c	R _{min} ^d	Source ^e
acetophenone	98-86-2	4.36	40.64	0.35	2
acetyl salicylic acid	50-78-2	5.28	0.08	0.53	2
2-acetylthiazole	24295-03-2	3.72	37.63	0.34	4
2-acetylthiophene	88-15-3	24.44	33.88	0.08	4
B-alanine	107-95-9	n.i	56.12	1.00	2
B-alanine, methyl ester	3196-73-4	21.17	33.60	0.63	2
2-amino benzyl alcohol	5344-90-1	3.31	38.20	0.27	2
2-amino-4,5-dimethoxyacetophenone	4101-30-8	0.29	9.76	0.26	2
2-amino-4,5-dimethoxybenzoic acid	5653-40-7	n.i.	7.15	1.00	2
p-aminoacetophenone	99-92-3	15.80	35.53	0.15	1
o-aminoacetophenone	551-93-9	1.70	36.98	0.16	1
m-aminoacetophenone	99-3-6	13.7	31.70	0.31	1
3-aminobenzoic acid	99-05-8	12.3	31.66	0.51	3
4-aminobenzoic acid	156-13-0	8.72	36.47	0.38	3
m-anisic acid	586-38-0	n.i.	7.80	1.00	3
p-anisic acid	100-9-4	n.i.	1.40	1.00	3
o-anisic acid	579-75-9	14.26	25.50	0.70	3
anthranil	271-58-9	5.34	40.17	0.08	2
anthranilic acid	118-92-3	n.i	36.50	1.00	3
o-anthranilic acid salt	118-92-3	55.90	36.47	0.79	4
benzaldehyde	100-52-7	5.17	42.08	0.09	4
benzamide	55-21-0	0.29	41.29	0.64	3
benzoic acid	65-85-0	9.48	38.33	0.87	3
benzothiole	41.689	17.31	0.15	0.47	4
benzyl acetate	140-11-4	10.23	14.28	0.41	4
capsaicin	404-86-4	n.i.	0.30	1.00	5
cinnamamide	621-79-4	75.71	20.38	0.57	4
N,N-dimethyl aniline	121-69-7	1.13	7.01	0.31	2
ethyl anthranilate	87-25-2	0.45	4.10	0.11	3

Compound Name	CAS ^a	C _{r.s.} ^b	C _{max} ^c	R _{min} ^d	Source ^e
p-hydroxyacetophenone	99-93-4	n.i.	32.57	1.00	1
o-hydroxyacetophenone	118-93-4	2.60	13.07	0.15	1
m-hydroxyacetophenone	121-71-1	n.i.	24.72	1.00	1
indole	120-72-9	4.61	20.26	0.16	4
isobutyl methyl anthranilate	65505-24-0	n.i.	0.24	1.00	3
isobutyl-N,N-dimethyl anthranilate	68480-21-7	0.66	0.36	0.62	3
isoquinoline	119-65-3	2.53	35.57	0.08	4
linalyl anthranilate	7149-26-0	0.47	6.70	0.22	3
m-methoxyacetophenone	586-37-8	5.90	17.78	0.12	1
p-methoxyacetophenone	100-6-1	1.80	22.27	0.05	1
o-methoxyacetophenone	4079-52-1	2.60	28.77	0.10	1
methyl anthranilate	134-20-3	3.51	17.33	0.01	3
methyl benzoate	93-58-3	1.36	12.27	0.61	2
methyl salicylate	119-36-8	1.91	5.19	0.48	3
methyl-2-methoxybenzoate	606-45-1	12.28	29.61	0.14	2
methyl-4-methoxybenzoate	121-98-2	0.10	4.66	0.82	2
phenethanol	60-12-8	8.62	40.93	0.46	4
phenyl ethyl anthranilate	133-18-6	0.03	0.04	0.03	3
piperazine	110-85-0	50.00	58.04	0.48	4
propionyl methyl anthranilate	25628-84-6	2.52	7.21	0.27	3
d-pulegone	89-82-7	0.58	0.87	0.03	4
pyrazine	1758-62-9	67.37	52.72	0.55	4
pyridine	110-86-1	10.72	53.16	0.23	4
pyrrole	109-92-7	6.57	62.08	0.35	4
salicylaldehyde	90-2-8	25.18	0.33	0.62	3
salicylic acid	69-72-7	n.i.	31.28	1.00	2
sodium benzoate	532-32-1	n.i.	34.69	1.00	2
sodium cyanide	143-33-9	18.48	1.02	0.18	4
5,6,7,8-tetrahydroquinoline	36556-6-6	2.38	35.14	0.23	4
1,2,3,4-tetrahydroisoquinoline	635-46-1	n.i.	14.64	1.00	4
thiazole	288-47-1	3.13	50.22	0.35	4
veratryl acetate	synthesized	4.01	21.02	0.55	6

- CAS (Chemical Abstract Service) registry number.
- The inflection point of the fitted four-parameter logistic dose-response curve. This is the concentration where 50% of the maximum suppressive response is observed. Note that this is different than a relative intake of 50% of the control condition. The two measures are equivalent only when R_{\min} is zero, which was generally not the case for the empirical tests.
- The maximum concentration tested. This value, in most cases, represents the maximum water solubility of the test compound.
- The minimum fluid intake relative to pretreatment water consumption as calculated by the minimum asymptotic parameter of the four-parameter logistic dose-response curve. Empirically, R_{\min} closely corresponds to the actual relative intake value at C_{\max} .
- (1) Clark and Shah (1991), (2) Clark and Shah (1994), (3) Clark et al. 1991), (4) Clark, unpublished, (5) Mason et al. (1991), (6) Shah et al. 1991.

Table 2. A summary of the relative fluid intake by European starlings measured at a single concentration for 25 compounds.^a

Compound ^b	CAS	Relative Intake ^c	Concentration (mM) ^d
allyl sulfide	592-88-1	0.57	5.69
anethole	4180-23-8	0.70	6.14
benzaldehyde	100-52-7	0.09	42.08
benzophenone	119-61-9	1.03	0.41
benzyl acetate	140-11-4	0.41	14.28
cinnamic alcohol	104-54-1	0.36	25.64
cinnamaldehyde	104-55-22	0.54	12.07
citral	5392-40-5	0.59	7.29
ethyl butyrate	105-54-4	0.66	0.30
ethylcinnamyl acetate	621-82-9	0.46	1.08
ethylphenyl acetate	101-97-3	0.54	7.22
farnesol	4602-84-0	0.75	0.09
hydrocinnamaldehyde	104-53-0	0.31	15.16
methylcinnamate	1754-62-7	1.08	2.16
methylphenyl acetate	101-41-7	0.46	14.65
o-anisaldehyde	135-02-4	0.17	17.78
o-tolualdehyde	529-20-4	0.28	13.44
phenethyl acetate	103-45-7	0.73	7.09
phenyl acetaldehyde	122-78-1	0.85	19.60
phenethanol	60-12-8	0.24	40.93
salicaldehyde	90-02-8	0.33	25.38
tannic acid	1401-55-4	0.41	2.58
o-toluidine	95-53-4	0.20	5.85
m-toluidine	108-44-1	0.23	6.43
p-toluidine	106-49-0	0.26	6.43

^a Data were derived from Clark (unpublished).

^b All compounds are used as human food and flavor additives (see Furia and Bellanca 1971).

^c Relative intakes were based upon standard one-bottle, 6-hr drinking assays. Scores were calculated by dividing the fluid intake of the day of treatment by the pretreatment baseline water consumption.

^d Concentrations were originally prepared as 0.5% (w/w) but were subsequently validated by spectrographic and HPLC assays to account for water solubility differences among compounds.

Table 3. Compounds tested (*N* = 19) for bird repellent activity where solubility was not determined.^a

Name	CAS	Relative Intake^{b,c}	Source^d
N-acetyl vanillyl amine	synthesized	0.38	3
N-acetyl veratryl amine	synthesized	0.32	3
aluminum ammonium sulfate dodecahydrate	7784-26-1	1.00	1
anthranilamide	88-68-6	0.38	2
anthraquinone-powder	84-65-1	0.83	1
anthraquinone-liquid (50% dispersion)	84-65-1	0.57	1
aspartame	22839-61-8	0.90	1
azodicarbonamide	123-77-3	1.20	1
dimethyl anthranilate	85-91-6	0.22	2
isatoic anhydride	118-48-9	0.69	2
isobutyl anthranilate	7779-77-1	0.45	2
4-ketobenztriazine	90-16-4	0.35	2
methyl capsaicin	synthesized	0.75	3
5-nitro anthranilic acid	616-79-5	0.90	2
sacharin	81-07-2	0.85	1
sodium sacharin	82385-42-0	0.92	1
veratryl alcohol	93-03-8	0.55	1
veratryl amine	5763-61-1	0.30	3
veratryl nonanoate	synthesized	1.02	1

^a Fluid intake data are derived from standard one-bottle, 6-hr drinking assays.

^b Relative intake values were calculated by dividing the 6-hr treatment day consumption by the 6-hr pretreatment day water intake. Group size per amino acid was *n* = 6. The maximum standard error recorded for relative intake was <5% of full scale.

^c Fluids were prepared on a wt/wt basis with a maximum theoretical concentration of 0.5%.

^d Source: (1) Clark, unpublished, (2) Clark et al. 1991, (3) Mason et al. 1991, (4) Shah et al. 1991.

Table 4. Intake of amino acid solutions by European starlings.^a

Amino Acid	CAS	Relative Intake ^b	Concentration ^c (mM)
L-alanine	56-41-7	0.988	6.830
L-arginine	74-79-3	1.094	4.310
L-asparagine	70-47-3	1.159	1.336
L-aspartic acid	56-84-8	0.820	0.169
glutamine	56-85-9	1.076	0.889
L-glutamic acid	56-86-0	0.882	0.294
glycine	56-40-6	1.037	16.650
histidine	71-00-1	0.979	1.350
L-methionine	63-68-3	0.916	1.323
L-phenylalanine	63-91-2	1.200	0.896
L-tryptophan	73-22-3	0.730	0.279
L-tyrosine	60-18-4	0.997	0.013

- ^a Fluid intake data are derived from standard one-bottle, 6-hr drinking assays (Clark, unpubl.).
- ^b Relative intake values were calculated by dividing the 6-hr treatment day consumption by the 6-hr pretreatment day water intake. Group size per amino acid was $n = 6$. The maximum standard error recorded for relative intake was <5% of full scale.
- ^c Fluids were prepared on a wt/wt basis with a maximum theoretical concentration of 0.5%. Actual molar concentrations were validated analytically, and different concentrations reported reflect different water solubilities of the amino acids.