REP 1993-012

CHEMICAL BIRD REPELLENTS: POSSIBLE USE IN CYANIDE PONDS

LARRY CLARK, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Science & Technology, Denver Wildlife Research Center, % Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308
PANKAJ S. SHAH,[1] Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308

Abstract: Regulatory agencies are pressuring the mining industry to protect wildlife from mortality associated with the consumption of dump leachate pond water containing cyanide. Using European starlings (*Sturnus vulgaris*) as an avian model, we tested the effectiveness of 5 chemical bird repellents at reducing consumption of pond water containing cyanide. The repellents, which were previously shown to be good bird repellents, were: o-aminoacetophenone (OAP), 2-amino-4,5-dimethoxyacetophenone (2A45DAP), methyl anthranilate (MA), 4-ketobenztriazine (4KBT), and veratryl amine (VA). Despite the high pH (10.6) and presence of chelating metals, conditions which we hypothesized might destroy the activity of repellents, each of the additives reduced pond water intake relative to controls for up to 5 weeks. The rank order (from best to worst) of repellents was: OAP, 2A45DAP, VA, MA, and 4KBT, although only OAP and 4KBT differed at the $P < 0.05$ level. These candidate repellents hold promise as a strategy to reduce bird losses at cyanide ponds and should be tested in the field.

J. WILDL. MANAGE. 57(3):657–664

Cyanide is used in the extraction of gold and silver from ore, and results in a leachate that is highly toxic to wildlife. Because the cyanide bearing solution is often stored in open impoundments, wildlife attracted to the toxic water are at risk (Hallock 1990). Eliminating cyanide from ponds via quenching (treatment with hydrogen peroxide) is expensive. Furthermore, quenching is not always desirable, especially in circumstances when recovery of the cyanide is desired.

Drift fences generally have prevented wildlife that do not fly from entering ponds or drinking from them; however, birds and bats remain at risk. Traditional methods of hazing to keep birds away from toxic ponds have not been sufficient to meet regulatory requirements of zero mortality. The most effective commercially available means of protecting birds from toxic ponds, and that recommended by the U.S. Fish and Wildlife Service, is to enclose the ponds with netting (Hallock 1990). This is expensive and requires considerable logistical effort in terms of installation and maintenance (Allen 1990). Despite these efforts and a substantial reduction in avian mortality, nets periodically fail. As a consequence, the goal of zero mortality is rarely realized (Hallock 1990, Jackson 1990). Clearly an economical ancillary strategy for keeping birds out of toxic free-standing water is needed as a fail safe strategy.

The development of chemical repellents represents one possible solution to the problem. If repellents are to be used as an ancillary strategy to reduce intake of toxic pond water, they must meet 3 criteria. First, they must be repellent upon initial exposure. Second, they must be effective even after repeated exposure. Third, any fluid intake by a bird (there will always be some) must fall safely below acute and chronic toxic exposure levels.

The Environmental Protection Agency currently has 95 compounds registered for bird control. Only 40% of these compounds are described as nonlethal chemical repellents, and none act solely on a sensory basis. Effectiveness depends upon ingestion, an undesirable characteristic when toxic, potentially lethal waters are involved. For this reason, we have begun to explore the development of sensory repellents that do not require a learning period to be effective. Birds are repelled by the chemical on initial exposure, and do not habituate to the repellent.

A series of studies and observations led us to concentrate our research on derivatives of a basic phenyl ring structure and formulate a model that accurately predicts bird repellents (Mason et al. 1989, 1991*b*; Clark and Shah 1991*a*; Clark et al. 1991; Shah et al. 1991). Briefly, sensory repellency is mediated via irritation of the trigeminal nerve and is associated with the specific chemical attributes of molecules (i.e., basicity, the presence of an electron-donating group in resonance with an electron-withdrawing group on a phenyl ring, and a heterocyclic ring in the

[1] Present address: Pfizer Chemical Company, Terre Haute, IN 47808.

same pi cloud plane as the phenyl ring). Molecules that combine the best of these features are the more potent sensory repellents.

We selected the best repellents predicted from the model and previously tested (Clark and Shah 1991*a*) with the goals of determining the initial levels of repellency for compounds presented in water derived from cyanide holding ponds; whether birds habituated to the repellent; and whether repellency persisted over time (i.e., weeks) once the compounds were placed in a hostile chemical environment (i.e., dump leachate pond water derived from a gold mining operation). Thus, we evaluated the repellency of solutions upon initial mixture and exposure, and after 2 and 5 weeks.

This study was funded by a United States Department of Agriculture cooperative agreement #12-34-41-0040 between the Monell Chemical Senses Center and the Denver Wildlife Research Center. D. W. Coleman provided assistance in the laboratory. We thank J. Arnold of Gold Field Operating Company for providing pond water from the Chimney Creek tailings ponds and J. R. Mason for reading a draft of the manuscript. All procedures outlined in this study were approved by Monell's Animal Use and Care Committee and are in compliance with the National Institutes of Health and United States Department of Agriculture's, Animal and Plant Health Inspection Service guidelines for experimental use of animals. Use of product name does not imply endorsement of that product by the United States government.

METHODS

Birds

We captured adult European starlings (hereafter called starlings) at the Philadelphia Zoo using funnel traps. Birds were transported from the zoo to the Monell Center via car. Upon arrival at the laboratory, the birds were individually caged (61 × 36 × 41 cm) under a 12:12 light : dark cycle. During a 2-week adaptation period prior to testing, all birds were given free access to Purina Flight Bird Conditioner (Purina Mills, St. Louis, Mo.), water, and medicated oyster shell grit (United Volunteer Aviaries, Nashville, Tenn.).

We chose starlings as test animals because previous experiments showed them to be good models of avian chemosensitivity (Clark and Smeraski 1990, Clark and Shah 1991*b*), and at least for methyl anthranilate, the concentrations required for repellency are similar in starlings, mallards (*Anas platyrhynchos*), and ring-billed gulls (*Larus delawarensis*) (cf. Clark et al. 1991, Dolbeer et al. 1992). Furthermore, starlings are remarkably resilient when challenged with sodium cyanide (Clark and Shah 1991*a*). Thus, starlings could be tested with actual pond water containing cyanide without undue risk of acute toxicosis. There is evidence that other birds, including waterfowl, are more sensitive to sodium cyanide (R. Clark, U.S. Fish and Wildl. Serv., Laurel, Md., pers. commun.). Finally, starlings are logistically more tractable in a laboratory setting.

Chemicals

Most of the chemicals we selected for our study are repellent to birds when presented in distilled water (Clark and Shah 1991*b*, Clark et al. 1991, Shah et al. 1991). However, if these repellents are to have utility in the field they must retain their repellent properties under hostile chemical conditions (e.g., dump leachate pond water containing cyanide [hereafter referred to as pond water]).

The chemicals included in our study were: o-aminoacetophenone (OAP; CAS # 551-93-9), 4-ketobenztriazine (4KBT; CAS # 90-16-4), methyl anthranilate (MA; CAS # 25628-84-6), veratryl amine (VA; CAS # 5763-61-1), 2-amino-4,5-dimethoxyacetophenone (2A45DAP; CAS # 4101-30-8). Untreated pond water served as the control. Chemicals were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin and PMC Specialties Company, Cincinnati, Ohio. Barren pond water was obtained from Gold Fields Operating Co., Chimney Creek operation in Nevada. The pond water was assayed by Gold Field Mines and found to have a pH of 10.6, 150 ppm sodium cyanide, and 0.009 g gold/907 kg ore.

A single concentration for each of the repellents (0.5% vol/vol or wt/vol), dissolved in pond water, was prepared at the beginning of the drinking trials. Stock solutions were stored at room temperature in sealed clear glass containers under laboratory lighting (L:D, 12:12). Concentrations of repellents in the test solutions were verified using ultraviolet spectroscopy. We prepared standards by dissolving measured quantities of chemical in distilled water to yield a concentration of 0.5% (vol/vol or wt/vol). The stock solution was then diluted serially to yield the remainder of the standard dilutions: 0.25, 0.13, 0.06, 0.03, 0.015, and 0.0075%. We tested each dilution for UV absorbance from a range of 200–400 nm at 2-nm intervals to yield an absorbance spectrum. Pond water was used as a blank. The wavelength that maximized the absorbance among the dilutions was selected as the wavelength at which assays of concentration were to be conducted. We evaluated concentrations of chemicals at: 300 nm (2A45DAP), 340 nm (4KBT), 280 nm (OAP), 270 nm (MA), and 252 nm (VA).

Behavioral Assay

Initially, we measured tap-water consumption by starlings for 6 hours, on each of 3 pretreatment days. Only those birds with consistent daily consumption were used. Thus, at the end of 3 days, an individual's variance for fluid intake was compared with the mean variance for intake for all birds. Individuals' intake whose variance deviated from the population's 3-day mean variance by more than ± 1 standard deviation were excluded from the trials ($n = 7$). We ranked those birds with stable daily water consumption according to mean water consumption and assigned them to the 6 treatment (chemical) groups. We assigned the bird with the highest water consumption to the first treatment group, the bird with the second highest consumption to the second treatment group, and so forth to the final group, followed by a series of assignments from the final group back to group 1. This procedure assured that all groups were balanced with respect to consumption (Mason et al. 1991*b*). We used 36 birds for the experiments, with 6 birds/treatment group. Groups were randomly assigned to receive chemical treatments.

After we assigned a treatment group, a 1-day pretreatment drinking trial was initiated. Beginning at 0930, the tap-water consumption was recorded every 2 hours for the next 6 hours. These timed observations allowed us to test for habituation effects. The treatment period began at 0930 the next day, when we gave birds their preassigned chemical treatment. Consumption was recorded every 2 hours for 6 hours for 1 day. After the test, we gave birds free access to tap water. Beginning at 0930 during the posttreatment period the following day, we recorded consumption of tap water every 2 hours for a total of 6 hours. We compared the mean within group, 6-hour posttreatment water consumption with the mean within group, 6-hour pretreatment water consumption to determine whether consumption returned to pretreatment levels. Experiments were conducted at 0, 2, and 5 weeks in a repeated measures fashion.

Statistical Analyses

We analyzed the total 6-hour water consumption data using a 3-factor ANOVA with repeated measures on days, with 3 levels (pretreatment, treatment, posttreatment); and weeks, with 3 levels (0, 2, 5 weeks). The between subjects factor was chemical treatment group, with 6 levels (groups were randomly assigned to one of the 5 repellents in pond water or the control). Post-hoc differences among means ($P < 0.05$) were determined using a Tukey's *B*-test.

We also tested whether 6-hour consumption of treated water differed from a theoretical value of zero consumption. This hypothesis was of practical interest because there may be times when a bird must be repelled absolutely from potentially lethal toxic waste water. The analysis was performed separately for each of the 6 treatment groups. Considering only the fluid intake on the day of treatment, we used repeated measures analysis over weeks. The constant term for the between subjects effects was used to test the hypothesis that consumption did not differ from zero. Estimates of the error term remained the same as in a standard between subjects repeated measures ANOVA. We made post-hoc comparisons to test the hypothesis that fluid intake was zero using a modification of Dunnett's (1955) *t*-test, using a theoretical value of zero rather than the mean, and comparing the resulting *t* for each compound to critical values in Dunnett's calculated distribution. Unless otherwise indicated, variances were tested and found to be homogeneous using Bartlett's-Box method.

We analyzed the within day, 2-hour consumption pattern during the treatment period using a 3-factor repeated ANOVA with repeated factors on hour, with 3 levels (2, 4, and 6 hours); and weeks, with 3 levels (0, 2, and 5 weeks). The between subjects factor was chemical treatment group, with 6 levels. The 2-hour drinking pattern was of interest because these data indicated whether repellency was learned (i.e., acquired over weeks, or over the course of a day), or was sensory (i.e., apparent within the first 2 hours).

RESULTS

Total Water Consumption

There was a significant day by chemical interaction ($F = 5.92$; 10, 60 df; $P < 0.001$), as well as a strong day effect ($F = 229.2$; 2, 60 df; $P < 0.001$). With the exception of the pond water control, each of the chemical treatment groups showed the same pattern (Fig. 1). Pretreatment and posttreatment tap-water consumption were higher than consumption of pond water bearing repellent. Pond water without repellent had little effect on the consumption of water in starlings. Consumption also differed across weeks ($F = 3.45$; 2, 60 df; $P = 0.038$). Overall, starlings consumed less water during the fifth week of the study relative to initial trials. The largest decrease in fluid intake over time occurred in birds exposed to 2A45DAP. Both VA and OAP showed good initial repellency, and this activity was maintained over the course of the test period. Neither 4KBT nor MA resulted in a steady rate of decrease in consumption, though birds exposed to MA did consume less fluid on the fifth week. At 0 weeks, pond water with no repellent appeared slightly aversive; however, the intake did not differ ($P > 0.05$) from the consumption of tap water, and the apparent aversion disappeared at weeks 2 and 5. No other terms in the 3-way repeated measures ANOVA achieved statistical significance.

Short Term Patterns of Consumption

Overall, there was an hour effect ($F = 8.36$; 2, 60 df; $P < 0.001$) for fluid intake. In post-hoc analyses of individual chemicals, 2A45DAP showed an hour effect (Fig. 2; $F = 8.29$; 2, 10 df; $P = 0.008$), with starlings consuming more during the first 2 hours of exposure and lower amounts during the fourth and sixth hour sampling intervals. The week by hour interaction for OAP was also significant ($F = 4.86$; 4, 20 df; $P = 0.007$). During the first week there was no substantial hour effect, but during the second and fifth weeks, consumption was highest during initial encounter and decreased thereafter. There were no other hour or week effects for any of the other compounds.

Bihourly intake differed (Fig. 3; $F = 27.7$; 5, 30 df; $P < 0.001$) among compounds. The post-hoc test showed that all groups receiving chemically treated pond water consumed less than the control group, which received only uncontaminated pond water (Fig. 3). OAP, 2A45DAP, VA, and MA were the most effective repellents.

Absolute Repellency

Even though 4KBT was repellent to starlings relative to the pond water control, the level of consumption was always greater ($F = 49.9$; 3, 18 df; $P < 0.001$) than zero consumption throughout the 5 weeks of testing. Consumption of pond water treated with 2A45DAP also was above zero levels ($F = 18.39$; 3, 18 df; $P = 0.008$); however, only the first week's consumption was above a theoretical value of zero ($P < 0.025$), with consumption during weeks 2 and 5 statistically indistinguishable from zero consumption. A similar pattern was observed for OAP ($F = 38.23$; 3, 18 df; $P < 0.002$); post-hoc Dunnett t-tests showed that the first week's consumption differed from a theoretical value of zero ($P < 0.025$), while consumption during weeks 2 and 5 was effectively zero. Consumption of MA treated pond water also differed from zero consumption ($F = 15.17$; 3, 18 df; $P = 0.011$), primarily due to increased consumption during week 2 ($P < 0.025$). Consumption of pond water was never above zero levels for veratryl amine ($F = 3.06$; 3, 18 df; $P = 0.054$). For the pond water without repellent, consumption exceeded zero levels for each of the 3 time periods tested ($F = 46.16$; 3, 18 df; $P < 0.001$).

Stability of Repellents

The UV absorbance of repellents did not change during the first 2 weeks of exposure of the repellents to pond water. There was apparently some degradation of repellents by the fifth week, with approximately an 80% decrease in concentration in some cases. Nonetheless, chemicals persisted in concentrations that were still repellent to starlings. In general, the least stable compounds were those with unprotected amines (i.e., OAP, 2A45DAP, VA). The most stable compound was 4KBT, where the electron donating group was contained within a cyclic ring structure.

DISCUSSION

All 5 candidate compounds were repellent to starlings upon first exposure. With the exception

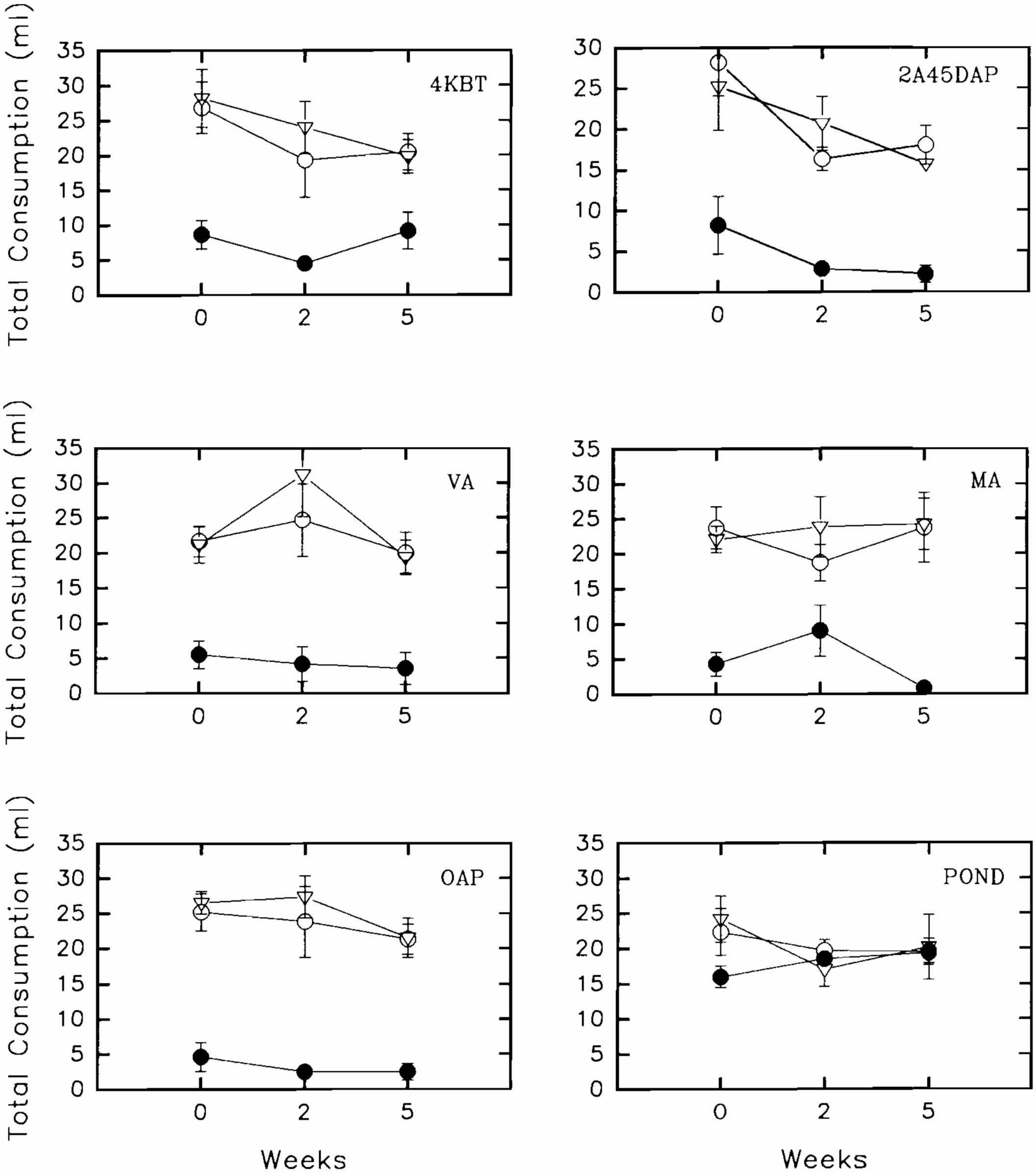

Fig. 1. Six-hour water consumption of European starlings for pretreatment (open circles), treatment (solid circles), and posttreatment (open triangles) periods. Pre- and posttreatment presentations were tap water. The treatment period presentation was a solution of repellent and cyanide bearing pond water. Vertical lines depict ±SE. Chemical codes are defined in the text.

of 2A45DAP, there was little evidence to suggest that postingestional factors contributed to repellency within any given test week. We attribute aversions to VA, OAP, MA, and 4KBT to nonlearned sensory effects (i.e., irritation). Some malaise-induced aversion may be attributable to 2A45DAP.

There was no evidence that starlings habituated to the repellents. For example, OAP and VA maintained a constant level of effectiveness throughout the test period. Some learning may have been associated with aversion to 2A45DAP because its repellency improved with time.

All of the tested repellents were stable in the pond water for up to 5 weeks. We suspected that water derived from dump-leach ponds

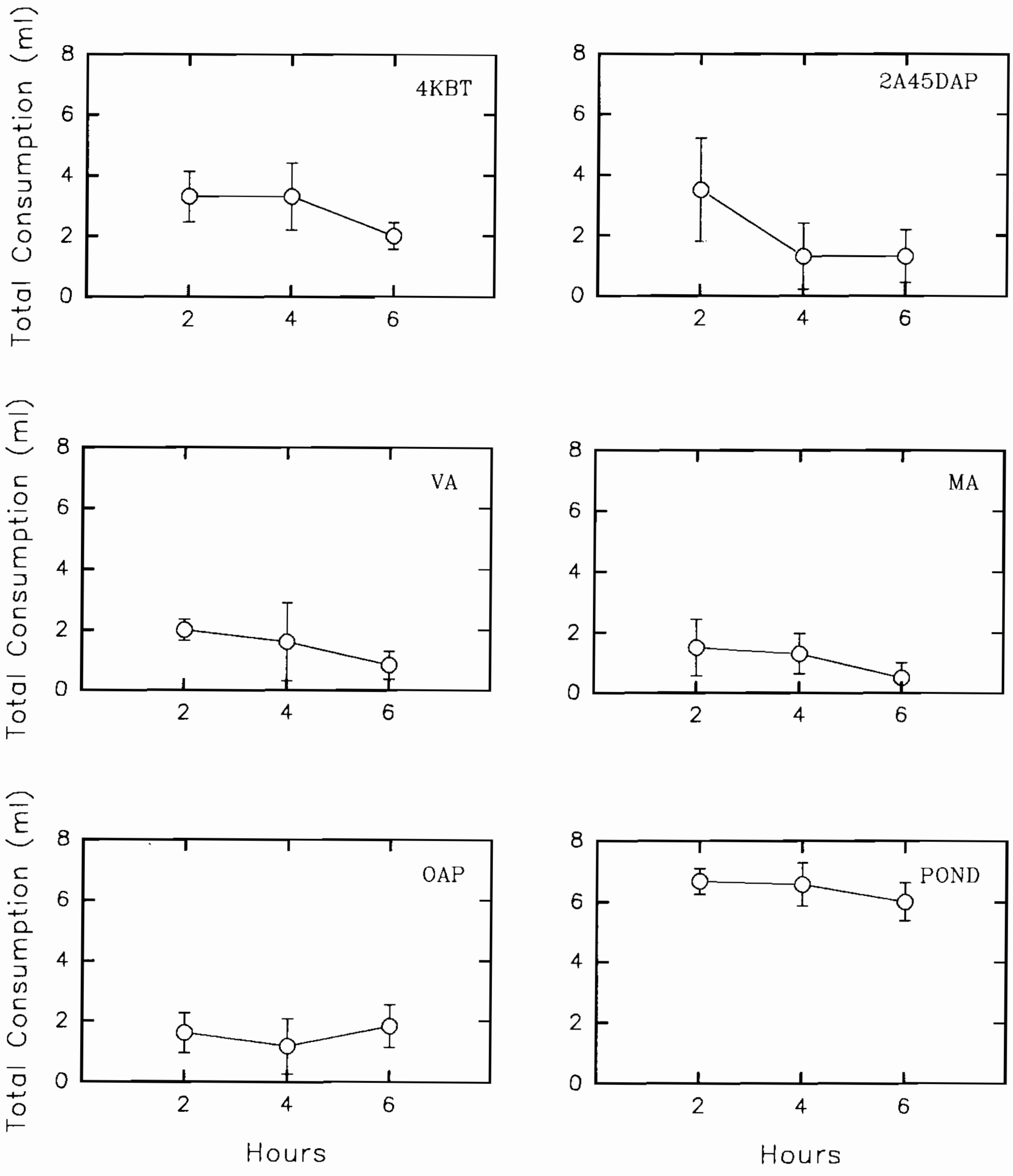

Fig. 2. Water consumption of European starlings for each 2-hour time block during the course of a 6-hour observation period averaged over the 3 weekly test periods. Chemical codes are defined in text.

might affect the stability of the repellents, hence repellency itself. The pond water was highly basic (pH 10.5). In addition, the water contained a variety of chelating metals and other contaminants that might affect repellency. The UV spectra of samples indicated that some degradation or loss of the repellents may have occurred by the fifth week. However, the decrease in active compound apparently was not sufficient to significantly affect repellency over time because in general, the lowest concentrations detected in the fifth week were above concentrations known to be repellent (Clark et al. 1991, Shah et al. 1991). Although not tested, we predict that repellency probably would not be maintained for longer periods of time, because the concentrations of the repellents would most likely fall below effective levels.

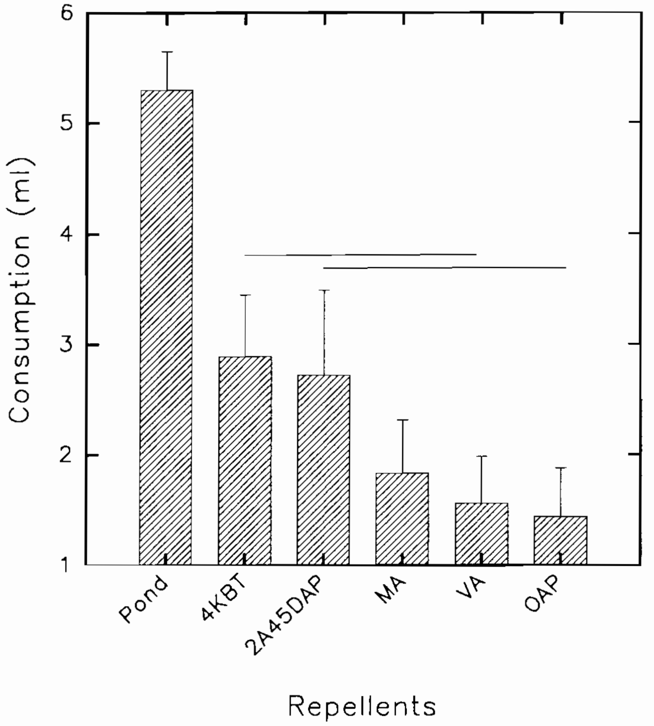

Fig. 3. Comparison of bihourly fluid intake of European starlings. Vertical bars depict ±SE. Horizontal lines depict similar means as determined by a Tukey post-hoc test. Chemical codes are defined in text.

Fluid intake by starlings was below toxic levels for the tested repellents. In addition, we provided a measure of statistical zero consumption to estimate the level of protection a repellent can provide. VA resulted in zero consumption for each of the test weeks. MA and OAP showed statistically zero consumption for 2 of the 3 weeks, as did 2A45DAP, but the latter compound showed strong evidence that post-ingestional learning was important in achieving zero consumption.

Because mallards, ring-billed gulls, and starlings are all repelled by similar levels of MA (Dolbeer et al. 1992, Clark and Shah 1991*b*), we believe that starlings are an adequate model for predicting avian sensitivity to sensory repellents. Thus, based upon the level of intake and the time course for repellency to starlings, these chemicals seem to hold promise as sensory bird repellents for use in waste pond water. However, managers must recognize that species do exhibit different sensitivity to cyanide (Fairchild 1977), with starlings being resistant to cyanide poisoning (Clark and Shah 1991*a*) and mallards being highly susceptible to cyanide poisoning (R. Clark, U.S. Fish and Wildl. Serv., Laurel, Md., pers. commun.).

MANAGEMENT AND RESEARCH IMPLICATIONS

In many circumstances open waste water ponds are an attractive resource to birds, but pose risks to individuals that utilize them. A reduced risk of morbidity or mortality is of direct benefit to wildlife, and also is a benefit to mine owner/operators because there is frequently no tolerance for mortality in statutes designed to protect wildlife, e.g., The Migratory Bird Treaty Act. Our data suggest that OAP, VA, and MA warrant further study as sensory repellents to be used in cyanide ponds and that such repellents might be applied to many waste water situations as an ancillary means to protect wildlife. We broadly define waste water as including, but not restricted to, water from tailing ponds, agricultural drainage ponds, water ponds associated with oil drilling operations, and standing water or runoff at airports.

If chemical repellents are to be used as an ancillary strategy in deterring holding pond water consumption, estimates of the effectiveness of a repellent must be evaluated for the species of primary concern. High sensitivity to repellents tends to minimize risk, but this can be offset by increased sensitivity to contaminants such as cyanide. Future studies must test sensitivity of species to specific contaminants to adequately estimate the level of protection a repellent can provide.

LITERATURE CITED

ALLEN, C. 1990. Mitigating impacts to wildlife at FMC Gold Company's Paradise Peak Mine. Pages 67–71 *in* R. McQuivey, coord. Proc. Nevada Wildlife/Mining Workshop. Nev. Mining Assoc., Nev. Dep. Minerals and Nev. Dep. Wildl., Reno.

CLARK, L., AND P. SHAH. 1991*a*. Chemical bird repellents: applicability for deterring use of waste water. Pages 157–164 *in* S. Foster, ed. Issues and technology in the management of impacted wildlife. Thorne Ecological Institute, Boulder, Colo.

———, AND ———. 1991*b*. Nonlethal bird repellents: in search of a general model relating repellency and chemical structure. J. Wildl. Manage. 55:538–545.

———, ———, AND J. R. MASON. 1991. Chemical repellency in birds: relationship between structure of anthranilate and benzoic acid derivatives and avoidance response. J. Exp. Zool. 260:310–322.

———, AND C. A. SMERASKI. 1990. Seasonal shifts in odor acuity by starlings. J. Exp. Zool. 255:22–29.

DOLBEER, R. A., P. P. WORONECKI, T. W. STEARNS, AND L. CLARK. 1992. Pen tests of methyl anthranilate as a bird repellent in water. Proc. Fifth East. Wildl. Damage Control Conf. 5:112–116.

DUNNETT, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096–1121.

FAIRCHILD, E. J. 1977. National Institute for Occupational Safety and Health, Registry of toxic effects of chemical substances. Vol. II. U.S. Department of Health, Education and Welfare, Cincinnati, Ohio. 987pp.

HALLOCK, R. J. 1990. Elimination of migratory bird mortality at gold and silver mines using cyanide extraction. Pages 9–17 *in* R. McQuivey, coord. Proc. Nevada Wildlife/Mining Workshop. Nev. Mining Assoc., Nev. Dep. Minerals, and Nev. Dep. Wildl., Reno.

JACKSON, W. B. 1990. Bird repelling techniques. Pages 46–50 *in* R. McQuivey, coord. Proc. Nevada Wildlife/Mining Workshop. Nev. Mining Assoc., Nev. Dep. Minerals, and Nev. Dep. Wildl., Reno.

KARE, M. R. 1965. The special senses. Pages 406–446 *in* P. D. Sturkie, ed. Avian physiology. Cornell Univ. Press, Ithaca.

MASON, J. R., M. A. ADAMS, AND L. CLARK. 1989. Anthranilate repellency to starlings: chemical correlates and sensory perception. J. Wildl. Manage. 53:55–64.

———, M. L. AVERY, J. F. GLAHN, D. L. OTIS, R. E. MATTESON, AND C. O. NELMS. 1991*a*. Evaluation of methyl anthranilate relative to starch-plated DMA as a bird repellent livestock feed additive. J. Wildl. Manage. 55:182–187.

———, L. CLARK, AND P. S. SHAH. 1991*b*. Ortho-aminoacetophenone repellency to birds: perceptual and chemical similarities to methyl anthranilate. J. Wildl. Manage. 55:335–340.

SHAH, P., L. CLARK, AND J. R. MASON. 1991. Prediction of avian repellency from chemical structure: the aversiveness of vanillin, vanillyl alcohol and veratryl alcohol. Pesticide Biochem. Physiol. 40:169–175.

WINDHOLZ, M. 1983. The Merck index. Merck & Co., Rahway, N.J. 1463pp.

Received 11 November 1991.
Accepted 23 December 1992.
Associate Editor: Fagerstone.