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Acute Toxicity of the Bird Repellent, Methyl Anthranilate, to Fry of *Salmo salar*, *Oncorhynus mykiss*, *Ictalurus punctatus* and *Lepomis macrochirus*

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Abstract: Several laboratory and field studies have shown methyl anthranilate to be an effective, non-toxic and non-lethal bird repellent, with application potential for protecting crops, seeds, turf and fish stocks from bird damage. Furthermore, methyl anthranilate can be added to liquids for the purposes of protecting migratory birds, e.g. addition to waste water associated with mining and to standing water pools at airports. Mammalian toxicity data are favorable. Methyl anthranilate is used as a fragrance and food flavoring and is GRAS listed by the US Food and Drug Administration. Despite the favorable outlook for methyl anthranilate's use as a safe repellent, no data exist on its environmental fate and effects. We have tested the acute toxicity of methyl anthranilate in a static system against the fry of four species of fish. The LC_{50} at 24 h for Atlantic salmon (*Salmo salar* L.) was 32·3 mg liter^{-1}, with the no observable effect limit at 6 mg liter^{-1}. The LC_{50} at 24 h for rainbow trout (*Oncorhynus mykiss* Richardson) was 23·5 mg liter^{-1}, with the no observable effect limit at 5 mg liter^{-1}. The LC_{50} at 24 h for channel catfish (*Ictalurus punctatus* Raf.) was estimated to be 20·1 mg liter^{-1}, with the no observable effect limit at 7 mg liter^{-1}. The LC_{50} at 24 h for bluegill sunfish (*Lepomis macrochirus* Raf.) was estimated to be 19·8 mg liter^{-1}, with the no observable effect limit at 7 mg liter^{-1}.

1 INTRODUCTION

Methyl anthranilate (CAS 134-20-3; methyl 2-aminobenzoate) was first described as a bird repellent by Kare.[1] Since that time a series of studies have focused on its efficacy as a non-lethal avian irritant.[2–4] In the field, methyl and dimethyl anthranilate have proved effective at reducing bird depredations of feed at cattle feed lots.[5,6] It also has potential for use in protecting orchard crops and seeds.[7,8] Formulated methyl anthranilate can minimize goose grazing damage to turf, and protect birds when included in granular pesticides.[9–11] Furthermore, methyl anthranilate incorporated into 'Concover'® dissuades gulls from using landfills.[12] Methyl anthranilate can reduce water consumption of ducks, gulls and passerines.[3,12–14] Without access to fresh free-standing water at airports, the risk of air collisions between aircraft and birds can potentially be reduced.[12,13] Decreasing the attractiveness of contaminated water (e.g. cyanide ponds associated with gold-mining operations) may reduce the risk of accidental kills of birds at tailing ponds.[15–17]

Contributing to the attractiveness of methyl anthranilate as a non-lethal bird control agent is its use in the fragrance and flavor industries.[18,19] Notwithstanding the favorable toxicity data for mammals (LC_{50} for mice is 3900 mg kg^{-1}), there are no data which address methyl anthranilate's effects on aquatic organisms or the environment.[20]

This study focuses on the acute toxicity of methyl

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anthranilate to fry in static tests.[21] The data herein are intended for use to evaluate whether methyl anthranilate is suitable for broad-scale environmental use as a non-lethal avian repellent.

2 EXPERIMENTAL METHODS

2.1 Test substance

The purity of the methyl anthranilate was specified as > 98% by GC (Lot # 271292387, Fluka Chemical Company). All test concentrations were based on the total compound, i.e. not corrected for sample purity.

2.2 Analytical method and validation

A concentrated standard was prepared by dissolving methyl anthranilate (0·1 ml) in doubly deionized distilled water (1000 ml) to give a nominal concentration of 100 mg liter^{-1}. The solution was sonicated for 30 min, then magnetically stirred for an additional 30 min. The concentrated solution was allowed to cool to room temperature and was checked for precipitates. Working standards were prepared to nominal concentrations by quantitative dilution of the concentrated standard solution with water.

We used an HPLC system to validate samples from the catfish and bluegill bioassays. The mobile phase was water + acetonitrile (1 + 1 by volume) at 1·0 ml min^{-1}. Injection volume was 120 μl at 25°C. The column was a Zorbax ODS 4·6 mm × 250 cm, configured with a Rainin HPXL pump, Rainin pressure module, Dynamax UV-M detector, Dynamax AI-2 autosampler, HP 3390A integrator and Perkin-Elmer Nelson 1020S computer integrator for archiving. The UV detector was set at 330 nm.

The relative standard deviation of the methyl anthranilate chromatographic peak response was not greater than 2·2% for six consecutive injections of a 50-mg liter^{-1} working standard. Chromatographic peak response and concentration were significantly correlated ($(r = 0{\cdot}99981)$ over the range of interest) (100, 50, 10, 5 mg liter^{-1}; slope $= 2{\cdot}93971 \times 10^{-6}$).

Control water samples were analyzed using the above method, as were water samples which had fish in them for several days. No chromatographic interferences were observed.

2.3 Test species

Fry were maintained in holding tanks on a 12-h daylight photoperiod for two weeks prior to testing.[22] During the holding period, fish received food once per day at a maintenance rate of 0·04 g (g fish)$^{-1}$. Fry were not fed 48 h prior to testing.

The 120 Atlantic salmon (*Salmo salar* L.) and the 160 rainbow trout (*Oncorhynus mykiss* Richardson) used in the test were hatchery spawned and of the same size and year class. Fry were obtained from The Tunison Laboratory of Fish Nutrition of the US Fish & Wildlife Service, 3075 Gracie Road, Cortland, NY 13045. Salmon were tested at nominal methyl anthranilate concentrations of 0, 1, 6, 13, 25, and 50 mg liter^{-1}. Trout were tested at nominal concentrations of 0, 1, 5, 10, 15, 20, 25 and 50 mg liter^{-1}. We did not validate these concentrations with quantitative procedures, other than visually observing whether methyl anthranilate was completely dissolved. The HPLC system was not available at the time of testing, and the gas chromatographic method available at the time proved too slow to process all samples before microbial degradation of methyl anthranilate influenced samples (Clark, unpublished data). Salmon were housed five animals to a 10-liter test vessel. Twenty salmon per concentration were tested. Trout were housed 10 animals to a 10-liter test vessel, with two replications per concentration.

The 280 channel catfish (*Ictalarus punctatus* Raf.) and the 280 bluegills (*Lepomis macrochirus* Raf.) used in the test were hatchery spawned and raised and were obtained from Delmarva Aquatics, PO Box 349, Odessa, DE 19730. All fry for each species were from the same source and year. Both catfish and bluegills were tested at nominal concentrations of 0, 5, 10, 20, 40, 50 and 100 mg liter^{-1}. Concentrations were validated using the HPLC method at 0 and 96 h of the test. Both catfish and bluegills were housed 10 animals to a 10-liter test vessel, with four replications per concentration.

2.4 Test conditions

The procedures for this static bioassay were generally those suggested by the EPA.[21,23,24] Dilution water was derived from well water and run through a series of sand and charcoal filters. Dissolved oxygen and pH were measured at 24 h and 96 h (Table 1). The temperature of the four test tanks within the water bath, as well as room temperature, was monitored every 30 min and recorded to a datalogger. Illuminance was maintained on a 12:12 h light:dark cycle and total illuminance was monitored using a photosensor and recorded to the datalogger every 30 min. The light source was a bank of overhead fluorescent lights suspended over the test tanks. Total illuminance at tank level was 1·8 W m^{-2}.

2.5 Analyses

As a precondition to initiating toxicity tests, similarities of mass among test groups were compared using a one-way analysis of variance (not reported). Dose-response curves and confidence intervals were generated using

TABLE 1
Mass of Fry Used and Summary of Water Conditions at 24 and 96 h

Species		*Mass*	*24 h*			*96 h*		
			Dissolved oxygen (g decaliter^{-1})	*pH*	*°C*[a]	*Dissolved oxygen (g decaliter^{-1})*	*pH*	*°C*[a]
Atlantic salmon	N	120	1	1		—	—	
	Mean	0·30	11·2	7·0	14·1	—	—	14·2
	SE	0·21	—	—	0·1	—	—	0·1
Rainbow trout	N	160	7	7		7	7	
	Mean	0·14	9·9	7·3	8·6	7·7	7·1	8·6
	SE	0·03	0·2	0·1	0·1	0·2	0·1	0·1
Channel catfish	N	280	28	28		28	28	
	Mean	0·12	7·2	7·8	22·7	4·4	7·5	22·7
	SE	< 0·00	0·1	< 0·0	< 0·1	0·5	0·1	< 0·1
Bluegill	N	280	28	28		28	28	
	Mean	0·62	7·2	7·4	22·7	2·4	7·2	22·7
	SE	0·24	0·2	0·1	< 0·1	0·4	< 0·0	< 0·1

[a] Water bath temperatures based on average of readings taken at 30-min intervals for a 24-h period.

logit and probit procedures.[25] The model which had the lowest confidence interval around the LC_{50} value was selected as the best descriptor of mortality.

3 RESULTS AND DISCUSSION

Methyl anthranilate is normally stable under standard laboratory conditions (Clark, pers. obs.). However, we found evidence that methyl anthranilate was subject to rapid decomposition after 24 h if dead or dying fry were present in the test vessel. Nonetheless, nominal concentrations were close to validated concentrations for periods less than 24 h, irrespective of the degree of mortality in a test vessel. The rapid loss of methyl anthranilate appears to be due to aerobic bacterial degradation (Clark, unpublished). The presence of these bacteria is associated with decomposition of fry, and the effect is exacerbated by higher temperatures. Because of the instability of methyl anthranilate beyond 24 h, we recommend that 96-h studies be conducted in flow-through systems. Herein we report LC_{50} and no observable effect limit (NOEL) over the course of 96 h, but descriptive emphasis is placed on behavior and mortality in the first 24 h only (Table 2).

3.1 Atlantic salmon

Upon initial contact with methyl anthranilate, mortality was swift at high concentrations. Salmon exhibited a loss of equilibrium (LOE) after 30 s at 50 mg liter^{-1}. Within 1 min, in addition to the LOE, all fry became dark in color (cyanotic). Pumping of the operculum during this period was more rapid and exaggerated than that seen for controls. Most fry were dead within 15 min, and all fry were dead within 3 h. At 25 mg liter^{-1}, salmon showed LOE after 2 min. Within 5 min all salmon were immobile on the bottom of the vessel, in darkened condition, and exhibited rapid opercular pumping. For test concentrations of 13 mg liter^{-1} the behavior and timing of toxicosis were similar to those reported for 25 mg liter^{-1}. For the 6 and 1 mg liter^{-1} tanks, mobility and color were similar to those of controls throughout the test period. The NOEL was estimated to be 6 mg liter^{-1} (nominal).

3.2 Rainbow trout

Initially, mortality was swift at high concentrations. Trout exhibited a LOE after 25 s at 50 mg liter^{-1}. Within 1 min all fry became dark in color. Relative to controls, opercular pumping was more rapid and exaggerated. All fry were dead within 15 min. At 25 mg liter^{-1} trout showed LOE and signs of cyanosis after 40 s. Within 70 s all trout were immobile on the bottom of the vessel, exhibiting rapid and exaggerated opercular pumping. All mortality recorded for this concentration occurred within the first 3 h of contact. For test concentrations of 20 and 15 mg liter^{-1} the behavior was similar to that reported for 25 mg liter^{-1}, with the exception that mortality was considerably less (i.e. 5% of the total tested). For 5 and 10 mg liter^{-1}, only a few fry (3 at 10 mg liter^{-1}) showed any LOE. Though darker in color than controls, fry were considerably lighter than those exposed to higher concentrations. Mobility was similar to that of controls. At 0 and 1 mg liter^{-1} all fry appeared normal, with good color and activity. The NOEL was 5 mg liter^{-1}.

TABLE 2
LC_{50} Values for Fry with Lower (LCL) and Upper (UCL) 95% Confidence Limits, along with Logistic and Probit Regression Parameters of Slope (B) and Intercept (I)

Species		*24 h* (*mg liter*$^{-1}$)	*48 h* (*mg liter*$^{-1}$)	*72 h* (*mg liter*$^{-1}$)	*96 h* (*mg liter*$^{-1}$)		*B*	*I*
Atlantic salmon	LC_{50}	34·28	33·31	32·35	32·35	Mean[a]	2·880	0·645
	LCL	—	—	—	—	SE	0·593	0·867
	UCL	—	—	—	—			
Rainbow trout	LC_{50}	23·47	23·19	23·19	22·91	Mean[a]	16·681	−17·693
	LCL	22·11	21·80	21·79	21·55	SE	3·708	5·060
	UCL	14·87	24·73	27·73	24·35			
Channel catfish	LC_{50}	20·08	17·35	16·94	16·23	Mean[b]	3·784	0·420
	LCL	12·77	12·17	11·88	11·57	SE	0·447	0·565
	UCL	30·35	24·40	23·95	22·47			
Bluegill	LC_{50}	19·80	9·12	9·12	9·12	Mean[b]	8·019	−2·698
	LCL	14·16	7·98	7·98	7·98	SE	1·131	1·087
	UCL	26·24	10·51	10·51	10·51			

[a] Parameters for the logit regression of the form: $\text{Log}(p/1-p))/2+5) = I+BX$.
[b] Parameters for the probit regression of the form: Probit $(p)+5 = I+BX$. X is concentration.

Surviving affected trout remained immobile and cyanotic throughout the test. After the test, and prior to sacrificing the fry, we placed affected trout in clean water. Within 30 min all behavior and coloration returned to levels similar to controls. Thus, short-term recovery was rapid once the methyl anthranilate was removed.

3.3 Catfish

The mortality pattern for this warm water species was similar to that seen for salmon and trout. At concentrations of 50 mg liter^{-1} or higher fry became immobile, showed evidence of exaggerated opercular pumping, became cyanotic and lost equilibrium within seconds. Fry did not respond to prodding. Death was estimated to have occurred within 30 s. Between 20 and 49 mg liter^{-1}, cyanosis, LOE, immobility and death took somewhat longer, approximately 1–2 min. Mortality for concentrations in the range of 7·5 to 20 mg liter^{-1} was variable. Generally, fry died within 12 h of introduction. Surviving fry within these concentrations showed signs of oxygen stress. They were immobile, staying near the surface, gulping air, and were dark in color relative to controls. Fry in vessels below 7·5 mg liter^{-1} did not show any signs of stress. Activity and color were normal. The NOEL was 7 mg liter^{-1}.

3.4 Bluegill

At concentrations of 50 mg liter^{-1} or greater, 100% mortality was observed within 10–30 s. Fry would rapidly swim from the bottom to the top of the tank and die. At approximately 30–49 mg liter^{-1}, mortality took several minutes. Fry would lose equilibrium, become darkened, lie on the bottom of the tank and die. At concentrations of 10–29 mg liter^{-1}, fry were observed to remain still, positioned near the surface and taking air at the surface. Mortality was variable within this range, taking from 12 to 24 h. The NOEL was 10 mg liter^{-1}.

4 CONCLUSIONS

Methyl anthranilate can be acutely toxic to fry at concentrations $\geqslant$ 20–35 mg liter^{-1}. The cyanotic condition and exaggerated opercular pumping of fry exposed to the higher concentrations of methyl anthranilate suggests that oxygen debt may be a cause of death. Methyl anthranilate is moderately lipophilic (it has an octanol:water partition coefficient of 83·4) and may easily be absorbed into gill lamellae membranes. Anthranilates are easily subject to metabolic hydrolysis.[26] While no assays for hydrolysis products in fry were performed, a likely hydrolytic product is anthranilic acid. If this were the case, and anthranilic acid was transported systemically, its presence might be sufficient to cause a pH shift of the plasma, thus affecting oxygen dissociation.

Only high concentrations of methyl anthranilate dissolved in water are of potential environmental concern. In separate experiments we have found that fry can consume up to 1000 mg kg^{-1} of methyl anthranilate incorporated into diet formulations without adverse effects (Clark & Aronov, unpublished). Because of the moderate affinity of methyl anthranilate for lipids, we

found that partition of methyl anthranilate from food into water yielded only 10 mg liter^{-1} under test conditions.

These observations will prove useful in setting guidelines for application rates of active ingredients. The impact of methyl anthranilate on fry may be reduced without affecting its bird-aversive qualities by using formulations which decrease partitioning into water. While formulations may protect methyl anthranilate from microbial and photodegradation, release of unprotected methyl anthranilate into water may expose it to microbial attack. Combined with its minimal impact on vertebrates when ingested, the outlook for methyl anthranilate as an environmentally safe repellent appears good.

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