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Stabilization of the avicide 3-chloro-*p*-toluidine (CPTH) on rice baits by pseudo latex polymeric coating and *β*-cyclodextin inclusion was investigated. When CPTH-treated rice baits were exposed to sunlight, the CPTH formed colored compounds, which exacerbated problems with bait acceptance and efficacy. Fluidized bed coating with controlled-release polymeric psuedo latexes (RS, RL, NE) reduced CPTH loss but did not eliminate color formation. Enteric pseudo latex coatings (CAP and 4110) gave mixed results. Coating 4110 reduced CPTH loss but still allowed color formation, whereas CAP allowed more CPTH loss than any other coating as well as increased color formation. Inclusion in *β*-cyclodextrin (molar ratio 1:1) led to enhanced retention of CPTH and minimal color formation. The CPT/*β*-cyclodextrin adduct increased retention of CPTH from 43 to 70% upon simulated weathering. The retention was independent of the adhesives used for attachment.

Keywords: *3-Chloro-p-toluidine, CPTH; pseudo latex coatings; β-cyclodextrin; inclusion adducts*

INTRODUCTION

Birds have long been noted to be in conflict with man's interests. Feral pigeons (*Columba livia*) induce losses due to crop depredation, contamination of feed and food products, and building deterioration and serve as a vector for disease transmission (Scott, 1961; Kreps, 1974; Krzysik, 1987). Massive flocks of birds that cause extensive crop losses include great-tailed grackle (*Quiscalus mexicanus*), which cause damage to citrus fruit in the Rio Grande Valley of Texas (Glahn et al., 1995), and red-wing blackbirds (*Agelaius phoeniceus*), which cause severe depredation to sprouting rice in Louisiana (Besser, 1985).

The avicide 3-chloro-4-methylbenzenamine hydrochloride (CPTH), has been used for >30 years for pest bird control (Decino et al., 1966). This chemical has high toxicity to most pest birds and low to moderate toxicity to most predatory birds and almost all mammalian species (Schafer et al., 1991). Unfortunately, CPTH also has a sporadic record of success ranging from excellent bait consumption and subsequent population suppression to rejection of treated bait and little effect on pest population (S. Blom, Pocatello Supply Depot, Pocatello, ID, personal communication, 1998). Anecdotally, the cause for rejection has been placed on either the strong odor of the treated baits, likely caused by the free amine (CPT), or the orange-red discoloration of bait caused by presumed degradation products. The standard approach to bait preparation is to apply the CPTH as an aqueous slurry or as a slurry in a viscous liquid such as syrup, corn oil, molasses, Alcolec soy lecithin or to utilize these liquids as adhesives for the powdered concentrate. This treated bait is often diluted with untreated bait varying with the bird species and the numbers of birds involved. This dilution aids in the return of birds to the feeding areas over extended time and ultimately increases efficacy. Observations of Wildlife Services personnel concerning their experiences with CPTH indicate that with smaller numbers of birds, the level and frequency of rejection are significant even when treated baits are not diluted with untreated bait. Massives flocks of birds generally show much less rejection, presumably due to competition for the food, and the treated material is diluted 25:1 with untreated bait for more efficient coverage. Degradation products of CPTH in soil, water, and watermelon have been studied. The reaction of CPTH with monosaccharides in watermelons at least partially explains a source of failure for some of the standard application methods that incorporate sugars as adhesives (Tawara et al., 1996; Primus et al., 1997). Colored degradation products include two fully characterized dimeric compounds and two partially characterized trimeric compounds (Smith 1978). This study was undertaken to develop a formulation with increased stability and improved acceptance by target species.

METHODS AND MATERIALS

Materials. The following chemicals were obtained from commercial suppliers and used as received or as noted: ethanol, acetonitrile, 2-chloro-4-aminotoluene (CPT), cellulose acetate hydrogen phthalate (CAP), cellulose acetate butyrate (CAB), acacia, poly(vinyl alcohol), triethyl citrate (Aldrich Chemical Co.); Eudragits RS30D, RL30D, NE30D, L-100, RS-100, and 4110 (Rohm); Tween 80 (Sigma Chemical Co.); Aquateric (FMC); Uvinul P-25 (BASF Corp.); Escalol 567 (ISP Van Dyk); AlphaFil 500 USP (Luzenac America); brown rice (local supplier); Alcolec S lecithin (American Lecithin Co.); *β*-cyclodextrin (Cerestar USA, Inc.); SE21 antifoam (Wacker); and mineral oil (Squib).

CPTH was prepared by neutralization of the free amine with hydrochloric acid, evaporation to dryness, and recrystallization from ethanol/acetonitrile over two to four cycles to remove all visible color.

Methods. A solution of CPTH (10 g) and acacia (2 g) in 100 mL of water was sprayed onto rice in a fluid-bed coater (Strea 1 Aeromatic Fielder, Wurster insert, Niro Inc., Columbia, MD;

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500 g charge; inlet temperature, 40 °C; spray rate, 2–5 g/min to obtain adduct layered rice at 2% nominal loading). Talc, emulsifier (Tween 80), and plasticizer (triethyl citrate) were dispersed in the dilution water, stirred for 5–10 min, and added into the appropriate polymer pseudo latex, and the combined suspension was poured through a 100 mesh screen to remove aggregates. The antifoam agent, SE21, was added last. The entire mixture was continuously stirred during the spray application period. The polymer pseudo latexes were sprayed onto the coated rice in the fluid-bed coater (500 g charge; inlet temperature for RS, RL, NE at 30–35 °C, for 4110 at 35–40 °C, and for Aquateric at 60–65 °C; spray rate, 3–5 mL/min; preheating time, 5 min; post-drying time, 5 min except Aquateric, which was 30 min) until the desired layer was achieved. The coated rice particles were oven-dried at 40 °C overnight. The concentrations, ratios, temperatures, and post-treatments for each coating were in accord with manufacturers' directions. The antioxidant and ultraviolet absorber were added to the CPTH/acacia solution prior to coating when these additives were evaluated.

The treated rice particles underwent simulated outdoor exposure in a Revco environmental chamber set at a 12/12 h day/night cycle, with day temperature of 35 °C and relative humidity of 80–90% and a night temperature of 20 °C and relative humidity of 65–75%. Samples were placed in plastic disposable weighing dishes. The light intensities as measured with a UVX Radiometer 97-0015-02 were 365 nm, 77.5 μW/cm^2; 310 nm, 27.0 μW/cm^2; and 254 nm, 3.6 μW/cm^2.

The β-cyclodextrin inclusion adducts were prepared by suspending the cyclodextrin in water, warming the water to 65–80 °C (until a clear solution was obtained), adding the CPT or CPTH to the solution with stirring, continued warming for 0.5 h, slow cooling to room temperature over 1–3 h, cooling to 5 °C, vacuum filtration, and drying at 100 °C to a constant weight. For example, 250 g of β-cyclodextrin was suspended in 1200 mL of water and warmed to 80 °C; 30 mL of CPT was stirred into the solution (initially a dispersion, but within 15 min was in solution and precipitation of the adduct began shortly afterward), which was stirred for an additional 30 min at the higher temperature, stirred for 3 h with a gradual return to room temperature, cooled in the refrigerator for ~4 h until the temperature reached 5 °C, vacuum filtered, and dried at 100 °C in an air flow oven to a constant weight. Cyclodextrin adduct (232 g) was recovered with 13.5% CPTH inclusion for an overall 82% recovery. Additional concentration of the filtrate gave more recovered product but with a much lower CPTH inclusion rate and was not continued in standard preparations.

The β-cyclodextrin–CPT adduct was coated onto the rice with either Alcolec 3S (3:1 mixture of Alcolec S and mineral oil) or Elmer's Glue All diluted with water 1:4. The rice was placed in a Kitchen Aid KS55 mixer equipped with a paddle stirrer. Approximately two-thirds of the sticker was added, and the rice–sticker mixture was stirred at a low speed for 3–5 min. The β-cyclodextrin–CPT powder was dusted in and stirred for 2 min. The remainder of the sticker was added and stirred for 3 min, and the coated rice was dried in a 40 °C oven for 2–3 h. This material was stored in sealed plastic bags in a refrigerator (5 °C) until use. Polymer coatings for waterproofing of these materials were done in the fluid-bed sprayer as delineated above. The exposure testing was carried out in the Revco environmental chamber, as above.

CPTH was recovered from samples by extraction into pH 2 acetonitrile/water (80:20–60:40). Samples were sonicated for 10 min, centrifuged as necessary, and diluted as appropriate in acetonitrile/water (80:20). Quantification of CPTH was accomplished using a Hewlett-Packard 1090M HPLC equipped with a Hewlett-Packard diode array detector at 241 nm and an Alltech Econosil C-18, 5 μm, 4.6 mm × 25 cm column. The injection volume was 10 μL. A mobile phase of 80:20 acetonitrile/water was used at a flow rate 1.0 mL/min. The column was at room temperature (~22 °C). Quantification of CPTH was determined by comparison of sample response to an external standard curve. Generally, three repetitions of each sample were analyzed.

Table 1. Exposure Stability of CPTH-Rice Coatings

treatment[b]	% CPTH retained[a] day 0	day 1	day 3	day 7	color intensity (1 = least)
water	100	87	53	44	7
acacia	100	89	75	36	8
PVAlc	100	91	64	47	6
standard (Alcolec 3S)	100	91	56	43	9
5% RS30D*	100	94	68	67	4
5% RS30D-P*	100	*c*	86	66	*c*
10% RS30D*	100	95	69	68	3
10% RS30D-P*	100	*c*	91	75	*c*
10% RS30D-E*	100	*c*	83	70	*c*
15% RS30D*	100	96	78	81	2
10% NE30D*	100	99	68	70	1
10% RL30D*	100	92	70	66	5
10% CAP	100	91	48	37	unique color[d]
10% 4110	100	93	74	78	unique color[d]

[a] Mean values of three experiments; standard deviations were ±3%. [b] RS and RL are water insoluble polyacrylic esters with 6 and 3% trialkylamino substitution; NE is a water insoluble polyacrylate ester. These three are controlled-release coatings. CAP is phthalate-substituted cellulose acetate, and 4110 is a partially esterified polyacrylic acid; both are enteric coatings. Standard is 0.75% Alcolec 3S. P is an UV absorber, and E is an antioxidant. All entries marked with an asterisk (*) had 30–50% talc in the resin coating matrix. [c] Data are from a separate series and were not analyzed on day 1 nor included in the color comparison. [d] The unique color was orange-red, but had a shiny surface, did not extract well, and was not included in the comparison.

RESULTS AND DISCUSSION

A preliminary investigation was done to determine an acceptable carrier/adhesive for the initial layering of the CPTH on the rice kernels. Under the same coating conditions (temperature, air flow, and spray rate), CPTH (10%) in 100 mL of water, 2% poly(vinyl alcohol), or 2% acacia was applied to 500 g of brown rice. The acacia solution gave the most efficient transfer of CPTH and was used as the standard application for all applications, except as noted. Interestingly, the acacia, when used alone, also allowed the greatest loss of CPTH of those three during environmental chamber stability testing.

Typically each batch of rice (500–2000 g) was sprayed with the CPTH–acacia solution just prior to use to avoid unnecessary degradation of CPTH. This method led to some variation in concentration of CPTH between batches, but, as we were more concerned with relative changes in concentration and color development, the differences in starting concentrations were deemed to be acceptable.

Coatings ranged from simple solutions of CPTH in water sprayed on the rice to fluidized bed coated layers of resins for controlled release or enteric style at several layering rates. Representative samples from each of the application modes were placed in plastic boats in an environmental chamber, and aliquots were removed at 0, 24, 72, and 168 h and stored in a freezer until analyzed.

As seen in Table 1, the treatments with the least retention of CPTH after 1 week of simulated weathering were water, acacia, poly(vinyl alcohol), standard Alcolec 3S, and CAP. These all are essentially equivalent and equated to no added protection. The CAP unique behavior might be due to the phthalic acid causing an acid/base surface capture of CPTH and ultimately enhanced loss. The controlled-release polymer coatings (RS, RL, NE) retained more CPTH and had less color development due to a combination of a less permeable

Table 2. Formation of the β-Cyclodextrin–CPT Adduct under Varying Molar Ratios during Preparation

β-cyclodextrin/CPT	recovery,[a] %	%CPT[b] (as CPTH)
1:1	84	13.5
1:2	93	13.5
1:4	89	13.4

[a] Mean values of two experiments; standard deviations were ±3%. [b] Mean values of two experiments; standard deviations were ±1.5%.

Table 3. Stability of the β-Cyclodextrin–CPT Adduct (Molar Ratio 1:1)

treatment	% CPTH remaining[a]
no exposure	100
1 week in refrigerator—open to air	96
1 week in window—open to air	93
20 weeks in closed bottle	91

[a] Mean values of two experiments; standard deviations were ±1.5%.

layer and, more likely, physical shielding by the talc as seen in the 5, 10, and 15% RS series. The addition of UV absorbent (Uvinol P-25) and antioxidant (Escalol 567) gave small, but not striking, increases in retention of CPTH. At this point, the retention of CPTH had been essentially doubled and progress looked favorable. However, when several of the better coatings were exposed under field conditions in Louisiana, all developed objectional levels of coloration. Unfortunately, CPTH was capable of color formation even when embedded in a polymer matrix with physical shielding by talc and protection by light absorbers and antioxidants.

The need for molecular isolation to eliminate intermolecular reactions appeared to be absolute. Precipitation with an acidic polymer (L-100) and microencapsulation in CAB, CAP, and RS-100 utilizing emulsion solvent evaporation were done. However, none removed the problem of coloration upon exposure to light and are not detailed here. Extensive literature reports for a wide variety of molecules suggested cyclodextrins should allow isolation of CPTH and therefore reduced photodegradation due to coupling (Albers and Muelller, 1995; Szejtli, 1984).

The inclusion of either CPT or CPTH in β-cyclodextrin was done using methods described earlier. The CPTH–cyclodextrin adduct was much more water soluble than the CPT–cyclodextrin and required the addition of isopropyl alcohol to give a reasonable yield. The CPTH incorporation was much lower at 6.2%, showed excessive coloration when exposed to light, and was discontinued. The CPT inclusion was generally 13–13.5% CPTH, which implied a 1:1 ratio. β-Cyclodextrin (1135 MW) plus CPTH (178 MW) in a 1:1 adduct (1313 MW) calculated to 13.6% based on CPTH. The CPT–cyclodextrin adduct was prepared under 1:1, 2:1, and 4:1 molar ratios. Comparable yields were recovered from all three sets, and the concentration of CPTH inclusion was constant at 13.4%, corresponding to a 1:1 molar ratio (Table 2). The CPT adduct only slowly released CPT even at room temperature in direct sunlight, had very low detectable odor, had no color development after 1 week of simulated exposure, and showed stability for at least 20 weeks in a sealed bottle at room temperature (Table 3).

Rice baits treated with the β-cyclodextrin–CPT adduct were prepared using our traditional adhesive of Alcolec 3S (a moderately nonpolar liquid). Rice baits were also prepared using diluted white glue (Elmer's Glue All), a more polar adhesive, to assess any effect of the adhesive on the opportunity for photocoupling and coloration. Results of 1 week of simulated weathering are listed in Table 4. The retention of CPT has been increased over our standard application and color formation decreased if not eliminated. The adhesives showed no difference in retention of CPTH or development of color.

Table 4. Exposure Stability under Simulated Weathering of the Rice Baits Treated with the β-Cyclodextrin–CPT Adduct (Molar Ratio 1:1)

	% CPTH remaining[a]				
treatment	day 0	day 1	day 3	day 7	color
rice–β-cyclodextrin–CPT–Alcolec 3S	100	97	83	70	no
rice–β-cyclodextrin–Elmer's glue	100	90	86	73	no
rice–CPTH–Alcolec 3S (standard)	100	91	56	43	yes

[a] Mean values of three experiments; standard deviations were ±3%.

Inclusion of CPT in β-cyclodextrin has proven to be a more stabilized form and to be compatible with application to rice bait under simulated weathering conditions. Recognizing that laboratory and field conditions differ, this CPT–β-cyclodextrin inclusion adduct will be coated onto rice bait and evaluated for bird acceptance and efficacy with both large and small flock situations through cooperation of Wildlife Services field personnel. Microcoating of the CPTH–β-cyclodextrin particles prior to application on baits to further reduce CPT loss and associated coloration will be investigated.

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