The Effect of Time on the Recovery of DRC-1339 Residues from Tissues Collected from Decomposing Mourning Dove Carcasses

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ABSTRACT: Due to the slow-acting nature of the avicide, DRC-1339 (3-chloro-*p*-toluidine hydrochloride), birds may travel considerable distances following ingestion of lethal quantities of treated bait. Confirmation of DRC-1339 bait consumption in bird carcasses collected temporally and spatially removed from a baiting site has been problematic, particularly after a prolonged period of time, due to the decomposition of the carcass. To establish a temporal baseline for analytical methods developed for determining DRC-1339 residues in bird tissues, mourning doves were fed 2% DRC-1339-treated rice or control untreated rice. Birds were euthanized after 24 hrs. Bird carcasses were allowed to decompose for 1, 2, 7, 14, or 21 days at ambient temperatures (22.4 ± 0.7°C). GI tract and breast tissue samples were collected and solvent extracted for analysis by GC/MS for DRC-1339 residues. Residues could be detected in the GI tract samples up to 14 days after dosing. Linear regression analysis of the data indicated the possibility of detecting residues in GI tract samples for up to 23 days under similar environmental conditions for carcass decomposition. These results provide a time frame under which it is possible to confirm DRC-1339 bait consumption in birds by analyzing for residues in tissues from carcasses collected after a baiting operation.

KEY WORDS: avicide, CPTH, DRC-1339, residue

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INTRODUCTION

DRC-1339 (3-chloro-*p*-toluidine hydrochloride, or CPTH) is registered for use as an avicide. Use practices include a 2% CPTH rice bait formulation. It can be used to control 18 primary target species of pest birds. Early methods for characterizing CPTH exposure in birds were based on dissecting the carcass and looking for physiological characteristics of CPTH exposure, including the accumulation of uric acid deposits in the peritoneal and pericardial cavities (DeCino *et al*. 1966, Schafer 1991, Johnston *et al*. 1999). Early analytical methods for assaying residues in tissues were perceived to be labor intensive, time consuming, and produce results that were highly variable (Cummings *et al*. 1994). Improvements in sensitivity and repeatability were made using a deuterated surrogate of CPTH (Hurlbut *et al*. 1998). This method suffered from poor recoveries of CPTH in tissues collected from birds in the wild (Stahl *et al*. 2002) or in tissues collected in a time frame exceeding 18 hrs following death (Johnston *et al*. 1999). To address these limitations, we developed and validated a method to accurately determine the CPTH concentration in bird tissues collected in the field following ingestion of CPTH-treated rice bait. This study was conducted to determine the time range following consumption of the bait in which a carcass had to be sampled for subsequent CPTH residue analysis.

METHODS

Mourning doves (*Zenaida macroura*) were collected by Wildlife Services personnel at Luke Air Force Base, Arizona, under the appropriate federal Fish and Wildlife Service permit and Arizona Game & Fish Department license. The birds were transported to Fort Collins, CO and upon arrival were banded, weighed, and dusted for mites. The birds were individually housed in cages in a climate-controlled room of the Animal Research Building, USDA National Wildlife Research Center, Fort Collins, CO. The target temperature was 23°C through the duration of this portion of the study. Lighting was set at 90% of full intensity and set to turn on at 0500 and turn off at 2050 with a 30-min fade up or down. Birds were fed *ad libitum* maintenance feed comprised of white millet, red millet, cracked corn, rape seed, canary seed, stripped sunflower seed, thistle seed, peanut pieces, and brown rice. The maintenance diet was removed the day before dosing to decrease the likelihood that the birds would regurgitate the treatment.

Fifteen birds of mixed gender were used for the study. The gender of a bird was determined post mortem by establishing the presence of testes during dissection. Five of the birds were randomly designated to receive a control treatment of 10 grains of rice, while the remaining 10 birds each received 10 grains of 2% CPTH-treated rice. The grains were individually placed in the throat of a bird and the throat massaged to encourage swallowing. Occasionally, during dosing, 1 or more seeds would be dropped on the floor; these were not recovered. The number of seeds a bird received was recorded. The birds were returned to their cage and observed for 1 hour. Regurgitated grains were counted to permit determination of dose. One hour after dosing, maintenance feed was returned to the cages. Birds were checked at 1100 and 1500 for mortality. After 24 hrs, all surviving birds were euthanized. Carcass weights were determined post mortem.

The 2% CPTH-treated rice was analyzed by reverse phase high performance liquid chromatography using an Agilent 1090 HPLC (Agilent, Palo Alto, CA) to establish the actual dose received by the birds. To allow for the calculation of amount of CPTH received by each bird, each treatment of 10 individual rice grains were weighed into vials. The contents of these vials were then used to dose the birds.

The carcasses were placed in plastic bags left open to the atmosphere in a fume hood. The plastic bags were used to aid in handling the carcasses and control body fluid loss either through evaporation or leakage. Temperature in the hood was monitored with a HOBO temperature monitor (Onset Computer Corp., Bourne, MA). Carcasses were randomly assigned to a decomposition interval of 1, 2, 7, 14, or 21 days. On the day of sampling, 1 control bird and 2 dosed bird carcasses were removed from the fume hood and placed in a freezer at -12°C until analyzed. Additionally, the carcass mass was determined at the decomposition intervals of 7, 14, and 21 days to assess the magnitude of dehydration occurring for the carcasses at these sampling intervals.

In preparation for extraction, the carcasses were thawed and the GI tract and breast tissue were removed and ground using a Waring blender. The mass of the GI tract was determined prior to grinding. Sub-samples of the tissues were weighed, extracted, and analyzed according to Stahl *et al*. (2002). Each GI tract sample from a bird was sub-sampled twice for analysis. To summarize the method: 2 g of breast tissue or 1 g of GI tract tissue was extracted with a weak acid, releasing the CPTH. The CPTH was converted to the free base (CPT) by the addition of sodium hydroxide, then CPT was solvent-extracted in hexane and concentrated on a silica solid phase extraction column. The CPT was eluted from the column in *n*-butyl acetate, diluted with a deuterated surrogate standard in *n*-butyl acetate, and analyzed with an Agilent 5890 gas chromatograph and Agilent 6890 mass spectrometer. Fortified tissue samples from pigeon (*Columba livia*) were also analyzed simultaneously to establish the method limit of detection and the method limit of quantitation. The method limit of detection was defined as a concentration of CPTH that would produce a signal 3 times the baseline noise. The method limit of quantitation was defined as the concentration of CPTH that would produce a signal 10 times the baseline noise.

RESULTS

Post-mortem examination established that the control group was comprised of 2 males and 3 females, and the treatment group was composed of 3 males and 7 females. The mean carcass weights, post mortem, were 118.5 ± 5.1 g for the control group and 113.8 ± 11.7 g for the treatment group. These were not significantly different at $\alpha = 0.05$ using Student's t-test assuming equal variance.

The treated birds were dosed with 0.187 ± 0.009 g (mean ± 1 std dev.) of treated rice. The measured concentration of CPTH on the rice averaged 2.1%. Thus, the mean dose a treated bird received was 3.64 ± 0.45 mg of CPTH or 32.6 ± 6.5 mg/kg on a body mass basis. Following dosing, 2 of the treated birds were observed to have regurgitated 3 rice grains each, and at 24 hrs both of these birds were listless with ruffled feathers. Three of the other treated birds were found dead at the end of the 24-hr observation period.

The bird carcasses were placed in a fume hood to mitigate undesirable odors during the decomposition process. Over the 21-day decomposition interval, the mean air temperature in the hood was 22.4 ± 0.7°C with a range of 21.3-28.3°C, and the relative humidity in the hood had a mean of 29.6 ± 6.9% with a range of 13.1-46.9%. Appreciable water loss was observed for carcasses over the 21 day period with mean losses of 4.9 ± 1.3 g at 7 days, 9.6 ± 3.3 g at 14 days, and 15.5 ± 7.6 g at 21 days. Dissection recovered an average of 26.1 ± 3.6 g of breast tissue and 6.3 ± 1.5 g of GI tract from all the birds for analysis.

The analysis of fortified pigeon tissues provided minimum limits of detection (MLOD) of 0.021 and 0.011 µg/g CPTH in the breast and GI tract samples, respectively. The CPTH concentrations for all the control samples (birds fed untreated rice) were below the MLOD for both the breast tissue samples and the GI tact samples. The CPTH concentrations at the decomposition intervals of 1, 2, 7, and 14 days were 0.022 ± 0.026, 0.043 ± 0.002, 0.033 ± 0.038, and 0.055 ± 0.015, respectively. The CPTH concentrations determined for the breast tissue samples were only used to infer qualitatively that the birds were exposed to CPTH as all the concentrations determined for CPTH in the breast tissue fell below 0.070 µg/g, the method limit of quantitation (MLOQ). For both the breast tissue and GI tract samples, the CPTH concentration after a 21-day decomposition interval was below the MLOD, 0.021 µg/g for the breast tissue, and 0.011 µg/g for the GI tract samples. The GI tract samples from tissues collected at decomposition intervals from 1 to 14 days had CPTH concentrations above 0.036 µg/g, the MLOQ for this matrix. The average CPTH concentrations observed for the decomposition intervals were 0.195 ± 0.105 at 1 day, 0.421 ± 0.235 at 2 days, 0.152 ± 0.177 at 7 days, and 0.055 ± 0.110 at 14 days.

A regression was performed on mean CPTH concentration and the decomposition interval (days). The resulting expression was:

$$CPTH\ concentration\ (\mu g/g) = 0.3704 - 0.01587 \times days,\quad r^2 = 0.6788$$

Solving this equation for the number of days for the CPTH concentration to reach an average MLOD of 0.011 µg/g (the x-intercept, y = 0.011) produced a result of 23 days. This was slightly longer than the time frame actually used in the study, but of similar magnitude.

DISCUSSION

The detectable levels of CPTH in the breast tissue over a 14-day decomposition interval qualitatively reflected the exposure of the birds to CPTH. The levels were below the MLOQ and were not accepted as accurate. Concentrations determined above the MLOD, but below the MLOQ, had a high degree of variability associated with them and were not accepted as true concentrations, but as approximations.

The concentrations of CPTH measured in the GI tract samples were relatively high and reflected the large doses of CPTH the birds received. The ability to actually quantitate the amount of CPTH in these samples after a 14-day decomposition interval was remarkable, but due in part to the high dose the birds received. Recoveries in birds collected in the field post baiting would probably have lower detectable levels of CPTH for a shorter period of time, due to the use of the treated rice in a dilution of approximately 1 treated grain to 24 untreated grains of rice. The usefulness of the results of this study were that

they provided an operational window for the use of this method to determine CPTH concentration in birds found in the field following a baiting operation. Birds located within a week or two, post baiting, probably can be assayed using this method with some possibility of detecting CPTH residues in the GI tract or breast tissue, reflecting exposure to CPTH. The levels observed would not necessarily reflect the consumption of a toxic dose of treated bait. Birds collected in an interval longer than 2 weeks would not likely contain detectable levels of CPTH using this method, even at very high levels of exposure.

The process responsible for the loss of recovery of CPTH in tissues is not known. CPTH is a reactive molecule containing an amine group that allows the molecule to be chemisorbed on charged sites, in which case it may not be extracted. CPTH may also be degraded during decomposition and would not be detected. The amine group may also allow CPTH to be incorporated into another compound by the formation of an amide linkage (Tawara *et al*. 1996). CPTH may also be rapidly metabolized after uptake in the bird and the metabolites would not be detected.

Understanding the upper limit on the time constraint for the detection of residues of CPTH in bird tissues is important for a forensic analysis to determine probable cause of death. The slow-acting nature of DRC-1339 as an avicide makes it difficult to establish efficacy. The bait matrix may also greatly impact the recovery of CPTH from exposed birds. Johnston *et al*. (1999) reported an 18-hr period for determining residues in common grackles (*Quiscalus quiscula*) fed CPTH-treated watermelon bait when quantified with the method developed by Hurlbut *et al*. (1998).

SUMMARY

The results of this study indicate that it was possible to quantitate CPTH in the GI tract of bird carcasses allowed to decompose under mild climatic conditions for a period up to 2 weeks after consumption of 2% CPTH-treated rice bait. It was also possible to qualitatively establish the presence of CPTH in the breast tissue of these birds over the same 14-day interval, however the levels were not quantifiable.

The results of this study led us to conclude that the method we use to quantify CPTH residues in bird carcasses can be used to assess exposure to CPTH in carcasses collected in the field, where the suspect birds have been dead for a period less than 2 weeks under moderate climatic conditions.

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