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Determination of residue levels of alpha-chloralose in duck tissues

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Abstract: Alpha-chloralose (AC) is used to capture nuisance waterfowl so that they can be relocated. Concerns for food safety limit its use prior to or during the waterfowl hunting season. To determine its half-life in tissue, we administered AC to adult male (n = 7) and female (n = 5) mallard ducks (*Anas platyrhynchos*) via oral gavage. A gel capsule containing 100 µCi of radioactive AC (^{14}C-AC) and sufficient nonlabeled AC to produce a dose of approximately 30 mg/kg was given to each duck.The ducks were euthanized at 2, 6, 10, and 18 hours post-dosing (n = 3 per exposure period), and tissue samples were collected for radioactive analysis. Residues observed in edible tissues from ducks dosed with radioactive AC demonstrated a delayed uptake as the duck was under the anesthetic effects of the AC. Following the period when the ducks were unconscious, most of the AC was rapidly excreted from the body with mean half-life ($t_{½}$) values of 9.0 hours, 9.8 hours, and 9.1 hours for breast muscle, liver, and skin, respectively. An approximation of total excretion (99%) was made by taking 7 times the $t_{½}$, resulting in values of between 27 to 33 hours post-exposure. These results suggest that tissues from mallard ducks are safe for human consumption 48 hours after dosing, a period that is significantly shorter than the FDA-required 30-day withdrawal period.

Key word: alpha-chloralose, disposition, ducks, human–wildlife conflicts, immobilizing agent, radio-labeled

Removal or relocation of nuisance wildlife is a continuing problem that is being addressed by the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Services Wildlife Services (WS). Humane removal or relocation of nuisance birds, such as Canada geese (*Branta canadensis*) and various species of ducks, continues to be a primary goal of WS. One tool available for the removal of these birds is an immobilizing drug known as alpha-chloralose (AC; Borg 1955, Belant et al. 1999). The drug is a chlorinated acetal derivative of glucose prepared by condensing chloral with pentose or hexose sugars in the presence of sulfuric acid. It has been in use as a human and veterinary anesthetic since the 1890s (Hanriot and Richet 1893). Although the specific mode of action of AC is not fully understood, it is assumed to cause depression of the cortical centers of the brain (Williams 1966, Lees 1972, Silverman and Muir 1993), but at low doses it also produces some hyperexcitability (Balis and Monroe 1964).

In 1992, the U.S. Food and Drug Administration (FDA) granted WS permission to use AC under an Investigational New Animal Drug application to capture waterfowl (*Anatidae* spp.), pigeons (*Columba livia*), and American coots (*Fulica americana*). Sandhill cranes (*Grus canadensis*) and common ravens (*Corvus corax*) were allowed by the FDA at a later date (Woronecki et al. 1992, O'Hare et al. 2007).

Widely used by the WS program to control pest populations of geese, such as Canada geese due to the absence of relevant excretion data, AC use is limited by a default moratorium for use 30 days before and during waterfowl hunting season. Birds captured within 30 days of or during open hunting season must either be euthanized or held in captivity for 30 days prior to release. An absorption, distribution, metabolism, and excretion (ADME) study conducted with radio-labeled AC is critical for generating data to determine a science-based use restriction period that protects food safety. If all AC derived residues are excreted prior to 30 days post dosing, it is likely that these data can be used to justify a reduction in the length of the pre-hunting season moratorium on AC

use. Also, if AC residues never reach significant concentrations in edible tissues, it may be possible eliminate the moratorium.

The objective of this study was to administer AC to mallard ducks (*Anas platyrhynchos*) and euthanize them at selected times post-dose and analyze edible tissues (breast muscle, liver, and skin) to determine residue levels. The data would be used to generate a half-life of elimination and to evaluate potential risk to humans from consumption of these tissues.

Methods

Test material

The test material for this study was AC obtained from TCI Chemicals (CAS# 15879-93-3; Lot# AT01; Tokyo, Japan). The material was a mix of the alpha and beta isomers and had a certified purity of 89.7%. The radiolabeled AC contained only the alpha-isomer and was ubiquitously labeled on the glucose ring ([glucosyl-^{14}C(U)]-alpha-chloralose; 283 mCi/mmol; 99% radiochemical purity; Perkin Elmer, Waltham, Mass.). The beta isomer of chloralose does not have significant narcotic activity and does not contribute to the dose delivered to each duck.

Test organism

AC was administered to adult male ($n = 7$) and female ($n = 5$) mallard ducks via oral gavage. The ducks were obtained from a commercial supplier and held in quarantine for 2 weeks prior to the start of the study. Each duck was weighed prior to receiving a dose of AC to ensure delivery of the correct dose. Average mass (±SD) of test animals was 1.2 ± 0.13 kg.

Test conditions

A gel capsule containing 100 μCi of ^{14}C-AC and sufficient nonlabeled AC to produce a nominal dose of 30 mg/kg body mass was given to each duck before placing it in a stainless steel metabolism cage. The stainless steel cage was placed inside a Plexiglas™ chamber and sealed shut. Air was delivered to the chamber by placing a vacuum pump on the exit tube from the chamber. Birds had free access to food and water for the duration of the test. All procedures involving animals were carried out with the approval of the National Wildlife Research Center (NWRC) Animal Care and Use Committee (Protocol QA-1465).

Sample collection

The time course was selected based on previous experiments that demonstrated that greater than 95% of AC in the feces of exposed ducks was excreted within 24 hours post dose (D. A. Goldade, National Wildlife Research Center Study Protocol QA-1060, unpublished report). For each time period (2, 6, 10, and 18 hours), ^{14}C-AC was administered to 3 ducks of mixed genders, as described in the section above, as a single dose. At the conclusion of each exposure time, the ducks were removed from their cages and euthanized via exposure to CO_2. Samples of breast muscle, liver and skin were removed from each duck. Tissue samples were cut into pieces <3-cm-long and placed in a -80° C ± 5° C freezer until frozen completely (typically for 2 to 3 hours). Each tissue sample was removed from the freezer and placed in a cold stainless steel Warring blender with between 1 and 2 times its weight of dry ice. The sample was blended to a powder and transferred to a Nalgene™ bottle of sufficient size to contain the entire sample.

Liquid scintillation counting analysis

Samples of breast muscle, liver, and skin were analyzed for extractable and total radioactive content. For the extractable fraction analysis, breast muscle, liver, and skin samples were removed from the freezer and weighed immediately to preserve them in a frozen state. A 1.0 g aliquot was weighed into a 10-mL screw cap glass test tube. Samples were extracted sequentially with acetonitrile (ACN), 0.1N hydrochloric acid (HCl), and 6N HCl.

Aliquots of the ACN, 0.1N HCl, and 6N HCl extracts were taken and placed into individual 20-mL plastic scintillation vials. Scintillation cocktail (20 mL of Scintiverse BD, Fisher Chemicals, Fair Lawn, N.J.) was added to all extracts, and radioactivity was determined using a Packard Tri-Carb 1600TR liquid scintillation counter (LSC; 4-156 kEV; 10-minute count time).

Total radioactive analysis

A slurry of each sample of tissue was

Table 1. Mean and standard deviations (n = 3) for total and extractable alpha-chloralose residue levels (mg/kg) in tissues of mallard ducks. N.D = not detectable.

Tissue	Time (hours)	Total		Extractable	
		$\bar{x}$	SD	$\bar{x}$	SD
Breast	2	14.4	1.3	13.6	1.4
	6	19.7	1.2	17.6	1.1
	10	9.7	1.6	8.3	1.9
	18	1.4	0.83	N.D.	
Liver	2	24.0	3.2	20.9	1.9
	6	32.2	1.3	24.1	0.69
	10	21.8	1.3	15.2	2.2
	18	5.2	3.7	N.D.	
Skin	2	6.9	2.3	6.2	2.0
	6	4.4	1.8	6.2	1.5
	10	3.3	0.78	3.5	0.71
	18	0.38	0.22	N.D.	

prepared by adding deionized water. The sample slurry was mixed, and an aliquot was weighed and combusted in an R. J. Harvey Model OX-600 Biological Oxidizer. Oxygen and nitrogen flows were 350 mL per minute. The combustion and catalyst zone temperatures were held at 900° C and 680° C, respectively; samples were combusted for 4 minutes. The CO_2 produced was trapped in a ^{14}C cocktail (R. J. Harvey, Tappan, N.Y.). The cocktail was transferred to a glass vial and counted on the LSC. Samples were counted according to the conditions listed above.

Statistical analysis

The Method Limit of Detection (MLOD) for each tissue was determined by following the general procedures provided by the Environmental Protection Agency (2011). The data were evaluated on the basis of concentration of AC and metabolites in parts per million of AC equivalents (Table 1; Figure 1). AC equivalents are defined as unmetabolized AC and all metabolites of AC containing ^{14}C. The results were log-transformed and subjected to a linear regression analysis. Results of this transformation were used to calculate elimination constants (K_{El}) and terminal half-life of elimination ($t_{½}$) values for each tissue between 6 and 18 hours post-dose.

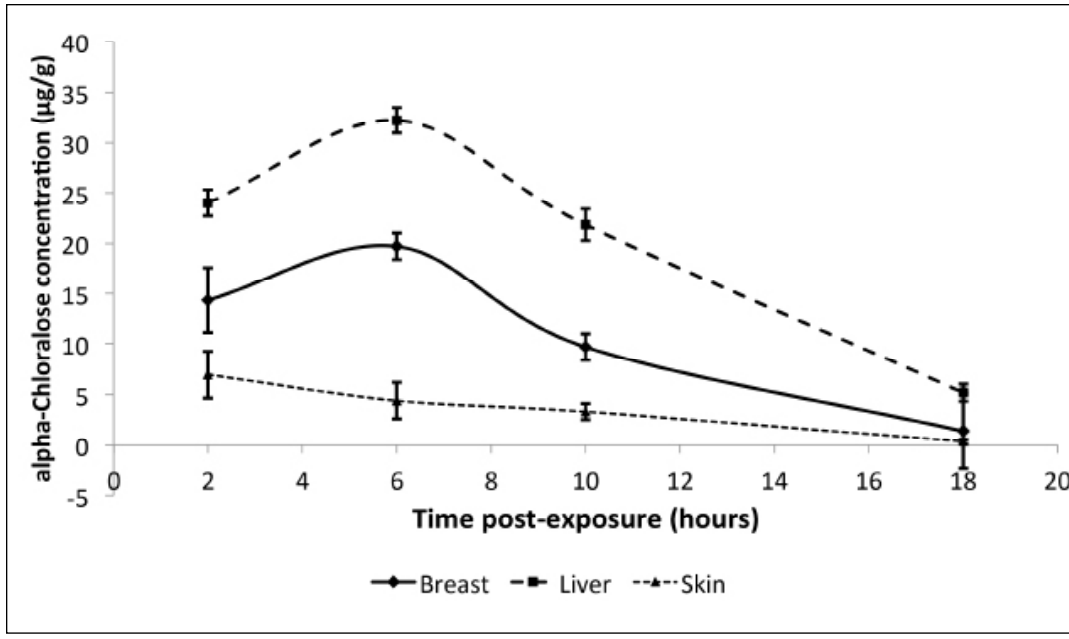

Figure 1. Elimination of radio-labeled alpha-chloralose (AC) and metabolites from mallard ducks (*Anas platyrhynchos*) following a single oral dose (n = 3/time point). Values plotted are means ±1 standard deviation.

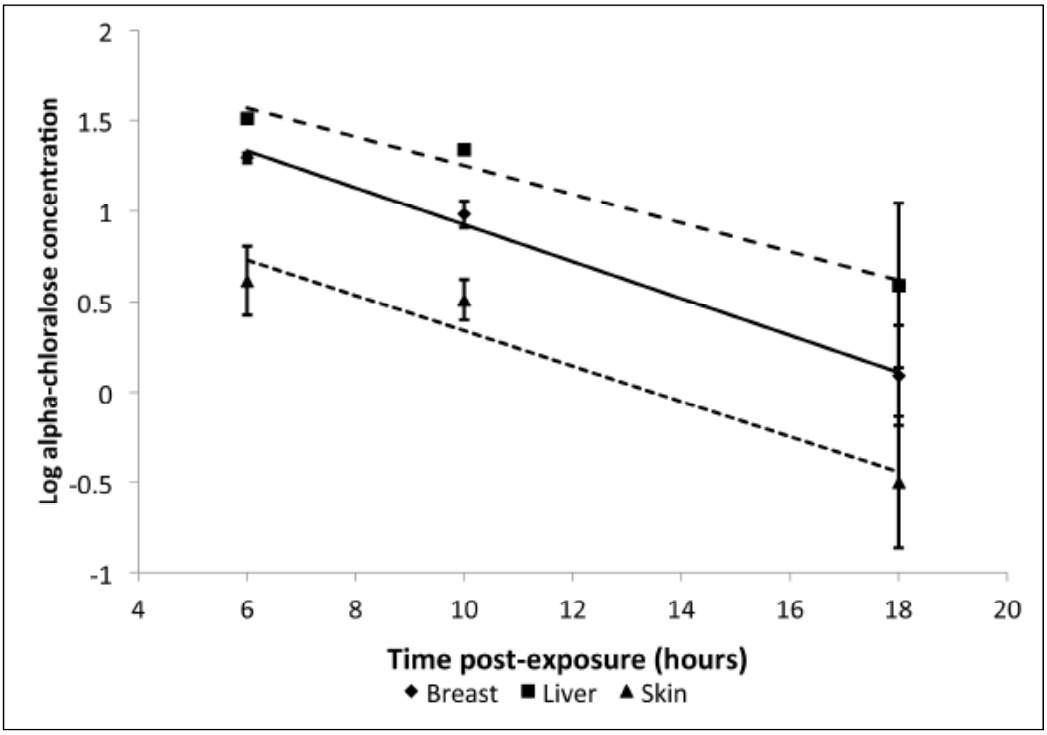

Figure 2. Logarithmic plot of elimination of radiolabeled alpha-chloralose (AC) and metabolites from mallard ducks (*Anas platyrhynchos*) following a single oral dose (*n* = 3/time point). Regression lines indicate the rate of elimination ±1 standard deviation.

The slope of this line yielded the K_{El}. From this value, the terminal half-life of elimination was determined. Because a half-life is commonly defined as the amount of time it takes for one-half of the remaining radioactivity to be excreted from the body, 100% elimination can never be mathematically achieved. A close approximation can be found using 7 times the value of $t_{½}$. This accounts for elimination of approximately 99% of the radioactivity.

Mean values from triplicate analyses were subjected to 2 analyses of variance. A mixed model (PROC MIXED; SAS, Cary, N.C.) was used to compare residues among the chemical extracts. Extract (ACN, HCl, reflux), matrix (breast, feces, liver, and skin), time (2, 6, 10, and 18 hours), all 2-way interactions, and the 3-way interaction were fixed effects. Subject was a random effect.

To compare AC residue data generated by extraction and combustion, values determined from all 3 extracts (ACN, HCl, reflux) were first summed for each sample to yield a total extract residue concentration. For analysis using a mixed model, a difference response was then calculated by subtracting the total extract AC residue concentration from the combustion value. Matrix (breast, feces, liver, and skin), time (2, 6, 10, and 18 hours), and the interaction were fixed effects. Subject was a random effect.

Exposure estimation

To estimate the magnitude of potential AC exposure from the consumption of tissues from AC-treated fowl, the maximum observed concentrations of total AC derived residues in breast tissue, liver, and skin were multiplied by the upper 99th percentile estimate of acute chicken meat, liver, and skin consumption, respectively. Human chicken consumption statistics were obtained from the USDA National Health and Nutrition Examination Survey (NHANES 2001 to 2006) using SUDAAN Proc DESCRIPT. To determine the potential for any adverse effects associated with consumption of AC residues in poultry, the estimated exposures were compared to the estimated acute human oral no observed effect level (NOEL) of 0.13 mg AC/kg body weight. Human NOEL was estimated by applying uncertainty factors

Table 2. Linear regression statistics for log transformed elimination of alpha-chloralose from mallard ducks (*Anas platyrhynchos*) for exposure time 6 hours to 18 hours.

Tissue	R^2	Slope	p	S.E.	K_{El}	$t_{½}$ (hours)	95% C.I. of $t_{½}$ (hours)	99% Elimination (hours)
Breast	0.928	-0.102	0.00003	0.0108	0.235	9.0	8.4–9.9	27
Liver	0.757	-0.0791	0.002	0.0169	0.182	9.8	8.6–14	33
Skin	0.810	-0.0977	0.0009	0.0179	0.225	9.1	8.2–11	28

of ten for interspecies extrapolation and ten for conversion of lowest observed effect level (LOEL) to NOEL to the LOEL for chickens (13.88 mg/kg bodyweight; Loibl et al. 1988).

Results

The MLOD values determined were between 0.06 and 0.47 µg/g, depending on the tissue. The elimination of radioactivity from the edible tissues (breast, liver, and skin) all followed a similar pattern. Following ingestion of the dose, the birds became lethargic as their respiration slowed, and they were rendered unconscious by the AC. As such, their digestion also slowed and the time to maximum AC concentration was delayed. The actual maximal value likely occurred between 2 and 6 hours post-dose, as most of the ducks had returned to an alert state 2 or 3 hours post-dose. Therefore, the half-life of elimination for AC in these tissues was calculated using the 6-, 10- and 18-hour observations only. The results reflect this treatment of the data and take into account the delay in uptake of the AC (Table 2). For any future studies, a time point between 2 and 6 hours might be used to elucidate the true maximum residue value. The elimination values for each tissue, as well as the *P* value for the slope of the regression lines are given (Figure 2; Table 2).

The presence of strongly bound residues in exposed tissues could indicate the presence of highly reactive metabolites. To assess any potential tissue-binding of AC, the data generated from solvent extraction of the tissues (with ACN, 0.1 N HCl, and 6 N HCl) were compared to the results from combustion of the same tissues. An ANOVA procedure was used to evaluate the difference between bound and extractable AC residues at each time point. Residue values determined by the 2 methods differed by matrix ($P < 0.0001$), but was influenced strongly by time (matrix × time; $P = 0.0002$).The main effect of time was not significant ($P = 0.09$). In breast tissue, the combustion value was significantly greater than the extraction value only at time 6 ($P = 0.01$). In liver, the combustion values were greater at all times ($P < 0.0001$). In skin, the extraction value was greater only at time 6 ($P = 0.04$). In all cases, by 16 hours post-dose the extractable content had decreased below MLOD levels and was only detectable via the more sensitive combustion analysis technique (Table 1).

All fixed effects were significant ($P < 0.0001$) except for time ($P = 0.05$) and time × extract ($P = 0.07$). Owing to the significant extract × matrix × time interaction, the differences in extractable residues for the edible tissues also demonstrated some interesting characteristics. In all 3 tissues, most of the extractable AC was found in the ACN extract. Smaller amounts were extracted in the subsequent 0.1 N HCl extraction. No additional radioactivity was found in the 6 N HCl reflux extraction of skin samples. There were detectable residue levels in reflux samples from breast muscle at the 6- and 10-hour time points. Significant residues were found in the liver at the 2-, 6-, and 10-hour time points in all 3 extracts. These residue levels were noticeably more concentrated than in any other tissue.

Discussion

The selected dose of AC (30 mg/kg) was representative of a dose that might be received by a bird in an actual field capture scenario (NWRC SOP WRC-284.R1, Preparing alpha-chloralose treated grain and bread baits for field trials involving waterfowl and other birds, 1992, unpublished data). The published LD_{50} for the test species is 54.6 mg/kg (Williams 1966). No mortalities were observed during the experiments.

Following AC administration, ducks were carefully observed. A very rapid loss of motor

control and lethargy was observed in all of the test animals. To minimize errors in preparation of the dose, the total mCi of radioactive AC was held constant for each experiment. The radioactive dose was sufficient to produce a detectable signal with as little as 1% of the dose present in any tissue analyzed.

The estimated upper bounds of AC exposure due to consumption of AC-treated fowl breast tissue (Table 1) was 0.081 mg AC/kg_{bw} per day. The potential additional consumption of poultry skin would contribute ≤0.005 mg AC/kg_{bw} per day. This combined (meat and skin) upper bound exposure is less than the estimated human acute oral no-effect level for AC. Estimated upper bound AC exposure from consumption of liver from AC treated fowl was less than the AC exposure associated with breast tissue consumption. As one would expect human ingestion of AC treated fowl to be infrequent, comparison of the upper bounds exposure estimate with the estimated acute human no-effect level of 0.13 mg AC/kg bodyweight suggests that the risk of adverse effects due to consumption of AC treated fowl approaches zero. Further, this worst case assessment assumed that the AC-derived residues were intact AC. It is likely that <10% of the tissue residues are AC, with the remainder being less potent metabolites. This suggests the probability of AC associated adverse effects from the consumption of AC treated poultry is of no public health concern.

The residues observed in edible tissues from ducks dosed with radioactive AC demonstrate a delayed uptake as the duck is under the effects of the AC and exposure from consuming treated ducks during this time period would be expected to be quite high. Following the duck regaining consciousness, most of the AC and metabolites are rapidly excreted from the body, with nearly 100% being excreted by 27 to 33 hours after exposure. The magnitude of AC-derived residues suggests that the risk of adverse effects due to consumption of AC treated fowl is negligible at the end of the excretion period. These results support shortening the 30-day moratorium on use of AC to a much shorter duration.

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