White Snakeroot Poisoning in Goats: Variations in Toxicity with Different Plant
Chemotypes

T.Z. Davis^{a*}, B.L. Stegelmeier^a, S.T. Lee^a, M.G. Collett^b, B.T. Green^a, J.A. Pfister^a, T.J.
Evans^c, D.S. Grum^a, and S. Buck^d

^aUnited States Department of Agriculture, Agricultural Research Service, Poisonous
Plant Research Laboratory, 1150 E. 1400 N., Logan, UT, USA

^bInstitute of Veterinary, Animal and Biomedical Sciences, Massey University, Private
Bag 11222, Palmerston North 4442, New Zealand

^cThe Veterinary Medical Diagnostic Laboratory at University of Missouri-Columbia ,
Columbia, Missouri, USA

^dUniversity of Illinois at Urbana-Champaign, 1816 S. Oak St., Champaign, Illinois, USA

* *Corresponding author.* Address: Poisonous Plant Research Laboratory, ARS/USDA,
1150 E. 1400 N., Logan, UT 84341, USA. Tel.: +1 435 752 2941x1112, *E-mail address:*
zane.davis@ars.usda.gov (T.Z. Davis)

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Abstract

Tremetone and possibly other benzofuran ketones are believed to be the toxic compounds in white snakeroot. However, disease has not been reproduced with purified toxins and the concentrations of the benzofuran ketones in white snakeroot populations that cause toxicosis have not been documented. The objectives of this study were to compare the toxicity of seven plant populations, better characterize the clinical and pathologic changes of poisoning, and correlate intoxication with benzofuran ketone content. Four of the seven white snakeroot collections were toxic at the dose and duration used in the study. Affected goats became exercise intolerant, had significant serum enzyme changes and histological lesions in the large appendicular muscles. The incidence and severity of poisoning was not correlated with total doses of tremetone or total benzofuran ketone concentrations suggesting they may not be closely involved in producing toxicity and the possible involvement of an unidentified toxin. The results also demonstrate that white snakeroot populations vary chemically and toxicologically.

Keywords: white snakeroot, tremetone, benzofuran ketones, goats

1. Introduction

White snakeroot [*Ageratina altissima* var. *altissima* previously known as *Eupatorium rugosum* Houtt. also known as *Ageratina altissima* (L.) R.M. King & H. Rob. or *Eupatorium urticaefolium* Reichard] is an herbaceous perennial with stiff stems that grow to be 0.3 to 1.5 m tall. White snakeroot leaves are 7.5 to 15 cm long, have three distinct veins, are opposite and sharply serrated while forming a pointed tip. The plant is easily identified during early fall by its small, bright, white flowers that are in composite heads of 10 to 30 flowers. White snakeroot is most commonly found in the eastern half of North America in moist, shaded, uncultivated areas along stream beds, near tree lines or on terraces (Burrows and Tyrl, 2001). Historically it is of considerable significance because of the suffering it caused for many of the midwest settlers in the United States in the early 1800's. It is reported that in some parts of Indiana and Ohio up to 50% of the deaths of early settlers was a result of white snakeroot-induced "milk sickness" (Wolf et al., 1918). The disease was so named as it was caused by drinking tainted milk from a cow that had ingested the plant. Livestock are also poisoned at white snakeroot doses of 1 to 1.5% of their body weight (BW) ingested daily for 1 to 3 weeks with the disease being known as "trembles". It is so named as intoxicated animals develop violent trembling when they are forced to move or are agitated (Kingsbury, 1964). Poisoning of sheep, goats (Reagor et al., 1989), cattle (Meyerholtz et al., 2011), horses (Olson et al., 1984, Smetzer et al., 1983; White et al., 1985), and even humans (Hartman et al., 1963) have been reported.

Initial signs of poisoning in most livestock are depression, reluctance to eat, and inactivity followed by fine muscle tremors of the nose, flanks, and legs especially after

exercise or activity. The affected animal will often have tachypnea, tachycardia, a stiff gait, and altered posture as poisoned animals are reluctant to move and stand hunched up with a flexed back.

In 1918 it was shown that extracts of white snakeroot caused milk sickness and trembles (Wolf et al., 1918). Later Couch (1929) identified a straw-colored oil from white snakeroot that he named tremetol. Initially tremetol was believed to be a pure compound with the chemical formula of $C_{16}H_{32}O_3$. However, later work demonstrated that it was a complex mixture of terpenes, sterol and ketones including the benzofuran ketones (BFKs) tremetone, dehydrotremetone, 6-hydroxytremetone and 3-hydroxytremetone (Bonner and Degraw, 1962; Bonner et al., 1961; Bowen et al., 1963). Additional work demonstrated that rayless goldenrod (*Isocoma pluriflora*) also contains the same BFKs and produces similar diseases (Couch 1929; Burke 1966).

Cases of white snakeroot poisoning are sporadic and unpredictable. However, *in vitro* cell culture studies suggested that tremetone was the likely toxin due to its cytotoxicity in several different cell lines after its microsomal activation (Beier et al., 1987; Beier et al., 1993). Nevertheless, tremetone toxicity in animal models has not been proven. In fact, neither, sheep or cockerels developed disease when treated with synthetic tremetone (Bowen et al., 1963). Correlating clinical poisoning with toxin presence and concentrations in the plant has been hindered by the inability to quantitatively detect the specific BFKs thought to be associated with these toxicoses. Recently Lee et al. (2009) developed a quantitative high performance liquid chromatography (HPLC) assay to measure the concentrations of the proposed toxins tremetone, dehydrotremetone, and 6-hydroxytremetone in white snakeroot. Analysis of

white snakeroot from different plant populations identified plant chemotypes with distinct benzofuran ketone types and concentrations (Lee et al., 2010). The differences in chemotypes may explain the sporadic incidence of poisoning and suggests that simply comparing doses based on percent of BW of plant material ingested is unreliable. The objectives of this study were to compare the toxicity of various white snakeroot populations, characterize the clinical and pathologic changes of poisoning in goats, and correlate intoxication with BFK content and concentrations.

2. Materials and methods

2.1. Collection of plant material

Seven collections of white snakeroot were made in four states (Missouri, Illinois, Indiana, and Ohio). The plant material was taxonomically identified as white snakeroot (*Ageratina altissima*) by laboratory taxonomists and by Dr. Stanley L. Welsh, curator of the Stanley L. Welsh Herbarium at Brigham Young University, Provo, UT. The collection site coordinates, dates of collection, and accession numbers of voucher specimens are shown in Table 1. The white snakeroot plant was air-dried and stored in a dry cool room until used. The day before beginning the study, the plant material was ground to pass through a 2.38 mm screen and mixed using a Gehl Mix-All, model 55 (Gehl Company, West Bend, WI).

2.2. Benzofuran ketone extraction and chemical analysis by analytical scale HPLC

Concentrations of the benzofuran ketones, chromenes, and phenols were determined by reversed phase HPLC on a Shimadzu LC-20AT equipped with an autosampler and photodiode array detector (PDA) from the same vendor and a 100 mm x 2 mm i.d., 5 μ m, Betasil C₁₈ column (Thermo Hypersil-Keysone, Bellefonte, PA) using the procedure

reported in Lee et al. (2009). The compounds were quantified against a seven-point standard curve using previously isolated compounds. The standard curve was prepared over the range of 1.56 µg/mL – 100.0 µg/mL by serial dilution.

2.3. Dosing of the goats

Thirty-two, yearling female Spanish goats in good body condition that weighed 22.6 ± 2.2 kg with a range of 17.4 to 29.0 kg were selected from the same herd. The goats were randomly divided into eight groups ($n = 4$). One goat from the Columbia group was removed from the study on the fourth day because of dosing difficulties. The animals were trained to lead and to run on a treadmill for two weeks before the start of the study. Four extra goats were trained for the study, two goats were eliminated because they would not run without stopping and two other goats were randomly eliminated so that there would be four animals per group. The day before the initial dosing, all animals were weighed, ran on treadmill for 5 min and bled by jugular venipuncture. All goats (except for 3 goats in each the Interstate 09 and Interstate 10 groups whose treatments were discontinued on days 8 or 9 to prevent further stress) were dosed intraruminally via oral gavage, for 9 days, with freshly ground dried white snakeroot at 2% of their body weight each day. The control group was dosed via oral gavage with similar amounts of ground alfalfa/grass hay. The dose was split and given twice per day at 1300 and 2200 hours. The goats had access to water and long stem alfalfa hay *ad libitum* during the study. When animals became clinically affected (developed reluctance to stand, anorexia, or exercise intolerance) dosing was discontinued. All animals were humanely euthanized during the morning of day 10 after 9 days of dosing. At necropsy, samples of skeletal muscle approximately 0.5 × 1.0 × 4 cm in size were collected, attached to wood tongue

depressors, and fixed in formalin. Skeletal muscles collected included: retro-ocular, tongue, masseter, cricothyroideus, pectoralis major, biceps brachii, triceps brachii, intercostals, diaphragm, longissimus dorsi, psoas major, biceps femoris, quadriceps femoris, semimembranous, semitendinosus, adductor, and gluteus medius. Other tissues collected, fixed, and examined included: brain, spinal cord, thyroid gland, heart, lung, liver, spleen, pancreas, rumen, abomasum, duodenum, jejunum, ileum, colon, mesenteric lymph node, adrenal gland, kidney, and urinary bladder. Lesions were subjectively scored by two pathologists (BLS, MGC) as : 0 = no change; 1 = minimal with myocyte degeneration characterized by rare myocyte swelling and hypereosinophilia; 2 = mild with occasional myocyte degeneration (myocyte swelling and hypereosinophilia) with rare myocyte necrosis; 3 = moderate with patchy myocyte degeneration and necrosis involving small portions (< 35%) of the skeletal muscle fibers or sample; and 4 = severe with extensive myocyte degeneration and necrosis with or without subsequent inflammation and regeneration with diffuse distribution involving 40% or more myocytes. All animal work was done under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee.

2.4. Measure of muscle strength and endurance

The ability of the goat to run on a treadmill was used as a measure of muscle strength and endurance. Goats were run on a treadmill (Horse Gym 2000 GmbH, GroBsorheim, Germany) starting at 0700 hours, at a 10 % incline, moving at approximately 12 kilometers per hour, for 5 minutes. If movement became labored and the animal was

unable to keep pace before the 5 minute running period was complete the animal was taken off of the treadmill and considered to be exercise intolerant.

2.5. Serum analyses

Hematology, serum biochemistries, and serum electrolyte analyses were performed using standard techniques with an automated hematology analyzer (Beckman Coulter Inc., Stak-S, Brea, CA) and a Hitachi 7180 biochemistry analyzer (Hitachi High Technologies Inc., Pleasanton, CA). Reagents and methodology recommended by the manufacturer were used. Analytes of clinical significance in these animals included the activities of lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK), as well as the concentration of cardiac troponin I (cTn I).

2.6. Statistical analyses

All serum biochemistry results from this study were analyzed as a completely random design with seven treatments. The model included treatments (Allerton, VRO, Interstate 2009, Interstate 2010, Columbia, Zanesville, and Wabash) with individual animals nested within treatments, and repeated measures over the multi-day experiment. Animals were a random factor in the mixed linear model analysis using the procedures of SAS, Version 9.1 for Windows (SAS Inst. Inc., Cary, NC). The primary independent variable in the study was the plant population. The variance-covariance matrix was chosen by an iterative process wherein the best fit was based on the Schwarz Bayesian criterion. The unstructured and compound symmetry covariance models were often the best fitting structures. Treatment differences were separated using predicted difference (PDIF option in SAS) for significant interactions ($P < 0.05$) in the model. Pre-planned

pairwise comparisons between treatment least squares means were considered different at $P < 0.05$. Mean histologic scores were compared and correlations made using the Mann-Whitney non-parametric test to analyze the ordinal score data with no assumption of a normal distribution. Differences were separated using a Kruskal-Wallis test and considered significant when $P < 0.05$. Total histologic scores were calculated and used to determine a correlation coefficient with CK activity or BFK concentrations.

3. Results

3.1. Dose determination

The concentrations of the benzofuran ketones (tremetone, dehydrotremetone, and 6-hydroxytremetone), chromenes, and phenols from the lipophilic extracts of the white snakeroot populations dosed in this study were determined by reversed phase HPLC (Table 2). No measureable amounts of 3-hydroxytremetone were found in any of the collections. As each collection was dosed to the goats at 2% of their BW and dosing was discontinued in animals that developed severe clinical disease on day 8 or 9, the resulting daily and total doses of BFKs and the individual compounds were unique for populations and individual animals (see Table 2).

3.2. Clinical signs

Three of four goats dosed with the Interstate 2009, Interstate 2010, and Wabash collections developed clinical signs of poisoning 6 to 7 days after the start of dosing of the white snakeroot plant material. By day 9, two of four goats dosed with the VRO collection exhibited clinical signs of poisoning. The clinical signs exhibited by these goats included decreased appetite, reluctance to stand, muscle fatigue and trembling which became much more severe after being forced to stand and exercise, muscle

soreness, and swelling of the large appendicular muscles. One goat from the Allerton group started to show some changes (exercise intolerance and increased CK activity) while all the other goats in the Allerton group and all goats in the Columbia and Zanesville groups did not develop clinical disease and their exercise tolerances, serum biochemical analyses, and histologic findings were similar to those of the controls.

3.3. Exercise intolerance

Typically, the clinically poisoned goat showed signs of muscle fatigue while running on the treadmill on approximately the same day that their serum CK activity began to increase. The following day the animal was generally unable to run for the entire 5 minutes and the treadmill was stopped before the exercise period was complete and the goat was determined to be exercise intolerant (Table 3). A general pattern was observed in which on the first day that the goat became exercise intolerant it would do so between four and five minutes of exercise. The following day the goat would be able to run for one to two minutes and the third day it would only be able to exercise for up to thirty seconds. On day 6, one goat from each the Interstate 2009, Interstate 2010, and Wabash groups became exercise intolerant. By day 9, three of four goats from each of the previously mentioned groups were exercise intolerant. Additionally, one goat from each the Allerton and VRO groups also became exercise intolerant on day 9.

3.4. Serum enzyme changes

Clinical disease was monitored biochemically during the study by assaying serum for the activities of specific enzymes (CK, AST, ALT, and LDH) and the concentration of cTn I in serum (Table 3). Noticeable differences in CK and LDH were observed in poisoned goats on day 6. However it wasn't until day 7 or 8 that the goats

dosed with the Interstate 2009, Interstate 2010, and Wabash collections had significant differences, in their CK, AST, ALT and LDH activities compared to the activities of the same enzymes on day 1. Two goats from the VRO group had elevated CK activities (7244 and 9926 U/L) on day 10, but they were not statistically significant for the group. There were noticeable changes in the cTn I concentrations in the serum of goats that were most severely poisoned from the Interstate 2009, Interstate 2010, and Wabash groups; however, the changes were not statistically significant. One goat from each the Interstate 2009 (3.67 ng/mL) and Wabash (4.12 ng/mL) group had very high cTn I concentrations. Most of the other goats that were clinically poisoned had cTn I concentrations in serum of 0.2 to 0.6 ng/mL.

3.5. Gross and histological lesions

At necropsy the animals that were clinically affected had skeletal muscle swelling and softness with occasional palor. Palor and pale streaking was most common in the large appendicular muscles. This was often difficult to differentiate from fatty infiltration that was often present in the axial muscles. No significant gross lesions were identified in the heart, kidneys, urinary bladder or any of the other visceral or neurologic tissues that were examined.

Histologically the affected animals had varying degrees of skeletal muscle degeneration and necrosis (Table 4). These severe lesions were most common in biceps brachii, biceps femoris, quadriceps femoris, gluteus maximus and psoas major. The lesions were monophasic within individual muscles but there was some polyphasia between different muscles. Lesions from different muscles from the same animal were often of different duration. Figure 1 demonstrates a section of biceps brachii from a goat

245 treated with white snakeroot from the Interstate 2010 collection. In this muscle there is
246 extensive myocyte necrosis with focally extensive inflammation and marked nuclear
247 proliferation and regeneration. The inflammation is primarily histiocytic with rare
248 lymphocytes and neutrophils. This more chronic and regenerative lesion is contrasted by
249 the changes from the biceps femoris from the same animal seen in Figure 2. In this
250 section the myocyte degeneration and necrosis is characterized by marked acute myocyte
251 swelling, hypereosinophilia with coagulation and clumping of sarcoplasmic proteins.
252 There is minimal inflammation (rare histiocytes) and nuclear proliferation.

253 Not all animals within groups developed clinical disease or histologic muscle
254 lesions. One goat treated with the Wabash collection and two of four goats treated with
255 the VRO collection were seemingly resistant to poisoning and their clinical findings,
256 exercise tolerance, serum biochemical analysis and histologic findings were
257 indistinguishable from those of the control goats. Some background histologic lesions
258 were found in all animals. These were most common in the tongue and were
259 characterized by rare and sporadic myocyte swelling and hypereosinophilia. As these
260 lesions were minimal and they never included secondary proliferative or inflammatory
261 responses, they are not treatment related and most likely artifact that may relate to muscle
262 physiologic state or agonal microfiber contracture at the time of euthanasia and fixation.
263 The myocardium of all of the animals was closely examined, especially in animals that
264 had elevated cTn I concentrations. Occasionally, the subendocardial and papillary
265 myocytes were swollen and hypereosinophilic, but these were found in all goats
266 including controls. No myocardial inflammation, fibrosis, or myocyte hypertrophy were
267 identified.

The liver of many of the clinically affected goats had minimal accumulations of lipid vacuoles in many hepatocytes. No other significant histologic lesions were observed in sections of kidney, urinary bladder, brain, spinal cord, lung, pancreas, stomach, intestine, colon, adrenal gland, thyroid gland, mesenteric lymph node or eye.

Clinical, biochemical and histologic changes were most closely associated with plant dehydrotremetone concentrations. The majority of animals that received total dehydrotremetone doses higher than 40 mg/kg BW over the 10 day study developed histologic lesions and when the total histologic score was plotted against dehydrotremetone dosed (Fig. 3) there was a correlation ($r^2 = 0.41$). However clinical disease and histologic lesions did not directly correspond to the total dose of total BFKs, tremetone, or 6-hydroxytremetone (Fig. 3). For example the VRO collection contained more than four times the total BFK concentration as the Wabash collection and only two of four goats dosed with the VRO collection developed the disease which also developed slower and less severely than those dosed with the Wabash collection. Additionally the concentration of tremetone was nearly twice as high in the Allerton collection as in the Wabash collection, however none of the goats dosed with the Allerton collection developed histologic lesions that were distinguishable from the control group and only one of the goats became exercise intolerant and had moderate elevations in CK activity.

The total histology grade and the highest CK activity of each goat dosed in the study were plotted and are shown in Figure 4. There was a good association ($r = 0.78$ and $r^2 = 0.60$) between the total histology score and the highest CK activity of each goat. Each goat that had a serum CK activity above 7,000 U/L and a total histology score above 15 was exercise intolerant with the exception of 1 goat from the VRO group. That

goat would have likely been exercise intolerant the following day as it began to show exercise fatigue on the last day that the goat ran on the treadmill before the study was terminated.

4. Discussion

White snakeroot has caused significant livestock losses and human poisoning in the United States since the early 1800s. However, poisoning has been sporadic and reproducing poisoning experimentally and correlating toxicity with the presence and concentrations of the proposed toxins has not been described. It is commonly accepted that the disease caused by rayless goldenrod is the same as the disease caused by white snakeroot. The BFKs are found in both plants at relatively high concentrations. In a recent report from our laboratory, rayless goldenrod dosed at 2 and 3% of the goats BW (40 and 60 mg BFK/kg BW respectively) for 7 days caused the disease (Stegelmeier et al., 2010; Davis et al., 2013b). The rayless goldenrod contained 0.53, 1.34, 2.39, and 4.26 mg/g plant of tremetone, dehydrotremetone, 3-oxyangeloyl-tremetone, and total BFKs, respectively. The rayless goldenrod did not contain 6-hydroxytremetone while none of the white snakeroot in the current study contained 3-oxyangeloyl-tremetone. The white snakeroot collections used in this study contained BFKs at similar concentrations (Table 2) as the rayless goldenrod used in previous reports (Stegelmeier et al., 2010; Davis et al., 2013b). In the rayless goldenrod study the goats dosed with 40 and 60 mg BFK/kg BW developed the clinical disease. The goats dosed with 10 and 20 mg BFK/kg BW developed less severe and more infrequent myocyte necrosis. Additionally they did not exhibit clinical signs of poisoning.

In this study there was no apparent association ($r^2 < 0.3$) between tremetone, 6-hydroxytremetone, or total BFKs with the histologic lesions (Fig. 3). However, there was an association with dehydrotremetone concentrations and histologic lesions ($r^2 = 0.41$). In the current study, the Allerton white snakeroot collection contained 1.3 mg tremetone/g plant which is much higher than many of the toxic collections in this study and the rayless goldenrod (0.53 mg tremetone/g plant) used in previous studies. This suggests that tremetone may not be as an important part in toxicity as previously thought. Additionally, the VRO collection contained 6.73 mg BFK/g plant, which was much higher than in any of the other plant populations dosed in this study or the rayless goldenrod used in the previous report, and yet it only poisoned two of the four goats in the group and the CK changes as a group were not statistically significant because the other two goats in the group did not have any increases in CK concentrations. Additionally, these goats were less severely poisoned than the goats in the Interstate 2009 and Interstate 2010 and Wabash groups (Tables 3 and 4).

Dehydrotremetone doses had a better association with both clinical disease and histologic scores (Fig. 3) than the other BFKs. The dehydrotremetone concentration in the rayless goldenrod used in the previous study was much higher than in any of the white snakeroot collections used in this study. As indicated previously microsomally activated tremetone was cytotoxic in several different cell cultures. In those same studies dehydrotremetone was not, and it was speculated that it might be a non-toxic metabolite of tremetone (Beier et al., 1987). The results from this study suggest that may not be the case. It should be noted that every white snakeroot population that resulted in poisoning contained relatively significant amounts of tremetone, and total BFK. It may be that

tremetone toxicity is only a partial component of white snakeroot toxicity. The lack of association of disease with the toxins that we are currently monitoring, suggest there may be additional toxins we have not yet identified. Alternatively it may be that there is a cumulative effect or synergism of multiple white snakeroot toxins. More work is needed to better identify the role that each of these compounds play in toxicity and if there are other toxins yet to be identified.

The lesions in the goats were most severe in the large appendicular muscles such as the biceps brachii, biceps femoris, quadriceps femoris, gluteus medius and psoas major. Conversely the tongue, masseter, retro-ocular, cricothyroideus and longissimus dorsi were relatively unaffected. This distribution is similar to those reported in goats dosed with rayless goldenrod (Stegelmeier et al., 2010). However, character and duration of the lesions in this study was different. Rayless goldenrod-induced myonecrosis was mostly monophasic and acute myocyte swelling, hypereosinophilia and necrosis with minimal nuclear proliferation. In this study the lesions were monophasic or of similar duration within muscles but between muscles there were predominantly polyphasic. When compared with similar lesions produced by rayless goldenrod these lesions were more chronic in nature with more extensive inflammation, nuclear proliferation and regeneration. As BFK doses and durations were similar, this suggests white snakeroot toxicity is slightly different and may not be totally attributed to the same BFK toxins.

It may be important to note that the large appendicular muscles that were affected have a large proportion of Type I muscle fibers with a high oxygen demand with potential for oxidative stress. Since the disease takes several days to develop it may be that an important component of poisoning is depletion of the reductive potential of the

cell resulting in oxidative damage. Individual animal resistance to oxidative stress may contribute to the variation seen in animal response to poisoning. A similar model may be found in livestock which are selenium deficient. Toxicity is a result of oxidative damage and as individual myocytes lose reducing capability they develop polyphasic lesions in large appendicular muscles (Arthur 1998). Certainly more work is needed to better characterize muscle and fiber type susceptibility to white snakeroot toxicity and to determine the contribution of oxidation in poisoning.

The myocardial lesions in goats with clinical disease were minimal in this study. Even in animals with elevated cTn I concentrations, there were no corresponding myocardial changes. As in most species cTn I is myocardial specific and cTn I concentrations were only elevated in severely poisoned animals late in the study, it may be that myocardial lesions in these goats did not have time to develop to the extent that they could be detected using light microscopy. Note that the group cTn I concentrations were never statistically different from controls. It may also be that goats are more resistant to white snakeroot myocardial toxicity. Previous rayless goldenrod studies found similar minimal myocardial lesions in goats with extensive myocardial damage in horses (Davis et al., 2013a). Myocardial damage in horses was seen as both marked elevation in cTn I and histologic myocardial degeneration and necrosis.

White snakeroot poisoning in goats has also been associated with lesions other than myopathy. Reagor et al. (1980) reported that a single dose of fresh white snakeroot from central Texas lethally poisoned Angora goats within 24 to 48 hours of dosing. In that study no clinical signs of trembles nor were skeletal muscle lesions observed. Reagor reported marked increases in serum LDH and SGOT activities and extensive para-acinar

hepatic necrosis. In this study there was minimal hepatocellular vacuolation. Though special stains were not performed the character and distribution of these vacuoles suggests that they are lipid vacuoles that are probably a result of the anorexia that was associated with clinical disease. Variability in toxins and toxin concentrations reported in white snakeroot chemotypes suggest that the plant used in Reagor's studies may be unique and could possibly contain unique unidentified hepatotoxins. Certainly this disparity suggests that the white snakeroot from Texas should be studied and chemically analyzed.

In this study there was a good association between clinical signs of poisoning, exercise intolerance, increased serum CK activity and histology scores (Table 4). This same association was previously observed in goats poisoned with rayless goldenrod (Stegelmeier et al., 2010; Davis et al., 2013b). In future studies exercise intolerance and serum chemistry changes can be used to diagnose and monitor poisoning to better study disease progression and resolution.

In conclusion white snakeroot is a relatively common toxic plant found in many parts of North America. Severe poisoning in goats is characterized by extensive skeletal muscle degeneration and necrosis; however, little is known of mild or subclinical poisoning and its affect on animal performance or production. As transmammary poisoning or "milk sickness" is possible, it also presents a risk for human poisoning. White snakeroot populations have very different toxicities and chemotypes (which are unique and contain varying concentrations of BFK). Some populations may be non-toxic. This study suggests the relative toxicity of the plant populations does not correlate well with the tremetone and total benzofuran ketone concentrations in the plant. This

suggests some toxic component or synergistic action of the BFKs has yet to be identified. More information is needed to better identify all the white snakeroot toxins, understand what controls toxin expression in plants, and understand the mechanism and progression of poisoning. Certainly identification of the unknown toxin(s) is crucial to determine which populations present a risk for further livestock and human poisoning and to better understand the pathogenesis of this disease.

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Table 1. White snakeroot collection sites, dates, and USDA-ARS-Poisonous Plant Research Laboratory herbarium accession numbers.

Collection Name	Collection Location (County, State)	Coordinates	Collection Date	Accession Number
Zanesville	Muskingum, OH	N 39°52'35", W 81°42'52"	9/20/2010	4229, 4230
Columbia	Boone, MO	"Spears" N 38°47'00", W 92°15'01"; "University" N 38°56'24", W 92°18'54"; "East 163" N 38°52'07", W 92°16'07"	9/18/2010	"Spears" 4222 "University" 4219, 4220 "East" 4217, 4218
Allerton	Piatt, IL	N 40°00'22", W 88°39'11"	9/17/2009	3560
VRO	Vermillion, IL	N 40°03.582', W 87°33.847'	9/15/2009	3550
Interstate 2009	Vermillion, IL	N 40°06'29", W 87°40'52"	9/18/2009	3562
Interstate 2010	Vermillion, IL	N 40°06'29", W 87°40'52"	9/23/2010	4241
Wabash	Tippecanoe, IN	"Wabash River" N 40°25'19", W 86°53'53" "Happy Hollow" N 40°26'05", W 86°53'54"	9/21/2010	"Wabash River" 4235, 4236 "Happy Hollow" 4237

Table 2. Concentrations (mg/g $\pm$ s.d. of plant dry weight) of benzofuran ketones, phenols, and chromenenes in white snakeroot collections dosed to goats.

	Zanesville	Columbia	Allerton	VRO	Interstate 2009	Interstate 2010	Wabash
Tremetone	0.004 $\pm$ 0.006	0.07 $\pm$ 0.01	1.3 $\pm$ 0	1.9 $\pm$ 0.3	1.9 $\pm$ 0.3	3.5 $\pm$ 0.4	0.8 $\pm$ 0.1
Dehydrotremetone	0.046 $\pm$ 0.005	0.033 $\pm$ 0.0098	0.19 $\pm$ 0	0.23 $\pm$ 0.02	0.370 $\pm$ 0.05	0.78 $\pm$ 0.11	0.47 $\pm$ 0.04
6-hydroxytremetone	0.6 $\pm$ 0.2	0.15 $\pm$ 0.03	0.44 $\pm$ 0.03	4.6 $\pm$ 0.3	1.5 $\pm$ 0.2	1.1 $\pm$ 0.1	0.24 $\pm$ 0.02
Total Benzofuran Ketones*	0.65	0.253	1.93	6.73	3.77	5.38	1.51
Unknown (5.80 min)		0.16 $\pm$ 0.03†					
6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene		1.01 $\pm$ 0.04†	0.036 $\pm$ 0.03†				0.067 $\pm$ 0.007†
Precocene II							
6,7-dimethoxy-2,2-dimethylchromene	1.2 $\pm$ 0.4						
Unknown (10.76 min)		0.024 $\pm$ 0.009†	0.26 $\pm$ 0.01†	0.065 $\pm$ 0.002†	0.36 $\pm$ 0.04†	0.28 $\pm$ 0.03†	0.43 $\pm$ 0.05†
Unknown (11.46 min)	0.02 $\pm$ 0.02†		0.2 $\pm$ 0.02†	0.014 $\pm$ 0.024†			
Encecaline							
6-acetyl-7-methoxy-2,2-dimethylchromene		1.33 $\pm$ 0.05	0.1 $\pm$ 0.0				0.180 $\pm$ 0.01
2-seneciyl-4-(1-methoxyethyl)phenol		0.334 $\pm$ 0.006					

* Total benzofuran ketones are the sum of the tremetone, dehydrotremetone, and 6-hydroxytremetone concentrations.
† The concentration of the compound is expressed in tremetone equivalents (see methods).

Table 3. Selected mean serum biochemical data (mean $\pm$ s.d.) from control and white snakeroot dosed goats*.

	Group/Dose	Day 1	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Alanine aminotransferase (<55 U/L)	Control	44 $\pm$ 3	33 $\pm$ 4	35 $\pm$ 4	38 $\pm$ 4	38 $\pm$ 5 ^a	33 $\pm$ 7 ^a	33 $\pm$ 4 ^a
	Zanesville	45 $\pm$ 4	45 $\pm$ 4	44 $\pm$ 4	35 $\pm$ 2	44 $\pm$ 3 ^a	40 $\pm$ 3 ^a	45 $\pm$ 4 ^a
	Columbia	48 $\pm$ 1	51 $\pm$ 9	46 $\pm$ 7	38 $\pm$ 3	47 $\pm$ 6 ^a	38 $\pm$ 3 ^a	46 $\pm$ 3 ^a
	Allerton	43 $\pm$ 5	33 $\pm$ 4	42 $\pm$ 3	42 $\pm$ 5	52 $\pm$ 13 ^a	71 $\pm$ 31 ^a	63 $\pm$ 27 ^a
	VRO	46 $\pm$ 21	36 $\pm$ 9	49 $\pm$ 14	55 $\pm$ 8	78 $\pm$ 34 ^a	122 $\pm$ 83 ^a	152 $\pm$ 137 ^{ab}
	Interstate 2009	29 $\pm$ 2	29 $\pm$ 3	53 $\pm$ 17	139 $\pm$ 114	279 $\pm$ 241 ^{†b}	380 $\pm$ 253 ^{†b}	312 $\pm$ 237 ^{†bc}
	Interstate 2010	33 $\pm$ 2	35 $\pm$ 3	48 $\pm$ 4	98 $\pm$ 54	348 $\pm$ 202 ^{†b}	446 $\pm$ 326 ^{†b}	410 $\pm$ 252 ^{†c}
	Wabash	46 $\pm$ 6	45 $\pm$ 5	56 $\pm$ 22	65 $\pm$ 53	174 $\pm$ 168 ^a	342 $\pm$ 229 ^{†b}	737 $\pm$ 583 ^{†d}
Aspartate aminotransferase (<125 U/L)	Control	91 $\pm$ 10	60 $\pm$ 19	86 $\pm$ 8	87 $\pm$ 9 ^a	81 $\pm$ 2 ^a	63 $\pm$ 8 ^a	74 $\pm$ 19 ^a
	Zanesville	89 $\pm$ 8	103 $\pm$ 19	97 $\pm$ 19	80 $\pm$ 31 ^a	136 $\pm$ 28 ^a	117 $\pm$ 17 ^a	226 $\pm$ 770 ^a
	Columbia	96 $\pm$ 9	140 $\pm$ 71	98 $\pm$ 35	84 $\pm$ 2 ^{ab}	115 $\pm$ 14 ^a	82 $\pm$ 5 ^a	113 $\pm$ 8 ^a
	Allerton	89 $\pm$ 6	49 $\pm$ 19	98 $\pm$ 16	101 $\pm$ 16 ^{ab}	209 $\pm$ 170 ^a	353 $\pm$ 262 ^a	260 $\pm$ 207 ^a
	VRO	150 $\pm$ 169	62 $\pm$ 45	147 $\pm$ 63	175 $\pm$ 57 ^a	388 $\pm$ 303 ^a	693 $\pm$ 664 ^a	1107 $\pm$ 1251 ^{ab}
	Interstate 2009	44 $\pm$ 6	52 $\pm$ 23	269 $\pm$ 252	1585 $\pm$ 1628 ^{†bc}	2978 $\pm$ 2996 ^{†b}	3652 $\pm$ 2726 ^{†b}	1862 $\pm$ 1196 ^{†bd}
	Interstate 2010	45 $\pm$ 5	89 $\pm$ 49	177 $\pm$ 83	745 $\pm$ 636 ^{ab}	3024 $\pm$ 2079 ^{†bc}	4006 $\pm$ 3200 ^{†b}	3089 $\pm$ 2076 ^{†cd}
	Wabash	89 $\pm$ 16	101 $\pm$ 20	211 $\pm$ 197	375 $\pm$ 527 ^{ab}	1518 $\pm$ 1713 ^{†b}	2848 $\pm$ 2146 ^{†b}	3948 $\pm$ 5412 ^{†c}
Creatine kinase (<350 U/L)	Control	166 $\pm$ 82	165 $\pm$ 181	82 $\pm$ 22	308 $\pm$ 413 ^a	75 $\pm$ 27 ^a	74 $\pm$ 14 ^a	394 $\pm$ 414 ^a
	Zanesville	166 $\pm$ 120	90 $\pm$ 23	82 $\pm$ 7	73 $\pm$ 16 ^a	137 $\pm$ 79 ^a	93 $\pm$ 14 ^a	114 $\pm$ 12 ^a
	Columbia	120 $\pm$ 11	87 $\pm$ 20	251 $\pm$ 266	224 $\pm$ 77 ^a	119 $\pm$ 19 ^a	72 $\pm$ 5 ^a	113 $\pm$ 29 ^a
	Allerton	107 $\pm$ 18	58 $\pm$ 23	142 $\pm$ 72	291 $\pm$ 374 ^a	1503 $\pm$ 2482 ^{ac}	540 $\pm$ 256 ^a	551 $\pm$ 508 ^a
	VRO	974 $\pm$ 1326	87 $\pm$ 19	421 $\pm$ 504	239 $\pm$ 144 ^a	1781 $\pm$ 1892 ^{ac}	2629 $\pm$ 3275 ^a	4352 $\pm$ 5009 ^a
	Interstate 2009	141 $\pm$ 105	105 $\pm$ 47	2607 $\pm$ 4392	6446 $\pm$ 10619 ^{†b}	9413 $\pm$ 10159 ^{†b}	9177 $\pm$ 5456 ^{†b}	5365 $\pm$ 3718 ^{†a}
	Interstate 2010	99 $\pm$ 39	137 $\pm$ 74	327 $\pm$ 247	2301 $\pm$ 3315 ^a	8025 $\pm$ 7242 ^{†b}	11560 $\pm$ 8001 ^{†b}	6328 $\pm$ 4694 ^{†a}
	Wabash	311 $\pm$ 364	177 $\pm$ 83	745 $\pm$ 1143	3851 $\pm$ 7200 ^a	6751 $\pm$ 5793 ^{†bc}	12280 $\pm$ 9198 ^{†b}	19606 $\pm$ 27558 ^{†b}
Lactate dehydrogenase ($<1,560$ U/L)	Control	953 $\pm$ 102	491 $\pm$ 240	818 $\pm$ 69	852 $\pm$ 220 ^a	731 $\pm$ 156 ^a	489 $\pm$ 86 ^a	483 $\pm$ 45 ^a
	Zanesville	1062 $\pm$ 190	838 $\pm$ 116	747 $\pm$ 89	527 $\pm$ 147 ^a	1053 $\pm$ 115 ^a	790 $\pm$ 192 ^a	1239 $\pm$ 104 ^{ab}
	Columbia	1031 $\pm$ 226	924 $\pm$ 191	741 $\pm$ 158	642 $\pm$ 116 ^a	908 $\pm$ 99 ^a	571 $\pm$ 43 ^a	888 $\pm$ 144 ^{ab}
	Allerton	1038 $\pm$ 452	444 $\pm$ 243	1133 $\pm$ 326	1149 $\pm$ 280 ^a	1376 $\pm$ 433 ^a	2092 $\pm$ 1274 ^a	1303 $\pm$ 762 ^{ab}
	VRO	1752 $\pm$ 1876	474 $\pm$ 271	1521 $\pm$ 587	1341 $\pm$ 286 ^a	2505 $\pm$ 1667 ^b	3526 $\pm$ 2903 ^a	2719 $\pm$ 2581 ^{ab}
	Interstate 2009	507 $\pm$ 182	429 $\pm$ 188	1574 $\pm$ 778	6641 $\pm$ 6316 ^{†b}	8335 $\pm$ 6845 ^{†c}	8219 $\pm$ 4342 ^{†b}	5029 $\pm$ 3478 ^{†bc}
	Interstate 2010	434 $\pm$ 81	617 $\pm$ 240	1268 $\pm$ 415	2449 $\pm$ 1267 ^{ab}	8808 $\pm$ 7017 ^{†c}	11480 $\pm$ 8033 ^{†b}	7310 $\pm$ 4483 ^{†c}
	Wabash	1009 $\pm$ 69	865 $\pm$ 79	1224 $\pm$ 899	2195 $\pm$ 3144 ^a	5713 $\pm$ 6621 ^{†bc}	9675 $\pm$ 6507 ^{†b}	13837 $\pm$ 17991 ^{†d}
Cardiac troponin I (<0.02 ng/ml)	Control	0.02 $\pm$ 0.03	0.02 $\pm$ 0.02	0.01 $\pm$ 0.02	0.01 $\pm$ 0.01	0.02 $\pm$ 0.02	0.01 $\pm$ 0.02	0.01 $\pm$ 0.02
	Zanesville	0.03 $\pm$ 0.01	0.02 $\pm$ 0.03	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02
	Columbia	0.02 $\pm$ 0.02	0.05 $\pm$ 0.04	0.07 $\pm$ 0.04	0.07 $\pm$ 0.02	0.03 $\pm$ 0.01	0.03 $\pm$ 0.02	0.03 $\pm$ 0.01
	Allerton	0.00 $\pm$ 0.00	0.01 $\pm$ 0.02	0.01 $\pm$ 0.01	0.01 $\pm$ 0.02	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02
	VRO	0.01 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	0.03 $\pm$ 0.03
	Interstate 2009	0.02 $\pm$ 0.01	0.01 $\pm$ 0.02	0.03 $\pm$ 0.03	0.07 $\pm$ 0.09	0.11 $\pm$ 0.17	0.44 $\pm$ 0.38	1.12 $\pm$ 1.71
	Interstate 2010	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.00 $\pm$ 0.00	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	0.13 $\pm$ 0.09	0.19 $\pm$ 0.17
	Wabash	0.03 $\pm$ 0.01	0.03 $\pm$ 0.03	0.03 $\pm$ 0.03	0.05 $\pm$ 0.03	0.05 $\pm$ 0.03	0.33 $\pm$ 0.42	1.17 $\pm$ 1.98
exercise intolerant	Control	0/4	0/4	0/4	0/4	0/4	0/4	NA
	Zanesville	0/4	0/4	0/4	0/4	0/4	0/4	NA
	Columbia	0/3	0/3	0/3	0/3	0/3	0/3	NA
	Allerton	0/4	0/4	0/4	0/4	0/4	1/4	NA
	VRO	0/4	0/4	0/4	0/4	0/4	1/4	NA
	Interstate 2009	0/4	0/4	1/4	3/4	3/4	3/4	NA
	Interstate 2010	0/4	0/4	1/4	2/4	3/4	3/4	NA
	Wabash	0/4	0/4	1/4	1/4	3/4	3/4	NA

*n = 4 except for the Columbia group in which n = 3. All goats were dosed by oral gavage at 2% of body weight with the respective plant population and dosing was discontinued for a poisoned animal, the day after the goat became exercise intolerant, but samples were still collected for analysis.

†The clinical sign that is measureable is exercise tolerance and goats that were exercise intolerant (could not run for 5 minutes on a treadmill moving at 12 kilometers/hour on a 10% incline) are expressed as a fraction of the total number per group. NA indicates that no data was available on the day.

†Significantly different means ($P < 0.05$) were determined by analysis of variance using a Schwarz Bayesian criterion. Significantly different populations on a given day are indicated by differing superscript letters (a-d).

*Means were significantly different ($P < 0.05$) from day 1 within the same group.

Table 4. Table of histologic scores* (mean $\pm$ s.d.) from skeletal muscles from goats treated with white snakeroot (*Ageratina altissima* var. *altissima* or *Eupatorium rugosum*). Significantly different plant populations are indicated with superscript letters[†].

Skeletal Muscle	Control (A)	Zanesville (B)	Columbia (C)	Allerton (D)	VRO (E)	Interstate 2009 (F)	Interstate 2010 (G)	Wabash (H)
Retro-ocular	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Tongue	1.0 $\pm$ 0.0	0.5 $\pm$ 0.6	0.3 $\pm$ 0.6	0.3 $\pm$ 0.5	1.3 $\pm$ 0.5	0.3 $\pm$ 0.5	0.5 $\pm$ 0.6	0.8 $\pm$ 0.5
Masseter	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.5 $\pm$ 1.0	0 $\pm$ 0	0.5 $\pm$ 0.6	0 $\pm$ 0
Cricothyroideus	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.3 $\pm$ 0.5	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Pectoralis major	0 $\pm$ 0 ^{E-H}	0.8 $\pm$ 1.0	0 $\pm$ 0 ^F	0.3 $\pm$ 0.5 ^F	1.3 $\pm$ 1.3 ^A	1.8 $\pm$ 1.5 ^{A,C,D}	2.0 $\pm$ 1.4 ^A	1.5 $\pm$ 1.3 ^A
Biceps brachii	0 $\pm$ 0 ^{E-H}	0 $\pm$ 0 ^{A,B}	0 $\pm$ 0 ^{F,G}	0.3 $\pm$ 0.5 ^{F,G}	2.3 $\pm$ 2.1 ^{A,B}	2.8 $\pm$ 1.3 ^{A-D}	1.5 $\pm$ 1.0 ^{A-D}	3.0 $\pm$ 2.0 ^{A,B}
Triceps brachii	0 $\pm$ 0 ^{F-H}	0.5 $\pm$ 0.6 ^G	0 $\pm$ 0 ^G	0.5 $\pm$ 0.6 ^G	0.3 $\pm$ 0.5 ^G	0.5 $\pm$ 0.6 ^G	1.5 $\pm$ 0.6 ^{A-F}	0.8 $\pm$ 0.5 ^A
Intercostal	0 $\pm$ 0 ^{F-H}	0 $\pm$ 0 ^{F-H}	0 $\pm$ 0	0.3 $\pm$ 0.5	0.3 $\pm$ 0.5	2.0 $\pm$ 1.4 ^{A,B}	2.0 $\pm$ 1.8 ^{A,B}	2.0 $\pm$ 1.4 ^{A,B}
Diaphragm	0 $\pm$ 0	0.3 $\pm$ 0.5	0.7 $\pm$ 0.6	0.5 $\pm$ 0.6	0.8 $\pm$ 0.5	3.0 $\pm$ 2.0	1.5 $\pm$ 1.3	0.8 $\pm$ 0.5
Longissimus dorsi	0 $\pm$ 0	0.3 $\pm$ 0.5	0 $\pm$ 0	0.3 $\pm$ 0.5	0 $\pm$ 0	0 $\pm$ 0	0.3 $\pm$ 0.5	0 $\pm$ 0
Psoas major	0 $\pm$ 0 ^{E-H}	0.3 $\pm$ 0.5 ^{F,G}	0.7 $\pm$ 0.6	0.3 $\pm$ 0.5	1.3 $\pm$ 1.3 ^A	3.0 $\pm$ 1.0 ^{A,D}	2.5 $\pm$ 1.9 ^A	2.0 $\pm$ 1.6 ^A
Biceps femoris	0 $\pm$ 0 ^{D-H}	0 $\pm$ 0 ^{D-H}	0 $\pm$ 0 ^G	1.3 $\pm$ 1.3 ^{A,B}	1.8 $\pm$ 1.5 ^{A,B}	2.0 $\pm$ 1.8 ^{A,B}	2.5 $\pm$ 1.0 ^{A-C}	2.5 $\pm$ 1.7 ^{A,B}
Quadriceps Femoris	0 $\pm$ 0 ^{F-H}	0.5 $\pm$ 1.0	0 $\pm$ 0 ^G	0.5 $\pm$ 0.06 ^G	1.0 $\pm$ 1.4	2.8 $\pm$ 1.9 ^A	2.5 $\pm$ 1.7 ^{A-D}	2.0 $\pm$ 1.4 ^A
Semimembranosus	0 $\pm$ 0 ^{D-H}	0 $\pm$ 0 ^{D-H}	0 $\pm$ 0 ^{F,G}	0.8 $\pm$ 0.5 ^{A,B,G}	1.5 $\pm$ 1.3 ^{A,B}	1.3 $\pm$ 0.5 ^{A-C,G}	2.0 $\pm$ 0 ^{A-D,H}	1.0 $\pm$ 0.8 ^{A,B,G}
Semitendinosus	0 $\pm$ 0	0.3 $\pm$ 0.5	0.7 $\pm$ 0.6	0 $\pm$ 0	1.0 $\pm$ 0	1.3 $\pm$ 1.9	0.5 $\pm$ 0.6	1.8 $\pm$ 1.5
Adductor	0 $\pm$ 0 ^{F-H}	0 $\pm$ 0 ^{F-H}	0 $\pm$ 0	0.3 $\pm$ 0.5	0.8 $\pm$ 0.9	2.0 $\pm$ 1.8 ^{A,B}	1.0 $\pm$ 0.8 ^{A,B}	1.8 $\pm$ 1.7 ^{A,B}
Gluteus medius	0 $\pm$ 0 ^{D-H}	0 $\pm$ 0 ^{D-H}	0.3 $\pm$ 0.6 ^{F,G}	1.0 $\pm$ 0.8 ^{A,B,F,G}	1.8 $\pm$ 1.7 ^{A,B}	3.5 $\pm$ 1.0 ^{A-D,H}	3.0 $\pm$ 0.8 ^{A-D,H}	1.5 $\pm$ 1.0 ^{A,B,F,G}
Total Grade[‡]	1.0 $\pm$ 0 ^{B,D-H}	3.3 $\pm$ 1.0 ^{E-H}	2.3 $\pm$ 1.5 ^{E-H}	6.3 $\pm$ 4.6 ^{A,F,G}	15.8 $\pm$ 11.2 ^{A-C}	26.0 $\pm$ 10.1 ^{A-D}	23.8 $\pm$ 11.0 ^{A-D}	21.3 $\pm$ 13.7 ^A
Mean Grade[‡]	0.1 $\pm$ 0 ^{B,D-H}	0.2 $\pm$ 0.1 ^{E-H}	0.1 $\pm$ 0.1 ^{E-H}	0.4 $\pm$ 0.3 ^{A,F,G}	0.9 $\pm$ 0.7 ^{A-C}	1.5 $\pm$ 0.6 ^{A-D}	1.4 $\pm$ 0.6 ^{A-D}	1.3 $\pm$ 0.8 ^A

* Histologic lesions were subjectively scored by two pathologists and the mean scores for each muscle were combined and compared. Scoring was: 0- no change; 1- minimal with myocyte degeneration characterized by rare myocyte swelling and hypereosinophilia; 2- mild with occasional myocyte degeneration (myocyte swelling and hypereosinophilia) with rare myocyte necrosis; 3- moderate with patchy myocyte degeneration and necrosis involving small portions (< 35%) of the skeletal muscle; and 4- severe with extensive myocyte degeneration, necrosis with or without subsequent inflammation and regeneration. Distribution is diffuse involving 40% or more of myocytes.

[†] Values with the letters above them differ ($P < 0.05$) within each muscle group from Control (A), Zanesville (B), Columbia (C), Allerton (D), VRO (E), Interstate 2009 (F), Interstate 2010 (G), and Wabash (H) groups.

[‡] Sum of histologic scores for all 17 skeletal muscles.

Figure Legends

Figure 1. (Necrotizing myopathy with extensive histiocytosis) Photomicrograph of the biceps femoris from goat 8693. The goat was treated with white snakeroot from the Interstate 2009 collection for 9 days. Notice the swollen, hypereosinophilic myocytes with loss of striation (arrowhead), coagulation and clumping of sarcoplasmic proteins (*) and focally extensive inflammation. The inset demonstrates the inflammatory reaction which is composed of histiocytes and macrophages with rare lymphocytes (arrow). H&E, Bar = 100u.

Figure 2. (Degenerative myopathy) Photomicrograph of biceps brachii from goat 8693. She was treated with white snakeroot from the Interstate 2009 collection for 10 days. Notice the swollen, hypereosinophilic myocytes (arrows), coagulation and clumping of sarcoplasmic proteins (arrow heads which is further demonstrated in the inset) and minimal inflammation (*). H&E, Bar = 100u.

Figure 3. Plot of the tremetone, dehydrotremetone, 6-hydroxytremetone, or total benzofuran ketones (sum of tremetone, dehydrotremetone, and 6-hydroxytremetone) concentrations versus the total pathology grades for each animal dosed with the respective white snakeroot collection.

Figure 4. Plot of the highest serum creatine kinase (CK) activity and total pathology score of each goat dosed with white snakeroot. Each goat to the right of the break in the x-axis

542 was exercise intolerant (i.e. could not run for 5 min on the treadmill at a 10 % incline
543 moving at 12 km/hr).

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Figure 1

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Figure 2

Figure 3

Figure 4.