

January 1, 2021

TO: All Veterinarians and Trainers

FROM: J. Michael Hopkins
Executive Director

RE: Clenbuterol – New Restrictions Effective March 1, 2021

Through the development of the Mid-Atlantic Strategic Plan with other racing jurisdictions in the region to enhance the safety and welfare of the horses competing in races, the Maryland Racing Commission has adopted the recommended requirement that effective March 1, 2021, Clenbuterol will not have a permissive threshold. The presence of Clenbuterol in a blood or urine sample, at any detectable level will be considered to be a violation. A horse found with any detectable level of Clenbuterol shall be disqualified and the trainer shall be subject to disciplinary action.

As guidance, to avoid a positive finding the Racing Medication and Testing Consortium (RMTC) has recommended that Clenbuterol not be administered to a horse, at a minimum, within 28 days of a race. Recognizing the therapeutic value of Clenbuterol, this medication may be used in accordance with the following:

1. Clenbuterol use is prohibited in racing and training unless the following conditions are met:
 - a. The prescription for clenbuterol is made for a specific horse based upon a specific diagnosis. The prescription and volume dispensed cannot exceed a treatment period of 30 days.
 - b. The veterinarian must provide a copy of the prescription and diagnosis to the Equine Medical Director for review and approval. The horse may not receive clenbuterol before this approval is issued.
 - c. Trainers must make daily notification to the official veterinarian of horse(s) in their custody having been administered clenbuterol. Notification shall be made on a form and by a deadline designated by the Commission.
 - d. A horse administered clenbuterol shall be placed on the official veterinarian's list. The horse must meet all conditions for removal from the list including a timed workout and blood and urine sampling. Both samples must have no detectable clenbuterol.
 - e. A horse may not enter to race until it has completed all the requirements in subdivision (d).
2. If a clenbuterol is detected in a horse's post-race or out of competition sample and appropriate notification as outlined in subdivision 1(a) or (b) was not completed, the horse shall immediately be placed on the Official Veterinarian's List pending the outcome of an investigation. The horse shall be required to meet all conditions for removal from the veterinarian's list outlined in subdivision 1(d), above.



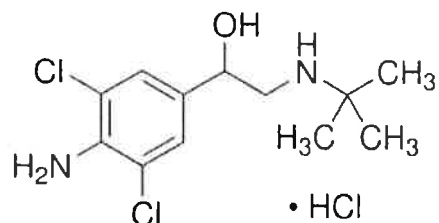


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Clenbuterol

Background

Clenbuterol is a β_2 -adrenoceptor agonist. It is assigned a 3/B in the ARCI's Uniform Classification of Foreign Substances. For Quarter Horse racing it is designated as a prohibited substance and assigned a Class A penalty. Clenbuterol is commonly administered orally as a bronchodilator and mucolytic agent in the management of various respiratory disorders, including recurrent airway obstruction (RAO).ⁱ



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Clenbuterol is a prescription medication and can only be dispensed by or upon the request of a veterinarian. It is commercially available as an oral syrup, Ventipulmin®.ⁱⁱ The lowest recommended dose of Clenbuterol is 0.8 mcg/kg however studies have indicated that only 25% of horses respond at this level of administration.ⁱⁱⁱ Doses of up to 3.2 mcg/kg have been used, to improve clinical efficacy, with dosing increased incrementally by 0.8 mcg/kg to avoid adverse effects. Clenbuterol's β_1 sympathomimetic activity can cause sweating, excitement, urticaria, trembling, and tachycardia when treatment is initiated or when a high dose is administered non-incrementally. Clenbuterol overdose can lead to complications including laminitis, acute renal failure, rhabdomyolysis, and cardiomyopathy, and death.^{iv}

Clenbuterol causes relaxation of airway smooth muscle by activating adenylyl cyclase and stimulating the production of cAMP. This increase in cAMP leads to activation of protein kinase A (PKA) that down regulates myosin light chain kinase activity and opens K^+ channels thus inhibiting smooth muscle contraction in the airways.^v Clenbuterol has also been determined to cause lipolysis and increase muscle mass in numerous species, including horses. Reports have shown a decrease in the percent body fat and increase in fat-free mass following the administration of high-dose clenbuterol.^{vi}

There is documented evidence that clenbuterol has been routinely administered absent any medical indication to racehorses, likely due to the increase in muscle mass associated with leptin- and adiponectin-mediated repartitioning effects. When examined closely, however the potential muscle building benefits are offset by the negative effects of long-term administration, including a decrease in aerobic capacity, time to fatigue, cardiac function, and maximal oxygen consumption.^{vii} Studies have shown that even minimal concentrations of clenbuterol adversely

affect aerobic performance, high-intensity exercise capacity, and the horse's ability to recover from exercise.^{viii} Clenbuterol has also been demonstrated to induce changes in the structural dimensions of the equine heart, including both cardiac remodeling and an enlarged aorta immediately after exercise. This increases the risk of aortic rupture and sudden death in the equine athlete.^{ix} Finally, studies have shown that clenbuterol becomes less effective as a bronchodilator when the treatment period extends beyond 14 consecutive days.^x

Administration Study

Clenbuterol was administered orally, at a dose of 0.8 mcg/kg, twice a day for a total of thirty days to 22 exercise-conditioned Thoroughbred horses (geldings and mares). A second group of six horses received clenbuterol according to the incremental dosing protocol outlined on the manufacturer's label: 0.8 mcg/kg twice daily for 3 days, 1.6 mcg/kg twice daily for 3 days, 2.4 mcg/kg twice daily for 3 days and 3.2 mcg/kg twice daily for the remainder of the 30-day dosing protocol. This study was performed at the University of California School of Veterinary Medicine (Davis, CA) by personnel in the Maddy Equine Analytical Pharmacology Laboratory under the direction of Dr. H. K. Knych.^{xi}

Blood samples were obtained immediately before dose administration and at the following times after dosing: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12 hours after the administration of the first and last dose and at 18, 24, 36, 48, 60, 72, 84, 98, 108, and 120 hours after the administration of the last dose. Additionally, blood samples were collected every 12 hours immediately prior to dosing throughout the entire course of the administrations.

Urine samples were collected at 12, 36, 60, 84, 108, 136, and 160 hours after the final administration and subsequently at 7-day intervals until the drug was below the limit of detection (LOD).

Extraction and Analysis Procedures

Quantification of clenbuterol in plasma and urine samples was performed at the Maddy Equine Analytical Pharmacology Laboratory (Davis, CA) using validated methods. Clenbuterol concentrations were determined in plasma and urine by liquid chromatography-mass spectrometry (LC-MS/MS) using a stock solution of clenbuterol-d9 as internal standard to verify quantitative accuracy and precision. The method was characterized by a Limit of Quantification (LOQ) of 10 pg/mL and 25 pg/mL in plasma and urine, respectively. The plasma LOD was 1 pg/mL.

Pharmacokinetic Modelling

Average clenbuterol plasma concentrations vs. time curves following chronic low-dose and escalating protocol administration are shown in Figure 1.1. Pharmacokinetic analysis was

performed on individual plasma concentrations vs. time data using Phoenix® WinNonlin® Version 6.0 pharmacokinetic analysis software (Pharsight Corporation, St. Louis, MO).

Results and Discussion

Clenbuterol plasma concentrations were below LOD in all horses in the low dose protocol and 5 of 6 horses in the escalating protocol by 7 days after the last administration. The terminal elimination half-life ranged from 5.5 to 21.5 hours with a mean of 10.4 (± 4) hours following low dose administration.

Urine clenbuterol concentrations were below the LOD between 21 and 28 days in all horses that received the low-dose administration and 5 out of 6 horses receiving the escalating protocol. Mean, median, and range of the urine concentrations at 14 days after low-dose administration in all twenty-two horses are shown in Table 1.1.

Figure 1.1 Mean \pm s.d. plasma clenbuterol concentrations vs. time following oral administration of 0.8 $\mu\text{g}/\text{kg}$ bwt Ventipulmin® b.i.d. for 30 days to 22 racing fit Thoroughbred horses. Inset graph depicts plasma concentrations following the final dose. LOD = limit of detection; LOQ = limit of quantification.

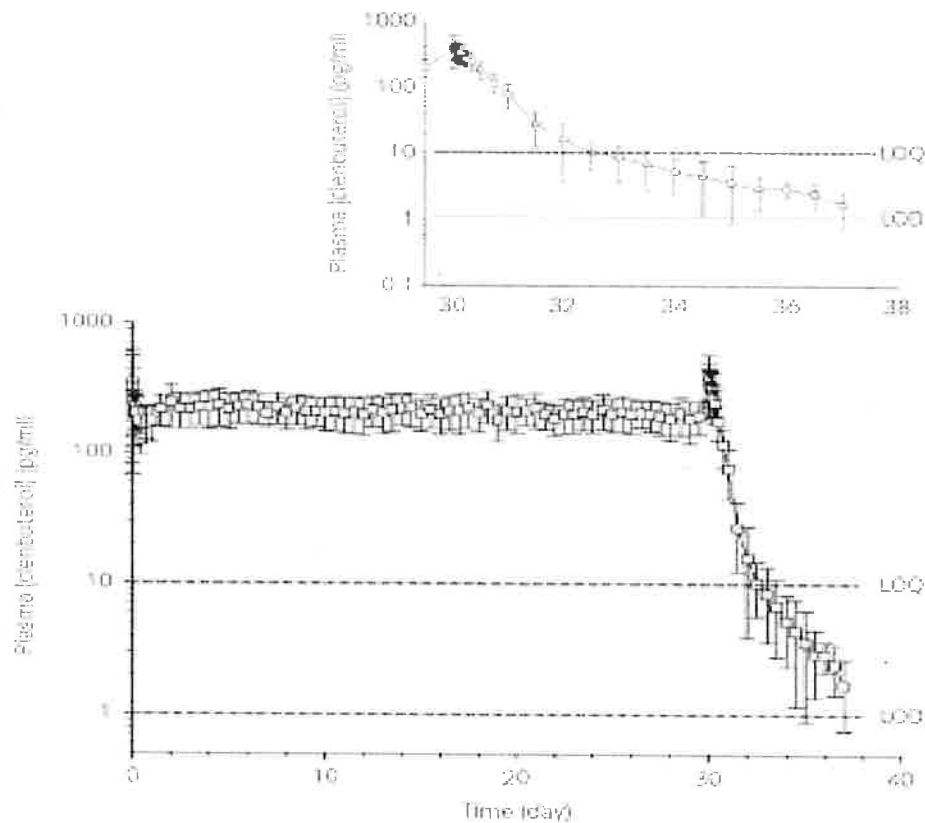


Table 1.1 Urine clenbuterol mean, median, and range at 14 days following last oral administration of 0.8 µg/kg BID of clenbuterol to 28 horses for 30 days.

Time after last administration (days)	Mean(±SD) pg/mL	Median pg/mL	Range pg/mL
14	35.28 ±20.61	33.25	12.5-76.1

Scientific Advisory Committee Recommendation

The 95/95 tolerance interval was calculated on the natural logarithmic (*i.e.*, ln) transformed urine concentration data from the 14-day collection time point for all 28 horses. and yielded a value of 136.7 pg/mL that was rounded to a threshold recommendation of 140 pg/mL of urine. Due to the concerns for the performance enhancing effects of race day administration, the RMTC Scientific Advisory Committee (SAC) recommended Limit of Detection for the threshold in serum or plasma (based upon the experimentally observed LOD).

Both thresholds must be met to ensure control of both serial oral administrations and a single race day administration of clenbuterol. To adequately control the use of clenbuterol it is important to submit paired (blood and urine) samples for analysis. The serum threshold is intended to control for race day administration. The urine threshold is intended to support the 14-day withdrawal interval. The submission of blood-only samples should be discouraged as it represents a vulnerability with respect to the control of the use of clenbuterol.

Practice Tips

Important Note: The recommended threshold and withdrawal guidance do NOT apply to Quarter Horse Racing where clenbuterol is a prohibited substance in recognition of its misuse as an anabolic agent. The use of clenbuterol in Quarter Horses is additionally controlled with hair testing.

The withdrawal guidance of 14 days is based upon a 0.8 mcg/kg twice daily, orally administered dose of clenbuterol given for a maximum of thirty days. Different formulations of clenbuterol, administration of higher doses, compounded products, or combinations of clenbuterol with other substances may result in concentrations above the threshold unless an extended withdrawal time is observed. Veterinarians are advised to assess the impact of these potential risk factors on the withdrawal time and to advise their clients accordingly.

Because there is an FDA-approved clenbuterol-containing product (Ventipulmin™) commercially available, the compounding of clenbuterol from any other source is not permitted pursuant to

FDA Guidelines. Illegally compounded clenbuterol has been acquired through investigations and in many cases laboratory analysis has determined that the concentration of clenbuterol differs widely from the labelled concentration. Administration of these illegal products has been associated with adverse events, including death.

Additionally, it is important to note that though clenbuterol can decrease fat mass percentage and increase muscle mass percentage multiple studies have shown that long term administration of clenbuterol causes a decrease in athletic performance from cardiac remodeling and decreased aerobic capacity, time to fatigue, cardiac function, and maximal oxygen consumption.^{xii}

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