RMTC
Racing Medication & Testing Consortium

June 19, 2012

RMTC DERMORPHIN BULLETIN

To stay ahead of participants in our sport who attempt to cheat, research to improve the detection of illegal substances is a perpetual effort of the Racing Medication and Testing Consortium (RMTC) and racing laboratories across the country. The RMTC, which is comprised of 25 industry stakeholder groups including the leading institutions in equine drug testing and research, is on the front line of the ongoing battle to strengthen the integrity of racing. Since 2001, it has been the research component for the racing industry, providing science-supported medication policies, rules and guidance to racing's regulatory authorities on all aspects of drug testing, as well as serving as a clearinghouse for the analysis and identification of unknown substances that pose potential threats. The RMTC also represents North American racing interests in the international community of horse racing regulatory authorities and drug testing advisory associations.

Attention Racing Commission Executive Staff:

This email is intended to serve as a notification of a recent threat to racing integrity.

Six months ago, Industrial Laboratories in Denver, Co., was told by state racing regulators to be vigilant for the highly potent opiate painkiller dermorphin – an alert that has resulted in a rash of post-race positives for the drug in at least two states. While no positive results were recorded in the first three months of testing, the laboratory began detecting the drug over two months ago, when the test was tweaked.

Believed to have 30 to 40 times more potency than morphine in similar concentrations, dermorphin is produced naturally as a skin secretion in certain species of South American frogs, but can also be produced synthetically. It is speculated that the drug is being manufactured synthetically due to the high doses that would be required to produce an effect in a racehorse.

Industrial Labs was the first to develop a method based on LC-MS for detecting and identifying dermorphin from official samples. TCC has had an ELISA test to detect dermorphin for several months but there are no reports documenting the validity of this ELISA test to detect dermorphin. Methods used to detect and confirm the identity of dermorphin in test samples or research samples have not been reported in any peer-reviewed publication, which could become an issue for concern as these findings move into administrative hearings and appeals.

Industrial Labs shared the testing method with LSU laboratory director Dr. Steve Barker, making it possible for his laboratory to confirm the detection of the drug in 15 split samples originating from Oklahoma. It is likely that this drug has been used for a substantial amount of time, as the LSU laboratory immediately began detecting positive samples after putting the new testing procedure in place.

Over the past week alone, 11 horses in Louisiana have tested positive for dermorphin, including both Thoroughbreds and Quarter Horses. The horses that tested positive in that state were competing at Delta Downs, Evangeline Downs and Louisiana Downs.

Industrial Labs conducts drug testing on behalf of racing commissions in Oklahoma, Colorado, Arizona, Minnesota and North Dakota, and has also shared the new testing method with a number of other laboratories across the U.S., introducing the possibility that more positives for the drug will be called in the next several weeks.

Currently, the recommended penalty for a dermorphin positive is a minimum one-year suspension, loss of purse and $10,000 fine.

The RMTC is committed to the long term integrity of horse racing and will be reaching out to each commission over the next two weeks to establish a dedicated commission contact to participate in secure monthly intelligence-sharing conference calls.

Thank you for your attention,

Jamie Haydon
RMTC Interim Executive Director

DERMORPHIN

A MONOGRAPH OF CURRENT KNOWLEDGE CONCERNING ITS USE AND DETECTION IN HORSES

PREPARED FOR

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Dermorphin

Dermorphin (figure 1) (CAS# 77614-16-5) is a heptapeptide that was first isolated and identified from the skin of South American tree frogs of the genus *Phyllomedusa* in the early 1980s (Montecucchi *et al*., 1981).

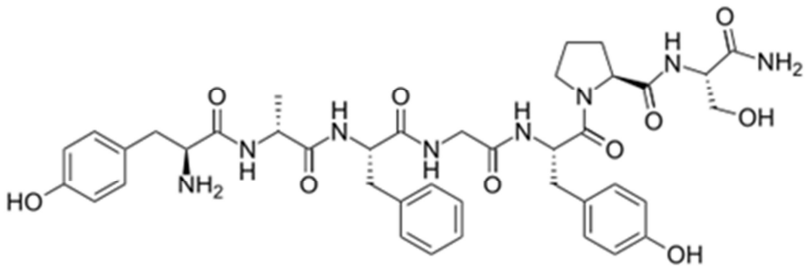

Figure 1. Chemical structure of dermorphin.

The Association of Racing Commissioners International, Inc. classified dermorphin as a Class 1 substance with Penalty Class A specifications one year ago when it was identified as a threat to racing integrity. Dermorphin has the potential to affect performance and has no legitimate use in horse racing. Dermorphin is an experimental substance that is not approved for any use in animals or humans in any part of the world.

Dermorphin is a potent analgesic substance that exerts its effects by selective activation of the mu opiate receptor in mammalian test models (Broccardo *et al*., 1981). Its potency may be as great as 30-40 times that of morphine depending on the model that is used to test it (Broccardo *et al*., 1981). The effects of dermorphin at the mu opiate receptor are antagonized by naloxone and other selective narcotic antagonists (Paakkari et al., 1990). Low doses of dermorphin cause respiratory and locomotor stimulation in mice that is attributed to interaction of dermorphin with a subclass of the mu receptor designated the mu1-receptor (Paakkari *et al*., 1990). Higher doses of dermorphin in mice produce respiratory depression that is attributed to activation of another class of mu receptor designated the mu2-receptor (Paakkari *et al*., 1990). The effects of dermorphin in the horse have not been documented at the time of this writing (June 2012).

Dermorphin is an unusual peptide in that the alanine residue is in the D-configuration whereas all amino acids in all mammalian proteins and peptides are exclusively in the L-configuration. The D-configuration is necessary for biological stability and pharmacologic activity. Studies have demonstrated that the pharmacologic activity of the peptide containing the D-alanine residue is approximately 4000 more potent than the synthetic analogue containing an L-alanine residue (Mizoguchi *et al*., 2011).

Dermorphin for use as a standard is available for purchase from a number of suppliers in the United States (see Appendix A). The purity and the identity of dermorphin purchased for use as a standard should be verified before use due to concerns about purity and identity.

Methods used to detect and confirm the identity of dermorphin in test samples or research samples have not been reported in any peer-reviewed publication as of this writing (June 2012). Industrial Laboratories was the first to report the presence of dermorphin from official samples.

They analyzed basic extracts of samples using a validated method based on liquid chromatography-mass spectrometry (LC-MS) to identify dermorphin. Other laboratories have also reported dermorphin from official samples including samples collected in Louisiana (http://www.nola.com/horseracing/index.ssf/2012/06/louisiana_state_racing_commiss_1.html). The number of reported findings from official samples nationwide is approximately thirty (June 2012).

Dermorphin is a heptapeptide (Tyr-D -Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) with molecular formula $C_{40}H_{50}N_8O_{10}$ and molecular weight of 802.87. The peptide can be extracted from samples using solid phase extraction techniques and analyzed by LC-MS without additional treatment such as trypsin digestion. The mass spectrum obtained on a dermorphin standard from GenScript is shown in figure 2.

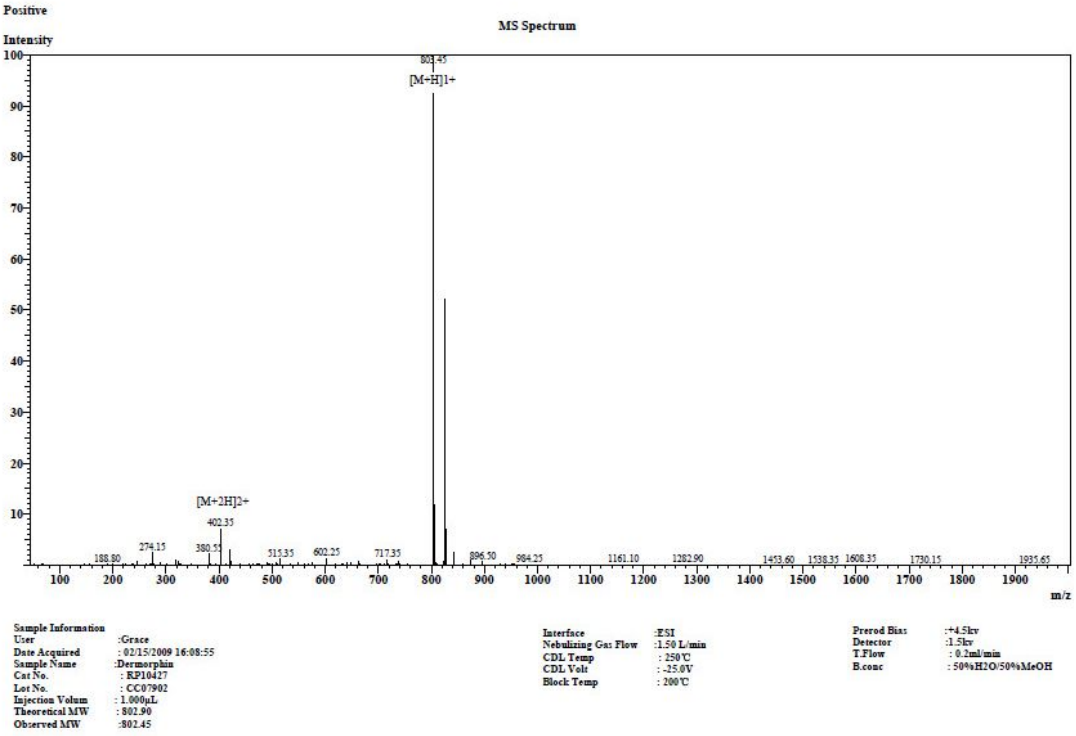

Figure 2. Mass spectrum of a standard of dermorphin from GenScript.

Peptides are often characterized by poor stability in blood due to the presence of enzymes such as proteases that cause their rapid degradation. However, incubation of dermorphin in mouse plasma and brain homogenate indicated excellent stability of dermorphin (Scalia *et al*., 1985; van Dorpe *et al*., 2010). Substitution of the D-alanyl residue for the D-alanyl residue in the native peptide dramatically reduces stability in blood (Scalia *et al*., 1985).

Professor Pat Colahan at the University of Florida (UF) administered dermorphin to horses in the UF research herd earlier this year at the request of the Racing Medication and Testing Consortium. HFL Sport Science, Inc. verified that the substance that was administered was dermorphin Dr. Colahan administered 7.5 mg of dermorphin IV and IM to each of three horses and collected blood and urine samples from them at specified times. These samples were

distributed to laboratories that requested them for use in method development. Samples from these studies remain at UF and can be obtained by contacting Professor Calahan (colahanp@ufl.edu).

TCC sells an ELISA test to detect dermorphin but there are no published reports documenting the validity of this ELISA test to detect dermorphin in test samples collected from horses.

References

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APPENDIX A – COMMERCIAL SOURCES OF DERMORPHIN

Abgent, Inc. 10239 Flanders Court San Diego, CA 92121	AnaSpec 34801 Campus Drive Fremont, CA 94555	GenScript USA Inc. 860 Centennial Ave. Piscataway, NJ 08854
LKT Laboratories, Inc. 545 Phalen Blvd. St. Paul, MN 55130	Phoenix Pharmaceuticals, Inc. 330 Beach Road Burlingame, CA 94010	