## *“Controlled administration trial of Oral-Turinabol metabolite confirmation and elimination profiles with special respect to long-term metabolites”*

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### Project Overview

Increasing numbers of adverse analytical findings were reported in the recent years due to the misuse of the anabolic androgenic steroid dehydrochloromethyltestosterone (DHCMT). Once developed in the former GDR for misuse in sports it regained enormous relevance especially in the samples from Bejing and London Olympic games. Several adverse analytical findings were reported after the samples had been retested for the newly reported long-term metabolite of DHCMT.

At present, the use of post administration urines instead of purified reference material has been accepted in confirmatory analyses. Very recently the currently used metabolites have been questioned in the literature. Thus a controlled administration trial in humans will be performed to provide further evidence for tracing back the long term metabolites 20ξOH-NorTHCMT and 20βOH-NorDHCMT to a DHCMT administration. Furthermore, the utilisation of in-vitro experiments will further broaden the scientific insights into metabolic pathways that lead to the generation of these metabolites.

### Results and Conclusions:

Dehydrochloromethyltestosterone (DHCMT) is an anabolic-androgenic steroid that was developed by Jenapharm in the 1960s and was marketed as Oral Turinabol®. It is prohibited in sports at all times. Even if discontinued as pharmaceutical in 1994, there are several adverse analytical findings by anti-doping laboratories every year. New long-term metabolites have been proposed in 2011/12, which resulted in adverse analytical findings in retests of the Olympic games of 2008 and 2012. However, no controlled administration trial monitoring these long-term metabolites was reported until now. In this study, a single oral dose of DHCMT (5 mg, p.o.) was administered to five healthy male volunteers and their urine samples were collected for a total of 60 days. The unconjugated and the glucuronidated fraction were analyzed separately by gas chromatography coupled to tandem mass spectrometry. The formation of the described long-term metabolites was verified, and their excretion monitored in detail.

Due to interindividual differences there were several varieties in the excretion profiles among the volunteers. The metabolite M3, which has a fully reduced A-ring and modified D-ring structure, was identified by comparison with reference material as 4α-chloro-17β-hydroxymethyl-17α-methyl-18-nor-5α-androstan-13-en-3α-ol. It was found to be suitable as long-term marker for the intake of DHCMT in four of the volunteers. In one of the volunteers, it was detectable for 45 days after single oral dose administration. However, in two of the volunteers M5 (already published as long-term metabolite in the 1990s) showed longer detection windows. In one volunteer M3 was undetectable but another metabolite, M2, was found as the longest detectable metabolite.

The last sample clearly identified as positive was collected between 9.9 and 44.9 days. Furthermore, the metabolite epiM4 (partially reduced A-ring and a modified D-ring structure which is epimerized in position 17 compared to M3) was identified in the urine of all volunteers with the help of chemically synthesized reference as 4-chloro-17α-hydroxymethyl-17β-methyl-18-nor-androsta-4,13-dien-3β-ol. It may serve as additional confirmatory metabolite.

To improve tracing of cheating athletes, it is highly recommended to screen for all known metabolites in both fractions, glucuronidated and unconjugated. This study also offers some deeper insights into the metabolism of DHCMT and of 17α-methyl steroids in general.