

## PROJECT REVIEW

### **“Characterization of Chemical and Pharmacological Properties of New Steroids Related to Doping of Athletes”**

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This project is aimed at providing rapid answers to testing authorities following the seizure and/or the discovery of use of a new steroid. As a matter of fact little can be done at the analytical level until the substance has been fully characterized, its urinary metabolites identified, the reference material made available and its potential performance-enhancing properties investigated.

Since 2000, the testing authorities have been alerted to the use of new steroids by certain athletes.

Public comments made by individuals involved in the Balco scandal are adding to the information obtained from other sources, mainly informants, to the effect that other “designer” potent and undetectable steroids have been prepared and would be available to some athletes. That has become a certainty in December 2003, following seizure at the Canadian border of hGH and two steroid products one of which being THG. The identification of the second one, DMT (17 $\alpha$ -methyl-5 $\alpha$ -androst-2-en-17 $\beta$ -ol) has been done within the scope of this grant application.

Lastly, urine samples of athletes at “high risks” have been found to contain metabolites of two isomers of a steroid which are not currently tested for.

Steroids or urinary steroid metabolites will be identified employing analytical techniques such as mass spectrometry and NMR; structures will be proposed. The synthesis of reference standards will permit the full characterization of the compounds (parent and metabolites). The hormonal properties of the steroids, androgenic/anabolic, estrogenic and progestagenic will be assayed. Studies will be conducted to enable the identification of the metabolites (phase I and II) which will permit ultimately their inclusion to testing methods

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### **Results and Conclusions**

We consider having fully met the objectives contained in the original application. New steroids were characterised: DMT1 - 17 $\alpha$ -methyl-5 $\alpha$ -androst-2-en-17 $\beta$ -ol major isomer along with the 3-en isomer, methyldrostanolone - 2 $\alpha$ ,17 $\alpha$ -dimethyl-5 $\alpha$ -androstan-17 $\beta$ -ol-3-one (sold under the names of Superdrol, Methasterone; and Guggulsterone -4,17(20)-pregnadiene-3,16-dione.

Anticipating what could be the new molecules introduced clandestinely we have synthesized 17-methylated derivatives of popular steroids such as methenolone and its isomer stenbolone.

The phase I metabolites were successfully produced from incubations with cryopreserved human hepatocytes for several steroids particularly drostanolone and its 17-methylated derivative, “Superdrol”. Structures were proposed for a novel metabolite hydroxylated in C-2 and confirmed by chemical synthesis. NMR and mass spectrometry were utilised to characterise the metabolites. The model failed however to produce conjugated phase II metabolites and significant amounts of phase 1 metabolites from desoxymethyltestosterone, DMT. In spite of these limitations, the identification of metabolites was much easier in incubation medium when compared to the heavily interfered urine matrix. The utilisation of human hepatocytes significantly reduces to the minimum the administration to human volunteers.

We have extended by the work in order to finish the characterisation of the metabolites of methyldrostanolone and drostanolone including new ones that were not reported previously.