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WADA Technical Letter – TL06

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Written by:	*WADA* LabEG	Approved by:	*WADA* LabEG*
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**The approval by the WADA Executive Committee is applicable only to Technical Letters issued after November 2019.*

POSSIBLE METABOLISM OF PROGUANIL INTO CHLORAZANIL

The *World Anti-Doping Agency* wishes to draw the attention of the Laboratories to the following remarks and instructions on the analysis and reporting of **chlorazanil**.

Scientific information and technical developments indicate that the anti-malarial drug proguanil can be metabolized under certain conditions into the prohibited diuretic chlorazanil. We therefore recommend that before chlorazanil is reported as an *Adverse Analytical Finding*, additional attention is given to testing the presence of proguanil or its *Metabolite(s)* in the *Sample* in order to exclude the possibility of proguanil as the primary source of chlorozanil.

Particular attention should be given to the *Doping Control* form for any relevant declaration of proguanil.

See complementary technical information below.

For further information, please also refer to: Thevis M, Geyer H, Thomas A, Tretzel L, Bailloux i, Buisson C, Lasne F, Schaefer MS, Kienbaum P, Mueller-Stoever I, Schänzer W: Formation of the diuretic chlorazanil from the antimalarial drug proguanil - Implications for sports drug testing. *Journal of Pharmaceutical and Biomedical Analysis* **115** (2015) 208–213.

Should you have any further questions, please do not hesitate to contact the *WADA* Science Department.

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SCIENTIFIC BACKGROUND*

Chlorazanil (Ordipan, N-(4-chlorophenyl)-1,3,5-triazine-2,4-diamine) is a diuretic agent and as such prohibited in sport according to the *International Standard* of the *List* of *Prohibited Substances* and *Prohibited Methods*. Despite its introduction into clinical practice in the late 1950s, the first two *Adverse Analytical Findings* were reported only in 2014. Such rare cases lead to further investigations as both *Athletes* denied the intake of the drug. However, the *Athletes* did declare the use of the antimalarial prophylactic agent proguanil due to temporary residences in African countries.

A structural similarity between chlorazanil and proguanil is given but no direct metabolic relation has been reported in the scientific literature. Moreover, chlorazanil has not been confirmed as a drug impurity of proguanil. Proguanil however is metabolized in humans to N-(4-chlorophenyl)-biguanide, which represents a chemical precursor in the synthesis of chlorazanil. In the presence of formic acid, formaldehyde, or formic acid esters, N-(4-chlorophenyl)-biguanide converts to chlorazanil.

In order to probe for potential sources of the chlorazanil detected in the *Samples*, drug formulations containing proguanil and urine samples of individuals using proguanil as antimalarial drug were subjected to liquid chromatography-high resolution/high accuracy mass spectrometry. In addition, in vitro simulations with 4-chlorophenyl-biguanide and respective reactants were conducted in urine and resulting specimens analyzed for the presence of chlorazanil.

While no chlorazanil was found in drug formulations, some of the urine samples of proguanil users returned findings for chlorazanil at low ng/mL levels, similar to the *Adverse Analytical Findings* in the *Samples*. Further, in the presence of formaldehyde, formic acid and related esters, 4-chlorophenyl-biguanide was found to produce chlorazanil in human urine, suggesting that the detection of this old diuretic agent was indeed the result of artefact formation and not of the illicit use of a *Prohibited Substance*.

Future chlorazanil findings in sports drug testing samples data interpretation in the light of the herein presented cases is recommended to avoid unwarranted accusations of athletes. In particular, presence of proguanil and *Metabolites* should be investigated, the *Doping Control* form and a note in the opinion box of the certificate of analysis should be used, in case chlorazanil is identified and reported to Testing Authorities.

* Based upon the research report provided by Wilhem Schänzer and Hans Geyer from the Cologne Laboratory