"Can the application of the anti-malaria drug proguanil lead to an adverse analytical finding for the diuretic agent chlorazanil?"

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Project Overview

Investigations in connection with an adverse analytical finding for the prohibited diuretic chlorazanil have raised the presumption that chlorazanil may originate from the application of the non-prohibited anti-malaria drug proguanil (ingredient e.g in medicament Malarone® from GSK). The structure of metabolites of the non-prohibited anti-malaria drug proguanil suggests that they can be converted to chlorazanil. To check this hypothesis it should be found out whether chlorazanil is a metabolite of proguanil or whether proguanil metabolites are converted in the urine to chlorazanil by chemical or bacterial influences or whether chlorazanil is a byproduct of the synthesis of proguanil.

Furthermore parameters should be identified which allow a discrimination between the application of the non-prohibited proguanil and the prohibited chlorazanil

Results and Conclusions

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 regulations of the World Anti-Doping Agency (WADA). Despite its introduction into clinical practice in the late 1950s, the worldwide very first two adverse analytical findings were registered only in 2014, being motive for an in-depth investigation of these cases. Both individuals 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 doping control 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, the urine samples of 2 out of 4 proguanil users returned findings for chlorazanil at low ng/mL levels, similar to the adverse analytical findings in the doping control 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 the obsolete diuretic agent was indeed the result of artefact formation and not of the illicit use of a prohibited substance.