WADA Technical Letter – TL26 Clomifene

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Written by: Reviewed by:	*WADA* Science *WADA* Laboratory Expert Advisory Group	Approved by:	*WADA* Executive Committee
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Clomifene

1.0 Introduction

WADA wishes to draw the attention of the Laboratories to the following observations and instructions on the analysis and reporting of clomifene (CLO) findings in urine *Samples*.

CLO is prohibited at all times (*In- and out-of-Competition)*, and as such is included in the *WADA Prohibited List* under section S4.2 Anti-estrogenic Substances [1].

However, CLO may be used as a fertility enhancer in hens to increase egg production [2-4]. Therefore, there is also the possibility that the presence of CLO and/or its *Metabolites* in urine *Samples* may result from food contamination, as CLO residues may be present in contaminated poultry meat and eggs [5].

1.1 Considerations on Pharmacokinetics of CLO from Contaminated Food Intake

After the consumption of CLO-containing poultry eggs or meat, (*Z*)-4-OH-CLO has been identified as the most abundant CLO *Metabolite* present in urine *Samples.* In contrast, (*Z*)-3-OH-CLO has been identified as the most abundant *Metabolite* after direct human intake of CLO [6]. In addition, it has been demonstrated that CLO parent compound can be detected in urine *Samples* after consumption of two (2) CLO-containing eggs in concentrations up to 25 pg/mL (*i.e.*, 0.025 ng/mL) [6].

1.2 Genetic Polymorphism Impact on CLO Urinary Excretion

The genetic polymorphism of CYP2D6 (the main CYP isozymes related to the CLO metabolism) or alterations in its enzymatic activity may influence CLO biotransformation, leading to variations in the expected presence/concentrations of (Z)-4-OH-CLO and (Z)-3-OH-CLO [7].

Furthermore, a two (2)-fold difference in CLO parent compound concentration in blood has been observed due to variations in CYP2D6 activity across different genotype groups [7]. Consequently, the concentration of the CLO parent compound in urine *Samples* may also be affected by variations in the CYP2D6 biotransformation process. This suggests that, in combination with the potential ingestion of large quantities of CLO-contaminated poultry products, individual differences could lead to elevated CLO urinary concentrations (beyond 25 pg/mL [6]) in food contamination scenarios.

[Comment to Article 1.2: The total CLO concentration observed in urine Samples resulting from food contamination may depend on the amount and intervals of ingestion of contaminated poultry meat and/or eggs, the extent of CLO contamination, and the genetic polymorphism of the individual.]

1.3 Analytical Considerations of CLO

The chromatographic and mass spectrometric differentiation of the two (2) hydroxy (OH)-CLO *Metabolites* mentioned above from other OH-CLO *Metabolites* can be challenging due to coelution [6], and unavailability of appropriate Reference Materials (RMs). Therefore, relying on a ratio between (*Z*)-4-OH-CLO and (*Z*)-3-OH-CLO concentrations to establish the source of CLO could be misleading.

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1.4 Further Challenges on CLO Metabolism Investigation

CLO has a complex metabolic pathway, and several other OH-CLO *Metabolites* have been described. For example, 4-OH-methoxy-CLO has also been suggested as a target Analyte for monitoring CLO misuse due to its longer detection window. However, different producers of RM have commercialized different isomers of 4-OH-methoxy-CLO under the same nomenclature, which negatively impacts the reliability of targeting this specific CLO *Metabolite* by the Laboratories [8-12].

1.5 Considerations on Pharmacokinetics of CLO Misuse in Sport

Studies have demonstrated the long-term detection of CLO parent compound in urine *Samples* in the absence of any other *Metabolites* [13-14]. In some other cases, OH-CLO *Metabolites* were observed together with the CLO parent compound, although in less abundance than the CLO parent compound [13-14].

Urinary elimination of CLO is nonuniform, and in most individuals in which CLO administration has been studied, the CLO parent compound concentration was detected at a concentration higher than (>) two (2) ng/mL for a prolonged period while taking the drug, followed by rapidly dropping excreted concentrations during the washout phase [15]. Therefore, the detection of CLO parent compound at concentrations lower than or equal to (≤) two (2) ng/mL may also be attributed to the washout phase of CLO administration, and not only due to consumption of contaminated food.

2.0 CLO Analytical *Testing* Strategy and Reporting Requirements

2.1 "A" *Sample*:

a) The Minimum Required Performance Level (MRPL) for CLO parent compound is set at 2.0 ng/mL Therefore, the Limit of Detection (LOD) of the Initial Testing Procedure (ITP), as estimated during Test Method validation, shall be not higher than (≤) 1.0 ng/mL (50% of MRPL).

b) The "A" Confirmation Procedure (CP) shall confirm the presence of the CLO parent compound in compliance with the effective *TD* IDCR [16].

[Comment to 2.1.a: The Laboratory may monitor the presence of CLO phase-I and phase-II Metabolites in the ITP and CP to strengthen the interpretation of the test results; however, the concentrations of the CLO phase-I and phase-II Metabolites shall not be considered for the application of the MRL.]

c) The Limit of Identification (LOI) of the CP, as estimated during Test Method validation, shall be less than (<) 2.0 ng/mL.

d) To exclude the potential food contamination scenario, a *Minimum Reporting Level* (*MRL*) in urine, applicable to the free-fraction CLO parent compound only (as the sum of both CLO isomers: zuclomifene and enclomifene), is set at the MRPL value of 2.0 ng/mL.

e) To estimate the concentration of the CLO parent compound in the "A" *Sample*, the "A" CP shall follow the requirements established in the effective *TD* MRPL [17] for Non-Threshold Substances

with an *MRL*.

f) *Adverse Analytical Findings* (*AAF*)

As per the *TD* MRPL reporting requirements for Non-Threshold Substances with an *MRL* [17], an *AAF f*or CLO shall be reported if the CLO parent compound is confirmed in the "A" *Sample* at an estimated concentration (adjusted for specific gravity (SG), if needed), which is confidently higher (as determined by comparison with a 120% *MRL* single point calibrator – see *TD* MRPL) than (>) the *MRL* of 2.0 ng/mL. This is applied irrespective of the presence of any other *Metabolites*.

[Comment to Article 2.1 f): This Technical Letter is an integral part of the International Standard for Laboratories (ISL) [18] and supersedes any previous publication on a similar topic, including Technical Document(s) (e.g., TD MRPL) and/or the ISL]

g) *Atypical Finding* (*ATF*)

In addition, the Laboratory shall report the presence of the CLO parent compound (as the sum of both CLO isomers: zuclomifene and enclomifene) in a urine *Sample* at an estimated concentration equal to or below (≤) the *MRL* of 2.0 ng/mL as an *ATF*. If an *ATF* is reported, the *Results Management* Authority (RMA) shall conduct a mandatory investigation to determine whether evidence exists that establishes that the consumption of contaminated poultry meat and/or eggs is more likely than not the explanation for the *ATF*. If such evidence exists, the RMA will take no further action in respect of the *ATF*. If such evidence does not exist, the RMA will progress the finding as an *AAF*.

[Comment to Article 2.1 g): Depending on the circumstances, the consumption of contaminated poultry meat and/or eggs may lead to very low concentrations of CLO parent compound in the urine of the consumer of the contaminated food. Therefore, the presence in urine of CLO parent compound at a concentration of 2.0 ng/mL or less (≤ 2 ng/mL) shall be reported as an ATF, even though the likelihood of a contaminated food consumption scenario decreases the closer the urinary concentration gets to that 2.0 ng/mL limit. Upon receipt of the ATF, the RMA shall conduct a mandatory investigation to determine whether there is sufficient evidence to support contaminated food consumption as the more likely than not explanation. This investigation may include, for example, the evaluation of the Athlete's longitudinal urinary T/E values and blood testosterone concentrations to assess a possible CLO doping scenario [15].

2.2 "B" *Sample*:

The "B" *Sample* CP shall only confirm the presence, at any concentration, of the CLO parent compound (in compliance with the *TD* IDCR [16]) for the *AAF* to be valid. No quantification or estimation of concentration is necessary.

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3.0 References

[1] *WADA Prohibited List*

[2] McGinnis Jr, C. H., and L. D. Wallace. "The effect of clomiphene citrate in chickens: 1. Androgenic and estrogenic activity." *Poultry science* 50.5 (1971): 1475-1480.

[3] Robinzon, B., et al. "The effect of clomiphene-citrate on broody turkey hens." *Poultry science* 63.11 (1984): 2268-2270.

[4] Goetting, Valerie, K. A. Lee, and L. A. Tell. "Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of the literature." *Journal of veterinary pharmacology and therapeutics* 34.6 (2011): 521-556.

[5] Seyerlein, L., Gillard, N., Delahaut, P., Pierret, G., Thomas, A. and Thevis, M., 2021. Depletion of clomiphene residues in eggs and muscle after oral administration to laying hens. *Food Additives & Contaminants: Part A*, *38*(11), pp.1875-1882.

[6] Euler, Luisa, et al. "Assessing human urinary clomiphene metabolites after consumption of eggs from clomiphene-treated laying hens using chromatographic-mass spectrometric approaches." *Analytica Chimica Acta* 1202 (2022): 339661.

[7] Kim, M.J., Byeon, J.Y., Kim, Y.H., Kim, S.H., Lee, C.M., Jung, E.H., Chae, W.K., Lee, Y.J., Jang, C.G., Lee, S.Y. and Choi, C.I., 2018. Effect of the CYP2D6* 10 allele on the pharmacokinetics of clomiphene and its active metabolites. *Archives of pharmacal research*, *41*, pp.347-353.

[8] Mazzarino, M., Fiacco, I., De La Torre, X. and Botrè, F., 2008. A mass spectrometric approach for the study of the metabolism of clomiphene, tamoxifen and toremifene by liquid chromatography time-of-flight spectroscopy. *European Journal of Mass Spectrometry*, *14*(3), pp.171-180.

[9] Mazzarino, M., Biava, M., de la Torre, X., Fiacco, I. and Botrè, F., 2013. Characterization of the biotransformation pathways of clomiphene, tamoxifen and toremifene as assessed by LC-MS/(MS) following in vitro and excretion studies. *Analytical and bioanalytical chemistry*, *405*, pp.5467-5487.

[10] Kröner, P., Heinkele, G., Kerb, R., Igel, S., Schwab, M. and Mürdter, T.E., 2021. Stereoselective quantification of phase 1 and 2 metabolites of clomiphene in human plasma and urine. *Talanta*, *221*, p.121658.

[11] Lu, J., He, G., Wang, X., Xu, Y., Wu, Y., Dong, Y., He, Z., Liu, X., Bo, T. and Ouyang, G., 2012. Mass spectrometric identification and characterization of new clomiphene metabolites in human urine by liquid chromatography–quadrupole time-of-flight tandem mass spectrometry. *Journal of Chromatography A*, *1243*, pp.23-32.

[12] Lu, J., He, G., Wang, X., Xu, Y., Wu, Y., Shen, L., Yan, K. and He, Z., 2013. Mass spectrometric analyses of urinary clomiphene and toremifene metabolites in doping control by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF). *Analytical Methods*, *5*(23), pp.6677-6681.

[13] Guddat, S., Görgens, C., Geyer, H., Pfanner, T. and Thevis, M., 2018. Clomiphene—targeting of the unchanged drug results in unusual, prolonged detection windows in urine. *Recent Advances in Doping Analysis*, *26*, pp.118-121.

[14] Ahi S, Beotra A, Upadhyay A, Bhardwaj A, Jain S.,2014. Excretion study of Clomiphene and its correlation with unusual findings in the routine doping control samples. *Recent Advances in Doping Analysis*, 22, pp.75-78

[15] Miller, G.D., Moore, C., Nair, V., Hill, B., Willick, S.E., Rogol, A.D. and Eichner, D., 2019. Hypothalamic-pituitary-testicular axis effects and urinary detection following clomiphene administration in males. *The Journal of Clinical Endocrinology & Metabolism*, *104*(3), pp.906-914.

[16] *WADA Technical Document TD* IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for *Doping Control* Purposes.

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[17] *WADA Technical Document TD* MRPL: Minimum Required Performance Levels and Applicable *Minimum Reporting Levels* for Non-Threshold Substances Analyzed by Chromatography-Mass Spectrometric Analytical Methods.

[18] The World Anti-Doping Code International Standard for Laboratories (ISL)