Document Number:	TL22	Version Number:	3.0
Written by: Reviewed by:	*WADA* Science *WADA* Laboratory Expert Group	Approved by:	*WADA* Executive Committee
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ETHYLMORPHINE

1.0 Introduction

WADA wishes to draw the attention of the Laboratories to the possible detection of the Threshold Substance **Morphine** (M) at a concentration higher than (>) the *Decision Limit* (*DL*) in urine *Samples*, which may result from the administration of the permitted drug **Ethylmorphine** (EtM).

In humans, EtM, which is the 3-ethoxy homologue of M, is mainly metabolized to ethylmorphine-6-glucuronide (EtM-6-glucuronide), and to M by O-deethylation by the microsomal enzyme CYP2D6. In addition, EtM is also biotransformed to **Norethylmorphine** (nor-EtM) by N-demethylation (Figure 1) [1,2,3].

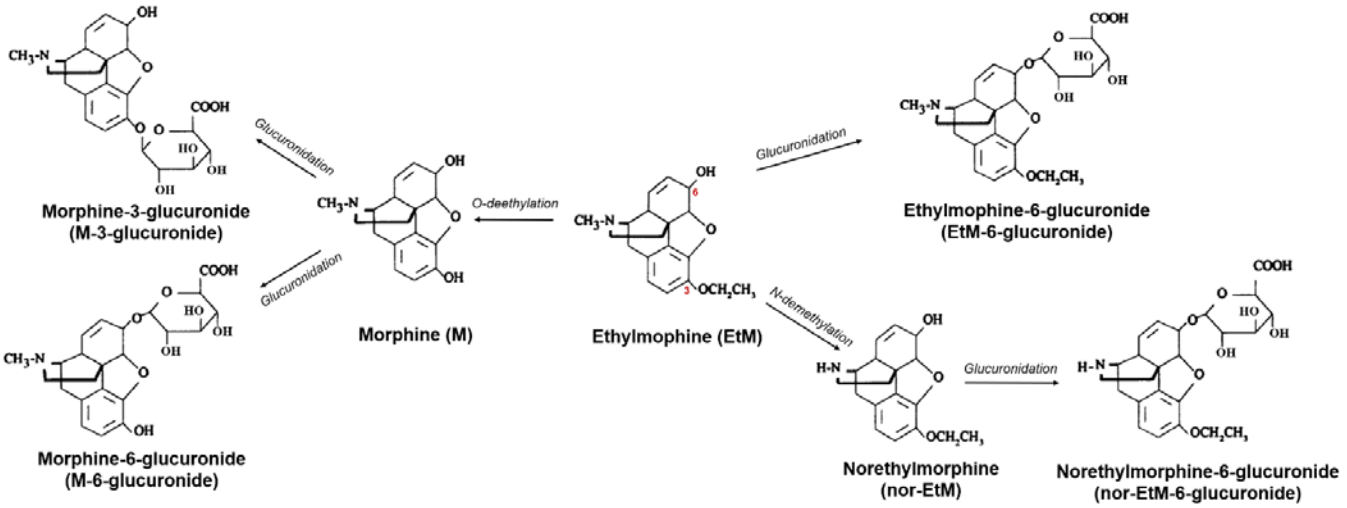

Figure 1. Metabolic pathways of ethylmorphine (EtM). Glucuronidation of ethylmorphine to ethylmorphine-6-glucuronide (EtM-6-glucuronide) is the major metabolic pathway followed by O-deethylation to morphine (M) and N-demethylation to norethylmorphine (nor-EtM).

The literature indicates that, following the administration of EtM, the urinary concentration of total EtM (free and conjugated forms) decreases more rapidly than the concentration of total M (free and conjugated forms) [4]: the calculated half-life ($t_{1/2}$) of total EtM in urine was 3-4 times shorter than the $t_{1/2}$ of total M. It has been also described that 24 hours after the administration of EtM, only M, M-3-glucuronide, M-6-glucuronide and N-demethylated *Metabolites* of EtM (nor-EtM and nor-EtM-6-glucuronide) were detected [2,4].However, a wide inter-individual variation was observed in the formation of M-3- and M-6-glucuronides, which does not allow to distinguish the source of M based solely on the concentration ratio of M to EtM [3,4]. Therefore, to determine the potential origin of M from a permitted

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administration of EtM, an additional test for the presence of EtM and nor-EtM shall be included in the Confirmation Procedure (CP) for M.

2.0 Analysis and Reporting Requirements

When a *Sample* with a total M concentration greater than (>) the *DL* (adjusted *DL* if the *Sample* SG > 1.018 [5]) includes the presence of EtM and/or nor-EtM, the Laboratories shall report the finding as follows:

- Negative Finding if:
  - The concentration of total M (free and conjugated forms) is equal to or lower than (≤) the concentration of EtM (free and conjugated forms);

    OR
  - The ratio of total M to total nor-EtM (free nor-EtM + nor-EtM-6-glucuronide) is equal to or lower than (≤) 20.

- *Adverse Analytical Finding* (*AAF*) for M if:
  - The concentration of total M (free and conjugated forms) is greater than (>) the concentration of EtM (free and conjugated forms);

    AND
  - The ratio of total M to total nor-EtM (free nor-EtM + nor-EtM-6-glucuronide) is higher than (>) 20.

  When reporting an *AAF* for M in the presence of EtM, a comment shall be included in the Test Report indicating that "Morphine was detected at a concentration greater than the *DL*, which was also higher than the concentration of total ethylmorphine detected in the *Sample*. In addition, the ratio of total morphine to total norethylmorphine was higher than 20. This is consistent with the mixed intake of morphine and ethylmorphine."

  [Comment: It is recommended that the Laboratories evaluate the rate of hydrolysis of EtM-glucuronide and nor-EtM-6-glucuronide (if the Reference Material is available) in their CP method validation, if applicable. The evaluation should also confirm the lack of artifact(s) formation. In the absence of nor-EtM-6-glucuronide Reference Material, the evaluation should consider a similar conjugate such as norcodeine-6-glucuronide]

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3.0 References

[1] Ripel, Å. *et al*. Morphine formation after intake of ethylmorphine. *Pharmacol Toxicol* **70**(3): 228-229, 1992.

[2] Aasmundstad, T. A. *et al*. Biotransformation and pharmacokinetics of ethylmorphine after a single oral dose. *Br. J. Clin. Pharmacol*. **39**(6): 611-620, 1995.

[3] Rane, A., Modiri A. R., and Gerdin E. Ethylmorphine O-deethylation cosegregates with the debrisoquin genetic metabolic polymorphism. *Clin. Pharmacol. Ther.* **52**(3): 257-264, 1992.

[4] Popa, C., Beck, O., and Brodin, K. Morphine formation from ethylmorphine: Implications for drugs-of-abuse testing in urine. *J. Anal. Toxicol.* **22**(2): 142-147, 1998.

[5] *WADA Technical Document* TD DL: *Decision Limits* for the Confirmatory Quantification of Exogenous Threshold Substances by Chromatography-based Analytical Methods.

[Current versions of WADA Technical Documents may be found at https://www.wada-ama.org/en/what-we-do/science-medical/laboratories]