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

1.0 Introduction

WADA wishes to draw the attention of the Laboratories to the following observations and instructions on the analysis, evaluation and reporting of analytical results for **Meclofenoxate**.

Meclofenoxate is rapidly degraded to **4-Chlorophenoxyacetic acid** (**4-CPA**) in biological fluids (*e.g.,* human plasma or urine) [1,2]. However, the presence of 4-CPA in urine may originate not only from meclofenoxate *Use*, but also from the following permitted administrations:

i. Food containing residues of 4-CPA, which is also used as an herbicide and a plant growth regulator in some countries or regions of the world [3].

[Comment: The substances from the chlorinated phenoxy acid herbicides (CPAHs) class, which includes 4- CPA, present similar pharmacokinetics, and after ingested, they are rapidly eliminated unchanged in the urine [3,4].]

ii. **Chlorphenesin** [3-(*p*-chlorophenoxy)-propane-1,2-diol], a non-prohibited substance that is used as a preserving agent in cosmetics and lotions or approved in selected countries, as **Chlorphenesin carbamate**, for the relief of muscle pain.

[Comment: Chlorphenesin functions as a biocide in cosmetics and is used at concentrations up to 0.32% in rinse-off products and up to 0.3% in leave-on products [5-7].

*Chlorphenesin is converted into 4-CPA after oral or transdermal administration, readily exceeding urinary concentrations of 5 µg/mL. Diagnostic Markers for the application of chlorphenesin-containing products are **3-(4-chlorophenoxy)-2-hydroxypropanoic acid** (**4-CPP**), **Chlorphenesin glucuronide** and **Chlorphenesin sulfate** (see Figure 1). These Metabolites are formed from chlorphenesin and chlorphenesin carbamate, but not from meclofenoxate.[8]]*

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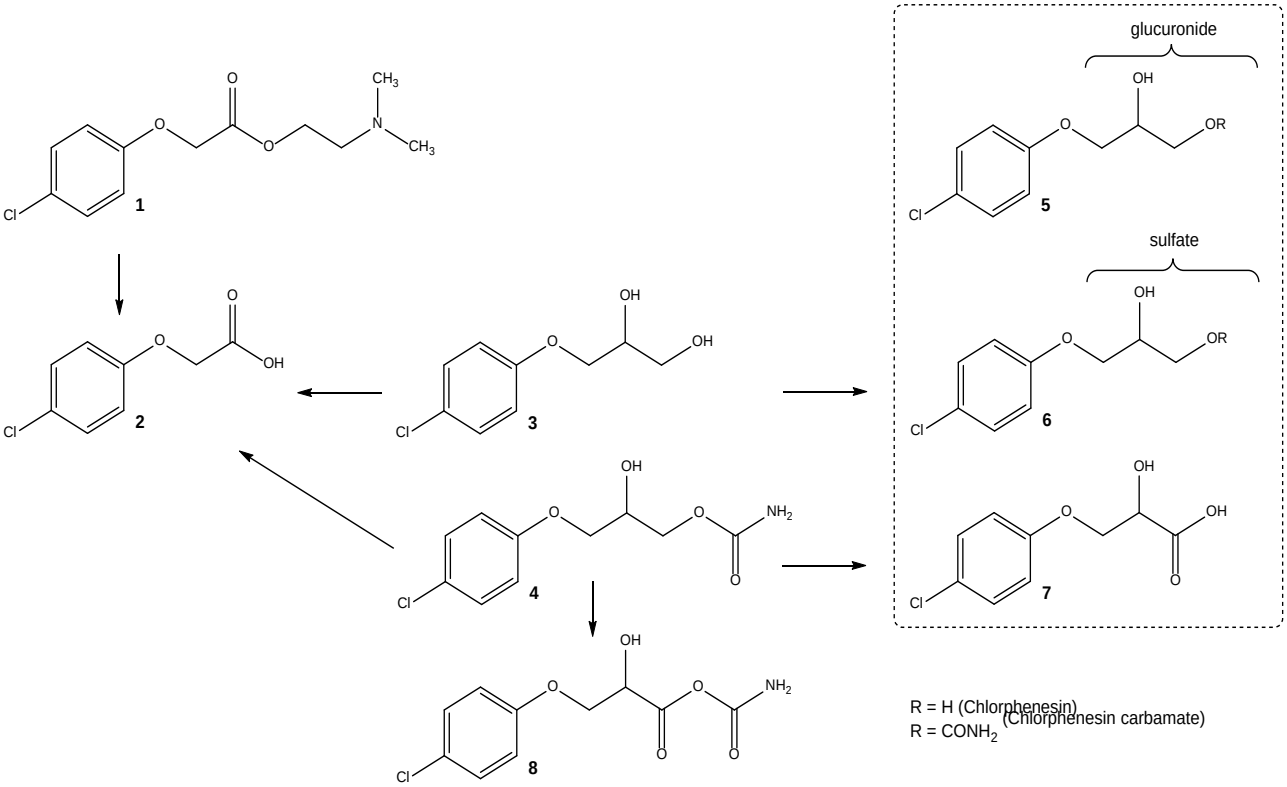

Figure 1. Chemical structures of meclofenoxate (**1**), 4-chlorophenoxy acetic acid (4-CPA, **2**), chlorphenesin (**3**), chlorphenesin carbamate (**4**), chlorphenesin glucuronide (**5**), chlorphenesin sulfate (**6**), 3-(4-chlorophenoxy)-2-hydroxypropanoic acid (4-CPP, **7**), and 4-CPP carbamate (**8**).

2.0 Analysis and Reporting Requirements

Before reporting a result as an *Adverse Analytical Finding* (*AAF*) for meclofenoxate, Laboratories shall exclude the consumption of 4-CPA contaminated food and/or exposure to chlorphenesin-containing products or chlorphenesin carbamate as the origin of the finding, as per Table 1 below.

In order to probe for the presence of chlorphenesin or chlorphenesin carbamate-derived *Metabolites* in urine *Samples*, which provides evidence for a permitted source of 4-CPA, both GC-MS(/MS) as well as LC-MS(/MS) are applicable Analytical Methods. Detecting either chlorphenesin or chlorphenesin carbamate after hydrolysis of the glucuronide and/or sulfate or targeting the intact chlorphenesin or chlorphenesin carbamate conjugates or 4-CPP can readily indicate whether the origin of the detected 4-CPA is related to the permissive use of chlorphenesin-containing products or chlorphenesin carbamate drugs. For more details refer to Rubio *et al* [8].

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Table 1. Conditions for reporting meclofenoxate findings ("A" *Sample*):

Condition	Meclofenoxate	4-CPA	Chlorphenesin or chlorphenesin carbamate *Metabolite*(s) [a]	Report finding as
1	**> 50 ng/mL**	[b]	**N/A**	***AAF***
2	**≤ 50 ng/mL or Not Detected**	A. ≤ 5 μg/mL	N/A	Negative Finding
		B. > 5 μg/mL	Detected	Negative Finding
		C. **> 5 μg/mL**	**Not Detected**	***AAF***

[a] This applies to any chlorphenesin or chlorphenesin carbamate *Metabolite* (see Figure 1).

[b] When meclofenoxate is detected at an estimated concentration higher than (>) 50 ng/mL in the "A" *Sample* and reported as an *AAF*, 4-CPA shall also be reported if its estimated concentration is higher than (>) 50 ng/mL.

For the "B" *Sample*, confirmation of the presence of either Analyte (in compliance with the TD IDCR [9]) is sufficient for the *AAF* to be valid. No quantification or estimation of the Analyte concentration is necessary.

N/A: Under this condition, presence or absence of this substance in the *Sample* is irrelevant.

2.1 "B" *Sample* Confirmation

- Due to the instability of meclofenoxate in urine, when condition 1 in Table 1 is met in the "A" *Sample*, the identification of meclofenoxate in the "B" *Sample* is not necessary. In such cases, the "B" Confirmation Procedure may only confirm the presence of 4-CPA (in compliance with the TD IDCR [9]) for the *AAF* to be valid. No quantification or estimation of concentrations of either meclofenoxate or 4-CPA is necessary;

- When condition 2C in Table 1 is met in the "A" *Sample*, the "B" *Sample* results shall only confirm the presence of 4-CPA (in compliance with the TD IDCR [9]) and the absence of chlorphenesin or chlorphenesin carbamate *Metabolite*(s) for the *AAF* to be valid. No quantification or estimation of the concentration of 4-CPA is necessary.

3.0 References

[1] Yoshioka S, Yukio A and Mitsuru U. Kinetics of hydrolysis of meclofenoxate hydrochloride in human plasma. *J Pharm Pharmacol.* **39**(3): 215, 1987.

[2] Guddat S *et al*. Detection of meclofenoxate and its degradation products Dimethylaminoethanol and p- chloro-phenoxyacetic acid. In Recent Advances in Doping Analysis (14), Schanzer W, Geyer H, GotzmannA, Mareck-Engelke U (eds). Sport und Buch Strauß: Koln, 399, 2006.

[3] Arnold E K and Beasley V R. The pharmacokinetics of chlorinated phenoxy acid herbicides: a literature review. *Vet Hum Toxicol.* **31**(2): 121, 1989.

[4] Aprea C, Sciarra G and Bozzi N. Analytical methods for the determination of urinary 2, 4-

dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid in occupationally exposed subjects and in the general population. *J Anal Toxicol.* **21**(4): 262, 1997.

[5] Johnson W *et al*. Safety Assessment of Chlorphenesin as Used in Cosmetics*. Int J Toxicol*. **33**(2 suppl): 5S-15S, 2014.

[6] Halla N *et al*. Cosmetics Preservation: A Review on Present Strategies. *Molecules* **23**(7):1571, 2018

[7] Panico A *et al*. Skin safety and health prevention: an overview of chemicals in cosmetic products. *J Prev Med Hyg.* **60**(1): E50-E57, 2019.

[8] Rubio A *et al*. Chromatographic-mass spectrometric analysis of the urinary metabolite profile of chlorphenesin observed after dermal application of chlorphenesin-containing sunscreen. *Rapid Commun. Mass Spectrom.* (DOI:10.1002/rcm.9183, 2021).

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

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