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SCCS OPINION on Prostaglandins and their analogues used in cosmetic products SCCS/1635/21 - Final version

U. Bernauer, L. Bodin, Q. Chaudhry, P.J. Coenraads, M. Dusinska, J.
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Scientific Committee on Consumer Safety
SCCS

OPINION
on Prostaglandins and prostaglandin-analogues
used in cosmetic products



The SCCS adopted this document
by written procedure on 3 February 2022

1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided, does the SCCS consider Isopropyl cloprostenate (CAS 157283-66-4) and Ethyl Tafluprostamide or DDDE (CAS 1185851-52-8) safe when used up to the concentrations provided in the respective dossiers (0.006% and 0.007% for Isopropyl cloprostenate and 0.018% for Ethyl Tafluprostamide)*

Having considered the limited data provided and the available information from published literature, the SCCS is not able to conclude on the safety of isopropyl cloprostenate and ethyl tafluprostamide when used up to the intended use concentrations indicated in the respective dossiers (0.006% and 0.007% for isopropyl cloprostenate and 0.018% for ethyl tafluprostamide).

2. *Does the SCCS have any further scientific concerns with regard to the use of Isopropyl cloprostenate (CAS 157283-66-4) and Ethyl Tafluprostamide / DDDE (CAS 1185851-52-8) in cosmetic products?*

The SCCS has noted concerns about the safety of isopropyl cloprostenate and ethyl tafluprostamide when used in cosmetic products - in particular those that are intended for use in the proximity of the eye. These concerns have been highlighted in more detail in Annex 1.

3. *In light of the available data, does the SCCS consider that the use in cosmetic products of prostaglandins analogues (listed in Table 1) raises safety concerns and might pose a risk to human health?*

Prostaglandins and synthetic analogues are widely known to be potent pharmacologically active substances. Due to these effects, other regulatory authorities have advised against, or have prohibited, their use in eyelash growth-promoting cosmetics. In view of the potential for causing effects at very low concentrations, and the intended use in the proximity of the eye, the SCCS has noted concerns over the safety of prostaglandin analogues when used in cosmetic products. These have been highlighted in Annex 1 to this Opinion.

Keywords: SCCS, scientific opinion, prostaglandins, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Prostaglandins and prostaglandin-analogues used in cosmetic products, preliminary version of 27 September 2021, final version of 3 February 2022, SCCS/1635/21

About the Scientific Committees

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members

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2. MANDATE FROM THE EUROPEAN COMMISSION

Request for a scientific opinion: Prostaglandins and their analogues (see Table 1 below).

	INCI / substance name	CAS Nr.
1	Benzothiophenyl dephenethylatanoprost	
2	Cyclopropylbimatoprost	1138395-12-6
3	Cyclopropylmethylbimatoprost	1138395-10-4
4	Dechloro cyclopropylcloprostenolamide	1138395-11-5
5	Dechloro cyclopropylmethylcloprostenolamide	1138395-09-1
6	Dechloro ethylcloprostenolamide	1005193-64-5
7	Dehydrolatanoprost	130209-76-6
8	Dihydroxypropyl dehydrolatanoprostamide	
9	Dihydroxypropyl didehydrolatanoprostamide	
10	Ethyl tafluprostamide	1185851-52-8
11	Ethyl travoprostamide	1005193-64-5
12	Isopropyl cloprostenate	157283-66-4
13	Keto travoprost	404830-45-1
14	Methyl travoprost	
15	Methyl bimatoprost acidate	38315-47-8
16	Norbimatoprost	
17	Nortafluprost	209860-89-9
18	Tafluprost	209860-87-7
19	Trifluoromethyl dehydrolatanoprost	

Table 1. List of prostaglandin analogues found in the 'CosIng' database described as 'a synthetic analogue of a prostaglandin' or found using the keyword 'prost' and relevant to the mentioned chemical family.

Background

In 2018, the German Federal Institute for Risk Assessment (BfR) informed the European Commission that cosmetic products intended to promote the growth of eyelashes were increasingly available on the market. These eyelash growth treatments contain substances such as prostaglandins or their analogues.

According to the BfR's Health assessment of eyelash growth treatments, the use of prostaglandins and their analogues as components of cosmetic products may include health risks for consumers. Prostaglandin analogues are also used in drugs to reduce ocular hypertension. Increased eyelash growth has been observed as a side effect, and furthermore, other adverse effects have been found.

As a result of this communication, EU countries' competent authorities were invited in 2019 to participate in a survey on products for eyelash growth containing prostaglandins and their analogues. The information collected helped to identify an uneven situation in terms of applicable regulatory framework (e.g. cosmetic products or medicinal products). Furthermore, several cases of serious undesirable health effects were recorded in many EU countries due to the use of cosmetic products containing prostaglandins or their analogues.

December 2019 the sub-working group on borderline products assessed this topic and considered that a SCCS opinion would be useful to assess the safety (lack of) of those products.

Finally, as a result of a [call for data](#) conducted in 2020, a number of contributions were provided on the safety of some substances belonging to this chemical family:

- Ethyl Tafluprostamide CAS 1185851-52-8 (also known as Dechloro Dihydroxy Difluoro Ethylcloprostenolamide or DDDE) and
- Isopropyl cloprostenate CAS 157283-66-4.

The Commission database for information on cosmetic substances and ingredients CosIng contains 14 cosmetic ingredients, each described as 'a synthetic analogue of a prostaglandin'; additional cosmetic ingredients relevant to the mentioned chemical family were also found using the keyword 'prost' (see Table 1 above and Paragraph 3.1.1 2018 BfR Health assessment).

In addition, the EU cosmetic products notification portal (CPNP) contains notifications of cosmetic products containing prostaglandins and their analogues placed on the EU market.

Prostaglandins and their analogues are not listed in the Annexes to the Cosmetic Regulation (EC) No. 1223/2009 and their use is not otherwise restricted in cosmetic products.

Therefore, the Commission requests the SCCS to carry out a safety assessment on Prostaglandins or their analogues in view of the information provided.

Terms of reference

1. *In light of the data provided, does the SCCS consider Isopropyl cloprostenate (CAS 157283-66-4) and Ethyl Tafluprostamide or DDDE (CAS 1185851-52-8) safe when used up to the concentrations provided in the respective dossiers (0.006% and 0.007% for Isopropyl cloprostenate and 0.018% for Ethyl Tafluprostamide)*
2. *Does the SCCS have any further scientific concerns with regard to the use of Isopropyl cloprostenate (CAS 157283-66-4) and Ethyl Tafluprostamide / DDDE (CAS 1185851-52-8) in cosmetic products?*
3. *In light of the available data, does the SCCS consider that the use in cosmetic products of prostaglandins analogues (listed in Table 1) raises safety concerns and might pose a risk to human health?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Isopropyl cloprostenate
Ethyl Tafluprostamide or Dechloro Dihydroxy Difluoro Ethylcloprostenolamide, DDDE

3.1.1.2 Chemical names

Isopropyl cloprostenate (PubChem):

IUPAC: propan-2-yl (Z)-7-[(1R,2R,3R,5S)-2-[(E,3R)-4-(3-chlorophenoxy)-3-hydroxybut-1-enyl]-3,5-dihydroxycyclopentyl]hept-5-enoate

(+)-Cloprostenol isopropyl ester
(+)-Cloprostenol isopropyl ester, ethanol solution
Cloprostenol isopropyl ester

isopropyl (Z)-7-((1R,2R,3R,5S)-2-((R,E)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-3,5-dihydroxycyclopentyl)hept-5-enoate

propan-2-yl (Z)-7-[(1R,2R,3R,5S)-2-[(E,3R)-4-(3-chlorophenoxy)-3-hydroxybut-1-enyl]-3,5-dihydroxycyclopentyl]hept-5-enoate

5-Heptenoic acid, 7-((1R,2R,3R,5S)-2-((1E,3R)-4-(3-chlorophenoxy)-3-hydroxy-1-buten-1-yl)-3,5-dihydroxycyclopentyl)-, 1-methylethyl ester, (5Z)-

5-Heptenoic acid, 7-((1R,2R,3R,5S)-2-((1E,3R)-4-(3-chlorophenoxy)-3-hydroxy-1-butenyl)-3,5-dihydroxycyclopentyl)-, 1-methylethyl ester, (5Z)-

5-Heptenoic acid, 7-(2-(4-(3-chlorophenoxy)-3-hydroxy-1-butenyl)-3,5-dihydroxycyclopentyl)-, 1-methylethyl ester, (1R-(1alpha(Z),2beta(1E,3R*),3alpha,5alpha))

propan-2-yl (5Z)-7-[(1R,2R,3R,5S)-2-[(1E,3R)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl]-3,5-dihydroxycyclopentyl]hept-5-enoate

Ethyl Tafluprostamide (PubChem):

IUPAC: (Z)-7-[(1R,2R,3R,5S)-2-[(E)-3,3-difluoro-4-phenoxybut-1-enyl]-3,5-dihydroxycyclopentyl]-N-ethylhept-5-enamide

Taflpostamide

Tafluprost ethyl amide

(Z)-7-((1R,2R,3R,5S)-2-((E)-3,3-difluoro-4-phenoxybut-1-en-1-yl)-3,5-dihydroxycyclopentyl)-N-ethylhept-5-enamide

Dechloro dihydroxy difluoro ethylcloprostenolamide [INCI]

Dechloro-Dihydroxy-Difluoro-Ethylcloprostenolamid

3.1.1.3 Trade names and abbreviations

Isopropyl cloprostenate:

/

Ethyl Tafluprostamide:

/

3.1.1.4 CAS / EC number

Isopropyl cloprostenate - CAS 157283-66-4 / EC 682-025-2

Ethyl Tafluprostamide - CAS 1185851-52-8

For other prostaglandin analogues (PGAs) please see Table 2 below.

3.1.1.5 Structural formula

The basic skeleton of all the naturally occurring prostaglandins consists of 20 carbon chain in which C-8 to C-12 is a cyclopentyl ring (Fig. 1). A carboxy group is present at C-1 terminal and an α -hydroxy group is present at C-15 position along with a double bond at C-13 position. This basic pharmacophore moiety is essential for its binding with the receptor. The chain containing carboxy group (C-1 to C-7) is known as the α -chain, and the chain containing hydroxyl group (C-13 to C-20) is termed as the β -chain (Piplani *et al.*, 2016).

Figure 1. The structural formula of a PG prototype. The basic structure is shown without the type-specific modifications (oxo-, hydroxy group(s)) of the cyclopentane ring and without the second double bond in the α -side chain (C5-C6) (Piplani *et al.* 2016).

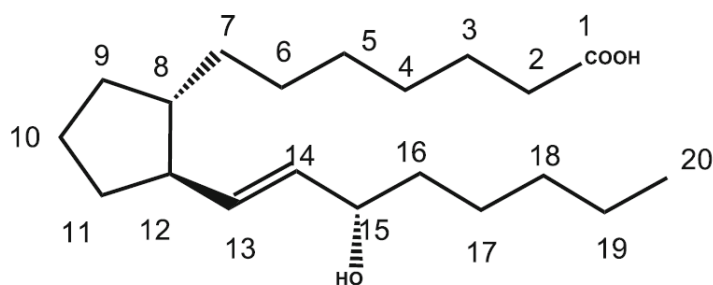
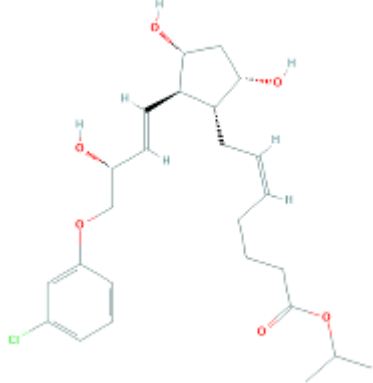
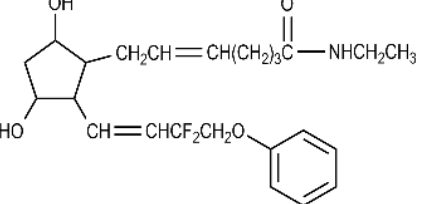
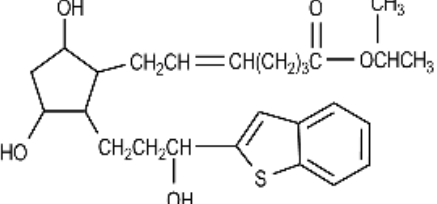
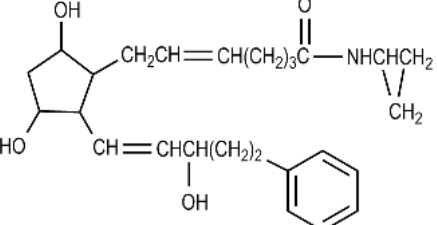
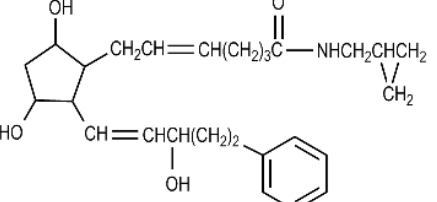
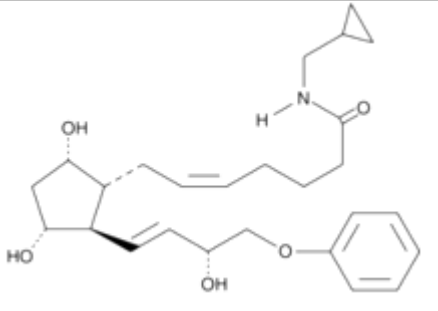
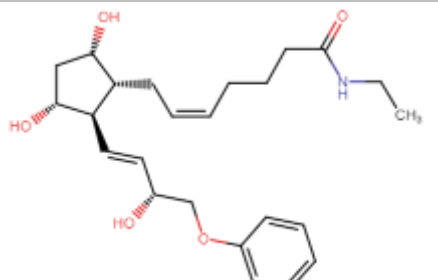
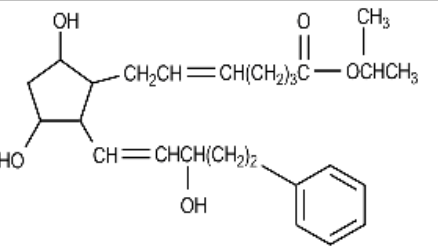
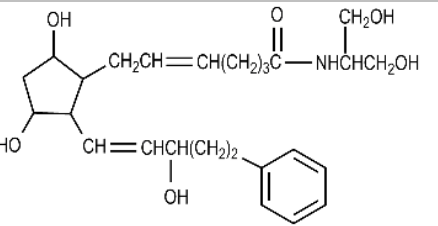
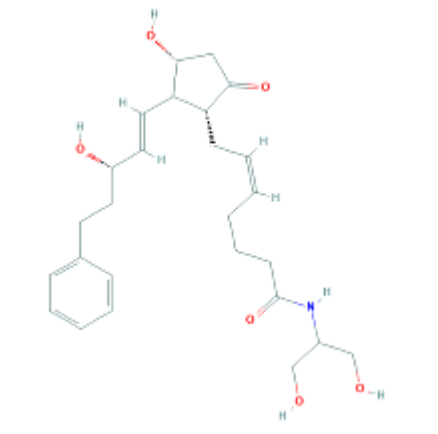


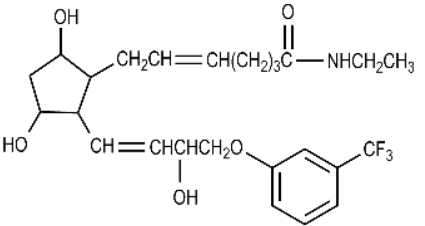
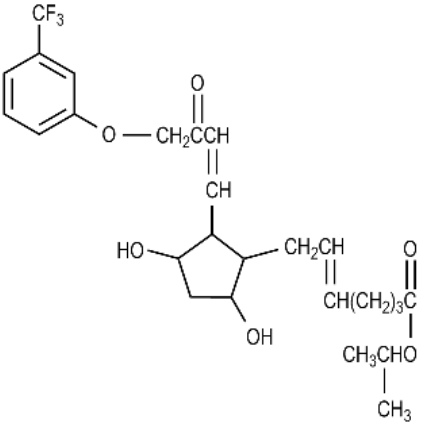
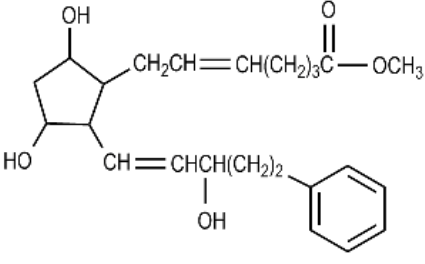
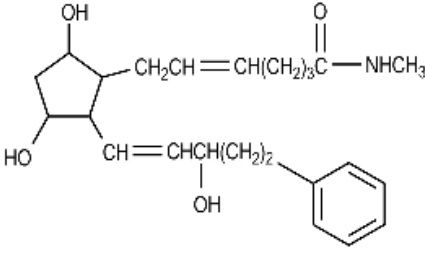
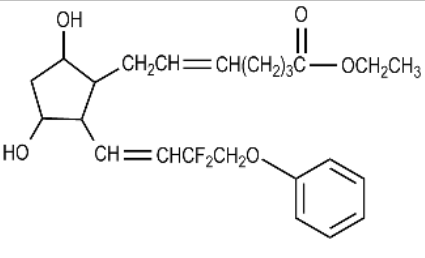
Table 2. Prostaglandin analogues identified in the CosIng Database. The CAS numbers and structural forms are from CosIng and the ChemIDplus website.

INCI Name/Substance Name	CAS No.	Structural formula
1 ISOPROPYL CLOPROSTENATE	157283-66-4	
2 ETHYL TAFLUPROSTAMIDE DDDE	1185851-52-8	
3 BENZOTHIOPHENYL DEPHENETHYLLATANOPROST		
4 CYCLOPROPYL BIMATOPROST	1138395-12-6	
5 CYCLOPROPYL METHYLBIMATOPROST	1138395-10-4	
6 DECHLORO CYCLOPROPYLCLOPROSTENOLAMIDE	1138395-11-5	

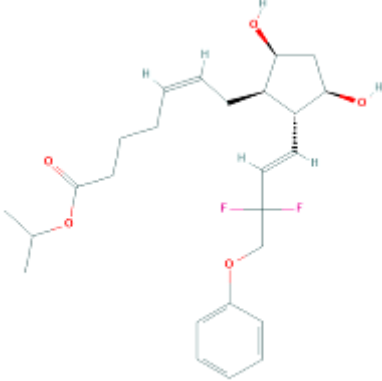
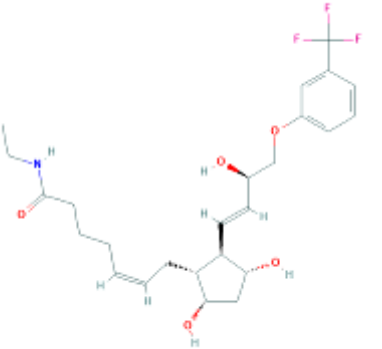
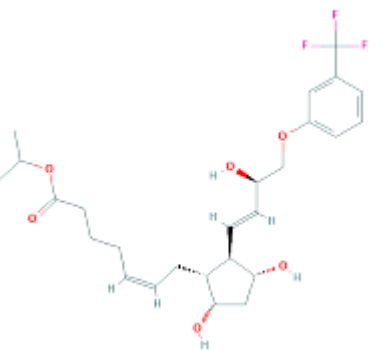
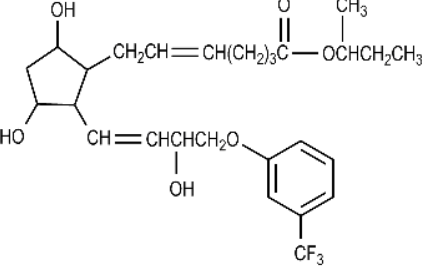
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7	DECHLORO CYCLOPROPYLMETHYLCLOPROSTENOLAMIDE	1138395-09-1	 <p>The structure shows a cyclopentane ring with two hydroxyl groups (one dashed, one wedged) and a side chain containing a double bond, a hydroxyl group, and a benzyl ether linkage. The side chain ends in a cyclopropylmethylamide group.</p>
8	DECHLORO ETHYLCLOPROSTENOLAMIDE	951319-59-8	 <p>The structure shows a cyclopentane ring with two hydroxyl groups (one dashed, one wedged) and a side chain with a double bond, a hydroxyl group, a benzyl ether linkage, and an ethylamide group.</p>
9	DEHYDROLATANOPROST	130209-76-6	 <p>The structure shows a cyclopentane ring with two hydroxyl groups (one dashed, one wedged) and two side chains: one with a double bond and a hydroxyl group, and another with a double bond and a methyl ester group.</p>
10	DIHYDROXYPROPYL DEHYDROLATANOPROSTAMIDE		 <p>The structure shows a cyclopentane ring with two hydroxyl groups (one dashed, one wedged) and two side chains: one with a double bond and a hydroxyl group, and another with a double bond and a hydroxymethylamide group.</p>
11	DIHYDROXYPROPYL DIDEHYDROLATANOPROSTAMIDE	ChemIDplus 1193782-16-9	 <p>The structure shows a cyclopentane ring with two hydroxyl groups (one dashed, one wedged) and two side chains: one with a double bond and a hydroxyl group, and another with a double bond and a hydroxymethylamide group.</p>

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12 ETHYL TRAVOPROSTAMIDE	1005193-64-5	
13 KETOTRAVOPROST	404830-45-1	
14 METHYL BIMATOPROST ACIDATE	38315-47-8	
15 NORBIMATOPROST	155206-01-2	
16 NORTAFLUPROST	209860-89-9	

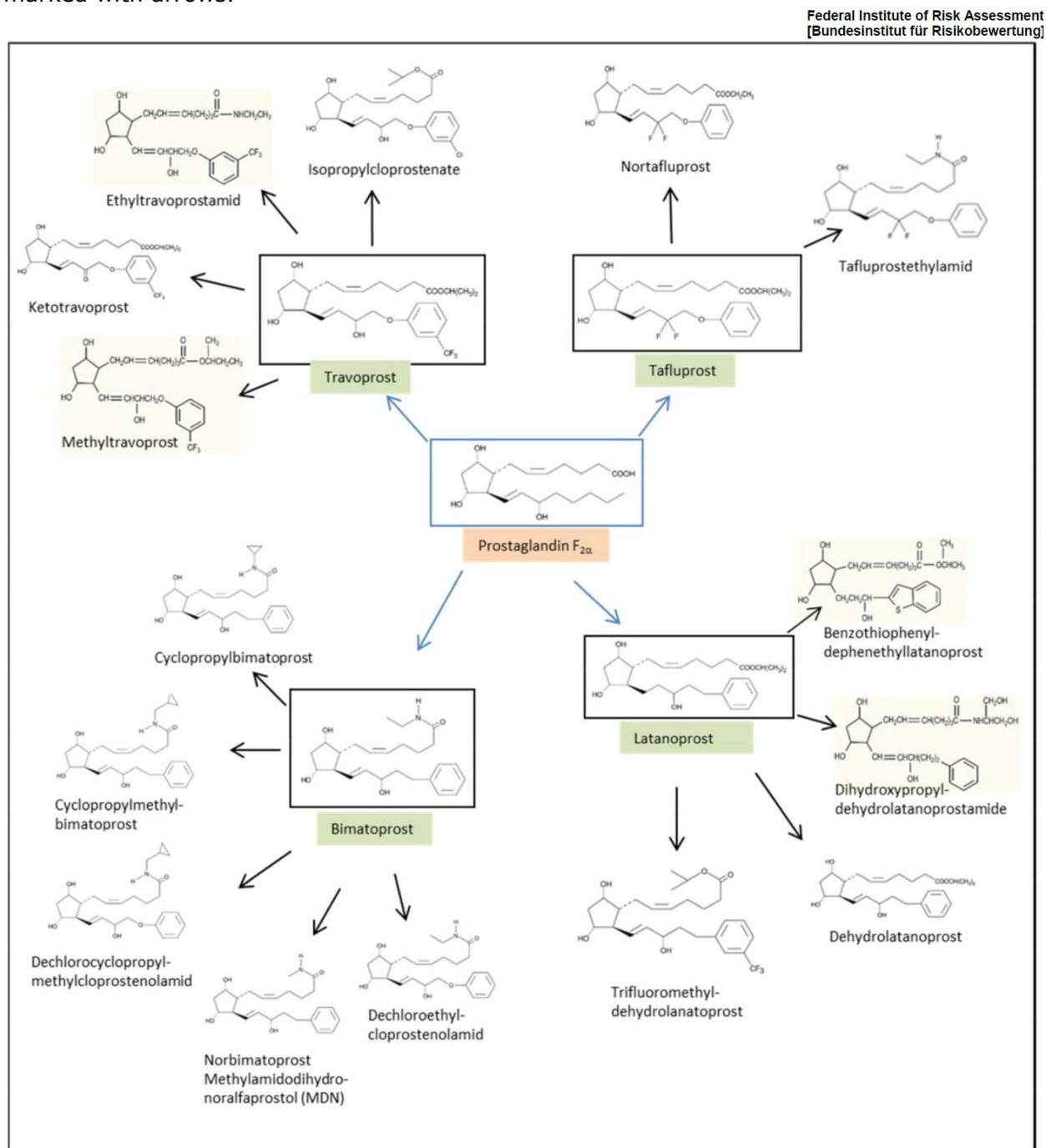
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17	TAFLUPROST	209860-87-7	
18	TRIFLUOROMETHYL DECHLORO ETHYLCLOPROSTENOLAMIDE	1005193-64-5	
19	TRIFLUOROMETHYL DEHYDROLATANOPROST / TRAVOPROST / TRAVATAN / Izba	157283-68-6	
20	METHYL TRAVOPROST		

No CAS number was found for: BENZOTHIOPHENYL DEPHENETHYLLATANOPROST (entry 3), DIHYDROXYPROPYL DEHYDROLATANOPROSTAMIDE (entry 10), METHYL TRAVOPROST (entry 20), and no structural formula could be found for dechloro cyclopropylcycloprostenolamide (entry 6).

The analogues can be categorised based on their structural relationship with four pharmaceutically active substances: tafluprost, bimatoprost, travoprost and latanoprost (Fig. 2) (BfR, 2018).

Figure 2. Structural formulae of PGF_{2α}, the pharmacologically active substances tafluprost, latanoprost, travoprost and bimatoprost, as well as other potential prostaglandin analogues identified in the CosIng database. The structural relationship between the substances is marked with arrows.



3.1.1.6 Empirical formula

Isopropyl cloprostenate: $C_{25}H_{35}ClO_6$
Ethyl tafluprostamide: $C_{24}H_{33}F_2NO_4$

3.1.2 Physical form**Isopropyl cloprostenate:**

No data provided

Ethyl Tafluprostamide:

DDDE is a colourless to pale yellow solution.

Ref.: Information provided in the ingredient dossier

3.1.3 Molecular weight

Isopropyl cloprostenate: 467 g/mol
Ethyl Tafluprostamide: 437.5 g/mol

3.1.4 Purity, composition and substance codes

Isopropyl cloprostenate: not less than 99.4%
DDDE: not less than 99.00%

Ref.: Information provided in the ingredient dossier

3.1.5 Impurities / accompanying contaminants

Isopropyl cloprostenate: water: 0.15%, 15-epimer: 0.25%, ethyl acetate: 0.2%
DDDE: not more than 1.00%

Ref.: Information provided in the ingredient dossier

3.1.6 Solubility

Isopropyl cloprostenate: Water solubility at 25°C: 0.047 mg/L (predicted using EPI Suite™ v.4.11)
Ethyl Tafluprostamide: Water solubility at 25°C: 0.09 mg/L (predicted using EPI Suite™ v.4.11)

SCCS comment

Both isopropyl cloprostenate and ethyl tafluprostamide are practically insoluble in water.

3.1.7 Partition coefficient (Log P_{ow})

Isopropyl cloprostenate: 5.15 (calculated by the Applicant using EPI Suite™ v.4.11)

Ethyl Tafluprostamide: 5.03 (calculated by the SCCS using EPI Suite™ v.4.11)

3.1.8 Additional physical and chemical specifications

No data provided on:

- organoleptic properties (colour, odour, taste if relevant)
- melting point
- boiling point
- flash point
- vapour pressure
- density
- viscosity
- pKa
- pH
- refractive index
- UV/visible light absorption spectrum

3.1.9 Homogeneity and Stability

/

SCCS comment

Some data have been provided on the stability of one final product, but not on the prostaglandin ingredient(s) assessed in this Opinion.

The limited physicochemical data provided indicate that both isopropyl cloprostenate and ethyl tafluprostamide are hydrophobic substances that are practically insoluble in water.

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS**3.2.1 Function and uses****Isopropyl cloprostenate:**

Isopropyl cloprostenate is used in cosmetic products as eyelash and/or eyebrow conditioners. These products are comprised of numerous cosmetic ingredients including isopropyl cloprostenate and are designed to enhance the appearance of natural eyelashes and eyebrows by nourishing, moisturising and protecting eyelashes and eyebrows from breakage.

As stated in the products' labels and information leaflets, the eyelash serum is intended to be used once daily, applied as a thin layer to the eyelid above the eyelash line (like a liquid eyeliner). As provided in the instructions for use, the serum should not be applied to the lower eyelid or the waterline, and users should not double dip. Similarly, the eyebrow serum is intended to be used daily to improve the appearance of the natural eyebrows.

Both products are for external use only and are designed not to come into contact with the eyes.

Isopropyl cloprostenate is the isopropyl ester of cloprostenate, which is converted under physiological conditions into cloprostenol, a synthetic prostaglandin analogue. Prostaglandins are endogenous tissue hormones that are approved as drugs for glaucoma therapy (increased intraocular pressure as a risk factor for glaucoma).

DDDE:

DDDE is used in RevitaLash® Advanced Eyelash Conditioner (RLA). In December 2007 the formulation of RevitaLash was changed and bimatoprost replaced with DDDE. DDDE is not an active ingredient in any FDA-approved drug.

Amount of DDDE applied to eyelashes: The concentration of DDDE in RevitaLash Advanced® is 0.018%. The amount of DDDE that on average is applied to the eyelashes is 0.018% DDDE x 2.4 mg = 0.00018 x 2.4 mg = 0.000432 mg or 0.432 µg. The maximum amount of DDDE applied per brush stroke is 4 mg x 0.018% = 0.00072 mg or 0.72 µg.

Ref.: Information provided in the ingredient dossier

3.2.2 Dermal / percutaneous absorption

The estimated dermal absorption of isopropyl cloprostenate was 10% on the basis of a molecular weight of 476 g/mol and a log POW of 5.15 (calculated via QSAR, Episuite 1.0).

Ref.: Information provided in the ingredient dossier

SCCS comment

No experimental data on percutaneous absorption of the prostaglandin analogues assessed in this Opinion have been provided. Only estimated values from QSAR models have been provided.

In view of the intended use of prostaglandin analogues in cosmetic products that will be applied on a sensitive area (i.e. proximity of eye), it is essential that experimental data are provided from reliable percutaneous absorption studies on each of the prostaglandin analogues.

3.2.3 Other studies on toxicokinetics

In rats, after subcutaneous administration of doses ranging from 20 to 200 µg/kg bw of cloprostenol, 60% of the administered radioactivity was recovered in urine and 14% in faeces within 48 hours. Excretion was complete in 7 days. Major urinary metabolites were identified by GC-MS: tetranor acid of 9-keto-cloprostenol, δ-lactone of the tetranor acid of cloprostenol. Unchanged cloprostenol (less than 10%) and an acid labile conjugate were minor components. No precise information was given about the relative percentage of metabolites.

In marmosets, 55% of the administered radioactivity was recovered in urine and 16% in faeces within 72 hours after subcutaneous administration of 100 µg of ¹⁴C-cloprostenol per kg bw. Unchanged cloprostenol (approximately 40%) was identified in urine together with its dinor acid metabolite (approximately 57%) formed by one stage of β-oxidation.

In pigs, after a single intramuscular administration of ¹⁴C-cloprostenol (acid in the form of sodium salt) at a dose of 200 µg, the highest plasma level of radioactivity (0.70 ± 0.14 µg/L) was measured at 1 hour after dosing. At 24 hours post dosing, the levels were close

to 0.04 µg/L. Fifty percent of the dose administered was recovered either via urine or faeces. In urine, the following compounds were identified: the parent cloprostenol (approximately 10-14%), the tetranor acid metabolite (approximately 37%) and polar compounds (26-32%).

After intramuscular administration of 75 µg of R-cloprostenol to sows, the maximum concentration of R-cloprostenol in plasma was close to 2 µg/L and appeared between 30 and 80 minutes after injection. The half-life of elimination $T_{1/2\beta}$ ¹ was estimated to be 3 h 10 min.

In dairy cows, after a single intramuscular injection of 500 µg of free acid ¹⁴C-cloprostenol (specific activity 122 µCi/mg free acid), the highest plasma level (0.43 ± 0.043 µg free acid equivalent/L) was reached within 30 minutes after dosing. The concentrations were lower than 0.01 µg free acid equivalents/L at 24 hours post dosing. The $T_{1/2\beta}$ was 3 hours. After 16 hours, 52.5 ± 4.8% of radioactivity was recovered from urine.

Cloprostenol was extensively metabolised in the cow by β-oxidation to give the tetranor acid of cloprostenol, isolated as δ-lactone and as glucuronide conjugates (44%). The parent compound represents 18% of the radioactivity excreted.

After intramuscular administration of 150 µg of R-cloprostenol to cows, the highest plasma concentration of R-cloprostenol was found at 90 minutes after injection (approximately 1.4 µg/L). The half-life of elimination $T_{1/2\beta}$ was estimated to be 1 h 37 min.

Residue depletion studies were performed in pigs and cattle. In pigs, 30 minutes after intramuscular administration of 200 µg ¹⁴C-cloprostenol (acid in the form of sodium salt), the highest concentrations of radioactivity were detected at the injection site and in kidneys (43.98 ± 6.90 and 19.00 ± 4.13 µg cloprostenol equivalent/kg respectively). At 24 hours post injection, the amounts were lower than the limit of quantification (0.04 µg cloprostenol equivalent/kg) in muscle and in fat. The residues in liver and kidney were of the same magnitude (close to 0.10 µg cloprostenol equivalent/kg). At the injection site the levels were still relatively high (0.83 ± 0.53 µg cloprostenol equivalent/kg).

A first tissue depletion study was carried out in cows dosed with 500 µg of ¹⁴C-cloprostenol (122 µCi/mg free acid) by single intramuscular injection. At 30 minutes post injection, residues of ¹⁴C-related cloprostenol radioactivity could only be detected in kidney (19.10 ± 2.73 µg cloprostenol equivalent/kg), in liver (7.25 ± 0.641 µg cloprostenol equivalent/kg) and at the injection site (162 ± 19.10 µg cloprostenol equivalent/kg). At 24 hours post dose, the amounts were much lower: 0.123 ± 0.019 µg cloprostenol equivalent/kg in kidney, 0.036 ± 0.010 µg cloprostenol equivalent/kg in liver and 0.493 ± 0.198 µg cloprostenol equivalent/kg at the injection site. No residues were detectable after 48 hours post administration.

A second depletion study, carried out with unlabelled compound at the same dose, showed that all the residues were below the limit of quantification of the radioimmunoassay method used at 24 hours post administration (0.3 or 0.5 µg cloprostenol/kg according to the tissue). In a third non-radiometric study, at 16 hours after a single administration of 150 µg of R-cloprostenol to cows, 0.092 ± 0.063 µg R-cloprostenol/kg was measured at the injection site, 0.051 ± 0.003 µg R-cloprostenol/kg in liver and 0.120 ± 0.021 µg R-cloprostenol/kg in kidney. Only traces of R-cloprostenol were noted in muscle.

Less than 1% of radioactivity administered was eliminated via cow's milk. After an intramuscular dose of 500 µg ¹⁴C-cloprostenol (specific activity 86.35 µCi/mg acid in the

¹ Some toxicokinetic studies present different half-lives. In the case of monophasic curve decay, only one half-life is reported. In the case of biphasic decay curve, this leads to calculation of initial half-life or alpha phase and terminal half-life or beta phase. This latter is referred to elimination half-life. In this opinion, the SCCS decided to present, when reported, the more relevant half-life for the risk assessment, e.g. the terminal half-life.

form of sodium salt - 22 µCi per animal) to cows, the highest levels, corresponding to 4 µg free acid equivalents/L, were found in samples collected over the 0-4 hour period. By 24 hours after dosing, levels had fallen below 0.012 µg/L.

In a second depletion study carried out with unlabelled compound no detectable cloprostenol residues (less than 0.1 µg cloprostenol/L) were found nine hours after dosing or in subsequent samples after single intramuscular dose of 500 µg cloprostenol.

In cows, the ratios of parent compound to total residues were estimated from tissue samples collected at 30 minutes after intramuscular administration of 500 µg ¹⁴C-cloprostenol. Ninety percent of the radioactivity could be extracted. The parent compound represented 85% of total residues in muscle, 35.5% in kidney and liver. In liver and kidney, the percentage of cloprostenol metabolites, lactone and its tetranor acid, was close to 20%. In milk, the ratio of cloprostenol to total radioactivity ranged from 65% (at 0 to 4 hours) to 16% (24 hours post dosing).

Twenty-four hours after intramuscular treatment tissue residues in cattle and pigs are generally only present at the injection site and to a lesser extent in the liver and kidney. Total radioactivity in cow's milk was less than 1% of the administered dose. The maximum intake of total residues that might be ingested from animals slaughtered 24 hours after treatment would be 6.7% of the ADI for pig meat (including injection site) plus cow's milk and 4.8% for cattle meat and milk. These values decrease to below 1% without the injection site. The available pharmacokinetic and residues depletion data do not indicate any significant variability between the mammalian species that have been investigated, therefore, any possible difference in pharmacokinetics in goats, including goat's milk, would not be expected to have a significant impact on this percentage.

Ref.: EMA, 2004

SCCS comment

No relevant data on toxicokinetics of the prostaglandin analogues assessed in this Opinion have been provided.

The SCCS is of the opinion that the toxicokinetics of isopropyl cloprostenate can be expected to be different from that of cloprostenol and R-cloprostenol. Cloprostenol (Log Kow=3.95) is less hydrophobic compared to isopropyl cloprostenate (Log Kow=5.15). Therefore, systemic uptake via the dermal or oral route can also be expected to be different. Although metabolic transformation of isopropyl cloprostenate will yield cloprostenol, it can be assumed that before the metabolism, systemically available isopropyl cloprostenate can be distributed differently in the organism compared with cloprostenol. Hence, drawing conclusions on the toxicokinetics profile of isopropyl cloprostenate from the toxicokinetics data on cloprostenol and R-cloprostenol is not appropriate.

3.2.4 Calculation of SED/LED

SCCS comment

Due to the lack of relevant data, SED/LED cannot be determined for any of the prostaglandin analogues assessed in this Opinion.

3.3 TOXICOLOGICAL EVALUATION

3.3.1. Irritation and corrosivity

3.3.1.1 Skin irritation

SCCS comment

No relevant experimental data on skin irritation of the prostaglandin analogues assessed in this Opinion have been provided.

3.3.1.2 Mucous membrane irritation / eye irritation

SCCS comment

Some experimental data on the *in vitro* Hen's egg chorioallantoic membrane (HET-CAM), MatTek Epiocular™ MIT viability assay, and conjunctival hyperaemia in rabbit and guinea pigs, have been provided but they are related to final products and not the ingredients. Within the remit of the SCCS, safety assessments are based on assessment of the ingredients and not cosmetic formulations. Test results relating to cosmetic formulations have therefore not been taken into consideration in this Opinion.

3.3.2 Skin sensitisation

SCCS comment

No relevant experimental data on skin sensitisation of the prostaglandin analogues assessed in this Opinion have been provided.

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

In the EMA 2004 report, the acute toxicity of cloprostenol and R-cloprostenol is considered low. In rats the oral LD50 values were higher than 25 mg/kg bw.

Ref.: EMA, 2004

SCCS comment

Some experimental data on acute oral toxicity of cloprostenol have been provided but not for the prostaglandin analogues assessed in this Opinion.

3.3.3.2 Acute dermal toxicity

Three male and three female rats received a topical dose of 250 µg of cloprostenol sodium in 1 mL sterile water on day 1 and day 4 (1.25 mg/kg b.w.) on the shaved area of skin. No particular adverse effects were shown.

Ref.: CNEVA, 1995

SCCS comment

Some experimental data on acute dermal toxicity of cloprostenol have been provided but not for the prostaglandin analogues assessed in this Opinion.

3.3.3.3 Acute inhalation toxicity**SCCS comment**

No relevant experimental data on acute inhalation toxicity of the prostaglandin analogues assessed in this Opinion have been provided.

3.3.3.4 Acute toxicity – other routes**SCCS overall comment on acute toxicity**

No relevant experimental data on acute toxicity of the prostaglandin analogues assessed in this Opinion have been provided.

3.3.4 Repeated dose toxicity**3.3.4.1 Repeated dose (28 days) oral / dermal / subcutaneous / inhalation toxicity**

After subcutaneous administration of cloprostenol at doses of 0, 12.5, 25, 50 µg/kg bw/day for one month in rats, vacuolisation of the luteal cells of the corpora lutea was the only significant consistent drug-related change observed for all the doses tested. This effect was reversible one month after the end of the treatment.

Ref.: EMA, 2004

SCCS comment

Some experimental data on subcutaneous administration have been provided for cloprostenol; the data show that even low doses of this analogue can exert a systemic effect.

3.3.4.2 Sub-chronic (90 days) oral / dermal / inhalation toxicity

In the 3-month oral toxicity study carried out in rats (0, 10, 50, 150 µg/kg bw/day of cloprostenol), 50 µg/kg bw was the NOEL, as ovarian vacuolisation has been observed at the highest dose.

In the 3-month oral repeated studies in marmosets (0, 10, 50, 150 µg/kg bw and 0, 10, 20, 50 µg/kg bw/day of cloprostenol), induction of myocardial changes and statistically significant increases in testicular weights were reported at 150 µg/kg bw/day. A NOEL of 50 µg/kg bw/day could be retained.

Ref.: EMA, 2004

SCCS comment

Some experimental data on sub-chronic oral administration are available for cloprostenol. Although not directly relevant, the data indicate systemic effects caused by very low doses of cloprostenol. This raises concern about the potential for similar effects by other prostaglandin analogues assessed in this Opinion.

3.3.4.3 Chronic (> 12 months) toxicity**SCCS comment**

No relevant experimental data on chronic toxicity of the prostaglandin analogues assessed in this Opinion have been provided.

SCCS overall comment on repeated dose toxicity

Some experimental data on sub-chronic oral administration are available for cloprostenol. No relevant experimental data on repeated dose toxicity of the prostaglandin analogues assessed in this Opinion via relevant routes (oral, dermal) have been provided. Although not directly relevant, the available data on cloprostenol indicate that even very low doses of prostaglandin analogues may have the potential to exert systemic effects.

3.3.5 Reproductive toxicity

3.3.5.1 Fertility and reproduction toxicity

Physiological effect of PGF2 α

The effect of PGF2 α on reproduction is particularly well investigated. PGF2 α induces, amongst other effects, luteolysis (degradation of the *corpus luteum*), which is an important process in the regulation of the sexual cycle and for the introduction of labour (Dukes et al. 1974). PGF2 α leads to contractions of the uterus muscles of pregnant and non-pregnant persons and is used in veterinary medicine to induce labour or abortion.

Ref.: BfR, 2018

A specific investigation carried out to evaluate the impact of isopropyl cloprostenate on the male reproductive system suggests significant histopathologic modifications in the testis of rats and mice following intraperitoneal exposure to 0.1 mg/kg bw/day for 28 days (Indrei et al., 2001).

Ref.: Indrei et al., 2001

In a 3 generation study carried out in rats, the oral administration of doses of 0, 10, 15, 20 and 40 μ g of cloprostenol/kg bw did not induce effects on reproductive performance of the animals. The only effects seen were the slight reduction in neonatal viability attributable to the prematurity² of the offspring. A NOEL of 15 μ g/kg bw/day for cloprostenol was retained.

In a series of reproductive studies performed with cloprostenol, it was shown that the sensitivity of the rat to termination of pregnancy resulting from luteolysis varies depending on the point in pregnancy when the compound is administered. The oral dose 25 μ g/kg bw of cloprostenol did not terminate pregnancy; the most sensitive period to luteolytic action of cloprostenol was just prior to the parturition.

Ref.: EMA, 2004

SCCS comment

The available data on cloprostenol effects on reproduction in rats indicate its activity at very low doses. This also indicates the possibility of similar strong activity of other prostaglandin analogues when systemically available.

No other relevant experimental data have been provided on the reproductive toxicity of the prostaglandin analogues assessed in this Opinion.

3.3.5.2 Developmental Toxicity

² Increased number of rats prematurely expelling their uterine contents was observed due to the luteolytic activity of cloprostenol.

No teratogenic properties of cloprostenol were reported in the two teratogenicity studies performed either in rats after oral administration of 0, 10, 25, 50 and 100 µg/kg bw/day or in rabbits after subcutaneous administration of 0, 0.025, 0.075 and 0.250 µg/kg bw/day.

Ref.: EMA, 2004

SCCS comment

No teratogenic effects were observed for cloprostenol, however, relevant experimental data on developmental toxicity of the prostaglandin analogues assessed in this Opinion have not been provided.

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

Isopropyl cloprostenate was predicted to be not mutagenic in Ames test according to the QSAR model Toxtree v. 3.1.0-1851 and the statistical method based model US EPA T.E.S.T v. 4.2.1. Further, isopropyl cloprostenate was assigned a Cramer class III toxicity classification by Toxtree v. 3.1.0-1851.

Ref.: Information provided with ingredient dossier

R-Cloprostenol was devoid of mutagenic activity in the bacterial Ames test and the *in vitro* mouse lymphoma L5178Y/TK+/- test. In an *in vitro* chromosomal aberration test in human lymphocytes, an increased frequency of aberrations were observed only at very high concentrations (2320 µg/mL).

Ref.: EMA, 2004

3.3.6.2 Mutagenicity / genotoxicity *in vivo*

R-Cloprostenol gave negative results in the *in vivo* bone marrow micronucleus test when administered by the intraperitoneal route to mice.

Ref.: EMA, 2004

SCCS overall comment on mutagenicity

No relevant experimental data on mutagenicity/genotoxicity of the prostaglandin analogues assessed in this Opinion have been provided.

Taking into account that a mutagenic effect was observed for cloprostenol in the chromosome aberration assay in human lymphocytes, although at high concentrations, the SCCS is of the opinion that a genotoxic hazard of other prostaglandin analogues cannot be excluded in absence of further data.

3.3.7 Carcinogenicity

SCCS comment

No relevant experimental data on the carcinogenicity of the prostaglandin analogues assessed in this Opinion have been provided.

A review of the data from available literature has indicated the involvement of prostaglandin E2 in tumorigenesis, mainly through interaction with E Prostaglandin receptors (EP) (Piotrowski *et al.*, 2020). EP2 receptor subtype is known to act as tumour promoter predominantly through activation of angiogenesis related to induction of Vascular Endothelial Growth Factor (VEGF). EP4 is another receptor with well-described connection to tumourigenesis. The PGE2-induced activation of EP4 receptor results in the development of pro-tumourigenic immune response. EP4 receptor pathway has been implicated in the activation of Treg lymphocytes, which inhibit the inflammatory response to the tumour or can promote growth and invasiveness of tumor cells.

Considering the limited available data, the SCCS cannot exclude the potential carcinogenicity of the prostaglandin analogues.

Ref.: Piotrowski *et al.*, 2020

3.3.8 Photo-induced toxicity

3.3.8.1 Phototoxicity / photo-irritation and photosensitisation

3.3.8.2 Photomutagenicity / photoclastogenicity

SCCS comment on photo-induced toxicity

No relevant experimental data on the photo-induced toxicity of the prostaglandin analogues assessed in this Opinion have been provided.

3.3.9 Human data

In a 4-week human eye irritation study on 27 subjects, a 10% Lash isopropyl cloprostenate formulation was found to be safe for daily use by both non-contact lens and contact lens wearers for 4 weeks. No signs of ocular irritation or any other discomfort or deposits were observed or reported by any of the subjects.

Ref.: The information provided with ingredient dossier: Lifetech response letter to FDA warning; May 10, 2011.

A three-month clinical trial was carried out in 23 glaucoma patients, aged between 41 and 67 years (average 54 years). The isopropyl ester of d-cloprostenol (0.1 mg/mL (or 0.01%) eyewash in phosphate buffer solution) was administered once daily for three months directly into the eye. The intraocular pressure (IOP), ophthalmoscopic appearance of the optic disc, visual field, visual acuity and the main side effects were assessed. No changes were observed in the appearance of the papilla (by direct and stereo ophthalmoscopy) and visual accuracy over the treatment period. The only local reaction observed was mild hyperemia of the bulbar conjunctiva, which was reported to disappear after two to three days of treatment. No systemic reactions were observed.

Ref.: The information provided with ingredient dossier: Apostol S *et al.*, 1995.

SCCS comment

In the submission, the applicants provided some studies on human subjects. The SCCS has considered each of these studies, but has regarded them as not relevant for the current assessment for the reasons given below:

- For human eye irritation, product-based information was provided. However, within the remit of the SCCS, safety assessment of cosmetic ingredients is based on assessment of the ingredients and not of cosmetic formulations.
- A clinical trial was submitted that evaluated various ophthalmological parameters, including intra-ocular pressure. This trial was performed with a topical ocular dose of 5 microgram of isopropyl ester of d-cloprostenol and postulates no effect in 23 glaucoma patients. While a full evaluation of a clinical trial is outside the remit of the SCCS, it is worth pointing out that this dose appears to be higher than (or at least in the same order of) the dose that produces significant lowering of intra-ocular pressure in animals (see 3.3.10 - Studies in animals).

3.3.10 Special investigations

Mechanisms of action

The pharmacological classification of prostanoid receptors was developed on the basis that each receptor preferentially recognises one of the major natural prostaglandins (PGs). Prostaglandins have the affinity to bind to the ocular receptors where PGD series preferentially activates the DP receptors (subtypes—DP1 and DP2), PGE series activates the EP receptors (subtypes—EP1, EP2, EP3 and EP4) and PGF2a series activates the FP receptors (Wright *et al.*, 1999; Reimer *et al.*, 1992). All prostanoid receptors belong to the G protein-coupled receptor superfamily containing rhodopsin-like seven transmembrane spanning segments and may couple to one or more signal transduction processes. These receptors induce the stimulation of adenylyl cyclase and hence increase the levels of cAMP and phospholipase C in turn resulting in increased levels of inositol triphosphate (IP3) which metabolizes Ca²⁺ from intracellular organellar depots. In addition, cAMP directly opens Ca²⁺ channels and this cytosolic Ca²⁺ is responsible for the activity at the receptor (Weinreb *et al.*, 2002).

Ref.: Piplani *et al.*, 2016

The mode of action (MoA) can be considered to be established for PGF2a analogues. Activation of the PGF (FP) and PGE (EP) receptors should be assumed, both at the eye as well as the eyelid. PGF2a analogues are available as prodrugs and the essential hydrolysis to the bioactive free acids takes place in the tissue. For bimatoprost, a different MoA is being discussed by Woodward's active substance development team. According to the team, bimatoprost activates a previously not yet clearly identified prostamide receptor that presumably consists of the chimera of an FP receptor and an FP receptor splice variant. Bimatoprost acid is a potent agonist of the FP receptor, and could be detected in pharmacologically sufficient quantities in analyses of the aqueous humour of patients. While the *in vitro* binding affinity of PG analogues to the FP receptor is well correlated with the *in vivo* intraocular pressure-reducing effect, corresponding data on adverse effects are lacking.

Ref.: BfR, 2018

Studies in animals

Isopropyl cloprostenate applied **directly into the eye** was evaluated in three animal models for the following endpoints: (i) conjunctival hyperemia, (ii) constriction of pupils and (iii) intra-ocular pressure (IOP) lowering efficacy (Klimko *et al.*, 2004) (Table 3).

The conjunctival hyperaemia was studied in New Zealand rabbits along with the determination of rabbit ocular irritancy (ROI15) which denoted the dose estimated to produce 15% incidence of hyperemia over the 4 h course of the study. The ROI15 for isopropyl cloprostenate was determined to be 0.3 µg (Klimko *et al.*, 2004).

The ability of isopropyl cloprostenate to constrict the cat pupil when applied directly into the eye over time was measured and was expressed as an ED₅ value, which indicated the dose required to produce a 5 unit area (mm h) in a graph of the difference in pupil diameter in the dosed eye versus time. The ED₅ for isopropyl cloprostenate was determined to be 0.013 µg (Klimko *et al.*, 2004).

Further, the acute IOP-lowering efficacy was measured for isopropyl cloprostenate in conscious ocular hypertensive cynomolgus monkeys. Isopropyl cloprostenate showed a 39% reduction in the intraocular pressure following application of a topical ocular (lasered right eyes) dose of 1 µg directly into the eye (Klimko *et al.*, 2004).

For comparison, other prostaglandin analogues such as Latanoprost and Travoprost were also evaluated in the above experiments and resulted in ROI₁₅ of 1.8 and 3 µg and ED₅ of 0.13 and 0.015 µg, respectively. Further, these analogues produced 27 and 39% IOP reduction at 3 and 0.3 µg doses, respectively.

Table 3. The effects of isopropyl cloprostenate applied directly into the eye evaluated in three animal models for the following endpoints: (i) conjunctival hyperemia, (ii) constriction of pupils and (iii) intra-ocular pressure (IOP) lowering efficacy.

Compound	ROI ₁₅ (µg)	CPD, ED ₅ (µg)	Monkey IOP, % change (dose in µg)
Isopropyl cloprostenate	0.3	0.013	-39% (1)
Latanoprost	1.8	0.13	-27% (3)
Travoprost	3	0.015	-29% (0.3)

ROI = Dose estimated to produce conjunctival hyperemia in 15% of the tested rabbits over 4 h

CPD = Cat pupil diameter constriction

IOP = Intra-ocular pressure

Based on this information, it appears that isopropyl cloprostenate (when applied directly into the eye) has 6-10 fold lower tendency to produce conjunctival hyperemia compared to Latanoprost (ROI₁₅ = 1.8 µg) and Travoprost (ROI₁₅ = 3 µg), while its ability to constrict pupils or reduce IOP was not consistent. The ability to constrict pupils occurred at almost equal doses for isopropyl cloprostenate (ED₅ = 0.013 µg) and Travoprost (ED₅ = 0.015 µg) and 10-fold lower compared to Latanoprost (ED₅ = 0.13 µg). The IOP lowering dose appeared to be more potent for isopropyl cloprostenate (3-fold lower, i.e., 1 µg) compared to Latanoprost (3 µg), but not when compared to Travoprost (3-fold higher, i.e., 0.3 µg). In each of these studies, isopropyl cloprostenate was inserted directly into the eye.

In another study, isopropyl cloprostenate was evaluated for incidence of conjunctival hyperemia in guinea pigs and intra-ocular pressure (IOP) lowering efficacy in monkeys, following topical ocular exposure to multiple doses (Bishop *et al.*, 1996).

Isopropyl cloprostenate applied directly into the eye was found to produce 0, 7, 13 and 19% incidence of hyperemia in upper bulbar conjunctiva following topical ocular administration of 10 µL of 0.03, 0.1, 0.3 and 1 µg doses in guinea pigs over the 4 h course of the study (Bishop *et al.*, 1996) (Table 4). The 15% incidence, similar to effects observed in New Zealand rabbits, was observed at doses ≥0.3 µg. Other prostaglandin analogues such as Travoprost and Bimatoprost isopropyl ester, which were also evaluated in the above experiments, showed a 29 and 27% incidence of hyperemia at a dose of 0.3 µg.

Table 4. Evaluation for incidence of conjunctival hyperemia in guinea pigs following topical ocular exposure to multiple doses.

	Guinea pig conjunctival hyperemia **																			
	Prostaglandin dose																			
	0.03 µg					0.1 µg					0.3 µg					1 µg				
	Score					Score					Score					Score				
	0	1	2	3	N*	0	1	2	3	N*	0	1	2	3	N*	0	1	2	3	N*
Isopropyl cloprostenate	40	60	0	0	5	60	33	7	0	23	23	61	13	3	21	18	59	19	4	23
Travoprost	17	70	13	0	6	12	88	0	0	6	17	50	29	4	6	21	60	13	6	12
Bimatoprost isopropyl ester	46	54	0	0	6	23	62	13	2	12	10	61	27	2	12	15	56	17	12	12

* Number of animals tested

** Numbers indicate percent incidence for that score

The IOP-lowering efficacy was measured for isopropyl cloprostenate following administration directly into the eye of 0.3 and 1 µg doses in conscious ocular hypertensive cynomolgus monkeys (Bishop *et al.*, 1996). The right eyes of the cynomolgus monkeys used in this study were previously given laser trabeculoplasty to induce ocular hypertension in the lasered eye. The test formulations were administered to the lasered right eyes and the normal left eyes remained untreated. The test protocol included a five-dose treatment regimen because of the typically delayed response to prostaglandins. Baseline IOP values were determined prior to treatment with the test formulation, and then IOP was determined from 1 to 7 hours after the first dose, 16 hours after the fourth dose, and 1 to 4 hours after the fifth dose.

Isopropyl cloprostenate at 4 hours after the fifth dose showed a maximum 31.2 and 38.7% reduction in the intraocular pressure following application of a topical ocular dose of 0.3 and 1 µg respectively (Table 5). Travoprost and Bimatoprost isopropyl ester produced maximum 31.2 and 39.8% IOP reduction at 0.3 µg doses, respectively.

Table 5. Evaluation for incidence of intra-ocular pressure (IOP) lowering efficacy in monkeys following topical ocular exposure to multiple doses.

Comparison of Percent IOP Reduction						
Compound	Dose (µg)	Baseline IOP (mm Hg)	Percent IOP reduction (Hours after last dose/dose #)			
			16/4	2/5	4/5	6/5
Isopropyl cloprostenate	1	39.6	34.8 ± 4.5	36.7 ± 5.8	38.7 ± 5.9	35.8 ± 5.1
	0.3	36.9	23.6 ± 3.3	30.2 ± 4.5	31.2 ± 6.8	24.4 ± 6.9
Travoprost	0.3	41.6	18.4 ± 5.9	31.2 ± 3.7	30.3 ± 3.8	26.6 ± 3.6
Bimatoprost isopropyl ester	0.3	40.8	25.6 ± 2.6	36.0 ± 2.4	39.8 ± 3.1	30.3 ± 2.8

Based on the above information, isopropyl cloprostenate was found to show the least percentage (13%) of hyperemia incidences compared to Travoprost (29%) and Bimatoprost isopropyl ester (27%), following exposure to the same dose level. For IOP reduction percentage, while Isopropyl cloprostenate and Travoprost showed comparable values, Bimatoprost isopropyl ester produced relatively higher reduction (39.8%) which was close to the percentage produced by a higher dose (1 µg) of isopropyl cloprostenate. This data indicates Bimatoprost isopropyl ester to have a relatively higher IOP reduction potential compared to isopropyl cloprostenate.

Ref.: Information provided with ingredient dossier

SCCS comment

The SCCS has noted that in a study on three animal models of conjunctival hyperemia, constriction of pupils and intra-ocular pressure lowering efficacy, in which isopropyl cloprostenate was applied directly into the eye, it induced similar effects (constriction of pupils and intra-ocular pressure lowering efficacy) to the reference prostaglandin analogues when used at comparable or several fold lower concentrations.

In another study, isopropyl cloprostenate, when evaluated for conjunctival hyperemia in guinea pigs, showed a concentration-dependent increase in incidence of hyperemia, which was slightly lower compared to reference prostaglandin analogues. Isopropyl cloprostenate induced IOP reduction percentage in monkeys that was comparable to reference prostaglandin analogues.

The SCCS is of the opinion that, although these results are not directly applicable for the safety assessment in this Opinion because of direct application to the eye, they do indicate that isopropyl cloprostenate can exert strong local adverse effects in the eye, and has a strong IOP lowering activity.

In silico assessment of toxicity

As noted above, the available information on prostaglandin analogues was either very patchy, or missing altogether. In view of this, the SCCS used *in silico* modelling approach to gain some insights into the potential toxicities of prostaglandin analogues using QSAR models and read-across. It needs to be stressed that the SCCS carried out this exercise only to identify the first level alerts for any potential harmful effects that might impact consumers' health from the use of cosmetic products containing prostaglandin analogues. The endpoints assessed included skin sensitisation, carcinogenicity, mutagenicity and reproductive toxicity. The *in silico* systems used included QSAR-based systems (VEGA-QSAR and US EPA-TEST) and read-across (TOXREAD).

The exercise showed that, out of the total 21 substances listed in Table 6, *in silico* analysis could only be carried out for fifteen (15) for which CAS numbers were available. Six of the compounds without CAS numbers could not be assessed because of non-availability of chemical structures.

Table 6. Prostaglandin analogues evaluated by the SCCS in *in silico* assessment of toxicity

	INCI / substance name	CAS Number
1	Benzothiophenyl dephenethylatanoprost	
2	Cyclopropylbimatoprost	1138395-12-6
3	Cyclopropylmethylbimatoprost	1138395-10-4
4	Dechloro cyclopropylcloprostenolamide	1138395-11-5
5	Dechloro cyclopropylmethylcloprostenolamide	1138395-09-1
6	Dechloro ethylcloprostenolamide	1005193-64-5
7	Dehydrolatanoprost	130209-76-6
8	Dihydroxypropyl dehydrolatanoprostamide	
9	Dihydroxypropyl didehydrolatanoprostamide	
10	Ethyl tafluprostamide	1185851-52-8
11	Ethyl travoprostamide	1005193-64-5
12	Isopropyl cloprostenate	157283-66-4
13	Keto travoprost	404830-45-1
14	Methyl travoprost	
15	Methyl bimatoprost acidate	38315-47-8
16	Norbimatoprost	155205-89-3*
17	Nortafluprost	209860-89-9
18	Tafluprost	209860-87-7
19	Trifluoromethyl dehydrolatanoprost	
20	Ethyl Tafluprostamide	1185851-52-8
21	Isopropyl cloprostenate	157283-66-4

* CAS number used for N-Norbimatoprost

The collective results from *in silico* assessment indicated that:

1. None of the prostaglandin analogues was predicted to be mutagenic or genotoxic. This is supported by some experimental data, e.g. on cloprostenol, which is an analogue of isopropyl cloprostenol. (www.ema.europa.eu/en/documents/mrl-report/cloprostenol-r-cloprostenol-extension-goats-summary-report-2-committee-veterinary-medicinal-products_en.pdf; <https://agris.fao.org/agris-search/search.do?recordID=YU1997000650>)
2. Almost all of the 15 PG analogues were flagged for potential carcinogenicity with a reasonable model certainty. This raises the concern that PG analogues may be non-genotoxic carcinogens.
3. All of the 15 analogues were flagged for reproductive and developmental toxicity with a reasonable model certainty, raising the concern that they are potentially reproductive/developmental toxicants.
4. In addition, several of the assessed analogues were predicted to be skin sensitizers.

SCCS comment

The first line of evidence obtained from *in silico* analysis of the 15 PG analogues suggests that they may not be mutagenic/genotoxic, but there is a strong indication for their potential carcinogenicity via non-genotoxic mechanisms. In addition, the analysis indicated potential reproductive/developmental and skin sensitisation effects of the assessed analogues. All of these endpoints are critical for safety assessment of a substance intended for use in cosmetic products, and therefore experimental data addressing these endpoints need to be provided to the SCCS to exclude the likelihood of adverse effects on the consumer health from the use of PGAs in cosmetic products.

3.4 SAFETY EVALUATION (including calculation of the MoS)

The Applicants provided the following safety evaluation of prostaglandin analogues:

ISOPROPYL CLOPROSTENATE – Applicant #1

Due to confidentiality issues, the SCCS cites only the main findings from the application.

Margin of safety

The Margin of Safety (MoS), which is the ratio between a POD_{sys} and an estimate of the exposure (SED) has been calculated for isopropyl cloprostenate according to the following formula:

$$\text{MoS} = \text{POD}_{\text{sys}} / \text{SED}$$

With:

- POD_{sys} = Point of Departure (mg/kg bw/day)
- SED = Systemic Exposure Dosage (mg/kg bw/day)

When performing a Toxicological Screening Value TSV-based safety evaluation³, it is generally accepted that the MoS should at least be 1, to conclude that there is no or low systemic safety concern for the respective ingredient.

Table 7. Calculation of Margin of safety by Applicant #1.

Product type	SED (mg/kg bw/day)	MoS >1 (TSV)
Eyelash	1.40E-07	15.0
Eyebrow	7.00E-07	3.0
Combined exposure	8.40E-07	2.5

Therefore, under the conservative consideration of a daily product use level of 0.00028 g/day for eye lash and 0.0014 g/day for eyebrow products each or combined uses, the safety

³ EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Bruschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vollmer G, Vleminckx C, Wallace H, Filipi_c M, Furst P, O’Keeffe M, Penninks A, Van Leeuwen R, Baert K and Hoogenboom LR, 2018. Guidance update: methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin. EFSA Journal 2018;16(7):5332, 25 pp. <https://doi.org/10.2903/j.efsa.2018.5332>

assessment concludes that the use of isopropyl cloprostenate in eye lash and eyebrow cosmetic products is safe for use.

Isopropyl cloprostenate - Applicant #2

Isopropyl cloprostenate is contained in the Long Lashes eyelash serum at 0.007%. A maximum of 0.07 g of the eyelash serum is applied to the lashes per day. This corresponds to an SED of 0.000008 mg/kg bw /d Isopropyl Cloprostenate at a dermal absorption of 10% and an average body weight of 60 kg. The dermal absorption of 10% was estimated on the basis of a molecular weight of 476 g/mol and a log POW of 5.15 (calculated via QSAR, Episuite 1.0).

For cloprostenol, a NOEL of 0.015 mg/kg bw/d was determined during a 3-generation feeding study in rats. Higher NOELs were also determined in 3-month feeding studies of 50 µg/kg bw/d each in rats and marmosets. An ADI value of 0.075 µg/kg bw/d has been established by the EMEA on the basis of the 3-generation NOEL used here for the MOS calculation.

This results in a MOS for the lash serum of 916.

Dechloro dihydroxy difluoro ethylcloprostenolamide (DDDE) - Applicant #3

Collectively, the biological tests have objectively and scientifically confirmed the safety of RLA. Highly qualified independent experts have assessed the safety of RLA and concluded in written reports that RLA is safe and that the presence of DDDE in RLA poses no local or ocular safety risk beyond any other cosmetic ingredient.

Note by the SCCS:

Several weeks before the SCCS issued its Opinion, the Applicant submitted a Position Paper on the Use of Prostaglandin Analogues in Cosmetics, in which he posited that the assessment of the safety of PGAs in cosmetic products must be product-specific (including looking at the site of application and overall formulation), not ingredient-specific.

SCCS comment

The SCCS was not provided with the data needed to assess the risk associated with the use of each of the prostaglandin analogues in cosmetic products. Although some experimental data were provided, these related to final products containing PGAs. The SCCS emphasises that risk assessment conducted within its remit is based on assessment of the ingredients and not of cosmetic formulations. Although the test results based on cosmetic formulations were analysed, they were considered of limited usefulness for the purpose of this Opinion.

The detailed analysis of the data provided in ingredient dossiers by all applicants, as well as the available literature data, indicates that the prostaglandins analogues are capable of exerting strong pharmacological effects at very low doses. Therefore, the SCCS is of the opinion that the PGAs pose a concern for adverse effects on human health when used in cosmetic products.

3.5 DISCUSSION

Prostaglandin analogues (PGAs) are used in cosmetic products that function as eyelash and/or eyebrow conditioners. These products are comprised of numerous cosmetic ingredients including isopropyl cloprostenate and ethyl tafluprostamide that are designed to enhance the appearance of natural eyelashes and eyebrows by nourishing, moisturising and protecting eyelashes and eyebrows from breakage. Due to the very limited data available for the others PGAs, isopropyl cloprostenate and ethyl tafluprostamide are the only analogues assessed in this opinion as requested in the mandate.

Physicochemical properties

From the limited physicochemical data provided (predicted using EPI Suite™), both isopropyl cloprostenate and ethyl tafluprostamide can be considered as hydrophobic substances that are practically insoluble in water.

Some data have been provided on stability of one final product, but not on the PGAs assessed in this Opinion.

Exposure assessment & Toxicokinetics

No experimental data on percutaneous absorption of the PGAs assessed in this Opinion have been provided.

The estimated dermal absorption of isopropyl cloprostenate was 10% on the basis of a molecular weight of 476 g/mol and a log POW of 5.15 (calculated via QSAR, Episuite 1.0).

The SCCS is of the opinion that the toxicokinetics of isopropyl cloprostenate will be different from that of cloprostenol and R-cloprostenol. Cloprostenol (Log Kow=3.95) is less hydrophobic compared to isopropyl cloprostenate (Log Kow=5.15). Therefore, systemic uptake via the dermal or oral route can be expected to be different. Although metabolic transformation of isopropyl cloprostenate will yield cloprostenol, it can be assumed that before the metabolism, systemically available isopropyl cloprostenate can be distributed differently in the organism compared with cloprostenol. Hence, drawing conclusions on the toxicokinetics profile of isopropyl cloprostenate based on the toxicokinetics data available for cloprostenol and R-cloprostenol is not appropriate.

No relevant data on toxicokinetics of the PGAs assessed in this Opinion have been provided.

Toxicological Evaluation

Irritation and corrosivity

Some experimental data on the *in vitro* Hen's egg chorioallantoic membrane (HET-CAM), MatTek Epiocular™ MIT viability assay, and conjunctival hyperaemia in rabbit and guinea pigs have been provided but they were related to final cosmetic products and not ingredients. No relevant experimental data on skin irritation of the PGAs assessed in this Opinion have been provided.

Within the remit of the SCCS, safety assessments are based on assessment of the ingredients and not cosmetic formulations. Test results relating to cosmetic formulations have therefore not been taken into consideration in this Opinion.

Skin sensitisation

No relevant experimental data on skin sensitisation of the PGAs assessed in this Opinion have been provided.

Acute toxicity

Some experimental data on acute oral and dermal toxicity of cloprostenol have been provided but not for the PGAs assessed in this Opinion.

Repeated dose toxicity

Some experimental data on sub-chronic oral administration are available for cloprostenol. Although not directly relevant, the data indicate that even low doses of the PGAs assessed in this Opinion may exert systemic effects.

No relevant experimental data on repeated dose toxicity of the PGAs assessed in this Opinion via relevant routes (oral, dermal) have been provided.

Reproductive toxicity

The available data on cloprostenol effects on reproduction in rats indicate activity at very low doses. No relevant experimental data on reproductive toxicity of the PGAs assessed in this Opinion have been provided.

In the available studies no teratogenic effects were observed for cloprostenol, however no relevant experimental data on developmental toxicity of the PGAs assessed in this Opinion have been provided.

Mutagenicity / genotoxicity

No relevant experimental data on mutagenicity/genotoxicity of the PGAs assessed in this Opinion have been provided.

Isopropyl cloprostenate was predicted to be not mutagenic in Ames test according to the QSAR model Toxtree v. 3.1.0-1851 and the statistical method based model US EPA T.E.S.T v. 4.2.1. Further, isopropyl cloprostenate was assigned a Cramer class III toxicity classification by Toxtree v. 3.1.0-1851.

R-Cloprostenol was devoid of mutagenic activity in the bacterial Ames test and the *in vitro* mouse lymphoma L5178Y/TK+/- test. In an *in vitro* chromosomal aberration test in human lymphocytes, increased frequencies of aberrations were observed only at very high concentrations (2320 µg/mL). R-Cloprostenol gave negative results in the *in vivo* bone marrow micronucleus test by the intraperitoneal route in mice.

Taking into account that for R-cloprostenol, a mutagenic effect was observed in chromosome aberration assay in human lymphocytes (although at high concentrations), the SCCS is of the opinion that a genotoxic hazard of other PGAs cannot be excluded.

Carcinogenicity

No relevant experimental data on carcinogenicity of the PGAs assessed in this Opinion have been provided.

Review of available literature data indicates involvement of prostaglandin E2 in tumorigenesis mainly through its interaction with EP receptors (Piotrowski *et al.*, 2020). EP2 receptor subtype is known to act as a tumour promoter predominantly through activation of angiogenesis related to induction of VEGF. EP4 is another receptor with well-described connection to tumourigenesis. The PGE2-induced activation of EP4 receptor results in the development of pro-tumourigenic immune response. EP4 receptor pathway has been implicated in the activation of Treg lymphocytes which inhibit the inflammatory response to the tumour or can promote growth and invasiveness of tumour cells.

Considering the available data, the SCCS cannot exclude the potential carcinogenicity of the PGAs.

Photo-induced toxicity

No relevant experimental data on photo-induced toxicity of the PGAs assessed in this Opinion have been provided.

Human data

In the submission, the applicants provided some studies on human subjects. The SCCS considered each of these studies but regarded them as not relevant to the current assessment.

For human eye irritation, product based information was submitted, whereas, risk assessment of cosmetic ingredients within the remit of the SCCS is based on assessment of the ingredient and not of cosmetic formulations.

A clinical trial was submitted, evaluating various ophthalmological parameters, including intra-ocular pressure. This trial was performed with a topical ocular dose of 5 microgram of isopropyl ester of d-cloprostenol, and postulated no effect in 23 glaucoma patients. While a full evaluation of a clinical trial is outside the remit of the SCCS, it would like to point out that this dose appears to be higher than (or at least in the same order of) the dose that produces significant lowering of intra-ocular pressure in animals.

Special investigation

The applicant provided a study on three animal models for: conjunctival hyperemia, constriction of pupils and intra-ocular pressure lowering efficacy, in which isopropyl cloprostenate was applied directly into the eye. The results showed similar effects of isopropyl cloprostenate to the reference PGAs, when it was used at comparable or several fold lower concentrations.

In another study, isopropyl cloprostenate, when evaluated for conjunctival hyperemia in guinea pigs, showed a concentration-dependent increase in incidence of hyperemia which was slightly lower compared to reference prostaglandin analogues. Isopropyl cloprostenate induced IOP reduction percentage in monkeys, comparable to reference prostaglandin analogues.

Although the test results based on direct eye application were considered of limited usefulness, they indicate that isopropyl cloprostenate after such application can exert strong local adverse effects and have a strong IOP lowering activity.

The SCCS carried out *in silico* assessment of toxicity on PGAs using QSAR models and read-across. The endpoints assessed included skin sensitisation, carcinogenicity, mutagenicity and reproductive toxicity. The *in silico* systems used included QSAR-based systems (VEGA-QSAR and US EPA-TEST) and read-across (TOXREAD).

The first line of evidence obtained from *in silico* analysis of the 15 PGAs suggests that they may not be mutagenic/genotoxic, but there are strong indications for their potential carcinogenicity via non-genotoxic mechanisms. In addition, the analysis indicated potential reproductive/developmental and skin sensitisation effects of the analogues. All of these endpoints are critical for the safety assessment of a substance intended for use in cosmetic products, and therefore experimental data addressing these endpoints need to be provided to the SCCS to exclude the potential adverse effects on consumer health from use of the PGAs in cosmetic products.

We will start with preparing a letter for the COM (GROW).

4. CONCLUSION

1. *In light of the data provided, does the SCCS consider Isopropyl cloprostenate (CAS 157283-66-4) and Ethyl Tafluprostamide or DDDE (CAS 1185851-52-8) safe when used up to the concentrations provided in the respective dossiers (0.006% and 0.007% for Isopropyl cloprostenate and 0.018% for Ethyl Tafluprostamide)*

Having considered the limited data provided and the available information from published literature, the SCCS is not able to conclude on the safety of isopropyl cloprostenate and ethyl tafluprostamide when used up to the intended use concentrations indicated in the respective dossiers (0.006% and 0.007% for isopropyl cloprostenate and 0.018% for ethyl tafluprostamide).

2. *Does the SCCS have any further scientific concerns with regard to the use of Isopropyl cloprostenate (CAS 157283-66-4) and Ethyl Tafluprostamide / DDDE (CAS 1185851-52-8) in cosmetic products?*

The SCCS has noted concerns about the safety of isopropyl cloprostenate and ethyl tafluprostamide when used in cosmetic products - in particular those that are intended for use in the proximity of the eye. These concerns have been highlighted in more detail in Annex 1.

3. *In light of the available data, does the SCCS consider that the use in cosmetic products of prostaglandins analogues (listed in Table 1) raises safety concerns and might pose a risk to human health?*

Prostaglandins and synthetic analogues are widely known to be potent pharmacologically active substances. Due to these effects, other regulatory authorities have advised against, or have prohibited, their use in eyelash growth-promoting cosmetics. In view of the potential for causing effects at very low concentrations, and the intended use in the proximity of the eye, the SCCS has noted concerns over the safety of prostaglandin analogues when used in cosmetic products. These have been highlighted in Annex 1 to this Opinion.

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181.

8. LIST OF ABBREVIATIONS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181.

ANNEX 1

SCCS concerns over the safety of the prostaglandin analogues (PGAs) in cosmetics

Prostaglandin analogues (PGAs) are registered drugs for glaucoma treatment, however, they are also used as ingredients in cosmetic products that are on the market for improving eyelash and eyebrow appearance. The review of the open literature indicates that the PGAs are very potent pharmacologically active substances for which serious adverse effects after direct eye applications have been reported.

In response to the EU call for data on prostaglandins and their analogues used in cosmetic products conducted in 2020, 3 dossiers were submitted, which gave very limited useful information that could help conducting an adequate risk assessment.

After evaluation of the available information, the SCCS has concluded that the use of the PGAs in cosmetic products constitutes a concern for consumer safety on the basis of the following:

PHYSICOCHEMICAL ASPECTS

The data provided on physicochemical properties of the PGAs is very limited and insufficient for use in safety assessment. The limited data provided on stability were for a final product not the PGAs. For adequate safety assessment, detailed physicochemical data as indicated in the SCCS NoG 2021 are essentially required.

TOXICOLOGICAL ASPECTS

In silico

In view of the data gaps, the SCCS conducted its own *in silico* assessment of the toxicity of PGAs using QSAR models and read-across. The endpoints assessed included skin sensitisation, carcinogenicity, mutagenicity and reproductive toxicity. The *in silico* systems used for QSAR and read-across assessments included VEGA-QSAR platform, TOXREAD, and EPA-TEST. The first line of evidence obtained from *in silico* analysis of the 15 PGAs suggested that although they are not likely to be mutagenic/genotoxic, there is a strong indication for their potential carcinogenicity via non-genotoxic mechanisms. In addition, the analysis has indicated potential reproductive/developmental and skin sensitisation effects of the PGA.

In vitro

The available experimental data are limited to *in vitro* Hen's egg chorioallantoic membrane (HET-CAM) and MatTek Epiocular™ MIT viability assay, which indicate low/no effects. However, the data relate to final products and not the ingredients, and as such are not useful for safety assessment of the PGAs.

Therefore, from the available data, the potential of the PGAs analogues to cause skin irritation cannot be excluded.

Genotoxicity

Taking into account that for cloprostenol a mutagenic effect has been reported in an *in vitro* chromosome aberration assay in human lymphocytes (although at high concentrations), the SCCS is of the opinion that a genotoxic potential of other PGAs cannot be excluded. Although the data for cloprostenol indicate no *in vitro* gene mutation and chromosomal aberration potential *in vivo* (EMA, 2004), the original study reports are not available to the SCCS to enable assessment of the quality of the studies. Similarly, data on the other PGAs are not available, and the SCCS is therefore of the opinion that the genotoxic potential of the PGAs cannot be excluded on the basis of the very limited available information.

Information from in vivo studies

1. Although available data for some PGAs indicate a rather short half-life after parenteral administration, no data are available on basic toxicokinetic parameters for most of the PGAs.
2. Although some data are available on eye and skin irritation/sensitisation, these concern the final product and not the cosmetic ingredient, or the data were obtained after direct eye application. Therefore, based on the analysis of available data, the potential of PGAs to cause eye and skin irritation/sensitisation cannot be excluded.
3. The data on acute, chronic, reproductive/developmental toxicity of the PGAs are also very limited and insufficient. The limited available data do not allow drawing conclusions on these endpoints.
4. In the EMA 2004 Report on cloprostenol and R-cloprostenol, an ADI of 0.075 µg/kg bw/day (i.e 4.5 µg/person/day) was established for cloprostenol, based on a NOEL of 15 µg/kg bw/day derived from a rat 3 generation oral reproductive study. The data indicate a potentially very high reproductive activity of the PGAs.

Human data

The SCCS review of the open literature has indicated that PGAs caused serious adverse effects in ocular and periocular tissues in some glaucoma patients after direct eye applications (Nakakura *et al.*, 2015, Shah *et al.*, 2013; Wang *et al.*, 2014). These data indicate a concern for the manifestation of serious and irreversible histological changes after consumer exposure to the PGAs in cosmetic products.

For pharmacological treatment of intra-ocular pressure, a daily dose of one drop with a PGA is prescribed. This implies, depending on the type of analogue, a dose of 0.75 – 2.5 µg per eye per day. In the absence of data on skin absorption from the application of an eyelash growth formulation, assuming a dermal absorption of 50% and full transfer from the eye-lid conjunctiva to the eye, a maximum exposure of the eye of 0.36 µg DDDE and 2.5 µg isopropyl cloprostenate can theoretically be estimated. These doses are in the same order of magnitude as those used for the epi-ocular pharmacological treatment of intra-ocular pressure.

EXPOSURE ASPECTS

Dermal uptake:

No reliable data on percutaneous absorption of the PGAs are available. Therefore, a potential of the PGAs in cosmetic products for interference with the intraocular pressure, induction of intraocular and periocular tissue histological alterations, as well as induction of systemic effects cannot be excluded.

The calculations by the SCCS of the maximum dose intake by a person per day indicate that the ADI value of 0.075 µg/kg bw/day as proposed by EMA is in the same order of magnitude to the doses potentially applied with an eyelash growth product containing isopropyl cloprostenate or DDDE. These calculations raise an additional concern over potential reproductive toxicity of the PGAs when used in cosmetic products.

OTHER ASPECTS

Since PGAs have strong physiological effects, some regulatory authorities have advised against or have prohibited its use in eyelash growth-promoting cosmetics.

CONCLUSION

With a collective consideration of the physicochemical, toxicological and exposure aspects noted above, the SCCS is of the view that there is a basis for a concern that the use of the

prostaglandin analogues, as notified through CPNP for use in cosmetic products, can pose a health risk to the consumer.

Considering the potent pharmacological activity and the scarcity/lack of safety data, the SCCS cannot advise on the concentrations of the PGAs that can be safely used in cosmetic products.

The SCCS will be ready to assess any evidence provided to support safe use of PGAs in cosmetic products.
