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THINKING AHEAD IN TOXICOLOGY

Safety assessment of Ethyl Tafluprostamide as used in in cosmetic products

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Background

- In 2018, the **German Federal Institute for Risk Assessment (BfR)** informed the European Commission (EC) that they were concerned that the use of prostaglandins (PG) and prostaglandin analogues (PGAs) as ingredients in cosmetic products may pose health risks for consumers
- In 2020, the EC issued a call for data and in 2022, the **EC requested the EU Scientific Committee for Consumer Safety (SCCS)** to provide a safety assessment of the use of PGs/PGAs in cosmetics
- In 2022, the **SCCS** released an **opinion on the use of PGs/PGAs in cosmetic products,** stating:
  - The SCCS could **not advise on safe use** concentrations due to the **lack of toxicology data**
  - The SCCS would assess **any new evidence** supporting the use of PGAs in cosmetic products
- In 2022-2023, on behalf of a cosmetic product manufacturer that uses **Ethyl Tafluprostamide (also known as Dechloro Dihydroxy Difluoro Ethylcloprostenolamide – DDDE)** as an ingredient, ToxMinds coordinated completion of a suite OECD-compliant toxicology studies and, in 2023 submitted to the EC a **safety dossier** (compliant with the SCCS Note of Guidance) supporting the safe use of **0.018% Ethyl Tafluprostamide in a cosmetic eyelash** product formulation
- This safety dossier has also been submitted **to the CIR Expert panel** in response to its Insufficient Data Announcement (IDA) for Ethyl Tafluprostamide in June 2023
- Core to the proposed strategy is the use of **systemic toxicity data available for the close structural analogue Tafluprost for the safety assessment of Ethyl Tafluprostamide by means of read across**
- The **suitability of the read across** has been demonstrated by rigorously **applying the principles of existing regulatory read across assessment frameworks** (i.e., OECD and European Chemicals Agency 'ECHA')

Overview of available data on Ethyl Tafluprostamide

Endpoint	Available data
UV/VIS absorption test	Molar extinction coefficients (MCEs): 1046 to 1306 L*Mol-1cm-1 (> 1000 L/mol-1cm-1 cut-off; likely to be photo-reactive); Maximum absorbance **226-276 nm** (<313 nm cut-off; **therefore, not phototoxic**)
Dermal penetration test	*In vitro* percutaneous absorption study with 0.018% Ethyl Tafluprostamide using human skin: **6.51% ± 2.16%**
Skin metabolism	*In vitro* dermal penetration test with fresh skin having metabolic activity demonstrated the formation of **65.8-71.2% free acid**
Skin irritation	*In vitro* EpiSkin™ RhE assay with neat Ethyl Tafluprostamide: **Not irritating** (OECD 439) HRIPT study with0.025% Ethyl Tafluprostamide in 51 panellists: **Not irritating**
Eye irritation	*In vitro* EpiOcular™ RhCE assay with neat Ethyl Tafluprostamide: **Not irritating** (OECD 492) *In vitro* HET-CAM assay with 0.025% Ethyl Tafluprostamide: **Not irritating**
Skin sensitisation	**Negative in Directive peptide reactivity assay (DPRA)** (OECD 442C) **Negative in KeratinoSens™** (OECD 442D)
Genotoxicity	**Negative in the Ames assay** with/without S9 mix (OECD 471) **Negative in the *in vitro* micronucleus test (MNT)** with/without S9 mix (OECD 487)

Systemic toxicity data gaps

Endpoints	Endpoint assessment strategy
Dermal absorption	Data available on Ethyl Tafluprostamide
Acute toxicity	No data
Skin irritation	Data available on Ethyl Tafluprostamide
Eye irritation	Data available on Ethyl Tafluprostamide
Skin sensitisation	Data available on Ethyl Tafluprostamide
Phototoxicity	Data available on Ethyl Tafluprostamide
Repeated dose toxicity	No data
Genotoxicity	Data available on Ethyl Tafluprostamide
Reproductive toxicity	No data
Developmental toxicity	No data

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Strategy to fill data gaps for systemic toxicity endpoints: Read-across

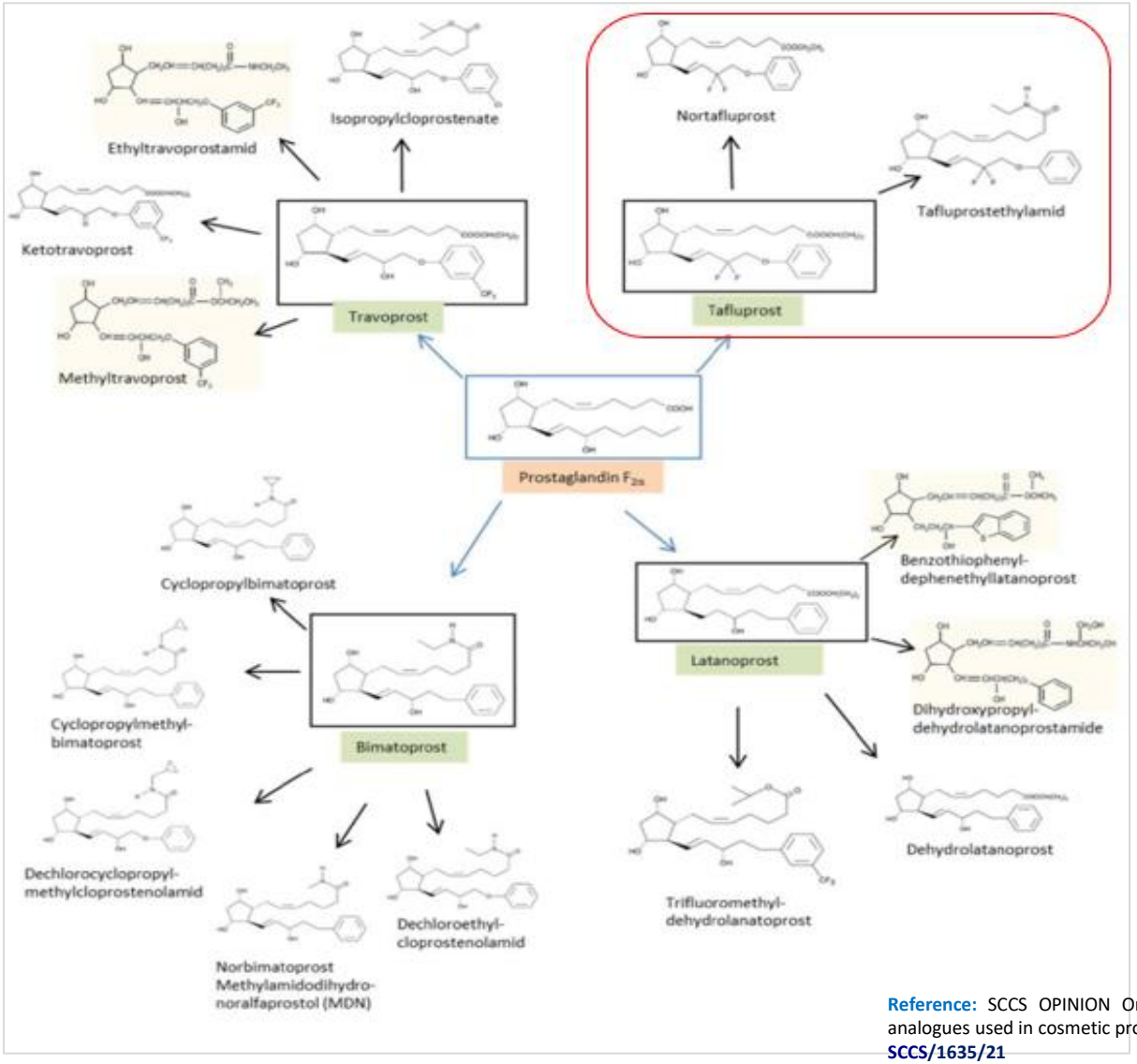
- Read across criteria
- Identification of suitable analogue (‘Tafluprost’)
- Justification of suitability for using Tafluprost data for systemic toxicity assessment of Ethyl Tafluprostamide

Strategy to fill systemic toxicity endpoint gaps

- Use of systemic toxicity data available for a close structural analogue for the safety assessment of Ethyl Tafluprostamide by means of read across
- The suitability of the read across for demonstrating similarity of the analogue has been demonstrated by rigidly applying the principles of the read across assessment framework by the OECD, developed further by ECHA
  - Common functional groups and structure
  - Similarity or trend in physico-chemical properties
  - Common reactivity/toxicity profiles (e.g., structural alerts, ‘anchor data’)
  - Likelihood of common breakdown products via biological processes

Identification of suitable analogues

4 Prostaglandins with systemic toxicity data

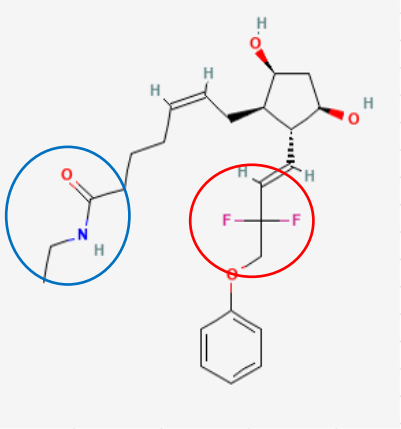


Reference: SCCS OPINION On Prostaglandins and prostaglandin-analogues used in cosmetic products
SCCS/1635/21

Identification of suitable analogues

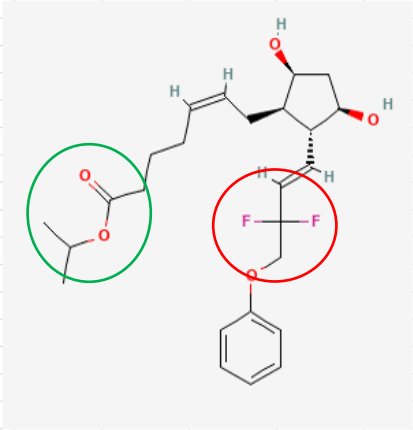
Comparison the structural similarity of the 4 prostaglandin analogues with data to Ethyl Tafluprostamide

TARGET SUBSTANCE



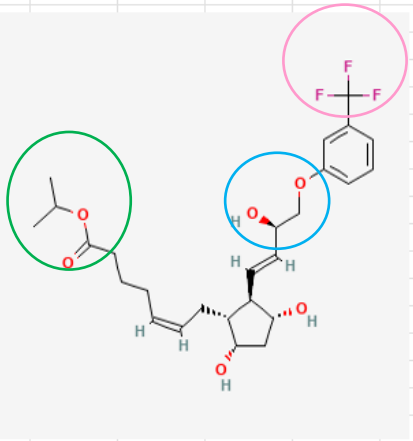
CAS No. 1185851-52-8

Tafluprost ethyl amide



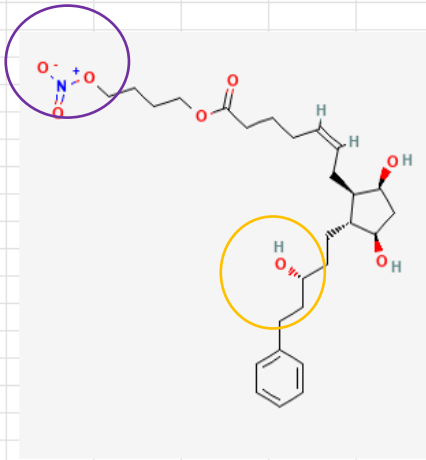
CAS No. 209860-87-7

Tafluprost



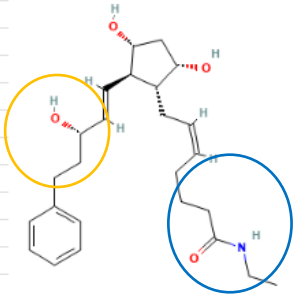
CAS No. 157283-68-6

Travoprost



CAS No. 157283-68-6

Latanoprostene BUNOD



CAS No. 155206-00-1

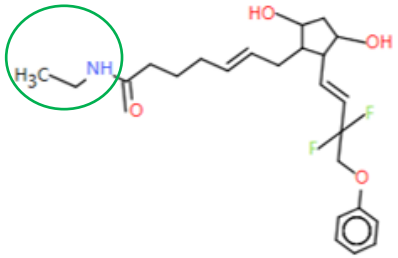
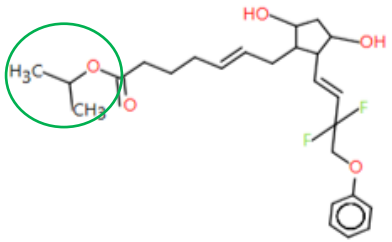
Bimatoprost

Structurally closest analogue to Ethyl Tafluprostamide: Tafluprost

(in line with BfR health assessment report on Ethyl Tafluprostamide)

Analogue suitability evaluation for read across

Common functional groups and structural similarity

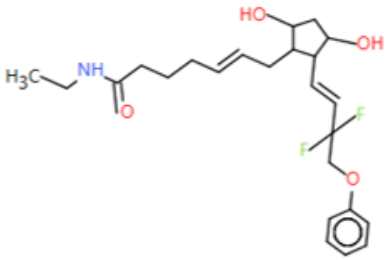
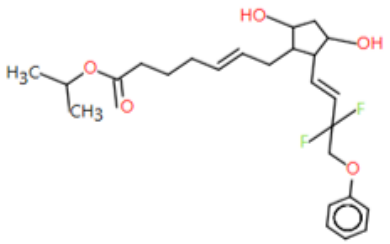
	Target substance	Analogue
CAS number	1185851-52-8	209860-87-7
Name	Dechloro dihydroxy difluoro ethylcloprostenolamide (DDDE) or Tafluprost ethyl amide	Tafluprost
2D Structure		
Common functional groups and structural similarity		
Organic groups from OECD TB v.4.5	**Organic functional groups** Alcohol Alkene moiety Alkyl halide Allyl Aryl Cycloalkane Dihydroxyl derivatives Ether moiety Organic amide and thioamide	**Organic functional groups** Alcohol Alkane, branched with secondary carbon Alkene moiety Alkyl halide Allyl Aryl Carboxylic acid ester Cycloalkane Dihydroxyl derivatives Ether moiety Isopropyl
Dice index from OECD Tool box v.4.5	-	0.86

Same functional groups; except it presents the **carboxylic acid ester group instead of the amide group** and it contains the **isopropyl group**

High Dice index

Analogue suitability evaluation for read across (cont.)

Similarity/trend of physico-chemical property

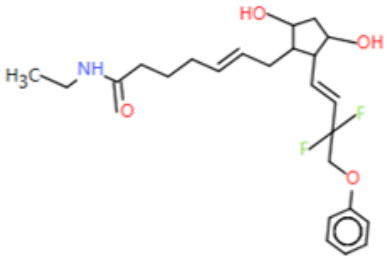
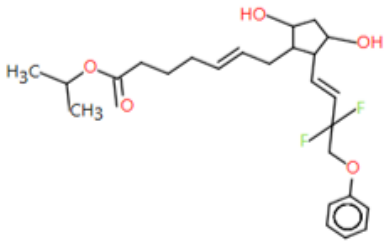
	Target substance	Analogue
CAS number	1185851-52-8	209860-87-7
Name	Dechloro dihydroxy difluoro ethylcloprostenolamide (DDDE) or Tafluprost ethyl amide	Tafluprost
2D Structure		
Similarity in physico-chemical properties		
Molecular weight (Da)	437.53	452.54
Melting Point (deg C)- Estimated Prediction software: MPBPWIN v.1.44	247.58	204.56
Boiling Point (deg C)- Estimated Prediction software: MPBPWIN v.1.44	574.90	509.44
Vapour Pressure (Pa, 25°C)- Estimated Prediction software: MPBPWIN v.1.44	1.2E-013	5.06E-011
Log Kow - Estimated (Experimental) Prediction software: KOWWIN v1.69	5.03	6.51
Water solubility (mg/L) - Estimated Prediction software: WSKOW v1.43	0.091	0.0039

Overall same range, with slight differences indicating the analogue Tafluprost is expected to be less bioavailable through the oral route compared to Ethyl Tafluprostamide

PoD study via IV route

Analogue suitability evaluation for read across (cont.)

Common reactivity/toxicity profile

	Target substance	Analogue
CAS number	1185851-52-8	209860-87-7
Name	Dechloro dihydroxy difluoro ethylcloprostenolamide (DDDE) or Tafluprost ethyl amide	Tafluprost
2D Structure		
Common reactivity/toxicity profile		
Structural alerts from OECD TB v.4.5	**Estrogen Receptor Binding:** Strong binder, OH group **Toxic hazard classification by Cramer:** High (Class III) **Toxic hazard classification by Cramer (extended):** High (Class III) **Oncologic Primary Classification:** Alpha- and beta-Haloether Reactive Functional Groups	**Estrogen Receptor Binding:** Strong binder, OH group **Toxic hazard classification by Cramer:** High (Class III) **Toxic hazard classification by Cramer (extended):** High (Class III) **Oncologic Primary Classification:** Alpha- and beta-Haloether Reactive Functional Groups
Toxicological properties (bridging data)	Not sensitising in *in vitro* sensitisation assays and HRIPT Not genotoxic in an *in vitro* Ames and micronucleus assays	Not sensitising in GPMT Not geotoxic in an *in vitro* Ames, *in vitro* chromosmal aberration **assay** and *in vivo* micronucleus **assay**assays

Same structural alerts and toxicological properties

Analogue suitability evaluation for read across (cont.)

Likelihood of common breakdown products

	Target substance	Analogue
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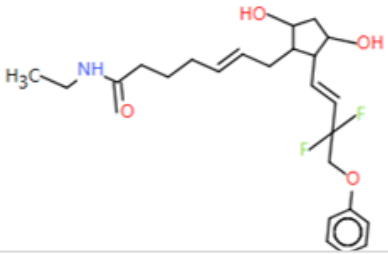
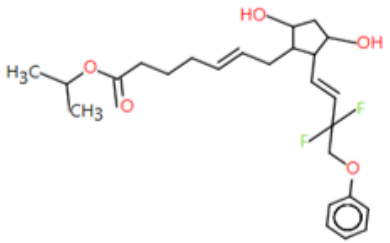
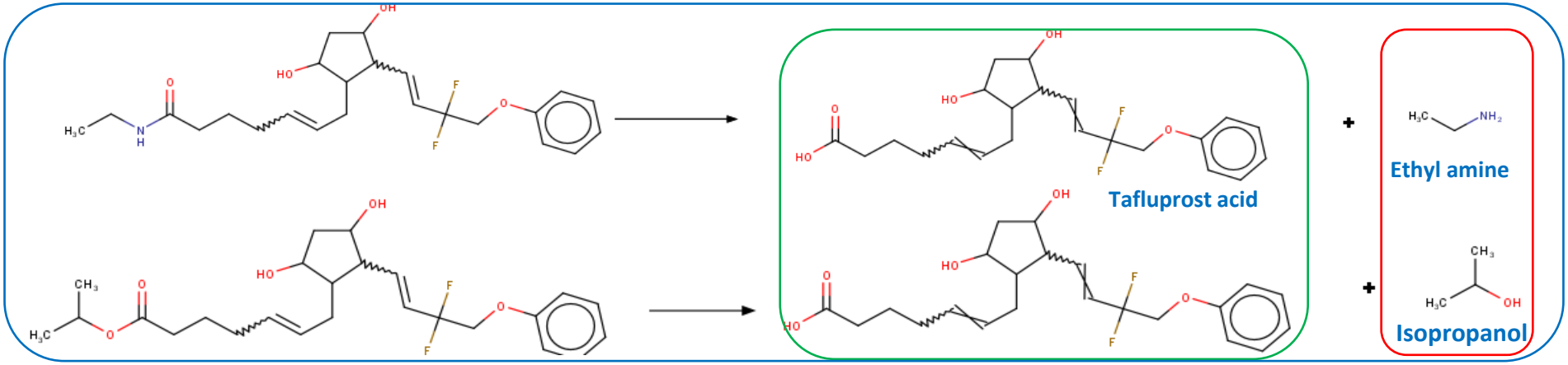
2D Structure		
Common breakdown products via biological processes (metabolism)		
Prediction of the firmetabolic pathway using Meteor Nexus v.3.1.0 and literature data	**Meteor:** Hydrolysis of Acyclic Carboxylic Amides with the formation of tafluprost acid and aromatic hydroxylation (see **Annex II**).	***Literature:*** Tafluprost is an ester prodrug which is rapidly hydrolysed by corneal esterases to form its biologically active acid metabolite. Tafluprost acid is further metabolized via fatty acid β-oxidation and phase II conjugation (CDER, 2011).

Predicted to undergo a **similar first metabolic reaction (hydrolysis)**



Analogue suitability evaluation for read across (cont.)

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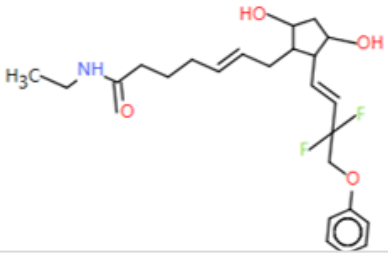
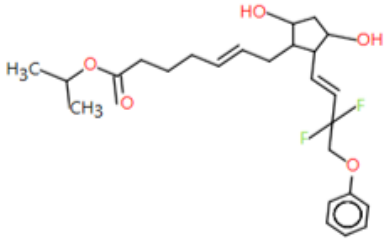
2D Structure		
Common breakdown products via biological processes (metabolism)		
Prediction of the firmetabolic pathway using Meteor Nexus v.3.1.0 and literature data	**Meteor:** Hydrolysis of Acyclic Carboxylic Amides with the formation of tafluprost acid and aromatic hydroxylation (see **Annex II**).	***Literature:*** Tafluprost is an ester prodrug which is rapidly hydrolysed by corneal esterases to form its biologically active acid metabolite. Tafluprost acid is further metabolized via fatty acid β-oxidation and phase II conjugation (CDER, 2011).

- The formation of the **uncommon metabolites (ethyl amine** for Ethyl Tafluprostamide and **isopropanol** for Tafluprost), was not assessed to have an impact on the read across strategy
- A comparison of available data on Tafluprost and the two uncommon metabolites demonstrates that **Tafluprost acid is the driver of toxicity**
- Formation of Tafluprost acid is demonstrated for Ethyl Tafluprostamide *in vitro* dermal penetration study with skin metabolic capacity

Summary: Justification for using Tafluprost data for assessing Ethyl Tafluprostamide

- Common functional groups and structure ✓
- Similarity or trend in physico-chemical properties ✓
- Common reactivity/toxicity profiles (e.g., structural alerts, 'anchor data') ✓
- Likelihood of common breakdown products via biological processes ✓

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Safety assessment of Ethyl Tafluprostamide for use in a cosmetic product

- Toxicological endpoint assessment strategy for Ethyl Tafluprostamide
- Derivation of Point of Departure (PoD) for systemic toxicity assessment
- Exposure Assessment
- MoS calculations

Toxicological endpoint assessment strategy for Ethyl Tafluprostamide

Toxicological endpoints	Endpoint assessment strategy
Dermal absorption	Data available on Ethyl Tafluprostamide
Acute toxicity	Read across to analogue Tafluprost
Skin irritation	Data available on Ethyl Tafluprostamide
Eye irritation	Data available on Ethyl Tafluprostamide
Skin sensitisation	Data available on Ethyl Tafluprostamide
Phototoxicity	Data available on Ethyl Tafluprostamide (UV absorbance data)
Repeated dose toxicity	Read across to analogue Tafluprost
Genotoxicity	Data available on Ethyl Tafluprostamide
Reproductive toxicity	Read across to analogue Tafluprost
Developmental toxicity	Read across to analogue Tafluprost

PoD selection and justification

- **Available systemic studies**
  - Repeated dose and chronic carcinogenicity studies in rats, mice, and dogs: NOAELs ranged from 1 to 100 µg/kg bw/day (IV route); <3 to 100 µg/kg bw/day (SC route)
  - Reproductive toxicity study in rats: NOAEL: 100 µg/kg bw/day (IV route) PND studies in rats: NOAEL: 0.3-3 µg/kg bw/day (IV route)
- **PoD study**
  - Pre-post-natal developmental toxicity study in rats dosed at 0, 0.3, 1, 3, and 10 µg/kg bw/day via IV route
  - NOAEL was based on decreased F1 offspring viability at ≥1 µg/kg bw/day and delayed pinna unfolding, increased F1 newborn mortality and decreased body weight at 10 µg/kg bw/day
- **PoD justification**
  - Lowest NOAEL established in the available set of well-conducted studies for Tafluprost
  - **Represents a very conservative and worst-case PoD** when compared with the relevant exposure route (i.e., dermal)
  - Topical ocular administration studies resulted in higher NOAELs (6.7 to 10 µg/kg bw/day) and did not show any systemic toxicity
  - Same PoD was selected by the BfR for the health assessment of Ethyl Tafluprostamide

PoD = 0.3 µg/kg bw/day (PND study in rats)

Dermal exposure considerations for eyelash product with 0.018% Ethyl Tafluprostamide

Directions For Use	Caution Statements
Once a day, apply a thin line of the cosmetic eyelash product directly to eyelashes, above the lash line. Let dry completely before applying additional beauty products.	Do not get in eye. Rinse immediately with water if eye contact occurs. If irritation develops, reduce frequency of use until irritation resolves. If irritation persists or is excessive, discontinue use and consult a physician. Some users have reported a faint darkening of the eyelash base (primarily with excessive use); if this is of concern, do not use. Keep out of reach of children.

- **Similar to mascara and eyeliner,** exposure is expected to be via the **dermal route**
- Similar to **eyeliner**, a **small amount of the eyelash product is applied with a fine brush applicator**. In contrast, a large amount of mascara is applied along the length of eyelashes.
- Similar to **mascara**, this eyelash product is **applied to eyelashes** (unlike eyeliner).
- Use of a **thickener** (cellulose gum) in the cosmetic eyelash product **minimizes the migration of the product to the eyelid skin or into the eye**.
- The amount of Ethyl Tafluprostamide that will to migrate to the eyelid and be available for skin penetration is **negligible**.
- Nevertheless, for a worst-case calculation, **50% of the applied amount** was **used for MoS calculations**.

Dermal penetration of Ethyl Tafluprostamide

- *In vitro* absorption of Ethyl Tafluprostamide present in a cosmetic eyelash product formulation was determined in an **OECD TG 428-compliant dermal penetration study** using healthy human skin tissue samples
- The **absorbed fraction** of the applied test substance was determined to be **6.51±2.16%** of the applied dose after 24 hours of exposure
- The **mean total recovery** was within the SCCS acceptance criteria (i.e., **85-115%**), validating the results obtained
- **Variability** of results was low and considered acceptable (SD = 2.16%)
- According to the SCCS criteria, a dermal penetration value of **8.67%** (i.e., 6.51+2.16%) was used for MoS calculations

Algorithm for systemic exposure calculation

$$SED = E_{product} \times \frac{C}{100} \times \frac{DA_p}{100} \quad (6)$$

Where:

SED (mg/kg bw/day)	Systemic Exposure Dose
$E_{product}$ (mg/kg bw/day)	Estimated daily exposure to a cosmetic product per kg body weight, based upon the amount applied and the frequency of application (for calculated relative daily exposure levels for different cosmetic product types, **Tables 3A** and **3B**, Section 3-3.4.2).
C (%)	Concentration of the substance under study in the finished cosmetic product on the application site
DA_p (%)	Dermal Absorption expressed as a percentage of the test dose assumed to be applied in real-life conditions

- **Exposure scenario**
  - $E_{product}$: Considers 50% of the measured 'average amount' or 'maximum amount' of the cosmetic product per kg bw is **0.04-0.067 mg/kg bw/day** for a 60 kg adult (i.e., 50% x 4.8 mg/day* ÷ 60 kg = 0.04 mg/kg bw/day; 50% x 8 mg/day* ÷ 60 kg bw = 0.067 mg/kg bw/day)
  - C (%): The concentration of the substance in the cosmetic product (C) (i.e., **0.018%**)
  - DA_p: Dermal absorption (**8.67%**)

The measured average and maximum amount of product per application are 2.4 mg and 4 mg per brush stroke (or 4.8 mg and 8 mg per day for both eyes) respectively

Derivation of Margin of Safety (MoS) for exposure scenario

$$MoS = PoD_{sys}/SED$$

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

- In accordance with SCCS NoG, to consider a substance to be safe for use or an acceptable risk assessment, the MoS for systemic toxicity should be ≥100

Product daily amount (mg)	SED (mg/kg bw/d)	PoD_{sys} (mg/kg bw/d)	MoS
Based on average daily amount of 4.8 mg the cosmetic eyelash product	6.24E-07	0.0003	481
Based on maximum daily amount of 8 mg the cosmetic eyelash product	1.04E-06	0.0003	288

Conclusions

- Existing local toxicity information on Ethyl Tafluprostamide - as a neat substance and in cosmetic eyelash formulations - supports the safe use of 0.018% Ethyl Tafluprostamide
- Read-across was applied to assess systemic toxicity using the close analogue, Tafluprost
- The suitability of the analogue Tafluprost has been demonstrated against the criteria of regulatory read across assessment frameworks developed by the OECD and ECHA
- A conservative point of departure has been derived for the risk assessment of Ethyl Tafluprostamide on the basis of an IV pre-post-natal developmental toxicity study with Tafluprost
- Even under worst-case exposure assumptions, the calculated MoS values are well above 100

Based on the MoS values for an eyelash product with 0.018% Ethyl Tafluprostamide, cosmetic eyelash products with up to and including 0.02% Ethyl Tafluprostamide are safe when applied as directed.

Thank you very much for your attention!

In case of any further questions, please contact us at: **+32 2 762 91 45**

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