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THINKING AHEAD IN TOXICOLOGY

# USE OF NEW APPROACH METHODOLOGIES (NAMs) TO ASSESS THE SAFETY OF PROSTAGLANDINS FOR USE IN COSMETICS

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## Introduction

In 2018, the German Federal Institute for Risk Assessment (BfR) informed the European Commission (EC) that they were concerned that the use of prostaglandins and their analogues in cosmetic products might pose risks for consumers (BfR, 2018; SCCS, 2022).

Following a call for data in 2020, the EC requested the Scientific Committee on Consumer Safety (SCCS) to carry out a safety assessment of the uses of prostaglandins and their analogues in cosmetic products. In February 2022, SCCS concluded that the safe use concentrations for prostaglandin-analogues ('PGAs') in cosmetic products could not be determined due to the scarcity of toxicological data on the ingredients. However, SCCS said that it would be ready to assess any new ingredient-based evidence provided to support safe use (SCCS, 2022).

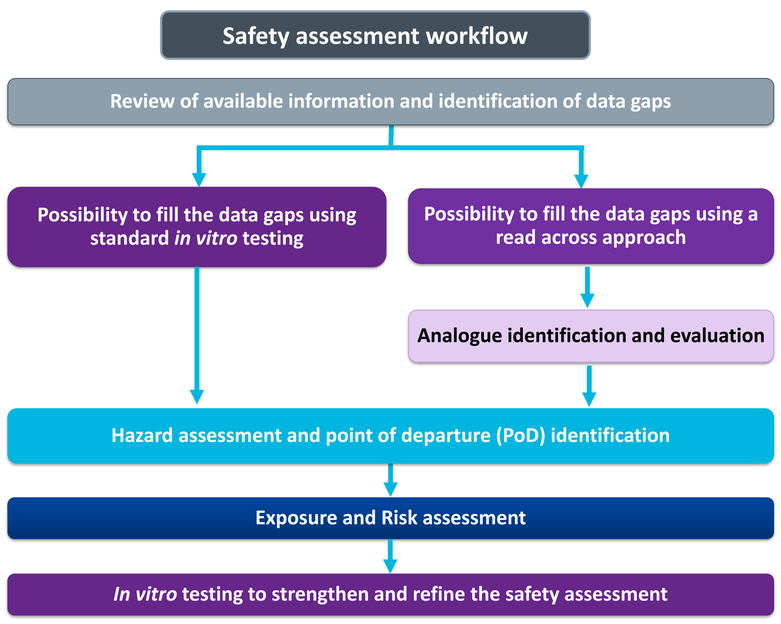
Bans on animal testing pose challenges to the generation of new ingredient-based toxicology data. This poster describes the application of 'new approach methodologies' (NAMs), including guideline-compliant *in vitro* testing for the different toxicological endpoints, to evaluate the safety of the prostaglandin, ethyl tafluprostamide, also known as dechloro dihydroxy difluoro ethylcloprostenolamide (DDDE), at use levels in a cosmetic eyelash product formulation.

The assessment of systemic endpoints such as acute toxicity, repeated dose toxicity, carcinogenicity, and developmental and reproductive toxicity was addressed through a read-across approach. A potential analogue for DDDE was identified and assessed using a stepwise process in line with the OECD guidelines and the ECHA's Read Across Assessment Framework (RAAF).

To strengthen the confidence in the safety assessment, NAMs based biological activity assays are in progress to provide mechanistic insights and support the read-across hypothesis with additional bridging data (acute toxicity).

## Safety assessment of prostaglandins in cosmetics

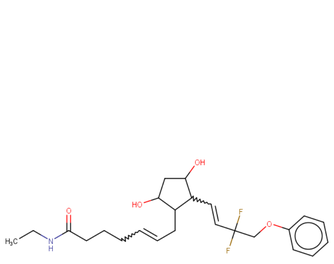
### Workflow of safety assessment and overview of data on DDDE



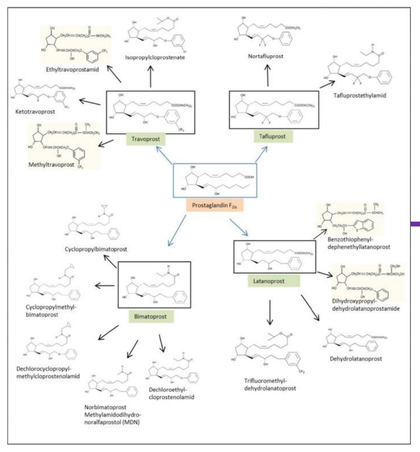

| Endpoint | Overview of existing and newly generated *in vitro* data |
|---|---|
| **UV/VIS absorption test** | Molar extinction coefficients: 1046 to 1306 L*Mol-1cm-1 (likely to be photo-reactive); maximum absorbance: 226-276 nm (not phototoxic, as it is <313 nm cut-off) – new study |
| **Dermal penetration test** | *In vitro* percutaneous absorption study using human skin: 6.51% ± 2.16% – new study |
| **Metabolism** | *In vitro* metabolism study using fresh human skin*:* formation of 65.8-71.2% tafluprost acid through hydrolysis reaction – new study |
| **Acute toxicity** | **No data** |
| **Skin irritation** | HRIPT study with 0.025% DDDE in 51 panellists: Not irritating<br>*In vitro* EpiSkin™ RhE assay with neat DDDE: Not irritating (OECD 439) – new study |
| **Eye irritation** | *In vitro* HET-CAM assay with 0.025% DDDE: Not irritating<br>*In vitro* EpiOcular™ RhCE assay with neat DDDE: Not irritating (OECD 492) – new study |
| **Skin sensitisation** | Negative in Directive peptide reactivity assay (OECD 442C) and KeratinoSens™ (OECD 442D) – new studies |
| **Repeated dose toxicity** | **No data** |
| **Genotoxicity** | Negative in the Ames assay (OECD 471); Negative in the *in vitro* micronucleus test (MNT) (OECD 487) – new studies |
| **Reproductive and developmental toxicity** | **No data** |
| **Special investigation (for ocular effects)** | No significant effect on intraocular pressure with 0.025% DDDE in 19 healthy human volunteers |

*HRIPT: human repeated insult patch test; RhE: reconstructed human epidermis; HET-CAM: Hen's egg test-chorio allantoic membrane; RhCE: reconstructed human cornea-like epithelium*

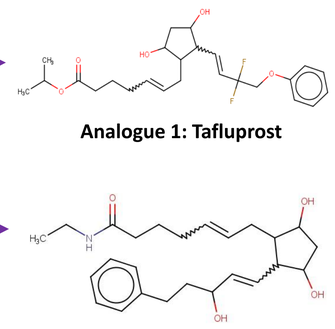
### Read across approach to fill data gaps for systemic endpoints



**Target substance: DDDE**




**Grouping of PGs and PGAs (SCCS, 2022)**



**Analogue 1: Tafluprost**

**Analogue 2: Bimatoprost**

**Selected analogues**

| Similarity criteria | Analogue evaluation |
|---|---|
| **Common functional groups and structure similarity** | • Both analogues have a high Dice index (>0.75)<br>• They share key functional groups (cycloalkane, ether moiety, alkyl halide and aryl groups); analogue 1 presents the carboxylic acid ester group instead of the amide group and contains the isopropyl group; analogue 2 does not contain ether and alkyl halide functional groups |
| **Similarity in physico-chemical properties** | • Both analogues have physico-chemical properties in the same range as compared to the target substance, suggesting low bioavailability |
| **Common reactivity/toxicity profiles (e.g., structural alerts and 'bridging data')** | • Both analogues presents the same structural alerts 'identified by Cramer classification', 'oncologic primary classification', 'estrogen receptor binding' profilers and toxicity in bridging endpoints (non-sensitising and non-genotoxic) |
| **Likelihood of common breakdown products via biological processes** | • Has been demonstrated to undergo hydrolysis as the first metabolic reaction; analogue 1 has been shown to give rise to a common metabolite, tafluprost acid |

### Safety assessment of DDDE

#### Hazard assessment

***Overview of studies***

- **Tafluprost**:
  - ✓ Based on the repeated dose toxicity (RDT) and carcinogenicity studies in rats, mice, and dogs, NOAELs: 0.001-0.1 mg/kg bw/day via the intravenous (i.v.) route and <0.003 -0.1 mg/kg bw/day via the subcutaneous route (CDER, 2011)
  - ✓ Based on the developmental and reproductive toxicity (DART) studies in rats: NOAELs: 0.0003-0.1 mg/kg bw/day via the i.v. route (CDER, 2011)
- **Bimatoprost**:
  - ✓ Based on RDT and carcinogenicity studies in rats, mice and monkeys, NOAELs: 0.1-250 mg/kg bw/day via the oral route and 0.3-10 mg/kg bw/day via the i.v. route (CDER, 2001)
  - ✓ Based on the DART studies in rats, NOAELs: 0.1-0.6 mg/kg bw/day via the oral route (CDER, 2001)

***PoD identification & justification***

**PoD = 0.3 µg/kg bw/day or 0.0003 mg/kg bw/day**

- ✓ It corresponds to the lowest NOAEL considering well-conducted studies available for both analogues
- ✓ It is derived based on the adverse effects observed in the developmental toxicity study with tafluprost via i.v. route in rats
- ✓ Topical ocular administration studies with the analogue tafluprost did not show systemic toxicity (NOAELs were 22-33-fold higher)
- ✓ The same PoD was selected by the BfR for the health assessment of PGs including DDDE (BfR, 2018)

#### Exposure & Risk assessment

***Exposure assessment***

The cosmetic product has a very specific application instruction. It is to be applied once a day as a thin line to eyelashes above the lash line; the maximum amount of the product applied per brush stroke is 4 mg (a total of 8 mg/day if applied to both eyes)

**Systemic exposure dose (SED) = $E_{product}$ x C/100 x $DA_p$/100**

**$E_{product}$** = Estimated daily exposure to the cosmetic product per kg bw (i.e., 50% x 8 mg/day ÷ 60 kg bw = 0.067 mg/kg bw/day, assuming only 50% of the applied product may migrate to the eyelid skin due to use of a thickener that prevents dripping)
**C** = Concentration of the ingredient in the finished cosmetic product (%) (i.e., 0.018%)
**$DA_p$** = Dermal Absorption expressed as a percentage of the test dose assumed to be applied in real-life conditions (%) (i.e., 8.67%)

***Risk assessment***

In accordance with the SCCS note of guidance, the margin of safety (MoS) for systemic toxicity should be ≥100

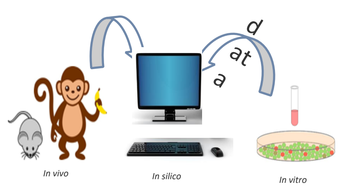
MoS = PoDsys/SED

PoDsys = Systemic Point of Departure (mg/kg bw/day)
SED = Systemic Exposure Dosage (mg/kg bw/day)

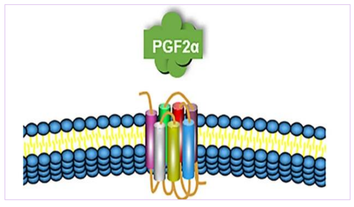
| SED (mg/kg bw/day) | PoDsys (mg/kg bw/day) | MoS |
|---|---|---|
| 1.04E-06 | 0.0003* | 288 |

**No correction is needed as the study is via the i.v. route*

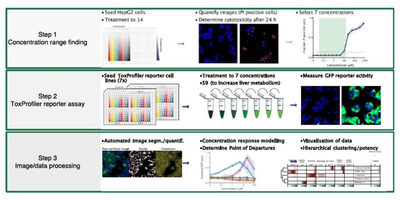
### Next steps: Additional NAMs data generation to strengthen the confidence in use of read across



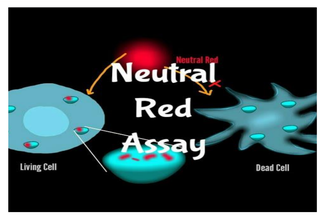

***in silico* endocrine receptor/activation predictions**



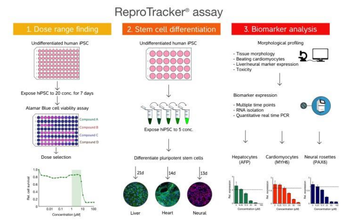

**Receptor binding potency studies**




**ToxProfiler® assay**




***In vitro* neutral red uptake assay**




**ReproTracker® assay**

## Conclusion

Based on the available data, the present safety assessment reveals a calculated MoS greater than 100 and thereby supports the safe use of DDDE at a concentration of up to 0.018% in cosmetic eyelash products under the conditions presented in this evaluation. Additional NAM-based testing is ongoing to strengthen the read-across hypothesis with additional bridging data.

www.toxminds.com

## References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