ToxMinds
THINKING AHEAD IN TOXICOLOGY

New data supporting the use of read across in the safety assessment of DDDE as a cosmetic ingredient

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Introduction

- **Initial ToxMinds submission (October 2023):** The dossier of DDDE safety data was submitted to the Cosmetic Ingredient Review (CIR) Expert Panel in response to the Insufficient Data Announcement (IDA #1) issued in June 2023
  - **Initial read across rationale :** In order to reduce animal testing, the data gaps for systemic endpoints were addressed using read across to tafluprost
- **CIR concern (December 2023):** The CIR Expert Panel raised concerns on the use of tafluprost as a read-across analogue, due to the absence of an amide functional group, which could be critical for receptor-mediated biological activity (specifically, the prostamide receptors)
  - **Alternative analogue:** Bimatoprost was proposed by the Expert Panel for its amide-driven prostamide receptor activity, acknowledging the lack of the 'geminal fluorine' functional group. A second IDA (IDA#2) was issued requesting data to support use of read-across and additional safety data
- **ToxMinds submitted an updated DDDE safety dossier (May 2025) in response to IDA# 2, including :**
  - An updated read across strategy based on the use of 2 complementary analogues to address CIR's concerns
    - **Tafluprost** as a structurally similar analogue with a conservative toxicological profile
    - **Bimatoprost** as a biologically similar analogue to address any additional toxicological concerns related to the prostamide receptor binding
  - Additional supportive evidence to strengthen the justification and to generate bridging data:
    - **New Approach Methodologies (NAMs) studies:** *in vitro* 3T3 NRU cytotoxicity, PGF2α receptor binding, ToxProfiler® and ReproTracker® assays
    - **Additional *in silico* analysis** to predict the interaction of DDDE with DART and ED-related receptors

Overview of safety endpoint data availability for DDDE – Initial submission

Endpoints	Endpoint assessment strategy
Dermal absorption	Data provided
Acute toxicity	**Data gap addressed using read across to tafluprost**
Skin irritation	Data provided
Eye irritation	Data provided
Skin sensitisation	Data available on DDDE (in vitro and human data)
Phototoxicity	Data provided
Repeated dose toxicity	**Data gap addressed using read across to tafluprost**
Genotoxicity	Data provided
Carcinogenicity	**Data gap addressed using read across to tafluprost**
Reproductive toxicity	**Data gap addressed using read across to tafluprost**
Developmental toxicity	**Data gap addressed using read across to tafluprost**
Other endpoints (intraocular pressure)	Data provided

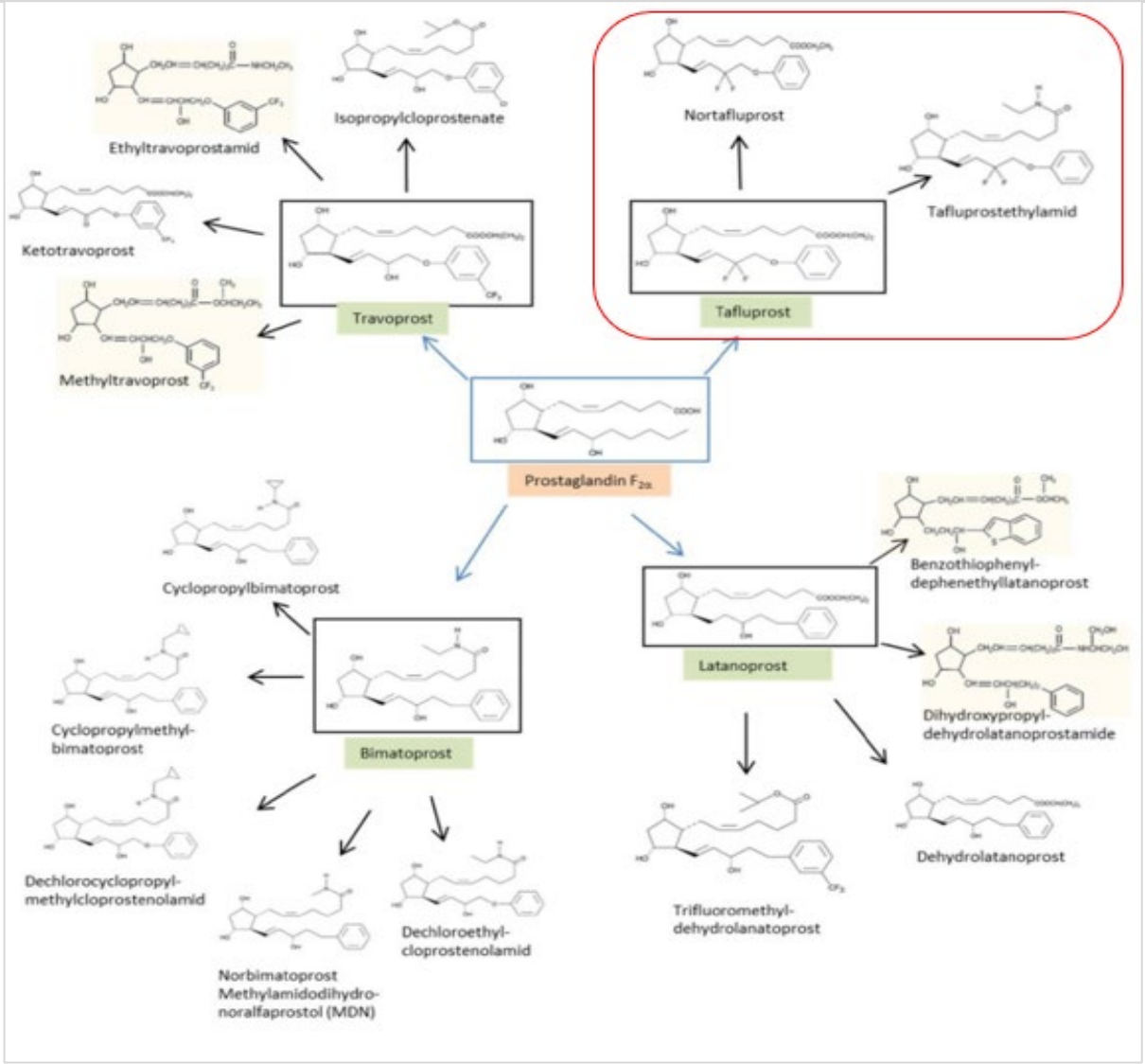
CIR IDA#2 request and testing roadmap

- Due to the concern with read across, CIR issued IDA#2 for DDDE and requested data on:
  - Acute toxicity
  - Repeated dose
  - Developmental and reproductive toxicity (DART)
  - *In vivo* genotoxicity
  - Targets and mechanism data (receptor interaction studies and downstream profiles of adverse event)
- Testing roadmap

Analyses	Rationale	How it addresses CIR concern
PGF2α binding assay	**Receptor interaction/mechanistic data:** To assess receptor binding potency using isolated FP receptors derived from human recombinant (HEK-293) cells	To support the read across (RA) by comparing the **mechanistic data at the molecular initiating event (MIE) level**
ToxProfiler®	**Mechanistic data:** To profile toxicological stress responses using 7 genetically engineered HepG2 cell lines containing fluorescent reporter genes	To support the RA by identifying similarities in **biological stress response pathways**
ReproTracker®	**Apical endpoint related data:** To assess key events during early embryonic development using human induced pluripotent stem cells (hiPSCs)	To support the RA by comparing the effects on expression profile of **developmental biomarkers**
***In vitro* 3T3 NRU cytotoxicity**	**Apical endpoint related data:** To evaluate the acute toxicity potential in Balb/c 3T3 fibroblasts using the 3T3 NRU test	To support the RA by providing **quantitative data for acute systemic toxicity potential**
***In silico* profiling (e.g. DART/ED receptor mapping)**	**Receptor interaction/mechanistic data:** To predict interactions with DART and endocrine-relevant receptors (ERα, ERβ, AR, GR, TRα, TRβ)	To support the RA by establishing mechanistic comparability across **DART-relevant molecular targets**

Updated read across strategy

Analogue selection



- Using **additional** recommended **tools** (GenRA, ToxEraser, AMBIT, ToxRead) in the **SCCS NoG and ECHA's Read Across Assessment Framework (RAAF)**, **tafluprost** still represents the **closest analogue** from a structural point of view
- **Bimatoprost** has also been considered to address any **additional toxicological concerns** related to the prostamide receptor binding
- The revised read across strategy is in line with the approach used by **Federal Institute for Risk Assessment of Germany (BfR)** for the safety assessment of DDDE

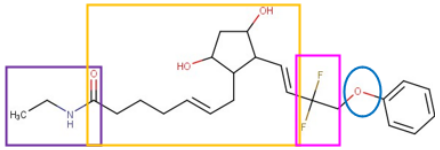
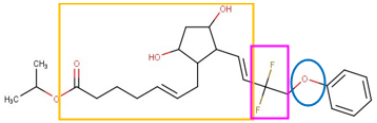
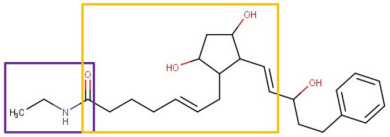
Reference: SCCS OPINION On Prostaglandins and prostaglandin analogues used in cosmetic products **SCCS/1635/21**

Read across criteria

- The suitability of the two analogues has been evaluated by addressing the following criteria in line with the principles of the OECD guidance and the read across assessment framework from ECHA (RAAF):
  - Common functional groups and structure
  - Common structural alerts or reactivity
  - Similarity in physico-chemical properties
  - Likelihood of common breakdown products via biological processes
  - Common biological activity
  - Comparable toxicological profile for endpoints having 'anchor/bridging' data

OECD guidance, 2017: https://www.oecd.org/en/publications/guidance-on-grouping-of-chemicals-second-edition_9789264274679-en.html
ECHA RAAF, 2017: https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a

Analogue suitability evaluation for read across

Similarity criteria	Comparison elements	DDDE	Tafluprost	Bimatoprost
Common functional groups and structure	2D structure			
	Dice index	-	0.86	0.79
	Common functional groups	Prostaglandin core Amide Geminal fluorine Ether moiety	✓ Prostaglandin core ✗ Amide ✓ Geminal fluorine ✓ Ether moiety	✓ Prostaglandin core ✓ Amide ✗ Geminal fluorine ✗ Ether moiety
Common structural alerts or reactivity	Structural alerts as per OECD QSAR Toolbox v.4.7	Cramer Class III No alerts for skin and eye irritation No alerts for skin sensitization No alerts for genotoxicity No alerts for carcinogenicity Strong binder, OH group	✓ Cramer Class III ✓ No alerts for skin and eye irritation ✓ No alerts for skin sensitization ✓ No alerts for genotoxicity ✓ No alerts for carcinogenicity ✓ Strong binder, OH group	✓ Cramer Class III ✓ No alerts for skin and eye irritation ✓ No alerts for skin sensitization ! One alert for genotoxicity (Michael addition) ✓ No alerts for carcinogenicity ✓ Strong binder, OH group

✓: Present/Similar ✗: Absent !: Dissimilar Δ: Partially similar

Conclusion: Tafluprost is relatively more similar to DDDE than bimatoprost with respect to structural similarity, functional groups and structural alerts

Analogue suitability evaluation for read across

Similarity criteria	Comparison elements	DDDE	Tafluprost	Bimatoprost
Physico-chemical (PC) properties	Key PC properties*: Log Kow Water solubility (WS), Vapour pressure (VP)	High log Kow (5) Poor WS (0.091 mg/L) Low VP (1.2E-013 Pa)	✓ High log Kow (6.5) ✓ Poor WS (0.0039 mg/L) ✓ Low VP (5.06E-011 Pa)	! Moderate log Kow (3.97) ✓ Low WS (0.9948 mg/L) ✓ Low VP (7.03E-16 Pa)
	Oral absorption	Low oral bioavailability potential (≥1% and <50%)	✓ Low oral bioavailability potential (≥1% and <50%)	! Moderate oral bioavailability potential (≥50% and <90%)
	Dermal penetration	Low dermal absorption potential (8.67% after 24 h; *in vitro* data)	✓ Low dermal absorption potential (≤10%)	✓ Low dermal absorption potential (≤10%)
Common breakdown products	Common metabolic pathways/key metabolites	Amide hydrolysis leading to the formation of **tafluprost acid** and **ethylamine** (*in vitro* data)	Δ Ester hydrolysis leading to the formation of **tafluprost acid** and **isopropanol** (*in vivo* data)	Δ Amide hydrolysis leading to the formation of **bimatoprost acid** and **ethylamine** (*in vivo* data)

✓: Similar !: Dissimilar Δ: Partially similar

* Predicted values

- **Conclusion for PC properties: Bimatoprost represents the worst case in terms of bioavailability**
- **Conclusion for metabolism: DDDE undergoes hydrolysis to form tafluprost acid (common metabolite with tafluprost) and ethylamine (common metabolite with bimatoprost)**

across – additional data generated to support read across

Similarity criteria	Comparison elements	DDDE	Tafluprost	Bimatoprost
Common biological activity	*In silico*: Receptor binding for DART/ED endpoints using recognised tools	No DART alerts EATS: Overall positive for Erα, AR, TRα and GR binding	✓ No DART alerts ✓ EATS: Overall positive for Erα, AR, TRα and GR binding	! 'Equivocal' teratogenicity alert ! E: Overall negative for ERα binding ✓ ATS: Overall positive for AR, TRα and GR binding
	In vitro receptor binding (PGF2α) assay	Binds to PGF2α Ki: 35 nM	✓ Binds to PGF2α Ki: 89 nM	✓ Binds to PGF2α Ki: 110 nM
	In vitro biological stress response (ToxProfiler®)	Oxidative & ER stress (detoxified by S9)	! Ion stress at cytotoxic concentrations (detoxified by S9)	✓ Oxidative & ER stress (mild detoxification by S9)
	In vitro developmental biomarker profile (ReproTracker®)	Altered expression of **neural** biomarkers but **no effects on morphology** Altered expression of cardiac biomarkers with **no functional effects** **No alteration** of hepatotocyte biomarker expression and morphology	! **No alteration** of neural biomarkers expression or morphology ! Altered expression of cardiac biomarker **only at highest dose** ✓ **No alteration** of hepatotocyte biomarker expression and on morphology	Δ Altered expression of **neural** biomarkers with **effects on morphology** Δ No effects on the cardiac biomarker expression and on **cardiac functionality** Δ No alteration of hepatocyte biomarker expression but with **effects on hepatocyte morphology**

✓: Similar !: Dissimilar Δ: Partially similar; EATS: Estrogen, androgen, thyroid and steroidogeneses modalities

Conclusions:

- Based on the *in silico* analysis, DDDE presents an **overall similar profile** to tafluprost and bimatoprost with respect to **receptor binding potential**
- DDDE, tafluprost and bimatoprost showed a **similar binding affinity to the PGF2α receptor** (*2- to 3-fold differences observed in IC_{50} and K_i values are within the accepted experimental variability of ±0.5 log units*)
- Based on **ToxProfiler** results, the stress response profile of DDDE and bimatoprost are more similar than those of DDDE and tafluprost
- Based on **ReproTracker** results, **Bimatoprost** represents a **worst-case** as compared to DDDE and tafluprost, when considering the effects on **developmental biomarkers/morphology** in the ***in vitro* conditions**

Analogue suitability evaluation for read across - with focus on systemic endpoints

Similarity criteria	Comparison elements	DDDE	Tafluprost	Bimatoprost
Comparable toxicological profile	Acute toxicity (*in vitro* 3T3 NRU) (quantitative bridging data)	LD50 ~880 mg/kg bw – ✓ moderate acute toxicity potential (**Acute Tox. 4 as per EU CLP/GHS**)	LD50 ~367 mg/kg bw – ✓ moderate acute toxicity potential (**Acute Tox. 4 as per EU CLP/GHS**)	LD50 ~1204 mg/kg bw – ✓ moderate acute toxicity potential (**Acute Tox. 4 as per EU CLP/GHS**)
	Genotoxicity – *in vitro* (qualitative bridging data)	✓ Non-genotoxic (Ames, *in vitro* MN test)	✓ Non-genotoxic (Ames, *in vitro* MN test)	✓ Non-genotoxic (Ames, *in vitro* gene mutation test)
	Genotoxicity – *in vivo*	-	Non-genotoxic (*in vivo* MN test)	Non-genotoxic (*in vivo* MN test)
	Repeated dose toxicity	-	NOAEL: 1–100 µg/kg bw/day (i.v.); severe effects at lower doses (subacute/subchronic studies in rats and dogs)	NOAEL: 300–1000 µg/kg bw/day (i.v.) (subacute/subchronic studies in rats and monkeys)

✓: Similar !: Dissimilar Δ: Partially similar

Conclusions:

- *In vitro* 3T3 NRU study indicates that all the three substances have a **similar moderate acute toxicity potential (qualitatively similar),** and **tafluprost** showed a relatively **lower oral LD50 value (representing a worst case)**
- *In vitro* genotoxicity studies showed that the three substances have **no genotoxicity potential - qualitatively similar**
- Common studies available on the two analogues via intravenous routes demonstrate that **tafluprost represents a worst case** in terms of severity of effects and the NOAEL value **(300x lower)**

Analogue suitability summary

Similarity criteria	Comparison elements	Tafluprost	Bimatoprost
Common functional groups and structure	Good structural similarity	✓	✓
	Common functional groups	Δ (except amide)	Δ (except geminal fluorine)
Common structural alerts or reactivity	Structural alerts	✓	✓! (except for one geno alert)
Similar PC properties	Comparable MW, logKow, water solubility (WS), VP	✓	Δ (worst case)
	Comparable oral absorption	✓	Δ (worst case)
	Comparable dermal penetration	✓	✓
Common breakdown products	Common metabolic pathways/key metabolites	Δ (common metabolite tafluprost acid)	Δ (common metabolite ethylamine)
Common biological activity	*In silico* receptor binding predictions for DART/ED endpoints	✓	✓! (except for ER binding)
	PGF2α receptor binding affinity	✓	✓
	ToxProfiler®	!	✓
	ReproTracker®	!	Δ (worst case)
Comparable toxicological profile	Acute Toxicity (*in vitro* NRU)	✓ (worst case)	✓
	Genotoxicity – *in vitro*	✓	✓

✓: Similar !: Dissimilar ✓!: Similar with minor deviations Δ: Partially similar

Conclusion: Tafluprost is structurally more similar to DDDE than bimatoprost, while bimatoprost is biologically more similar to DDDE than tafluprost

Conclusions on the IDA#2 and the overall safety

Endpoint / Data Type	Data Received / Available?	Notes
Acute toxicity data	✓	*In vitro* 3T3 NRU assay
Repeated dose toxicity data	✓*	*Based on proposed read across to tafluprost and bimatoprost
Developmental and reproductive toxicity (DART)	✓*	*Based on proposed read across to tafluprost and bimatoprost
***In vivo* genotoxicity data**	✓*	*Based on proposed read across to tafluprost and bimatoprost
Targets and mechanistic data	✓	Mechanistic in vitro assays conducted
Receptor binding / potency / Ki values	✓	PGF2α binding assay data available

The read across approach is supported by the fact that:

- Tafluprost **represents the worst -case in comparable i.v. repeated dose toxicity studies**, with NOAEL values being lower up to 300 times than those of bimatoprost
- The PoD based on developmental toxicity observed in **parenteral studies** with tafluprost, is considered **overly conservative given the use scenario in cosmetics**
- Despite this **conservative PoD**, the calculated **MoS exceeds 100**, which is generally considered acceptable for systemic toxicity
- The use of the **EFSA CONTAM Panel's toxicological screening value (TSV) of 0.0042 µg/kg bw/day for PGF2α receptor -acting substances** supports the safety of DDDE at the current use concentration of **0.018%**, with a resulting **MoS >1**

Therefore, taking into consideration all the information, the **CIR IDA#2 endpoints** can be considered to be **addressed using a WoE approach**

Comments on the Draft SCCS opinion

Important context:

- This is a DRAFT opinion that is based on an incomplete data set – the same data set the CIR considered in December 2023
- ToxMinds will submit the additional data presented to the CIR in May 2025 (and summarised in this presentation) to the SCCS within the review process

- Transparent analogue selection
  - The updated dossier explains the inclusion and exclusion criteria for those analogues with a similarity index ≥0.7 in a stand-alone read across justification report
- Inclusion of a weight-of-evidence approach
  - 2024 NAM results: 3T3-NRU (acute cytotoxicity), ToxProfiler and ReproTracker (developmental toxicity)
  - Read across from tafluprost and bimatoprost for RDT, DART and carcinogenicity data
- Conservative PoD
  - Parenteral-route NOAEL exceeds any dermal exposure, giving an intrinsic safety buffer—an approach already accepted by SCCS in its opinion for daidzein (SCCS/1641/22)
  - The oral route studies on bimatoprost showed NOAEL values higher >300x compared to tafluprost
- Confidence in DART threshold can be supported via EFSA TSV limits
  - EFSA TSV Group II limit (0.0042 µg kg^{-1} bw day^{-1}) provides a stringent, exposure-based screen for potent PG analogues, covering SCCS concerns about systemic potency
- Addressing non-genotoxic carcinogenicity concern
  - Tafluprost and bimatoprost read-across data satisfy the endpoint

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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