WC11
Abstract ID 1097
Poster

AN *IN VITRO* PANEL FOR COSMETIC CHEMICAL SYSTEMIC TOXICITY SAFETY TESTING

Cosmetics Europe
the personal care association

*C. Mahony[1], G. Ouedraogo[2], P. Kukic[3], M. Dent[3], I. Willox[4], D. Wei[4], F. Sadler[4], A. Otto-Bruc[4], A. Schepky[5], M. Böttcher[5], J. Ebmeyer[5], D. Armstrong[6], G. Kenna[7], N.J. Hewitt[8], **M. Varcin**[8]*

[1] P&G, Procter & Gamble Technical Centres Ltd, Egham, UK, [2] L'Oreal Life Sciences Research, Aulnay-Sous-Bois cedex, France; [3] Unilever, Safety & Environmental Assurance Centre, Sharnbrook, UK; [4] Eurofins Cerep S.A., at Le Bois L'Evêque, Celle L'Evescault, France; [5] Beiersdorf AG, R & D, Hamburg, Germany; [6] Duncan Armstrong, Armstrong Pharmacology Ltd., Macclesfield, UK; [7] Gerry Kenna Consulting, Macclesfield, UK [8] Cosmetics Europe, Brussels, Belgium

The Cosmetics Europe Systemic Toxicity Task Force has led the Pharmacology Profiling project, which aims to provide a screening approach using *in vitro* binding and enzymatic assays to identify potential bioactivity of cosmetic-relevant chemicals. This approach is based on the knowledge that various targets of pharmacological interest have been linked to human adverse drug reactions (ADRs), and the screening of these has helped the pharma industry in identifying drug candidates, as well as off-target and potential adverse effects. In a feasibility study, a set of 100 cosmetically relevant chemicals will be profiled in the assays to develop a benchmark dataset. The aim of this work is to contribute to a practical, initial resource for Next Generation Risk Assessment (NGRA) to inform a possibly relevant systemic toxicity Mode of Action (MoA) of the cosmetic ingredient. Here, we describe the selection of chemicals and targets.

Target selection

1. Target candidates with possible safety liabilities

2. Literature curation based on *in vivo* and clinical evidence

Selection of a set of pharmacology targets known to be linked to human adverse effects of pharmaceuticals

3. (a) Toxicity pathways and (b) qualification of target

Benchmark chemicals

4. ~250 cosmetic-relevant chemicals

5. Filter according to selection criteria to 100 chemicals

Selection of a representative set of 100 test compounds, for target assay benchmarking

6. Confirm feasibility

Feasibility test

7. Stability & solubility test

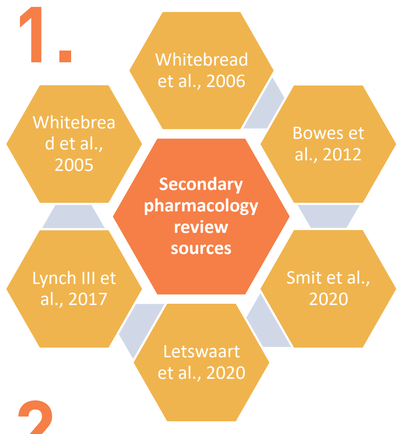
Testing of benchmark chemicals in target assays

8. Two-step testing of chemicals in target assays

Case study chemicals

9. Test LRSS Case Study chemicals plus relevant chemical analogues (n=26)

1.



Collective experience from the cosmetics industry

- **Addition of targets outside the safety pharmacology core battery that are often flagged for cosmetics but not previously mapped in the core battery**

Examples:

- **Nuclear receptors (endocrine disruption, development, metabolism, immune function, reproduction, etc.)**
- ***Tar*gets from the DART and LIVT framework (development, reproduction, hepatotoxicity)**
- **Aromatase CYP19, 5alpha reductase, neurokinin NK2, etc.**

Example of a novel target selected (outside the Bowes 44 list) and all information collated: Estrogen Receptor (ER)

Literature evidence: Estrogen can cause hepatotoxicity. the extent and severity of hepatic damage are dose and time dependent (Pandey et al., 2011). ERa(-/-) mice are resistant to synthetic estrogen, 17alpha-ethynylestradiol (EE2)-induced hepatotoxicity (Yamamoto et al., 2006). DDT and its isomers and metabolites, exhibit hormonal activity by binding and activating the ER. The mechanisms are associated with steroidogenic pathway, receptor mediated changes in protein synthesis or estrogenic actions. Studies in adult mice showed that DDT induced a dose-dependent effect on estrogen receptor activity in liver (Mrema et al., 2013). An epidemiological study also showed an increasing risk of liver cancer among individuals with values in the highest vs the lowest quintile of serum DDT concentration adjusted for DDE level

Compelling data for adversity coming from animal and human studies but N.B. receptor interaction through dietary consumption of isoflavones.

Endpoint linked to target: 17-β-estradiol controls development and maintenance of female sex characteristics and is an endogenous ER ligand. Non-steroidal ER binding compounds e.g. tamoxifen, bisphenol-A, diethylstilbesterol analogues may cause reproductive abnormalities and decrease fertility. These chemicals activate the ER with an efficacy comparable to that of 17-β-estradiol and exert a variety of DART effects in animals.

Expression: ERα expression continued to be an independent predicting factor of liver fibrosis in patients infected with chronic HCV genotype 1b (Cengiz et al. Hepat Mon. 2014 Sep; 14(9): e21885). higher mRNA expression of ERα in HCV-related HCC liver tissues as compared to normals ($P < 0.05$) and ERβ in livers of HCV-related cirrhosis and HCV-related HCC subjects ($P < 0.05$). (Iyer et al., 2017). The embryotoxicity and teratogenicity of estradiol were reviewed by a working group convened by IARC (1979), which concluded that "Oestradiol-17ß has teratogenic actions on the genital tract and possibly on other organs and impairs fertility.

Protein Atlas: RNA Detected in many tissues, enhanced in cervix, uterine, endometrium) https://www.proteinatlas.org/ENSG00000091831-ESR1

AOP exists: https://aopwiki.org/aops/43 N.B. Various other AOPs mention estrogen and ER but are not sub-type specific

2.

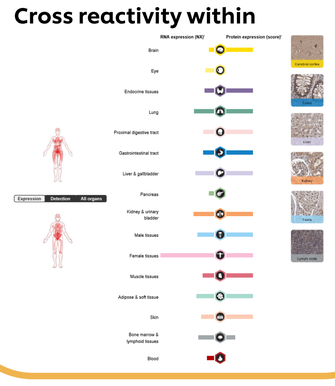
Literature types and curation

- **Toxicology studies - time/dose response**
- ***In vivo* safety pharmacology studies**
- **Human clinical and post marketing adverse drug reactions**
- **Literature review undertaken to determine and summarise the most likely pathological and physiological outcomes of the targets using:**
  - **Articles cited in secondary pharmacology reviews**
  - **PubMed search using key terms: agonist, agonism, activator, activation, antagonist, antagonism, inhibitor, inhibition, toxicity, null, knockout, adverse, safety pharmacology, toxicity**

3a

Toxicity pathways and tissue expression

- **Mechanism-based linkage of targets to established toxicities (AOPs)**
- **Tissue expression (Human Protein Atlas)**
- **Pharmacological promiscuity, evidence from Eurofins**
- **Cross reactivity within**



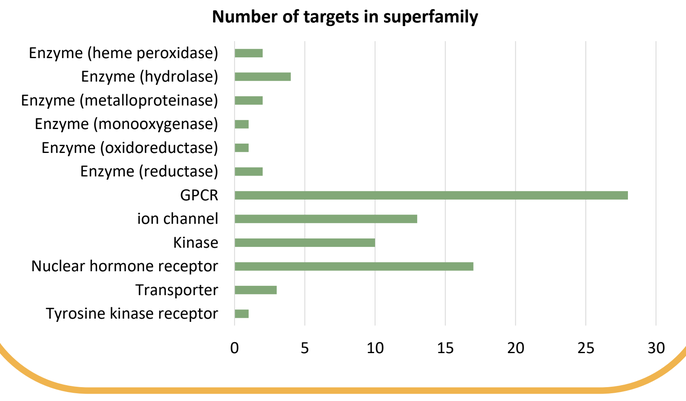
3b

Questions to qualify target

- **How is the tissue expression profile related to frequently reported adverse reactions and pathological effects?**
- **Toxicity pathways - are there any well-established AOPs associated with the target?**
- **Is there high homology and reported cross reactivity within the subfamily (e.g. Carbonic Anhydrase, Dopamine receptors D4-D2, D5-D1, kinases, etc.)?**

Final *In Vitro* Pharmacological Profiling Panel

- **44 targets from Bowes et al. (2012), which contains sufficient evidence to be included in the safety panel**
- **A literature review carried out for 78 additional targets found in at least two separate sources (secondary pharmacology reviews, legacy data from companies)**
- **33 out of the 78 targets were included in the final panel**
- **Additional enzyme targets (kinases) were included to broaden proposed target panel**



4.

Cosmetic-relevant chemicals:

- Preservatives, fragrances, hair dyes, UV filters, masking agents, conditioners, buffers, antioxidants
- Wide range of physicochemical properties and organ toxicities

5.

Benchmark chemical selection criteria

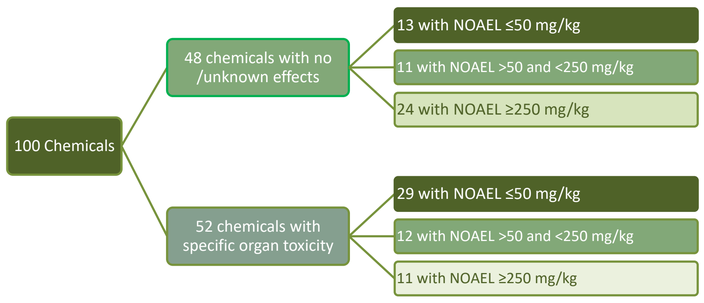
Include:	Exclude:
• Chemicals with NO(A)EL and LO(A)EL values	• Botanicals and extracts
• Study duration >14 days, unless no data available otherwise	• Genotoxic compounds - MoA would be captured by other assays
• Preference for 90-day studies over a cancer bioassay	• Chemicals with biokinetic problems expected from physicochemical properties
• Oral preferred but i.v. and i.p. if not available	• Chemicals producing only local effects (e.g. hydrogen peroxide)
• Rat and mouse data preferred over other species	• Chemicals with no effect at highest dose lower than 250 mg/kg
• Include chemicals with ToxCast data	• Chemicals without data on repeated dose toxicity
• Skin sensitisers e.g. hair dyes included (for systemic, not local effects)	• Avoid chemicals that might be hazardous to researchers in the laboratory e.g. explosive
• Positive controls (drugs) used in toxicogenomics assays	
• CMR chemicals (mentioned in SCCS annexes) considered	

6.

Confirm feasibility and note:

- In absence of NOAEL divide LOEL by three (according to Yang et al., 2017)
- Pricing and availability (e.g. start with Sigma-Aldrich and/or other suppliers)
- NO(A)EL and LO(A)EL values only (not DEL, LD_{50} etc)

Chemical distribution: even spread with respect to specific organ effects vs no or non-specific toxicity and with respect to the NOAELs within each category



7.

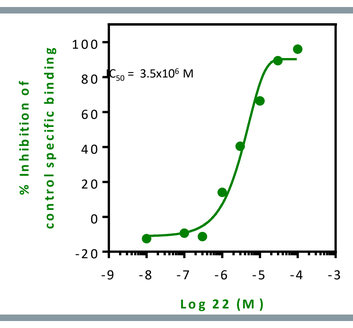
Stability and solubility tests

- Confirm that the test chemicals are stable under the assay conditions
- Confirm that the test chemicals are soluble at 10 µM → If not, consider lower test concentration if feasible.

8.

Testing

- Initial test at 10 mM nominal concentration.
- When activity observed, potency quantified in follow-up dose-response testing



9.

Next Steps

- This panel of 83 target assays will be used to complement other data streams used in NGRA. These include in silico MoA tools to assess Structure Activity Relationships and support read-across, as well High Throughput Transcriptomics (HTTr) to assess biological effects in cells in vitro.
- We anticipate that use of these different approaches will enable identification of systemic toxicity relevant MoA in a comprehensive manner that is consistent with current scientific knowledge

Summary

- In a feasibility study, a set of 100 cosmetically relevant chemicals will be profiled in the target assays to develop a benchmark dataset. All chemicals were selected according to specific criteria, including the availability of legacy repeated dose toxicity data, critical effects, suitable biokinetic properties and availability of in vitro data (e.g., ToxCast).
- Relevant literature was reviewed to produce a tentative list of targets of pharmacological interest that have been linked to human adverse drug reactions (ADRs) that could be suitable for cosmetic chemical safety testing.
- The final list of targets includes receptors, ion channels, transporters and enzymes identified by the pharma industry as linked to human ADRs, as well as additional targets that have been reasoned to link to pathological effects.
- The main targets and considerations could aid safety decision making based on either a lack of response on systemic toxicity relevant targets or, by way of comparisons to substances with a similar MoA.
- We do not yet know all MoAs relevant to systemic toxicity but we do aim to harness specific knowledge where it exists and, using a combination of in silico and in vitro testing approaches, provide a wide coverage of upstream molecular and cellular targets that could possibly associate with eventual toxicity if perturbed at a significant level and/or for an appropriate length of time.

This work was sponsored by:
Cosmetics Europe
the personal care association

References:
Bowes et al., Nat Rev Drug Discov. 2012 Dec;11(12):909-22. doi: 10.1038/nrd3845; Cengiz M, Ozenirler S, Yılmaz G. Hepat Mon. 2014 Sep 24;14(9):e21885. doi: 10.5812/hepatmon.21885; Letswaart et al. EBioMedicine. 2020 Jul;57:102837. doi: 10.1016/j.ebiom.2020.102837; Lynch JJ 3rd et al., J Pharmacol Toxicol Methods. 2017 Sep;87:108-126. doi: 10.1016/j.vascn.2017.02.020; Mrema et al., Hum Exp Toxicol. 2013 Dec;32(12):1323-39. doi: 10.1177/0960327113485255; Lyer JK, Kalra M, Kaul A, Payton ME, Kaul R. Estrogen receptor expression in chronic hepatitis C and hepatocellular carcinoma pathogenesis. World J Gastroenterol. 2017 Oct 7;23(37):6802-6816. doi: 10.3748/wjg.v23.i37.6802. PMID: 29085224;
Pandey G, Pandey SP, Sharma M. Toxicol Int. 2011 Jul;18(2):160-2. doi: 10.4103/0971-6580.84270; Smit et al., Chem Res Toxicol. 2021 Feb 15;34(2):365-384. doi: 10.1021/acs.chemrestox.0c00294; Whitebread et al., Drug Discov Today. 2005 Nov 1;10(21):1421-33; Whitebread et al.. Drug Discov Today. 2016 Aug;21(8):1232-42. doi: 10.1016/j.drudis.2016.04.021; Yamamoto et al., J Biol Chem. 2006 Jun 16;281(24):16625-31. doi: 10.1074/jbc.M602723200.