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in native marker expression such as cytokeratin 3 and 12 to the primary human epidermal keratinocytes derived models. Secondly, by employing a non-destructive measuring system based on impedance spectroscopy, we could increase the sensitivity of the test system. Moreover, the impedance measurement allowed for the first time to detect the persistence of irritative effects by repeated measurements in an *in vitro* model and thus to distinguish between all GHS categories. Substances that do not need to be labeled stayed above 60% normalized to the negative control. Category 1 substances reduced the tissue integrity after application below 6% and the effect did persist over a period of 7 days. Category 2 substances however, could be identified by a decrease below 60% after the application of a category 2 chemical such as ethanol and increased again above 50% after 7 days. Thereby, all GHS categories of eye irritation could be identified by repeated measurements over a period of 7 days. Based on a novel prediction model we achieved an accuracy of 78% with a reproducibility of 88.9% to determine all three categories of eye irritation in one single test. This could pave the way according to the 3R principle to replace the Draize eye test.

References

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P-Late-27
Toxicological approach in the safety assessment of novel foods in the European Union (EU)

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According to the European legislation, novel foods (NF) are foods that were not consumed to a significant degree by humans within the European Union before 15 May 1997. NF are foods consisting of, isolated from or produced from different sources (e.g. microorganisms, fungi, plants or animals and their parts), produced by new processes or technologies or newly synthetized compounds which have not been previously consumed in our diet to a significant degree. Although the history of safe use within a third country may be relevant, the safety of such foodstuff has to be assessed before the marketing authorisation can be granted.

The role of EFSA is to assess the safety of NF and provide scientific advice to the competent EU regulatory bodies. Since the entry into force of the relevant regulation ((EU) No 2015/2283) of January 2018 more than two hundred applications for NF have been received by the European Commission (EC) and depending on the complexity of the dossier and the characteristics of the NF an assessment by EFSA has been requested. The Authority shall complete a safety assessment within nine months. The scientific opinion of EFSA is then considered by the EC during the authorization process of the NF.

The experts of EFSA's Panel on Nutrition, Novel Foods and Food Allergens (NDA) follow a multifaceted approach to carry out the safety assessment of the NF under the proposed uses and use levels. The assessment is based on dossiers provided by applicants. Dossiers need to contain data on the compositional, nutritional, toxicological and allergenic properties of the NF as well as information on respective production processes, and the proposed uses and use levels, as specified in the relevant EFSA guidance (EFSA NDA 2016). The toxicological assessment is based on the whole set of data provided and particularly on ADME and *in vitro* and *in vivo* toxicity studies that shall provide insight on kinetics, genotoxicity, sub-chronic/chronic toxicity, and reproductive and developmental toxicity. A tiered toxicity testing approach is implemented with the aim of limiting the use of animals and resources. The results provided may trigger the need for further specific testing.

A thorough assessment by EFSA and its experts on the full set of data of the NF with particular focus on the available toxicological information helps to ensure a high level of food safety for the consumers within the European Union.

References

EFSA NDA Panel 2016 "Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283" EFSA Journal 2016;14(11):4594 - DOI:10.2903/j.efsa.2016.4594

P-Late-28
Assay-Ready Use of KeratinoSens® Cells in Skin Sensitization

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The reproducibility of cell-based assays strongly depends on the cell quality, which in turn is influenced by multiple factors such as the choice of the culture media and sera, the source and passage number of the cell line, or even slight differences in cell handling by different operators. Thereby, all these parameters need to be optimally standardized. For this, the use of pre-made and pre-qualified assay-ready cells, which can be applied in a cellular assay basically like a reagent without prior cultivation or passaging, can minimize the variability related to cell culture.

To evaluate the skin sensitizing potential of chemicals, reporter skin cell lines are used to measure the activation of the ARE/Nrf2 pathway, which is one of the key events of this complex cascade. Within the context of the keratinocyte activation, the KeratinoSens® cell line has been developed by Givaudan and validated by the ECCVAM. Here we demonstrate that the use of these in an assay-ready format to test the proficiency substances according to the OECD guideline 442D leads to equivalent results as compared to continuously cultured cells.

P-Late-29
A two-year carcinogenicity study of the new opioid receptor antagonist ondelopran in rats

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Purpose: New drug Odelepran (INN: ondelopran) with a unique binding profile to all three types of human opioid receptors (μ, κ, δ) is being developed by R-Pharm. Ondelopran is intended for the treatment of alcohol dependence [1,2]. Since the drug is intended for the long-term treatment, the study of carcinogenic potential of chronic (two years) administration to rats is required to be approved by regulatory authority.

Method: The study was performed in male and female Wistar rats at the age of 8-10 weeks at the start of experiment. There were four groups of male and four groups of female, 50 animals each. Test item (ondelopran film-coated tablets, 125 mg), was administered to the animals intragastrically (vechicle - 1% starch solution) daily, 5 days a week for 24 months in two doses: 10 mg/kg (equivalent therapeutic dose for humans) and 100 mg/kg. The amount of placebo (tablet excipients) administered intragastrically equated to the amount of excipients contained in the tablet mass proportional to the 100 mg/kg ondeloprane dose. The control group was administered with the

vehicle (1% starch solution). Clinical observation and examination were conducted weekly to detect any signs of toxicity; mortality; dynamics of the body weight. At the end of the treatment period all animals in the study had been subjected to a full, detailed gross necropsy with subsequent histopathological study.

Results: During the study the mortality rates did not differ between the groups. Changes in the body weight ranged within the normal values. There were no any signs of toxicity in groups treated with tested items. Neoplastic lesions were found in all groups of animals. More than 30 types of neoplasms were identified upon pathomorphological examination, icluding follicular thyroid cancer (11/164 in males and 10/169 in females) and malignant non-Hodgkin's lung tissue lymphoma (17/164 in males and 20/169 in females) as the most frequent cases. The identified tumors are typical for rats and considered as spontaneous age-related pathology. There was no statistically significant differences between groups in the total incidence of tumors and the incidence of specific types of tumors. To conclude the above said, the test item of the ondelopran film-coated tablets, 125 mg, has no carcinogenic potencial.

References

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P-Late-30
Assessing reactive oxygen species produced by nanomaterials and their consequences for cells: contribution to a testing strategy for grouping approaches

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The large variety of nanomaterials (NMs) entering the EU market poses the issue of performing a robust risk assessment without performing a huge amount of time-consuming and costly animal experiments. The use of alternative approaches to overcome the case-by-case risk assessment would permit not only the reduction of work load, but also allow a targeted, prioritized and more reliable risk assessment. Grouping approaches represent a valid alternative to the case-by-case assessment and several approaches have already been proposed. The existing grouping approaches would benefit greatly from the inclusion of the toxicity mode of actions in the framework.

The observed toxicity of NMs can often be evaluated considering the production of reactive oxygen species at the surface of NMs, which can trigger oxidative stress and thus irreversible modifications of proteins, DNA and lipid oxidation and further lead to apoptosis and inflammation. Assessing the oxidative potential of NMs using functional assays would permit grouping of NMs, provide a deeper insight into the mode of action of the cellular toxicity, and support prioritization of NMs for further testing. This strategy could moreover represent a first step into a safer–by-design approach.

Within the GRACIOUS project we tested and optimized several assays to assess the oxidative potential of NMs in different environments of increasing complexity: Electron Paramagnetic Resonance (EPR), dichlorodihydrofluorescin diacetate ($DCFH_2$-DA) assay, Ferric Reduction Ability of Serum (FRAS) assay and protein carbonylation, focusing on proposed benchmark materials and on different variants of several classes of NMs. The results from each assay were compared.

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P-Late-31
The development of an *inhouse* reconstructed human epidermis (RhE) and performance as a skin irritation model.

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Reconstructed Human Epidermis (RhE) as recommended by OECD TG 439 is one of the best alternatives for *in vitro* skin irritation evaluation, since it mimics skin barrier function and is histologically similar to native human epidermis. However, in some countries it is difficult and expensive to import the commercially available models recommended by the guideline. To overcome this limitation and increase the accessibility of *in vitro* skin irritation testing, we developed a novel *in-house* RhE model and verified its potential use based on the OECD TG 439. For RhE construction, primary keratinocytes (KCs) derived from neonatal donors were seeded upon collagen IV-coated inserts and kept under submerged condition for cell proliferation, followed by an air-liquid interface condition for differentiation and stratification. RhE was characterized regarding morphological and biochemical features by standard H&E staining and immunofluorescence against cytokeratin-10, cytokeratin-14, filaggrin and involucrin. Quality control was verified by quantification of cell viability in the control RhEs (570nm O.D.) and evaluation of barrier function integrity after RhE exposure to four different SDS (sodium dodecyl sulfate) concentrations. To validate our RhE model, the irritation potential of different chemicals listed in OECD TG 439 was evaluated by topical application for 42 min followed by a 42 h post-incubation. Cell viability was subsequently measured by the MTT assay. Our *in-house* RhE model presented a multilayered epidermis with a mature *stratum corneum* and a pattern of differentiation markers similar to that of the native human epidermis. The developed model presented a mean O.D. value of 1,57 and barrier function parameters in accordance to OECD TG 439. Moreover, the *in-house* RhE-based skin irritant test was able to discriminate between skin irritating and non-irritating substances. Taken together, our data pointed out the promise use of the *in-house* RhE on OECD TG 439 in countries in which validated RhEs are not available for purchase due to customs barriers.

References

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P-Late-32
Immune response of the sea urchin *Paracentrotus lividus* to contaminated marine sediments

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