CEC04-02
The multiple facets of skin inflammation: from direct toxic insult to specific immune responses

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Skin inflammation is a sign of an immune response (innate of adaptive) in the body. Symptoms can include redness, heat, itching, sensitivity, and swelling. The cause or trigger of skin inflammation may be acute, such as a skin infection, chemical insult, or chronic, such as an autoimmune condition like psoriasis. Allergic mechanisms are often a cause of skin inflammation including Allergic Contact Dermatitis (ACD) and Atopic Dermatitis (AD). Skin inflammation can be the consequences of multiple agents from pathogens to chemicals.

Contact dermatitis (CD) is one of the most common inflammatory dermatological conditions and is caused by the exposure to exogenous substances that elicit an immune response resulting in inflammation in the skin. Categories of CD include ACD, photoallergic CD (PACD), irritant CDD (ICD) and photoirritant CD (PICD, or phototoxic CD). ICD is caused by direct cellular toxicity leading to the inflammation and activation of the innate immune system, whereas ACD results from adaptive immunity involving innate immunity and T lymphocytes. Individuals who develop ACD acquire a specific immunity against the chemical to which they have been exposed.

ACD and ICD are both inflammation of the skin due to a contact with chemicals in the environment, but ICD is a non-specific skin response and is consequence of direct damage of the skin due to the irritating nature of the substance. Another difference between ICD and ACD is that, in ACD, symptoms do not appear after the first exposure, but rather usually within a few days after a re-exposure to the allergen.

CEC04-03
Innate immune inflammatory signaling in glial cells modulates chemical neurotoxicity

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Risk for developing Alzheimer's and Parkinson's disease is associated with aging, genetics and exposure to environmental agents such as infectious pathogens and neurotoxic chemicals. Neuroinflammation represents a common mechanism of toxicity for each of these factors, resulting in inflammatory activation of microglia and astrocytes, the principal non-neuronal cells of the brain. Transition of these cell types from a trophic or supportive phenotype to a damaging inflammatory phenotype during neurotoxic stress promotes both aggregation of proteins such as amyloid beta and alpha-synuclein, as well as injury to neurons. Repeated exposure to infectious pathogens and other neurotoxic agents is therefore implicated in the onset and progression of prodromal and clinical neurodegenerative disease. One of the central molecular pathways regulating innate immune inflammatory signaling in glial cells is the transcription factor, nuclear factor kappa B (NFκB), which is activated by multiple cellular stress signals through the kinase complex, IKK2. Stimulation of NFκB in microglia and astrocytes activates coordinate expression of numerous inflammatory cytokines and chemokines, as well as reactive oxygen and nitrogen species and inflammatory lipid mediators. Neurotoxic chemical agents including heavy metals, pesticides and drugs of abuse can stimulate inflammatory activation of glial cells resulting in neuronal injury. Selective genetic and pharmacologic inhibition of NFκB in microglia and astrocytes can mitigate neuronal injury from these agents, suggesting that innate immune inflammatory signaling in glial cells is critical to the progression of pathology resulting from exposure to these agents. Data from animal models of neurodegeneration are extremely informative in determining the role of inflammatory signaling in glial cells as a mediator of neuropathology relevant to chronic neurodegeneration.

CEC04-04
Evaluating cytokines in immunotoxicity testing

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Cytokines are secreted proteins released by cells with specific effect on the interactions and communications between cells. Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action). Different cell types can secrete the same cytokine and a single cytokine can act on several different cell types (pleiotropy). In addition, cytokines may be redundant in their activity, and similar functions can be stimulated by different cytokines. They are usually produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines. Finally, cytokines can act synergistically or antagonistically. They mediate all immunological and inflammatory reactions.

In the evaluation of the adverse effects on the immune system, cytokines provide important evidence to support or refute the biological plausibility of chemical-induced immunotoxicity. Therefore, their measurement represents an important tool that can be applied both to *in vivo* as well as to *in vitro* models. There are a multitude of examples on the use of cytokines as parameters in the assessment of immunotoxicity, both for immunosuppression and inappropriate immunostimulation, both in animals and human studies as well as in non-animal models. Examples will be provided in the presentation, as method available to assess cytokine production.

CEC05 | Nanotoxicology

CEC05-01
Genotoxicity of nanomaterials

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A main safety concern related to nanomaterials is their possible genotoxicity. Genotoxicity describes the capacity of a chemical to produce genetic damage that, if it is not repaired, can lead to mutations and, eventually, to cancer. Therefore, every mutagen is considered to be potentially carcinogenic. Furthermore, mutagenicity is also involved in reproductive and developmental abnormalities.

Due to the important consequences to human health, mutagenicity is a hazard endpoint required in all product regulations (REACH, biocides, pharmaceuticals, medical devices, food additives, cosmetics, etc.). On the other hand, genotoxicity assessment at an early stage of innovation is highly advised as part of the safe-by-design strategies during product development. The mutagenicity of chemicals is usually evaluated on the basis of a battery of standard *in vitro* assays, which can be followed up by validated *in vivo* assays. However, it is presently not clear to which extent genotoxicity assays can be applied to test nanomaterials, and how well such data could be utilized in predicting the carcinogenicity of these materials. We will provide an overview on the limitations of genotoxicity assays and how much of

the available genotoxicity data on nanomaterials are relevant from the regulatory perspective. It is also unclear how different physicochemical properties of nanomaterials can modulate their genotoxic potential. The effect of size, surface chemistry and biopersistence will be discussed.

Nanomaterials can be genotoxic through a primary mechanism, executed by the substance itself, or a secondary mechanism involving an inflammatory response. The primary mechanism can involve a direct interaction with the DNA, or an indirect effect mediated by other molecules, such as the induction of reactive oxygen species. Traditional *in vitro* genotoxicity tests are considered to detect primary genotoxicity, whereas *in vivo* assays are required to reveal secondary genotoxicity. Current genotoxicity assessment is based on identifying a substance as genotoxic or non-genotoxic, assuming that genotoxicity does not have a threshold value. Hence, no dose can be considered as 'safe'. However, recent findings in genetic toxicology are moving this paradigm forward to a more semi-quantitative approach, where a threshold mechanism of action (MoA) is assumed when genotoxicity is mediated by secondary mechanisms. Several *in vitro* co-culture systems are presently being developed and may provide a way of differentiating between primary and secondary MoAs. Finally, new approaches on genetic toxicology, aiming at reducing animal experiments while providing more mechanistical based information on the molecular changes involved in the induction of genotoxic effects, will be discussed.

CEC05-02
Nanomaterial-induced inflammation, acute phase response and risk of cardiovascular disesase

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Inhalation studies of insoluble nanomaterials show that they induce pulmonary inflammation that is predicted by total deposited surface area. Transcriptomics studies of mice show the induction of pulmonary acute phase response in parallel with the inflammatory response. Acute phase response is a well-established risk factor for cardiovascular disease. Pulmonary exposure to soluble and insoluble nanomaterials induce dose-dependent acute phase response.

The lecture will give an overview of the scientific evidence linking nanomaterial exposure to risk of cardiovascular disease, the biological mechanism of action of the acute phase response and provide examples of occupational biomonitoring studies showing correlation between particle exposure levels and systemic acute phase response. The pathway has been submitted as adverse outcome pathway AOP237 in Aopwiki.

CEC05-03
Toxicity of nanomaterials in the user phase

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Nanomaterials are increasingly used in different product groups due to improvement of characteristics of the products. The consumer is potentially exposed to nanomaterials in their final, intended use, i.e. when the nanomaterials are part of a matrix. Consumers may be exposed by inhalation, dermal exposure and oral intake. Occupational exposure may also occur in the end-of-life phase.

Based on different cases, this lecture will focus on how the toxicity of nanomaterials is affected when nanomaterials are part of a consumer matrix. Cases will include the toxicity of inhalation of dust obtained by sanding of paint and epoxy containing nanomaterials and dermal exposure due to use of nanomaterial containing sunscreen and other cosmetics. Because free nanomaterials may be liberated during the use phase, the hazard of pristine nanomaterials will also be discussed.

CEC05-04
In vitro-based high-throughput screening and toxicogenomics to support effective safety evaluation of engineered nanomaterials

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High-throughput screening (HTS) technology allows for rapid first-level safety evaluations of large numbers of engineered nanomaterials. A wide variety of cell culture-based, data-rich HTS approaches, including those that build on "omics", are in development to keep pace with the steadily increasing innovations that involve nanomaterials, and which have potential to efficiently serve for toxicity-based ranking, grouping, read across and prioritizations for deepened studies. Generation of large toxic mode of action (MoA)-informative data sets are coupled to the need for harmonized interpretation, integration, and data management approaches in order to modernize current risk assessment practices. This educational lecture will introduce recent state-of-the-art HTS testing methodologies which permit collective interpretation and visualization of diverse types of relevant data for nanomaterials, including from applying the US-EPA developed Toxicological Priority Index tool (ToxPi). Examples include combining basic toxicity indicators into scores as well as new strategies for assessment of direct and indirect (secondary) genotoxic mechanisms. Further concepts to be discussed include applying targeted or genome-wide transcriptomics data-driven predictions of toxic MoA coupled to Adverse Outcome Pathway-driven hypothesis-generation for guiding further testing needs. Finally, to be presented are current strategies towards making existing toxicity and safety-testing data "findable, accessible, interoperable and reusable" according to the FAIR principles.

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