Proposed Amendments to OECD Subacute and Subchronic Inhalation Test Guidelines

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Proposed Changes to TG 412 (28 day inhalation) and TG 413 (90 day inhalation)

- Change the upper limit for MMAD of the aerosol for rodent inhalation studies
- Addition of bronchoalveolar lavage (BAL)
- Recommend measurement of lung burden and particle clearance

I. Mass Median Aerodynamic Diameter

- Current: MMAD in the range of 1 – 4 um
- Proposed: MMAD limit of 2 um with a GD of < 3 um
- Reason: To achieve an alveolar deposition representative to human exposure.

Respirability in Humans vs. Rodents

- MPPD models indicate that a significant fraction of inhaled particles with a MMAD between 1 – 4 um deposit in the alveolar region of the human lung (RIVM, 2002).
- In rodents, MPPD models indicate very low alveolar deposition of particles with a MMAD > 2 um (Rabbe et al., 1988).

II. BAL Endpoints

- Current: Pulmonary response to particle inhalation by histopathology of lung tissue
- Proposed: Addition of measurement of indicators of cytotoxicity, inflammatory, and alveolar air/blood barrier damage using BAL samples

Bronchoalveolar Lavage (BAL)
I. Background

1. **BAL has been used in pulmonary toxicology studies for more than 40 years.**
2. **BAL is relatively simple and low cost.**
3. **BAL has been used to monitor dose and time dependence of lung damage and inflammation, resulting from exposure to particles, chemicals and gases.**
4. **BAL levels of mediators identify mechanisms for initiation and progression of lung pathology.**

Reasons for Requiring BAL

1. **BAL parameters are reproducible, objective and quantifiable measures amenable to statistical analyses and comparisons among studies using different particles and from various labs.**
2. **In contrast, histopathological results are not continuously quantified, i.e., employing a 1-5 scale, and are subjective.**
3. **Significant changes in BAL parameters often occur before pathological changes are discernable.**

BAL Methods

1. There is general commonality in methods used in various labs.
2. BAL methods to monitor lung injury and inflammation are relatively simple and do not require complex instrumentation.
3. Recommended BAL endpoints :
   a. LDH activity for cytotoxicity
   b. Albumin or protein for air/blood barrier integrity
   c. Cell counts and differentials for inflammation
4. Optional BAL endpoints: Inflammatory, proliferative and fibrogenic mediators.

Human Relevance of BAL

1. Segmental BAL in human subjects by bronchoscopy is common in the literature.
2. Sizable database exists relating BAL cells and mediators with lung pathogenesis in humans
3. BAL data have been used to elucidate mechanisms for disease initiation and progression.

Animal Welfare

1. Can do BAL of the left lung and use the right lung for histopathology.
2. Thus, no additional animals would be required.
3. BAL would not alter anesthesia or euthanasia protocols currently used for histopathology.

III. Measurement of Lung Burden and Particle Clearance

- Current: Lung dose from particle inhalation is calculated using MPPD models.
- Proposed: Measurement of initial particle lung burden and the rate of particle clearance (recommended).

Lung Burden
I. Background

1. Determination of a NOAEL, LOAEL, and MTD is critical to hazard assessment and ranking the bioactivity of various nanomaterials in relationship to low and high toxicity benchmark particles for which human hazard is known.
2. Lung burden data are needed to determine clearance rates following inhalation exposure and improve translational dosimetry.

Reason for Recommending Measurement of Lung Burden

1. The theoretical calculation of lung burden:

lung burden = (aerosol conc.) (min, ventilation) (duration) (deposition fraction)

2. Uncertainties with this estimate:
   a. Deposition varies with inflammation
   b. Minute ventilation in rodents is highly variable
   c. Deposition fraction for a given mass median aerodynamic diameter is modeled not measured.
3. The above calculation is not based on actual minute ventilation or clearance rates.

Lung Burden Methods

1. Published methods are available to quantify nanoparticles in lung tissue.
2. Metals and metal oxides can be quantified by ICP-MS.
3. Carbonaceous nanoparticles have been quantified by several published methods, such as, analysis of elemental carbon.
4. A few unexposed lungs should be spiked with nanoparticles to validate specificity and recovery

Human Relevance

1. Measured rat lung burden can be compared to a projected human lung burden by normalizing data to mass burden/alveolar epithelial surface area (Stone et al., 1992; Miller et al., 2011).

Animal Welfare

1. A separate set of animals (5 rats/group)should be dedicated to lung measurement to avoid:
   a. Loss of sensitivity due to sampling only a small portion of the lung
   b. Uneven regional particle deposition due to particle-induced inflammation.
2. Accurate lung burden measurement using validated methods would decrease animal use in the long run by avoiding repeat measurements due to low sensitivity and reducing the need for confirmatory studies where estimated lung burdens are in question.
3. Validated lung burden measurements would support comparison of studies with different nanoparticles from different labs.