Phospholipid Lung Surfactant Dispersion of NP for Retention of Size and Structural Properties in Bioassay: Lessons from Diesel Particulate Material in Vitro Genotoxicity

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There is growing evidence suggesting that particle number concentration or size itself, or some associated structural properties, may affect nano-particle (NP) toxicity in comparison with larger respirable particles of the same composition (Donaldson et al., Phil. Trans. R. Soc. Lond. A 358: 2741-2749, 2000; Oberdorster et al., Phil. Trans. Roy. Soc. London Series A 358: 2719-2740, 2000). Thus, NP material ideally should not be altered in size, morphology, surface properties, and aggregation state as administered for cellular or animal model bioassay. This has been addressed for the case of in vitro genotoxicity assay of diesel exhaust particulate material (DPM), a nano-particulate material. It was recognized that the conventional procedure of testing an organic solvent extract of DPM did not necessarily provide a physiologically reasonable model of genotoxicant biological availability as manifest by intact particles deposited in the lung. Therefore, NIOSH research has examined, as a medium for in vitro cellular challenge, the mixture of whole collected DPM into a model of the surfactant that coats the deep lung, using a primary surfactant component of the respiratory bronchiolar and alveolar surface coating, diacyl phosphatidylcholines dispersed into physiological saline (Wallace et al., Journal of Toxicology and Environmental Health, 21 163-171, 1987; Keane et al., Mutation Research 260 233-238, 1991; Gu et al., Mutation Research.279: 55-60, 1992; Annals of Occupational Hygiene 38: 345-349, 1994; J Toxicology & Environmental Health, Part A, 68: 431-444, 2005). The purpose is to avoid artifactual modification of collected NP structure, while permitting conditioning that is representative of surfactant dispersion effects for a particle deposited on the pulmonary alveolar hypophase surfactant interface of the lung. Filter collected DPM has been dispersed into surfactant, usually dipalmitoyl phosphatidylcholine (DPPC) in saline, and assayed in vitro for clastogenic, DNA damage, and mutagenic activities. The research has found that DPPC dispersion does not extract genotoxicants from the particles; rather, the phospholipid coats and "solubilizes" (not "dissolves") the DPM, providing a hydrophilic coating and permitting the dispersion of the surfactant-coated DPM in aqueous media of the culture medium. Comparisons made between surfactant-dispersed and solvent-extracted DPM show a comparable but slightly diminished activity for surfactant-dispersed samples on a mass of sample basis. Assays included salmonella mutagenicity (the Ames test) and mammalian cell activities for micronucleus induction, chromosomal aberrations, and DNA single and double strand breaks measured by single-cell gel electrophoresis. Surfactant solubilization also has been examined for bioassay of mineral particles, albeit respirable but not nano-sized, resulting in suppression of otherwise prompt cytotoxicities followed by cellular digestive removal of surfactant and restoration of toxicities over time. Particle surface area-normalized requirements on surfactant solubilization are extrapolated from the data for NP experimental design.

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