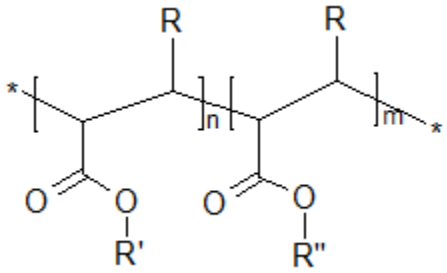
Category: **Polymer Lung Overload** Human Health

Definition. This category includes a variety of poorly soluble polymers, and specifically insoluble/non-water absorbing ("non-swellable") high molecular weight materials typically formed through a free-radical polymerization process. Substances included are branched and linear polymers, as well as co-polymers produced by random, block, graft, or other techniques. Crosslinked polymers are included in the category, but crosslinking is not necessary for inclusion.

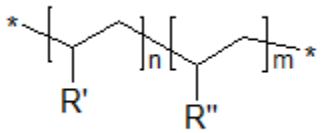
Representative polymers in this category include the following:

Polyacrylates and methacrylates



Where R is H or methyl; R' and R'' are typically alkyl or substituted alkyl, although there are currently no limits on the substituents. R' may be the same as R'' or different. This example represents a polymer containing one or two monomers, although sub-category members may comprise any number of monomers.

Polyvinyls



Where R is H or C1-C > 20. R' is typically methyl, CN, acetyl, Ph or Cl, although there are no current limits on R'. R' may be the same as R'' or different. This example represents a polymer containing one or two monomers, although sub-category members may comprise any number of monomers.

Polymers including both acrylate/methacrylate and vinyl monomers are also members of this category.

Hazard Concerns. The category concerns discussed here are limited to effects on the lung as a result of inhaling particles. Broadly, as shown in rat inhalation studies (*e.g.*, for polymers, Muhle et al., 1990a, 1990b, 1991; Bellmann et al., 1991, 1992), these effects range from inflammation to fibrosis to, potentially, cancer (demonstrated for some poorly soluble inorganic particulates, but not specifically for poorly soluble polymers). Because it is not known with certainty whether high lung burdens of poorly soluble polymers can lead to lung cancer in humans via mechanisms similar to those for poorly soluble inorganic particulates in the rat, in the absence of mechanistic data to the contrary, it must be assumed that the rat model can identify potential carcinogenic hazards to humans. Since the apparent responsiveness of the rat model at overload is dependent on coexistent chronic active inflammation and cell proliferation, at lower lung doses in which chronic active inflammation and cell proliferation are not present, no lung cancer hazard is anticipated based on that mode of action (ILSI, 2000).

Supporting Data. A series of sub-chronic and chronic studies were performed to test the effects of inhalation of respirable particles of a water-insoluble styrene/l-butylmethacrylate polymer (the primary component of toner used in copy machines) of MW 70,000 in rats. In a subchronic 13-week study in which exposure concentrations of 0, 1, 4, 16 and 64 mg/m^3 were tested, dose-related increased lung weight and histological lesions (thickening of alveolar structure due to hypertrophy and hyperplasia of Type II cells) were seen at 16 and 64 mg/m^3, exposure concentrations that also resulted in dose-related decreased lung clearance and increased lung particulate burden (Muhle et al., 1990a). The NOAEL from this study was 4 mg/m^3. A second rat study with sub-chronic exposure, but an extended 15-month monitoring period, found that impaired clearance of particulates and pulmonary effects (evidenced by increases in protein and enzyme markers of tissue damage in bronchiolar lavage fluid [BALF]) from sub-chronic exposure was not reversible at 40 mg/m^3 and only partially reversible at 10 mg/m^3 (Bellmann et al., 1992). Chronic 24-month exposure to toner resulted in dose-related impaired particle clearance, elevated lung particle burden, and lung effects (fibrosis, BALF markers of tissue damage, and increased lung weight) at 4 and 16 mg/m^3, with a NOAEL of 1 mg/m^3 (Muhle et al., 1991; Bellmann et al., 1991). There was no evidence of carcinogenicity in this study. More limited studies of polyvinyl chloride (PVC) powder and polystyrene spheres have also demonstrated the impairment of lung clearance mechanisms by insoluble polymers (Muhle et al., 1990b; Oberdorster et al., 1992 [as cited in Borm et al., 2015]).

Boundaries. There is a potential for respirability if there are any particles $\leq$10 μm in diameter in the material being handled. For insoluble polymers, there is a data gap on the potential for lung overload for those materials with MWs at or above 1,000 Daltons. EPA is interested in test data that address lung burden measurements and lung clearance kinetics of insoluble polymers as it relates to particle size, mass, volume, surface area, shape, or other factors. Thus, this category may be modified as new information becomes available to the Agency.

General Testing Strategy[1]

Consistent with the amended Toxic Substances Control Act (TSCA), the multi-tiered testing methods below employ new approach methodologies (NAMs) to reduce the use of vertebrate animals in chemical testing. It incorporates *in chemico* characterization of the chemical substance in Tier I (particle size distribution, biosolubility measurements) and structured *in vivo* testing from acute testing, sub-acute testing and sub-chronic testing in Tier II. It is recommended that any questions on the test strategy should be directed to the Agency.

Tier 1 – Use physical-chemical properties to demonstrate no lung exposure:

- Particle Size Distribution or Aerosolized Droplet Size [*i.e.*, cascade impactor, laser methods; OECD TG 110, OPPTS 830.7520, OECD Guidance Document (GD) 39]

- Bio-solubility Testing (*i.e.*, solubility in Gamble's solution and/or phagolysosomal simulant fluid)

If respirable and poorly soluble particles can be generated during manufacturing, processing, or any of the uses, proceed to Tier II. If not, then determine if Tier II Testing is needed

Tier II- *In Vivo* Studies

- Step 1: OECD Acute Test Guideline (TG) 403 (modified) ** featuring rats exposed for 4 hours and observed for 2 weeks. Proceed to step 2 if test chemical is retained in the lung.

- Step 2: 5-Day inhalation study with a 14-day recovery period ** to evaluate lung burden and to inform pulmonary deposition and retention of particles in the lung (use OECD TG 412, but conduct exposure duration for at least 5 days). Proceed to step 3 if multiple post-exposure sacrifices demonstrate lung burden over time.

- Step 3: OECD TG 412**: 28-day inhalation study in rats with 14-day recovery period to evaluate lung burden, clearance, and translocation. It is recommended to include multiple post-exposure sacrifices that demonstrate the relationship between lung burden and lung clearance kinetics over time.

Tier III – Proposed *In Vivo* Studies

- 90-day inhalation toxicity test** (Harmonized Test Guideline OECD TG 413) in rats with a recovery period of 60 days be included to assess the progression or regression of any lesions.

[1] Submitters may request to use comparable test guidelines other than those listed, pending EPA's review and approval.

Tier III – Proposed *In Vivo* Studies

- If the results of the subchronic 90-day study indicate particles have carcinogenic potential (*e.g*. increased inflammation), a 2-year inhalation bioassay** in rats may be warranted (exposure concentration high enough to impair pulmonary clearance of particles and lead to an "overload" condition).

** Modifications to all above studies should include special attention to pulmonary function tests; blood oxygen (pO_2) ; lung burden measurements and lung clearance kinetics;[2] collection of bronchoalveolar lavage fluid (BALF) for assessment of marker enzyme activities, total protein content, and cell counts; lung retention and clearance; lung weight; and lung histopathology (inflammation and cell proliferation). It is not necessary to look at internal organs. OECD 413 and OECD GD 39 should be consulted.

All studies should include an exposure concentration high enough to impair pulmonary clearance of particles and lead to an "overload" condition. It has been shown that in rats impaired clearance starts when phagocytized particle volume exceeds 6% of normal alveolar macrophage volume and clearance stops altogether when phagocytized volume reaches 60% of normal macrophage volume (*e.g.*, see Borm et al., 2015).

In vitro tests, such as those performed in NR8383 cells derived from rat alveolar macrophages, may be useful in preliminary screening of chemicals to be tested and setting exposure concentrations for *in vivo* testing, but do not, by themselves, constitute adequate tests for lung overload potential of these chemicals.

Furthermore, the following *in vitro* test methods for cytotoxicity and irritation can provide potentially useful information:

- ICCVAM Recommended Protocol for the BALB/c 3T3/A549 lung cells Neutral Red Uptake (NRU) Cytotoxicity Test - A Test for Basal Cytotoxicity (https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-appxc1-508.pdf)
- ICCVAM Recommended Protocol for the Normal Human Epidermal Keratinocyte (NHK) Neutral Red Uptake (NRU) Cytotoxicity Test - A Test for Basal Cytotoxicity (https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-appxc2-508.pdf)
- OECD *In vitro* Skin Irritation (Test 439) – reconstructed human epidermis test method (Note: Test 404 for skin irritation and corrosion is *in vivo*).

Supporting Data. The available studies provide some guidance for testing of poorly soluble polymers for pulmonary effects by inhalation exposure (Muhle et al., 1990a, 1990b, 1991; Bellmann et al., 1991, 1992). Duration of exposure in these studies was 90 days or longer. Observation periods were 60 days or more. Endpoints examined included clinical signs, mortality, hematology, clinical chemistry, pulmonary function tests, assessments of bronchiolar lavage fluid for cell counts and enzyme levels, lung retention and clearance, weights of lungs and

[2] See OECD (2017) *OECD TG 413: 90-days (subchronic) inhalation toxicity study*

other organs, and histopathology of the respiratory tract other organs, tissues and gross lesions. No effects were seen outside of the respiratory tract in these studies.

The mechanism for pulmonary effects and carcinogenicity of poorly soluble polymers involves impairment of alveolar macrophage-mediated clearance of particulates from the lungs. Wiemann et al. (2016) have developed an *in vitro* assay using NR8383 cells derived from rat alveolar macrophages. Responses of the NR8383 cells to particulates in the culture medium were used to differentiate biologically active particulates with specific toxicity (*e.g.*, quartz DQ12, CeO2, ZnO) from passive particulates that produce only non-specific cellular overload effects (*e.g.*, $BaSO_4$, graphite). The *in vitro* results accurately predicted the *in vivo* effects of the 20 nanoparticles tested. Although only nanoparticles were tested by these authors, the assay could be evaluated for use with poorly soluble polymers that can be dispersed in the culture medium. This assay may be useful in screening out poorly soluble polymers with specific toxicities prior to *in vivo* testing and setting exposure concentrations for *in vivo* testing (*e.g.*, inclusion of an "overload" condition for passive polymers).

References.

Bellmann B, Muhle H, Creutzenberg O, et al. 1991. Lung clearance and retention of toner, utilizing a tracer technique during a long-term inhalation study in rats. Fundam Appl Toxicol 17: 300-313.

Bellmann, B, Muhle H, Creutzenberg O, and Mermelstein R. 1992. Irreversible pulmonary changes induced in rat lung by dust overload. Environ Health Perspectives 97: 189-191.

Borm P, Cassee F and Oberdorster G. 2015. Lung particle overload: old school – new insights? Particle and Fibre Toxicol 12: 10-14.

ILSI. 2000. International Life Sciences Institute. Workshop: The relevance of the rat lung response to particle overload for human risk assessment. Inhal Toxicol 12: 1-17.

Muhle H, Bellman B, Creutzenberg O, et al. 1990a. Subchronic inhalation study of toner in rats. Inhal Toxicol 2: 341-360.

Muhle H, Bellman B, Creutzenberg O, et al. 1990b. Dust overloading of lungs after exposure of rats to particles of low solubility: comparative studies. J Aerosol Science 21: 374-377.

Muhle H, Bellmann B, Creutzenberg O, et al. 1991. Pulmonary response to toner upon chronic inhalation exposure in rats. Fundam Appl Toxicol 17: 280-299.

Oberdorster G, Ferlin J, and Morrow PE. 1992. Volumetric loading of alveolar macrophages (AM): a possible basis for diminished AM-mediated particle clearance. Exp Lung Res 18: 87-104. [as cited in Borm et al., 2015]

Wiemann M, Vennemann A, Sauer U, et al. 2016. An *in vitro* alveolar macrophage assay for predicting the short-term inhalation toxicity of nanomaterials. Journal of Nanobiotechnology 14: 16.

APPENDIX 1: Search Strategy

Computerized literature searches were conducted in PubMed in November 2016 to obtain studies related to lung overload from inhalation of insoluble polymers. The search query string is presented in Table 1.

Table 1. Summary of detailed search strategies for lung overload from insoluble polymers

Database Search Date	**Query String**
PubMed	
11/15/2016	(Aerosols[mh] OR particulate matter[mh] OR dust[mh] OR "Lung"[mh] OR "Lung diseases/chemically induced"[mh]) AND overload[tw]) NOT (iron[mh]OR calcium[mh] OR heart[mh] OR cardiac[tw])

Screening methods for this search include manual screening of titles/abstracts and screening of full text articles using the eligibility criteria shown in Table 2.

Table 2. Eligibility criteria for screening of literature search results for lung overload from insoluble polymers

PECO element	Evidence
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung) and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (e.g., case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

The results of the literature screening for lung overload from insoluble polymers are presented graphically in Figure 1. The database search results were supplemented by a review of the reference lists from relevant publications (*i.e.,* tree searching[3], which identified 7 selected references). The studies that were excluded after full-text review (n=29) were primarily reviews and studies of lung overload by materials other than insoluble polymers.

[3] This is also referred as backward reference searching.

Figure 1. Literature search and screening flow diagram