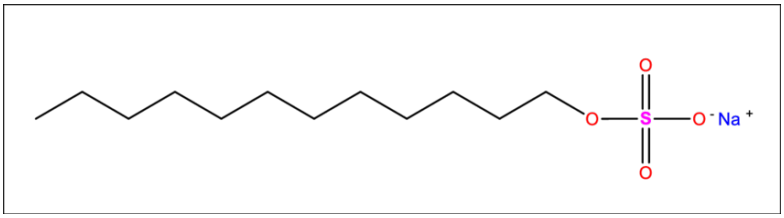
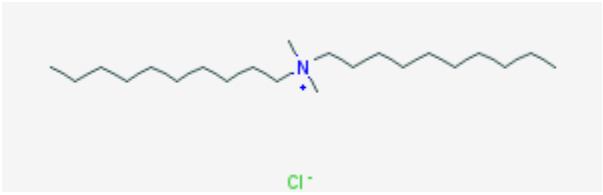
Category: **General Surfactants** Human Health

Definition. This category includes anionic, cationic, and non-ionic surfactants, which lower the surface tension of water.

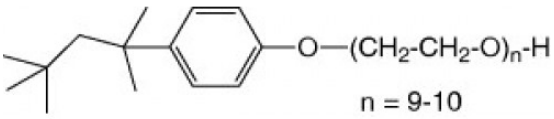
Anionic Surfactants - Any molecular structure with a net negative charge and having surfactant activity is a member of this category. The category includes for example, alkyl sulfonates, alkyl benzene sulfonates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups, *e.g.*, alkyl sulfonate with carboxylic acid substitutions. The structure of the common anionic surfactant sodium dodecyl sulfate (SDS, sodium laurel sulfate) is shown below. The surface tension of SDS is 39.5 milliNewtons/meter (mN/m) at 25° C.



Cationic Surfactants - Any cationic surfactant is a member of this category, for example: didecyldimethyl ammonium chloride (DDAC). The surface tension of DDAC is 27.0 mN/m at 1.0 gram/liter of water.



Non-ionic Surfactants - Any neutral structure having surfactant activity (*e.g.*, Triton X-100, a polyether shown below) is considered a member of this category. The surface tension of Triton X-100 is 30-31 mN/m at a concentration of 1.0 gram/liter in water. The surface tension of water is 72 mN/m.



Hazard Concerns. There is concern for dysfunction of natural surfactant in the lung from inhalation of chemicals with surfactant properties. The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in human volunteers and in laboratory animals. The pulmonary response to surfactant aerosol is in proportion to the exposure concentration and duration, but available data are inadequate to identify effect levels, which in any case are likely to vary not only with the specific chemical surfactant, but also with the exposure method (*e.g.*, aerosol droplet size).

Supporting Data. Healthy human volunteers showed significantly decreased pulmonary compliance following acute inhalation of a nonionic siliconized superinone respiratory detergent (Defomaire) beyond that produced by the distilled water control (Obenour et al., 1963). The authors suggested that the observed decrease in pulmonary compliance was due to an increase in surface tension in the alveoli in the presence of detergent. Decreased pulmonary compliance has also been used to indicate loss of natural alveolar surfactant function in animal studies (*e.g.*, Nieman and Bredenberg, 1985). Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid was observed in dogs and sheep following *in vivo* aerosol exposure to the anionic detergent dioctyl sodium sulfosuccinate (DOSS) in 1:1 mixture of ethanol and saline for 30 – 60 minutes (estimated dose of 15 mg detergent/kg body weight) (Nieman and Bredenberg, 1985; Wang et al., 1993). Increased minimum surface tension due to detergent was also demonstrated, and shown to be dose-dependent, using pulmonary surfactant extracted from dogs and mixed in vitro with the nonionic surfactant tyloxapol (Alevaire) (Modell et al., 1969). *In vivo* exposure of dogs in this study (8h aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed increased minimum surface tension), which the authors took to support the dose-dependence of the effect and to indicate that small amounts of detergent can be present in the lungs without detectably altering surfactant function (Modell et al., 1969).

Other pulmonary effects in dogs and/or sheep exposed to DOSS or tyloxapol included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). In the study by Modell et al. (1969), no gross pathology differences were seen in detergent-exposed vs. control lungs of dogs, although both control and exposed lungs were heavy and discolored reddish-purple, suggesting fluid accumulation. Nieman and Bredenberg (1985) performed light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol and found no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. Transitory inflammatory and necrotic changes in alveoli and terminal bronchioles were seen in hamsters shortly after *in vivo* lavage with the nonionic surfactant Triton X-100 (0.01 – 0.1% by wt.), but the relevance of these findings to inhalation exposure is unclear due to the greater intensity of exposure to these areas by lavage (Damon et al., 1982). In hamsters exposed to Triton X-100 by inhalation at 3000 mg/m^3 for up to 37 min (MMAD of 1.47 – 1.51 micrometers) as measured by an Andersen cascade impactor, there were no gross lesions detected in the lower trachea, bronchi or lungs. There were, however, exposure duration-related increases in intensity of clinical signs (*e.g.*, dyspnea) and mortality in the hamsters (death was by obstructive asphyxiation secondary to severe laryngeal and epiglottic edema leading to a marked reduction of the laryngeal lumen) (Damon et al., 1982). There is a single report, which comes from the Japanese literature, of two cases of severe effects occurring in people exposed to detergent aerosols. According to the English language abstract of the report, symptoms of dyspnea, bloody cough, and hypoxemia were reported in two persons apparently sickened by use of spray detergent (not further described) for household cleaning (Ohta et al., 2001). Chest radiographs showed diffuse infiltrative shadows in the lungs, and both patients were diagnosed by bronchiolar lavage with alveolar hemorrhage.

The alveolar-capillary barrier consists of the surfactant layer, the alveolar epithelium, the basement membrane and the capillary endothelium. Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. Inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, reflecting increased alveolar permeability after detergent exposure (Nieman et al., 1990; Nilsson and Wollmer, 1992, 1993; Evander et al., 1994; Tasker et al., 1996; Nilsson et al., 1997). In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occur with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in one study in which multiple dilutions of the liquid detergent were nebulized (Evander et al., 1994). Some studies also evaluated the clearance of radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA (Nilsson and Wollmer, 1992; John et al., 1997). Wang et al. (1993) observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which the authors attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies has been hypothesized to result from increased alveolar surface tension, which could cause increased permeability either by opening previously closed pores (through which solutes pass) in the membrane or stretching already open pores (Nieman et al., 1990; Wang et al., 1993).

Surfactant effect on cell membranes has been studied in vitro. Warisnoicharoen et al. (2003) evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$), and N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$) to cultured human bronchial epithelium cells (16-HBE14o-) in vitro, using the MTT cell viability assay. All of the surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that surfactant toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

In vitro tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals.

Boundaries. There are no known boundaries for surfactants with regard to acute pulmonary effects by inhalation. To better define the boundaries within this category, EPA may seek testing on individual members of this category. 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, surface tension 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 I – Use physical-chemical properties to characterize lung exposure/disruption

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

- Surface Tension Measurement (tensiometer using the ring, stirrup or plate method or the capillary surface tension method with appropriate positive controls; ASTM D1331, ASTM D7490, ASTM D3825, OECD TG115). The test concentrations should be 0.1%, 0.5% and 1.0%. Test concentrations representative of those at the unit operation should be tested. In addition, surface tension measurements should be done for the 90% saturated solution concentration for chemical substances with low solubility, if appropriate.

If respirable particles or aerosols can be generated during manufacturing, processing, or any of the uses and surface tension decreases are observed, proceed to Tier II. If not, then determine if Tier II testing is needed

Tier II- *In Vivo* Studies

- Step 1: OECD Acute TG 403 (modified)** featuring rats exposed for 4 hours and observed for 2 weeks. Proceed to step 2 if LOAEC < 2000 mg/m^3.

- Step 2: 5-Day study with a 14-day recovery period ** to address progression of effects (use OECD TG 412, but conduct exposure duration for at least 5 days). Proceed to step 3 if study reports substantial decrease in the point of departure or increase in severity at the same concentration over time relative to the acute study.

- Step 3: OECD TG 412**: 28-day inhalation study in rats with a 14-day recovery period

** Modifications to all above studies should include pulmonary function testing, analysis of BALF, LDH release and blood oxygen (pO_2)content, and satellite reversibility. OECD TG 412 and OECD GD 39 should be consulted.

[1] Submitters may request to use comparable test guidelines (*e.g.*, *in vitro* assays) other than those listed, pending EPA's review and approval.

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 surfactants for pulmonary effects by inhalation exposure. In all cases, duration of exposure was acute, primarily between 5 and 60 min (Damon et al., 1982; Nieman and Bredenberg, 1985; Nieman et al., 1990; Nilsson and Wollmer, 1993, 1993; Wang et al., 1993; Evander et al., 1994; Nilsson et al., 1997), although one study included an 8 hr exposure period (Modell et al., 1969). Observation periods were 24 hr or less in studies focused exclusively on pulmonary endpoints (most performed in ventilated, anesthetized animals) (Modell et al., 1969; Nieman and Bredenberg, 1985; Nilsson and Wollmer, 1993, 1993; Wang et al., 1993; Evander et al., 1994; Tasker et al., 1996; John et al., 1997; Nilsson et al., 1997; Liu et al., 2001), but extended to 7 days for one study of intact animals that included monitoring for mortality (Damon et al., 1982). Endpoints examined in these studies included clinical signs, mortality and pathology in intact animals and pulmonary and cardiovascular physiology, blood gas data, surface tension of BALF and lung extracts, lung weight and pathology, and tracer studies of pulmonary clearance in animals handled with surgical intervention.

Studies have been performed with the goal of developing *ex vivo* or *in vitro* models for effects of surface-active chemicals on pulmonary surfactant in order to reduce the number of animals subjected to product testing, with varying degrees of success (Fischer et al., 2012; Sorli et al., 2015). Although the specific materials tested in these studies were limited to waterproofing sprays and fluorocarbons, the approaches described are broadly applicable to chemical surfactants in general. Sorli et al. (2015) tested 9 commercially available waterproofing sprays (at multiple dilutions) for effects on function of bovine surfactant, as evaluated in a capillary surfactometer, and compared the results to *in vivo* plethysmograph findings. They found that the *in vitro* results were highly predictive of *in vivo* effects. All 4 materials that were found to be toxic *in vivo* also affected surfactant function *in vitro*, and at low concentrations. Among the 5 materials that were not active *in vivo*, 2 were identified as inactive *in vitro* and 2 others were active only at high concentrations not likely to be achieved in the alveoli *in vivo*. The authors offer that this study presents a proof-of-principle for using pulmonary surfactant inhibition *in vitro* as a predictor for effects of inhaled waterproofing products *in vivo*, while also noting limitations of the method used (*e.g.*, limitation to water-based or water-soluble products). They suggest that *in vitro* screening might be useful as a preliminary step in advance of *in vivo*

screening during product formulation, with products that fail the *in vitro* screen going back for reformulation and only products that pass the *in vitro* screen going on to *in vivo* testing.

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APPENDIX 1: Search Strategy

Computerized literature searches were conducted in PubMed in November 2016 to obtain studies related to the pulmonary effects of general surfactants. The search query string is presented in Table 1.

Table 1. Summary of detailed search strategies for general surfactants

Database Search Date	**Query String**
PubMed	
11/15/2016	("surface-active agents"[mh] AND lung[mh]) AND ((detergents[mh] OR aerosols[mh] OR "pulmonary surfactants"[mh]) OR (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]))

Screening methods for this search included 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 general surfactants

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 general surfactants 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)[2] and addition of relevant studies from the literature search for waterproofing chemicals (2 references). The studies that were excluded after full-text review (n=29) were primarily animal studies by routes other than inhalation and inconclusive epidemiological studies, neither of which were considered to provide useful information.

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

Figure 1. Literature search and screening flow diagram