#2583 | Surfactants Category: An Integrated Approach to Testing and Assessment (IATA) Including New Approach Methods (NAMs) for Assessing Inhalation Risks under the Toxic Substances Control Act (TSCA)

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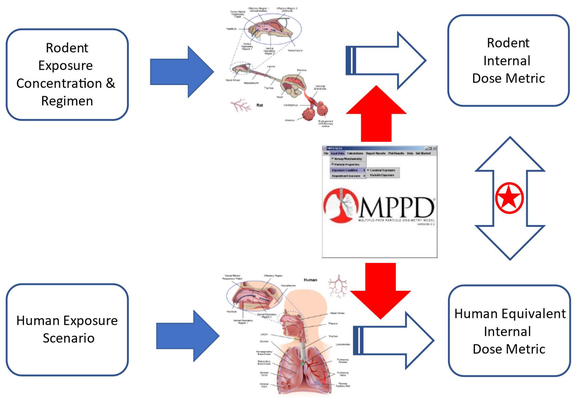
Motivation

- Section 5 of TSCA including pre-manufacturing notification (PMN) does not require testing for new chemical substances (NCS); only extant health or environmental effects data need to be submitted.
- EPA uses various methods to assess risks of NCS with limited data, including chemical categories and "read across" based on analogs. Chemical categories have specific chemical definitions, categorical boundaries, representative analogs, and testing recommendations to aid submitters in understanding potential hazard concerns and to facilitate EPA's review and evaluation of NCS.
- Surfactants may pose a potential inhalation hazard to humans, depending on their conditions of use, chemistry, or size characteristics, because they can disrupt the epithelial lining or perturb cell membranes.
- EPA has authority to require testing on NCS, but also must consider TSCA's mandate to reduce or replace the use of vertebrate animal testing. We present an IATA that supports consideration of physicochemical (PC) properties of the category and facilitates use of NAMs to screen for potential key events and adverse outcomes associated with inhaled surfactants.

Methods

- Inhalable surfactants were defined as those NCS that have the PC properties and meet the functional criteria for inclusion/exclusion shown in Figure 1. Subcategories considered are amphoteric, nonionic, cationic, and anionic surfactants.
- Systematic review methods were applied using population/exposure/comparator/outcome (PECO) statements that considered PC properties and key events (KEs) of potential adverse outcome pathways (AOPs) to identify relevant toxicity data and NAMs.
- The multi-path particle dosimetry (MPPD) model was used to translate observed effect levels from rodent studies to human equivalent concentrations (HECs) as depicted in Figure 2.
  - Due to potential direct interaction with epithelial lining and cells of the entire respiratory tract, the dose metric chosen was the daily deposited mass in each respiratory region normalized to its surface area.
  - The MPPD model may also be deployed to simulate target human exposure scenarios.
- Potential NAMs to inform screening were identified based on KEs and AOP that characterize KE of epithelial lining disruption or cell membrane perturbation.
- Figure 3 depicts the IATA as the resultant strategy for evidence integration and evaluation of inhaled surfactants.

Figure 2. Schematic of MPPD model deployed to extrapolate a rodent exposure regimen to an HEC. Default exposure assumptions or parameters specific to the target human exposure such as: particle size distribution, ventilation rate or pattern, and duration can be used to tailor simulations



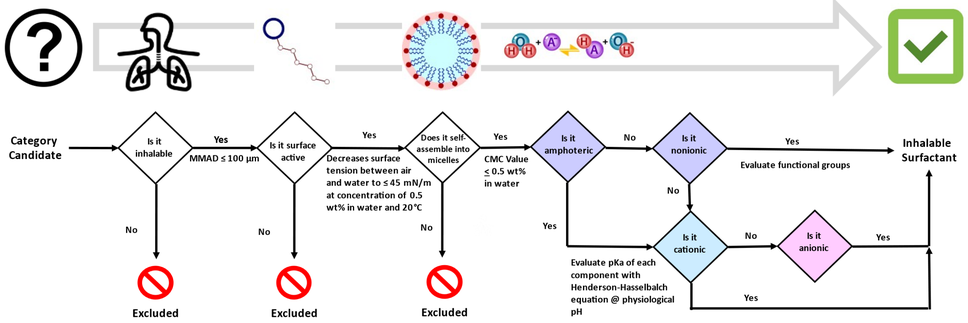


Figure 1. Decision strategy and criteria to consider critical PC properties of candidates for inhalable surfactants category

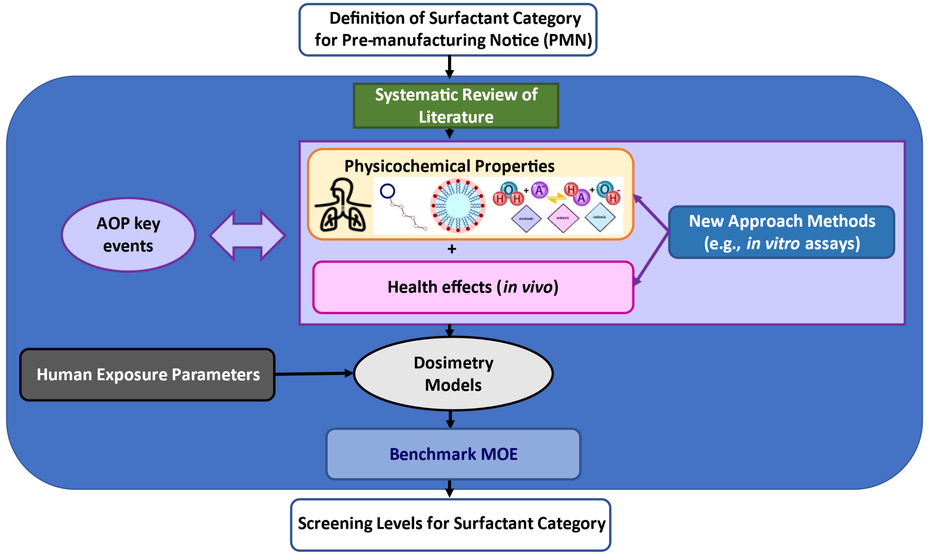


Figure 3. IATA for evidence integration and evaluation of available data on inhalable surfactants

Results

- Table 1 lists identified NAMs relevant to inhalable surfactants.
- Table 2 provides the HEC values to use in assessing risks of surfactants that meet the category definition.

Table 1. NAMs to Inform PC Properties and Evaluate Health Effects of Inhalable Surfactants

PC Properties		
Assessment Questions	Inclusion Criteria	Assessment Methods
Is it inhalable under the conditions of use?	MMAD ≤ 100 µm	The intended conditions of use will determine the need to generate data. The use form (e.g., powder) will determine the appropriate analytical techniques (e.g., sieving, optical microscopy, or laser diffraction).
Is it surface active?	Surface tension ≤ 45 mN/m at concentration of 0.5 wt% in water and 20 °C	e.g., ASTM D1331 (Methods A and C) "Standard test methods for surface and interfacial tension of solutions of paints, solvents, solutions of surface-active agents, and related materials" or OH 63914 "Determining the surface tension of surfactants"
Does it self-assemble into micelles?	CMC value ≤ 0.5 wt% in water	e.g., ISO 4311 "Anionic and non-ionic surface active agents – Determination of the critical micellization concentration – Method by measuring surface tension with a plate, stirrup or ring".
Health Effects		
Level of Biological Organization	Key Events	In Vitro Assays / Test System
Molecular Initiating Events (MIEs)	Interaction with pulmonary surfactant	In Vitro Respiratory Toxicity Assays: • In vitro lung surfactant interaction, e.g., Sodi et al. (2018) and Guzman and Santini (2018)
	Interaction with cell membrane and cell membrane components and interaction	Hemoglobin Denaturation Assay, Liposome Assay, and In Vitro/Ex Vivo Irritation Assays: • Hemoglobin denaturation assay, e.g., Hayashi et al. (1994) • Liposome assay, e.g., Dapper et al. (2020) • In vitro/ex vivo eye irritation tests for penetrance, e.g., Reconstructed human Cornea-like Epithelium (OECD TG 492), Bovine Corneal Opacity and Permeability Test (OECD TG 437), and Isolated Chicken Eye Test (OECD TG 438)
Cellular Level Events (CLEs)	Loss of membrane integrity/general cytotoxicity	In Vitro/Ex Vivo Cytotoxicity Assays: • In vitro/ex vivo eye irritation tests for cytotoxicity, e.g., Reconstructed human Cornea-like Epithelium (OECD TG 492), Bovine Corneal Opacity and Permeability Test (OECD TG 437), and Isolated Chicken Eye Test (OECD TG 438) • Cell membrane integrity test (LDH-cytotoxicity assay), cell viability assays (e.g., MTT, resazurin, and ATP), TEER, or lysosomal membrane integrity test • BALB/c3T3A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (ICCVAM, 2006)
Tissue or Organ Level Events (OLEs)	Tissue level events	Human organotypic Airway Cultures: • 3D constructs of human-derived cell cultures of differentiated airway epithelial cells (e.g., EpiAirway™, MucilAir™, SmallAir™, EpiAlveolar™, etc.) using the cell membrane integrity and viability assays described under cellular level events.
	Tissue level events	Specific Ex Vivo Respiratory Toxicity Assays: • Precision-cut lung slice test, e.g., Hess et al. (2016) and Neuhaus et al. (2017, 2018)

Table 2. Calculated HEC Values of Daily Regional Deposited Mass / Regional SA to Use in Evaluating Surfactants

Surfactant Type	Chemical Substance	Inhalation Exposure Regimen	Species Strain Sex	Body Weight (g)	Study POD Analytical [Nominal] (mg/m^3)	Density (g/cm^3) at 20 °C[1]	MMAD (µm) GSD	HEC (mg/m^3)
Nonionic	Octylphenoxypoly-ethoxyethanol (CASRN 9002-93-1)	14-day, 6 hr/d, 5 d/wk; whole body (Butterfield et al., 2003)	Rats Sprague Dawley M/F	M: 273.80 +/- 6.6 F: 177.2 +/- 8.2	LOAEC 5.3 ± 0.7 [275 ± 62]	1.002 water vehicle 5% dose solution	1.80 1.80	HEC_{ET} = 0.06924 HEC_{TB} = 2.32087 HEC_{PU} = 0.52777 HEC_{TH} = 1.72397 HEC_{TOT} = 0.7690
Anionic	Oleoyl sarcosine (CASRN 110-25-8)	28-day, 6 hr/d, 5 d/wk; nose-only (ECHA submission, 2010)	Rats F344 M/F	M: 215.14 +/- 8.76 F: 142.5 +/- 7.91	LOAEC < 6	0.806 ethanol vehicle 10% dose solution	1.11 2.12	HEC_{ET} = 0.97670 HEC_{TB} = 1.08077 HEC_{PU} = 0.26859 HEC_{TH} = 0.90333 HEC_{TOT} = 0.76081
Cationic	Didecyldimethyl ammonium chloride (DDAC) (CASRN 7173-51-5)	4-week, 6 hr/d, 5 d/wk; nose-only (EPA submission, 2016)	Rats Sprague Dawley M/F	M: 302.20 +/- 19.98 F: 202.20 +/- 17.6	LOAEC (lung effects) 0.091 [0.08]	0.997 water	1.5 1.83	HEC_{ET} = 0.09679 HEC_{TB} = 2.49104 HEC_{PU} = 0.47637 HEC_{TH} = 1.83732 HEC_{TOT} = 0.98992
	Benzalkonium chloride (BAC) (CASRN 8001-54-5)	14-day, 6 hr/d, 7 d/wk; whole body (Choi et al., 2020)	Rats F344 M/F	M: 173.78 +/- 15.96 F: 134.22 +/- 17.6	LOAEC (nasal effects) 0.84 ± 0.09	0.997 water vehicle 2% dose solution	1.614 2.00	HEC_{ET} = 0.10248 HEC_{TB} = 2.59640 HEC_{PU} = 0.58588 HEC_{TH} = 1.97504 HEC_{TOT} = 1.01034

Impact

- IATA represents a strategy to evidence integration and evaluation to aid assessment of surfactants with minimal available test data.
- Consideration of PC properties and NAMs aimed at KEs of AOPs creates context for evaluation of the need and strategy for higher-tiered testing based on mechanistic responses, dosimetry, and exposure information.
- Emphasis on development of mechanistic data will advance understanding of the potential inhalation toxicity of surfactants to drive the development of newer and safer chemistries.

References

- Available in the Notes pane.

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