Poorly Soluble, Low Toxicity (PSLT) Polymer Category: A Case Study for An Integrated Approach to Testing and Assessment (IATA) under the Toxic Substances Control Act (TSCA)

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Inhalation Webinar Series

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Topics Covered

- TSCA Reform
- High-molecular weight (HMW) Polymers
  - Default approaches
  - TSCA New Chemical Categories
- TSCA Section 4 (h) – Reduction of Testing on Vertebrates
- Case Study on Biosolubility: α-1,3-glucan
- Updates to the TSCA New Chemical Categories

TSCA Reform

- On June 22, 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Lautenberg Chemical Safety Act) was signed into law. The Lautenberg Chemical Safety Act amended the Toxic Substances Control Act (TSCA), the nation's primary chemicals management law.

President Obama signs TSCA reform into law. Photo courtesy of the White House via YouTube.

- Prior to June 2016, certain new chemical substances were "dropped" during EPA's 90-day review period
- After June 2016, EPA applied default approaches to hazard and exposure for these same types of substances that led to unreasonable risks

HMW Polymers

- High-molecular weight (HMW) polymers (*i.e.*, NAMW ≥ 10,000 Daltons) are those substances that meet the E2 criteria and are manufactured or used as particles with sizes in the respirable range (*i.e.*, ≤ 10 µm)

- May cause inhalation hazards, including lung overload *via* a specific mode of action (*i.e.*, impairment of alveolar-macrophage [AM] mediated clearance), as identified in rat inhalation studies

- Overload is defined in this presentation as when the exposure concentration is sufficiently high or the duration sufficiently long to overwhelm AM-mediated clearance.

HMW Polymers – Default Approach

Toxicological analogues

Test material	Strain, Species, Sex, Exposure frequency and duration, Recovery	Exposure Concentrations (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3)	BMCL (mg/m^3)	Lung Effects at LOAEC
Polyacrylates and Methacrylates Sub-category						
9000 Toner (styrene/butylmethacrylate random copolymer)	SPF F344 rats, male and female (288/group); 24 months (6 hr/d, 5 d/wk), 2 months recovery	0, 1, 4, or 16	1	4	2.5 (fibrosis)	Significantly decreased macrophages and increased PMN and lymphocytes in BAL; significantly increased incidence of minimal to mild pulmonary fibrosis
Polyvinyls Sub-Category						
Polyvinyl chloride Powder	Rat, female (F344); group sizes not reported; 8 months (25 hr/wk); up to 100 d recovery	0, 3.3, 8.3 or 20.2	ND	3.3	Not derived; missing SD/SE	Significantly decreased alveolar clearance; and dose-dependent increase in PMNs at 8 months.

Worker Margin of Exposure (MOE) Calculations

	Animal POD			Worker Exposure				Human Breathing Rates						Benchmark MOE (Default)	Respirator Fold Factor
Exposure Route	POD Conc. mg/m^3	POD Period hrs/day	POD Duration days/wk	Exposure mg/day Potential Dose Rate (PDR) (mg/day)	Total Worker Breathing Volume for PDR Exposure Period m^3	Worker Exposure Duration Hours/Day	Exposure Duration Days/Wk	Default	Worker	Structural Alert as % of new chemical substance	POD Conc - Duration & Breathing Rate Correction Scenario mg/m^3	Exposure TWA mg/m^3	**Margin of Exposure MOE**	100 or 1000	
Inhalation	2.5E+00	5.00	5	5.0E+01	10.0	8.00	5	4.90	10.00	100%	7.7E-01	5.0E+00	**1.5E-01**	Fold Factor =	653
	3.3E+00	5.00	5	5.0E+01	10.0	8.00	5	4.90	10.00	100%	1.0E+00	5.0E+00	**2.0E-01**		4947

- Benchmark MOE consisted of 10x for intraspecies, 10x for interspecies, and 1x for BMDL or 10x for LOAEC = 100 or 1000
- If the calculated MOE is lower than the benchmark MOE, then EPA interprets this as an unreasonable risk

HMW Polymers – Default Approach

- For findings of unreasonable risk, EPA is required to take risk management actions (*e.g.*, consent orders with testing requirements, restrictions on manufacturing, processing, use, disposal, *etc*.) to address unreasonable risks before a company may commence manufacture or processing of the new chemical substance.

- The default approach was applied to a novel *α*-1,3-glucan, which resulted in risk findings and risk management actions:

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 9 and 721

[EPA–HQ–OPPT–2017–0575; FRL–9991–19–OCSPP]

RIN 2070–AB27

Significant New Use Rules on Certain Chemical Substances

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

fiber additive. Based on analogy to high molecular weight polymers, EPA has identified concerns for lung effects if the chemical is not used following the limitations noted below. The conditions of use of the PMN substance as described in the PMN include the following protective measures:

1. No use of the substance other than the uses described in the PMN; and
2. No manufacture, processing, or use with particle size less than 10 micrometers.

The SNUR designates as a "significant new use" the absence of these protective measures.

Potentially useful information: EPA has determined that certain information about the toxicity of the PMN substance may be potentially useful to characterize the health effects of the PMN substance if a manufacturer or processor is considering submitting a SNUN for a significant new use designated by this SNUR. EPA has determined that the results of pulmonary effects toxicity testing of the PMN substance may be potentially useful in characterizing the health effects of the PMN substance.

HMW Polymers – Default Approach

- The SNUR requirements were consistent with EPA's hazard concerns and testing recommendations for the Respirable, Poorly Soluble Particulates Chemical Category

2002 Respirable, Poorly Soluble Particulates Category

Category: Respirable, Poorly Soluble Particulates Health Only

Definition. This category includes a variety of inorganic, poorly soluble (as designated in ILSI 2000) particulates. Typically, they are oxides of various metals or nonmetals (i.e., silicon)

Boundaries. There is a potential for respirability if there are any particles ≤10 μ in diameter in the material being handled by workers. Summarized below are currently available test data on five different poorly soluble particulates: silica, talc, titanium dioxide, PMN 96-175 (lithium manganese oxide), and carbon black. The suitability of one or more of these analogues for a particular PMN particulate must be determined on a case-by-case basis. Risk is to be assessed by the margin of exposure method for the reason stated in the next paragraph.

→ **Limited to MMAD, no wt% cutoff**

Hazard Concerns. The category concerns discussed here are limited to effects on the lung as a result of inhaling the particles. Broadly, as shown in rat inhalation studies, these effects range from inflammation to fibrosis to, potentially, cancer. Because it is still not known with certainty whether high lung burdens of poorly soluble particulates can lead to lung cancer in

→ **Limited to effects on the lung**

General Testing Strategy

A 90-day inhalation toxicity test (Harmonized Test Guideline 870.3465) in rats with special attention to histopathology (inflammation and cell proliferation) of the lung tissues and to various parameters of the bronchoalveolar lavage fluid (BALF), e.g., marker enzyme activities, total protein content, total cell count, cell differential, and cell viability. It is not necessary to look at internal organs. It is recommended that a recovery period of 60 days be included to assess the progression or regression of any lesions. If the results of the subchronic study indicate that the PMN particles have carcinogenic potential, a 2-year inhalation bioassay in rats may be warranted.

→ **Limited to *in vivo* testing**

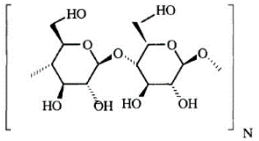
Amended TSCA: Section 4(h)

- However, the amended TSCA states “The Administrator shall reduce and replace, to the extent practicable, scientifically justified, and consistent with the policies of this title, the use of vertebrate animals in the testing of chemical substances or mixtures…” (Section 4(h)(1))

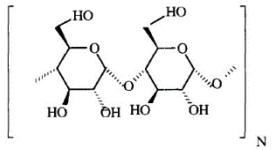
Case Study: α-1,3-glucan

Polysaccharides extracted from plants are widely used as industrial 'bulk' polymers to provide unique end-use applications but are limited by processes and raw material source. Nature often produces mixtures.

Cellulose
β(1,4) linkage

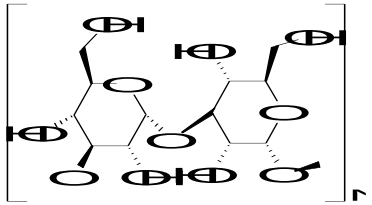


Starch
α(1,4) linkage



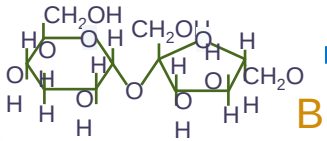
Case Study: α-1,3-glucan

Glucan
α (1,3) linkage



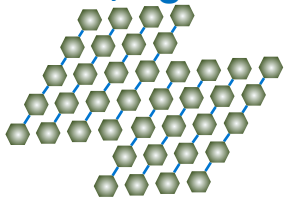
Using enzymatic polymerization to create a variety of polymer structures with unique product features and application benefits

SUCROSE



BIOCATALYST

α-1,3-glucan



Polysaccharide Applications

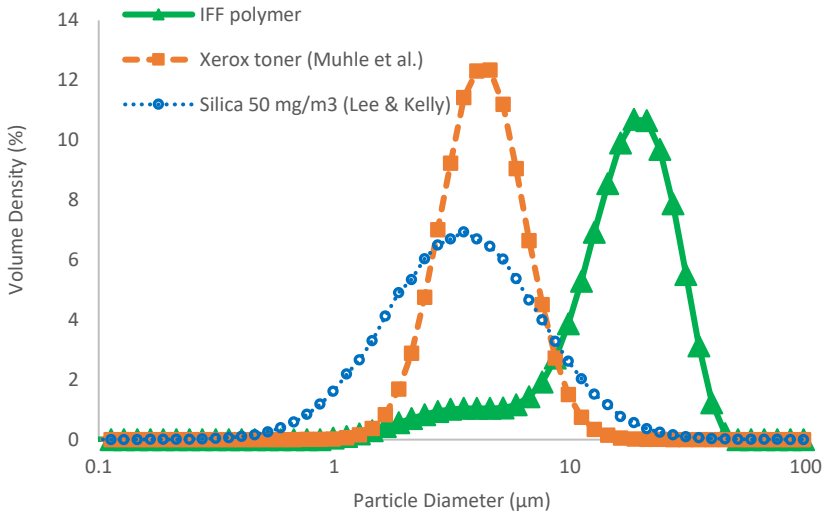
Powder

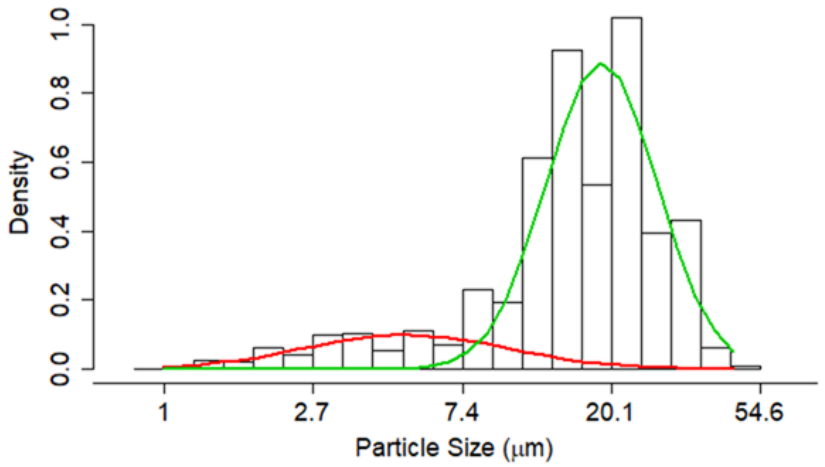
α-1,3-glucan
88% solids
Particle size control:
Unground - 150 micron
40 micron
17 micron

Case Study: α-1,3-glucan

Multi-faceted Strategic Approach in response to EPA's Risk Assessment taking into consideration *in chemico*, *in silico* and *in vitro* alternatives to animal studies

- Exposure monitoring results for manufacturing including grinding
- Demonstrated no reduction in PSD upon cross country transport
- **Re-examined Solubility using SELF according to Boisa et al**
- **Performed MPPD modeling to predict deposition, clearance and lung burden over a simulated long term exposure to the IFF polymer**
- Explored *in vitro* alternatives to characterize AOP





Source: Ladics *et al*. (2021) CHEMICAL RESEARCH IN TOXICOLOGY, available at: A Weight-of-the-Evidence Approach for Evaluating in Lieu of Animal Studies, the Potential of a Novel Polysaccharide Polymer to Produce Lung Overload

Particle size distribution of ground α-1,3-glucan

% Distribution	Size (µm)
d10	3.1
d50	9.2
d90	392.8
0.0	<0.2

→ **Solubility Determination**

Bimodal size distribution characterization for ground α-1,3-glucan

	Single Mode	Bimodal Mode 1	Bimodal Mode 2
MMD (µm)	16.2	5.0	18.8
GSD	1.71	1.99	1.45
Total Mass (%)	100	17	83

Characterization for native and ground α-1,3-glucan

Parameter	Value
Density	1.5 kg/m^3
Circularity	0.875 native, 0.84 ground
Aspect ratio	1.355 native, 1.354 ground
Zeta Potential	~0

Case Study: α-1,3-glucan

- ***In chemico*** **biosolubility tests were performed in water, Gamble solution, and simulated epithelial lung fluid (SELF) at 37 °C and pH 7.0-7.5 for 10- or 30-days**
- **α-1,3-glucan was found to be biosoluble in SELF, but not in water or Original Gamble's solution**

Solvent	Duration of Experiment (days)	weight loss (grams, dry basis)	% loss (average)	Solubility (grams/L)
Water	10	-0.01 ± 0.024	-0.60%	-0.08
Original Gamble	10	0.00 ± 0.006	-0.28%	-0.04
SELF	10	0.14 ± 0.054	9.98%	1.35
Water	30	-0.01 ± 0.020	-0.93%	-0.12
SELF	30	0.37 ± 0.016	26.95%	3.63

Source: Ladics *et al*. (2021) CHEMICAL RESEARCH IN TOXICOLOGY, at p. "G" (Table 5), available at: A Weight-of-the-Evidence Approach for Evaluating in Lieu of Animal Studies, the Potential of a Novel Polysaccharide Polymer to Produce Lung Overload

Case Study: α-1,3-glucan

- A simple exponential decay model was applied to the 10-day test data set to predict solubility in SELF:

$$P(t) = P_0 e^{-\lambda_D t}$$

- Where $P(t)$ is the amount of some quantity at time t, P_0 is the initial amount at time $t = 0$, λ_D is the decay rate, and t is time
- The half-life was estimated to be 66 days (time at which 50% of the polymer is dissolved in SELF)

Case Study: α-1,3-glucan

In silico modeling was performed using the Multiple-Path Particle Dosimetry (MPPD) model to predict deposition, clearance, and lung burden over the course of a simulated long-term exposure to the α-1,3 glucan

MPPD predictions were validated with experimental measurements of lung burden in rats exposed to 9000 Toner (see slide 5)

The empirical clearance model in MPPD was modified to account for the dissolution rate of the α-1,3-glucan using the following:

$$\lambda_A = ae^{-bm_A^c} + d + \lambda_D$$

- Where *a*, *b*, *c*, and *d* are fitted model coefficients, m_A is the lung burden in the alveolar airways, and λ_D is the dissolution rate for the α-1,3-glucan obtained from the biosolubility experiments

Case Study: α-1,3-glucan

Where science & creativity meet

Human equivalent concentrations (HECs) were calculated for the 9000 Toner exposure concentrations and for the α-1,3-glucan at the same concentration

HECs for the α-1,3-glucan polysaccharide were about 100 times larger than those for the 9000 Toner

Xerox Toner Rat Exposure Concentration (mg/m^3)	Measured** Xerox Toner Lung Burden (mg)	Lung Surface Area Normalized Xerox Toner Lung Burden (mg/m^2)	Equivalent Human Lung Burden (mg)	HEC for IFF Polysaccharide 40-Year Occupational Exposure (mg/m^3)	MPPD HEC for Xerox Toner 40-Year Occupational Exposure (mg/m^3)
1	0.22	0.51	35.8	5.7	0.043
2.5	0.50	1.16	81.4	13.0	0.098
4.1	1.73	4.02	281.6	44.9	0.338
16	15.6	36.28	2539	404.5	3.05

Source: Ladics *et al*. (2021) CHEMICAL RESEARCH IN TOXICOLOGY, at pp. "I" and "J" (Tables 8 and 9), available at: https://doi.org/10/1021/acs.chemrestox.0c00301 or A Weight-of-the-Evidence Approach for Evaluating in Lieu of Animal Studies, the Potential of a Novel Polysaccharide Polymer to Produce Lung Overload

Case Study: α-1,3-glucan

EPA concluded:

The biosolubility testing was conducted using a conservative respiratory tract fluid volume of 0.3 mL/kg bw (rounded down to 20 mL for a 70 kg individual). This equated to a loading concentration of 15 mg of the PMN substance per mL of simulated epithelial lung fluid (SELF). The SELF represented the intraluminal volume of respiratory tract fluid, without consideration of the daily turnover volume. The estimated average alveolar fluid volume is approximately 37 mL, nearly double the volume used for the biosolubility testing. In comparison, the normal reference range for extra vascular lung water (EVLW) index in humans is 7.3 ± 2.8 mL/kg bw (n = 534) or 511 mL for a 70 kg individual. EVLW index corresponds to the "sum of

interstitial, intracellular, alveolar, and lymphatic fluid, not including pleural effusions." Therefore, the solubility of the PMN substance in SELF represented a worst-case loading concentration for the PMN substance in the intraluminal compartment, assuming an equivalent static volume of 20 mL. Given that humans accumulate respirable, poorly soluble particles in the intra-alveolar, interstitial, subpleural, and broncho-vascular bundle compartments, with a predominance of particles eventually being found in the interstitium, the extrapolated *in vitro* to *in vivo* concentration of the PMN substance would equal a loading concentration of approximately 3 mg/mL of EVLW (*i.e.*, 1,500 mg/(511 mL for EVLW—37 mL for alveolar volume)) approximately 5 times lower than the loading concentration tested in the biosolubility study.

This information supports EPA's determination that the substance has inherently low toxicity and should not be considered a poorly soluble particle with the associated hazard concern for lung overload. Therefore, EPA proposes that the SNUR for this chemical substance be revoked pursuant to 40 CFR 721.185(a)(1).

Case Study: α-1,3-glucan

- Based on these findings, EPA determined that the α-1,3-glucan would not overload the lungs at the predicted worker exposure levels and concluded there was no need to conduct animal studies
- EPA revoked the SNUR restricting the manufacturing and use of the α-1,3-glucan, effective September 24, 2020

Federal Register / Vol. 85, No. 165 / Tuesday, August 25, 2020 / Rules and Regulations

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 9 and 721

[EPA–HQ–OPPT–2017–0575; FRL–10012–90]

RIN 2070–AB27

Revocation of Significant New Use Rule for a Certain Chemical Substance (P–16–581)

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: EPA is revoking the significant new use rule (SNUR) issued under the Toxic Substances Control Act (TSCA) for the chemical substance identified generically as alpha 1-, 3-polysaccharide, which was the subject of premanufacture notice (PMN) P–16–581. EPA issued a SNUR based on this PMN which designated certain activities as significant new uses. EPA is revoking the SNUR based on new test data for the chemical substance.

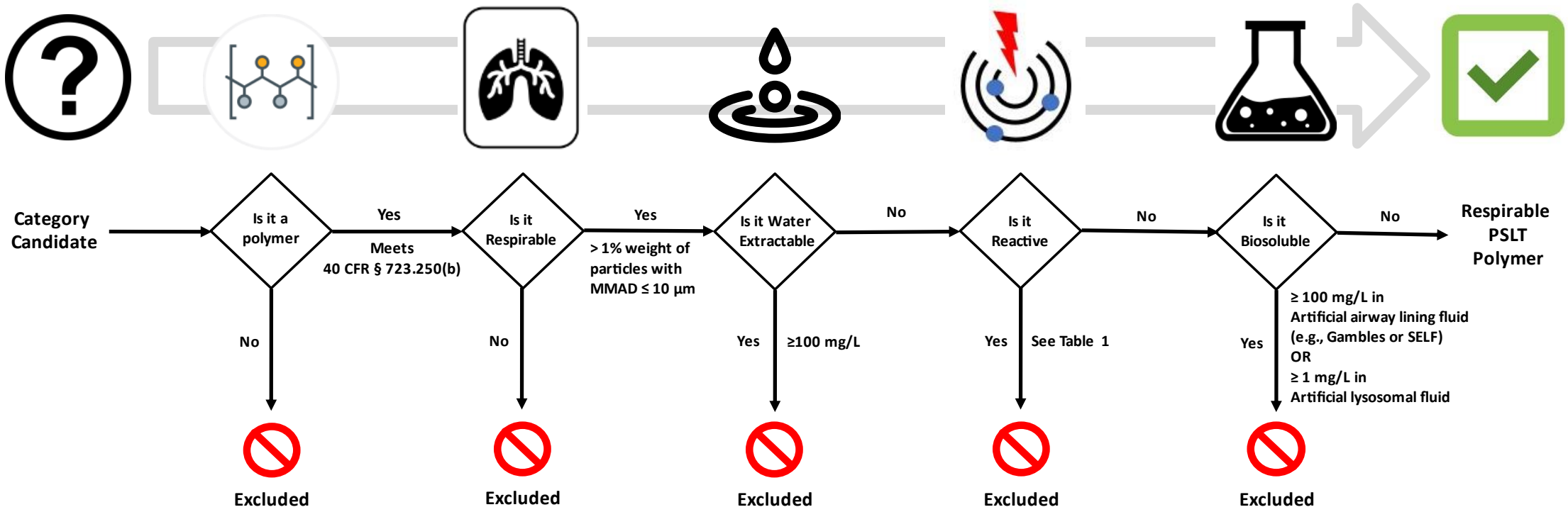
DATES: This rule is effective September 24, 2020. For purposes of judicial review, this rule shall be promulgated at 1 p.m. (EST) on September 8, 2020.

Amended TSCA: Section 4(h)

- The IFF contributions laid the groundwork for further refinements to EPA's evaluations on HMW polymers
- EPA collaborated with external partners, including NGOs and members of industry to further determine the breadth of non-animal test methods that could be used that provide information of equivalent or better scientific quality and relevance in accordance with Section 4(h) of TSCA
- This collaboration led to refinements to the HMW polymer category and inclusion/exclusion criteria, which removed the MW cutoff and included respirability, solubility, reactivity, and biosolubility.

TSCA New Chemicals Program (NCP) Chemical Categories

2021 Draft PSLT Polymer Category



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& creativity meet

Thank you!