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# A quantitative definition for poorly soluble particles

Lan Ma-Hock<sup>1\*†</sup>, Heidi Stratmann<sup>2†</sup>, Matthias Hufnagel<sup>3†</sup>, Anthony M. Bowden<sup>4</sup>, Blanca Serrano Ramón<sup>5</sup>, David Warheit<sup>6</sup>, Rüdiger Battersby<sup>7</sup>, Shannon M. Krieger<sup>8</sup>, Marco Kellert<sup>9</sup>, Nils Krueger<sup>10</sup>, Bennard van Ravenzwaay<sup>11</sup>, Robert Landsiedel<sup>1,12</sup> and Helmut Greim<sup>13</sup>

## Abstract

Poorly soluble low toxicity particles (PSLT) have long been a concept in particle toxicology and regulatory frameworks addressing inhalation hazards. The term PSLT refers to particles that exhibit low toxicity and minimal solubility in biological fluids, leading to prolonged retention in the lungs and potential overload effects. Historically, PSLT has been used to distinguish materials whose adverse effects are primarily driven by particle burden rather than intrinsic chemical toxicity. While the “low toxicity” (LT) component of the definition has been examined to a certain degree (Driscoll and Borm in *Inhal Toxicol* 32(2):53-62, 2020), the poorly soluble (PS)-criterion does not yet have a precise definition although it is critically influencing the interpretation of toxicological inhalation repeated dose studies and hazard classifications. An ECETOC Task Force (TF) was formed to define criteria for “poorly soluble” particles (PSPs). This paper presents a quantitative non-animal approach for defining PSP using a model particle, with a focus on its potential to cause volumetric lung overload and affect macrophage clearance mechanisms. The analysis allows to calculate a dissolution rate that would lead to a lung burden of about 1  $\mu\text{L/g}$  of lung tissue (lung overload threshold according to Morrow (Morrow in *Fundam Appl Toxicol* 10(3):369, 1988)). Below this threshold dissolution rate, this specific model particle would qualify as PSP. In addition, a formula was presented to translate abiotic dissolution rates into biotic (rat) dissolution rates to allow a PS-assessment in an animal-free system. As proof of concept, the TF collected existing *in vivo* data from member companies, publicly available literature of presumed PS-substances, and reference materials. The collected data revealed that most substances exhibited dissolution rates below the critical threshold and that the lung burden at no observed adverse effect concentrations (NOAECs) remained below the lung overload limit. Importantly, this threshold dissolution rate can differ from particle to particle, depending on factors such as agglomerate density, particle size distribution, and expected concentration. Thus, it should be evaluated on a case-by-case basis.

<sup>†</sup>Lan Ma-Hock, Heidi Stratmann and Matthias Hufnagel have contributed equally to this work.

\*Correspondence:  
Lan Ma-Hock  
Lan.ma-hock@basf.com

Full list of author information is available at the end of the article



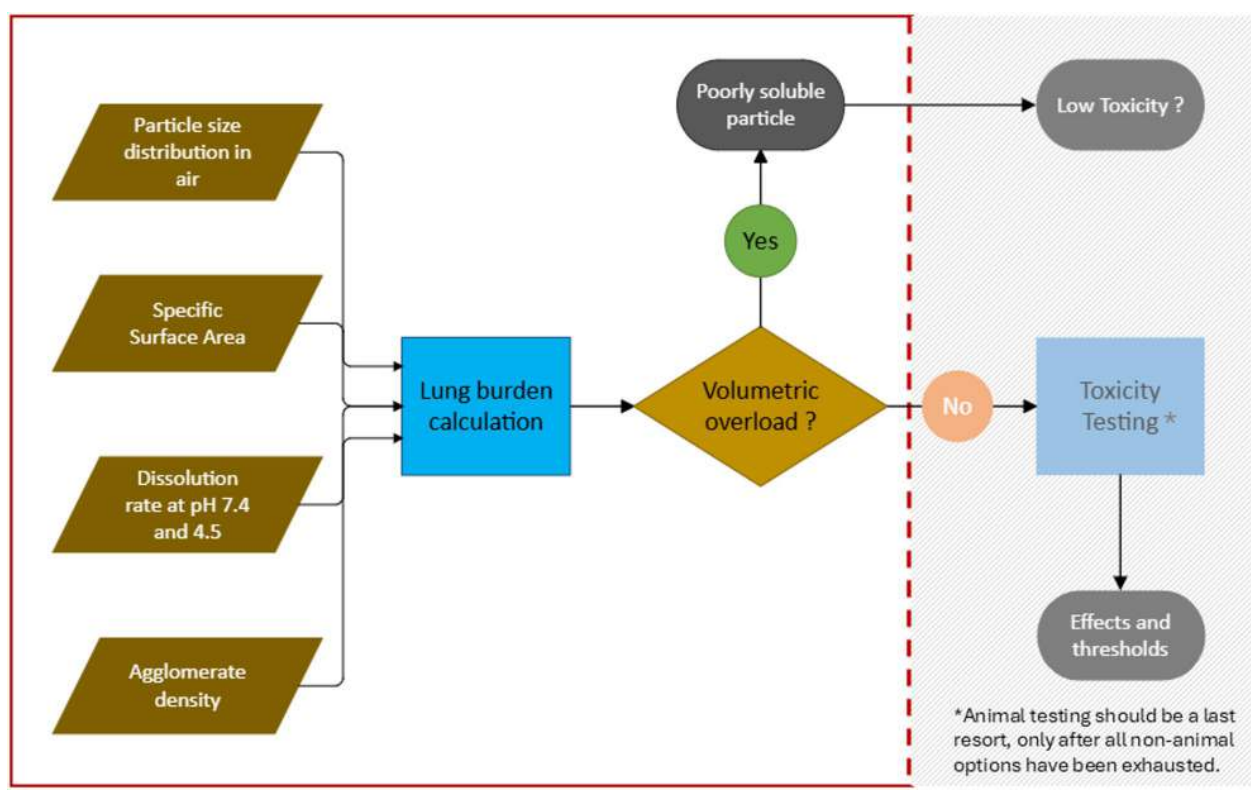
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## Highlights

- Dissolution rate threshold: For a model particle, a dissolution rate threshold was calculated for PSP. This dissolution threshold for PSP varies on the basis of several factors and should be assessed on a case-by-case basis.
- Animal-free PS-assessment: a formula was designed to translate abiotic dissolution rates into biotic (rat) dissolution rates to allow a PS-assessment in an animal free system.
- Lung burden assessment: Comparing the determined and modeled lung burdens of the collected dataset with the respective NOAEC/LOAEC mostly revealed relevant effects above a lung burden threshold of 1  $\mu\text{L/g}$  lung.
- Common Toxicological Pattern: 28- and 90-day inhalation toxicity data of particles with different chemistries revealed pulmonary foreign body inflammatory reactions as a prevalent toxicological response.

**Keywords** ECETOC, Poorly soluble particle, Dissolution, Lung burden, Inflammation, Clearance (max 7)

## Graphical Abstract



## Introduction

It is widely recognized that prolonged exposure to poorly soluble particles (PSP) can lead to harmful changes when the lungs are overloaded. The process of particle lung overload was examined by Morrow in 1988 [2], who observed that alveolar macrophage (AM) clearance, not dissolution, determines the particle residence time in the lung after inhalation of PSP. He described particle lung overload as a condition in which the clearance capacity of alveolar macrophages (AM) is exceeded, causing particle clearance to slow or fail. This results in extended particle residence time, chronic inflammation, and downstream adverse outcomes. In rats, lung overload is associated

with nonspecific lung responses, including the accumulation of particle-laden macrophages, persistent neutrophilic inflammation, epithelial hyperplasia, metaplasia, and, in extreme cases, lung tumors [1]. This is a mechanistic, capacity-limited phenomenon that can produce threshold-like behavior at the organ level because clearance capacity is finite. According to Morrow, impairment of clearance begins when the average composite phagocytized volume exceeds 6% of the normal AM volume, and complete cessation of clearance occurs when this phagocytized volume reaches 60% of the normal AM volume (Supplement C).

To distinguish materials whose adverse effects are primarily driven by particle burden rather than intrinsic chemical toxicity, authorities such as the American Conference of Governmental and Industrial Hygienists (ACGIH) and the German MAK introduced the terms “inert” or “nuisance” dust, or “biopersistent granular dust”, along with the respective dust limits and broad applicability descriptions. To date, the more often used term is poorly soluble particles of low toxicity or PSLT. It refers to particles that exhibit low toxicity and minimal solubility in biological fluids, leading to prolonged retention in the lungs and potential overload effects.

While the discussion within the scientific community about particle-related, adaptive and adverse effects is ongoing, the pursuit of a harmonized exposure level by European bodies has accelerated. Thus, there is an increasing demand for an applicable technical definition of PSLT particles that quantifies properties and indicates selection criteria to allow a reasonable identification of affected chemicals and a meaningful exposure level setting [3]. While the “low toxicity” (LT) component of the definition has been examined to some extent [1], the poorly soluble (PS)-criterion does not yet have a precise definition, although it is critically influencing the interpretation of toxicological repeated dose inhalation studies and hazard classifications.

The quantification of the properties, specifically the PS term, is inevitably linked with dissolution and lung clearance mechanisms. The dissolution of particles can be mimicked by, for example, in chemico tests in biologically relevant simulants [4–6] and allows classification of the substance according to their solubility under dynamic conditions. A particle with a low dissolution potential is supposed to remain in the lung for a longer time period and to have a greater contribution to the lung burden than an instantly soluble chemical [7].

The ECETOC Task Force (TF) on Inhalation Toxicological Properties of Low Soluble Particles, established in 2022, proposed a non-animal quantitative approach to establish a dissolution threshold, defining the term “poorly soluble” in the context of whether a specific particle would accumulate in the lung. The approach was exemplified using a hypothetical particle with predefined concentration, deposition rate, and agglomerate density in a model rat system. It is important to note that a dissolution threshold for one particle cannot be universally applied to all particles. The threshold depends strongly on additional physicochemical parameters, such as particle size distribution or agglomerate density. As only respirable particles deposit in the deep lung [8], particle size distribution determines their ability to reach this region and influences the daily mass deposition rate, while agglomerate density controls the volume-based lung burden. Thus, the dissolution threshold is not a fixed

value, but a particle-specific value shaped by its physical and chemical characteristics and their interaction with the biological system of interest (e.g., human or rat).

Furthermore, an equation was presented to convert abiotic dissolution rates into biotic (rat) dissolution rates enabling PS-assessments in an animal-free system. ECETOC also compiled existing 90-day inhalation study data from member companies, supplemented by documented literature, to evaluate the overload hypothesis comparing adverse outcomes in rat inhalation studies across different PSP chemistries. Finally, the applicability of the calculated dissolution threshold was evaluated by comparing it with existing substances described in the literature and in the ECETOC dataset containing supposedly PSP. Taken together, the proposed method offers a potential approach to shift ingredient risk assessment toward non-animal methods and reduce reliance on traditional toxicity testing.

The “low-toxicity” aspect of PSLT is still insufficiently defined and requires further efforts, as the regulatory need for clarity is just as high as for the “poorly soluble” aspect. The “low toxicity” topic is, however, out of scope of this TF and this paper and will be handled elsewhere.

## Materials and methods

### Derivation of a threshold of $k_{\text{dissolution}}$ for PSPs

In the literature, the half-life of macrophage-assisted clearance in rats is reported to be between 60 and 80 days [2]. Considering the dynamic dissolution rate, the calculated half-life should still be within this range for PSP, whereas particle accumulation upon inhalation exposure is possible. In this section, the authors propose a quantitative approach to define PS. The approach is exemplified using a standardized particle, characterized by a predefined deposition rate and a unit density of 1 g/mL, as well as in a standardized rat model (Box 1). It should be emphasized that the threshold for identifying a particle as PS is not constant; rather, it is influenced by particle size, agglomerate density and the species for which it is determined.

Kinetically, the clearance of dust from the alveolar or pulmonary region of the lungs has usually been treated as a first-order process [9].

The deposition fraction depends strongly on the particle size distribution and the density of the particles/agglomerates. In this example, a 10% respirable deposition fraction is applied. The rate of dust deposition is equal to the amount of air breathed by the rat multiplied by the exposure concentration ( $C$ ) and by the deposition fraction ( $D = 0.072 \text{ m}^3 \times C \times 10\%$ ).

Since a true steady state is never fully reached due to its asymptotic nature, it is reasonable to assume that after 5 half-lives 97% of the steady state lung burden is achieved,

**Box 1** Equations used for the calculation of the dissolution rate

$$1.1 L(t) = D - k * L(t - 1)$$

$$1.2 D = K_{ss} * L_{ss}$$

$$1.3 D = k_{MA-assisted} + k_{dissolution}$$

$$1.4 k_{dissolution} = \frac{D}{L_{ss}} - k_{MA-assisted}$$

In a single-compartment, first-order clearance model:

C	Exposure concentration (mg/m <sup>3</sup> )
D	Rate of pulmonary dust deposition (mass per day), product of the steady-state clearance rate $K_{ss}$ and the steady-state lung burden $L_{ss}$
L(t)	Build-up rate, under steady-state conditions, clearance and build-up rates are equal $L(\text{steady-state})=0$
$L_{ss}$	Steady state lung burden (g or mg)
$K_{ss}$	Clearance rate coefficient at steady-state, sum of $k_{ma-assisted}$ and $k_{dissolution}$
$k_{MA-assisted}$	Assumed alveolar macrophage-assisted clearance retention half time of 60 days $k$ is $\ln 2/60=0.0116$ / day
$k_{dissolution}$	Coefficient of dissolution triggered clearance difference between the deposition to lung burden ratio and the macrophage-assisted dissolution coefficient ( $k_{MA-assisted}$ )

Predefined parameters suggested for an initial calculation of  $k_{dissolution}$  to achieve a steady-state lung burden of 1 µl/g lung for standardized particles in the Wistar rat:

Exposure period	6 h
Ventilation rate	200 mL/min × 360 min/ day = 72 L = 0.072 m <sup>3</sup> per day
Respirable deposition fraction	10%
Continuous daily exposure	Usually limited to 5 days a week, the exposure could be adjusted to a value of 5/7
t (days) to achieve steady state	5*t/2 (5 half-life)
Lung weight	1.5 g
Clearance model	Constant macrophage-assisted clearance

which can be used as surrogate for the time required to reach steady state.

Moreover, to standardize the lung burden, we adopted a lung weight of 1.5 g, a value proposed by Snipes [10], based on adaptations from Phalen’s [11] work. Previous studies, such as those by Tillery and Lehnert [12], demonstrated that the lung weights of F344 rats increase with body weight, stabilizing at approximately 1.5 g for rats weighing approximately 385 g.

**Volumetric lung burden**

For PSPs with low toxicity, Morrow [2] proposed the volumetric overload of alveolar macrophages as the

**Box 2** Equations used for the conversion of abiotic dissolution into  $k_{dissolution}$

$$k_{dissolution} = k_{SSA} * SSA * \frac{24h}{day}$$

$k_{dissolution}$	Dissolution coefficient (day <sup>-1</sup> )
SSA	Specific surface area (m <sup>2</sup> /g)
$k_{SSA}$	Specific surface area-normalized dissolution rate constant (e.g. ng/cm <sup>2</sup> /h)

Depending on the units used for  $k_{SSA}$  and SSA, the corresponding unit conversions must be applied to ensure dimensional consistency of  $k_{dissolution}$

underlying mechanism for their toxicity. After reviewing the existing data, he determined that an average volumetric loading of 60 µm<sup>3</sup> per alveolar macrophage was the threshold at which macrophage-mediated clearance was impaired. The calculation was based on a uniform distribution of particles over the pool of 2.5 × 10<sup>7</sup> in total per rat lung. A particle with a unit density of 1 g/cm<sup>3</sup> refers to a lung burden of 1 mg/g lung. Thus, based on the lung overload hypothesis, PSP should not cause any adverse effects below an average volumetric lung burden of 1 µL/g lung.

**Translation of the abiotic dissolution rate into  $k_{dissolution}$**

In this work, the authors suggest denoting inhaled particles as PS when their dissolution half-life measured in artificial lung fluids (i.e., interstitial fluid (pH 7.4), artificial lysosomal fluid (pH 4.5), and artificial alveolar fluid (pH 7.4)) is significantly greater than the macrophage-mediated clearance time. This ensures that macrophage clearance, not dissolution, determines the particle residence time in the lung. Building on the before mentioned dissolution coefficient, we now translate the abiotic dissolution rate into  $k_{dissolution}$  —a key parameter, together with particle size distribution and agglomerate density, for identifying PSPs. This step serves as the bridge from abiotic data to assessing whether a substance may accumulate in vivo, ultimately enabling lung burden calculations for PSPs. For those particles with first-order dissolution kinetics, the rate law is expressed in terms of the specific surface area (SSA) and in terms of the concentration (mass per unit volume) of the particles. The surface area normalized first-order rate law is therefore given in Box 2 [13], . Further explanatory information on the equations used and the calculations done are provided in Supplement C.

**Data collection and study selection criteria**

After outlining the approach to define poorly soluble particles by calculating whether pulmonary overload would occur, the next step is to test its applicability. The TF employed a systematic approach to collect data on particles considered to be PS. As a first step, a comprehensive literature search was conducted to gather publicly

available information on subacute and subchronic inhalation toxicity studies with PS materials. In addition to the literature search, the TF proactively reached out to ECETOC member companies to obtain existing unpublished data. To facilitate this process, a questionnaire was developed and issued, which can be found in Supplement D.

The initial pool of presumed PS materials comprised of substances provided by member companies as well as chemicals that were indicated as PS(LT) particles. For example, the MAK document on the General Threshold Limit Value for Dust (respirable fraction) [14], the European High Court Judgment on TiO<sub>2</sub> [15] or, the opinion on priority chemicals for new or revised occupational exposure limit values, by the Advisory Committee on Safety and Health at Work (ACSH) [16] were taken into account. Furthermore, from the extensive dataset on TiO<sub>2</sub>, the TF selected TiO<sub>2</sub> P25 (NM105) to be added to our dataset used as a reference PS-material. Naturally occurring complex inorganic materials such as talc, attapulgite or aluminum oxide were, however, not included. These materials vary in purity depending on the mining area, and some may include fibers or needle-shaped particles.

The selection criteria for inclusion in the data set required that the published data originated from rat repeated dose inhalation toxicity studies conducted in accordance with either OECD guidelines 412 or 413. These studies were expected to provide detailed descriptions of the methods employed, as well as the results obtained, with a particular emphasis on lung burden data, if available. In cases where lung burden data were not provided, the availability of particle size distribution data was considered crucial, as it allowed for the calculation of the deposited dose using the multiple-path particle dosimetry (MPPD) model (MPPD v 3.04). Studies with a data quality below Klimisch 2 were not considered reliable and were not considered. Furthermore, only tests on untreated (i.e., uncoated, no surface-treatment) particles were selected, preferably in the microscale range.

The data sources used were the ECETOC collection as well as the ECHA Chem database and publicly available, peer-reviewed data. The selected data, both from published sources and from member companies, are presented in two tables in Supplement A. An overview of the substances assessed is provided in Supplement A, Table S4.

#### Calculation of lung burdens by the MPPD model

The TF collected and evaluated 28-day and 90-day inhalation studies in rats performed for regulatory purposes. For those substances for which the cascade impactor measurement could be performed, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) data are presented in Tables S1 and S2

(Supplement A). In the second step, based on the MMAD and GSD, the lung burdens were calculated by MPPD modeling (version 3.40, deposition and clearance model) for pulmonary retention using physiological respiratory parameters. To simplify the calculation, the asymmetric Sprague-Dawley rat model with a body weight of 450 g was used. The calculation anticipated that physiological macrophage-associated lung clearance occurred, but dissolution-related clearance can be neglected. The exposure settings chosen were 5 days/week, 6 h/day for 13 weeks. If multiple particle size measurements were performed during a study, and if the upper and lower ranges were close to each other, the means of the MMAD and GSD were taken for the calculation. When the range was too large, the lower and higher values were calculated separately to depict the range of the calculated pulmonary deposition.

There is only a limited number of substances for which agglomerate density has been measured using both mercury pycnometry [17] and centrifugation methods [18] (Supplement C). For MPPD modeling, the measured agglomerate density was utilized when available. Where the measured data was not accessible, a unit density (1 g/cm<sup>3</sup>) was employed for organic particles, as well as inorganic nanoparticles associated with a large surface area. In other cases, physical density was used.

## Results

### Threshold of $k_{\text{dissolution}}$ for PSP

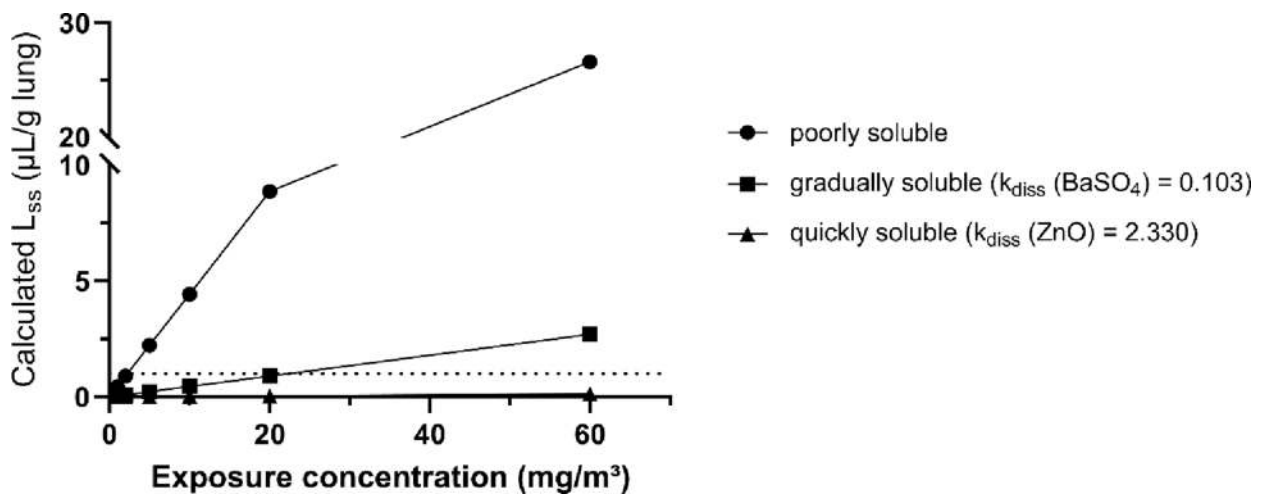
In Table 1, various atmospheric concentrations for the standardized particles were examined to determine the point at which a steady-state lung burden would be attained without accounting for dissolution, along with the corresponding time period to reach the steady-state condition. The required coefficient for dissolution ( $k_{\text{dissolution}}$ ) was calculated for each of the exemplified atmospheric concentrations to achieve the steady-state lung burden of 1  $\mu\text{L/g}$  lung ( $\rho = 1 \text{ g/cm}^3$ ). The exposure levels indicated reflect the average concentrations that were applied in the 90-day rat inhalation studies of our dataset. As a result, 2.25  $\text{mg/m}^3$  represents the critical boundary between poorly soluble and non-poorly soluble particles based on rat data.

According to this calculation, a concentration of 0.3  $\text{mg/m}^3$  of a particle with an agglomerate density of 1  $\text{g/cm}^3$  led to steady-state lung burdens below 1  $\mu\text{L/g}$  lung without dissolution clearance. At 2.25  $\text{mg/m}^3$ , a steady state would be achieved after 404 days of exposure when dissolution does not contribute to clearance. This exposure concentration serves as a marker of lung overload: macrophage-assisted clearance is impaired at this point, and exposure to higher levels results in lung overload in chronic studies. Studies for regulatory purposes, however, cover only a period of 28 or 90 days. Thus,

**Table 1** Calculated threshold dissolution rate ( $k_{\text{dissolution}}$ ) to achieve a steady-state lung burden of 1  $\mu\text{L/g}$  lung for rats

Atmospheric concentration ( $\text{mg}/\text{m}^3$ )	Daily deposition ( $\text{mg}/\text{day}$ )	K steady state for $L_{\text{ss}} = 1 \mu\text{L}/\text{g}$ lung	$k_{\text{MA-assisted}}$	$k_{\text{dissolution}}$ for $L_{\text{ss}} = 1 \mu\text{L}/\text{g}$ lung	Calculated $L_{\text{ss}}$ without considering dissolution	t (days) achieves steady state
C	D	$K_{\text{ss}}$	$k_{\text{MA-assisted}}$	$k_{\text{Dissolution}}$	$L_{\text{ss MA-assisted}}$	$5 * t_{1/2}$
0.3*	0.0015	0.0010	0.0116	-0.011	0.133	3369
2.25*	0.0116	0.0086	0.0116	-0.003	1.108	404
3*	0.0154	0.0103	0.0116	-0.001	1.330	337
20*	0.1029	0.0686	0.0116	0.057	8.867	51
60	0.3086	0.2057	0.0116	0.194	26.601	17

\* 0.3  $\text{mg}/\text{m}^3$  corresponds to the German MAK threshold for granular poorly soluble low toxicity particles, 2.25  $\text{mg}/\text{m}^3$  represents the critical boundary between poorly soluble and non-poorly soluble particles based on rat data, 3  $\text{mg}/\text{m}^3$  aligns with ECHA occupation exposure limit, 20  $\text{mg}/\text{m}^3$  is the threshold for CLP STOT RE1 according to GHS



**Fig. 1** Calculated lung burden at steady state ( $L_{\text{ss}}$ ) dependent on the solubility when different dissolution rates are applied. No impact of particle dissolution or only macrophage-assisted clearance was used for PSP (●), whereas dissolution rates of 0.103 and 2.330 were applied for gradually (■) and instantly (▲) soluble particles, respectively. The dotted line represents the threshold of 1  $\mu\text{L}/\text{g}$  lung

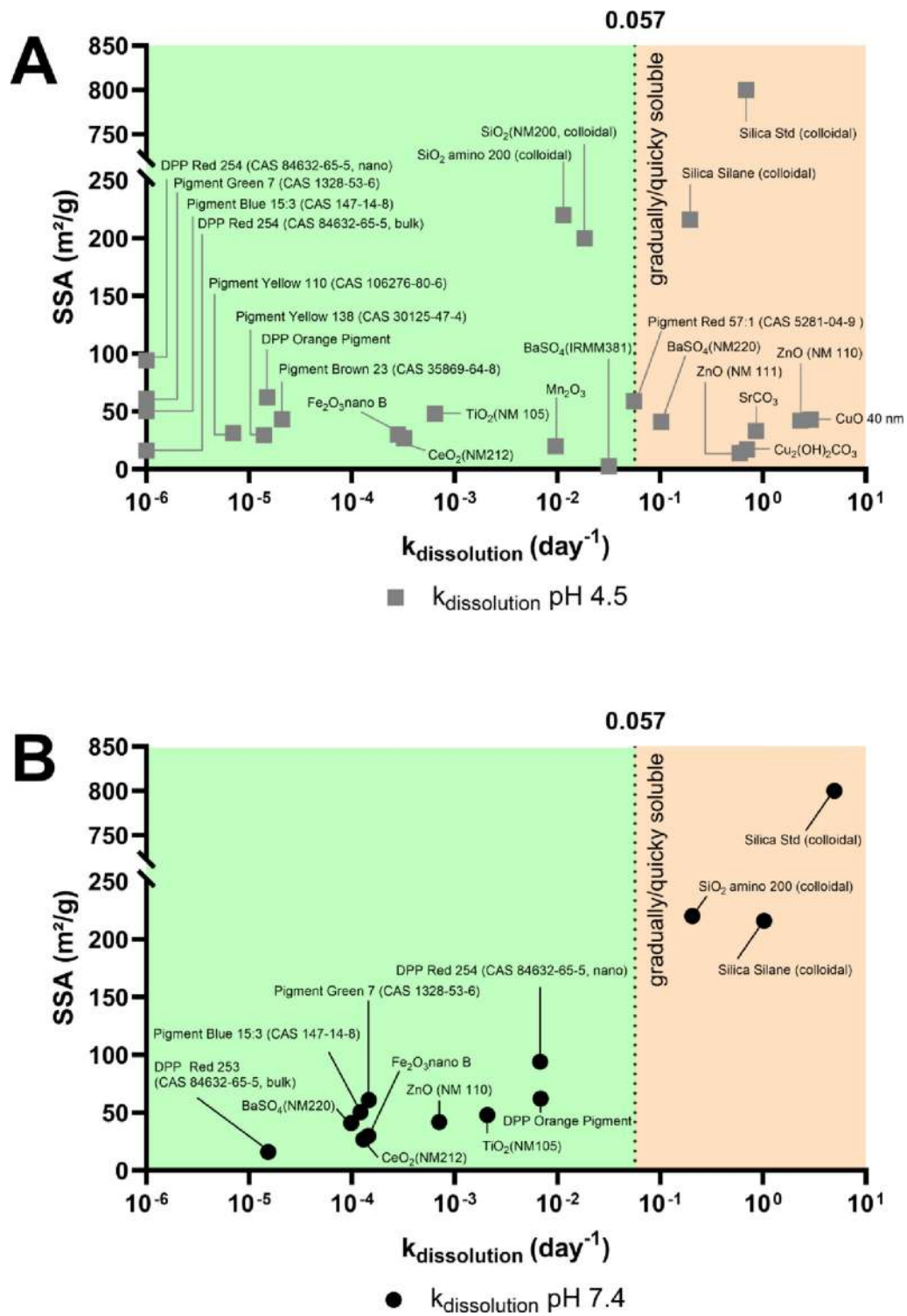
higher concentrations are necessary to achieve a steady-state lung burden of 1  $\mu\text{L}/\text{lung}$  within the framed exposure period. It is therefore proposed to set the threshold at 20  $\text{mg}/\text{m}^3$ , leading to a steady state within 51 days and allowing reasonable dose spacing. Thus, a substance with  $k_{\text{dissolution}} < 0.057/\text{day}$  is regarded as a PS for the model substance.

**Contextualizing  $k_{\text{dissolution}}$  with existing data on particle dissolution**

The  $k_{\text{dissolution}}$  is a key determinant of whether long-term inhalation exposure will result in particle accumulation in the lung. Figure 1 illustrates the calculated steady state lung burden ( $L_{\text{ss}}$ ) as a function of exposure concentration under varying dissolution rates. For PSP dissolution was not considered, while  $\text{BaSO}_4$  and  $\text{ZnO}$  serve as representative materials for gradually soluble and quickly soluble materials, respectively. For the quickly soluble particles the calculated lung burden does not increase above the threshold of 1  $\mu\text{L}/\text{g}$  lung, whereas it would achieve lung overload for gradually soluble particles.

Various chemicals have been examined to determine their dissolution rates, for example, flow-through systems using either Gambles solution, phagolysosomal lung fluid simulant (PSF) or artificial lung fluid (ALF) ([4–6] nanoGRAVUR, ECHA Chem; Supplement A, Tab. S3). Applying the equation used to translate abiotic dissolution rates into  $k_{\text{dissolution}}$  and assessing  $k_{\text{dissolution}}$  in relation to the respective particle-specific surface identifies chemicals that fall below the threshold of 0.057 and are therefore regarded as PS (Fig. 2). This threshold was calculated based on predefined parameters of a hypothetical model substance.

The calculation of  $k_{\text{dissolution}}$  from these substances shows that materials such as  $\text{TiO}_2$ ,  $\text{Fe}_3\text{O}_2$ , organic pigments or micro  $\text{CeO}_2$  can be assigned as PS, whereas  $\text{CuO}$ ,  $\text{SrCO}_3$ ,  $\text{BaSO}_4$  (NM 220), colloidal silica and  $\text{ZnO}$  revealed values above the threshold of 0.057/day and are therefore in the category of gradually or quickly soluble. Although this value was calculated based on predefined parameters of the hypothetical substance for rat 90-day inhalation studies, the assignment to PS, gradually or



**Fig. 2** Solubility of selected materials in artificial PSF at pH 4.5 and Gamble’s solution at pH 7.4 according to their calculated dissolution coefficient (based on the abiotic dissolution rate) and the dependence of their SSA. **A:** Materials with data in PSF. **B:** Materials with data in Gamble’s solution

quickly dissolving particle is in alignment with the definition by Braakhius et al. within the EU project GRACIOUS [19].

#### **Proof of concept: evaluation of effects observed in animal test with suggested PSPs**

The evaluation of the dataset revealed that the pulmonary changes observed had evolved from subacute (28-day) to subchronic (90-day) exposure, becoming more pronounced after 90 days. The nature of these changes remained comparable between the two exposure durations. Furthermore, the effects were comparable within this dataset, and a pattern of major findings was identified as particle-related effects. Changes in bronchoalveolar lavage fluid (BALF) usually included elevated total protein, LDH, GLU, alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) levels as well as increased total cells, neutrophils and macrophages (absolute). A word cloud depicts the most common effects, including histological findings, whereby terms with enlarged characters were mentioned more often than terms with smaller characters (Fig. 3). Notably, these studies were conducted over the past few decades and were carried out at various test facilities, and the histological assessments were performed by different pathologists. Throughout this long-term span, there have been continuous changes in the terminologies used to describe the same findings. As a result, there were several similar, but not identical, terminologies. Findings indicated with an asterisk were observed within BALF analysis, whereby

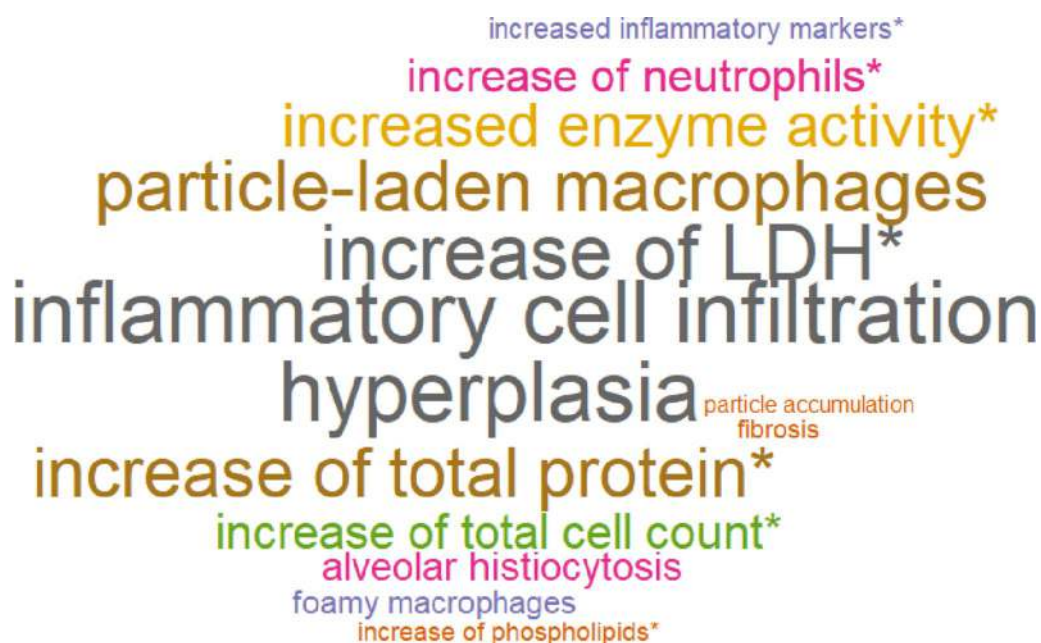
only effects on the absolute counts of respective cell populations of BALF cytology were used for the word cloud. The list of summarized findings to one terminology is provided in Supplement B.

All of these findings are also described and evaluated in detail for their pathogeneses and adversity by Weber et al. [20]. Although Weber et al. focused on 14 other inorganic particles, the similarity of the findings demonstrated again that these were not substance specific. Moreover, Stratmann et al. [21] and Herrmann et al. [22] demonstrated that organic pigments from different chemical classes produced the same effect pattern in both 5-day and 90-day inhalation studies as Weber et al. reported for inorganic particles. This finding reinforces that the observed effects were unspecific and not triggered by the chemical identity of the substances.

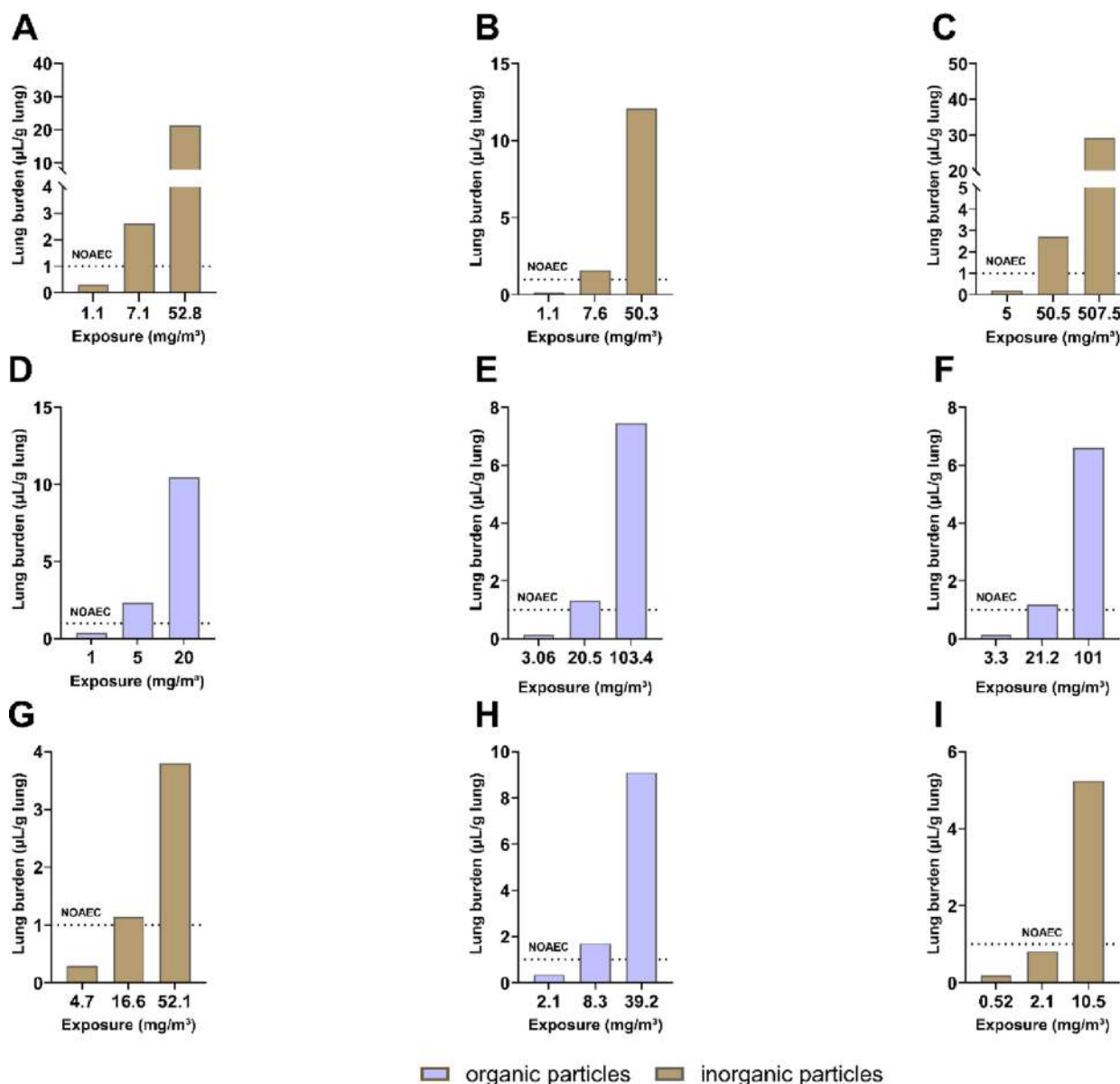
#### **Challenging the collected studies with obtained PS definition**

The calculated lung burdens for each substance at which they caused adverse effects are presented in Fig. 2. The results were plotted on graphs to visualize the relationship between concentration and lung burden (Figs. 4 and 5) as mass or volumetric load, with the NOAEC or LOAEC indicated. The threshold that was proposed by Morrow [2] is indicated as dotted lines.

The lung burdens were calculated for the majority of the compiled test substances as the lung burden for the entire organ. The lung burdens of ZnO, TiO<sub>2</sub>, BaSO<sub>4</sub> and synthetic amorphous silica (SAS) NM200 were



**Fig. 3** Word cloud of the most commonly observed effects, including histological findings and findings in bronchoalveolar lavage. The larger the characters, the more often the findings were described within the reports. Only findings that were observed at least six times within the analyzed dataset are shown within this word cloud



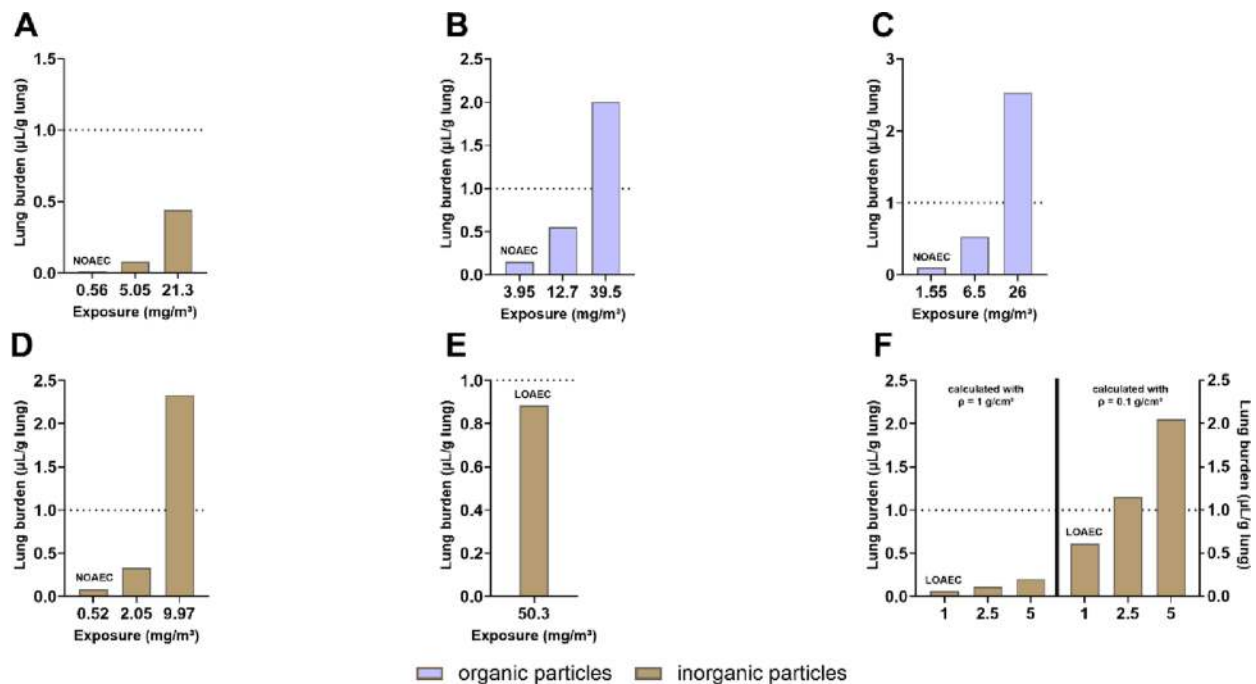
**Fig. 4** MPPD-based lung burdens shown as burdens per g lung applying a representative rat lung weight of 1.5 g for particles showing no adverse effects below a lung burden of 1  $\mu\text{L/g}$  lung (dashed line). **A:** Carbon black MMAD 0.88, **B:** Carbon black MMAD 1.4–1.6, **C:** Microscale  $\text{CeO}_2$ , **D:** 2,9-bis[4-(phenyldiazenyl)phenyl]isoquino-[4',5',6':6,5,10]anthra[2,1,9-def]isoquinoline-1,3,8,10(2 H,9 H)-tetrone, **E:** octadecanoic acid, reaction product (CAS 100545-48-0), **F:** 12-hydroxyocatcoic acid reaction product, **G:**  $\text{Fe}_2\text{O}_3$  [23], **H:** 3,6-diphenyl-1 H,2 H,4 H,5 H-pyrrolo[3,4-c]pyrrole-1,4-dione, **I:**  $\text{TiO}_2$  (NM105 [24]),

chemically analyzed in studies published by Thoma et al. [25], Bermudez et al., [24], Schwotzer et al. [26] and Dekant et al. [27], respectively. The normalized mass and volumetric lung burdens were calculated using a representative rat lung weight of 1.5 g [10].

Notably, as depicted in Fig. 4, the examined substances did not produce adverse effects at lung burdens below the normalized threshold of 1  $\mu\text{L}$  per gram of lung (dashed line). Adverse local effects were observed only when this threshold was exceeded, supporting the use of volumetric lung burden as a reliable indicator of potential risk. These

substances may be assigned to the group of PSLT according to Morrow's overload hypothesis.

Figure 5 presents studies on a few substances that cannot be clearly assigned as PSLT based on the available data. Manganese (Fig. 5A), the reaction product CAS 198028-14-7 (Fig. 5B), the reaction product EC 434-430-9 (Fig. 5C), ZnO (Fig. 5D), and  $\text{SiO}_2$  (Fig. 5F) showed adverse effects below 1  $\mu\text{L/g}$  lung. Dynamic dissolution data indicates that ZnO dissolves rapidly. For manganese and the two reaction products, no dissolution data and agglomerate density are available; if they were considered



**Fig. 5** MPPD-based lung burdens shown as burdens per g lung applying a representative rat lung weight of 1.5 g for particles showing adverse effects below a lung burden of 1 µL/g lung (dashed line). **A:** Mn, **B:** octadecanoic acid, reaction product (CAS 198028-14-7), **C:** reaction product of 1,6-hexanediamine and 12-hydroxyoctadecanoic acid (EC 434-430-9), **D:** ZnO [25], **E:** BaSO<sub>4</sub> [26], **F:** SiO<sub>2</sub> (NM200)

PS, they may not qualify as LT. In the case of BaSO<sub>4</sub>, only a high concentration of 50 mg/m<sup>3</sup> was tested, which was considered the LOAEC. The determined lung burden for BaSO<sub>4</sub> was lower than 1 µL/g lung (Fig. 5E). As shown in the dynamic dissolution, BaSO<sub>4</sub> is partially dissolving. SiO<sub>2</sub> has a very low volumetric lung burden, with an assumed agglomerate density of 1 g/cm<sup>3</sup>. However, the bulk density (density of the bulk of the particles, including the voids between each particle) of some high surface SiO<sub>2</sub> was as low as 0.1 g/cm<sup>3</sup>. When this density is used, SiO<sub>2</sub> still has adverse effects at a volumetric burden lower than 1 µL/lung, but the lung burden at the LOAEC was increased by a factor of 10. This underscores the fact that agglomerate density is a significant source of uncertainty when predicting volumetric loading. In this case, due to uncertainty of the agglomerate density and the dissolution rate, the SiO<sub>2</sub> in this study cannot be clearly assigned to either of the groups.

## Discussion

In this paper, the authors aim to define poorly soluble particles (PSPs) in a scientifically justified manner based on the overload hypothesis of Morrow [2]. A quantitative approach was then established to assess whether a particle qualifies as a PSP by calculating its potential to cause lung overload using several predefined parameters. Here, the possibilities and considerations associated with applying this approach, as well as its limitations, are addressed.

The applicability of the overload hypothesis enables the establishment of a quantitative threshold for PSP. This is based on the assumption that the clearance of dust from the alveolar region of the lung follows a first-order kinetic process. Morrow demonstrated that this model is advantageous because of its simplicity and is adequate for making initial assessments, provided that the AM-assisted clearance constant remains consistent regardless of total deposition. As shown in the equation, the threshold for dissolution coefficient is significantly influenced by the daily deposition dose. This dose is, in turn, affected by the exposure concentration, duration, particle size distribution, and respiratory mode. It should be noted that only respirable particles can deposit in the lungs. For human exposure, the threshold should be calculated using all human-relevant parameters.

In our example, some predefined parameters, including an assumed respiratory volume, a deposition fraction of 10%, and the unit agglomerate density, were used. The agglomerate density is a major uncertainty factor. Known methods for determining agglomerate density include mercury pycnometry [28] and volumetric centrifugation [18, 29]. However, there is no consensus within the scientific community. Therefore, we assumed unit density for the formed agglomerates and were aware of an underestimation of the volumetric load for materials with very low agglomerate density and an overestimation for high-density materials (details see Supplement C). The daily deposited dose is strongly influenced by the particle

properties and respiratory parameters. Given the numerous variables that need to be considered, this assessment should be calculated specifically for each individual particle under scrutiny.

With respect to solubility and dissolution, data on dynamic dissolution in PSF were available for seven substances in the dataset, whereas static water solubility data were provided for the other substances. Both dynamic dissolution in PSF and water solubility data show that most of the examined particles, including  $\text{TiO}_2$ , exhibit very low solubilities. This confirms their categorization as PS (Fig. 2).  $\text{ZnO}$  has a high dissolution rate in PSF (pH 4.5) and can be regarded as quickly soluble, whereas  $\text{BaSO}_4$  belongs to the gradually soluble category according to the GRACIOUS criteria (Fig. 2). In contrast to  $\text{TiO}_2$ ,  $\text{BaSO}_4$  should be regarded as a borderline material, where accumulation in the lung is possible despite its dissolution properties. At low concentration less than  $50 \text{ mg/m}^3$  and exposure duration less than 90 days, accumulation is unlikely to occur. This conclusion was confirmed by an inhalation study in rats [26]. Microscale  $\text{CeO}_2$  also seems to be a borderline case. Solely based on dissolution,  $\text{CeO}_2$  is a PSP. However, considering the nanoform, the NOAEC was far below the lung overload threshold. It was assumed that the toxicity was due to surface catalytic activity. The same may be applicable for manganese, which is widely recognized for its susceptibility to oxidation. However, dissolution data for manganese are not available. Overall, the *in vivo* inhalation studies in rats provided data that support the plausibility of the dissolution threshold derived from hypothetical parameters.

The analysis of the observed effects in subacute and subchronic studies, along with the measured and calculated lung burdens, revealed that most substances share a common effects pattern. Adverse effects were observed only when the lung burden exceeded  $1 \text{ }\mu\text{L/g}$  lung as shown in Fig. 4. The dataset analyzed included a broad range of chemical identities, from organic to inorganic particles, suggesting that the toxicological profile is actually particle-triggered and independent of the respective chemical or molecular structure. None of the study reports included any instances of systemic toxicity, indicating again that PSP does not cause chemical-specific effects and that their impact is limited to local effects within the respiratory tract. This observation calls into question the added value of inhalation studies with PSLT.

Furthermore, Fig. 4 clearly shows that atmospheric concentrations of  $5 \text{ mg/m}^3$  and above, sustained over 90 days, result in a rat lung burden exceeding the proposed threshold of  $1 \text{ mg/g}$  lung tissue for a particle with agglomerate density of  $1 \text{ g/cm}^3$ . This raises questions about the suitability of the CLP guidance values and their relevance for hazard assessments for PS chemicals. The guidance value for a STOT RE 1 classification for mists

and dusts after sub-chronic exposure is  $\leq 20 \text{ mg/m}^3$ , and for a STOT RE 2 classification this is between 20 and  $200 \text{ mg/m}^3$ . Both concentrations are many times above the dose levels that are toxicologically reasonable and appropriate from an animal welfare point of view for PSP.

In addition to the limited suitability of the CLP guidance values, species-specific differences, and the questionable need for animal tests with PSP, the absence of secondary and non-intrinsic effects in parts of the regulation are a general problem making it difficult to apply the given criteria for a hazard assessment. A more pragmatic approach to protect human health is a dust limit for PSP.

Several human dust limits exist aiming to protect workers and downstream users from workplace-related dust. PS dust limits are, however, only explicitly mentioned in the ECHA DNEL guidance [30] and bio-persistent, granular dusts in the MAK recommendation [14]. In a proof-of-concept approach both indicated values,  $3 \text{ mg/m}^3$  and  $0.3 \text{ mg/m}^3$  as proposed by ECHA and MAK, respectively, are challenged regarding their suitability for PSP using the derived PS-threshold and MPPD calculated human lung burden.

As the respirable fraction is the fraction of concern for the overload-related effects of PSP, the focus in the following calculation is on this fraction using the MMAD of  $3 \text{ }\mu\text{m}$  as respirable for adults according to the measurement by Brown [8]. Using MPPD modeling, the alveolar deposition fraction was calculated for granular particles with a density of  $1 \text{ g/cm}^3$ , MMAD of  $3 \text{ }\mu\text{m}$  and GSD of 2.5. The calculation resulted in 14% alveolar deposition for oronasal-mouth breathing. At the MAK-derived concentration of  $0.3 \text{ mg/m}^3$ , a steady state lung burden of  $1 \text{ }\mu\text{L/g}$  lung will never be achieved even without considering dissolution. In the case of  $3 \text{ mg/m}^3$ , steady state would be achieved after 330 days of exposure without the contribution of dissolution. With dissolution contribution (using the previously calculated  $k_{\text{dissolution}} = 0.057/\text{day}$  in the hypothetical case), a steady state lung burden of about  $0.2 \text{ }\mu\text{L/g}$  lung would be achieved for a lung of 400 g, corresponding to about  $80 \text{ mg/lung}$  for unit density particles. In human coal workers for lifetime exposure of 45 years to an average of  $2 \text{ mg/m}^3$  coal dust, an average of 5.4 to  $15.0 \text{ mg/g}$  lung was found [31]. Thus, both the MAK recommendation of  $0.3 \text{ mg/m}^3$  and the ECHA Occupational Exposure Limit of  $3 \text{ mg/m}^3$  for respirable particles, are considered safe for PSP, provided that these particles are also of low toxicity. A definition of “low toxicity” has not yet been established. Providing such a definition is beyond the scope of this paper.

Importantly, this concept applies only to poorly soluble particles, organic or inorganic, that exhibit low toxicity. To illustrate how this concept can be applied in defining poorly soluble particles, we developed a decision tree, presented as the graphical abstract. This decision tree

outlines the process for using relevant physicochemical data to determine whether a particle qualifies as a PSP. If the decision tree indicates that a substance is PS, it recommends proceeding with subsequent steps to evaluate its low toxicity. Because testing strategies for low toxicity are still under development, no information on LT is included in this decision tree. We want to emphasize, whenever possible, non-animal methods—commonly referred to as New Approach Methodologies (NAMs)—should be prioritized and given maximum effort. Animal testing should only be considered as a last resort, and only after all feasible NAM options have been fully explored to meet regulatory requirements. By introducing this tool, we aim to promote a more systematic and comprehensive approach to identify PSP with low toxicity and reduce animal testing.

## Conclusion

The overload hypothesis by Morrow as an underlying mechanism is applicable for PSP-induced inflammation in the lungs. Adverse effects after repeated exposure occur at a lung burden higher than 1  $\mu\text{L/g}$  lung and share a common effect pattern independent of the respective chemical structure. In this paper, a systematic approach was demonstrated to scientifically justify the classification of a particle as a PS on the basis of a standardized particle with predefined characteristics. The established PS threshold combined with abiotic dissolution tests and MPPD calculations allows the prediction and identification of PSP as visualized in the graphical abstract.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12989-026-00670-z>.

Supplementary Material 1.  
Supplementary Material 2.  
Supplementary Material 3.  
Supplementary Material 4.

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## Author contributions

Lan Ma-Hock and Heidi developed the initial concept and drafted the manuscript. Matthias conducted the data analysis. Heidi and Matthias also contributed to the creation of tables and figures. Helmut Greim was the group leader and senior author. He coordinated the work and provided input on the overall concept. The remaining authors supplied data, assisted in writing the manuscript, contributed to the final formulation, and made linguistic adjustments.

## Data availability

All publicly available data are referenced within the paper. Data included in the manuscript or supplementary information files consists of both publicly available and proprietary data provided by member companies. The proprietary data are presented in condensed form in the paper and in the

supplementary information, and more detailed information can be made available upon request.

## Declarations

### Competing interests

Most of the authors, including LMH, RL, HS, AB, NK, MK and SK are employees of chemical companies that manufacture products containing poorly soluble particles. The other authors not employed by a chemical company declare that they have no competing interests.

### Author details

- <sup>1</sup>Experimental Toxicology and Ecology BASF SE, Carl-Bosch-Strasse 38, 67056 Ludwigshafen, Germany
- <sup>2</sup>Colors&Effects Switzerland AG, Efringerstrasse 32, Basel 4057, Switzerland
- <sup>3</sup>Engelhard Arzneimittel GmbH & Co. KG, Herzbergstrasse 3, 61138 Niederdorfelden, Germany
- <sup>4</sup>Safety, Environmental & Regulatory Science, Unilever, Colworth Science Park, Bedfordshire, Sharnbrook MK44 1LQ, UK
- <sup>5</sup>European Centre for Ecotoxicology and Toxicology of Chemicals, Rue Belliard 40, Brussels 1040, Belgium
- <sup>6</sup>Warheit Scientific LLC, Wilmington, USA
- <sup>7</sup>EBRC Consulting, Hannover, Germany
- <sup>8</sup>Dow, Inc., Midland, USA
- <sup>9</sup>Wacker Chemie AG, Burghausen, Germany
- <sup>10</sup>Evonik Industries AG, Hanau-Wolfgang, Germany
- <sup>11</sup>Environmental Science Consulting, Altrip, Germany
- <sup>12</sup>Pharmacy, Pharmacology and Toxicology, Freie Universität Berlin, Berlin, Germany
- <sup>13</sup>Technical University of Munich, Munich, Germany

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