A weight-of-evidence approach for setting OELs for poorly soluble, low-toxicity nano-particles

Jürgen Pauluhn

Bayer HealthCare
Bayer Schering Pharma

Germany

Washington, DC – Sept. 10, 2012
Outline

- Principal Mode of Action of low-toxicity PSPs
- Pharmacokinetic model to interrelate the cumulative lung dose with associated chronic changes
- Verification of kinetic modelling by retrospective analysis of inhalation studies with fine and ultrafine PSPs
- The kinetic cornerstones of low-toxicity PSPs must be observed for designing long-term inhalation studies
- Derivation of Occupational Exposure Levels (OELs/DNELs) based on a unifying rat-human overload-threshold concept
- Summary and conclusions
Chronic Exposure Paradigms require focus on the Chronic Mechanisms of inhaled low-toxicity PSPs

The metric of dose depends on the chronic Mode of Action:

- Instant effect at the initial site of deposition.
- Transient surfactant dysfunction, coating and opsonation of PSPs.
- Phagocytosis of PSPs by alveolar macrophages (AM).
- Chemotactic stimuli for AMs and PMNs to migrate into the alveoli.
- Any increased pool of AMs increases the lung burden and residence time of retained PSPs.
- OELs are designed for preventing overload-like effects to occur.
**Putative Mechanisms of inhaled low-toxicity Nanoparticles**

Direct mode of action (acute)

- Dissolution/bioavailability
- Surfactant dysfunction
- Type II Cell activation

Retention-related indirect mode of action (cumulative, chronic)

- Substance-specific

System variables:
- Size of AM
- Total number of AM
- Retained volume dose per AM

\[ \text{Lung Burden} \ 	ext{[mg Substance.resp./kg rat]} \]

\[ \text{Study Day} 0 \ 10 \ 20 \ 30 \ 40 \ 50 \ 60 \]

\[ \text{MoA}_I \]

\[ \text{MoA}_{II} \]

\[ \text{time} \]
The Principal chronic Mode of Action and Lung-Overload are interrelated

- Low-toxicity PSPs do not show any (subcompartmental) solubility.
- Increasing cumulative lung burdens cause particle-displacement volume-dependent increase in total lung cells ($V_d$).
- Prevailing experimental evidence suggests that the particle volume is a lead metric to understand and model the AM-mediated kinetics of PSPs.
- Kinetic hallmarks can differentiate adaptive from overload effects as well as particle-specific effects.
**Particle Translocation to the LALNs occurs secondary to Overload-dependent Inflammation**

Baytubes $(\rho: 0.2 \text{ g/cm}^3)$ 13-wks+26 wks postexposure

Magnetite $(\rho: 4.6 \text{ g/cm}^3)$ 4-wks+26 wks postexposure

As long as the overload-threshold is not exceeded, the kinetics of PSPs appears to be predominated by the AM-mediated clearance.
Volumetric Capacity of Alveolar Macrophages and Overload-Threshold (MoA\text{\textsubscript{II}})

\begin{align*}
\text{Overload threshold AM}_{v-6\%_{kg-rat}} &= 7 \times 10^{10} \times \frac{6}{100} = 0.42 \times 10^{10} \, \mu m^3 \\
&= 4.2 \, \mu l \, PM_{\text{resp}} / kg_{rat} \, \text{cumulative pulmonary dose}
\end{align*}

Volume-load of AM-pool ≤ 6%: no change in pool-size (= kinetic NOAEL)

**Parameterization of \( V_d \):**

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*: empirically confirmed in the current 13-week rat inhalation study
**Volumetric Capacity of Alveolar Macrophages and Overload-Threshold (MoA_{ll})**

Cumulative alveolar threshold volume dose:

\[ = 4.2 \mu l \text{ PM}_{pulm} / \text{kg}_{rat} \]

Constrains: \( \Sigma \text{AM} = f(C \times t); \phi \text{AM} = \text{constant} \)

Alveolar ventilation:

\[ 0.29 \times \frac{2}{3} = 0.19 \frac{m^3}{\text{kg}_{rat-day}} \]

Chronic inhalation exposure concentration (6 h/day, 5-times/wk):

\[ \Rightarrow \text{Daily dose}_{lung} = \frac{0.069}{0.19} \times \frac{3}{2} \mu l \frac{\text{PM}_{pulm} \times \text{kg}_{rat-day}}{m^3 \times \text{kg}_{rat-day}} \approx 0.54 \mu l \frac{\text{PM}_{resp}}{m^3} \]

The kinetic threshold of PM-volume-dependent lung overload is the theoretical volumetric NOAEL for any poorly soluble, granular low-toxicity particulate.
**Cumulative Threshold Dose: The Transition from Homeostasis to Overload (increased $V_d$)**

\[
\frac{4.2 \, \mu l_{PM}}{kg - rat} \approx \frac{4.2 \, \mu l_{PM}}{lung_{kg-rat}} \approx 4.5 \left[ g - lung_{kg-rat} \right] \times f_{cum} \left[ \frac{day}{g - lung_{kg-rat}} \right] \times V_L \left[ m^3/\text{day} \right]
\]

\[
\Rightarrow \frac{4.2 \, \mu l_{PM}}{lung_{kg-rat}} \approx 4.5 \times f_{cum} \times 0.29 \, m^3 \Rightarrow \frac{1 \, \mu l_{PM}}{f_{cum} \times 0.29 \, m^3}
\]

Daily exposure dose to attain the overload threshold:

\[
NOAEL_{pred} = \frac{1 \, \mu l_{PM}}{f_{cum} \times 0.29 \, m^3} \times \rho \left[ mg/\mu l_{PM} \right] \times \frac{100}{Depos_{pulmonary}} \left[ mg/m^3 \right]
\]

Starting point for dose selection for repeated inhalation exposure studies

PM: respirable fraction of particulate matter likely to be deposited in the pulmonary region
Estimation of generic No-observed-adverse Effect Levels (NOAELs) for any PSP and Study Type

Daily dose to attain threshold

\[
f_{\text{cum-104wks}} = \frac{4.2}{0.069} = 61
\]

\[
f_{\text{cum-13weks}} = \frac{4.2}{0.105} = 40
\]

\[
f_{\text{cum-4wks}} = \frac{4.2}{0.24} = 17.5
\]

Dose-Time\text{adj}

+ 1.5

3.4

2.3

Not necessarily applicable to 1-wk studies since MoA-related acute effects may confound retention-related chronic effects.
The kinetics of adaptive changes in the $V_d$ and increased PMNs are interrelated

1. Kinetic threshold (homeostasis) precedes the effect threshold (inflammation, PMN)
2. Any elimination $t_{1/2} > 1$ year due to kinetic overload does deliver relevant information for hazard identification
The cumulative Lung Dose, not duration, determines the terminal $t_{1/2}$ and the Study Outcome

1. $C \times \Sigma t$ determines the cumulative lung burden, $t_{1/2}$, and the associated intensity of effect.
2. Time-related aggravations of effect did not occur.
Proof-of-Principle for low Density PSPs

13-Week Rat Baytubes Inhalation Study (Pauluhn, 2010)

\[
NO(A)EL_{predicted} = \frac{1 \mu l}{0.29 m^3} \times \frac{0.2}{40} \times \frac{100}{8} = 0.2 \left[ \frac{mg}{m^3} \right]
\]

NOAEL_{B-BAL-PMN\%} = 0.16 mg/m³ (BMDL)

MWCNT

Lung Burden [µl PM_{resp}/m³]

Study Day

Concentration [mg/m³]

Cytodifferentials [x10⁴ cells/lung]

Macrophages
non classifiable
PMN
Lymphocytes
foamy cells

Overload-Threshold

13 weeks

39 weeks
**Proof-of-Principle for high Density PSPs**

**13-Week Rat Magnetite Inhalation Study (Pauluhn, 2012)**

\[ \text{NO(A)EL}_{predicted} = \frac{1 \mu l}{0.29 m^3} \times \frac{4.6}{40} \times \frac{100}{8} = 5 \left[ \frac{mg}{m^3} \right] \]

**Lung Burden [\mu l Fe_3O_4-Resp/m^3]**

- 5 mg/m³ - \( t_{1/2} = 94 \text{ days} \)
- 15 mg/m³ - \( t_{1/2} = 177 \text{ days} \)
- 50 mg/m³ - \( t_{1/2} = 392 \text{ days} \)
- Overload threshold

**Study Day**

0 50 100 150 200 250 300

**Concentration [mg/m³]**

0 5 15 50

**Cytodifferentials [%]**

0 20 40 60 80 100 120

- Macrophages
- non classifiable
- PMN
- Lymphocytes
- foamy cells

**BAL-Total Cell Count measurements most sensitive to detect changes in \( V_d (t_{1/2}) \)**
**Proof-of-Principle for unit Density PSPs**

**2 Year Toner Rat Inhalation Study (Muhle, 1991)**

Empirical $t_{1/2}$ at 1, 4 and 16 mg/m$^3$: 81 (75-88), 173 (135-241) and 568 (306-1000 days)

$\text{NOAEL} = \frac{1 \mu l_{PM}}{f_{\text{cum}} \times 0.29 \ m^3} \times \frac{\rho \ [mg]}{\mu l_{PM}} \times \frac{100}{\text{Depos}_{\text{pulmonary}}} [mg/m^3]$

where:
- $\rho = 1.2 \ g/cm^3$ (toner)
- $f_{vi} = 61$
- $PM_{\text{resp}} = 5.3$

$\text{NO(A)EL}_{\text{predicted}} = \frac{1 \mu l}{0.29 \ m^3} \times \frac{1.2}{61} \times \frac{100}{5.3} = 1.3 [mg/m^3]$

$\text{NO(A)EL}_{\text{observed}} = 1.0 [mg/m^3]$ Based on BAL_PMN

Hypothesis verified
Repeated Exposure Inhalation Studies: Comparison of Predicted and empirical NOAELs

\[
\text{NOAEL} = \frac{1 \mu l_{PM}}{f_{\text{cum}} \times 0.29 \, m^3} \times \rho \left[ \frac{mg}{\mu l_{PM}} \right] \times \frac{100}{Depos_{\text{pulmonary}}} [mg/m^3]
\]
Kinetic Modeling-based Study Design for Inhalation Testing of low-toxicity (Nano)Particles

Long-term Inhalation Studies with PSPs should not exceed the MTD (= $t_{1/2} >1$ year)
**Kinetic Modeling-based Study Design for Inhalation Testing of low-toxicity (Nano)Particles**

There is an apparent dependence of BAL-PMN on TCC ($V_d$) and the cumulative volumetric particle lung burden. In the absence of any increased TCC, BAL-PMN% < 4% appear to be toxicologically insignificant.
Volumetric Capacity of Alveolar Macrophages and Overload-Threshold (MoA\textsubscript{\text{II}}) in Human Workers

Overload threshold AM\textsubscript{\text{v-6\%kg\textsubscript{human}}} = 5 \times 10^{11} \times \frac{6}{100} = 3 \times 10^{10} \mu m^3 \\
= 30 \mu l PM\textsubscript{pulm} / kg\textsubscript{human} cumulative pulmonary dose

Volume-load of AM-pool ≤ 6%: no change in pool-size (= kinetic NOAEL)

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- Respirability
- Inhaled volume

- t\textsubscript{1/2} should be 7-times higher due to 7-times higher V\textsubscript{d} which means t\textsubscript{1/2} = 420 days

\[ t_{1/2} = \ln(2) \times \frac{V_d}{CL} \]
When accounting for the species-specific differences in the kinetic hallmarks of PSP (AM-mediated clearance only), the cumulatively retained pulmonary dose for attaining the overload-threshold is similar in rats and humans. Hence, following adjustment for ventilation and respirability, the paradigm devised for rats are also applicable to humans.
Estimation of Human Equivalent Lung Dose – Rat- or Human-based (MWCNT)

Ventilation rat (0.8 L/kg-min) = 240 ml/min (300 g rat) x 360 min = 0.086 m³/day
Ventilation human: 10 m³/day

Ventilation x Deposition:

$$AF_{lungburden-A/H} = \frac{V_{E-A} \times F_{a-A}}{V_{E-H} \times F_{a-H}} \times \frac{BW_H}{BW_A} = \frac{0.086}{10} \times \frac{0.075}{0.164} \times \frac{70}{0.3} = \frac{0.452}{0.492} = 0.92$$

Clearance:

$$AF_{clearance-A/H} = \frac{V_{d-A} \times t_{1/2-H} \times \ln 2}{V_{d-H} \times t_{1/2-A} \times \ln 2} \times \frac{BW_H}{BW_A} = \frac{2.1 \times 10^{10}}{3.5 \times 10^{13}} \times \frac{400}{60} \times \frac{70}{0.3} = \frac{59 \times 10^{13}}{63 \times 10^{13}} = 0.94$$

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per rat = 2.1 x 10¹⁰ (300 g)

per human = 3.5 x 10¹³
Derivation of OEL (MWCNT): Empirical Approach

\[
OEL = NO(A)EL_A \times \frac{AF_{\text{lungburden-A/H}}}{AF_{\text{clearance-A/H}}} \times \frac{1}{AF_{\text{StudyDuration}}}
\]

\[
AF_{\text{StudyDuration}} = \frac{0.105}{0.069} = 1.5 \quad 90\text{days} \rightarrow 2\text{years}
\]

\[
OEL = 0.16_{BMDL} \frac{mg}{m^3} \times \frac{0.92}{0.94 \times 1.5} = 0.1 \frac{mg}{m^3} \quad (\text{Baytubes})
\]

(chronic exposure)
OEL Calculation for MWCNT (Baytubes): Theoretical Approach

\[ OEL_{\text{generic}} = \frac{0.5 \, \mu l \, PM_{\text{respirable}}}{m^3} \times \left[ \frac{AF_{\text{lungburden-A/H}}}{AF_{\text{clearance-A/H}}} \approx 1 \right] \times \delta_{\text{agglomerate}} \]

Generic threshold rat
6h/day-5-times/week

Rat-to-human differences in lung dosimetry-kinetics-exposure duration/day.
Assumption: aerosol size in inhalation chamber = workplace

\[ OEL_{\text{generic}} = 0.5 \frac{\mu l}{m^3} \times 0.92 \times 0.2 \frac{mg}{\mu l} \approx 0.1 \frac{mg}{m^3} \] (chronic exposure)

Which means the theoretical mechanism-based approach verifies the empirical approach and \textit{vice versa}. The NOAEL depends on kinetic factors (dosimetry) and not yet unknown Mode of Actions.
**OEL Calculation for Fe₃O₄: Empirical Approach**

\[
OEL = NO(A)\, EL_A \times \frac{AF_{\text{l lung burden}}}{AF_{\text{clearance}}} \times \frac{1}{AF_{\text{StudyDuration}}}
\]

\[
OEL = 5.6_{BMDL} \frac{mg}{m^3} \times \frac{0.92}{0.94 \times 0.105} \approx 4 \frac{mg}{m^3} (Fe₃O₄)
\]

(chronic exposure based on 13 wk study & prediction)

\[
OEL = 15.1_{BMDL} \frac{mg}{m^3} \times \frac{0.92}{0.94 \times 0.24} \approx 4 \frac{mg}{m^3} (Fe₃O₄)
\]

(chronic exposure based on 4 wk study & prediction)
Summary: Computational Design of Repeated Exposure Inhalation Studies

- Anticipation of threshold Cxt for overload (= no-observed-adverse effect concentration, NOAEL).
- NOAEL, LOAEL and MTD can be rationalized based on the dynamic overload-dependent increased $V_d$ and associated $t_{1/2}$.
- Appropriate postexposure period can be selected to verify simulated clearance and reversibility for particles being ‘inactivated’ ($\text{MoA}_i \rightarrow 0$).

**Principal hypothesis**: the effect follows the cumulative dose and ‘acute-on-chronic effects’ should be negligible.
Inhalation studies should be designed to meet the kinetic cornerstones of generic PSPs and observing the kinetic MTD ($t_{1/2} > 1$ year).

The metric of dose must be defined by the mechanism of target organ injury which is the volumetric overload of alveolar macrophages.

Therefore, the most important metrics for the OEL-derivation of Poorly Soluble Particle-like structures is the agglomerate/aggregate volume.

Adequately designed and executed 4-week inhalation studies can reliably predict the chronic OELs of low-toxicity PSPs in the absence of overriding lung-overload-related confounders.