Society of Toxicology

Inhalation and Respiratory Specialty Section

48th Annual Meeting of the Society of Toxicology to be held in Baltimore, Maryland on March 15-19, 2009

Baltimore Convention Center

INHALATION AND RESPIRATORY SPECIALTY SECTION (ISS) NEWSLETTER — MARCH 2009

Dear IRSS members:

I am anxiously awaiting our upcoming meetings in Baltimore. This newsletter is to refresh your memory of what occurred last year, and to update you on any new happenings. This year the technical committee will be meeting on Tuesday at 7:00am to 8:30am in room 345 of the Baltimore Convention Center. We have scheduled 3 of our members to make presentations. John Whalan of EPA will update us on the OECD guidelines, George Woodall of EPA's National Center for Environmental Assessment will discuss their centers initiatives to standardize the development of arrays that compare inhalation health affects reference values and Juergen Pauluhn will discuss the particle size distribution for repeated exposure inhalation studies as well as to evaluate substances with hepatic or other first-pass metabolism results in toxifying or detoxifying. Please see the attachment following the minutes of the Technical Meeting of last year.

I look forward to seeing you in Baltimore and having a very productive meeting.

Have a safe trip,
Harry Salem

PRESIDENT'S MESSAGE

PRESIDENT'S 2009 FEBRUARY/MARCH MESSAGE.

Dear IRSS members:

As the 48th Annual SOT meeting is fast approaching, I only have a brief message for our section members. As I stated in the last message, we have only one symposium that was accepted for this year's annual meeting. This could be due to the fact that IRSS no longer has a representative on the SOT Program Committee; John Morris's past presence in the Program Committee is greatly missed. It is my understanding that the SOT President appoints members to the Program Committee. Together with our Executive Committee, I will try to push National SOT to consider having a representative of specialty sections with large memberships, such as ours, consistently represented on this important committee.
With the passage of the economic stimulus package, the research community should be receiving funding announcements in the coming days, including some with a short turnaround time. Everyone should gear up to take the greatest advantage of the opportunities ahead. The establishment of the NIH Special Emphasis Panel on "Systemic Injury by Environmental Exposure" (SIEE) should also enhance our members' chances in gaining more research support.

Finally, it has been an honor and a privilege to serve as the President of this outstanding Specialty Section. Speaking for the Executive Committee, we deeply appreciate the outstanding contributions from the out-going Secretary-Treasurer, Alison Elder, and Councilors Jeff Tepper and Matt Campen. As always, we owe our gratitude to Harry Salem for his leadership on the Technical Committee over all these years as well as his organizing of this newsletter. I fully expect that under the leadership of both Jinkle Seagrave and Vince Castranova, IRSS will continue to grow and make significant contributions to Toxicology.

Until then, I look forward to seeing you all in Baltimore.

Sincerely,
Lung Chi Chen
President

EXECUTIVE MEETING MINUTES

Minutes, IRSS Executive Committee Meeting

March 19, 2008 7 am
Seattle, WA

Present: James Antonini, Deepak Bhalla, Matthew Campen, Vincent Castranova, Lung Chi Chen, Alison Elder, Annette Rohr, JeanClare Seagrave, Jeffrey Tepper, James Wagner
Guests: Flemming Cassee, Miriam Gerlofs-Nijland (RIVM); John Morris (UConn)

1. It has become clear that there is some ambiguity, not in the practice, but in the description of the IRSS **awards selection process**. The process for selecting the career achievement (CA) award is different than for other awards, so this needs careful attention. The executive committee affirmed that *it is the responsibility of the Vice-President to chair and form an ad hoc selection committee for the CA award. This committee will have five members, including the chair, and will be comprised of past IRSS Presidents and past CA awardees*.

In terms of candidacy, the CA award again is somewhat different than the others. *All awards candidates, except for the CA award, must be IRSS members. For the CA award, the purpose is to recognize excellent achievement in the field of inhalation and respiratory toxicology. Although emphasis is placed on membership in IRSS, exceptional outside candidates may be considered.*

2. Questions have also arisen about the distinctions amongst the **student awards**, in particular between the specialty section and the Mary Amdur student awards. The executive committee felt that there should be no impression that the student award is first place and the Amdur award is second place because of the way the selections are made. The Amdur award is given to a candidate whose research focuses more specifically on environmental toxicology and inhalation exposure technology.

Action items: JeanClare will work on the descriptions of all awards as they appear on the SOT website and send to the committee for review, with particular attention to the student awards.

3. We discussed **membership and budget issues**, particularly our sources of income. Concern was expressed that this year's meeting was more costly than last year's. We discussed international collaborations (e.g. RIVM) for sponsorship of meetings or awards.

Action item: Alison will contact SOT HQ about specialty section income so that a firmer budget can be reported.

4. The strategic plan for the SOT includes the aim to be more vocal in the public arena. Concern has been voiced by IRSS members regarding the **ozone NAAQS** that was recently released by US EPA. At issue is that the recommendations of the CASAC were ignored (see also minutes, technical committee). The executive committee discussed the possibility of sending a letter to EPA; however, most felt that such a letter needs to come from SOT Council. John Morris and Rogene Henderson (CASAC chair) drafted a letter about the use of science in policy decisions, on which the committee commented. By common consent, the committee decided that the edited letter will be forwarded to Rogene, Deepak, Dan Costa, and SOT Council.

5. The idea of having an IRSS **postdoctoral student representative** was discussed. This issue was tabled until we receive a formal proposal from student association.

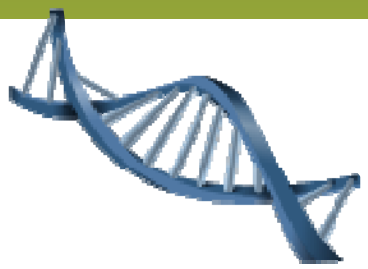
6. Leah Mitchell's nomination as the next IRSS **student representative** was moved, seconded, and passed by a unanimous vote of the councilors.

Action item: Lung Chi will inform Leah and Christina Hickey of the committee's decision.

The meeting was adjourned at 8:30 am.

Respectfully submitted,
Alison Elder

Agenda of Technical Committee Meeting



Tues March 17, 7-8:30am Baltimore Convention Center (Rm 345)

7:00 Meeting open
7:10 Status of OECD Guidelines—John Whalan (EPA-IRIS)
7:30 Questions & answer session
7:40 Comparative arrays of Inhalation Health Effects Reference Values –George Woodall (EPA-NCEA)
8:00 Questions & answer session
8:10 Repeated Inhalation Studies with Aerosols –Juergen Pauluhn
8:20 Questions & answer session

Minutes, Technical Committee Meeting

March 18, 2008 7:30 am
Seattle, WA

Present: Deepak Bhalla, Rogene Henderson, John Morris, Jürgen Pauluhn, John Whalan, George Woodall, Jan Moser, Alison Elder (IRSS sec'ty/treas)

George Woodall discussed the inclusion of genomics and proteomics data in the risk assessment process. This discussion was expanded to focus more generally on the development of a broad agency database for risk assessment purposes:

The idea of developing this database is the product of several interagency meetings and discussions. The idea is to create a response database, similar to an SAR database, so that users can access omics data from various studies.

There was a session at the SOT meeting on Tuesday afternoon that was intended to publicize the project and get input from the toxicology community. Dr. Woodall participated as a presenter in this session.

It would certainly be helpful if several agencies could share toxicological data. An example that was given was the use of the real data from the database to assess the performance of the C x T dose-response protocol.

Across-agency access is estimated within ~2.5 years, with targeted public access to follow shortly thereafter.

The OECD shared database needs to be updated (most recent literature is from 1995) and does not include omics data.

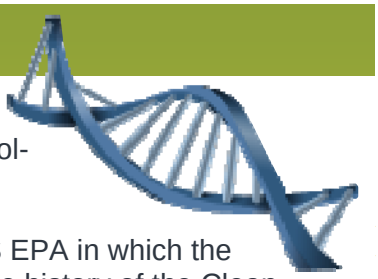
Technical Guidelines (TG) Updates:

TG433 – Issue is death as an endpoint in a toxicological study. The UK guideline states that evident toxicity instead of mortality be used as an endpoint, which can leave some uncertainty regarding dose- and time-response characterizations. TG433 was proposed as a compromise, stating that moribundity (death as a certainty) be used as an endpoint. It was, however, withdrawn when the UK guidelines reverted to evident toxicity.

TG436 – This was developed in Germany and is intended as a compromise between TG403 (LC_{50} testing guideline) and TG433. The hope is that acceptance will grow for the use of a modified C x T protocol, the purpose of which is to classify and label compounds during toxicological assessments. Such a protocol is described in TG436.

TG412, 413 – Describe guidelines for subacute (20 day) and subchronic (90 day) studies and will go to OECD soon for approval.

TG451, 452, 453 – These guidelines for carcinogenicity, chronic, and chronic carcinogenicity studies are being developed, mainly for oral exposures, but also for other exposure routes.



There was also some discussion about the development of TG for histopathology.

Lastly, Dr. Henderson raised concerns about a recent NAAQS review by the US EPA in which the agency failed to incorporate the advice of the CASAC. This is the first time in the history of the Clean Air Act that this has occurred (see also minutes, IRSS Executive Committee). Dr. Henderson proposed that IRSS write a position paper on the use of science and only science in policy decisions. Dr. Morris proposed that this might better be addressed by the Society as a whole. Drs. Henderson and Morris drafted a letter to be considered by the IRSS executive committee.

Respectfully submitted,

Alison Elder

Update of OECD-GD39: Repeated Exposure Inhalation Studies with Aerosols – Considerations on Particle size

John Whalan

The team charged with the guidance document OECD GD39 for TGs #403, #436, #412, and #413 considered an adoption of the preferable range of MMADs for repeated inhalation exposure studies. These recommendations focus on poorly soluble particulates. The reasoning for this modification is as follows:

1. The focus of inhalation studies is to optimize/maximize dosing of the lung. This requires that aerosols be tested in bioassays in a manner that optimizes deposition in the rodent lung. For particles in the range of 4 µm or greater, the majority of effects may be attributed to upper respiratory tract deposition. A higher degree of pulmonary deposition relative to upper respiratory tract deposition appears to be better achieved using MMADs in the range of ≈1 µm (GSDs up to 2).
2. High upper respiratory tract deposition is often accompanied by laryngeal changes (e.g., epithelial metaplasias at the ventral aspects of the rats' larynx) in the absence of irritation-related changes in the nasal passages and trachea proximal or distal to this location. This is due to the inertia of larger particles (due to the laryngeal jet) as well as the specific anatomical arrangement of the upper airways and the larynx. These features render obligate nasal breathing rodents not only particularly sensitive to larger particles it also complicates the interpretation of results in regard to human significance. Experimentally, larger aerosols have multiple other disadvantages in inhalation toxicology (e.g., anisokinetic sampling problems due to inappropriate aspiration efficiencies, loss of particles in tubing and/or dilution systems, toxicity due to dermal contact; overloading of sensitive particle detection equipment). Many of these problems can be readily overcome by optimizing aerosol size distributions towards an MMAD of ≈1 µm instead of ≈3 µm.
3. Hence, in order to maximize pulmonary deposition and to minimize extrathoracic deposition, aerosols in repeated inhalation toxicity studies should be optimized to the bioassay (species) used. In repeated exposure inhalation studies in rats, this objective appears to be achieved best by using aerosols with an MMAD in the range of 0.1-2 µm and a GSD of equal to or smaller than ≈2. Ideally, MMADs in the range of 0.5-1 µm may be most suitable to attain cumulative lung burdens of poorly soluble particles in the absence of an unbalanced overloading of the upper airways in obligate nasal breathing species, such as the rat.
4. Based on the deliberations of the GD39 drafting team the following recommendation is given: For substances not accumulating over time in the lung, lung burdens are not a concern. Absorption of aerosol may not necessarily be restricted to the pulmonary region alone. To prevent overestimation of toxicity to occur due to high upper respiratory tract deposition of aerosol, an MMAD range of 1-3 µm/GSD 1.5-3.0 is generally considered adequate. However, an MMAD range of 0.1 to 2 µm/GSD 1.5-2 should be given preference when technically possible and when "toxic effects by inhalation" can be shown.

Comparative Arrays of Inhalation Health Effects Reference Values

George M. Woodall, Jr., PhD

The US EPA's National Center for Environmental Assessment (NCEA) has undertaken a project to standardize the development of arrays that compare inhalation health effect reference values (i.e., RfCs, AEGLs, etc) across durations, populations (e.g., general public vs. healthy workers), and intended use (e.g., public health protective vs. emergency response vs. repeated occupational vs. occupational ceiling values). A number of program offices within the Agency, as well as other agencies, have an interest in having these types of arrays available. The audience for these arrays and accompanying documentation includes risk assessment professionals, decision makers (risk managers), and the general public. Introductory text will need to be provided with all arrays to provide an adequate foundation for understanding the arrays, to enable an appropriate comparison of the displayed reference values, and to clearly indicate that the various reference values are not "one-size-fits-all." Tables will also be provided that include the numerical values, along with the details on derivation of the values (i.e., critical study[ies], point of departure, uncertainty factors, duration extrapolations, etc). Examples of these comparative arrays, accompanying tables, and the plans for this project will be discussed during the Technical Committee Meeting of the Inhalation and Respiratory Specialty Section, which will be held in conjunction with the annual SOT Meeting in Baltimore on Tuesday morning, March 17. [This presentation does not necessarily reflect EPA policy.]

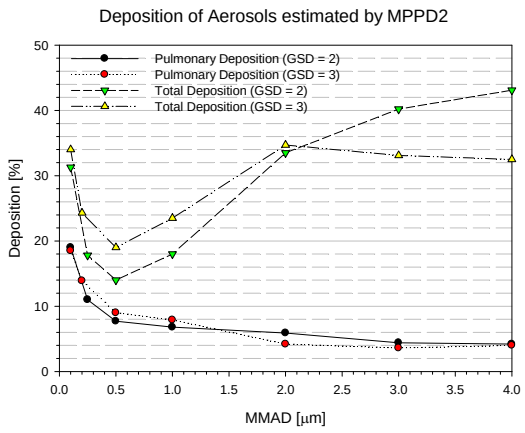
Repeated Inhalation Studies with Aerosols – Considerations on Particle size

Juergen Pauluhn

There are two major objectives of repeated exposure inhalation studies. The first objective is to evaluate the portal-of-entry specific toxicity of inhaled aerosols for substances not bioavailable to any appreciable extent by non-inhalation routes. The absorption of these substances is limited via other routes because they may decompose in the gastrointestinal tract or are of limited solubility and bioavailability. The second objective is to evaluate substances with hepatic and other types of first-pass metabolism which may be more or less toxic by inhalation depending on whether metabolism results in toxifying or detoxifying. Repeated exposure studies are suitable for revealing and quantify these differences in toxic potencies.

The focus of inhalation studies is to optimize/maximize dosing of the lung. This requires that aerosols be tested in bioassays in a manner that optimizes deposition in the rodent lung. As illustrated in Fig. 1, when the MMAD is 4 µm or greater, the majority of effects may be attributed to upper respiratory tract deposition (especially when there is absorption into the systemic circulation). The objective of the test is better achieved using MMADs in the range of ≈1 µm.

Figure 1: Deposition efficiencies in the entire (total) respiratory tract and pulmonary region as a function of aerodynamic particle size. Deposition efficiencies were calculated using the MPPD2 model.'



High upper respiratory tract deposition is often accompanied by laryngeal changes (e.g., epithelial metaplasias at the ventral aspects of the rats' larynx) in the absence of irritation-related changes in the nasal passages and trachea proximal or distal to this location. The apposition of the epiglottis to the soft palate in the resting condition isolates the oral cavity from the respiratory airways and makes the rat an obligatory nose breather (virtually no oropharynx). Thus, the direction of flow in rats is almost linear whereas in humans it is rectangular (for more details see DeSesso, 1993). It appears that the inertia of especially larger particles (due to the laryngeal jet) as well as the specific anatomical arrangement of the upper airways and the larynx renders obligate nasal breathing rodents particularly sensitive to larger particles as exemplified in Fig. 2. Experimentally, larger aerosols have

multiple other disadvantages in inhalation toxicology (e.g., anisokinetic sampling problems due to inappropriate aspiration efficiencies, loss of particles in tubing and/or dilution systems, toxicity due to dermal contact; overloading of sensitive particle detection equipment). Many of these problems can be readily overcome by optimizing aerosol size distributions towards an MMAD of ≈1 µm instead of ≈3 µm (Fig. 2).

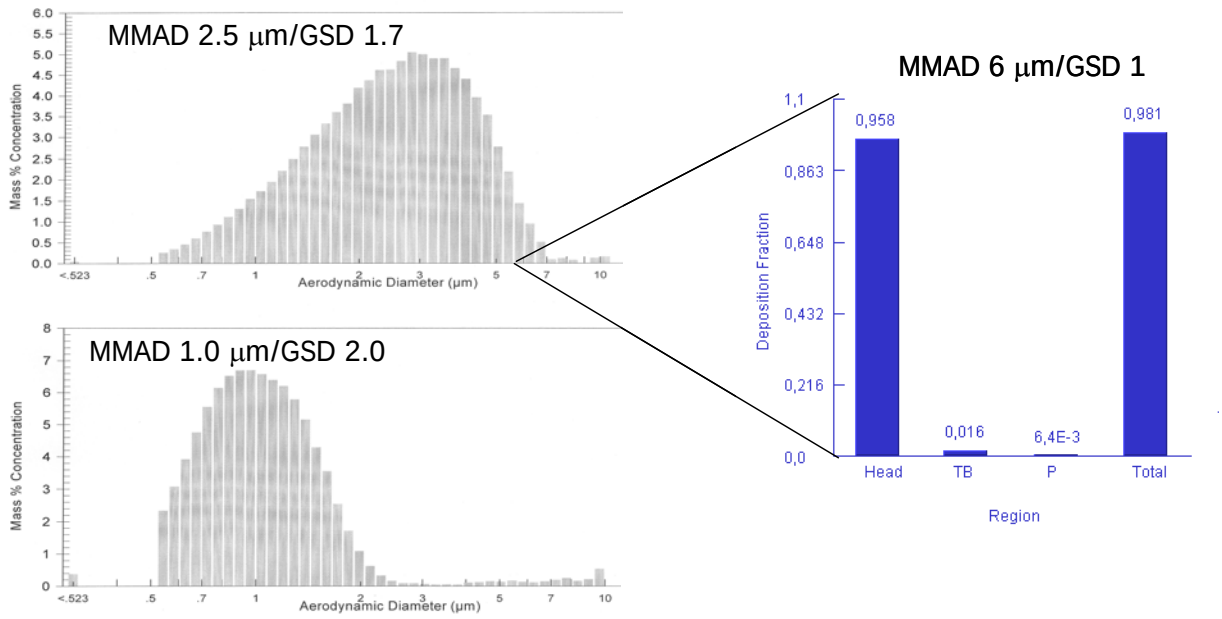


Figure 2: Aerodynamic particle size at an MMAD and GSD frequently used in repeated inhalation studies (MMAD 2.5 µm / GSD 1.7) and following aerodynamic optimization (cyclone).

Although particle size distributions can be readily optimized toward smaller particles, the trade-off is that the loss of larger particles required to achieve a smaller MMAD invariably results in a decrease in concentration. Therefore, especially for repeated inhalation toxicity studies, the MMAD must be seen in context with pulmonary dose.

For innocuous, poorly soluble particles, the “MTD”, due to their lack of systemic toxicity, adversity is commonly defined based on fate and pulmonary inflammation which occur at overloading conditions. Based on 4-week inhalation studies with poorly soluble powders (specific density 3-5 g/cm³) a clear interrelationship of lung burden-dependent increase in clearance due to lung overload could be demonstrated (Fig. 3). Likewise pulmonary inflammation, phenotypically evidenced by increased PMNs in bronchoalveolar lavage (BAL), correlated also with the mass-based pulmonary burden of particulate matter (Fig. 3). Inhibition of macrophage-mediated clearance is estimated to start at ≈60 µm³ per alveolar macrophage or at lung burdens of ≈1 mg particles/g lung or greater (Morrow, 1988, 1992). The elimination half-time for alveolar clearance in the non-overloading state (rats) has been reported to be in the range of 50-65 days.

Based on the interrelationship of overload, delayed clearance, and pulmonary inflammation, rats exposed to such particles should accumulate lung burdens up to levels which do not cause a delay in clearance or elicit pulmonary inflammation (0.4-0.6 mg PM/lung), which cause minimal effects (prolongation of particle clearance from $t_{1/2}$ ≈50 to ≈70 days; PMN influx of ≈5-10%) slightly above the overload threshold (2-3 mg PM/lung), and unequivocal effects (prolongation of particle clearance from $t_{1/2}$ ≈50 to ≈150 days; PMN influx of ≈20-25%) approximately 10-times above the overload threshold (10-12 mg PM/lung). This spacing of lung burdens provides a means to examine differences in toxic potencies relative to lung overloading.

Figure 3: Toxicokinetic and toxicodynamic effect parameters as a function of lung particulate matter (PM) burden. Open symbols represent primary particles in the 10 nm range. All lung burdens represent actually measured data.

The lung burdens depicted in Fig. 3 are translated to exposure concentrations to be used in 4- or 13-week inhalation studies at four different MMADs (0.5, 1, 2, and 4 µm / GSD 2) (Fig. 4). The simulated lung bur-

dens demonstrate that in regard to pulmonary dosimetry, the objectives can be met best with aerosols having MMADs in the range of 0.5 to 2 µm while potential for pulmonary "underdosing" prevails at higher MMADs. Indeed, the lower lung burden at higher MMADs can be compensated for by higher concentrations. However, this may shift aerosol distributions to even larger particles with increased probability of upper respiratory tract / laryngeal side effects which can be difficult to translate to human toxicological significance.

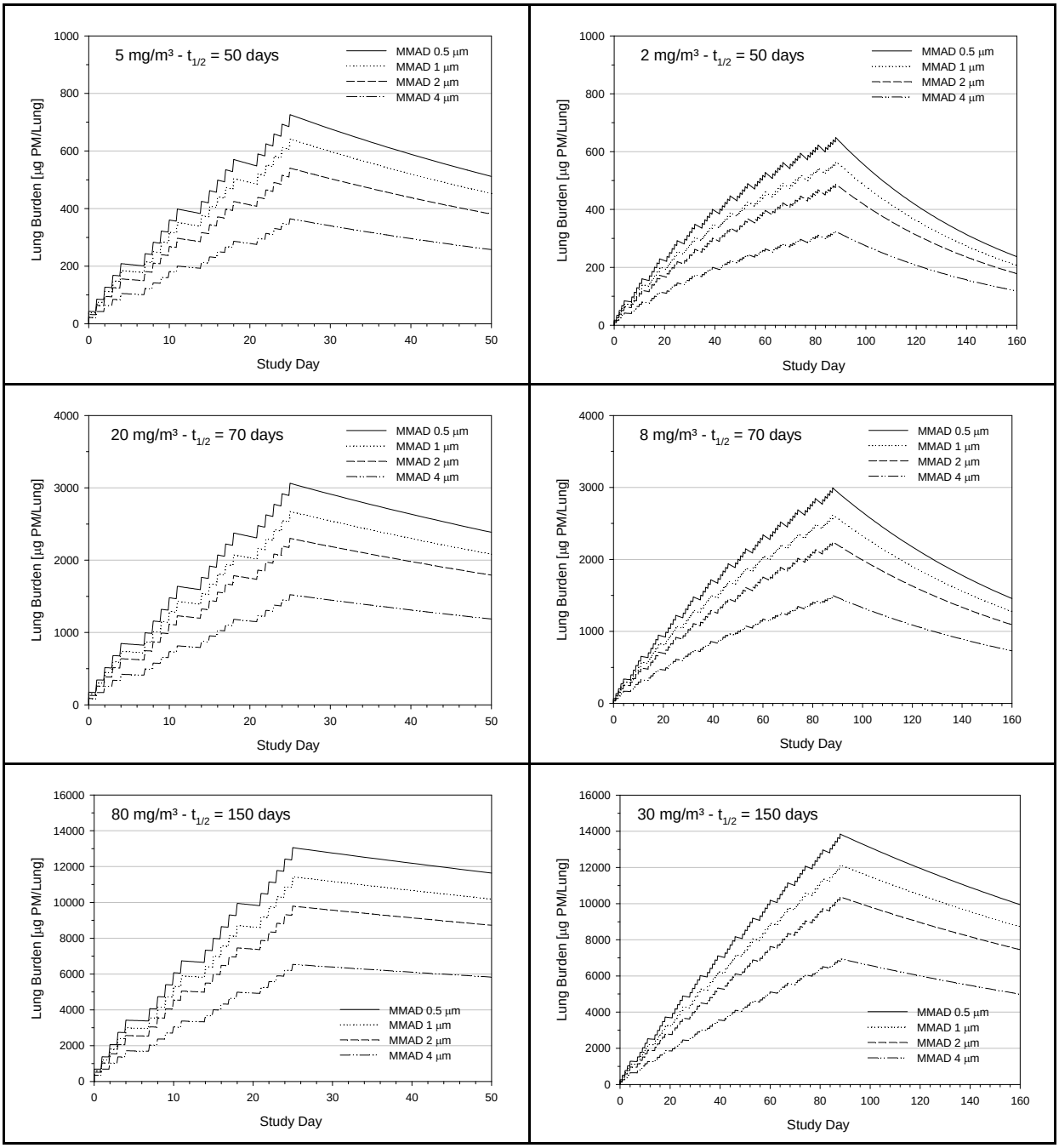


Figure 4: Toxicokinetic parameters as a function of MMAD (GSD 2) and inhalation chamber concentrations for rats exposed 6 h/day on 5 consecutive days/week. Pulmonary deposition efficiencies of aerosols were calculated by the MPPD2 model.

Summary and Conclusion

In order to maximize pulmonary deposition and to minimize extrathoracic deposition, aerosols in repeated inhalation toxicity studies should be optimized to the bioassay (species) used. In repeated exposure inhalation studies in rats, this objective appears to be achieved best by using aerosols with an MMAD in the

range of 0.1-2 µm and a GSD of equal to or smaller than ≈2. In case adequate pulmonary dosing well above the overloading threshold of poorly soluble particulates (≈1 mg/g lung) can be demonstrated, MMADs in the range of 2-3 µm may also serve the objective of the test. However, this may lead to an unbalanced overloading of the upper airways in obligate nasal breathing species, such as the rat. Such findings may be difficult to translate to similar human responses.
For substances not accumulating over time in the lung (i.e., systemically acting, soluble substances), lung burdens are not a concern because they are rapidly cleared from the lung. Absorption of aerosol may not necessarily be restricted to the pulmonary region alone. To prevent overestimation of toxicity to occur due to high upper respiratory tract deposition of aerosol, an MMAD range of 1-3 µm/GSD 1.5-3.0 is generally considered adequate. However, an MMAD range of 0.1 to 2 µm/GSD 1.5-2 should be given preference when technically possible and when “toxic effects by inhalation” can be shown.

National Institute for Public Health and the Environment (RIVM). Multiple Path Particle Dosimetry Model (MPPD2 v. 1.0): A Model for Human and Rat Airway Particle Dosimetry. Bilthoven, The Netherlands.

Anjilvel, S. and Asgharian, B. (1995). A multiple-path model of particle deposition in the rat lung. Fundam. Appl. Toxicol. **28,** 41-50.

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Morrow PE. (1988). Possible mechanisms to explain dust overloading of the lungs. *Fundam. Appl. Toxicol.* **10**, 369-84.

Morrow PE. (1992). Dust overloading in the lungs. *Toxicol. Appl. Toxicol.* **113**, 1-12.