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Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies

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Background:

Particle size and limit concentration requirements have been a major point of misunderstanding and contention within the Agency, and with registrants and laboratories who must meet our requirements. A recent examination of the OPP Guideline Rejection Factors revealed that of 754 acute inhalation toxicity studies reviewed between 1985 and 1991 (inclusive), 131 (17.4%) were rejected. Of those rejected, 55% failed to meet the criteria for having 25% of the particles <1 μm. There was an unknown number of other studies that also failed to meet this criteria, but were nevertheless accepted by the reviewers. In addition, 29% of the rejected studies could not define an LC_{50} and/or failed to achieve a limit concentration.

In 1991, HED requested public comments on its Inhalation Guidelines. These critiques, along with the results of recent interviews with several inhalation toxicologists, indicate these issues are universal concerns. Of the 9 responses, 9 considered the particle size criteria (25% <1 μm) to be unrealistic, and 6 considered the 5 mg/l limit concentration to be excessive (3 had no comment). Their recommendations are summarized below:

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Recommended Particle Size

1 μm MMAD	2-3 μm MMAD	3 μm MMAD	1-4 μm MMAD	Relax Requirement	Define by Species
Acute Studies					
	h	e	a,b,f*,g,h,i	c	d
Subchronic Studies					
	h	e,f†	a,b,i	c	d

Recommended Limit Concentration

0.5-1 mg/l	2 mg/l	5 mg/l	5 mg/l is unrealistic
Acute Studies			
g	a,f,i		b,d

a = National Agricultural Chemical Association (NACA)
b = Harry Salem, Aberdeen Proving Grounds, U.S. Army
c = Jellinek, Schwartz, Connolly & Freshman, Inc.
d = Wil Research Laboratories, Inc.
e = ICI Agricultural Products
f = CIBA-GEIGY
g = Lilly Research Laboratories
h = Hsu-Chi Yeh, Lovelace Inhalation Toxicology Research Institute
i = Kenneth Nitschke and Richard Corley, The Dow Chemical Company

* CIBA-Geigy also recommended that an MMAD of up to 10 μm should be acceptable in those situations where the physical characteristics of the test material prevent reducing particle size any further.

† CIBA-Geigy suggested that having >90% particle mass less than 5 μm and 50% less than 3 μm is more appropriate.

There are currently three documents available to registrants, laboratories, and HED reviewers which describe the conduct and interpretation of inhalation toxicity studies:

1. **Subdivision F Guidelines (1984)**

2. **Hazard Evaluation Division Standard Evaluation Procedure: Inhalation Toxicity Testing,** (EPA-540/09-88-101; August 1988) written by Stanley B. Gross and Frank J. Vocci.

3. **Comments on Standard Evaluation Procedure. Inhalation Toxicology Testing (SEP/Inhalation),** a memorandum from Stanley B. Gross (April 18, 1989), which added, "...some historical clarifications concerning particle testing sizes and the limit testing which have apparently caused some confusion with testing requirements."

These documents convey the following guidance regarding particle sizes and limit testing:

Aerodynamic Particle Sizes

Subdivision F Guidelines (1984):

The Guidelines do not offer any direction on particle sizes.

HED Standard Evaluation Procedure (1988):

"It is possible to generate chamber aerosols of high concentrations with particles that are so large that very few will gain access to the pulmonary system during the test procedures. It is important that the aerosol particle sizes are small enough that the inhaled particles can reach the deeper portions of the lung, that is the alveoli." [Page 14, paragraph 1]

"It would seem appropriate that at least 25% of the particle distribution used in these studies should be in the submicron range for acute and repeat exposure studies."

"When studies are carried out using large particle distributions (median diameters greater than 3.0 μm), judgment is necessary in determining whether the study should be repeated using a smaller particle size range. If the chemical proved quite toxic (Toxicity Category I, 40 CFR 162.10), no further acute testing is necessary as the chemical will already require the strictest labeling. If the test results show minimal toxicity by the inhalation route while showing significant toxicity via other routes, then the acute inhalation testing should be repeated using smaller particle sizes." [Page 15, paragraphs 3 & 4]

Memorandum from Stanley B. Gross (1989):

> "If the mass median aerodynamic diameter reported in a study is larger than 1 μm, we can accept the study if at least 25% of the particles are 1 μm or less. If the laboratory is having difficulty in achieving the required diameters, the study needs to indicate what they did and why they were unable to provide the small particles." [Page 2, item B]

HED considered the lungs to be the target organ in inhalation studies. A respirable particle was defined as having an aerodynamic diameter <1 μm, both in humans and laboratory animals. Since most pesticide toxicity studies describe MMAD values significantly greater than 1 μm, the 25% criteria has been applied to nearly every study. The upper airways were essentially disregarded, even though this is the most likely region of "real-world" exposure in humans.

Limit Concentration Testing

Subdivision F Guidelines (1984):

> "If a test at an exposure of 5 mg/l (actual concentration of respirable substances) for 4 hours or, where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration, produces no compound-related mortality, then a full study using three dose levels might not be necessary." [Page 51, item (3)(g)]

HED Standard Evaluation Procedure (1988):

> None

Memorandum from Stanley B. Gross (1989):

> "The limit test usually applies to the acute 4 hour inhalation test. This limit is set at the Toxicity Category IV in which the material would be considered to have minimal adverse effects during an acute exposure."
>
> "In order to favor a reduced use of animals during toxicity testing, the Agency has suggested the use of limit test (when such a test seems appropriate). If deaths are seen during the limit test, a full LC50 test as described in the Guidelines is still required. However, a number of registrants have used the limit test as the only test, as a 'yes/no test' and usually at levels below the 5 mg/L concentrations. This does not fulfill the testing requirements for this guideline."
>
> "Further, the limit test can be carried out at the maximum attainable concentration. A number of registrants have reported test results from a limit test

at concentrations below 5 mg/L which did not cause any deaths. The concentration was reported as a maximum attainable concentration without any documentation to support this conclusion. This has not been accepted. In order to declare the concentrations as the maximum attainable, the registrant needs to indicate what efforts were made to reach the 5 mg/L concentrations, what problems were encountered and, if possible, try to explain why higher concentrations were not achievable." [Page 2, item C]

This guidance acknowledges that submicron particles and limit concentrations cannot always be achieved. Although some contingencies are provided, experience has shown that a hard line has generally been applied by HED in judging the adequacy of studies, perhaps because the guidelines were not fully understood. This has complicated the performance and interpretation of inhalation toxicity studies for the following reasons:

- Studies have been rejected if the limit concentration could not be attained, or if fewer than 25% of the particles were <1 μm. Consequently, laboratories have often undertaken costly, difficult, and time consuming efforts to satisfy HED's rigorous requirements.

- Inhalation toxicity laboratories may find it impossible to generate a sufficient quota of submicron particles while trying to achieve a limit concentration of 5 mg/l. It may be impossible to mill a solid material to a submicron size. As nebulizers are pushed to higher output levels, particles become bigger and they are more likely to agglomerate. Depending on the nature of the test article, it can be impossible to generate submicron particles even at low concentrations.

- When a laboratory can only generate 5 or 10% of the particles <1 μm, they are left with the task of convincing an HED reviewer that smaller particles could not be generated. The HED reviewer must decide whether to accept the laboratory's explanation or request another study. This decision is complicated by Agency policy which urges the acceptance of less-than-adequate studies to avoid wasting life.

- Aerodynamic particle sizes are presented in two ways - particle size distribution, which reports the percentage of particles deposited in each stage (size range) of a cascade impactor; and the mass median aerodynamic diameter (MMAD) which represents the range of particle sizes as a median value with a geometric standard deviation (σ_g). If a study report gives the MMAD (e.g. 2.8 μm), but lacks distribution data, there is no way of knowing whether 25% of the particles were <1 μm.

- If <u>any</u> animals die in a limit test, current guidelines require a repeat study using 3 concentrations. Actually, if the mortality data are adequate to define a Toxicity Category, a repeat study becomes a waste of animals and resources.

- Many studies have been rejected because no mortality was seen at the maximum attainable concentration, simply because that concentration was less than 5 mg/l.

- The limit concentration for a formulation has never been defined. If, for example, a formulation contains 10% active ingredient dissolved in 90% xylene vehicle, the 5 mg/l limit concentration could conceivably be for the active ingredient alone, or for the active and inert ingredients combined. The fallacy in measuring only the active ingredient is that the test would mimic a study of the technical, except that the test animals would additionally be exposed to a huge quantity of the vehicle. This could make an inherently less toxic formulation appear more toxic than the technical.

- The 4-hour, 5 mg/l limit test bears no resemblance to human exposure. This concentration results in an aerosol cloud so dense that in-chamber observations may be impossible. During whole-body exposure, the animal's fur will be coated with dust or soaked with liquid. If airways become physically clogged, death by suffocation may be misconstrued as toxicity. If a human were accidentally exposed to such a high concentration, it would probably be for a matter of seconds or minutes.

On September 6, 1991, a contingent representing the Society of Toxicology (SOT), Inhalation Toxicology Specialty Section, and the National Agricultural Chemical Association (NACA) Toxicology Roundtable met with HED representatives to present a consensus position paper entitled, ***SOT Inhalation Specialty Section Position Paper - Recommendations for the Conduct of Acute Inhalation Limit Tests***. This document was written by the Technical Committee of the Inhalation Specialty Section (G.L. Kennedy, J.B. Morris, M.V. Roloff, H. Salem, C.E. Ulrich, R. Valentine, and R.K. Wolff), and approved by the Executive Committee. This paper was later published as a Commentary in *Fundamental and Applied Toxicology*. The abstract is reproduced below:

> ***Recommendations for the Conduct of Acute Inhalation Limit Tests, Prepared by the Technical Committee of the Inhalation Specialty Section, Society of Toxicology.*** Fundamental and Applied Toxicology. Volume 18. Pages 321-327. 1992.
>
> "This paper reviews the scientific issues related to exposure concentration and particle sizes used in acute inhalation limit tests. The current United States Environmental Protection Agency (USEPA) recommended exposure concen-tration for such tests is 5 mg/liter; while this level is very high, it is often achievable. On the other hand, its toxicological relevance is questionable. The USEPA recommendation that 25% of the particle distribution be less than 1 μm is a more difficult issue to address. Physical laws for aerosol particle generation and behavior limit the minimum size of particles in an exposure atmosphere at a concentration of 5 mg/liter. Particle size also influences deposition site in the respiratory tract. Since damage to any region of the respiratory tract can produce lethality, and it is not possible to predict, *a priori*, the most responsive region of the tract or the most harmful particle size of an untested agent, acute limit testing should employ particles

in a size range that deposits throughout the entire rodent respiratory tract. Particles between 1 and 4 μm mass median aerodynamic diameter (MMAD) are well suited for such studies. It is, therefore, recommended that the limit test concentration should be the highest concentration (up to 5 mg/liter) that can be achieved while still maintaining a particle size distribution having an MMAD between 1 and 4 μm."

Based on findings by Mauderly *et al.*, 1987; Raabe *et al.*, 1988; and USEPA, 1982, the SOT has recommended accepting acute studies with MMAD's of 1-4 μm for the following reasons (page 322, last paragraph):

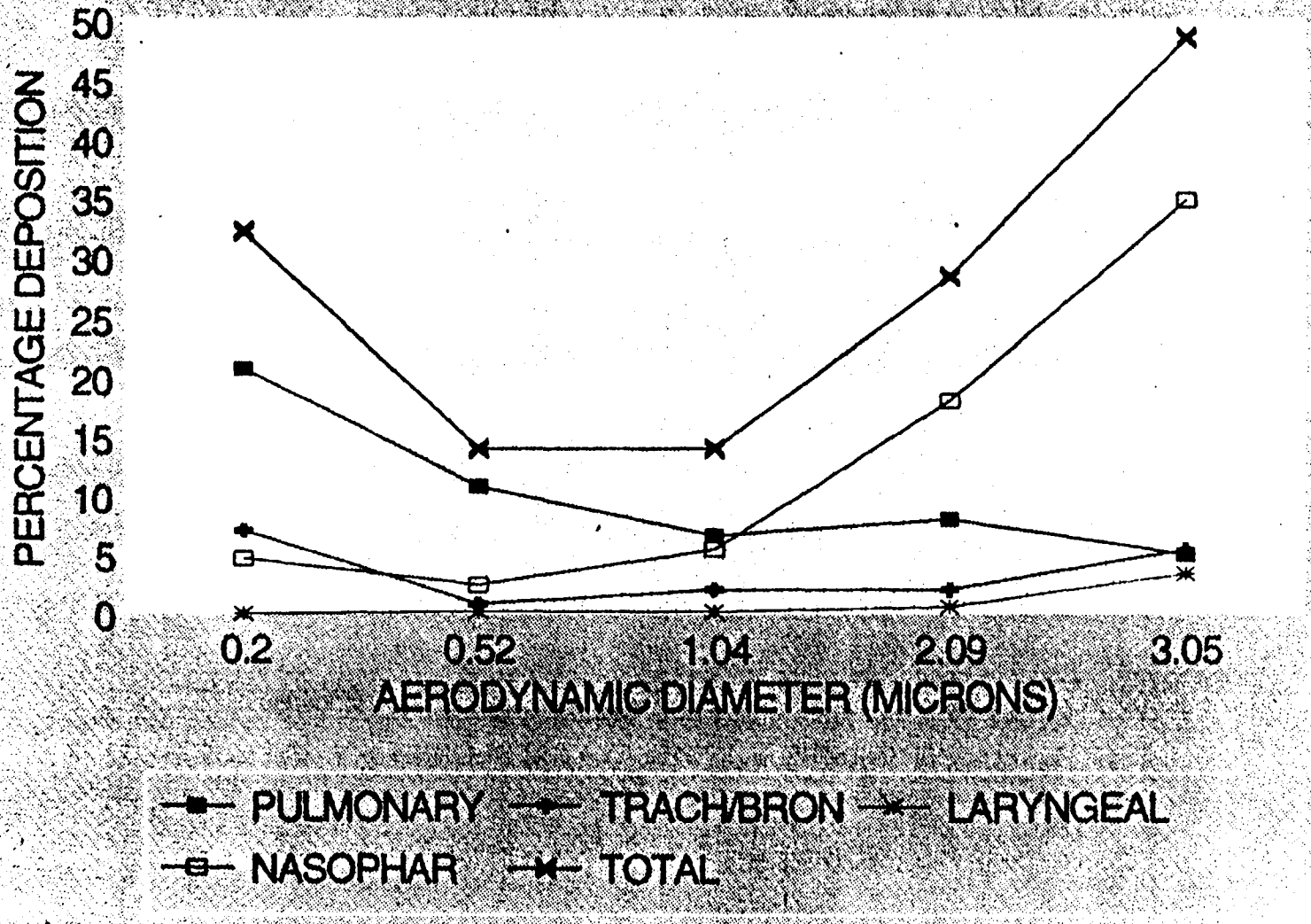
"...inhaled particles between 1 and 4 μm MMAD will deposit within all regions of the rat respiratory tract. Within this size range, nasopharyngeal and tracheo-bronchial deposition increase as particle size increases, but pulmonary deposition remains relatively constant. Based merely on pulmonary region deposition efficiencies, 1 μm particles offer no distinct advantage over 4 μm particles. Thus, because 1-4 μm particles will likely deposit in all regions of the respiratory tract, this size range is highly desirable for acute limit testing."

Pesticide aerosols contain polydisperse particles (geometric standard deviation $\sigma_g > 1.2$) which can be deposited to varying degrees throughout the respiratory tract. As particles are inhaled, a portion is deposited in the airways, and the balance is exhaled. A particle's aerodynamic diameter determines where it is most likely to be deposited in the respiratory tract. The deposition mechanisms include impaction (inertial deposition), sedimentation (gravitational deposition), Brownian diffusion, interception, and electrostatic precipitation. (Schlesinger, 1985)

These mechanisms are further affected by breathing patterns and respiratory tract anatomy. Deposition increases in the upper respiratory tract during rapid breathing. Deep lung deposition increases during slow, deep breathing, and during oral breathing in humans. Particle impaction in the convoluted nasal turbinates of small animals results in highly efficient nasal capture. The major difference in lung anatomy between humans and small animals is in airway branching. Humans tend to have a regular dichotomous pattern in which an airway gives rise to two branches, equal in diameter and length, that branch off at equivalent angles. Small animals have irregular dichotomous patterns in which the two branches differ in diameter, length, and departure angle. (Schlesinger, 1985)

Recent studies with radiolabelled particles have clarified the correlation between aerodynamic particle sizes and deposition sites. Raabe *et. al.* (1977) studied anesthetized Long Evans rats and Syrian hamsters exposed nose-only to ^{169}Ytterbium-labelled monodisperse spherical aluminosilicate particles with aerodynamic diameters of 0.2, 0.52, 1.04, 2.09, and 3.05 μm. Figure 1 depicts total and regional respiratory deposition in the rat (based on report Table 3).

Figure 1: Long Evans Hooded Rats
(Raabe 1977)



This graph shows that the majority of inhaled submicron particles were removed with the exhaled air. Only 7-21% of submicron particles were deposited in the pulmonary region, and 3-12% were deposited elsewhere in the respiratory tract. Total deposition decreased from 32% at 0.2 μm, to 14% at 0.52 μm and 1.04 μm, then increased to 28% at 2.09 μm, and 48% at 3.05 μm. The major deposition site for the larger particles was in the nasopharyngeal region. Pulmonary deposition decreased from 21% at 0.2 μm to a plateau of 5-10% for particles ranging from 0.52 to 3.05 μm. These data demonstrate that minimal respiratory tract deposition and toxicity should be expected with submicron particles unless, as is rarely the case for pesticides, the particles are <0.2 μm.

As particle size increases beyond 1 μm, the number of particles reaching the pulmonary region decreases, but these larger particles have a greater mass. For example, a 3 μm particle has 27-times the mass of a 1 μm particle, and thus 27-times the toxic potential if toxicity is mediated by mass. This explains why the overall percentage of deposition, (i.e. mass) in the pulmonary region is nearly the same whether the particles are 1.04, 2.09, or 3.05 μm.

A newer study (Raabe *et. al.*, 1988) was performed in unanesthetized Fischer 344 rats, golden Syrian hamsters, CF_1 mice, Hartley guinea pigs, and New Zealand rabbits exposed nose-only to ^{169}Ytterbium-labelled monodisperse spherical aluminosilicate particles. Aerodynamic diameters ranged from 0.18 to 10.16 μm with geometric standard deviations <1.3 (submicron particles had slightly greater deviation, but were still considered monodisperse). This study is especially useful since it compares 5 species, and encompasses the range of particle sizes typically encountered with pesticide products.

Nasopharyngeal deposition in **Fischer 344 rats** (Figure 2) dramatically increased from 6.9% for 1 μm particles, to 86.5% for 3 μm particles. Pulmonary deposition gradually decreased from 13.3% for 0.29 μm particles, to 4.8% for 4 μm particles, to nearly 0% for 10 μm particles. Nasopharyngeal and pulmonary deposition in the **golden Syrian hamsters** (Figure 3) resembled that in the Fischer 344 rats.

There are several reasons why the rat nasopharyngeal deposition data from the 1977 and 1988 Raabe studies differ. Two strains were used. The rats in the first study were anesthetized, and thus had different respiratory patterns. They were also incapable of swallowing particles cleared by mucociliary transport.

The **CF_1 mice** (Figure 4) were unique among the 5 species tested with regards to the rapid rise in nasopharyngeal deposition, and the equally rapid drop in pulmonary deposition as particle size increased. Nasopharyngeal deposition rose from 9.7% for 0.27 μm particles, to 42.6% for 1 μm particles, to 87.8% for 3.45 μm particles. Pulmonary deposition, which was 45.4% for submicron particles, dropped to 9.7% for 1 μm particles, and <1% for particles ≥3.45 μm.

Figure 2: Fischer 344 Rats
(Raabe 1988)

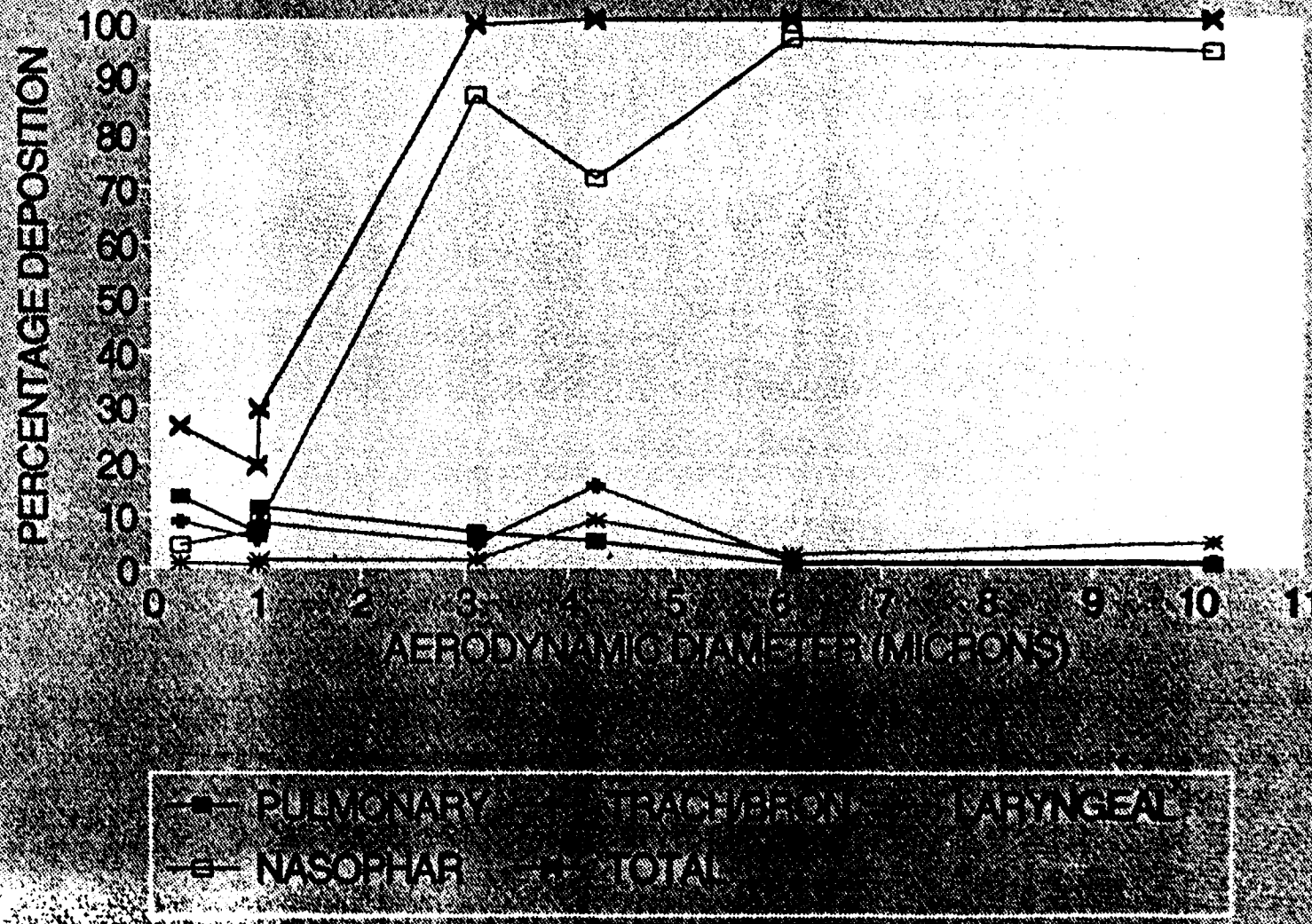


Figure 3: Golden Syrian Hamsters
(Raabe 1988)

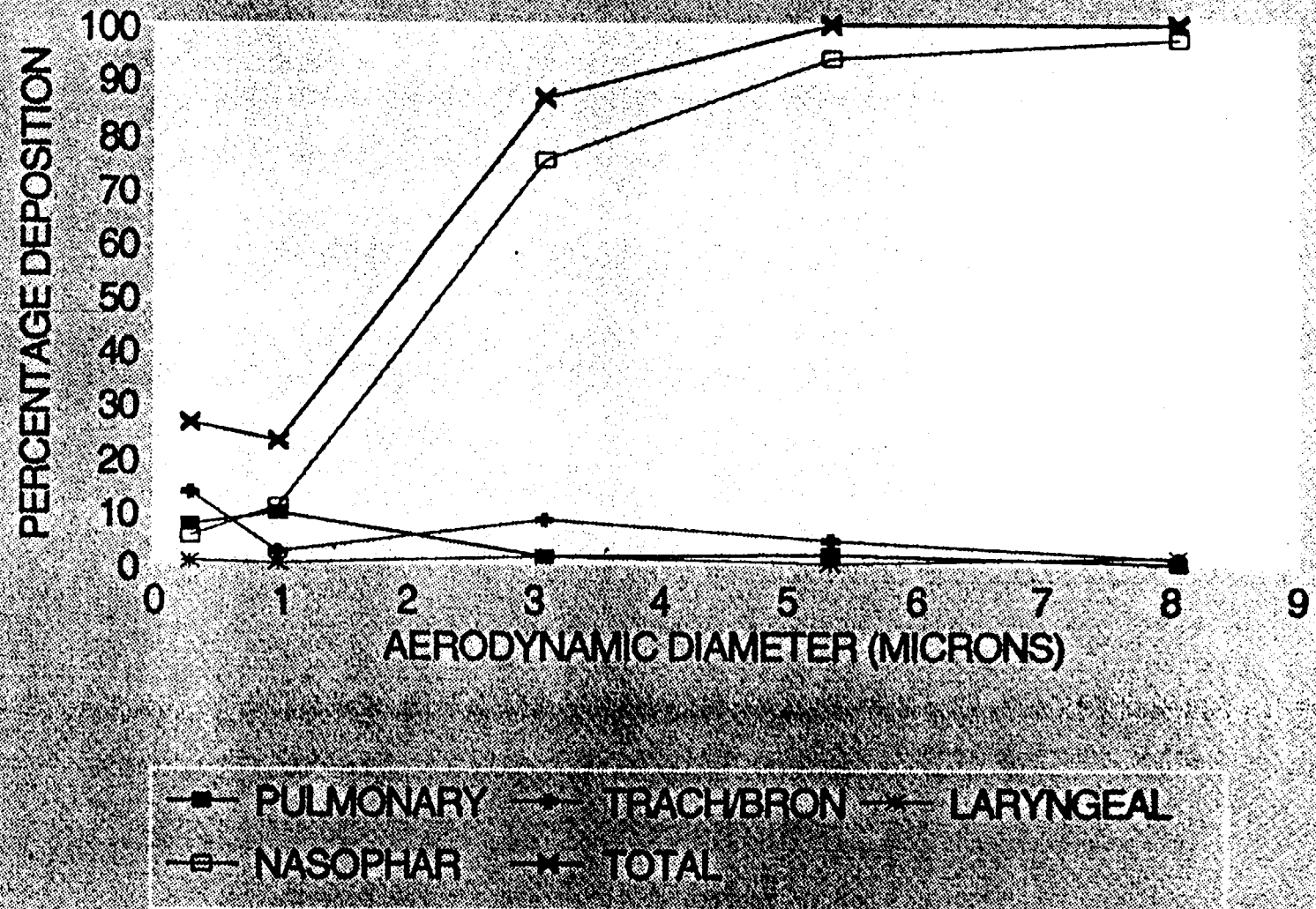


Figure 4: CF1 Mice
(Raabe 1988)

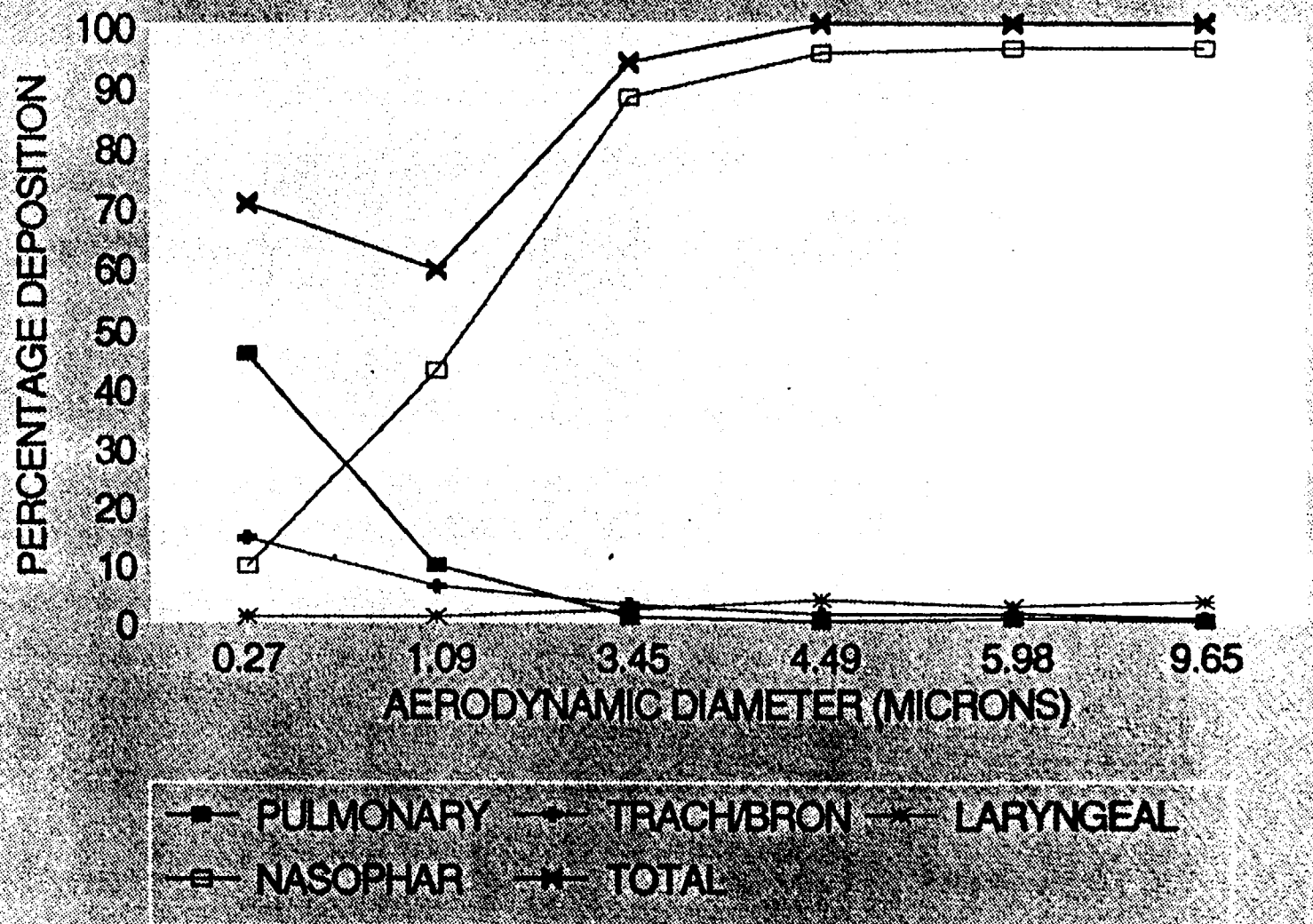


Figure 5: Hartley Guinea Pigs
(Raabe 1988)

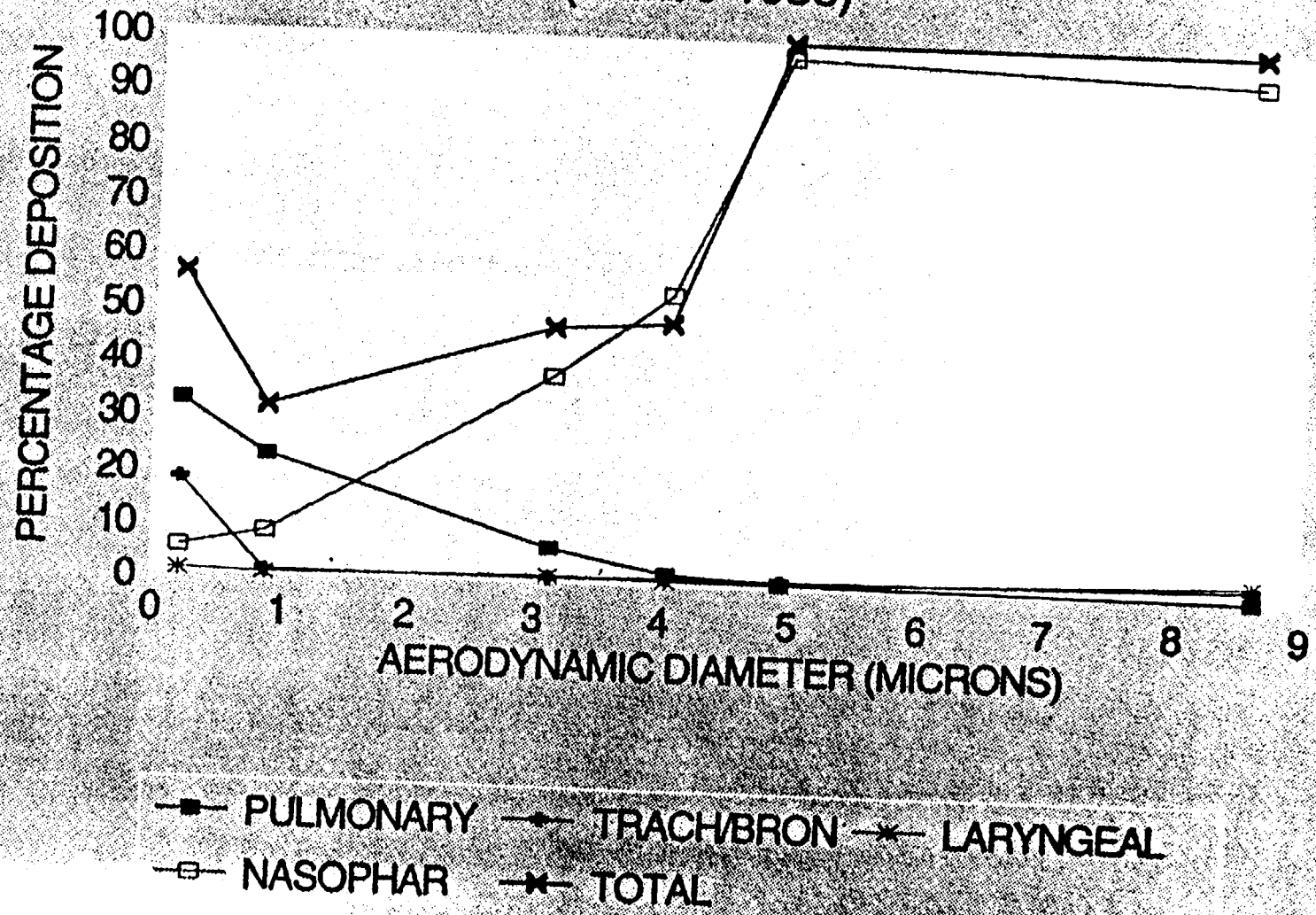
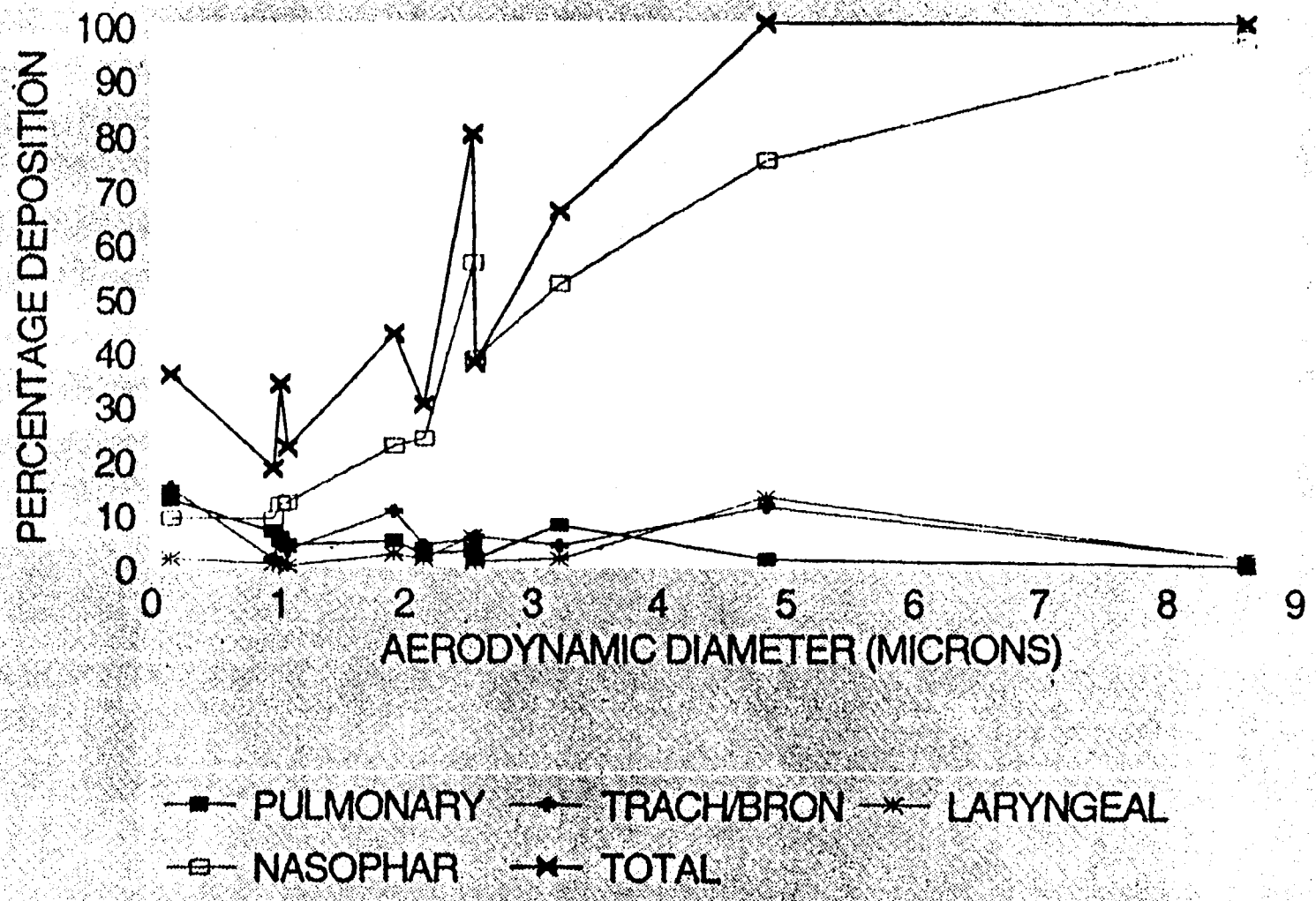


Figure 6: New Zealand White Rabbits
(Raabe 1988)



Nasopharyngeal deposition in the **Hartley guinea pigs** (Figure 5) was only 38.2% for 3 μm particles, and 53.3% for 4 μm particles, but nearly 100% for particles ≥5 μm. Pulmonary deposition gradually decreased from 32.4% for 0.24 μm particles to 22.3% for 1 μm particles, to 6.4% for 3 μm particles.

Nasopharyngeal deposition in **New Zealand rabbits** (Figure 6) increased more gradually than in the other species - 9% for 0.18 μm particles, 22.5% for 2 μm particles, 52.9% for 3 μm particles, 75.1% for 5 μm particles, and 97.2% for 9 μm particles. Pulmonary deposition, which was only 12.7% for 0.18 μm particles, gradually decreased as particle size increased.

These data show that as particle size increases beyond approximately 3 μm in rodents and rabbits, pulmonary deposition sharply decreases due to nasopharyngeal deposition (Raabe, *et. al.*, 1988). These animals, which are dedicated nose-breathers, effectively protect their lungs by capturing large particles in the nasopharyngeal region. In humans, large particles can still reach the lungs because of less efficient capture in the nasopharyngeal region, and mouth-breathing, which bypasses the nose. It is often necessary to compensate for rodent vs. human deposition differences by generating finer aerosol particles (via milling and the use of nebulizers) than would be found in real-world exposure. This artificial situation is necessary to assure deposition throughout the respiratory tract of rodents.

Nearly all particles having aerodynamic diameters >5 μm are deposited in the nasopharyngeal region in rats, compared to >10 μm in humans (Hsu-Chi Yeh, personal communication). Those materials not absorbed in the upper airway are eliminated by sneezing, mucociliary transport, and swallowing (the latter two result in oral exposure).

In order to maximize the percentage of particles reaching the alveoli, HED has always requested submicron particles. Particles with aerodynamic diameters <1 μm are able to reach the alveoli in humans and rodents because they are small enough to avoid inertial impaction in the turbulent air of the upper airways. Particles that are not absorbed through the alveolar walls are slowly removed by macrophages; alveolar lesions may ensue.

According to Dr. Hsu-Chi Yeh of Lovelace Inhalation Toxicology Research Institute (personal communication), the pulmonary deposition curve is biphasic in rats, with a major peak at 0.05 μm, and a minor peak around 2.5 μm. Minimal deep lung deposition occurs between 0.3 and 0.7 μm. Significant pulmonary deposition occurs when particle size is <0.2 μm. Cascade impactors cannot measure particles this small since the final stage cutoff is, at best, about 0.5 μm. Smaller particles are collected on an absolute filter. This has never been a problem for HED since submicron particles are usually a small fraction of the total. Thus, HED has been requesting a particle size range which is difficult to measure, and which results in minimal pulmonary deposition.

In order to maximize deep lung deposition for pesticides, Dr. Yeh has recommended using an MMAD of 2-3 μm. This recommendation concurs with that offered by Trent R. Lewis, *et. al.* (1989):

"For inhalation toxicity evaluations with rodents, the test aerosols should typically have a MMAD of 3 μm or less with a geometric standard deviation of no greater than three to maximize alveolar deposition. Aerosols with even a smaller MMAD of 1 to 2 μm and a tighter size distribution, i.e., a geometric standard deviation of less than two, would be preferable. However, this is sometimes not possible due to the nature of the material required in large quantity for chronic toxicologic study. For hygroscopic aerosols a smaller size, approximately 1 μm MMAD, is preferred recognizing that the particle will enlarge in the humid environment of the respiratory tract."

"Some materials for which toxicity or carcinogenicity testing evaluations are desired may normally occur as particles with sizes greater than a few micrometers and perhaps as large as several hundred micrometers. Such powders may be physically altered to produce aerosols with MMAD's of a few micrometers to facilitate the experimental study of their toxicity."

The SOT recommendation to accept particles with MMAD's of 1-4 μm means that studies will be accepted with the majority of particles ranging from submicron size to perhaps 6 or 7 μm. As the MMAD increases, so does the percentage of nasal and total respiratory tract deposition. The SOT proposal would provide the following benefits and disadvantages:

Benefits

1. It will be easier to perform an inhalation study for troublesome chemicals because extraordinary efforts to achieve submicron particles will no longer be necessary.

2. The lower concentrations used in repeated-exposure studies should make it easier to generate finer particles than in an acute study.

3. Very few studies will be rejected for failure to achieve the desired particle size range.

4. It will be easier to achieve a limit concentration.

5. If larger particles are used, more of the inhaled chemical will be retained throughout the respiratory tract, especially in the nasopharyngeal region. Increased toxicity and lower LC_{50}'s would be expected for larger particles. This will be more protective from a regulatory standpoint.

6. The use of larger particles will more closely resemble human exposure because <u>all</u> regions of the respiratory tract are potential deposition sites.

Disadvantages

1. If the particle size requirements are relaxed, there will be a tendency for laboratories to generate larger particles (i.e. MMAD of 4). The majority of particle deposition will then be in the nasopharyngeal region rather than in the pulmonary (lung) region.

2. Of the particles retained in the upper airways, an unknown portion will be eliminated by sneezing. The balance will contribute to systemic toxicity via absorption through the mucous membranes, or by swallowing of contaminated mucus (i.e. oral exposure). If the range in particle sizes is broad, pulmonary absorption may be only a minor contributor to the overall toxicity seen in a study.

It is reasonable to expect particle sizes to be smaller in repeated-exposure studies because the physical constraints are not as demanding as in acute studies performed at high concentrations. The SOT stated that,

> "Chronic respiratory tract toxicity often results from the accumulation of insoluble particles within the pulmonary region. The use of particle sizes to maximize deposition in this region may be desirable for assessing chronic effects but, ...may not be ideal for acute testing because the use of small particle sizes to maximize pulmonary region deposition minimizes nasal deposition, enhancing the possibility of failing to detect potential nasal toxicity."

The SOT, commenting on particle sizes for acute and repeated-exposure studies, explained that,

> "Because acute limit tests are designed to provide only an approximate index of toxicity, and because nasal effects can be of considerable importance, upper particle size cutoffs need not be so stringent as recommended for chronic inhalation toxicity studies."

The SOT did not recommend a particle size range for repeated-exposure studies. HED concurs with SOT's rationale, and proposes an MMAD range of 1-3 μm for repeated-exposure studies in order to maximize deep lung deposition and avoid excessive nasopharyngeal deposition. In later discussions, the SOT concurred with the 1-3 μm range, provided it can be adjusted based on future consideration.

Recommendations:

HED recommends the following interim guidelines be used in conducting and evaluating inhalation toxicity studies:

1. Aerodynamic particle sizes are acceptable if MMAD's are 1-4 μm in an acute study, and 1-3 μm in a subchronic or chronic study (the latter range is based on limited data, and may be adjusted in the future). It is expected that repeated-exposure studies should attain a particle size that will maximize deep lung deposition. The particle size of hygroscopic materials should be small enough to allow for pulmonary deposition once the particles swell in the moist environment of the respiratory tract. The study report should include particle size distribution data, MMAD and geometric standard deviation values, and a description of the generation methods and equipment. If the MMAD guidelines cannot be met, the study report should explain why. A reasonable effort to meet these guidelines is expected, but extraordinary measures are not required.

2. The analytical limit concentration for aerosols, gases, and vapors in an acute study is 2 mg/l (based on the recommendations of the SOT Inhalation Specialty Section, and several other inhalation toxicologists, see table on page 2). This concentration is generally achievable for aerosols. Although gases and vapors can often be generated at much higher concentrations than aerosols, they are inherently more toxic than aerosols because they are more bioavailable. In most cases, a concentration of 2 mg/l will be achievable, but if not, the maximum attainable concentration should be used, and the study report should provide reasons why a higher concentration could not be attained. A reasonable effort to achieve a limit concentration is expected, but extraordinary measures are not required. The Toxicity Category Criteria will be changed as follows:

Hazard Indicators	Category I (mg/l)	Category II (mg/l)	Category III (mg/l)	Category IV (mg/l)
Current Toxicity Categories				
Inhalation LC_{50} (analytical concentration; 4-hour exposure)	≤0.05	>0.05 - 0.5	>0.5 - 5	>5
Revised Toxicity Categories				
Inhalation LC_{50} (analytical concentration; 4-hour exposure)	≤0.05	>0.05 - 0.5	>0.5 - 2	>2

3. The selection of a dynamic inhalation chamber should be appropriate for the test article and test system. It is recommended, but not required, that nose-only or head-only exposure be used for aerosol studies in order to minimize oral exposure due to animals licking compound off their fur. The animals should be acclimated, and heat stress should be minimized. Individual housing must be used during whole-body exposure to prevent filtering by the fur due to animals huddling together.

4. Whenever the test article is a formulation, the analytical concentration must be reported for the total formulation, and not just for the active ingredient (a.i.). If, for example, a formulation contains 10% a.i. and 90% inerts, a chamber analytical limit concentration of 2 mg/l would consist of 0.2 mg/l of the active ingredient. It is not necessary to analyze inert components provided the mixture at the animals' breathing zone is analogous to the formulation; the grounds for this conclusion must be provided in the study report. If there is some difficulty in measuring chamber analytical concentration due to precipitation, nonhomogeneous mixtures, volatile components, or other factors, additional analyses of inert components may be necessary.

These recommendations were designed to reflect the current state of the science, to be realistic in the laboratory environment, and to satisfy regulatory requirements. They were presented to a Science Advisory Panel on December 15, 1993 for comment. The *ad hoc* inhalation toxicology experts were Drs. Joe L. Mauderly, Roger O. McClellan, and Maurice Weeks. The ***Final Report of the Joint FIFRA Scientific Advisory Panel and Science Advisory Board Meeting*** states that, "The Panel concurs with the Agency's recommendations and further that these guideline revisions reflect the current state-of-the-art for inhalation toxicity tests which are consistent with aerosol toxicology."

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