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Intratracheal Instillation as an Exposure Technique for the Evaluation of Respiratory Tract Toxicity: Uses and Limitations

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The evaluation of respiratory tract toxicity from airborne materials frequently involves exposure of animals via inhalation. This provides a natural route of entry into the host and, as such, is the preferred method for the introduction of toxicants into the lungs. However, for various reasons, this technique cannot always be used, and the direct instillation of a test material into the lungs via the trachea has been employed in many studies as an alternative exposure procedure. Intratracheal instillation has become sufficiently widely used that the Inhalation Specialty Section of the Society of Toxicology elected to develop this document to summarize some key issues concerning the use of this exposure procedure. Although there are distinct differences in the distribution, clearance, and retention of materials when administered by instillation compared to inhalation, the former can be a useful and cost-effective procedure for addressing specific questions regarding the respiratory toxicity of chemicals, as long as certain caveats are clearly understood and certain guidelines are carefully followed.

Key Words: **intratracheal instillation; inhalation; toxicant exposure.**

Introduction

Evaluation of the respiratory tract toxicity of airborne materials frequently involves exposure of animals via inhalation. Inhalation provides a natural route of entry into the host and, as such, is preferable for the introduction of toxicants into the lungs. However, this technique cannot always be used. Designing and building an inhalation exposure system with appropriate generation and characterization of exposure atmospheres involves special expertise and equipment that are not available at many institutions and are expensive to acquire and maintain. Even when such facilities are available, other factors may make inhalation exposures unfeasible. For example, the amount of test material available may be too limited for generation of atmospheres at sufficient concentrations for an adequate duration so as to allow testing by inhalation, or the test material may be so highly toxic that safety issues such as dermal/fur contamination and subsequent handling hazards preclude its atmospheric generation. As a result of these restrictions, the direct instillation of a test material into the lungs via the trachea has been employed in many studies as an alternative exposure procedure to inhalation. Intratracheal instillation has become sufficiently widely used that the Inhalation Specialty Section of the Society of Toxicology elected to develop this document to summarize some key issues concerning the use of this exposure procedure and to provide recommendations and guidelines as to its appropriate applications and specific limitations.

Instillation has certain advantages over inhalation, as discussed in detail by Brain *et al*. (1976). Briefly, with instillation, the actual dose delivered to the lungs of each animal can be essentially assured. The technique is simpler than inhalation exposure procedures and minimizes risks to laboratory workers from highly toxic, carcinogenic, or radioactive materials. Intratracheal instillation permits the introduction of a range of doses to the lungs within a short time, and avoids exposure to the skin and pelt that can occur with inhalation exposure. In larger animals and when using certain types of test material, highly localized exposures to specific lobes of the lung can be performed, with one lobe serving as a control for another. The response of the lungs to test materials that are of concern in human exposures but that are not readily respirable by many laboratory animals can also be evaluated by intratracheal instillation. Among such materials would be long fibers which are respirable by humans but not by rodents, or reactive or water-soluble compounds that would be removed in the nasal passages of obligate nasal breathing rodents but are of concern

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for oral-breathing humans. Furthermore, intratracheal instillation has been used as a screening tool to determine the approximate dose range that may be appropriate for later inhalation studies, or to determine the ranking of toxicity for a series of structurally similar chemical agents. Instillation can also be of use in identifying specific components of a complex mixture that may be most toxicologically relevant, thus focusing future research efforts.

Despite certain advantages of instillation over inhalation exposure, a number of concerns exist regarding use of the former technique. Primary among these is that the introduction of the toxicant is nonphysiologic, involving invasive delivery, usually at a dose and/or dose rate substantially greater than that which would have occurred during inhalation. In addition, the distribution of an instilled material within the respiratory tract will likely differ from the distribution of an inhaled material. Furthermore, the upper respiratory tract (URT; i.e., the nasal passages, oral passages, pharynx, and larynx), which can be a potentially important target site for an inhaled test material, is bypassed by intratracheal instillation. Additionally, the influence of the instilled vehicle in which the test material is suspended or dissolved may have an impact on the distribution of the test substance within the lung, produce effects itself or, if it alters the physicochemical nature of the test material, may alter the effects of the material on the lungs. Of secondary concern is the potential confounding effect of anesthesia, which could influence the initial effect of the instillate material on the lung surface, as well as test material retention and clearance. These concerns have tempered the adoption of intratracheal instillation as an acceptable alternative to inhalation.

The objective of this document is to provide a perspective on the use in respiratory toxicology of intratracheal instillation for the administration of solids or liquids to the lungs. Although instillation techniques have been used for the treatment of disease, i.e., surfactant replacement and transfection techniques, such applications will not be covered in this document. The use of intratracheal instillation solely as a means for the study of systemically distributed toxicants or their pharmacokinetics, for which this procedure may also have some utility, will not be covered here. Finally, this document does not discuss variations on classical intratracheal instillation procedures, such as intranasal instillation or intratracheal nebulization (e.g., Leong *et al*., 1998; Wheeldon *et al*., 1992).

The organization of this document is as follows. First, the methodology of intratracheal instillation in rodents, the most commonly used animals in this regard, is briefly described. Second, studies in which inhalation and instillation techniques have been used to study deposition, clearance, and biopersistence of test materials are summarized, comparisons between the two exposure modes are provided, and the limitations of instillation for such studies are discussed. Third, the merits and limitations of instillation studies designed to assess the lung toxicity of specific classes of materials are reviewed. Finally, recommendations and guidelines are provided as to the appropriate use of the instillation technique.

Intratracheal Instillation Methodology

Kimura (1923) first described studies in which coal tar was administered to rabbits and guinea pigs by a variety of noninhalation routes, including intratracheal instillation, in an attempt to induce tumors. Later, the use of intratracheal instillation was reported by Henry *et al.* (1981), Ho and Furst (1973), Kouri *et al.* (1976, 1980), and Nettesheim and Hammons (1971) for mice; by Blair (1974), Brain *et al.* (1976) and Henderson *et al.* (1979a) for rats; and by Brain *et al.* (1976), Kennedy and Little (1975), and Saffiotti *et al.* (1968, 1972) for hamsters. The procedures are basically similar in all of these species; the major difference is the volume of test material delivered.

Experimental Procedure

Important factors to consider when performing intratracheal instillations are as follows: the method of intubation; the specific vehicle containing the test material; the total liquid volume used; the dose of test material administered; and the method of anesthesia.

Test materials are instilled into small laboratory rodents usually by inserting a catheter or needle transorally into the tracheal lumen (Brain *et al*., 1976; Saffiotti *et al*., 1968, 1972). In larger species, test agents can be instilled directly into a specific lung lobe (Bice *et al*., 1982). Another delivery approach is transtracheal instillation (Thrall *et al.*, 1978), whereby the trachea is exposed surgically on the ventral side of the neck, and a needle is inserted through the tracheal wall into the lumen just below the larynx.

Clearly, instillation through the oropharynx during anesthesia is the easiest and least invasive method for delivery of test material to the lungs of small animals (Brain *et al.*, 1976). A fiberoptic laryngoscope can be easily fabricated to view the epiglottis of a number of species, such as rats, hamsters, guinea pigs, and mice (Costa *et al.*, 1986; Lindenschmidt *et al*., 1990). In hamsters, due to the wide cheek pouches, the mouth can be opened widely to directly visualize the epiglottis (Brain *et al.*, 1976; Saffiotti *et al.*, 1968). A speculum to hold the mouth open is easily made and facilitates the instillation procedure (Mauderly, 1977).

Most instillation procedures involve positioning of the animal against an angled restraining stand. A cannula is then inserted into the mouth and placed between the vocal cords and into the lumen of the trachea. Illumination can be provided by a directed light source (Brain *et al.*, 1976; Costa *et al.*, 1986). It is also possible to use a syringe to perform instillations without using a laryngoscope (Hatch *et al.*, 1981). A ball tipped needle can be maneuvered through the epiglottis, after which contact with the tracheal rings provides confirmation that the needle is, in fact, within the trachea. Visualization of

the vocal cords can facilitate this effort and ensure proper dosing. Another technique to determine if the intubation device is in the respiratory tract, rather than the gastrointestinal tract, is to gently introduce air through a syringe and observe if the lungs inflate (Mauderly, 1977).

The selection of the appropriate anesthetic for instillation procedures can help ensure the successful administration of a test material. Generally for rodents, short acting anesthetics, which suppress reflexes for a minimal period of time and allow the animal to recover quickly and regain normal respiration, are preferred. Halothane, metaphane, and enflurane are inhaled anesthetics frequently used. Injectable barbiturates may have special applications or uses (e.g., in dogs), but they have undesirable suppressive actions on the central nervous system that can impair recovery. Diethyl ether has been used for guinea pigs because this species has a unique sensitivity to cardiac arrest induced by some halogenated anesthetics (Costa *et al.,* 1986).

The selection of the appropriate vehicle is an important consideration for some test materials. Although saline is by far the most commonly used vehicle, the process of saline instillation may evoke responses in the lungs, such as a mild transient inflammation, even when the saline used is pyrogen-free (Driscoll *et al.,* 1991; Hatch *et al*, 1981; Henderson and Lowrey, 1983). Dispersing agents (e.g., Tween 80) are sometimes added to the saline to keep suspended particles from agglomerating or settling. However, such agents can disrupt endogenous surfactant and can even elicit adverse effects of their own (Damon *et al.,* 1982; Schermuly *et al.,* 1997).

The volume of instillate for injection is an important factor in determining toxicant distribution within the lungs. Smaller volumes have been shown to distribute test material less evenly than larger volumes (Baxter and Port, 1974), but excess fluid injection into the lung is not desirable; the typical instillation volume is 1–2 ml/kg body weight. However, the appropriate normalization metric (e.g., body weight, lung weight, surface area) may depend upon the objective of the study. For example, lung to body weight ratios differ between species and with the age of the animal, making body weight an inappropriate factor to normalize dose when making comparisons across species and age groups. Injection of large volumes ($>$2 ml/kg) should generally be avoided to limit potential vehicle effects (Costa *et al*., 1986), as well as to avoid any potential coughing in some species and subsequent loss of instilled test material.

Quality Assurance Considerations

Specific questions regarding the reproducibility of the intratracheal instillation methodology have been addressed in a number of studies. These indicate that when the instillation procedure is properly executed, the distribution of instillate to the lung lobes is quite reproducible, and appears to be proportional to the volume or mass of each lung (Costa *et al.,* 1986; Pritchard *et al.,* 1985). However, reproducibility of delivery is often quite dependent upon the experience of the individual performing the procedure, and appropriate training is required. Even with such training, it should be realized that results can vary from laboratory to laboratory, often making it difficult to compare interlaboratory results.

The use of a tracer of some type to detect the effectiveness of the instillation, as well as to check the distribution of the test material, is considered a valuable training and quality assurance tool. India ink or fluorescent latex beads can be good qualitative markers for visual examination of distribution for some particle types. Furthermore, the lungs can be inflated following the instillation procedure, removed from the body, frozen, lyophilized, and then sectioned to examine the instillate distribution, or the lung lobes can be homogenized and extracted for fluorescence quantitation. The reproducibility of the biologic effect observed in the lungs may provide sufficient evidence that there is a reproducible instillation of the agent into the lungs, but this is *post facto* confirmation and can be problematic.

Injury to the trachea caused by needle insertion is a possibility that is rarely specifically considered. However, the use of a separate vehicle control, which involves needle insertion, is generally performed to account for any response to the administration procedure itself. Characteristics of the needle in relation to instillation have been discussed elsewhere (Mauderly, 1977).

Using intratracheal instillation, there is the possibility that some of the injected material could be either coughed up in those species with such capability, especially if recovery from anesthesia is too rapid or if the volume of instillate is too large, or could be quickly cleared from the trachea. Two studies attempted to quantify particle retention in the early postexposure period. In one, mice retained 70% of instilled radiolabeled albumin in the lungs 2 h following instillation, while the head and carcass (mainly the stomach) contained the other 30% (Hatch *et al.,* 1981). In another study (Costa *et al.,* 1986) that employed labeled carbon particles (suspended in 0.1% Tween 80), rats, mice, and guinea pigs retained $>$ 90% of the instilled dose in the lungs immediately (i.e., $<$ 30 min) postexposure, whereas hamsters retained about 83%.

Deposition/Clearance/Retention of Test Material

Numerous studies have examined biologic responses to particles delivered by inhalation, while others have examined similar endpoints, but with particles delivered by instillation. In most cases, the effects of the method of administration upon particle deposition/retention/clearance *per se* have not been examined in a manner that allows easy comparison. Typically, differences in the experimental procedures and in the manner by which particle disposition was quantitated precluded any direct correlation. Studies that have compared the effects of different exposure modes upon the handling of particles by the lungs are summarized in this section.

Effect of Exposure Technique upon Initial Deposition and Early Intrapulmonary Distribution of Particles

An obvious major difference between intratracheal instillation and inhalation is that the former procedure bypasses the upper respiratory tract (URT), whereas with the latter, various fractions of the exposure dose will deposit in the URT. The exact amount, if any, depends largely upon the size of the particles in the exposure atmosphere and the animal species being used (Schlesinger, 1995).

There are data to assess the effects of exposure method upon the early intrapulmonary distribution of particles. This phase of particle disposition would potentially affect routes and rates of clearance from the lungs and the dose delivered to specific sites within the respiratory tract or to extrapulmonary organs. The distribution of particles within the lungs is certainly influenced by the exposure protocol.

In an extensive study, Brain *et al.* (1976) examined the intrapulmonary distribution in rats and hamsters of labeled particles of different sizes delivered by inhalation or intratracheal instillation. Inhalation resulted in greater deposition in apical areas of the lungs compared with basal areas, whereas instillation resulted in less deposition in apical areas and greater amounts in basal regions. However, this difference was influenced by the positioning of the animal during the instillation and inhalation exposures. Consistent with other studies discussed below, instillation produced less uniform deposition than did inhalation, although the nonhomogeneous distribution of a single instillation exposure could be reduced by multiple exposures. Instillation resulted in heavier and more centralized particle deposition, perhaps due to the bolus delivery, whereas inhalation resulted in a wider and more even distribution of particles throughout the lungs. Similarly, hamsters exposed to insoluble Fe_2O_3 (0.7 μm) particles showed greater peripheral lung burdens immediately following inhalation than following intratracheal instillation, and inhalation resulted in a greater variation in particle burden between different animals (Dorries and Valberg, 1992).

The issue of instillation-related variability was also addressed by Pritchard *et al.* (1985), who noted that the variability in retention of CeO_2 (2.2 μm) within a specific lobe between different rats was much greater with intratracheal instillation than with inhalation. However, relative deposition fractions between the lobes were similar for both exposure techniques. Likewise, Müller *et al.* (1989) reported that the mode of administration did not affect the relative interlobar distribution of insoluble particles (U, PuO_2) in the rat lung. Drew *et al.* (1987) found that the distribution of short and long glass fibers in rats exposed via intratracheal instillation and a transoral inhalation procedure (to bypass the nose) was similar within the alveolar region for both exposure techniques. Costa *et al.* (1986) reported the interlobular distribution pattern of a single dose of instilled particles in rodents (except the guinea pig) to be essentially proportional to lobe mass (and volume); intralobular distribution, however, was heterogeneous.

Differences in relative deposition between the alveolar and tracheobronchial regions of the lungs related to exposure mode have been noted in some studies. Using soluble materials ($CdCl_2$; 0.34 μm), Oberdörster *et al.* (1980) reported that the amount of material depositing within the alveolar region relative to the bronchial region of the rat lung was greater with inhalation than with intratracheal instillation. Likewise, the inhalation of diazo ink by rats resulted in a relatively even distribution of particles throughout the lungs, whereas instillation resulted in an even distribution of particles in the major conducting airways but an uneven distribution within the alveolar region (Leong *et al.*, 1986).

Effect of Exposure Technique upon Particle Retention and Clearance Kinetics

Most of the studies directly comparing different modes of particle administration have examined overall particle retention during some period postexposure. In one such study, both inhalation and intratracheal instillation resulted in similar clearance kinetics of a deposited soluble material ($CdCl_2$; 0.34 μm) in rats up to 100 days postexposure (Oberdörster *et al.*, 1980). Dahl *et al.* (1983), using another soluble particle, H_2S0_4 (0.4–1.2 μm), exposed dogs via inhalation or intratracheal instillation. The retention half-time for the material delivered by inhalation ranged from similar to twice as long as that of the material delivered by instillation, depending upon the actual site of deposition of the instillate.

Pritchard *et al.* (1985), using insoluble CeO_2 (2.2 μm) particles, noted that by 48 h postexposure, 87% of particles delivered to rats by intratracheal instillation was retained in the lungs, whereas only 22% of material delivered by inhalation was so retained, even though total initial lung burdens were similar. Müller *et al.* (1989) exposed rats to either spherical or irregular insoluble particles (UO_2, PuO_2; 0.9–1.5 μm) and examined retention through 200–300 days postexposure. There was no difference in lung retention between the two particle types when administered by inhalation, even though there was some difference in the amount initially depositing. Although irregular particles given by instillation cleared the lungs at a rate similar to that when the particles were administered by inhalation, clearance of instilled spherical particles was slower than that of inhaled spherical particles.

Most studies that have examined different exposure techniques involved a single administration of particles. However, some have employed repeated exposures. Driscoll *et al.* (1990a,b; 1991) evaluated pulmonary retention in rats of inhaled and instilled SiO_2 (1.6 μm) and TiO_2 (1.0 μm) particles. Repeated exposures were used for inhalation (6 h/day, 5 days); only a single exposure was used for intratracheal administration. The percentage retention was assessed 28 days following the last exposure. At similar deep lung burdens, SiO_2 retention

was 72% and 87% for inhalation and instillation, respectively. Values for TiO_2 were 39% and 54% for inhalation and instillation, respectively. This indicates that instillation exposure resulted in somewhat greater lung retention than did inhalation.

Thus, in general, it appears that clearance of instilled particles tends to be slower that that of comparable particles that are inhaled, but the results are influenced by the location of particle delivery and by particle characteristics such as shape.

Effect of Exposure Technique upon Pulmonary Clearance Pathways

There are a few studies that allow the assessment of differences in clearance pathways for particles delivered by different experimental procedures. Henderson *et al*. (1995) examined clearance of quartz (1.7 μm) in rats. Particles delivered via both intratracheal instillation and inhalation were found in bronchus-associated lymphoid tissue (BALT), but only those delivered by inhalation appeared to reach the pleura (likely via pleural lymphatics) in sufficient amounts to produce a biologic response (i.e., pleural granuloma). This may suggest that the pathway of lymphatic clearance can differ for particles delivered to the lungs via the two exposure techniques.

Particles depositing within the lungs may be subject to systemic absorption, the extent of which depends upon clearance routes and rates. Chui *et al*. (1988) noted in rats that absorption of a paraquat aerosol (1.2 μm) delivered via inhalation was somewhat greater than for the same material delivered by intratracheal instillation. The half-time reported for elimination from blood was over twice as long for instillation as for inhalation. Although the exact dose delivered via inhalation was not known, the study suggests that the route of administration can affect the rate and extent of systemic bioavailability of a toxicant.

One of the major respiratory tract clearance pathways involves particle uptake and removal via pulmonary macrophages. Pritchard *et al.* (1985) noted that intratracheal instillation resulted in a large number of particles within macrophages, whereas with inhalation, macrophages contained only a few ingested particles. Furthermore, with intratracheal instillation, macrophages at the lung periphery contained few, if any, particles, and those cells in the regions of highest deposition were overloaded, reflecting the inhomogeneity of particle distribution when the test material was administered by instillation. Similarly, using Fe_2O_3, Dorries and Valberg (1992) noted that inhalation resulted in a lower percentage of lung-associated iron recovered in lavaged cells and a more even distribution of particles among macrophages. More individual cells received measurable amounts of particles via inhalation than via intratracheal instillation. With the latter, many cells received little or no particles and others received very high burdens. The distribution of particles among macrophages was more homogeneous with inhalation than with instillation. Thus, the route of exposure influenced the particle distribution in the macrophage population and could, therefore, influence clearance pathways and clearance kinetics. Particle loading also influences the number of macrophages in the lungs.

Toxicology of Specific Test Materials

Although, as noted previously, the intratracheal instillation method has been used extensively to characterize the toxicity of materials to the lung, only a few studies have directly compared biologic responses to materials administered by intratracheal instillation and inhalation. This section provides a summary of studies that have made direct comparisons of responses following administration of various test particles via the two exposure methods.

Soluble Materials

In early studies by Henderson *et al.* (1979a), intratracheal instillation was used in Syrian hamsters to determine doses of soluble metal salts for subsequent inhalation studies. The technique used was a lavage; 4 ml (80% of total lung capacity) of a saline solution containing various concentrations of the salts was instilled, the excess saline containing the test material immediately withdrawn, and the difference between the wash volume delivered and recovered used to estimate the dose of the metal salt remaining in the lung. The range of lung doses delivered was 0.5 μg to 100 μg. One day after instillation, the animals were evaluated by histopathology and analysis of bronchoalveolar lavage fluid (BALF). The results indicated that the toxicity of metal salts could be ranked as follows: $CdCl_2 > SeO_3$, NH_4VO_3, $NiCl_2 > CrCl_3$. Inhalation studies on $CdCl_2$ and $CrCl_3$ resulted in deposition in the lungs of 0.6 and 4.4 μg $CdCl_2$ and 0.7 and 20 μg $CrCl_3$ (Henderson *et al.*, 1979b). The histologic responses and the alterations in BALF parameters were similar to those observed in the instillation studies. For example, a lung burden of 4.4 μg of inhaled $CdCl_2$ resulted in a 6-fold increase in BALF lactate dehydrogenase (LDH) activity, whereas an instilled dose of 5–10 μg induced a 7-fold increase in LDH. $CrCl_3$ did not cause alterations in BALF fluid parameters over the dose range studied by either inhalation or instillation. Thus, the relative ranking of the toxicity of $CdCl_2$ and $CrCl_3$ was the same following administration by either instillation or inhalation.

Damon *et al.* (1982) characterized the acute toxicity of the nonionic surfactant, polyethylene glycol p-isooctylphenyl ether (Triton X-100), in hamsters after exposure via an intratracheal instillation procedure or by inhalation. For comparison, a single inhalation exposure to Triton X-100 was performed for differing periods of time to obtain total respiratory tract burdens similar to those obtained after instillation exposure. The respiratory tract burdens obtained after inhalation were predicted based on the particle size of the Triton X-100 aerosol. Exposure to Triton X-100 by either instillation or inhalation resulted in dose-related lethality, with the LC50 for the two methods being similar. Differences were detected in the slope

of the mortality dose-response curve and, at the high doses, animals died sooner after instillation (5 h) than following inhalation (19 h). Histopathology demonstrated exposure method-dependent differences in the site of injury. After intratracheal instillation, the deep lung was the primary site of injury; that after inhalation was the larynx and epiglottis. This difference in site of action is not surprising, because the instillation procedure would, as noted previously, circumvent the upper respiratory tract.

Hatch *et al.* (1981) investigated the influence of exposure method on chemically induced changes in susceptibility to respiratory infection. Exposures were to a panel of materials that included both soluble and poorly soluble (e.g., carbon black) agents. In these studies, mice were intratracheally instilled at a dosing volume of 10 μl. Twenty different test materials that had been previously investigated by acute 2- to 3-h inhalation exposures for their effects on susceptibility to infection were examined. To determine doses for the instillation studies, lung doses delivered in the previous inhalation studies were calculated based upon air concentrations, duration of exposure, and the deposited fraction of the inhaled particles reaching the lungs of mice (20%). Additionally, an experiment was performed using intratracheally instilled radiolabeled protein to determine the fraction of the instilled dose that was retained within the lung. This fraction, determined to be 70%, was used to adjust the intratracheally instilled dose to the lung dose calculated to have occurred after inhalation. One to 2 h after exposure to the test materials, the animals were exposed for 20 min to an aerosol of *Streptococcus pyogenes*, and mortality was tracked during a 15-day postexposure period. The authors reported that characteristics of the intratracheal instillation technique, such as brief halothane anesthesia, needle insertion into the trachea, and injection of saline, did not alter infectivity. There was agreement between dose-response curves generated either by inhalation or intratracheal instillation of the more toxic materials (i.e., cadmium, copper, and zinc sulfates). Comparison of materials with intermediate toxicity appeared to show some discrepancy between the two methods. For example, ammonium bisulfate, ferric ammonium sulfate, and aluminum ammonium sulfate showed moderate effects by intratracheal instillation, but no significant effects after inhalation exposure. Overall, however, the authors concluded that intratracheal instillation of a given amount of material yielded results qualitatively similar to those following the deposition of the same amount of material by inhalation.

Fibers

Although the intratracheal instillation technique has been used quite extensively for administering fibers to the lungs of rats, hamsters, and mice, only one published study has been reported in which lung responses after intratracheal instillation and inhalation exposure were directly compared. Drew *et al.* (1987) investigated the response of rats to long and short glass fibers after a single bolus intratracheal instillation of 2 and 20 mg fiber (in 0.5 ml saline) or 10 weekly intratracheal instillations of 0.1 mg/0.5 ml. The latter repeated exposure protocol was compared to an intratracheal inhalation exposure (which involved a transoral tracheal cannula) to fiber concentrations predicted to result in a lung burden similar to that after repeated instillation. Specifically, rats were exposed via intratracheal inhalation 1 h/week for 10 weeks to either 260 mg/m^3 long fibers, 1300 mg/m^3 short fibers, or air. These exposures were estimated to result in a weekly deep lung deposition of 0.15 mg and 0.75 mg of the long and short fibers, respectively. The introduction of both long and short glass fibers by intratracheal instillation as a bolus of 20 mg produced a granulomatous response. Within the granulomas, dense focal aggregates of fibers could be seen. In contrast, when the fibers were instilled at lower concentrations (0.1 mg/0.5 ml) in repeated doses, a macrophage response was observed. Few fibers were seen in the lungs after 10 weeks of repeated intratracheal instillation or inhalation of short glass fibers. After repeated inhalation or instillation of long glass fibers, a diffuse distribution of fibers was seen with little or no accumulation of fibers in the terminal bronchioles or the alveolar region. The authors concluded that single boluses of high doses (2 and 20 mg per rat) of fibers produced an artifactual granulomatous lesion in the rat lung, whereas repeated exposure to low doses of fiber (0.1 mg per rat) resulted in a fiber distribution and response similar to that after inhalation via tracheal cannula. It should be noted, however, that only very limited information was provided regarding the comparative histopathology for the two exposure methods.

Poorly Soluble Particles

Two studies have compared responses of the rat lung to inhaled and intratracheally instilled mineral dusts. In a study by Driscoll *et al.* (1991), rats were exposed via inhalation to quartz or titanium dioxide at 53 and 51 mg/m^3, respectively. The exposures were in whole-body dynamic exposure chambers (6 h/day for 5 days). Lung burdens, determined 24 h after the final exposure, were 1.9 and 1.8 mg/lung for quartz and titanium dioxide, respectively. Respiratory tract responses to the dusts were characterized at 7, 14, 28, and 63 days after exposure by analysis of bronchoalveolar lavage fluid (BALF), by the release of inflammatory mediators by alveolar macrophages, and by histopathology. The responses in this study were compared to studies in which rats were intratracheally instilled with 1.8 mg of the same quartz and titanium dioxide material (Driscoll *et al.*, 1990a,b). After instillation, quartz increased the BALF markers of lung injury (lactate dehydrogenase, total protein, and neutrophils) and alveolar macrophage fibronectin release by 7 days, and this response persisted to 63 days post-treatment. After inhalation exposure, the same endpoints were increased; however, significant changes were not detected until 63 days for BALF lactate dehydrogenase and macrophage fibronectin, 28 days for total protein, and 14 days

for neutrophils. Quartz increased endotoxin-stimulated alveolar macrophage interleukin-1 (IL-1) production by 7 days after both inhalation exposure and intratracheal instillation. Thus, after inhalation of quartz, it appeared that a longer time was required before significant adverse effects developed, and the responses were generally less severe than those seen after instillation. Overall, however, the types of responses were similar irrespective of the exposure method. Regarding titanium dioxide, both qualitative and quantitative differences in lung responses were observed for instillation versus inhalation exposure. Intratracheal instillation elicited transient increases in BALF lactate dehydrogenase, total protein, neutrophils, and macrophage fibronectin, as well as a persistent increase in endotoxin-stimulated IL-1 release. In contrast, after inhalation of titanium dioxide, no significant effects were detected. Given that similar deep lung burdens were obtained after inhalation and instillation of titanium dioxide, the greater responses after instillation were likely due to differences in the dose rate and/or distribution of material in the lungs. Overall, this comparison of responses suggests that, at the lung burdens examined, intratracheal instillation resulted in greater responses than did inhalation. Regarding relative toxicity to the lungs, both inhalation and instillation of quartz resulted in markedly more toxicity than did exposure to titanium dioxide.

Henderson *et al.* (1995) compared the rat lung response to intratracheally instilled quartz and titanium dioxide with that occurring after 4-week inhalation exposure to these same materials. Animals were exposed via nose only inhalation (6 h/day, 5 days/week for 4 weeks) to 0, 0.1, 1.0, and 10 mg/m^3 quartz or titanium dioxide. Lung responses were characterized by analysis of BALF for lactate dehydrogenase, total protein, acid protease, β-glucuronidase, and inflammatory cells, and histopathology at 1, 8, and 24 weeks after the end of exposure. The doses of the two mineral dusts instilled corresponded to the lung burdens of quartz determined 7 days after the end of the 4-week inhalation exposure (specifically, 750, 200, and 50 μg of dust). Responses were characterized in the same manner as for the animals exposed by inhalation. Exposure of rats by intratracheal instillation to lung burdens of up to 750 μg/rat titanium dioxide by either method produced no changes in BALF parameters or histopathology. The BALF changes after quartz exposure were indicative of progressive inflammation and tissue injury, and were of a similar magnitude and time course for the two methods of exposure. At the high dose of quartz, histopathologic observation revealed microgranulomas in the bronchial-associated lymphoid tissue after both instillation and inhalation exposure. However, microgranulomas associated with the pleural surface were seen only in animals exposed by inhalation. Overall, the results of this study indicated that the degree of lung inflammation and tissue injury characterized by BALF analysis and histopathology was similar after both methods of exposure. The localization of granulomas on the pleural surface only after inhalation exposure may reflect a difference in lymphatic clearance of quartz particles when delivered at the relatively low dose rate in the 4-week inhalation exposure compared to the dose rate with a single bolus instillation. Alternatively, differences in intrapulmonary distribution of particles or degree of acute injury may be factors contributing to the difference in granuloma formation. Another observation from this study was the difference in neutrophils in the air and saline control rats. At 7 days after exposure, there was a greater number of neutrophils in the BALF of the saline-instilled control rats compared to the air-exposed inhalation controls. An increase in BALF neutrophils shortly after intratracheal instillation of saline into rat lungs had been reported previously (Driscoll *et al.,* 1990a). Additionally, lung lavage with sterile saline had been reported to elicit transient inflammation (Kazmierowski *et al.,* 1977).

Protein Allergens

Intratracheal instillation has been used as an alternate method to inhalation for delivery of allergens to the respiratory tract of guinea pigs. In a study by Ritz *et al.* (1993), a direct comparison of the allergic antibody response of animals exposed to protein allergen by intratracheal instillation with responses of animals exposed by inhalation showed a similar dose-response relationship over 10 weeks of exposure. The kinetics of the antibody response, the percentage of animals responding, and the time to onset of immediate respiratory reactions were similar for both methods of exposure. In addition, the onset of the respiratory reactions occurred by 4 weeks of intratracheal instillation or inhalation exposure to comparable doses of allergen.

In another study, an interaction between protease enzyme allergens and nonprotease enzyme allergens was observed following both intratracheal and inhalation exposure of the protein (Sarlo *et al.,* 1997b). This interaction also occurred in animals exposed to a similar mixture of enzyme allergens by inhalation. Finally, the extent of the immune response to different enzyme allergens following intratracheal instillation in the guinea pig has been used to rank these enzymes as more potent, less potent, or equipotent to the enzyme Alcalase (Sarlo *et al.,* 1997a). These data have been used to establish exposure guidelines in the workplace where workers are monitored for the development of allergic antibody to enzymes via the skin prick test. Good concordance has been observed between the relative potency of enzymes for causing respiratory sensitization upon intratracheal instillation of guinea pigs and potency for eliciting allergic antibody in the workplace (Sarlo *et al*., 1997a,b). Overall, the data on enzymes and sensitization indicate that when intratracheal instillation is used to sensitize and to induce allergic responses (both antibody and immediate-onset respiratory symptoms) to protein allergens, responses observed are similar to those found after inhalation.

Other Comparisons Regarding Toxicity of Test Materials

The above discussion focused on studies that have directly compared biologic responses from test materials using intra-

tracheal instillation and inhalation exposure methods. However, even when the comparison of the two methods is expanded beyond these directly comparable reports, it appears there are many qualitative similarities in the types of lung tissue responses occurring after inhalation and intratracheal instillation of the same test materials. For example, comparison of non-neoplastic responses in the rodent lung seen after exposure to crocidolite asbestos (Begin *et al*., 1991; Driscoll *et al*., 1995; Quinlan *et al.,* 1994), cadmium chloride (Driscoll *et al.,* 1992; Henderson *et al.,* 1979a,b; Kutzman *et al.,* 1986), volcanic ash (Beck *et al.,* 1981; Martin *et al.,* 1983), crystalline silica (Beck *et al*., 1981; Driscoll *et al*., 1991), and titanium dioxide (Ferin *et al.,* 1992) suggest similarities in the nature of the inflammatory and fibrotic responses in the lungs after exposure by either method. Additionally, both intratracheal instillation and inhalation exposures to poorly soluble particles have been shown to result in carcinomas in the lungs of rats (Heinrich *et al.,* 1986, 1995; Heinrich, 1994; Mühle *et al.,* 1991; Pott *et al.,* 1994; Saffiotti and Stinson 1988). In addition, the rat lung tumor response to particles and the apparent lack of responsiveness of the hamster, at least to quartz, is observed after either intratracheal instillation or inhalation exposure (Holland *et al.,* 1986; Mühle *et al.,* 1991; Pott *et al.,* 1994; Saffiotti and Stinson 1988). The apparent concordance in the qualitative nature of lung responses indicates that the instillation technique, when properly conducted, may be useful as a screening tool to determine the effects that could occur after inhalation exposure, as well as for comparing one material to another in an effort to assess relative toxicity.

Summary

Intratracheal instillation is a widely used procedure to deliver materials into the lungs. Reasons for employing this method instead of the more physiologic inhalation exposure include its simplicity; its relatively low cost; the ability to deliver a well-defined dose; the ability to deliver rodent nonrespirable but human health-relevant particles (e.g., long fibers) to a rodent lung; and practical and safety reasons, i.e., limited material available for study, or highly toxic or radioactive materials. However, there are several issues that need to be considered when using intratracheal instillation for the evaluation of pulmonary toxicity.

Some of these considerations relate to the method of intubation: the vehicle; the volume of material administered; the dose; and the anesthetic agent. Most commonly, intratracheal instillation is performed using a short-acting anesthetic and transoral intubation of the trachea followed by instillation of the test agent dissolved or suspended in physiologic saline. The volume of vehicle is commonly 1–2 ml/kg body weight, although normalization of instilled volume to other parameters may be more appropriate when comparing different species or animals covering a wide range in age. The procedure of intratracheal instillation requires technical skills that must be acquired and validated by appropriate training of personnel, and its use must also encompass quality assurance measures to ensure appropriate performance.

A thorough understanding of the differences between the instillation and inhalation methods is necessary to avoid misinterpretation of instillation-derived results. A key difference between the two exposure methods is the dose rate, i.e., administration of a dose within a few seconds with intratracheal instillation, as opposed to minutes, hours, days, weeks, or even months when the material is inhaled. The possibility of delivering excessive doses to the lungs because of the bolus delivery inherent in an instillation exposure poses the risk of overwhelming lung defenses and causing effects that are not relevant to those which may occur at lower doses or dose rates. The potential for altering lung defenses needs to be considered when designing and interpreting studies using instillation. Another important difference between the two exposure techniques is that inhalation can result in deposition within the upper respiratory tract, the extent of which depends upon the characteristics of the airborne material. Because instillation bypasses this portion of the respiratory tract, it is not a viable technique if responses in this region are endpoints of concern.

Perhaps the most consistently reported disparity between inhalation and intratracheal instillation methods relates to the intrapulmonary distribution of particles. Inhalation results in a relatively homogeneous distribution of particles throughout the lungs, whereas instillation generally results in less homogeneity of dose distribution in the alveolar region and can result in focally high doses of material. These differences in dose distribution can influence clearance pathways, doses to certain cells and to tissues, and the degree and site of systemic absorption.

The influence of instillation exposure on the clearance/retention of test materials in the lower respiratory tract is not entirely certain. Whereas some studies indicate that short (0–2 days) and long (100–300 days) phases of clearance of insoluble particles delivered either by inhalation or intratracheal instillation are similar, other studies indicate that retention of particles delivered by instillation is greater than that for inhalation. An important factor that may influence these differences appears to be the dose delivered by instillation, i.e., low doses (< 100 mg) are cleared with a similar rate as would occur following inhalation, whereas larger instilled doses (> 100 mg) are generally cleared more slowly. Furthermore, it appears that the method of administration can affect the systemic bioavailability of soluble particles, with inhaled materials being more bioavailable than those delivered by instillation.

The use of intratracheal instillation for evaluating the biopersistence of fibrous particles allows administration of long fibers that may be respirable by humans into the lungs of rodents. However, results must be interpreted with caution. Pulmonary retention half-times for instilled fibers can be greater than those that would occur after short-term inhalation, especially for less soluble materials. The greater retention is likely due to the high dose typically administered as a bolus by

instillation. Administration of low doses may not alter fiber biopersistence from that seen after inhalation, but this needs to be verified.

Although differences exist in the dose rate and distribution of material delivered by instillation versus inhalation, the database on pulmonary toxicity of various particulate materials indicates that the two modes of exposure yield qualitatively similar results for a variety of biologic endpoints, including pulmonary inflammation, fibrosis, susceptibility to infection, allergic sensitization, and lung cancer in rats after exposure to poorly soluble particles. However, some differences have been reported in the distribution of lesions in the lungs with instillation compared to inhalation exposure. Although some studies indicate that adverse pulmonary responses to particles may be exaggerated when using intratracheal instillation, this may be due to high dose rate, focally high lung burdens, and/or vehicle effects. However, any exaggeration of response after instillation appears to be minimized when low doses are used.

Recommendations

The database on intratracheal instillation supports the use of this method of exposure to address certain questions important to pulmonary toxicology. The situations where intratracheal instillation could be considered as a method of exposure are indicated below. Also noted are those instances where significant concerns exist regarding the relevance of observations made using intratracheal instillation compared to inhalation. Finally, several key guidelines that must be considered for appropriate use of intratracheal instillation are presented.

Applications of Intratracheal Instillation

Evaluation of comparative toxicity. Intratracheal instillation is a useful method for studies designed to screen panels of test materials for their relative potential to produce toxicity in the lower respiratory tract or to screen for effects over a range of doses. Another potential application of the instillation technique is to compare the effects of a new material to similar materials for which inhalation data are available. In addition, by comparing the toxicity of fractionated components of a mixture with toxicity of the whole mixture, information can be obtained on which constituents may be most toxicologically relevant. In such screening and comparative studies, it is important to characterize both the dose-related and temporal patterns of response.

There are significant concerns with extrapolating directly from a lung dose administered by intratracheal instillation to an inhalation exposure concentration that could be expected to result in a similar response. However, inferences on how materials may behave in the lung under certain inhalation exposure conditions can be developed from intratracheal instillation studies by inclusion of appropriate control materials. For example, an intratracheal instillation exposure study may be used to compare the lung effects of a new material against physically and chemically similar materials that have an extensive inhalation database. If such a comparative instillation study indicates that the nature, magnitude, and temporal pattern of response to the new material is similar to a control (or reference) material, then, by extrapolation from the database for the control, inferences can be made regarding the inhalation conditions under which the new material may produce adverse effects.

Evaluation of material not respirable by rodents. Because of significant differences between humans and rodents with respect to the dependence of respirability and regional deposition on the size of airborne particles, some inhaled toxicants, depending upon their particle size, can reach the deep lung regions in humans but would not reach these regions in rodent lungs, or only to a very limited extent. If such materials cannot be delivered by inhalation in sufficient amounts to the alveolar region in the animal model used, then intratracheal instillation may be a viable alternative for dosing of the peripheral lung to evaluate the retention and/or effects of these materials for human hazard evaluation. The advantage of administering such material in such cases has to be weighed against the disadvantages of creating particle clumping and local inflammatory responses by instillation, which can affect local lung clearance processes that are determinants of the biopersistence of particles. In order to minimize the interference of clumping and localized inflammatory responses in a biopersistence study, only intratracheal doses below approximately 100 μg/rat should be used. The degree of potential inflammatory response should also be evaluated by using histopathology and/or lung lavage parameters, and appropriate vehicle controls. More information is needed to validate results from intratracheal instillation in fiber biopersistence studies against those from short-term (i.e., 1 week) inhalation studies. Results from instillation studies with low doses of nonfibrous particles have demonstrated good agreement of retention half-times compared to inhalation studies.

Situations Where Intratracheal Instillation Should Not Be Considered

Because of the nonphysiologic nature of particle delivery using intratracheal instillation, the technique cannot be used to determine particle deposition patterns in the lungs that would occur following inhalation. Similarly, intratracheal instillation will not provide information on the upper respiratory tract toxicity of a material. Results of short-term clearance assays, i.e., mucociliary transport rates, obtained by intratracheal instillation may not reflect those obtained with inhaled materials. Finally, the technique should not be used for materials that react with the vehicle, or that change in the vehicle in a manner that may alter their toxicity.

Guidelines for the Conduct and Design of Studies Using Intratracheal Instillation

Validation. If intratracheal instillation studies are to be conducted, it is imperative that the procedure be appropriately

validated in the laboratory. For example, preliminary tests should be conducted to demonstrate that the test material can be reproducibly delivered to the lungs (e.g., fraction delivered, distribution between lobes) and to establish that the test material is not altered by the vehicle.

Study design. The design of studies using intratracheal instillation will depend on the question being addressed. However, certain general statements can be made about special concerns related to study design. The method of instillation that is least invasive should be chosen; in most cases, this choice will be a transoral, rather than a transtracheal, procedure. However, other techniques are beyond the scope of this document. The choice of vehicle will usually be endotoxin-free saline solution; any deviation from a saline vehicle must take into account the effect of the vehicle on the lung lining fluid and on the interaction of the test substance with the lung. In addition, the effect of the vehicle on potential toxicologic properties of the test material must be considered.

The dosing volume for the instillation must not be so large as to damage the lung, or so small as to preclude adequate distribution of test material within the lung. The design for toxicity studies should encompass a range of doses and a range of observation times after instillation. At the low end of the dose-response curve, the design should include a control group exposed to vehicle only. At the high-dose end, care must be taken to avoid doses that are excessive and that may result in immediate toxic effects to the lung due to mechanisms not expected to occur during dosing at a lower rate, or in exaggerated responses due to a large bolus delivery. The data generated from instillation studies can provide information on the nature of effects a material will have after inhalation exposure, provided that the instilled doses are reasonable.

Intratracheal instillation should be considered generally as a method for single exposure of the lungs to characterize potential toxicity. There are little data on effect of repeated instillation, and more baseline work needs to be performed in this regard. If repeated dosing is to be performed, special consideration must be given to the frequency of the instillation.

The observation times should include an early time after instillation (e.g., 0–3 days) to detect an immediate response to the test material compared to the response to vehicle alone; a slightly longer time after instillation (e.g., 7–10 days) to determine if the immediate response is persistent; and a later observation time (e.g., 1–3 months) to determine if long-term effects have resulted from the test agent.

Dose metric. The dose metric used in instillation studies is normally based on body weight (mass), i.e., it is expressed in terms of total mass of test material instilled per kilogram body weight. This metric is adequate for studies within one species and/or one age group. For studies seeking to compare responses between species or between age groups within a species, other metrics should be considered. In such cases, the dose normalized to such parameters as estimates of lung surface area or lung weight (estimated from known ratios of lung weight/body weight for the species or age) may be more appropriate.

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