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Retrospective analysis of acute inhalation toxicity studies: Comparison of actual concentrations by filter and cascade impactor analyses

Jürgen Pauluhn *

Institute of Toxicology, Bayer HealthCare, 42096 Wuppertal, Germany

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Abstract

Determination of acute inhalation toxicity is usually the initial step in the assessment and evaluation of the toxic characteristic of a substance that may be inhaled. Commonly, data from this bioassays may serve as a basis for classification and labeling and may also be used for the derivation of Emergency Response Guidance Levels. The focus of this analysis is on the comparative measurement of actual total mass concentrations in inhalation chambers obtained from independent filter (or alternative) analyses and cascade impactor analyses and whether the similarity/disparity of concentration measurements found by different equipment and sampling strategies could serve as robust criterion for the identification of inconclusive measurements. Potential artifacts leading to erroneous concentrations include anisokinetic sampling errors, obstructions of filters, errors related to the calculation/measurement of the sampled volume of atmospheres, wall losses or evaporation. The outcome of this analysis supports the conclusion that the mass concentrations obtained by the commonly performed cascade impactor analysis provide an important adjunct to the established procedures. In summary, the similarity of mass concentrations obtained independently by cascade impactor and filter analyses, i.e., sampling equipment with different aspiration efficiencies and collection media, improve the judgment whether the results from atmosphere characterization are 'conclusive' or 'inconclusive.'

Keywords: Aerosols; Collection efficiency; Atmosphere characterization; Particle size; Collection efficiencies; Ranking of inhalation studies; Acceptance criteria

1. Introduction

Especially in acute inhalation toxicology, testing guidelines, and available technologies have improved significantly over time both in terms of the more defined exposure of experimental animals and the characterization of test atmospheres. Accordingly, especially for short-term inhalation studies, exposure paradigms have shifted from whole-body to nose-only modes with novel and more refined procedures to minimize the re-breathing of atmospheres, a faster attainment of inhalation chamber equilibrium concentrations, and optimized uniformity or degree of dynamic mixing of flows within an inhalation chamber. To date, the availability of computer-supported real-time monitoring devices and increased analytical sensitivity allows a better appreciation of a uniform, spatial dispersion and temporal stability of test materials in an inhalation chamber or at different nose-only exposure ports.

This dependence on available technologies to the dosing of experimental animals is somewhat unique to inhalation toxicology. Therefore, the comparative assessment of inhalation studies with the same test material from different laboratories often requires a critical analysis to give preference to studies of highest

* Fax: +49 202 364589
E-mail address: juergen.pauluhn@bayerhealthcare.com

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quality, which often means most conclusive exposure data. Most contemporary testing guidelines attempt to make this judgement based on the comparison of nominal concentrations (mass of test material disseminated into the exposure system during the generation period divided by the total airflow through the inhalation system during the same time period) and actual concentrations (mass concentration of test material recovered from the breathing zone of the exposed animal). Indeed, this approach appears to be valid for gases and vapors because the nominal flow-rates used to meter the neat test material (gas) or the weight-loss of the test substance from reservoirs (e.g., bubblers) predict with high precision what should be present analytically within the exposure atmosphere. In contrast, for liquid and solid aerosols technically demanding measures have to be taken to effectively remove larger particle-size fractions from the air stream and actual concentrations are increasingly discordant from nominal concentrations. Device-specific default ratios of nominal to actual concentrations have little relevance as the physico-chemical properties of test substances per se (e.g., viscosity, volatility, ability to sublimate or to co-distillate with any carrier material, dustiness, stickiness, and coagulation) as well as the methodology used for aerosol generation affect this ratio, at high concentrations even in a concentration-dependent manner. For instance, Collison nebulizers operate using an atomizer tube dipped into a fluid reservoir and baffles intercept the majority of droplets having diameters greater than about 10 μm. Accordingly, the majority of the atomized liquid refluxes back into the reservoir. Depending on the volatility of the fluid appreciable amounts might evaporate with subsequent changes of the remaining liquid. Similar artifacts are not occurring in atomizing systems. Thus, each generation system might not only have its own typical ratio of actual to nominal concentrations it is commonly also affected by the concentration and specific physical characteristics of the test substance examined.

The objective of this analysis is to assess retrospectively data related to the characterization of atmospheres from acute inhalation studies with chemicals (active ingredients, intermediates) and agrochemical or biocidal mixtures (liquid, pure materials—27; liquid mixtures—112; solid, pure materials—28; solid mixtures—10; and fumes and spray-cans—6) over a time period of approximately one decade. The focus is on the measurements of actual mass concentrations obtained from filter (or alternative) analyses and cascade impactor analyses (total mass collected) taken during the same exposure and whether the similarity/disparity of concentration measurements found by two the different pieces of equipment used in standard inhalation toxicity testing can serve as criterion to distinguish 'conclusive' from 'inconclusive' results. All studies utilized the same type of *directed-flow* nose-only exposure system and equipment for atmosphere characterization.

2. Methods

2.1. Test guidelines

The data from the studies analyzed was carried out in accordance with OECD Guideline No. 403 and the study conditions were adjusted to fulfill other testing guidelines, such as Council Directive 92/69/EEC, OPPTS (1998) or Japan MAFF, Notification No. 12 Nousan-8147 (2000).

2.2. Aerosol generation and exposure technique

Animals (rats) were exposed to the aerosolized test materials in Plexiglas exposure restrainers using a commercially available *directed-flow* nose-only exposure system (TSE Systems GmbH, Bad Homburg, Germany). The validation of this chamber system has been published elsewhere (Pauluhn, 1994; Pauluhn and Mohr, 2000; Pauluhn et al., 2000).

Pre-tests (without animals) were always conducted with first preference to maximize the output of the aerosolization system and secondly to generate airborne particulates targeted at a mass median aerodynamic diameter (MMAD) $\leqslant 4$ μm (OPPTS, 1998; SOT, 1992). Liquid aerosol generation utilized either a nozzle atomization (TSE-binary jet nozzle or Schlick-nozzle Type 970, form-S 3; Schlick GmbH, Coburg, Germany) or *a* modified BGI 3-nozzle Collison nebulizer (Type CN-25 MRE, BGI, Waltham MA, USA) system. The temperature of liquid reservoirs and/or nozzles were maintained at 25–40 °C using a digitally controlled thermostat to increase the aerosolization efficiency for viscous materials. During the course of the exposure period, the reservoir of nebulizers was exchanged hourly to avoid appreciable changes in concentration of test material in the reservoir. Most of the solid aerosol studies utilized either a Wright Dust Feeder (BGI) or an EXACTOMAT 4200 (TSE). For powder dispersion, the operating principle of the *Exactomat 4200* was the following: the test substance is filled into a glass reservoir, then the powder is fed (by suction) into the orifice of a venturi tube and conveyed by a high air-flow rate into the inhalation chamber. Reproducible and temporally stable dosing into the orifice is commonly achieved by an oscillating orifice between the exit of the dust reservoir and orifice of the venturi tube. The principle performance of the Wright Dust Feeder dust generating system can be described as follows: the test substance is filled into the reservoir of the dust generator, is then