mask and virtual mask systems produced similar results of 3 to 5 ml of exhaled breath condensate collected per hour per pig for pigs weighing approximately 8 kg. (This work was performed under a subcontract to Applied Physics Laboratory, APL No 864736, as part of a DARPA funded project MDA972-01-D-005)

2123 EFFECTS OF 1, 3-BUTADIENE, ISOPRENE, AND THEIR PHOTOCHEMICAL DEGRADATION PRODUCTS ON HUMAN LUNG CELLS.

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Due to potential exposure in both workplace and ambient air, the known carcinogen 1, 3-Butadiene (BT) is considered to be a priority hazardous air pollutant (HAPS). Most of the studies investigating BT toxicity have examined carcinogenic endpoints. BT and its 2-methyl analog, Isoprene (ISO) are chemically similar but have very different toxicity, with ISO showing no significant carcinogenesis. The degradation chemistry of these compounds induced by sunlight is not fully understood, resulting in many unknown degradation products, which makes it difficult to identify their full toxic potential. In this study, we determined the relative toxicity and inflammatory gene expression induced by BT, ISO, and their photochemical, degradation products in A549 cells, a human alveolar type II-like cell line. A concentration of 200 ppb BT or ISO in the presence of 50 ppb NO were injected into separate photochemical smog chambers and allowed to photochemically react for approximately 4 hours. At sundown, A549 cells grown on membraneous support were exposed for 5 hours to either the gaseous precursor mixtures, i.e. BT + NO or ISO + NO, or their gaseous photochemical reaction products. GC and GC/MS initial analysis indicates that the primary photochemical products produced during these experiments for BT are acrolein, acetaldehyde, and formaldehyde, while photochemical reactions of ISO generated methacrolein, methylvinylketone, and formaldehyde (both formed ozone ≤ 200ppb). Approximately 9 hours post-exposure, the cells were examined for cytotoxicity and IL-8 gene expression. BT induced a 2-3 fold greater cytotoxicity and IL-8 gene expression as compared to its photochemical reaction products. In contrast, ISO photochemical reaction products caused a significantly greater cytotoxicity and IL-8 gene expression as compared to the unreacted ISO precursor. Taken together, these data suggest that biogenic ISO becomes increasingly more toxic as it reacts within the natural atmosphere, unlike BT, which appears to become less toxic as it reacts with sunlight.

2124 ACUTE, FOUR-WEEK, AND MICRONUCLEUS INHALATION STUDIES IN RATS WITH HEXAFLUOROISOBUTENE EPOXIDE.

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Hexafluoroisobutene epoxide (HFIBO) is an intermediate used in the preparation of perfluorinated compounds and it is considered to be moderately toxic following inhalation. A single 4-hr exposure to 820 ppm was lethal in 3 of 6 male (m) rats; ataxia was noted at 600 ppm, and weight loss at 600 and 200 ppm. After positive findings (strains TA 100 and WP2 uvrA) in an Ames Salmonella assay, HFIBO was found to be negative in an *in-vivo* bone marrow micronucleus study where m and f rats were exposed to 25, 125 or 350 ppm of HFIBO for 6 hr/day for 2 days. However, at 350 ppm, 3 of 12 f rats died on the 2nd exposure day. In a 4 wk study, groups of 20 m and 20 f rats were exposed for 6 hrs/day, 5 days/wk to 5, 25, or 125 ppm of HFIBO followed by a 4 wk recovery. No deleterious effects were observed in clinical observations or body weights during the exposure or recovery periods. Neurobehavioral tests (including grip strength, FOB, and motor activity tests) at the end of the 4-week exposure period showed no effects. Clinical pathology evaluation showed minimal changes in rats exposed to 125 ppm, including decreased platelets (m only), decreased mean cell hemoglobin concentration and red cell distribution width (f only), increased serum bilirubin and urea nitrogen (f only), and increased urine volume/decreased urine osmolality (m only). Urinary fluoride was increased only at 125 ppm, indicating metabolism of HFIBO. None of the clinical pathology findings were considered necessarily adverse; rather the changes were minimal, likely unrelated to the test substance, and within the range of normal values. A slight reversible increase in heart weights (but no corresponding histological observations) was seen in m and f rats in the 125 ppm groups. No other anatomic pathologic effects were observed in this study. Based on the increased heart weights at 125 ppm, the no-observed-adverse-effect-level (NOAEL) for repeated inhalation exposure was 25 ppm.

2125 SINGLE-PASS BUBBLE AEROSOL GENERATOR FOR INHALATION STUDIES.

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Inhalation exposure studies of pathogens require stable and reliable aerosol generators. As fragile organisms are aerosolized, their structural and biological integrity must be preserved. Air jet as well as ultrasonic nebulizers induce significant stress and reduced *via*bility due to forceful agitation, high shear force and repeated "recycling" of the carrier media. Recently, an aerosol generator that produces particles from a bubbling liquid was reported. This technique promised low shear force but still recycled the fluid. We describe an improvement in particle generation from a bubbling liquid by eliminating fluid reuse. In this device, a thin film of liquid is pumped onto the upper surface of an encapsulated porous stainless steel disk. Porosity can be set from 0.2 to 100 microns. Air is forced through the disk from below and breaks the liquid film into bubbles that grow and subsequently burst, releasing aerosol particles to the air. The particles are gently stirred and entrained by eight or more streams of dry air parallel to the surface. These radial streams are above the disk. Particles not entrained collect in the glass vessel and play no further role. We aerosolized P. fluorescens bacteria and polystyrene latex particles (1 micron) and determined that the particle concentration increased as the airflow increased from 2 to 5 L/min. Drying airflow from 10 to 30 L/min increased aerosol output. Distance above the porous disk had little effect. Constant generation rate over one hour demonstrated stability. Since organisms go through the aerosolization process only once, no significant decrease in bacterial *via*bility is expected during prolonged generation. As such, this instrument is useful in inhalation studies where extended delivery of stable and undamaged biological aerosols is required.

2126 COLLECTION, VALIDATION AND GENERATION OF BITUMEN FUMES FOR CHRONIC INHALATION STUDIES IN RATS.

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The objective of the study was to develop a laboratory generated exposure atmosphere to be used for chronic inhalation toxicity studies in rats that resembles, as closely as possible, personal exposures seen in workers during road paving operations. To achieve this, bitumen fume condensate (BFC) samples were collected from hot bitumen storage tanks and compared analytically with atmospheric workplace samples collected by different sampling devices and strategies. Two different laboratories analyzed personal and static samples taken at road paving worksites by different methods. Parameters determined were: Total Particulate Matter, Benzene Soluble Matter, Semi-Volatiles and Total Organic Matter, Boiling Point Distribution (BPD), Polycyclic Aromatic Hydrocarbons and UV Fluorescence (UVF). Different sampling methods were used to allow comparison of the standard German sampling method with the most commonly used industrial methods. A collecting procedure was developed that allowed sampling from hot bitumen storage tanks in an operational asphalt mixing plant. The sampling procedure has been optimized to collect material that matches the workplace samples as closely as possible. The validation procedure has been performed using a range of parameters that could be analyzed in both the workplace samples and the BFC collected from the tanks. BPD, UVF and content of individual PAHs were selected as parameters. For example the BPD of the collected sample did not differ more than 17 degree C from the average boiling point distribution of the work place samples, in the range from 5 to 95%. UVF of the BFC nearly exactly matched the average fluorescence of the workplace samples (105%). As a result, approximately 16 liters of BFC have been collected. A laboratory set-up for the re-generation of the airborne fume at three different exposure concentrations but with a similar composition was developed and has been running successfully for several months. The study was sponsored by the Bitumen industry assocaitions, ARBIT, Germany and Eurobitume, EU.

2127 INHALATION TOXICITY OF THE FLAVORING AGENT, DIACETYL (2, 3-BUTANEDIONE), IN THE UPPER RESPIRATORY TRACT OF RATS.

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Diacetyl (2, 3-butanedione) is a diketone found naturally in foods such as butter and "generally recognized as safe" for use in low concentrations as a food additive. Diacetyl imparts the odor and flavor of butter to foods and also has industrial applications. Recently, an increased prevalence of fixed airways obstruction was reported in workers at a microwave popcorn plant and the lung disease correlated

with diacetyl exposure. In a previous study, inhalation of diacetyl-containing artificial butter flavoring caused necrosis of the nasal, bronchial, and bronchiolar epithelium in rats. We have now investigated the hypothesis that inhalation of diacetyl produces epithelial injury. Therefore, male Sprague-Dawley rats were exposed in a whole-body inhalation chamber for 6 hours to 0, 99.3 ± 0.07, 198.4 ± 0.10, or 294.6 ± 0.20 ppm diacetyl and euthanized the next day. Four levels of nose, three levels of trachea, and two lung sections were examined by light microscopy. In addition, the nose was examined by transmission electron microscopy (TEM) and the trachea was examined by TEM and scanning electron microscopy (SEM). At 198.4 ppm or higher, diacetyl inhalation resulted in significant necrosis of nasal epithelium with associated neutrophilic inflammation. At 294.6 ppm, diacetyl inhalation also caused significant necrosis of tracheal epithelium with associated neutrophilic inflammation. By SEM, diacetyl-induced tracheal changes included multifocal denuding of basement membrane with cell swelling, loss of microvilli, and loss of ciliated cells in the remaining epithelium. By TEM, tracheal changes included epithelial necrosis, denuded basement membrane, and elongation of epithelial cells near foci of exposed basement membrane. Diacetyl did not produce significant changes in the lung under these exposure conditions. These findings suggest that acute exposure to diacetyl alone is sufficient to cause upper respiratory tract epithelial necrosis in rats at concentrations of 198.4 ppm or higher.

2128 THE EVALUATION OF TWO ANESTHETICS FOR USE IN A BRONCHODILATOR SCREEN MODEL.

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The goal of this study was to develop a model for screening bronchodilator drugs using propofol and isoflurane as alternative anesthetics to morphine-chloralose. The first phase involved comparing 2 models: one using propofol and the other using isoflurane anesthesia, when performing methacholine (Mch) challenge experiments (using escalating graded-doses of Mch) in 3 canines. In establishing a bronchoconstrictive model, it was found that with both anesthetic models, the plateau dose-response (seen through dynamic lung resistance measurements) was not consistent even though the peak response was very stable. Shifting to the second phase of the study, the propofol-anesthetized model was chosen over the isoflurane-anesthetized model as it resulted in a lower hypotensive response to the Mch challenge. The second phase used 6 canines and implemented measuring the peak response only, performing a second challenge (directly following the first challenge) to evaluate short-term repeatability of the model, and repeating these experiments using morphine-chloralose anesthesia as a standard for comparison. Following the morphine-chloralose experiments, animals were euthanized due to the known long-term neurological side effects resulting after chloralose anesthesia. Results demonstrated that the propofol model did not produce consistent results to repeat Mch challenge whereas the morphine-chloralose model was repeatable. In conclusion, it is suggested that the morphine-chloralose anesthetized bronchoconstrictive dog model may have potential as a screen for bronchodilator drugs.

2129 VALIDATION OF AN ISO-KINETIC DILUTOR FOR USE WITH AN ANDERSON CASCADE IMPACTOR.

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The Anderson Cascade Impactor (ACI) is one of the most widely used and accepted devices employed by the Pharmaceutical industry for characterizing particle size distribution (PSD) of aerosols. For animal based pre-clinical toxicology studies, use of an ACI for PSD measurements is impractical due to the inlet flow rate required for operation (28.3 L/min). It is necessary to match impactor inlet flow rates to the aerosol available at the animal model-breathing zone. To accommodate the different inlet flows an iso-kinetic diluter was constructed for use with the ACI. The diluter allows the ACI to operate at its designed flow yet draw a low flow sample from the breathing zone in the exposure apparatus. The dilutor sample inlet tube cross-sectional face velocity was maintained equal to the ACI inlet cross-sectional face velocity, accommodating mixing sample aerosol with the dilution air in an iso-kinetic stream. Dilution air was added from a filtered compressed air source. Flow rates of the ACI and the dilution air were controlled and measured with calibrated mass flow meters. Two sample flow rates, representing small (0.5 L/min) and large (3.0 L/min) animal species were selected for validation. Jet nebulizers and solutions containing Certified Particle Size Standards (micro-spheres) were used to produce nearly mono-disperse aerosols in a holding plenum. The aerosol was dried in process before collection with the ACI. For each flow rate, three impactor collection samples were obtained at each of three particle sizes. Impactor stage masses were measured gravimetrically and the results analyzed for MMAD and GSD for each sample. Review and comparison of the results indicate good correlation between particle size samples collected at lower flow rates with the diluter and the nominal particle size used in aerosol generation. This diluter allows the use of the ACI to collect particle size samples from pre-clinical animal toxicology models at sample collection flow rates similar to production rates without significant alteration of PSD

2130 ACUTE RESPIRATORY RESPONSES OF THE MOUSE TO CHLORINE.

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In human subjects, fifteen minute exposure to 0.5 - 1.0 ppm chlorine gas causes a nasal obstructive response in the absence of a marked sensation of irritation. For comparative purposes, the current investigation was designed to assess the respiratory responses of the mouse to this irritant gas. Towards this end, respiratory physiological responses were measured in female C57Bl/6J mice exposed to 0.6 to 3.0 ppm chlorine. Chlorine was a potent sensory irritant, with an RD50 of 2.3 ppm. Chlorine exposure produced airway obstruction as indicated by a concentration dependent increase in specific airways resistance (sRaw) during exposure, which persisted for fifteen minutes post exposure. At 0.6 ppm, chlorine produced mild sensory irritation (<20% change in breathing frequency) and significant obstruction (65% increase in sRaw). Pretreatment with atropine was without effect on the obstructive response suggesting the lack of involvement of cholinergic pathways. Pretreatment with the sensory nerve toxin, capsaicin, dramatically reduced both the sensory irritation and obstructive responses, strongly suggesting the involvement of sensory nerves. Studies were also performed using the surgically isolated upper respiratory tract of the anesthetized mouse. Chlorine was efficiently scrubbed from the airstream (>95%) in that site and produced an obstructive response that was of sufficient magnitude to account for the entire response observed in the intact animal. In summary, chlorine gas produces an immediate nasal obstructive response in the mouse that appears to be similar to that in the human.

2131 COMPARISON OF DOSE AND TOXICITY AFTER ADMINSTRATION OF A FLUOROALKYLETHYL PHOSPHATE SURFACTANT BY DERMAL AND INHALATION ROUTES IN RATS.

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Fluorosurfactants are used as specialty wetting and leveling additives in cleaning and coatings formulations. Previously, this fluoroalkylethyl phosphate surfactant was evaluated for subchronic, developmental, and reproductive toxicity in gavage studies; the liver was the most sensitive target organ. Since the intended uses of this surfactant would predict that dermal or respiratory tract exposures are likely, studies were conducted to assess internal exposure and toxicity by these routes. In the 28-day study, the test substance was applied to the skin of male rats each day for 6 hours at doses of 0, 10, 100, and 1000 mg/kg/day (35% ai). There were no adverse effects on mortality, clinical signs, body weights, food parameters, anatomic pathology, or hematology. Aspartate- and alanine-aminotransferases and sorbitol dehydrogenase activities were increased at 1000 mg/kg/day. The NOEL for dermal exposure was 100 mg/kg/day. In the inhalation study, male rats were exposed nose-only to 0, 0.2, 2, or 20 mg/m3 of aerosol for 6 hrs/day, 5 days/wk for 2 wks. There were no effects on body weights, clinical signs, or clinical pathology parameters. The only histological effects were mild inflammation in the larynx and lung at 2 and 20 mg/m3. The NOEL for inhalation was 0.2 mg/m3. Internal exposures by the dermal, inhalation, and oral routes were compared by fluorine blood analysis (Wickbold Torch Combustion Method). Minimal levels of fluorine were detected at the two lowest doses administered dermally. Fluorine levels were apparent in all rats exposed by inhalation, however, the levels were equal to or less than levels in rats exposed orally. Based on the fluorine dosage results in the above studies and the findings in the respiratory tract after inhalation exposure, inhalation appears to be a more sensitive route of exposure than the dermal or oral routes for this fluorosurfactant.

2132 DEVELOPMENT OF A METHOD FOR THE SIMULTANEOUS ANALYSIS OF VINYL ACETATE AND ACETALDEHYDE CONCENTRATIONS IN THE NASOPHARYNGEAL CAVITY AND EXHALED BREATH OF HUMAN VOLUNTEERS.

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Vinyl acetate is a volatile monomer used in the plastics, coatings, and polymer industries. Inhaled vinyl acetate is absorbed by the nasal mucosa and rapidly hydrolyzed to acetaldehyde and acetic acid. A PBPK model has been developed to describe the uptake of vinyl acetate in the rat and human nasal cavity based on

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