

intervention and at wide ranges of aerosol concentration. The EDPDS is sufficiently versatile to establish stable powder aerosol concentrations of different magnitudes (ie. 5 mg/L to 2000 mg/L) and for different lengths of time (ie. 10 minutes to 4 hours) without having to replenish the supply of test article and interrupt the exposure. Its modular design allows the system to be easily adapted by changing the size of the feed components and by controlling the feed rate with a variable speed motor which can be set to as low as 1 RPM to aerosolize minute quantities of test powder into the exposure chamber. The EDPDS has been used successfully on 5-day, 14-day and 28-day rodent inhalation toxicity studies. The system proved to be an efficient and highly reproducible means of delivering a range of powder concentrations on subacute studies that could be readily applied to subchronic, chronic and carcinogenicity inhalation studies.

971 SPECIES COMPARISON OF METHEMOGLOBIN REDUCTASE.

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Methemoglobin (Mhb) formation is an effective strategy for treating cyanide (CN) poisoning. Methemoglobin reductase (MR) is the enzyme that reduces Mhb back to hemoglobin (Hb). The endogenous, circulating, normal level of MR reflects the capacity to reduce Mhb and is therefore an important factor in evaluating the anti-CN efficacy of Mhb formers. To help determine which animal species would most accurately predict the human response to Mhb formers, the activity of MR was measured (mean±SEM) in whole blood of several species following established analytical methods. The animals employed included rhesus monkeys (*Macaca mulatta*; samples collected at USAMRICD), African green monkeys (*Chlorocebus aethiops*; samples collected at USAMRICD), and beagle dogs (*Canis familiaris*; samples collected at WRAIR). The MR values in these animal species were compared with normal human values. The human range for MR (n = 30) was 12.6 - 24.2 IU/g Hb, with a mean of 16.6±0.44 IU/g Hb (Mayo Medical Laboratories, Rochester, MN). The mean MR level in rhesus monkeys (n = 15) was 20.2±0.74 IU/g Hb, falling within the normal human range. For African green monkeys (n = 19) the mean was 26.0±0.76 IU/g Hb, slightly higher than the normal human upper limit. For beagle dogs (n = 15) the mean MR level was 9.5±0.22 IU/g Hb, approximately 25% lower than the normal human lower limit. Although the beagle dog has been used extensively in the study of Mhb formers, these data suggest that the rhesus monkey may also be an appropriate model.

972 COMPARISON OF RESULTS FROM CROSS-SITE VALIDATION OF CONTINUOUS INFUSION PROCEDURES, EVALUATING TWO ROUTES OF INTRAVENOUS ADMINISTRATION AND PRECANNULATED CRL:CD(SD)IGS BR AND FISHER CDF (F-344)CRLBR RATS.

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Identical validation studies were conducted at our Vienna, VA and Madison, WI laboratories, to determine the feasibility of using vendor-supplied precannulated rats, to compare the use and manageability of Fisher-344 and CD[®] rats, to evaluate the jugular and femoral routes of intravenous infusion, and to assess survival when a known vascular irritant is infused. The results of the two studies were compared to determine the similarities and differences between sites when a common protocol is followed and a harmonized technology is utilized. The rats were precannulated by the supplier (Charles River Laboratories) and eighty animals (10/sex/strain/route) were assigned to each study. Saline (0.9%) was administered to all rats at a rate of 15 mL/kg/1 hour daily, for Days 1-15; from Days 16-30 a 7.5% solution of Solutol HS was infused at the same rate to equal numbers of each sex/strain/route. A standard battery of ante- and post-mortem evaluations were performed, to include mortality, clinical signs, body weights, food consumption, clinical pathology, necropsy findings, and histopathology. Ten rats died, were sacrificed moribund due to sepsis and inflammation of the infusion site, or were removed from each study; the majority (70%) were male CD[®] rats exposed to Solutol with no predilection for route. Clinical observations included swelling at the cannulation site, primarily in the jugular CD[®] rats exposed to Solutol. Males of both strains exposed to Solutol had a slight decrease in body weights and food consumption with a less apparent effect in females during Weeks 3 and 4 of treatment. Clinical pathology changes were similar between studies and

were primarily related to chronic cannulation (decreased red blood cell mass, increased neutrophil count, and increased globulin). In addition, lower cholesterol values were found in both strains of rats given Solutol. Microscopic evidence of catheter-site abscessation were more frequent in jugular CD[®] rats at the Vienna site, with no appreciable difference in lesion incidence among groups at the Madison site. These data indicate that similar results were obtained for both studies, precannulated rats are acceptable for use, femoral intravenous infusion is preferred for irritating formulations, and the Fisher rat is a superior model for chronic infusion.

973 ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (ESI-MS/MS) ANALYSIS OF URINARY METABOLITES OF 1,1,2,2-TETRACHLOROETHYLENE USING AN AUTOMATED SPE SAMPLE PREPARATION SYSTEM.

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1,1,2,2-Tetrachloroethylene [Perchloroethylene] (PERC, CAS 127-18-4), used extensively as a dry cleaning agent and in industrial degreasing processes, has been reported to increase the incidence of liver tumors in mice or of nephrotoxicity and renal tumors in male rats. Bioactivation of PERC is reported to occur by two pathways, oxidation by CYP2E1 and glutathione S-conjugation. Trichloroacetic acid (TCA, CAS 76-03-9) and dichloroacetic acid (DCA, CAS 79-43-6) are reported urinary metabolites of PERC oxidation whereas the glutathione conjugate, S-(1,2,2-trichlorovinyl)-L-glutathione (TCVG) is further cleaved to S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC) and acetylated to N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine (N-ac-TCVC). A potential biomonitoring method was developed to measure urinary levels of TCA, DCA, TCVC and N-ac-TCVC. Stable isotope-labeled analogs of TCVC and N-ac-TCVC were prepared (Bartles and Miner, 1989) and deuterated DCA was purchased as internal standards. A BenchMate[®] II robotic workstation was used to automate sample preparation. PERC metabolites (>90% recovery) were analyzed using a ThermoQuest Finnegan LCQ tandem mass spectrometer. Chromatographic standards TCVC and N-ac-TCVC were synthesized as above. Compounds of interest were eluted within 12 min and detected using ESI-MS/MS in multiple segments, initially in the negative ion mode for detection of TCA and DCA, and subsequently in the positive ion mode for TCVC and N-ac-TCVC. Urine samples from rats exposed to 100, 500, or 1000 mg/kg PERC contained detectable amounts of PERC metabolites in urine over a 48-hr period. The LOD per injection was 30 fmol for TCVC, 800 fmol for N-ac-TCVC, 10 pmol for DCA, and 75 pmol for TCA. This procedure appears to offer significant advantages over typical extraction and derivatization methods for the same compounds by GC-MS. Thus, PERC internal dose for various exposures can be rapidly quantified using metabolites in a single assay using an automated sample preparation system.

974 INDUSTRIAL COMBUSTOR VALIDATION TEST MEASURING AIR EMISSIONS OF DIOXINS AND FURANS ON A TEQ BASIS USING EPA METHOD 23 AND HRGC/HRMS VERSUS THE AMBSTACK SAMPLER AND CALUX BIOASSAY.

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The analysis of polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) in gaseous samples by EPA method 23 and analysis by high resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS) is expensive and complex. We have designed a low cost unitized sampling system, the (AmbStack Sampler) and combined it with a reporter gene bioassay system, the Chemical Activated Luciferase Expression method (CALUX), to provide direct TEQ estimates of PCDD/PCDF contamination in air. The current study was designed to compare PCDD/PCDF determinations on a toxic equivalency (TEQ) basis by EPA method 23 and HRGC/HRMS and the AmbStack/CALUX system. In the current experiments simultaneous sampling by both methods were performed on a North American Package Boiler (NAPB) combusting a dopant (a mixture of 1,2-dichlorobenzene and copper naphthenate) at a flow rate adjusted to yield a HCl concentration at the stack of approximately 500 ppm at 7% O₂. The flue gas stream for this experiment was stable at a temperature of 140°C with a moisture level of about 11%. Samples were collected for three hours at a flow rate of 3.58 M³ and analyzed by both HRGC/HRMS and CALUX analysis. HRGC/HRMS results were 2.75 ng TEQ/dscm (% O₂) versus 3.4 ng

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