effect that is traditionally assessed using animal-based methods and low throughput cell culture methods. High-throughput, non-animal assays are critically needed in order to advance our understanding of MONP toxicity while minimizing the overall use of animals, in line with the principle of 3 Rs in toxicology. The 96-well CometChip® assay is a relatively new, high-throughput, cell-based assay for *in vitro* measurement of DNA damage. The purpose of this study was to assess its utility for nanomaterial genotoxicity screening. To test its utility with respect to nanomaterials, the CometChip® assay was utilized, alongside benchmark concentration modelling, to assess the DNA damage-inducing potential of pristine copper oxide (CuO), zinc oxide (ZnO), and titanium dioxide (TiO_2) MONPs in mouse lung cells. These MONPs show varying solubility, which is due to their chemical composition and size. To test the contribution of the dissolved species on DNA damage, as well as particle size induced differences, microparticle (MPs) analogues of each MONP, as well as soluble Zn (II) and Cu (II) salts, were also examined. The most potent inducers of DNA damage were CuO MONPs, ZnO MONPs, and ZnO MPs, which induced both dose and time-dependant increases in DNA damage, without significantly decreasing cell viability. Benchmark modelling was used to derive the following DNA damage potency ranking: CuO MONPs > ZnO MONPs > ZnO MPs > $ZnCl_2$ >> TiO_2 MONPs, TiO_2 MPs, CuO MPs, $CuCl_2$. Results from the DNA damage screening align with previously reported toxicity profiles of these compounds, and provide support for the use of the CometChip® assay for high-throughput screening of nanomaterials. This is one of the first studies to use the CometChip® assay for potency ranking of nanoparticle genotoxicity.

PL 2025 Toxicity of Fluorinated and Fluorine-Free Foams: Potential Implications on Firefighter Renal Health

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Military Specification aqueous film forming foams (AFFF)—typically comprised of complex mixtures of per- and polyfluorinated alkyl substances (PFAS), solvents, stabilizers, and wetting agents—are used to extinguish liquid fuel fires. Health concerns from use of AFFF have emerged because of studies documenting elevated PFAS serum levels in some firefighter populations, including firefighters with known exposure to AFFF, compared to the public. PFAS exposure is associated with increased risk for chronic conditions, including decreased kidney function and cancer. With a scheduled phase out of AFFF containing PFAS in the US by 2024, synthetic fluorine-free foams (SFFF) are an attractive replacement; however, little is known about their potential occupational health risks. This project's objective was to conduct rapid high-throughput screening of cultured human kidney cells to identify potential adverse outcome pathways associated with renal injury. Commercial AFFF and SFFF were analyzed for total fluorine using combustion ion chromatography. Human renal proximal tubule epithelial cells with over-expressed organic anion transporter 1 (RPTEC-OAT1) were exposed to serial dilutions of 5 AFFF (A-E), 5 SFFF (F-J), and 7 frequently detected PFAS in cell culture media for 6 - 24 hours. High-throughput screening for cytotoxicity, mitochondrial polarization, and intracellular reactive oxygen species (ROS) levels of exposed cells was performed by multiplex fluorescence and image analysis using a high content imaging platform. All tested AFFF resulted in acute cytotoxicity (IC_{50}) at concentrations ranging from 109-262 ppm (v:v) while SFFF F exhibited the greatest toxicity at IC_{50} = 3.3 ppm (v:v). Toxicity in SFFF G - J ranged from 33 to 278 ppm. Perfluorooctane sulfonate (PFOS) and perfluorooctanoate acid displayed IC_{50} at 74 and 200 ppm (m:v), respectively, while all C4 to C6 PFAS were not acutely cytotoxic. A majority of AFFF, perfluorohexanesulfonic acid (PHFxS), and PFOS caused decreases in mitochondrial depolarization at sub-toxic doses. SFFF F enhanced intracellular ROS dose-dependently 6 and 24 hours post-exposure while the other treatments did not differ from unexposed controls. In summary, AFFF showed similar kidney cell cytotoxicity to C8 PFAS-exposed cells suggesting PFAS within AFFF contributes to kidney cell cytotoxicity. Effects of SFFF varied substantially, signifying additional need for future hazard characterization.

PL 2026 Development and Validation of an Analytical Method for Quantitation of PFAS Constituents in Rat Plasma, Urine, and Liver by UPLC-MS/MS

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Aqueous film-forming foams (AFFF) containing per- and polyfluoroalkyl substances (PFAS) used during firefighting training exercises have been released into the environment and have contaminated drinking water supplies. In support of studies investigating bioaccumulation of PFAS following exposure of rats to AFFF formulations, an analytical method was developed and validated to quantitate major PFAS present in AFFF formulations. The PFAS investigated were 6:2 fluorotelomer sulfonic acid (FTS), N,N-dimethyl-3-((perfluorohexyl)ethylsulfonyl)amino-propanamine N-oxide (FTNO), 6:2 fluorotelomer sulfonamide betaine (FtSaB), 6:2 fluorotelomer sulfinyl amido sulfonic acid (FtSiAoS), and 6:2 fluorotelomer thioether amido sulfonic acid (FtTAoS). The method was validated in rat plasma for FTS, FTNO, and FtSaB. Since standards were not available for FtSiAoS and FtTAoS, they were quantitated using FTS calibration curves. Standards were prepared by fortifying 100 µL plasma with FTS/FTNO/FtSaB and internal standard (FTS-$^{13}C,d_4$). Samples were extracted with 300 µL methanol, frozen at -80°C to separate phospholipids, and analyzed by UPLC-MS/MS in positive ion mode for the first 6 min (FTNO and FtSaB), then switched to negative ion mode for the remaining 12 min (FTS, FtSiAoS, and FtTAoS). The method was linear over the range 0.25-100 ng/mL, accurate (mean RE≤±12.0%), and precise (RSD≤8.0%). Mean recoveries were ≥81%; the limits of detection were 0.0507, 0.0354, and 0.118 ng/mL for FTS, FTNO, and FtSaB, respectively. Since standards of FtSiAoS and FtTAoS were not available, matrix QC standards prepared using AFFF formulation containing all five components were used to assess precision. Intra- and inter-day RSD values were ≤13.2% for all components, except inter-day precision for FtTAoS, which showed day-to-day variation in chromatography. The method was evaluated in male Hsd:Sprague Dawley®SD® rat plasma, urine, and liver homogenate (mean %RE≤±18.7 and %RSD≤7.2 for FTS, FTNO, and FtSaB). Stability of FTS, FTNO, and FtSaB in extracted samples was demonstrated for 5 d at ambient and refrigerated temperatures, as well as in plasma, urine, and liver homogenate stored at -80°C for 63 d (86.5-116% of day 0 concentrations), except for FTNO in liver homogenate (59.8-62.6% of day 0). These data demonstrate that this simple method is suitable for determination of PFAS in rat matrices following exposure of rats to AFFF, but the method could be improved with use of standards for FtSiAoS and FtTAoS.

PL 2027 Untargeted Mass Spectrometric Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Rat Matrices following Exposure to Aqueous Film-Forming Foams (AFFFs)

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AFFFs contain proprietary mixes of surfactants and numerous PFAS. PFAS are released into ground water and drinking water supplies and hence are ubiquitous contaminants. To investigate which PFAS bioaccumulate in rats following exposure to AFFF formulations, untargeted analysis was conducted using high resolution mass spectrometry following liquid chromatographic separation. Male Hsd: Sprague Dawley SD rats were administered a daily dose of 1% dilution of 5 MIL-SPEC-qualified AFFF formulations for 1 or 14 days. Plasma and liver were collected 24 h after the last dose, and urine was collected at 24 h after the first and seventh dose. A pilot assessment was done using a limited number of liver and plasma samples followed by a definitive assessment using liver from 5 animals per exposure duration (i.e. control, single dose, 14 doses). Samples were extracted with 300 µL of methanol, frozen at -80°C to separate phospholipids prior to analysis of the supernatant in both positive and negative ion modes. Data were processed with Compound Discoverer 3.2 using an untargeted approach against a library of standards, and against libraries including PFAS compounds from US EPA's Chemical Dashboard, from Luo et al. (2020), McDonough et al. (2020) and Ruyle et al. (2020). Compounds were identified/annotated using exact mass, isotope ratio, adducts, and comparison of retention time with standards, including 6:2 fluorotelomer sulfonic acid (FTS), N,N-dimethyl-3-((perfluorohexyl)ethylsulfonyl)amino-propanamine N-oxide (FTNO), and 6:2 fluorotelomer sulfonamide betaine, as well as perfluorocarboxylic acids and sulfonates. A limited number of PFAS were detected in liver with levels increasing with exposure duration. FTS was readily detected in all matrices from exposed animals. FTS bioaccumulated in liver from rats administered each AFFF, accumulating 3.6-7.1 fold between 1 and 14 days. FTS was the major analyte (highest peak area) detected in urine, with all AFFFs investigated. FTNO was found in urine from one AFFF. FTNO was not detected in liver, but several possible metabolites were detected. Other compounds detected in liver and plasma included 6:2 fluorotelomer sulfinyl amido sulfonic acid, 6:2 fluorotelomer thioether amido sulfonic acid, and 6:2 fluorotelomer thiohydroxyl ammonium. Perfluoropentanoic, -hexanoic, and -heptanoic acids, which may arise from metabolism of 6:2 fluorotelomers, were found in urine samples from all treated rats. FTS was the major component found to bioaccumulate in liver.

PL 2028 Comparative Accumulation and Biological Activity of Polyfluorinated Chemicals in Rats Varies with Exposure to Different Aqueous Film-Forming Foams

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Aqueous film-forming foams (AFFFs) are used to suppress fires that are difficult to contain. Per- and poly-fluorinated substances (PFAS) in AFFFs have beneficial chemical properties, essential for some firefighting situations, but are of high concern for environmental and human health as they can contaminate soil and water. To investigate possible differences in accumulation or toxicity, we compared five different PFAS-containing AFFFs (AFFF1-5) that are currently in use. Male Hsd:Sprague-Dawley®SD® rats were administered 0, 0.03, 0.3, or 1% v/v dilutions

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