THREAT AGENT DEFEAT MODELING AND TESTING USING WMD SIMULANTS

Stable Isotope Tracer Method To Monitor Methyl Salicylate In Biological Matrices For Assessing Mustard Gas Exposure During Protective Clothing Testing

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All chemical protective apparel for the military and first responders must be tested prior to use in the field. However, because of the toxicity of chemical warfare agents, surrogate compounds with similar properties are used instead. For assessing mustard gas exposure, methyl salicylate (MeS) is used as the surrogate and is currently measured by passive adsorbent samplers placed on the participant's skin. This does not accurately represent what gets absorbed into the body. Additionally, MeS is present in plant-based foods, medicines, etc. so testing a whole-body exposure dose is challenging. No method currently exists to associate a measured biological fluid concentration within an initial exposure dosage of MeS. A method was developed that can measure and distinguish between exposure and endogenous levels of MeS and its metabolites [salicylic acid (SA), salicyluric acid (SU), salicylate glucuronide (SPG), salicylic acyl glucuronide (SAG) and gentisic acid (GA)] in biological samples by leveraging isotopologues.

Sample preparation methods were developed to extract MeS and its metabolites from human and porcine matrices: urine & saliva via "dilute-and-shoot" methodologies, & plasma via a cold methanol crash. All samples were analyzed using an Agilent 1290 UPLC and 6495c QqQ. The analytes were separated on a Phenomenex Kinetex F5 analytical column. A dynamic multiple reaction monitoring method was used to measure two transitions for all analytes and each isotope version. The data was analyzed using Skyline®.

Using a neat solvent preparation, the following limits of quantification were determined: MeS & GA at 10 ng/mL, SA at 2 ng/mL, & SAG, SPG & SU at 1 ng/mL. The endogenous forms of these analytes were successfully detected in both porcine and human matrices. A 13C6 labeled ring version of MeS was used for the exposure analogue to avoid biotransformation of a deuterium labeled compound. This allowed us to track multiple transitions for each isotope analogue; light (endogenous), exposure (13C6 labeled), and internal standard (deuterium or 15N). The final component of this study involved a human exposure trial to produce biological samples that contained the metabolic products that were generated in vivo from a 13C6-MeS exposure due to only MeS and SA having commercially available 13C6 analogues. Precursors and potential product ions were predicted based on fragmentation patterns of the non-labeled versions while also using in-silico predictions (Chemdraw version 19.1.1.21). Exposure precursor masses were confirmed on a high-resolution mass spectrometer, Thermo Orbitrap 240. Following human exposure to 13C6-MeS, 30-44% of that metabolite is converted into 13C6-SU in urine. A small percentage ~2% is converted into 13C6-SA in blood. The remaining exposure metabolites were detected but not measurable. Endogenous MeS metabolite levels were found to be highly variable across the two human participants which highlights the need to validate this method in a larger human trial to establish background concentrations of MeS metabolites in test subjects' pre- and post-exposure biological media. Use of 13C6-MeS as the exposure analogue allowed for measurement of biological uptake post exposure which will be used to develop modeling tools that can be implemented in larger field scale testing.