MITIGATION - SCIENCE AND TECHNOLOGY ADVANCES FOR CHEMICAL AND BIOLOGICAL HAZARD MITIGATION

Exploration Of In Vitro Methods For Investigating Mechanism Of Action Of Sulfur Mustard (HD)

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HD exposure produces a lesion characterized by erythema, itching, and sensitivity to touch after a latent period, with subsequent blistering and necrosis with severity depending on dose. A host of inflammatory mediators have been observed both in vitro and in vivo in response to percutaneous HD exposure, though upstream targets have not been identified.

Modern in vitro methods, including microphysiological systems (MPS), provide potential avenues for new discoveries around a century old chemical. In this work, we aim to answer the question “which modern in vitro methods are useful in understanding mechanism of action (MOA) of HD?” Results of this work may provide validation of new models for applications in toxicology, decontamination, medical countermeasure development, and protection.

Exploratory research was conducted to identify promising New Approach Methods (NAMs) for probing MOA of HD. A thorough review of HD literature and interviews with subject matter experts were conducted. A variety of cell types and diverse culture techniques, including 2D culture, 3D culture, and MPS, were exposed to acutely toxic doses of HD.

Testing and optimization of analysis techniques demonstrated that dermal primary cells do not exhibit acceptable viability when using standard serum starvation. AlamarBlue™ assay using fluorescence appears most promising for viability analysis in 2D and 3D cultures. Dermal cell types exhibited unique morphologies after exposure, agreeing with known phenomenon of basal keratinocytes degrading at a faster rate than other skin cells in vivo.

This work will inform the DTRA JSTO mission and the Joint Force by providing an updated recommendation for conducting in vitro HD studies, provide updated toxicity data using modern techniques and NAMs, and may provide information on MOA of HD revealing druggable targets for HD prophylaxis or treatment. The results from this work will yield to more effective ways to leverage MPS as the field expands within the DoD.

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