HR-MAS Analysis of Skin Exposed to Chemical Warfare Agents

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Abstract

In the event of a chemical warfare agent (CWA) release, there is a likelihood of CWA deposition on the exposed skin of an unprotected population. Therefore, it is vital to understand the interaction of CWAs and skin to design and evaluate successful decontamination techniques and technologies. This research uses HR-MAS NMR to analyze pig skin samples before and after exposure to chemical warfare agents. The degradation of the agents was followed using this nondestructive technique, and breakdown products and the rates of degradation were measured. Nerve agents and blister agents can be investigated using this technique. Further research will allow for evaluation of effectiveness of decontamination methods at different time points.

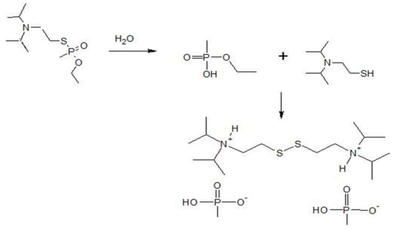
Why Pig Skin?

Pig skin, also known as porcine skin, is used in many experiments as a substitute for human skin. This is because it is the closest in chemical and physical likeness. Human and pig skin share a firm skin attachment, thick epidermis and dermis, sparse hair coverage and re-epithelialization as a healing mechanism. While guinea pig, mouse and rat skin share some of these similarities with human skin, pig skin is the only kind that shares them all (Summerfield, 2015).

Summerfield, A.; Meurens, F.; Ricklin, M. E. The Immunology of the Porcine Skin and Its Value as a Model for Human Skin. Molecular Immunology 2015, 66 (1), 14–21.

VX Hydrolysis

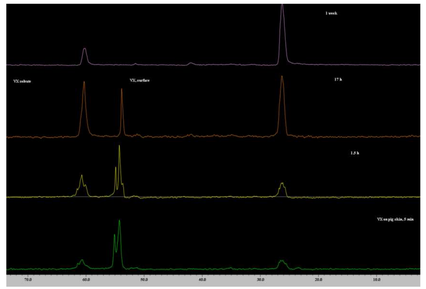
Pig skin samples were cut into small pieces, and exposed to 10 ul of VX. The structure of VX and its typical hydrolysis pathways are shown below.



The hydrolysis process typically starts with cleavage of the P-S bond, and loss of the diisopropylamino sidechain. Under certain conditions, the ethyl group can be lost, resulting in a highly toxic byproduct referred to as EA-2192. When the ethyl group is lost as a second hydrolysis product, the much-less-toxic methyl phosphonic acid is formed

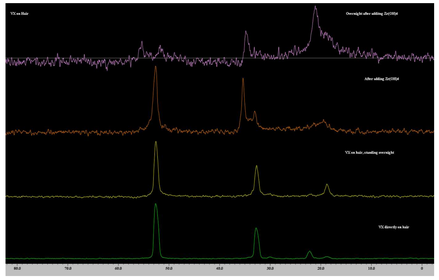
P31 Solids NMR of Pig Skin

Solids NMR experiments were performed using a Doty HR-MAS probe. Pig skin was added to the solids rotor followed by 10 ul of VX. Solids NMR experiments often produce spectra with broad peaks that can be difficult to interpret. These spectra are very detailed for solids spectra, allowing for much easier identification and quantification of the remaining chemical agent, and the reaction products formed.



The bottom spectra shown was taken just five minutes after spiking the VX onto the skin samples. The peak at 54 ppm represents free VX, and the peak at 55 ppm is assigned to protonated free VX. The broader peak at 62 ppm is assigned to VX that has been absorbed into the skin. At 26 ppm, the first hydrolysis product, ethyl methylphosphonic acid is observed. By 17 hours, substantial breakdown of the VX can be seen, and after one week, the hydrolysis product is the predominant compound.

An experiment was also done with human hair as a precursor to studying both the hair and skin together as a substitute for areas of the body with hairy surfaces. These results are seen in the spectra to the right. In the hair samples, we can see the formation of the secondary hydrolysis product, methylphosphonic acid, at 19 ppm, as well as the previously seen product. Adding Zr(OH)4 to the spiked hair sample greatly accelerated the formation of the hydrolysis products, and may indicate potential for this compound as a decontaminating agent.



Conclusions

These experiments have shown that HR-MAS solids NMR analysis shows great potential as a technique for the study of skin and hair exposed to chemical agents. The in vitro methods developed can produce highly interpretable spectra, and the non-destructive nature of the NMR experiment allows for repeated analysis of the samples to obtain kinetic data, and the identification of breakdown products formed. This technique offers significant advantages over extraction techniques that may miss absorbed agent, or will disrupt a particular experiment, creating only a single time-point analysis for each sample prepared. Further experiments will be performed to evaluate the degradation of agents under a variety of conditions, and using other chemical agents, such as mustard, and G-series agents like Sarin and Soman.

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