USAMRICD-TR-15-01

Confocal Raman Microspectroscopy: The Measurement of VX Depth Profiles in Hairless Guinea Pig Skin and the Evaluation of RSDL

Ernest H. Braue, Jr.
Bryce F. Doxzon
Horace L. Lumpkin
Joseph D. Boecker
Richard E. Sweeney
John I. Azeke

February 2015

US Army Medical Research Institute of Chemical Defense
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

an element of the

US Army Medical Research and Materiel Command

DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The views expressed in this manuscript are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
February 2015	Technical Report	August 2011 to August 2012

4. TITLE AND SUBTITLE
Confocal Raman microspectroscopy: The measurement of VX depth profiles in guinea pig skin and the evaluation of RSDL

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Braue, EH. Jr., Doxzon, BF, Lumpkin, HL, Boecker, JD, Sweeney, RE, Azeke, JI

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDT-T
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER
USAMRICD-TR-15-01

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Defense Threat Reduction Agency
8725 John J. Kingman Road STOP
Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
DTRA proposal #2H.857_08_RC_C

14. ABSTRACT
VX depth profiles were obtained using a confocal Raman microspectrometer (River Diagnostics) with a specially designed microscope objective to focus low power laser light into the skin. Noninvasively and in real-time, the scattered light was collected to a depth of about 100 µm with an axial resolution of approximately 4 µm. The objectives of this study were to use confocal Raman microspectroscopy to investigate the fate of percutaneously applied VX in the upper skin layers of hairless guinea pigs and to determine the ability of Reactive Skin Decontamination Lotion (RSDL) to remove or degrade VX from the skin surface and from any depots forming below the skin surface. VX was observed to remain in the stratum corneum, 0-20 µm deep, at about 10-20 minutes postexposure. This postexposure VX concentration in this stratum corneum depot decreased with time but was still detectable at 48 hr postexposure. RSDL was observed to remove or degrade VX from both the skin surface and the depot below the skin surface. Our conclusions are that confocal Raman microspectroscopy is an effective tool to obtain real-time, non-invasive analytical data on the concentration of chemicals in the upper skin layers and that RSDL effectively removes or degrades VX from the skin surface and from the depot formed in the upper skin layers following percutaneous exposure.

15. SUBJECT TERMS
confocal Raman spectroscopy, VX nerve agent, hairless guinea pig, Reactive Skin Decontamination Lotion (RSDL), noninvasive real-time skin depth profiles

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Ernest H. Braue, Jr.
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	97	**19b. TELEPHONE NUMBER** *(include area code)* 410-436-2848

ACKNOWLEDGMENTS

I would like to thank Mr. Christopher Byers and his team at the U.S. Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, Maryland, for analyzing our samples for the presence of VX.

This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

EXECUTIVE SUMMARY

The nerve agent VX is a potent organophosphorous compound that is extremely toxic. VX depth profiles were obtained using a confocal Raman microspectrometer (River Diagnostics) that used a specially designed microscope objective to focus low power laser light into the skin. Noninvasively and in real-time, the scattered light was collected to a depth of about 100 µm with an axial resolution of approximately 4 µm. The objectives of this study were to use confocal Raman microspectroscopy to investigate the fate of percutaneously applied VX in the upper skin layers of hairless guinea pigs and to determine the ability of Reactive Skin Decontamination Lotion (RSDL) to remove or degrade VX from the skin surface and from any depots forming below the skin surface. A total of 20 anesthetized hairless guinea pigs (337-456 g) were exposed to neat VX (0.3 µl, 13-14 x LD_{50}), using a specially designed template that allowed repeated Raman measurements on the same skin location. Animals were given a bioscavenger to protect against signs of VX toxicity. Raman depth profiles were recorded preexposure, at various times postexposure, and following decontamination with RSDL, ranging between 2 minutes and 48 hours after VX application. Animals were euthanized no later than about 50 hr postexposure. In some animals, exposure site skin punches were collected after euthanasia and analyzed for VX. VX was observed to remain in the stratum corneum, 0-20 µm deep, at about 10-20 minutes postexposure. This postexposure VX concentration in this stratum corneum depot decreased with time but was still detectable at 48 hr postexposure. RSDL was observed to remove or degrade VX from both the skin surface and the depot below the skin surface. Our conclusions are that confocal Raman microspectroscopy is an effective tool to obtain real-time, non-invasive analytical data on the concentration of chemicals in the upper skin layers and that RSDL effectively removes or degrades VX from the skin surface and from the depot formed in the upper skin layers following percutaneous exposure.

INTRODUCTION

The nerve agent VX (o-ethyl S-[2-(diisopropylamino)ethyl]methylphosphonothiolate) is an organophosphorous compound that acts by inhibiting acetylcholinesterase, an enzyme that aids in the breakdown of the neurotransmitter acetylcholine. This neurotransmitter then accumulates at synaptic sites, causing cholinergic system hyperactivity. Symptoms of VX poisoning include miosis (pinpoint pupils), rhinorrhea (runny nose), lacrimation (watery eyes), vomiting, bronchial constriction, muscle fasciculations, seizures, and ultimately death.

Percutaneous exposure represents the most likely real-world threat scenario for persistent nerve agents like VX. The use of confocal Raman microspectroscopy to investigate the fate of percutaneously applied VX in the upper skin layers has direct application to the evaluation of candidate decontamination products and postexposure treatments. Studies of anesthetized swine percutaneously exposed to VX have indicated that decontamination can be delayed for up to 30 minutes and still be effective, suggesting that VX does not completely distribute from the upper layers of skin (Bjarnason, 2008). Studies in guinea pigs involving VX (Braue, 2009 and Braue, 2011) have indicated that decontamination can be delayed for up to 25-30 minutes and still be effective. These observations suggest that VX slowly distributes from the upper layers of skin, making the skin a temporary reservoir or depot for VX. Until the development of confocal Raman microspectroscopy technology, however, the extent and distribution of agent in the skin could not be determined non-invasively in real time. Understanding the time course of agent distribution in and through the skin and the extent of its axial distribution with time is critical to the development of effective therapeutics and decontamination products and to the timing of their administration. The River Diagnostics Raman Skin Analyzer uses a specially designed microscope objective to focus low power laser light into the skin. The scattered light is collected at a depth resolution of approximately 4 µm. The use of confocal Raman microspectroscopy to measure molecular concentration profiles in the skin is described in a paper by Caspers (Caspers et al., 2001).

Reactive Skin Decontamination Lotion (RSDL) was approved by the FDA in 2003 for the removal and neutralization of vesicants and nerve agents. The Joint Services of the United States (U.S.) established an operational requirement in 2004 (Joint Requirements Office, 2004) for a new skin decontaminant that could be used effectively on the skin, near eyes, around wounds, and on equipment against all chemical, biological, radiological, and nuclear (CBRN) agents as well as against other toxic industrial materials. RSDL was selected as the Joint Service Personnel Decontamination System (JSPDS) by the Joint Program Executive Office for Chemical and Biological Defense at the Milestone C review in March 2007. A manufacturing contract was established and RSDL began to replace the M291 SDK. In this kit, a sponge is saturated with RSDL and sealed in an aluminum-coated packet ready for service members or civilians to apply to the contaminated skin. RSDL is a mixture of potassium 2,3-butanedione monoximate (KBDO), potassium 2,3 butanedione

monoxime (also called diacetylmonoxime, DAM) in a solvent of polyethylene glycol monomethyl ether (MPEG) and water. The RSDL formulation is 1.25 molal KBDO in 9:1 MPEG:water with about 5 per cent DAM added to the solution (Bide, 1996 and 2002). The nominal molecular weight of MPEG is 550 Daltons. This product acts to remove and neutralize the agent on the exposed skin. However, it is not approved for use in wounds or in the eyes. Also, it should not be used in combination with bleach.

OBJECTIVE

There were two objectives to this project.

1. Determine the time course of agent penetration into guinea pig skin and the extent of its axial distribution to a depth of 100 µm.

2. Evaluate the ability of RSDL to remove VX from the skin surface and below the skin surface following topical application.

MATERIALS AND METHODS

Experimental Groups

This study involved four experimental groups. The first group was model development. We used a total of five animals (HGPs 1, 2, 5, 6, and 30). Several animals did not come with catheters or the catheters were not patent. These animals (HGPs 5, 10, 30, 31, and 32) were used for model development or modified experiments in other groups. Prior to VX exposure, several model development animals were used to determine a variety of experimental parameters as well as the best skin location for VX exposure.

The second experimental group was the determination of VX distribution in skin for a period of about 48 hours postexposure. We used a total of four animals (HGPs 3, 4, 7, and 8). These animals were exposed to VX, and Raman depth profiles were recorded at various times (described with each animal summary) postexposure. Animals were euthanized about 50 hours post-exposure. After about 48 hours, two animals (HGPs 7 and 8) were decontaminated with RSDL.

The third group used 10 animals to evaluate the ability of RSDL to remove VX from the skin surface and from the epidermis at various postexposure times (HGP 10 at 2 minutes; HGPs 9, 11, 12, 31, and 32 at about 1 hour; HGPs 35 and 36 about 2 hr, and HGPs 7and 8 at about 48 hours). We only had one animal at 2 minutes postexposure (PE) because this was a modified experiment making use of an animal with a catheter that was not patent.

The fourth group used a total of three animals (HGPs 27, 28, and 29) to evaluate a new model developed to estimate the total amount of VX in the upper layers of skin following exposure using the recorded Raman depth profiles. These experiments also collected 8 mm skin punch biopsies from the exposure site following euthanasia to compare the VX totals estimated from the Raman data to the analyzed skin punches.

Animals

The 20 animals used in this study were hairless guinea pigs [IAF-HA/HO) obtained from Charles Rivers Labs. Most animals came with implanted catheters for easy IV injections. The animals were all males ranging in weight from 337 to 456 g. The animals were maintained under an AAALAC-accredited animal care and use program. The animals were observed for evidence of disease during the acclimation period prior to protocol use. They were housed and maintained under USAMRICD SOP-VMSB-203, titled "Guinea Pig Husbandry." During quarantine the animals were housed two per polycarbonate cage on corncob bedding that was changed twice weekly. The animals were provided commercial guinea pig ration (Harlan Teklad guinea pig Diet, W, #7006) as appropriate and tap water *ad libitum*. Animal holding rooms were maintained at 20-26^{o} ± 2^{o}C with 30-70% relative humidity using at least 10 complete air changes of 100% conditioned fresh air per hour. All animals were on a 12-hour light/dark, full-spectrum lighting cycle with no twilight. During the experimentation the animals were housed singly in polycarbonate containers containing contact bedding and kept in the exposure hoods during the postexposure holding period. Animals were observed periodically throughout the normal work day (0800 to 1700) until euthanasia.

About 24 hours prior to exposure the dosing area on the animal was cleaned by being wiped four to six times with a gauze pad soaked with warm soapy water (Ivory unscented bar soap). The yellowish skin oil was removed. After the cleaning, the soap was removed by gentle wiping with a gauze pad wetted with warm tap water. After rinsing, the site was gently wiped with a dry gauze pad, and the animal was returned to the holding cage.

On the morning of the experiment, animals (typically one or two) were weighed, assigned animal numbers (ear marked with permanent marker) and transported from the animal holding room to the exposure laboratory. Some animals were injected with bioscavenger via catheter to protect them from the toxic effects of VX. Animals were anesthetized, and once they were under anesthesia a small amount of Puralube Vet Ointment (Pharmaderm, Melville, NY 11747) was placed in each eye to prevent the eyes from drying out while the animals were under anesthesia. Before placing the dosing template on the shoulder of the animal Raman spectra were collected on the proposed site to be sure good skin contact was obtainable. Once good contact was established the dosing template was place on the shoulder with the aid of mastasol. Preexposure Raman depth profiles were recorded on most but not all animals. After collecting preexposure depth profiles the animal was challenged with VX and returned to the hood holding cage until it was time for a postexposure Raman depth profile.

All exposed guinea pigs remained in the chemical fume hood throughout the 0- to 48-hour observation period. They were provided with food and water *ad libitum*. The animals were observed for signs of toxicity periodically for as long as they were in the chemical fume hood. At the end of the experiment, guinea pigs were euthanized.

Anesthesia

All animals were fully anesthetized using isoflurane (ISO, GE Healthcare Avance Carestation 6.X anesthesia system, Madison, WI) during VX exposure and the recording of Raman depth profiles. Anesthesia was initiated using an induction chamber with 3% ISO, 100% oxygen, and a total volume of 0.8 ml/min. Anesthesia was sustained using a nose cone with 3% ISO, 100% oxygen, and a total volume of 0.8 ml/min (Figure 1). Once the animal was under anesthesia, a small amount of Puralube Vet Ointment (Pharmaderm, Melville, NY 11747) was placed in each eye to prevent the eyes from drying out while the animal was anesthetized.

Figure 1. Isoflurane anesthesia

Euthanasia

All animals were euthanized 24 hours postexposure in a halothane or isoflurane-filled chamber IAW USAMRICD SOP-VMSB-301, titled “Animal Euthanasia.” After euthanasia, the area of skin receiving VX was excised down to the fat layer and placed in 5% bleach. The carcasses were disposed of IAW USAMRICD SOP-VMSB-301.

VX Dosing Template

A unique dosing template was designed to allow the collection of replicate Raman depth profiles from the same skin site following VX exposure. The dosing template was a 2.38 x 2.75 inch Tegaderm sheet with a 9/16 inch hole punched in the center. A 1.12 inch diameter Teflon disk was placed on the top sticky side of the Tegaderm sheet, and a 3 mm diameter hole was punched in the center of the Teflon disk. This template assembly was placed on the shoulder of the animal with the hole near the backbone (Figure 2).

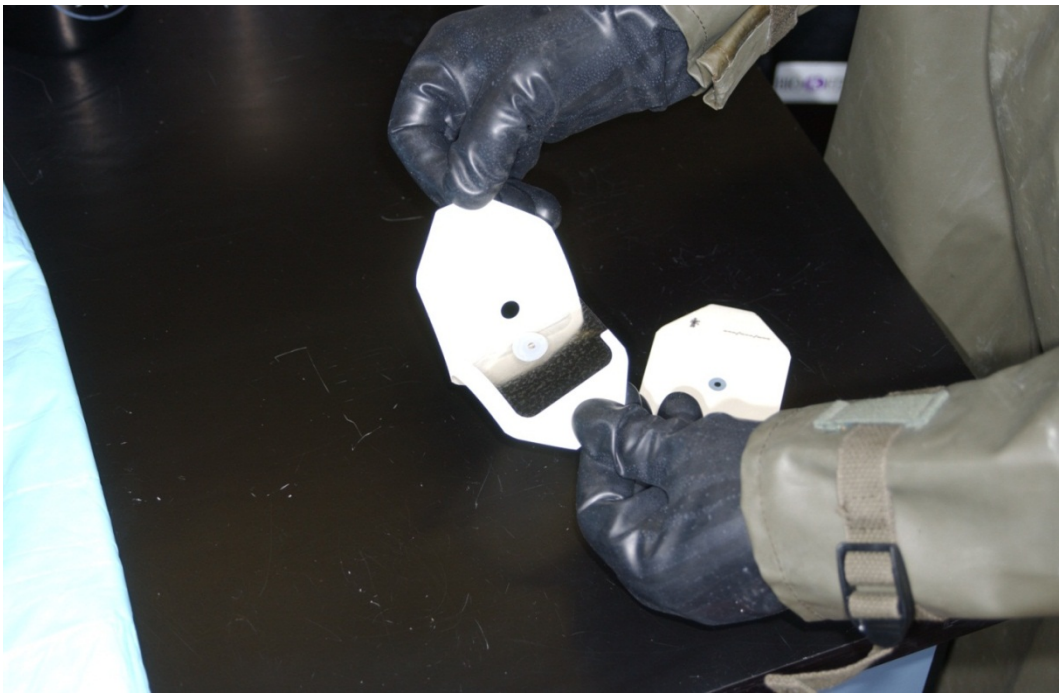


Figure 2. VX dosing template with polytetrafluoroethylene disk. The hole is 3 mm diameter.

Mastisol (Ferndale Laboratories, Ferndale, MI) was applied to the Tegaderm adhesive to assist in keeping the template in place for 48 hours. The mastisol was allowed to dry for 1 min before the template was placed onto the animal (Figure 4).

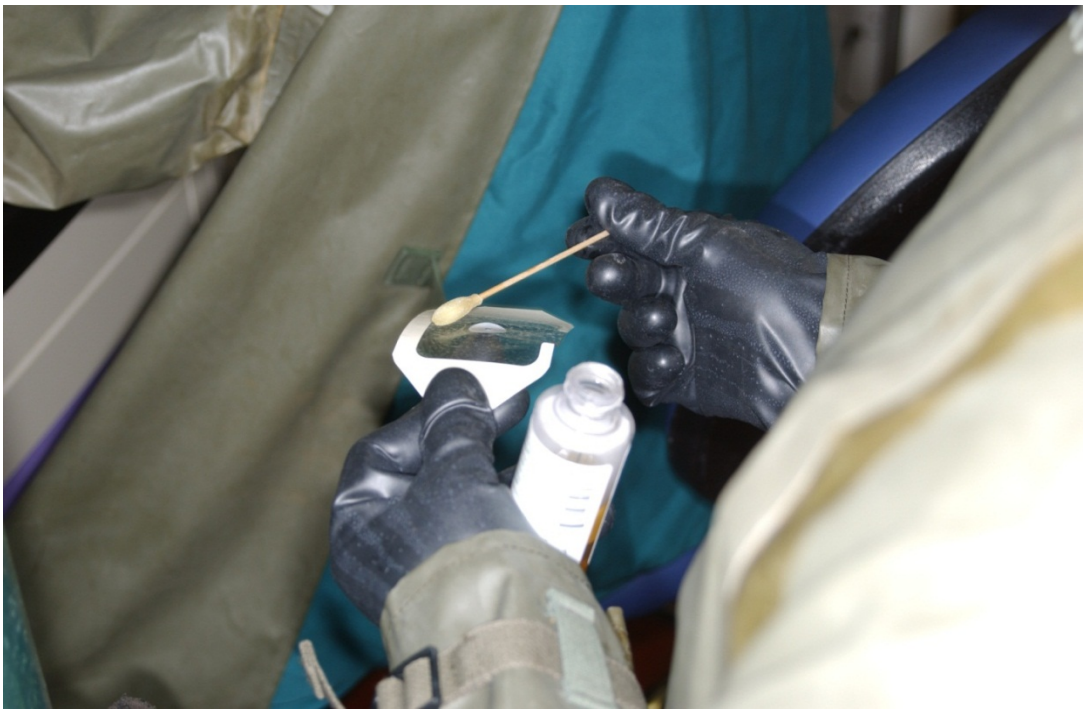


Figure 4. Mastisol application to dosing template.

Agent Application

VX (O-ethyl S-[2-(diisopropylamino)ethyl]methylphosphonothiolate) was obtained from the U.S. Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD. The VX (lot number was VX-U-4076) had a purity of 94% as determined by NMR spectroscopy. The minimum volume that could be pipetted and spread evenly over the 3.2 mm diameter hole in the dosing template was determined to be 0.30 µl using DMSO as simulant. The LD_{50} value for hairless guinea pigs challenged percutaneously with VX was estimated to be 0.056 mg/kg body weight (Battelle, personal communication). The 0.30 µl (284 µg) neat VX dose represents about 13 times the LD_{50} for VX in a hairless guinea weighing 388 g. We used a digital pipette (Hamilton model 7000.5KH 0.5µl PT3 Digital syringe with S/N 07779) to apply the agent (Figure 5).

Figure 5. Hamilton model 7000.5KH 0.5µl PT3 digital syringe.

Bioscavenger Protection

To protect animals from signs of nerve agent poisoning, a human butyrylcholinesterase (HuBuChE) bioscavenger (Protexia, lot #20-08-002, 100 mg/ml HuBuChE) was injected into an external catheter, which was surgically placed into the animals by Charles Rivers technicians prior to delivery (Figure 6). The first animal received 200 µl of HuBuChE solution (Baxter, 20 mg/ml), which provided 4.3% of the stoichiometric amount needed. This animal died 1.47 hr after VX exposure. The concentration of this lot of bioscavenger was too low to provide protection. All other animals received the Protexia HuBuChE product at a stoichiometric amount of 32 to 50%, which provided protection.

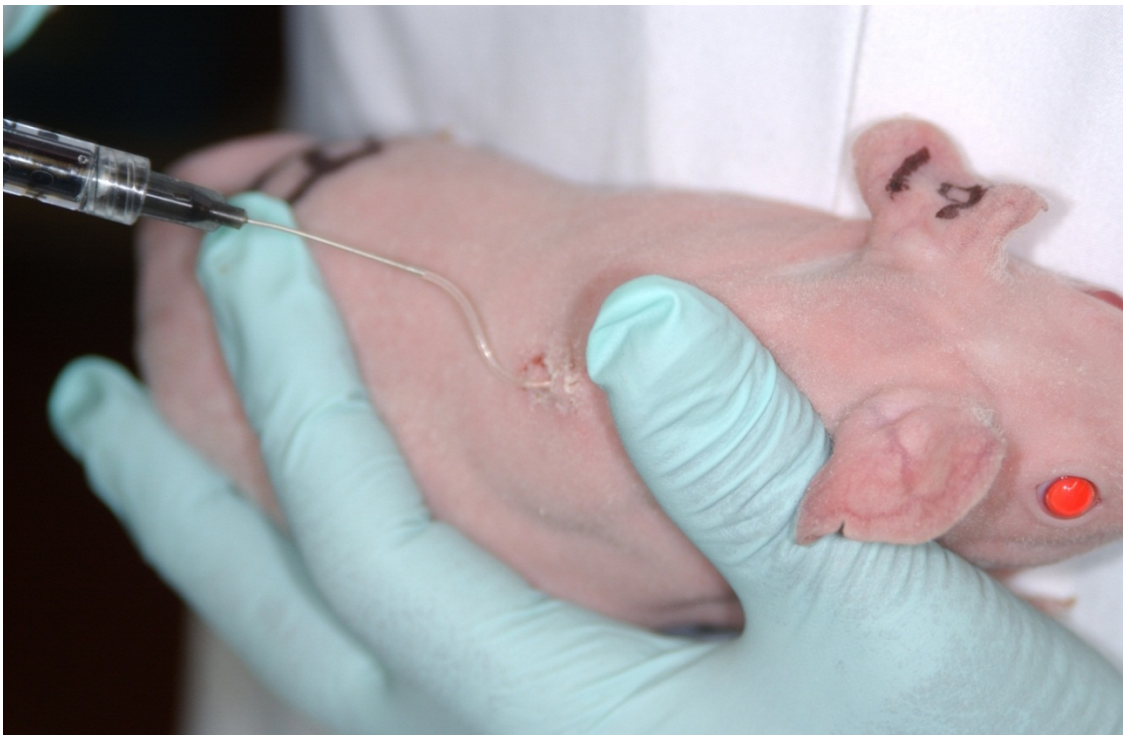


Figure 6. Injection of human butyrylcholinesterase (HuBuChE) bioscavenger.

Confocal Raman Microspectroscopy

Raman depth profiles were recorded in a chemical fume hood using confocal Raman microspectroscopy (Figure 7, River Diagnostics Skin Analyzer model 3510). All depth profiles used the 785 nm laser. At the start of each day, following the manufacturer's instructions (User's manual 3510 V0903), the 785 nm laser power was measured (Ophir-Spiricon power meter using Ophir-Spiricon PD 300 laser detector) at the Raman window and a new calibration was performed.

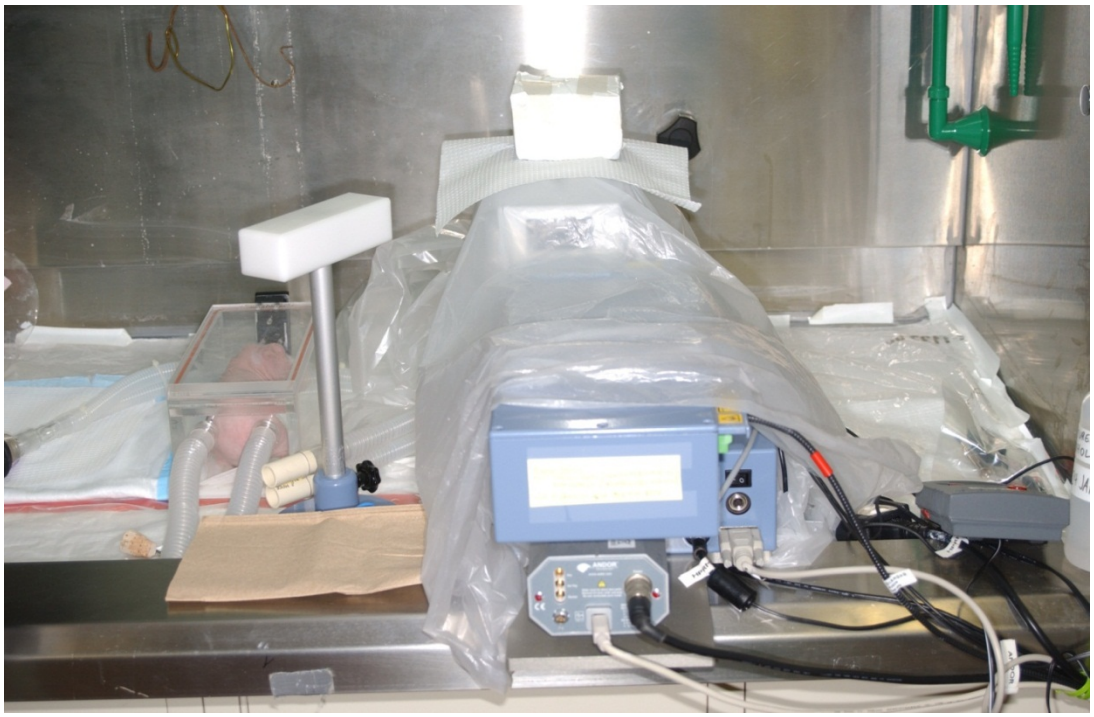


Figure 7. River Diagnostics Skin Analyzer model 3510.

Raman depth profile collection parameters were determined and optimized using animals in the model development phase of this project. Several different parameter sets were used at different times and are summarized in Table 1. Animals were positioned over the Raman window so that the laser was focused within the small hole in the dosing template. In preparation for recording Raman profiles, animals were placed under anesthesia and hand held on the recording window. Recording a typical profile took about four to five minutes.

Template Name	Profile Collection Time (m:s)	Track No.	Z Offset (um)	Frame Duration (s)	Z Start (um)	Z Stop (um)	Z Step (um)
T1	4:45	1	23	10	0	40	4
		2	23	25	40	100	10
T2	3:35	1	23	5	0	15	4
		2	23	10	15	35	10
		3	23	15	35	50	10
		4	23	45	50	150	50
T3	6:25	1	23	10	0	40	4
		2	23	25	40	80	4
T4	4:45	1	50	10	0	40	4
		2	50	25	40	100	10
T5	4:45	1	5	10	0	40	4
		2	5	25	40	100	10
T6	5:25	1	5	5	0	45	10
		2	5	10	45	105	20

Table 1. Skin Analyzer's Raman depth profile collection parameters. Notes:

T1 = gpT1_Raman_Profile_Fingerprint_110428
T2 = hgpT2_Raman_Profile_Fingerprint_110616
T3 = hgp_T3_Raman_Profile_Fingerprint_110825
T4 = hgp_T4_Raman_Profile_Fingerprint_110928
T5 = hgp_T5_Raman_Profile_Fingerprint_111020
T6 = hgp_T6_Raman_Profile_Fingerprint_111109

Figure 8. Recording Raman depth profile with hairless guinea pig.

RSDL Decontamination Procedure

RSDL packets were procured from E-Z-EM, Inc. (Lake Success, NY 11042). The RSDL decontamination applicator was made by opening the RSDL packet and trimming the enclosed pad to form four pads, approximately 2.5 x 6.0 cm. Individual pads were attached to wooden tongue depressors with ½-inch staples. Applicators were prepared the morning of the experiment. The decontamination applicator was held by the end of the tongue depressor opposite the attached pad to position the pad over the exposure site. The first step of the decontamination process (Figure 9) involved ten strokes across the dosing template followed by wipes with dry gauze. The second step involved holding a new applicator on the exposure site for an additional 10 minutes followed by wipes with dry gauze. Sometimes the RSDL pads were held with forceps instead of being stapled to a tongue depressor.

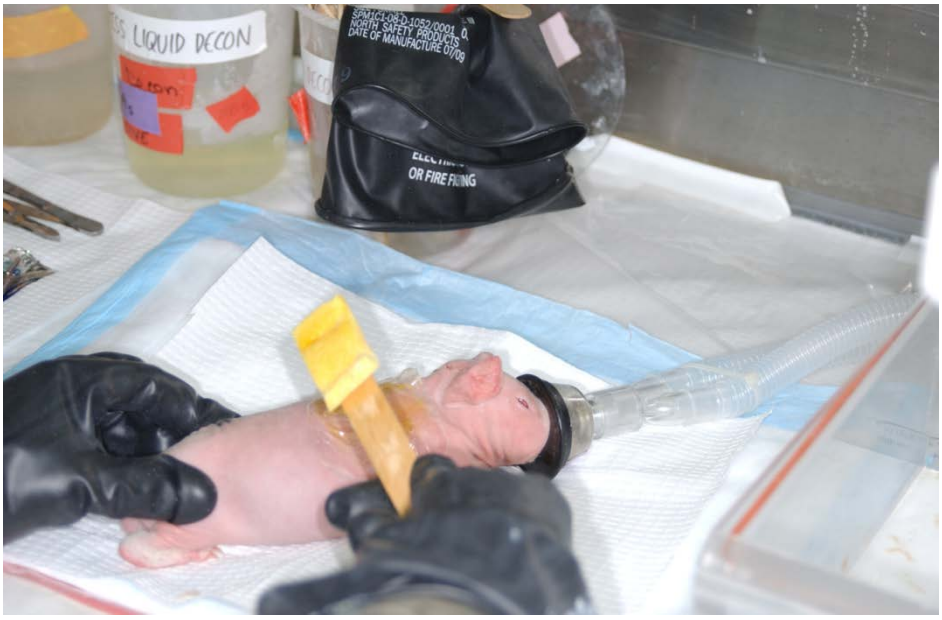


Figure 9. RSDL decontamination.

Animals were prepared and exposed to VX as described in the Agent Application section. At the specified postexposure time, several Raman depth profiles were recorded prior to RSDL decontamination to confirm that VX was present on and in the skin. After RSDL decontamination, Raman depth profiles were again recorded and the data analyzed to determine if any VX remained in or on the skin.

Semi-Quantitative Estimate of VX from Raman Depth Profiles

A total of three animals (HGPs 27, 28, and 29) were used to evaluate a new model developed to estimate the total amount of VX in the skin following exposure, using the recorded Raman depth profiles. These experiments also collected 8 mm skin punch biopsies from the exposure site following euthanasia to compare with the VX totals estimated from the Raman data.

Analytical Procedure for Determining VX in Skin Punches

Skin samples were analyzed by ECBC, Aberdeen Proving Ground, MD. For selected animals (HGPs 27, 28, and 29), 8 mm punch biopsy tissue samples of the exposure site were collected immediately after euthanasia. Since the skin exposure area was only a 3.2 mm diameter circle, the collected skin was assumed to include all the VX that remained in the skin. The samples were placed into tared polypropylene tubes and stored at -80°C. These tissues were placed in separate Mylar bags which were then subjected to freeze-fracture pulverization under cryogenic temperatures employing a Tissue CryoPrep™ system (Covaris, Woburn, MA). Pulverized samples were placed into tared borosilicate test tubes, and tissue weights were recorded.

Samples were further processed utilizing the S-series focused acoustic energy system (Covaris, Woburn, MA). To each borosilicate tube containing a weighed tissue sample, 1.0 ml of 0.69 mM phosphate buffer (pH 7.4) was added, and the mixture homogenized using the Adaptive Focused Acoustics process for 1 min. Next, 1.0 ml of additional phosphate buffer, 400 µl of 6M KF, and 1 µl of the appropriate internal standard were added. This mixture was vortexed for 30 sec and centrifuged at 4400 rpm for 10 minutes using an IEC Centra GP8R. The supernatant was then loaded onto a previously conditioned 3 cc Oasis® WCX (Weak Cation eXchange) solid phase extraction (SPE) cartridge (Waters Corporation, Milford, MA). Conditioning consisted of eluting 1 mL of methanol, followed by 1 mL of deionized water. The pellet from the centrifuged sample was re-suspended with an additional 2 ml of phosphate buffer, vortexed, and centrifuged as before. The additional supernatant was added to the SPE cartridge, and the entire mixture allowed to elute through the SPE cartridge. This was followed by an addition of 500 µl of 5% ammonium hydroxide, which ionizes weak cation-exchange sites to retain the target compound and internal standard while removing proteins and salts. Then 1 ml of deionized water followed by 1 ml of 10% methanol:90% deionized water was eluted through the column. The SPE cartridge was then dried under vacuum for five minutes. Using a gentle vacuum, 1 mL of methanol was used to elute VX and the internal standard. This fraction was then transferred to a GC autosampler vial where it was concentrated under a stream of nitrogen at 40°C to an approximate volume of 50 µl for analysis.

The analysis was carried out on an Agilent Technologies 1200 series liquid chromatograph interfaced to an Agilent Technologies 6410 triple quadrupole mass spectrometer (Agilent Technologies, Wilmington, DE). Samples were analyzed under reversed phase conditions using 1 µl injections of the previously prepared extracts at a constant flow of 1 ml/min through a ZORBAX Eclipse XDB-C18, 4.6 x 150 mm, 5 µm particle size analytical HPLC column equipped with a C18 precolumn filter. Mobile phases A and B consisted of 0.1% formic acid in LC/MS grade water and 0.1% formic acid in methanol, respectively. Samples were analyzed using a 5-95% solvent B linear gradient over 10 min with a 1 min hold at 95% B. The column was then reconditioned with 99% solvent A for 1 minute prior to the next injection. The target compound and internal standard peak eluted between 8 and 9 min. The sample flow was delivered to the mass spectrometer via an electrospray ionization source under positive polarity. The capillary voltage was 4000V, and the nebulizing gas temperature was 350°C with a flow of 10 L/min and pressure of 35 psi. The triple quadrupole mass spectrometer was operated in multiple reaction monitoring (MRM) mode. For the target analyte the MRM program monitored one transition for quantitation and one for confirmation, and for the internal standard only one transition was monitored. The MassHunter Quantitative Analysis software provided automated peak detection, calibration, and quantification. A calibration curve with standards ranging from 0.5 to 250,000 ng/mL of VX and 50 ng/mL of internal standard was used for quantification. The detector relative response with respect to the relative concentration was linear with a correlation coefficient value of 0.9997. Separate quality control and matrix spike check standards were analyzed before each batch of samples.

The VX tissue concentration (ng/g) was determined from the sample concentration-to-pulverized sample weight ratio. These measurements for each animal were then multiplied by the total weight (g) of the exposed skin. This resulting product represented the total dose (ng) recovered from the homogenized sample.

RESULTS

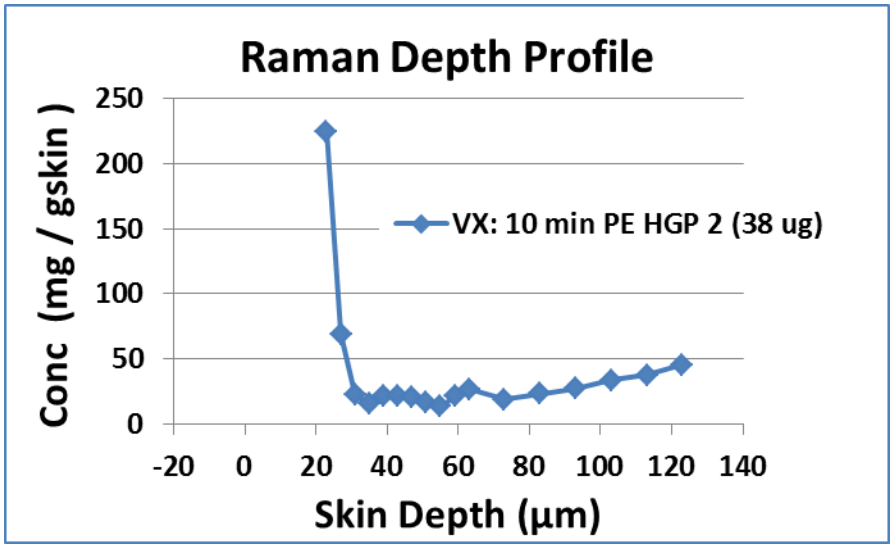
Notes on terms used

1. The Skin Analyzer software supplies a number, which is automatically incremented in the file name for each new Raman depth profile recorded on any given day. This "p" value allows easy reference to the file names in the lab notebook.
2. "PE" = postexposure time.
3. "Mechanical" refers to depth profiles that do not have the skin surface value automatically determined by the system software.
4. For a given profile, each spectrum is numbered. The spectrum number increases with skin depth.
5. Cumulative constant mass values are the estimated total VX quantities in skin determined by the mass estimate model. Values were calculated using the constant mass option. Total mass of VX was determined using one of two methods. The first method used all spectra in the profile (all peaks). The second method used only the spectra with observable VX peaks.
6. Occasional random cosmic rays were observed in some spectra as a large sharp spike.

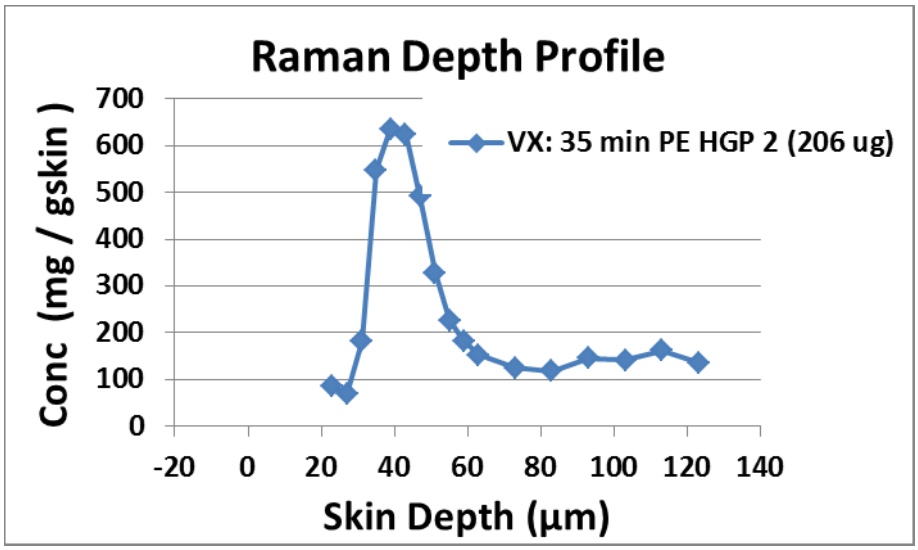
HGP 1 Observations 24 August 2011: Model Development; VX distribution

1. (p1,2) Blank (mechanical): Normal blank skin profile and spectra.
2. (p3) 10 min PE (mechanical): No VX peaks observed in any spectra of the depth profile.
3. (p4) 58 min PE (mechanical): VX peak observed only in spectrum 1.
4. (p5) 73 min PE (mechanical): Small VX peaks possibly observed in spectra 1 and 2.
5. (p6) 98 min PE (mechanical): Possible VX peak in spectrum 1.
6. Animal died 1.78 hr PE.
7. Animal received a small dose of HuBuChE (Stoichiometric ratio = 0.043).
8. This animal will not be used in summary data because all profiles were mechanical depth.

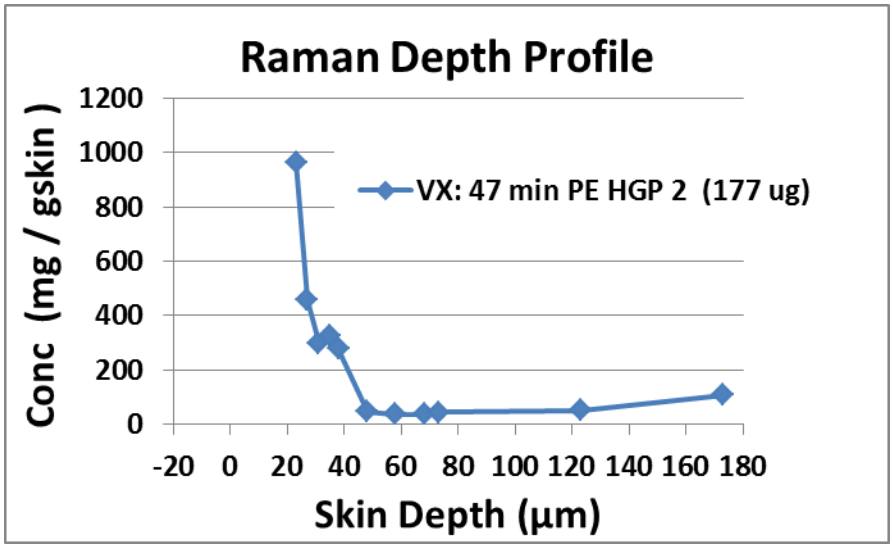
HGP 2 Observations 25 August 2011: Model Development; VX Distribution

1. As an example of the breadth of data available, all the usable depth profiles for HGP 2 will be included in this section. Other sections provide representative examples.
2. (p1) Blank: Normal blank skin profile and spectra; no VX peaks.
3. (p2) 10 min PE (mechanical): Template (T1) 17 spectra
    a. Profile starts in skin.
    b. VX peaks only in spectrum 1 (already in skin).
    c. 

    d. Figure 10. Raman depth profile 2 in HGP 2.
    e. Cumulative constant mass of VX using all peaks = 37.5 µg.
    f. Cumulative constant mass of VX using only peaks with VX = 16.9 µg
4. (p3) 33 min: animal moved, collection stopped.
5. (p4) 35 min PE (mechanical): Template (T1) 17 spectra
    a. Profile starts in skin.
    b. VX peaks in spectra 1-13 (spectrum 1 is in skin).

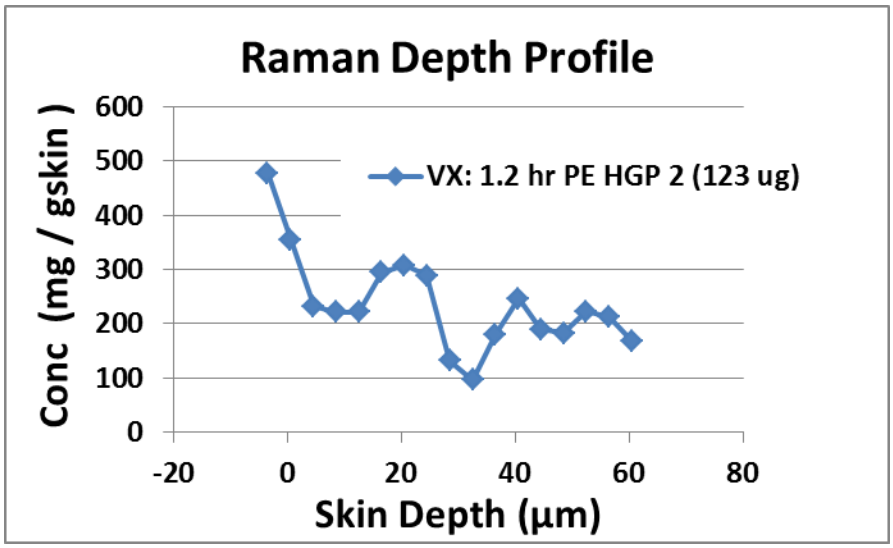
c. 

d. Figure 11. Raman depth profile 4 in HGP 2.
e. Cumulative constant mass of VX using all peaks = 206 µg.
f. Cumulative constant mass of VX using only peaks with VX = 166 µg.
g. Limit of detection (LOD) estimated to be 120 mg VX/g skin.

6. (p5) 47 min PE (mechanical): Template (T2) 11 spectra.
   a. Profile starts in skin.
   b. VX peaks in spectra 1-5 (spectrum 1 in skin).
   c. 

   d. Figure 12. Raman depth profile 5 in HGP 2.
   e. Cumulative constant mass of VX using all peaks = 177 µg.
   f. Cumulative constant mass of VX using only peaks with VX = 120 µg.

7. (p6) 73 min PE: Used another new collection template with 21 spectra.
   a. VX peaks in spectra 2, 4-16 but difficult to interpret spectra because of sloping baseline.

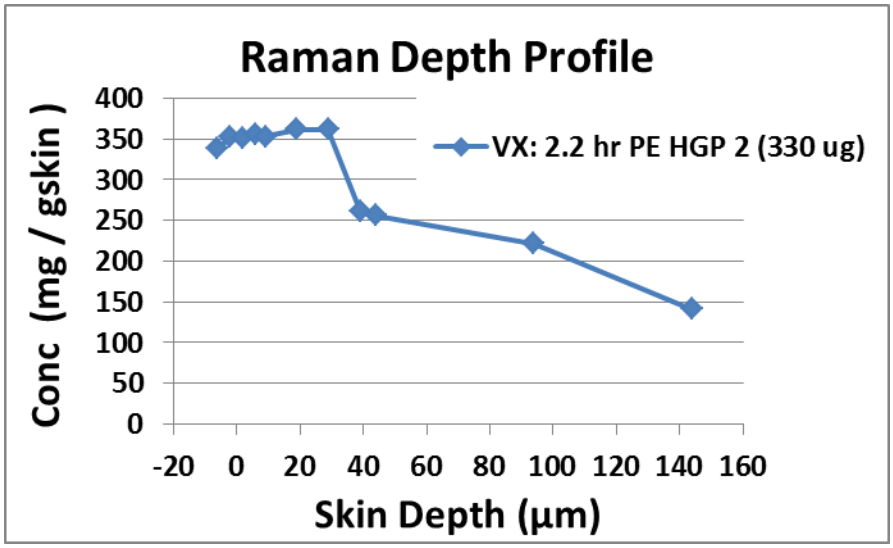
b. 

c. Figure 13. Raman depth profile 6 in HGP 2.

d. Only spectra 1-17 shown in graph.

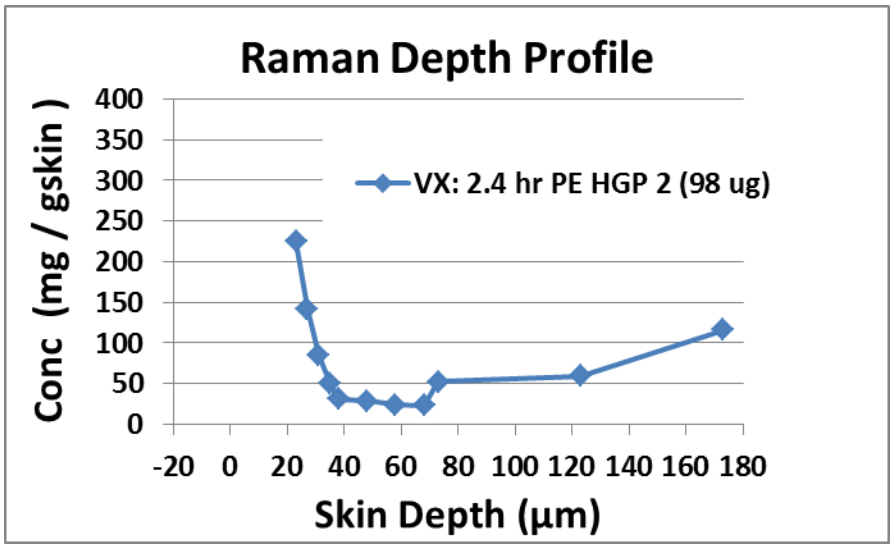
e. Cumulative constant mass of VX using all peaks = 123 µg.

f. Cumulative constant mass of VX using only peaks with VX = 116 µg.

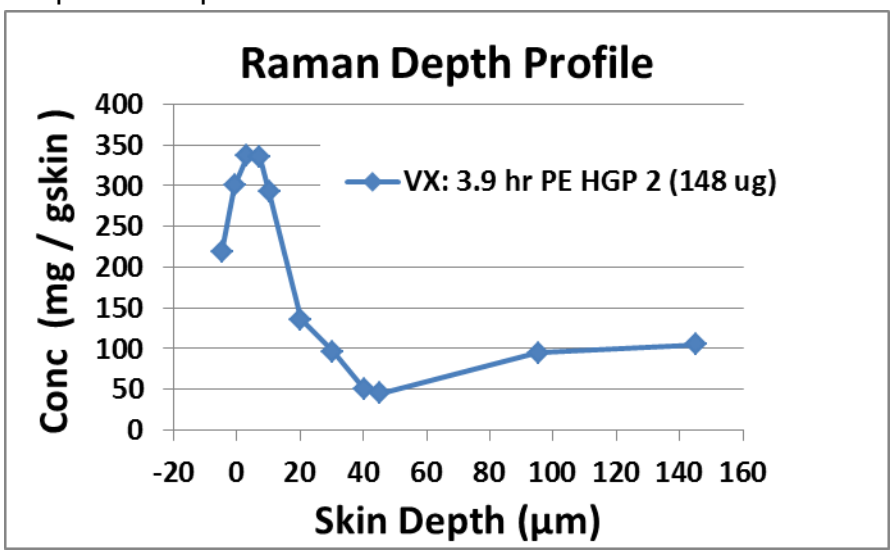
8. (p7) 2.2 hr PE: Used another new collection template with 11 spectra.
   a. VX peaks in spectra 1-9 (very large peaks).
   b. 

   c. Figure 14. Raman depth profile 7 in HGP 2.
   d. Cumulative constant mass of VX using all peaks = 330 µg.
   e. Cumulative constant mass of VX using only peaks with VX = 136 µg.

9. (p8) 2.4 hr PE (mechanical): Template (T2) 11 spectra.
   a. Profile starts in skin.
   b. VX peaks in spectra 1-2.

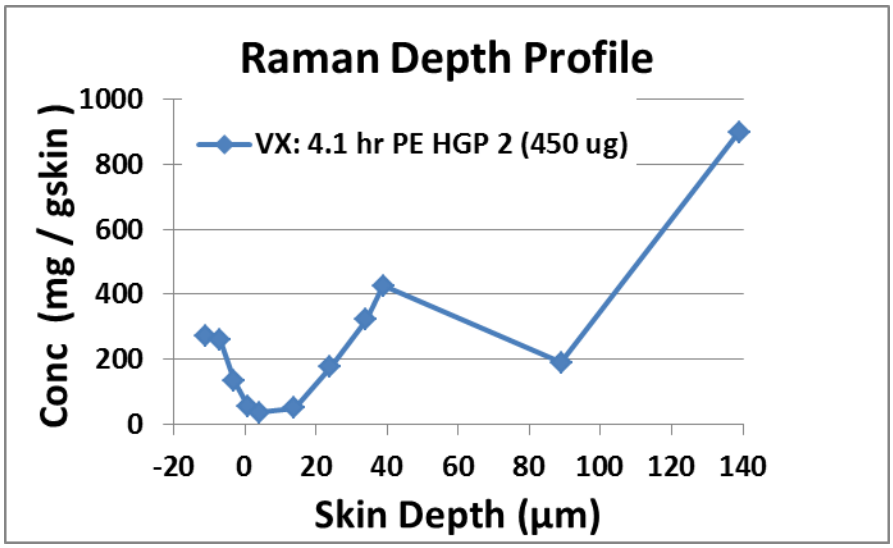
c. 

d. Figure 15. Raman depth profile 8 in HGP 2.
e. Cumulative constant mass of VX using all peaks = 98 µg.
f. Cumulative constant mass of VX using only peaks with VX = 15 µg.

10. (p9) 3.9 hr PE: Used another new collection template (T3) with 11 spectra.
    a. VX peaks in spectra 2-6.
    b. 

    c. Figure 16. Raman depth profile 9 in HGP 2.
    d. Cumulative constant mass of VX using all peaks = 148 µg.
    e. Cumulative constant mass of VX using only peaks with VX = 50 µg.

11. (p10) 4.1 hr PE: Used another new collection template (T3) with 11 spectra.
    a. All spectra looked very strange.
    b. VX peaks not observed in any spectra.

c. 

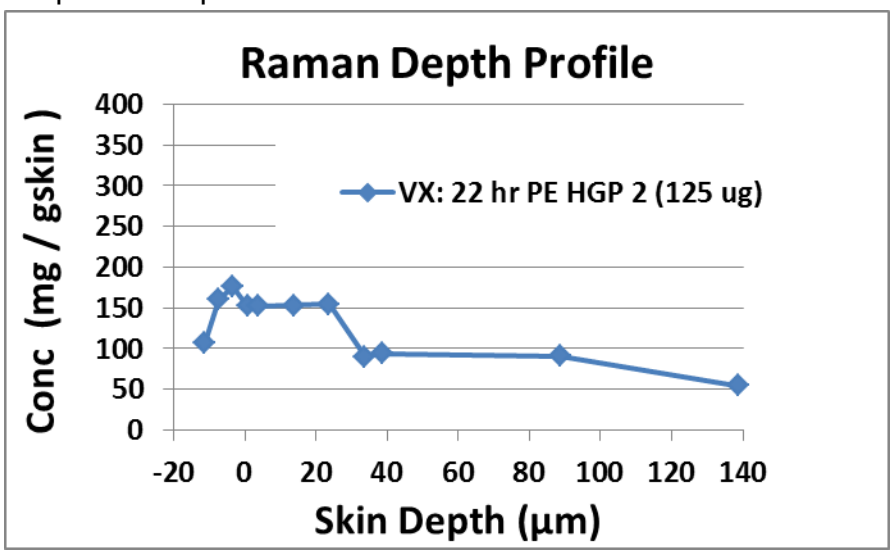
d. Figure 17. Raman depth profile 10 in HGP 2.

e. Cumulative constant mass of VX using all peaks = 450 µg.

f. Cumulative constant mass of VX using only peaks with VX = 0 µg.

HGP 2 Observations 26 August 2011: Model Development; VX Distribution

1. (p1) 22 hr PE: Template (T2) 11 spectra.
   a. VX peaks in spectra 3-7.

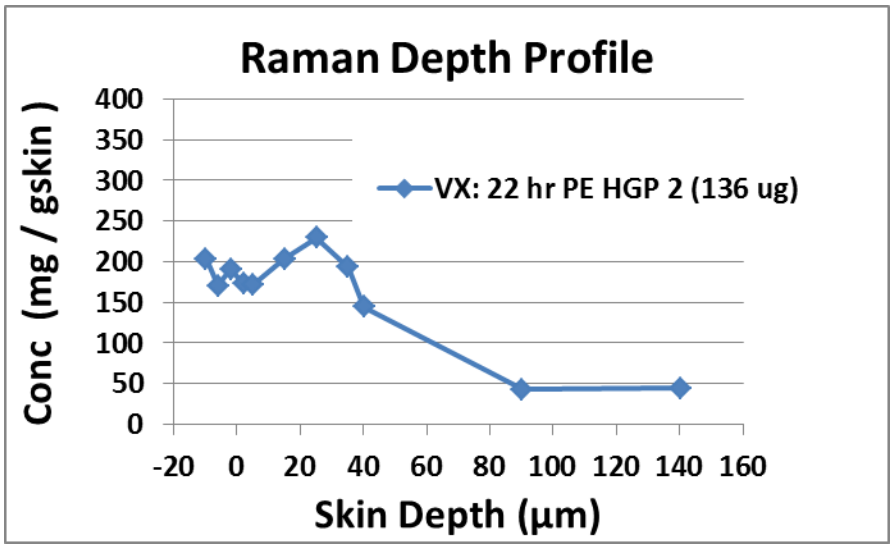
b. 

c. Figure 18. Raman depth profile 1 in HGP 2.

d. Cumulative constant mass of VX using all peaks = 125 µg.

e. Cumulative constant mass of VX using only peaks with VX = 33 µg.

2. (p2) 22 hr PE: Template (T2) 11 spectra.
   a. VX peaks in spectra 3-9 (large peaks).

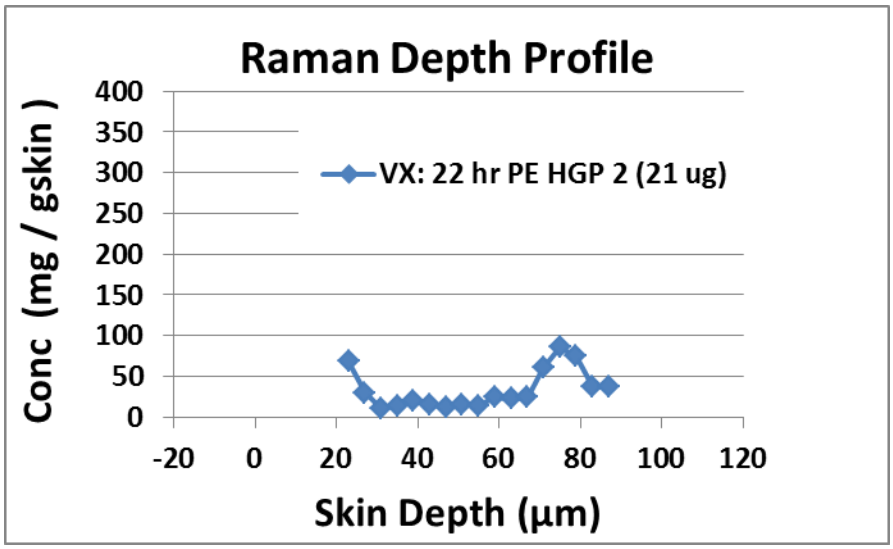
b. 

c. Figure 19. Raman depth profile 2 in HGP 2.

d. Cumulative constant mass of VX using all peaks = 136 µg.

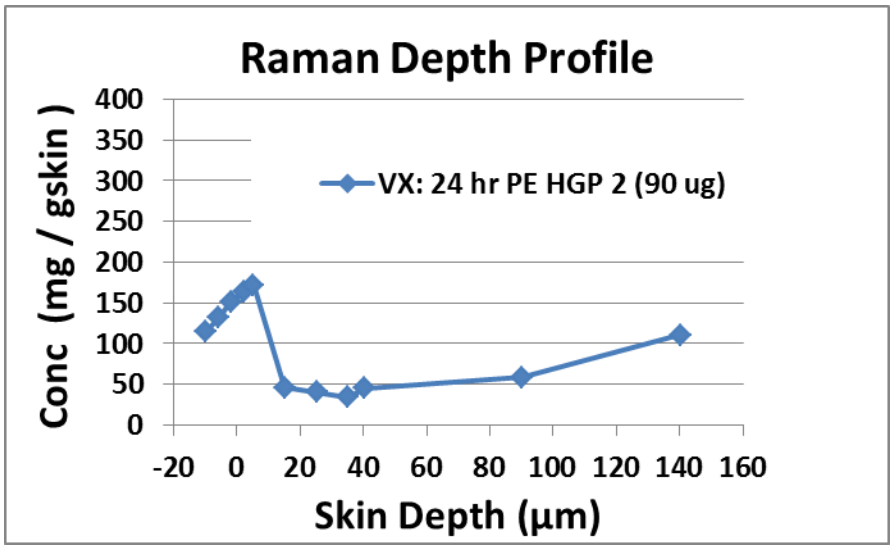
e. Cumulative constant mass of VX using only peaks with VX = 73 µg.

3. (p3) 22 hr PE (mechanical): Template (T3) 21 spectra (all steps 4 µm).
   a. Profile starts in skin.
   b. VX peaks only in spectrum 1.
   c. Cosmic ray peaks in spectra 7 and 14.
   d. Missing graph.

4. (p4) 22 hr PE (mechanical): Template (T3 modified) 21 spectra (all steps 4 µm).
   a. Profile starts in skin.
   b. Possible VX peaks only in spectrum 1.
   c. 

   d. Figure 20. Raman depth profile 4 in HGP 2.
   e. Cumulative constant mass of VX using all peaks = 21 µg.

f. Cumulative constant mass of VX using only peaks with VX = 0 µg.

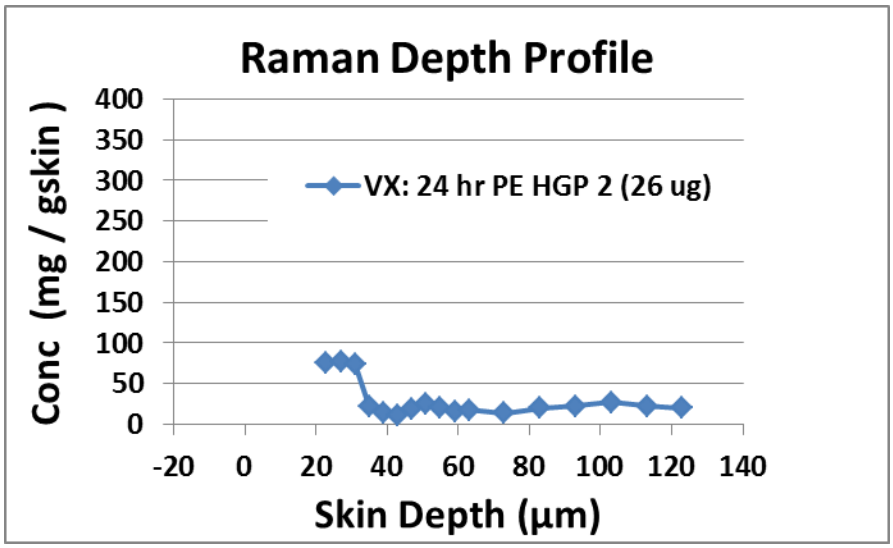
5. (p5) 24 hr PE: Template (T2) 11 spectra.
   a. VX peaks in spectra 4-5 (large peaks).
   b. 

   c. Figure 21. Raman depth profile 5 in HGP 2.
   d. Cumulative constant mass of VX using all peaks = 90 µg.
   e. Cumulative constant mass of VX using only peaks with VX = 18 µg.

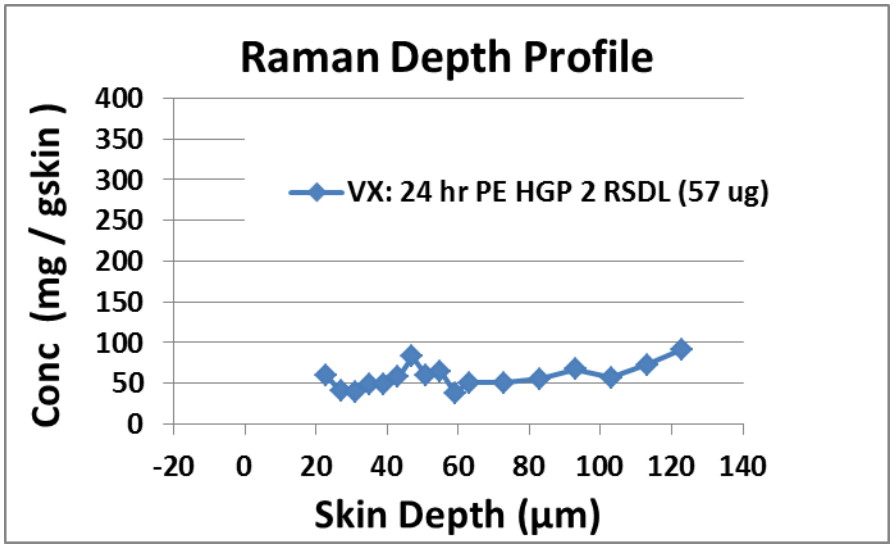
6. (p6) 24 hr PE: Template (T2) 11 spectra; after RSDL decontamination.
   a. Animal decontaminated with RSDL (template in place) 10 wipes and contact for 10 min.
   b. No VX peaks observed in any spectra.
   c. VX profile variable, mostly likely from RSDL.

7. (p7) 24 hr PE: Template (T2) 11 spectra; after RSDL decontamination.
   a. No VX peaks observed in any spectra.
   b. VX profile variable, mostly likely from RSDL.

8. (p8) 24 hr PE: (mechanical) Template (T1) 17 spectra; after RSDL decontamination.
   a. Profile started in skin.
   b. VX peaks in spectra 1-3.

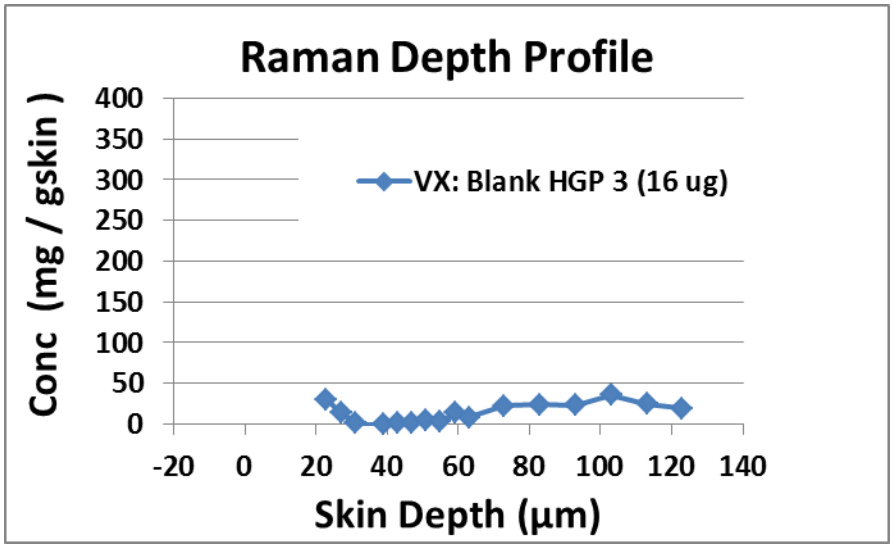
c. 

d. Figure 22. Raman depth profile 8 in HGP 2.
e. Cumulative constant mass of VX using all peaks = 26 µg.
f. Cumulative constant mass of VX using only peaks with VX = 8.4 µg.
g. This was one of the few Raman profiles that demonstrated VX present after RSDL decontamination.

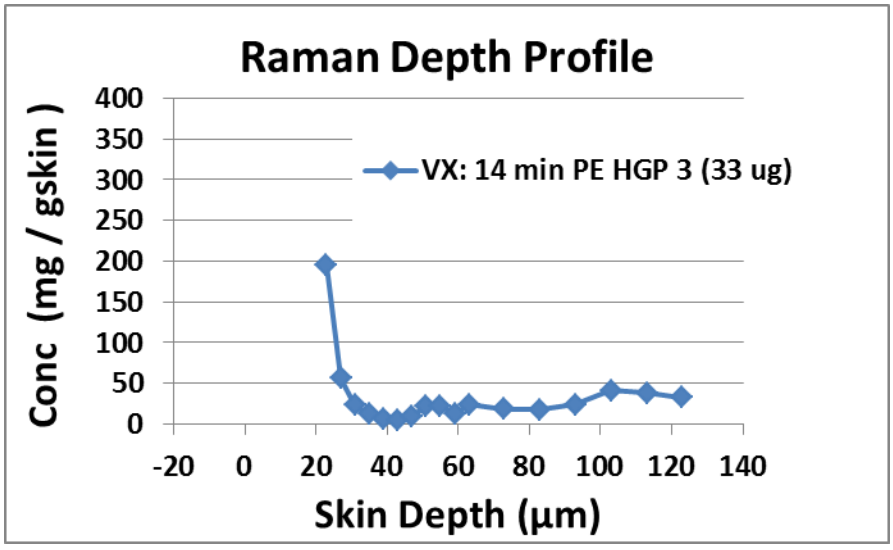
9. (p9) 24 hr PE (mechanical): Template (T1) 17 spectra; after RSDL decontamination.
   a. Profile started in skin.
   b. No VX peaks in any spectra.
   c. 

   d. Figure 23. Raman depth profile 9 in HGP 2.
   e. Cumulative constant mass of VX using all peaks = 57 µg.
   f. Cumulative constant mass of VX using only peaks with VX = 0 µg.
   g. VX not detected following RSDL treatment.

HGP #3 Observations 31 August 2011: VX Distribution

1. (p1) Blank: Template (T1) 17 spectra.
    a. Normal blank skin profile and spectra; no VX peaks.
    b. 

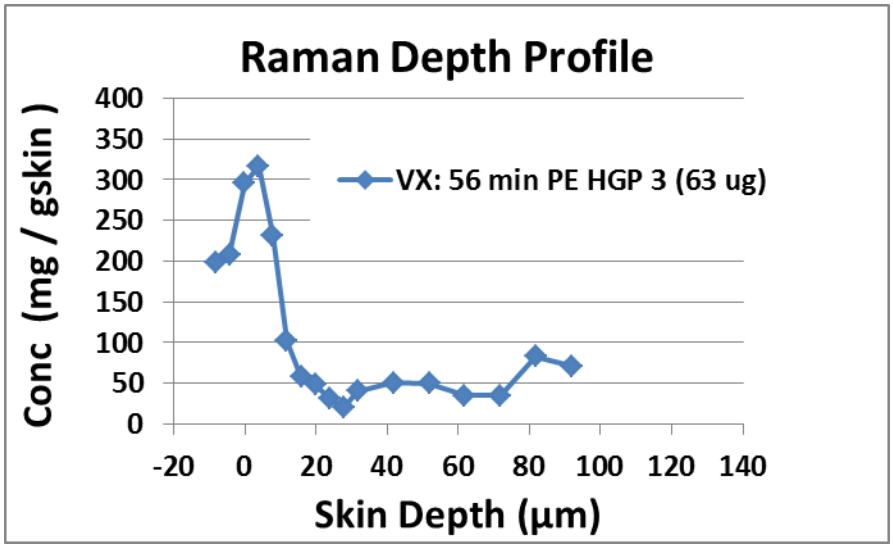
    c. Figure 24. Raman depth profile 1 in HGP 3.
    d. Cumulative constant mass of VX using all peaks = 16 µg.

2. (p2) 14 min PE (mechanical): Template (T1) 17 spectra.
    a. Profile started in skin.
    b. VX peaks in spectrum 1.
    c. 

    d. Figure 25. Raman depth profile 2 in HGP 3.
    e. Cumulative constant mass of VX using all peaks = 33 µg.
    f. Cumulative constant mass of VX using only peaks with VX = 7.2 µg.

3. (p3) 56 min PE: Template (T1) 17 spectra.

a. VX peaks in spectra 2-8.

b. 

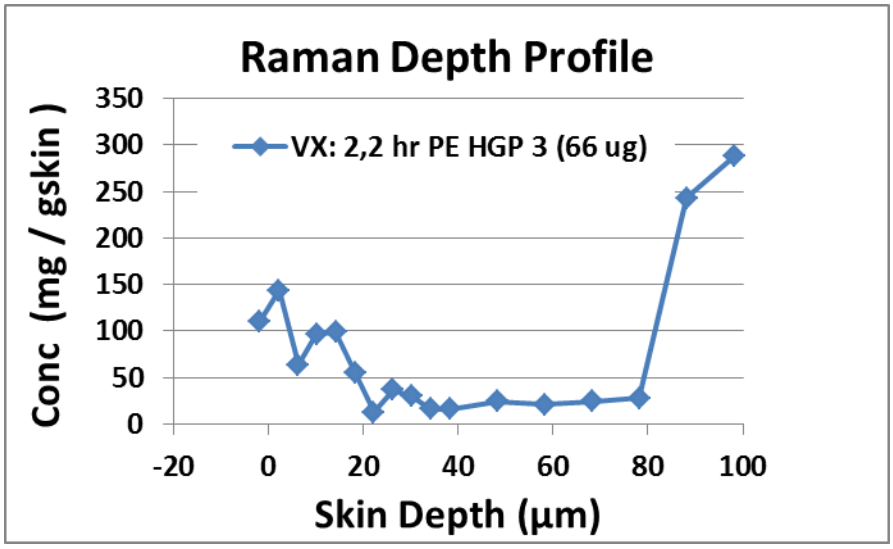
c. Figure 26. Raman depth profile 3 in HGP 3.

d. Cumulative constant mass of VX using all peaks = 63 µg.

e. Cumulative constant mass of VX using only peaks with VX = 31 µg.

4. (p7) 2.2 hr PE: Template (T1) 17 spectra.

a. VX peaks in spectra 2-5 and 16,17.

b. 

c. Figure 27. Raman depth profile 7 in HGP 3.

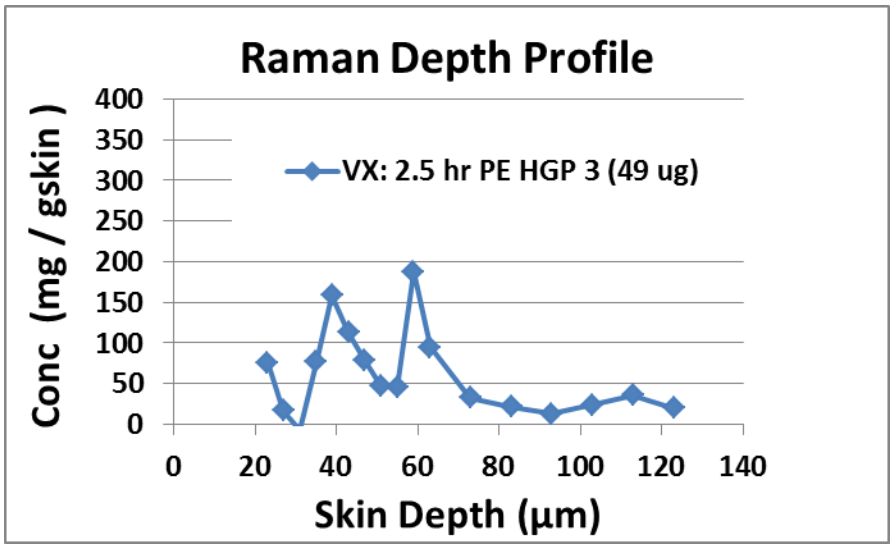
d. Cumulative constant mass of VX using all peaks = 66 µg.

e. Cumulative constant mass of VX using only peaks with VX = 53 µg.

5. (p8) 2.5 hr PE (mechanical): Template (T1) 17 spectra.

a. Profile started in skin.

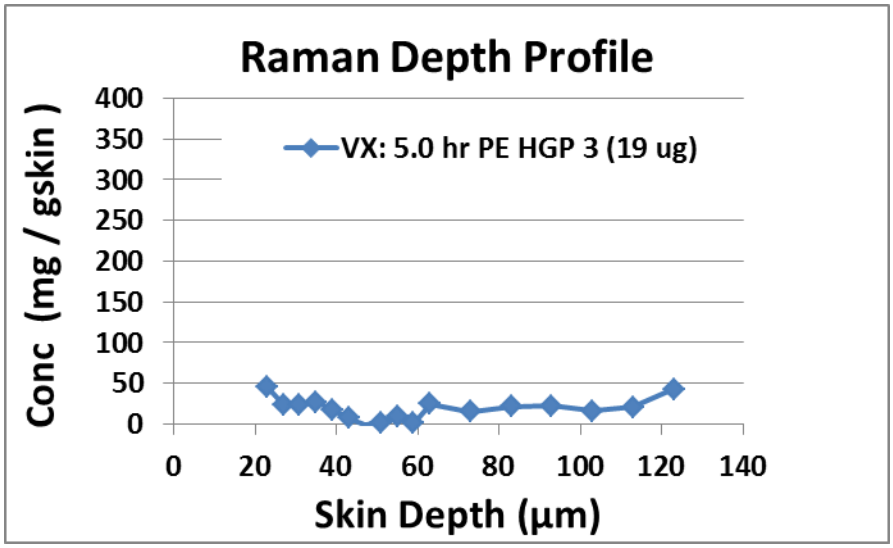
b. VX peaks in spectra 1, 4-7, 10-11.

c. 

d. Figure 28. Raman depth profile 8 in HGP 3.

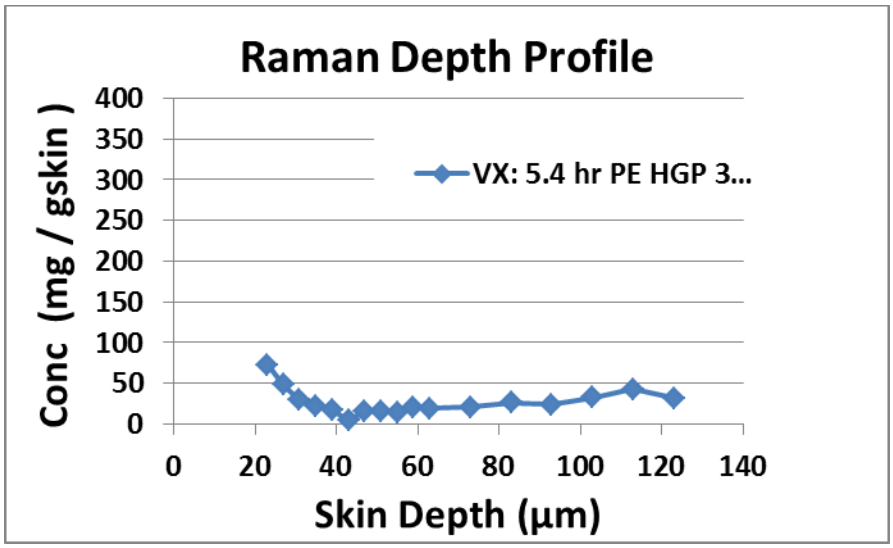
e. Cumulative constant mass of VX using all peaks = 49 µg.

f. Cumulative constant mass of VX using only peaks with VX = 27 µg.

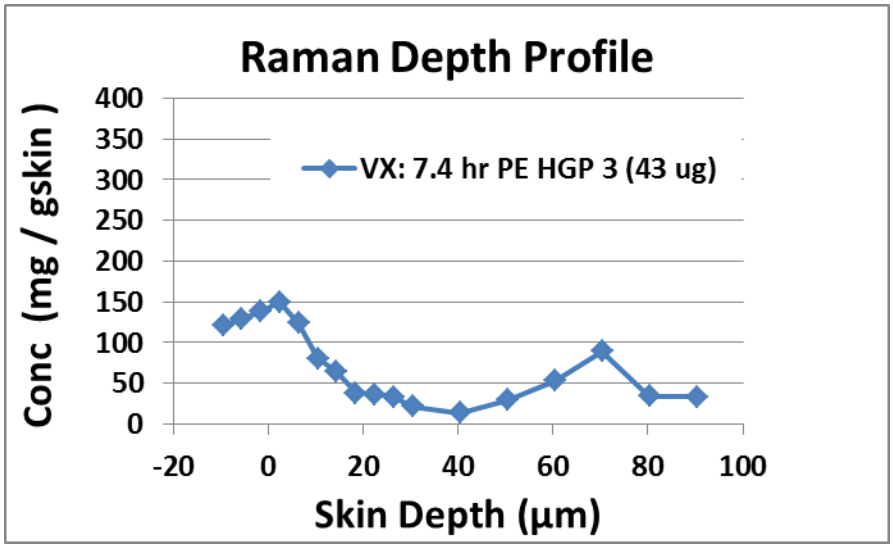
6. (p10) 5.0 hr PE (mechanical): Template (T1) 17 spectra.
   a. Profile started in skin.
   b. No VX peaks in spectra.
   c. There was some uncertainty as to whether this HGP was 3 or 4 (best guess 3).
   d. 

   e. Figure 29. Raman depth profile 10 in HGP 3.
   f. Cumulative constant mass of VX using all peaks = 19 µg.
   g. Cumulative constant mass of VX using only peaks with VX = 0 µg.

7. (p11) 5.4 hr PE (mechanical): Template (T1) 17 spectra.
   a. Profile started in skin.

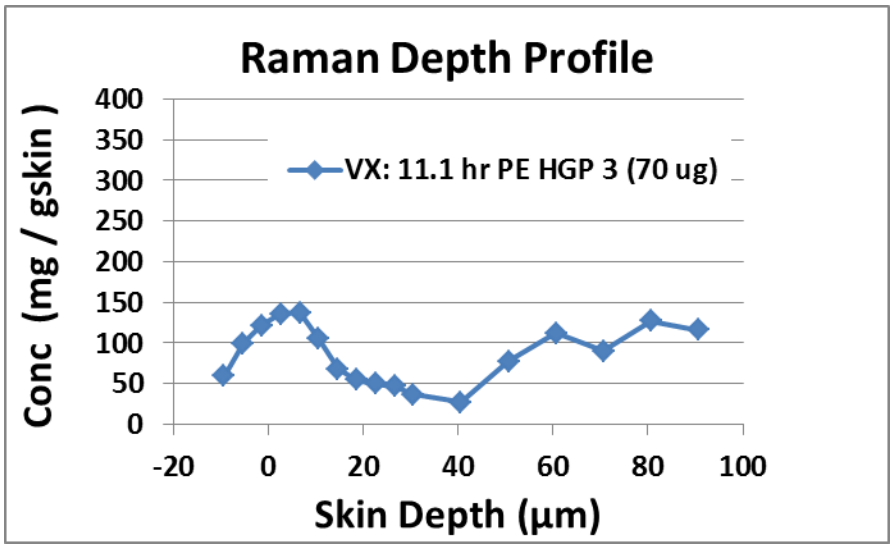
b. No VX peaks in spectra.
c. There was some uncertainty as to whether this HGP was 3 or 4 (best guess 3).
d. 

e. Figure 30. Raman depth profile 11 in HGP 3.
f. Cumulative constant mass of VX using all peaks = 27 µg.
g. Cumulative constant mass of VX using only peaks with VX = 6.4 µg.

8. (p14) 7.4 hr PE: Template (T1) 17 spectra.
   a. VX peaks in spectra 2-7.
   b. 

   c. Figure 31. Raman depth profile 14 in HGP 3.
   d. Cumulative constant mass of VX using all peaks = 43 µg.
   e. Cumulative constant mass of VX using only peaks with VX = 14 µg.

9. (p16) 11.1 hr PE: Template (T1) 17 spectra.
   a. VX peaks in spectra 3-8, 14.



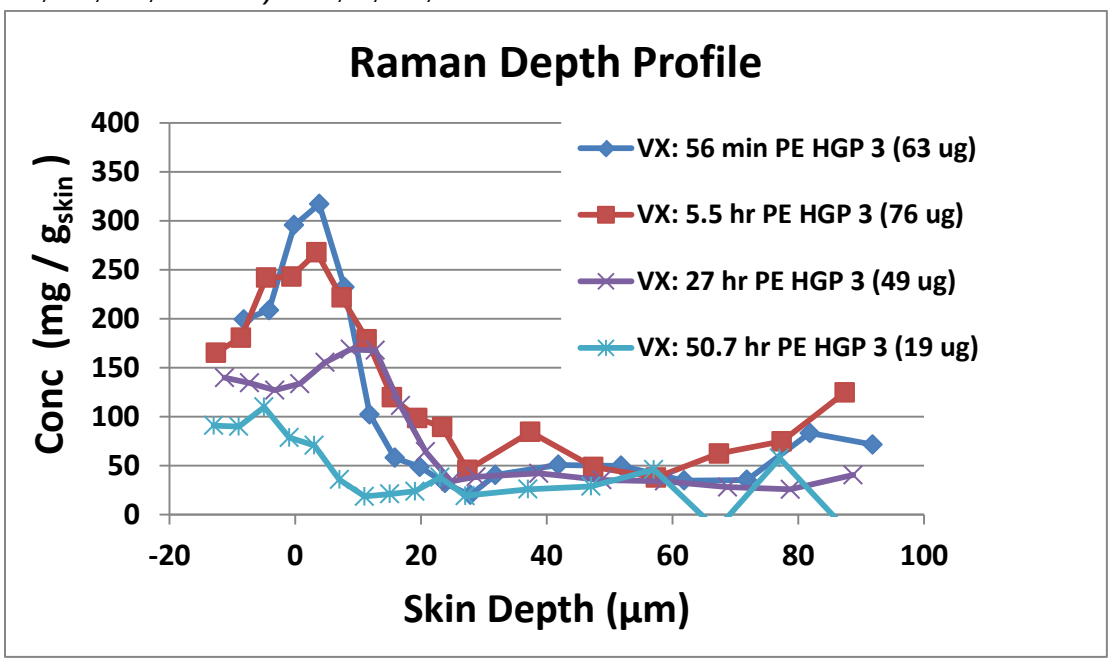
b.

c. Figure 32. Raman depth profile 16 in HGP 3.

d. Cumulative constant mass of VX using all peaks = 70 µg.

e. Cumulative constant mass of VX using only peaks with VX = 26 µg.

10. As representative examples, the next figure compares depth profiles over time (p = 3, 13, 29, and 36) at 1, 6, 27, and 50 hr PE.



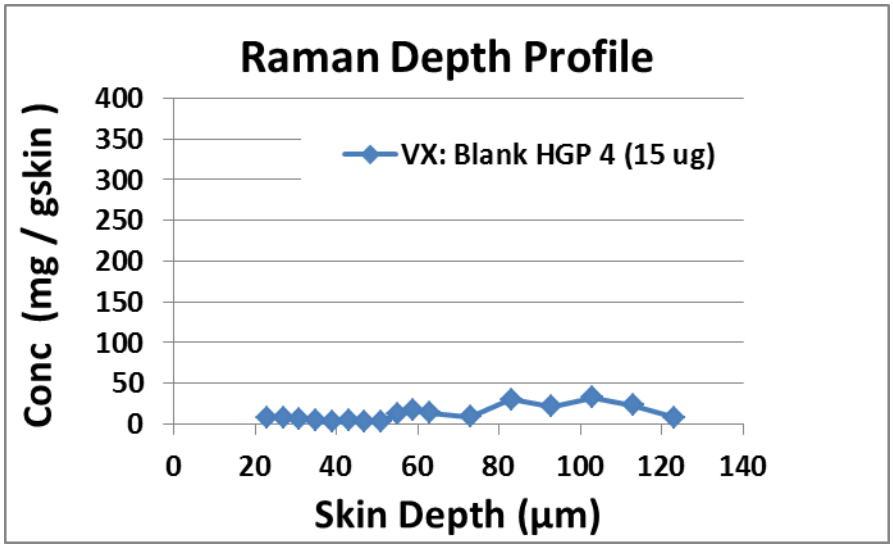
a.

b. Figure 33. Raman depth profiles for HGP 3.

c. Cumulative total VX values using all peaks are given in figure legend.

HGP 4 Observations 31 August 2011: VX Distribution

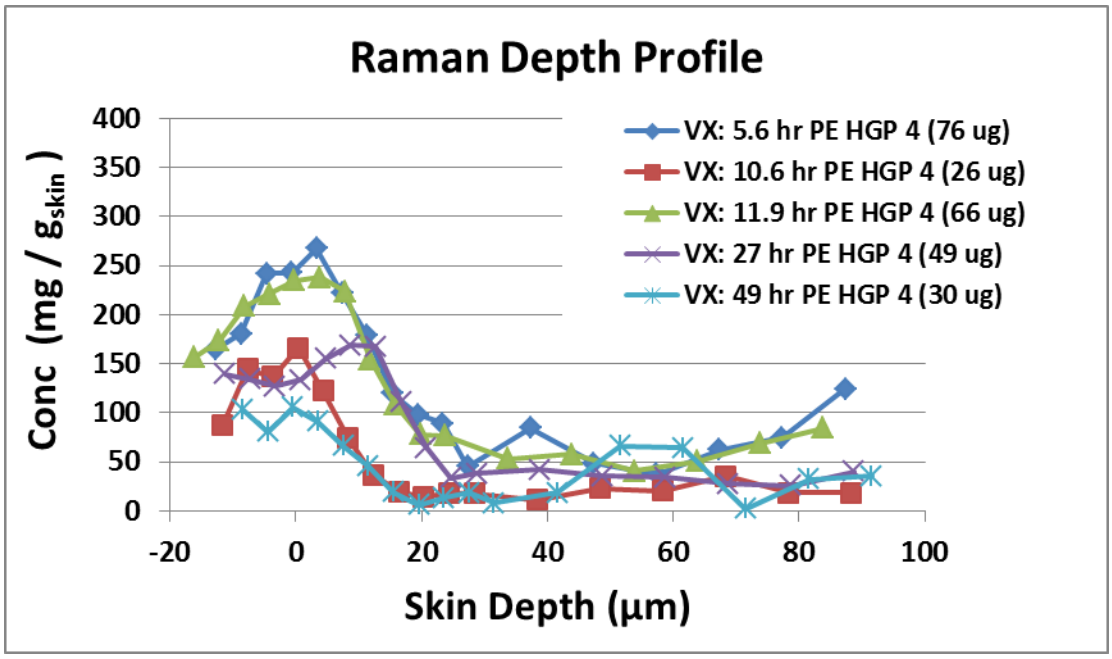
1. (p4) Blank: Template (T1) 17 spectra (mechanical).

a. Normal blank skin profile; no VX peaks.
b. Spectrum 1 already in skin.
c. 

d. Figure 34. Raman depth profile 4 in HGP 4.
e. Cumulative constant mass of VX using all peaks = 15 µg.

2. Notes: for VX profiles.
   a. Profiles 4 (blank), 5 (11 min), 6 (20 min), 9 (2.9 hr), 12 (5.2 hr), 24 (22 hr), and 27 (27 hr) were all mechanical depth with no surface calculation.
   b. Mechanical depth profiles, except for the blank, were not used in the comparison.

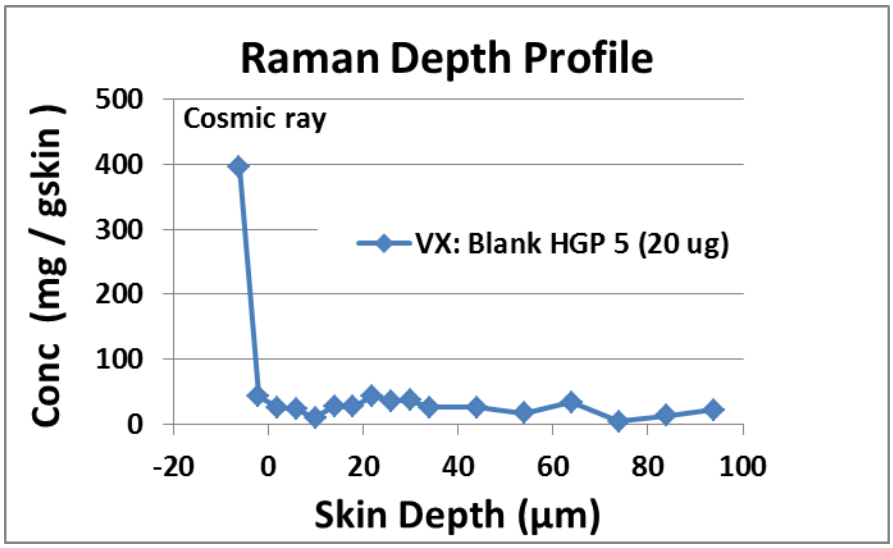
3. Compare (p 13, 18, 22, 29, and 35) at 6, 11, 12, 27, and 49 hr PE.



d. Figure 35. Raman depth profiles in HGP 4.
e. Cumulative total VX values using all peaks are given in figure legend.

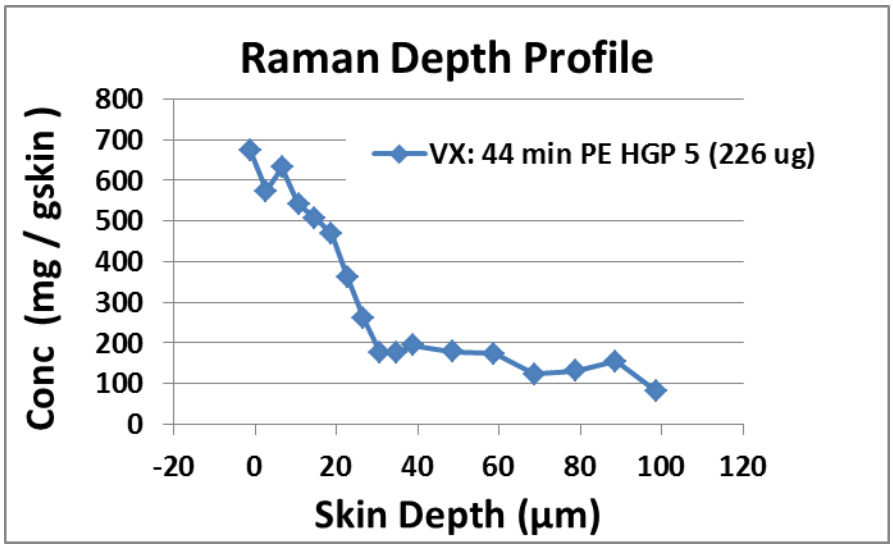
HGPs 5 and 6 Observations 7 September 2011: Model Development

1. The catheter in HGP 5 was not patent so no HuBuChE was injected. Following VX exposure, Raman depth profiles were collected at 12 (p2), 33 (p3) and 44 (p4) min PE. As expected, VX peaks were observed in spectra from all profiles; however, profiles 2 and 3 were mechanical and the skin entry was not recorded. HGP 5 died 64 min PE.

2. (p1) Blank (mechanical): Template (T1) 17 spectra.
   a. Normal blank skin profile; no VX peaks.
   b. Cosmic ray caused high VX value for spectrum 1.
   c. 

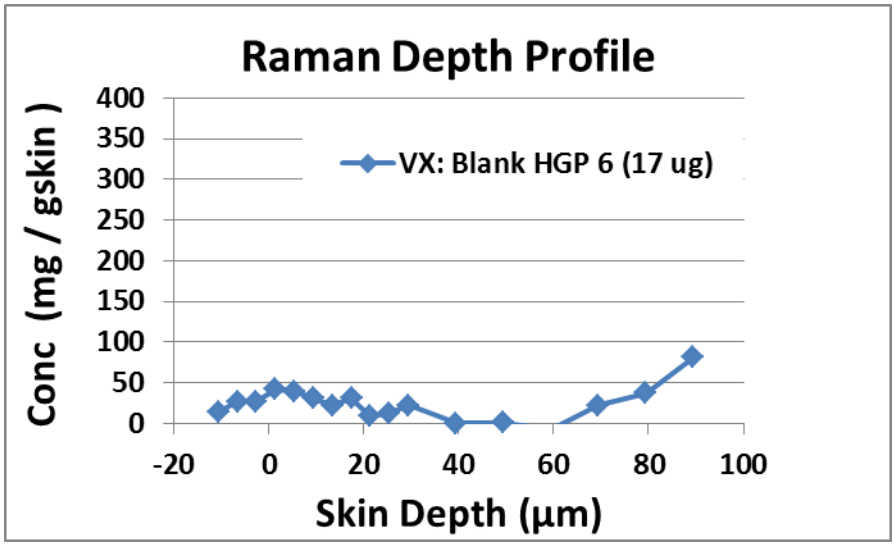
   d. Figure 36a. Raman depth profile 1 in HGP 5.
   e. Cumulative constant mass of VX using all peaks = 20 µg.

3. (p4) 44 min PE: Template (T1) 17 spectra
   a. VX peaks (extremely large) observed in spectra 1-12.

b. 

c. Figure 36b. Raman depth profile 4 in HGP 5.
d. Cumulative constant mass of VX using all peaks = 226 µg.
e. Cumulative constant mass of VX using only peaks with VX = 160 µg.

4. HGP 6 had a normal catheter and received 33.5% of the stoichiometric amount of HuBuChE.
   a. Raman profiles were collected at 10 (p6), 32 (p7), and 76 (p9) min PE.
   b. Additional Raman profiles were collected at 3.2 (p10), 4.0 (p11), 4.2 (p12), 4.4 (p13), 6.5 (p14), and 6.9 (p15) hr PE.
   c. Profiles 6 and 14 were mechanical depths and not used in the analysis.
   d. Unexpectedly, HGP 6 died at 6.9 hr PE. Very few animals given HuBuChE showed any signs of VX toxicity. Perhaps it was from prolonged anesthesia.

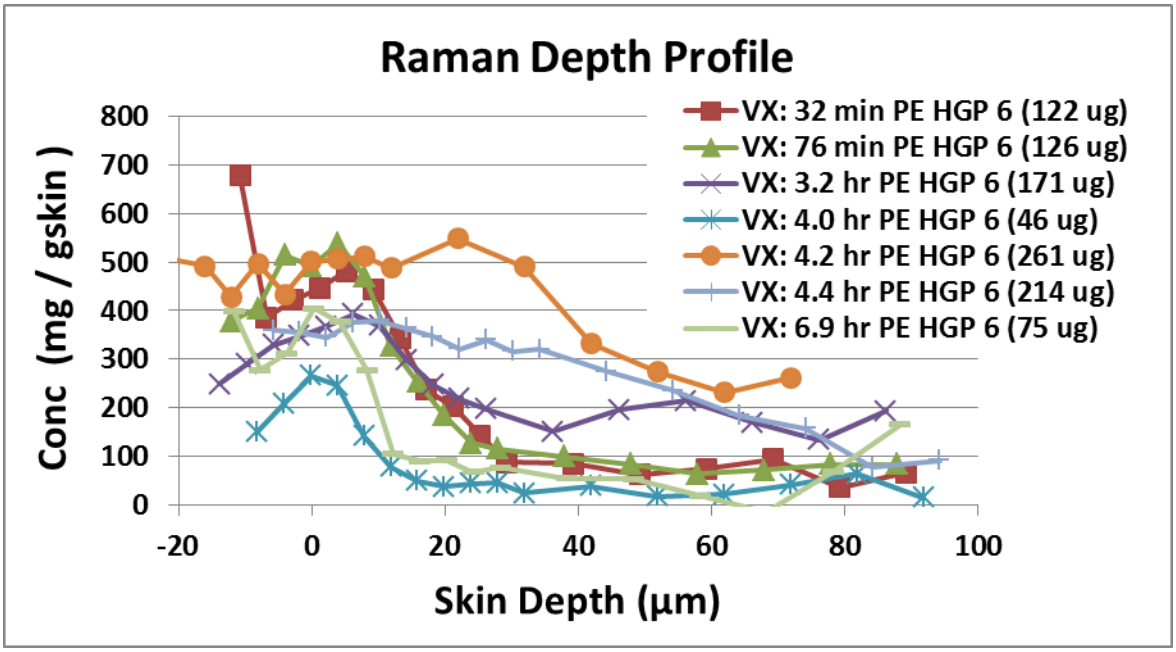
5. (p5) Blank: Template (T1) 17 spectra.
   a. Normal blank skin profile; no VX peaks.
   b. 

c. Figure 37. Raman depth profile 5 in HGP 6.

d. Cumulative constant mass of VX using all peaks = 17 µg.

6. Compare (p = 7,10,11,12,13, and 15) at 32 and 76 min and 3.2, 4.0, 4.2, 4.4, and 6.9 hr PE.

a.



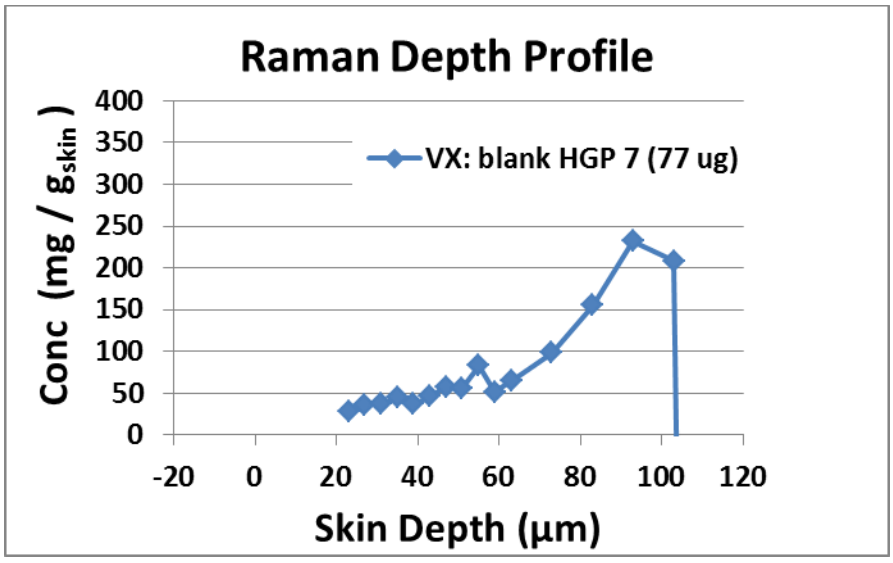
b. Figure 38. Raman depth profiles in HGP 6.

c. Cumulative total VX values using all peaks are given in figure legend.

d. Some of the profiles show VX deeper than the typical 0-20 µm.

HGP 7 Observations 21-23 September 2011: RSDL Decontamination

1. (p1) Blank (mechanical): Template (T1) 17 spectra.
   a. Abnormal blank skin profile and spectra.
   b. Depth profile slopes up at deeper depths, but no VX peaks were observed in any spectra.

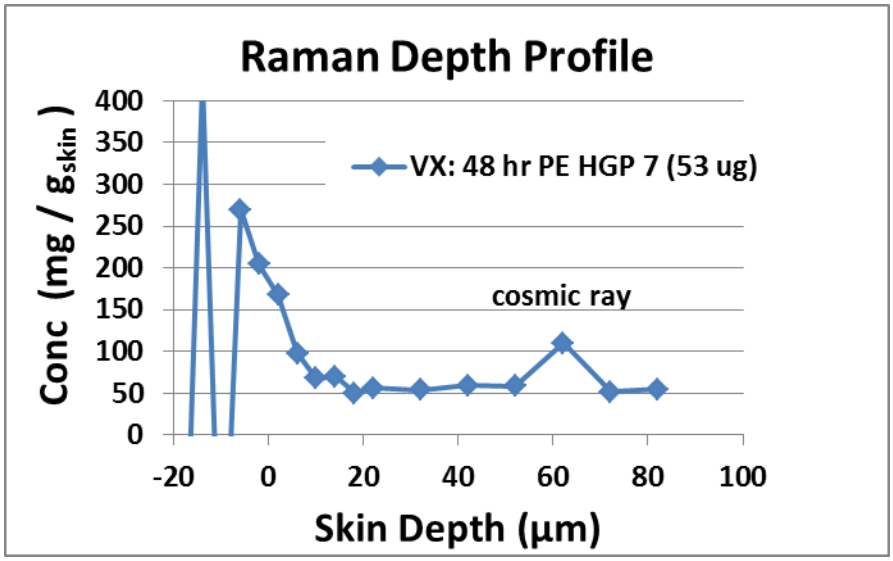
c. 

d. Figure 39a. Raman depth profile 1 in HGP 7.

e. Cumulative constant mass of VX using all peaks = 77 µg.

2. No Raman profiles recorded until > 1 hr PE.
   a. Profiles at 1, 2, 4, 6, 9, 10, 12, 24, 25, and 48 hr PE.
   b. Most show spectra with VX peaks, but not all of them.
   c. RSDL decontamination at approximately 51 hr PE: 10 wipes, small droplet for 10 min, and dry gauze wipes.

3. (p37) 48 hr PE: Template (T1) 17 spectra.
   a. VX peaks in spectra 5-7.

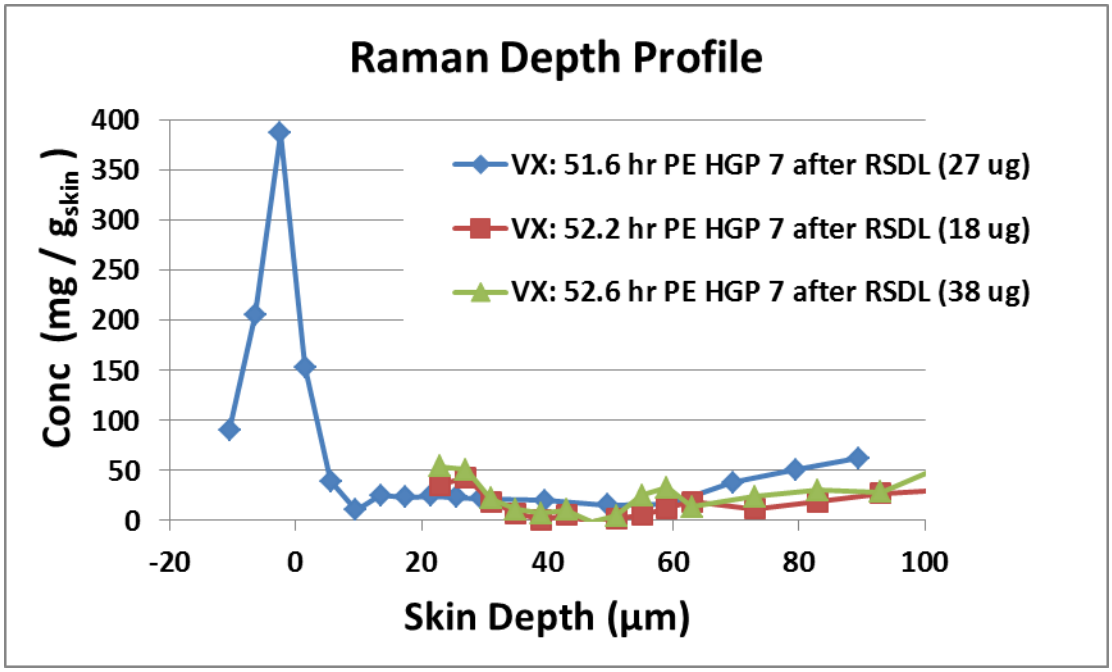
b. 

c. Figure 39b. Raman depth profile 37 in HGP 7.

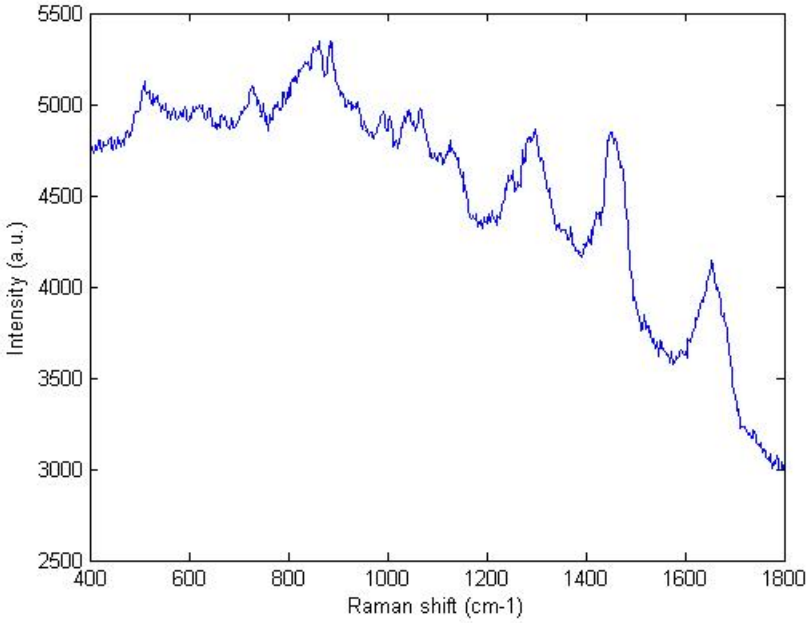
d. Cumulative constant mass of VX using all peaks = 53 µg.

e. Spectra 1,2, and 3 off scale and above skin surface.

f. Spectrum 15 cosmic ray.

4. A total of three Raman profiles were recorded after RSDL decontamination at 51.6 hr PE (profile 44), 52.2 hr PE (profile 45), and 52.6 hr PE (profile 46).
   a. All three profiles used T1 collection parameters.
   b. The initial spectra in all three profiles show peaks associated with RSDL.
   c. 

   d. Figure 40: Raman depth profiles in HGP 7.
   e. Profiles 45 and 46 were mechanical with no established skin entrance.
   f. Profiles 45 and 46 had no spectra with VX peaks.
   g. Profile 44 had a possible VX peak in spectrum 4, but the spectrum was poor quality, and all points were at or below the LOD line established by the instrument.



h.

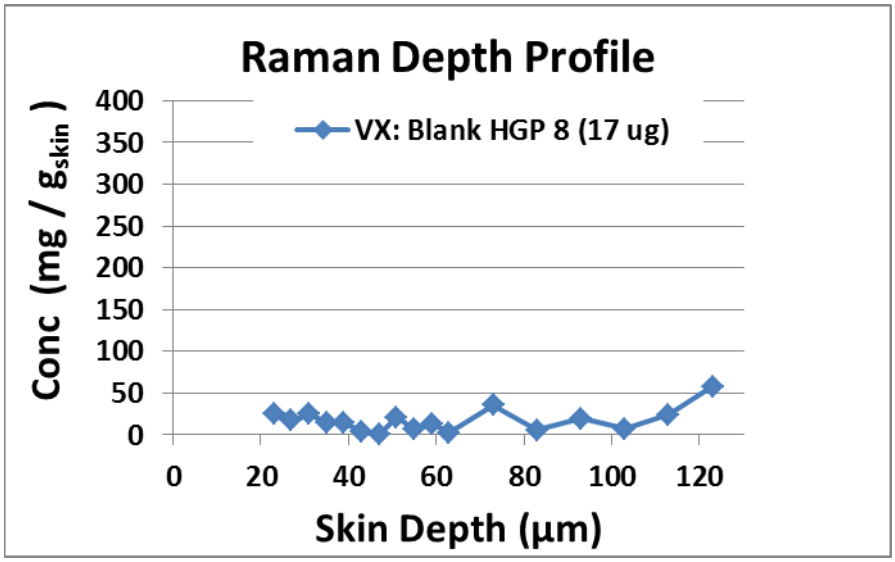
i. Figure 41. Raman spectrum 4 for profile 44 (just inside the skin surface).

j. Possible VX (spectrum 4, peak at 730 cm^{-1}).

k. The estimated total VX mass from that one spectrum was 1.9 µg.

HGP 8 Observations 21-23 September 2011: RSDL Decontamination

1. (p2) Blank (mechanical): Template (T1) 17 spectra.
   a. Blank with no skin surface recorded. Normal spectra; no VX peaks.

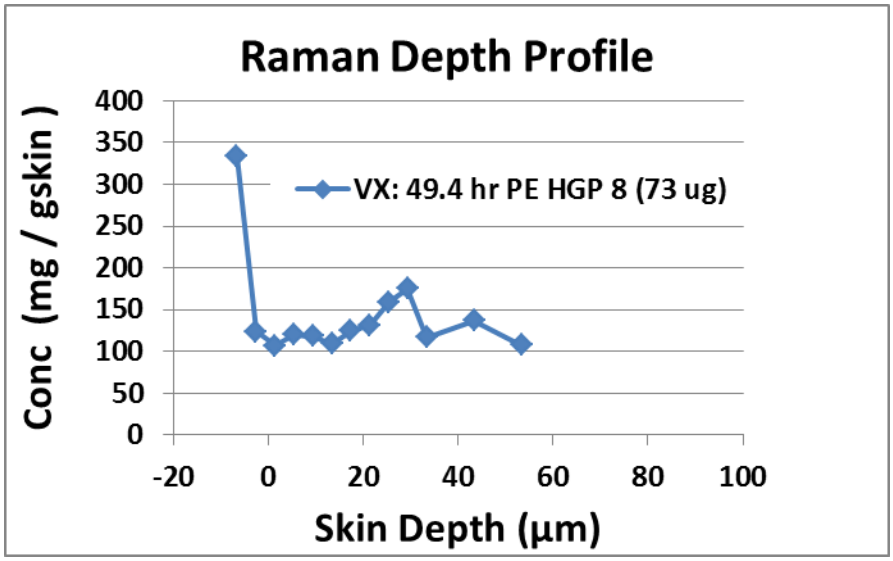


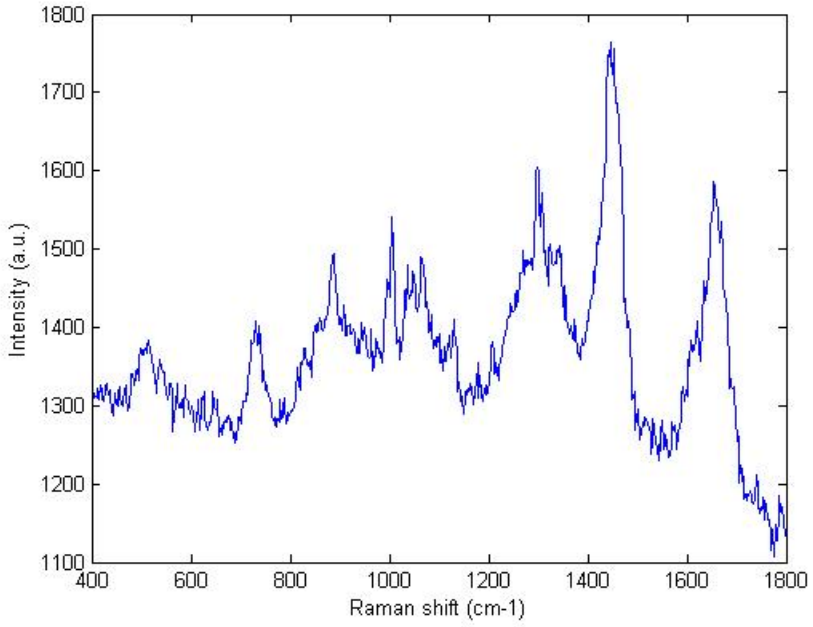
b.

c. Figure 42: Raman depth profile 2 in HGP 8.

d. Cumulative constant mass of VX using all peaks = 17 µg.

2. No Raman profiles recorded until > 1 hr PE.
    a. VX peaks at: 1.1, 4, 6 ,9,10,12, 24, 25, 49, and 50 hr PE.
    b. No VX peaks at: 1, 2.5, 2.9, 4.4, 48.5, 48.9, and 49.5 hr PE.
    c. Several profiles were mechanical. Generally the first spectrum is already in the skin.

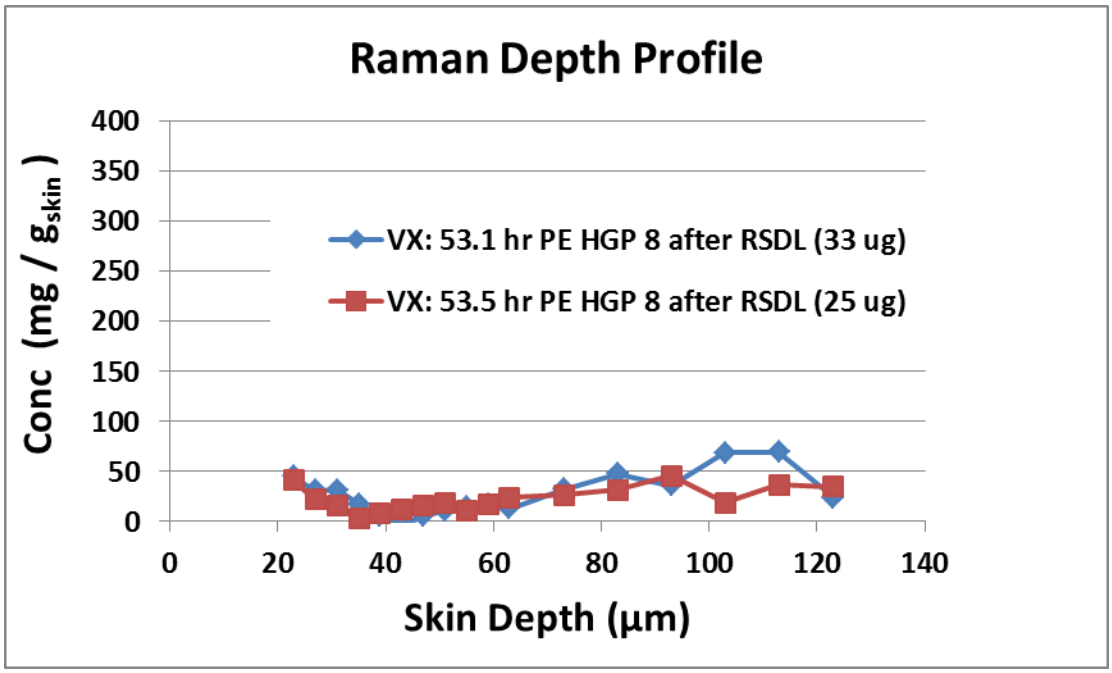
3. (p41) 49.4 hr PE: (T1) 17 spectra; before RSDL decontamination.
    a. VX peaks in spectra 3-6.
    b. Spectra 14-17 poor quality and omitted.
    c. 

    d. Figure 43: Raman depth profile 41 in HGP 8.
    e. Cumulative constant mass of VX using all peaks = 73 µg.
    f. 

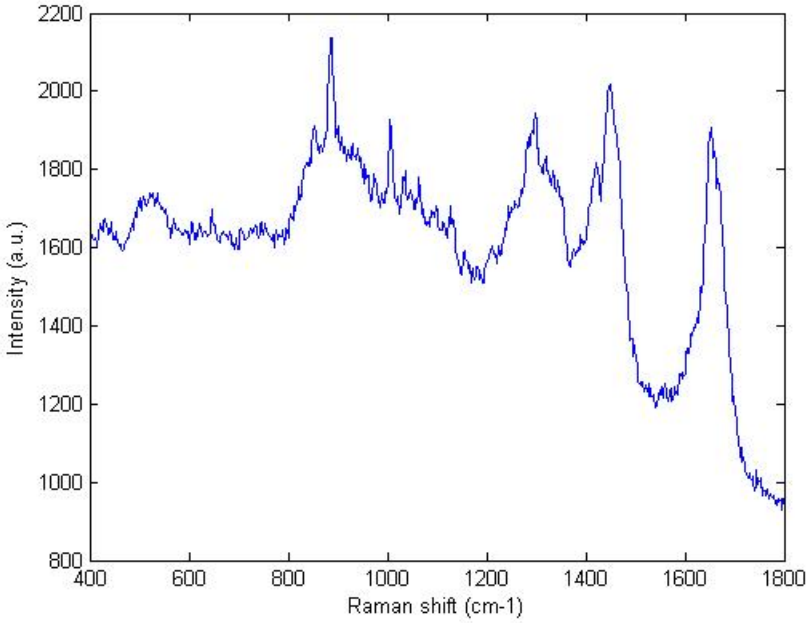
g. Figure 44. Raman spectrum 3 for profile 41 in HGP 8 (about 5 µm deep in skin).
h. VX peak observed at 730 cm^{-1}.

4. RSDL decontamination at approximately 52 hr PE: 10 wipes, wait 75 min, and 10 dry gauze wipes.
    a. No VX observed after RSDL in profiles at 53.1 and 53.5 hr PE.

5. Compare (p48, 49) 53.1 and 53.5 hr PE: (both mechanical) Template (T1) 17 spectra.

    a. Skin surface not auto-calculated and 1st spectrum is already in skin.
    b. Skin surface is at about 20 µm in profiles below.
    c. No VX peaks observed in any spectra from both profiles.
    d. 

    e. Figure 45: Raman depth profiles after RSDL in HGP 8.
    f. Cumulative constant mass of VX using all peaks (p48) = 33 µg.
    g. Cumulative constant mass of VX using all peaks (p49) = 25 µg.



h.

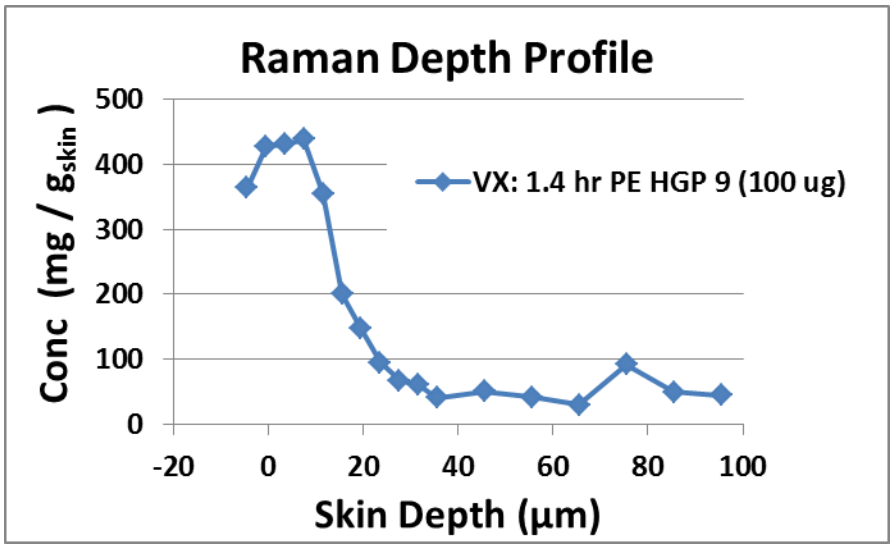
i. Figure 46. Raman spectrum 1 in profile 49 in HGP 8.
j. No VX peak observed at 730 cm^{-1} about 5 µm deep into skin.

HGP 9 Observations 28-29 September 2011: RSDL Decontamination

1. We had a problem with the Raman window position, and it was re-adjusted during the experiment.

2. (p1) Blank was recorded after VX exposure remote from the exposure site: Template (T4) 17 spectra.
    a. Skin surface not observed until the 9^{th} spectrum.
    b. No VX peaks observed.

3. (p2) 1.4 hr PE: Template (T4) 17 spectra.
    a. No Raman measurements made until after 1 hr PE.
    b. VX peaks in spectra 2-8 (large peaks).

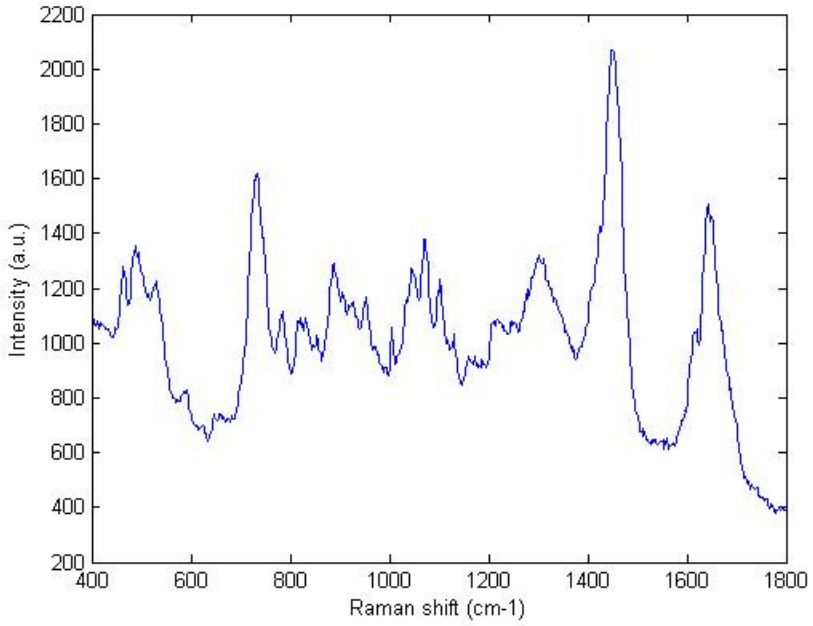


c.

d. Figure 47. Raman depth profile 2 in HGP 9.

e. Cumulative constant mass of VX using all peaks = 100 µg.

f. Cumulative constant mass of VX using only peaks with VX = 64 µg.

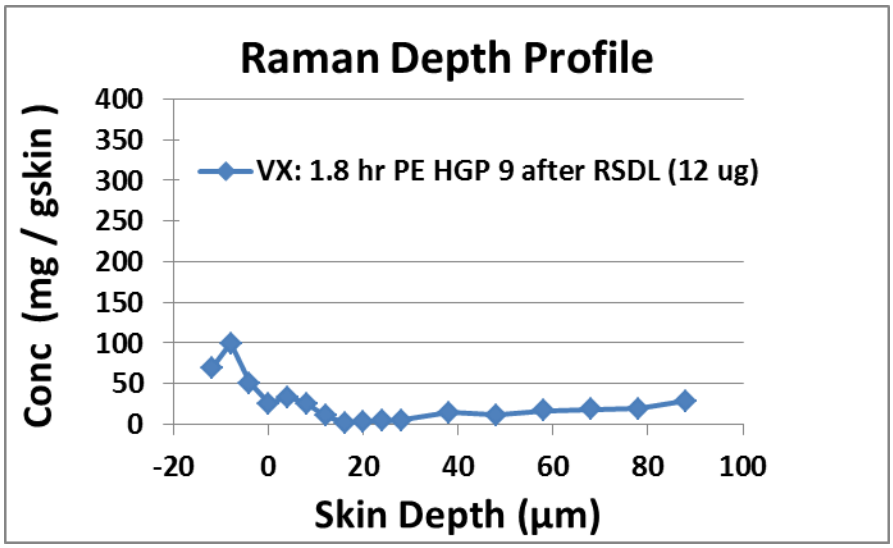


g.

h. Figure 48. Raman spectrum 3 for profile 2 in HGP 9.

i. Observed large VX peak at 730 cm^{-1} about 5 µm deep into skin.

4. (p3) 1.8 hr PE: Template (T4) 17 spectra.
   a. RSDL at 1.5 hr PE: 10 wipes, new pad for 10 min, 10 gauze wipes.



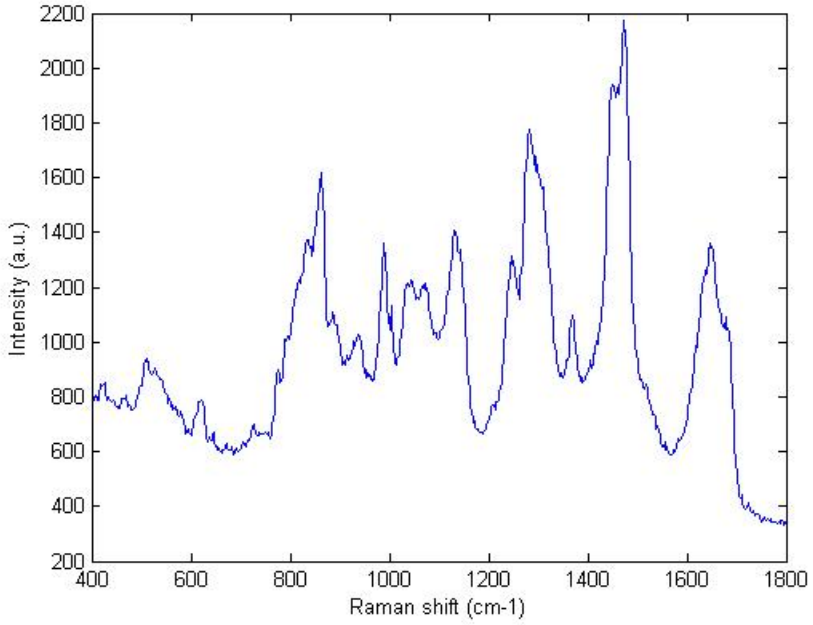
b.

c. Figure 49. Raman depth profile 3 in HGP 9 after RSDL.

d. Cumulative constant mass of VX using all peaks = 12 µg.

e. Cumulative constant mass of VX using only peaks with VX = 0 µg.

f. No VX peaks in spectra after RSDL decontamination.



g.

h. Figure 50. Raman spectrum 4 for profile 3 in HGP 9 after RSDL.

i. Observed no VX peak at 730 cm^{-1} just below the skin surface.

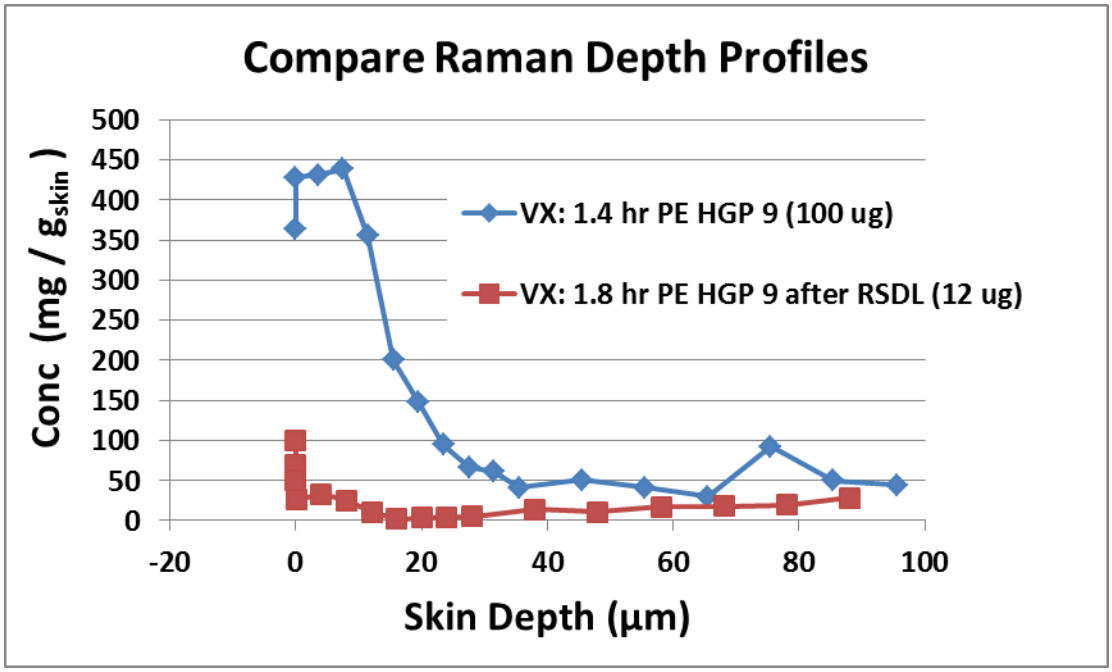
j. No VX peaks observed in any spectra for all profiles after RSDL decontamination.

5. (p2,3) Compare before and after RSDL decontamination; Template (T4) 17 spectra.

   a. Large VX peaks before RSDL decontamination.

b. No VX peaks observed in any spectra for profiles after RSDL decontamination.

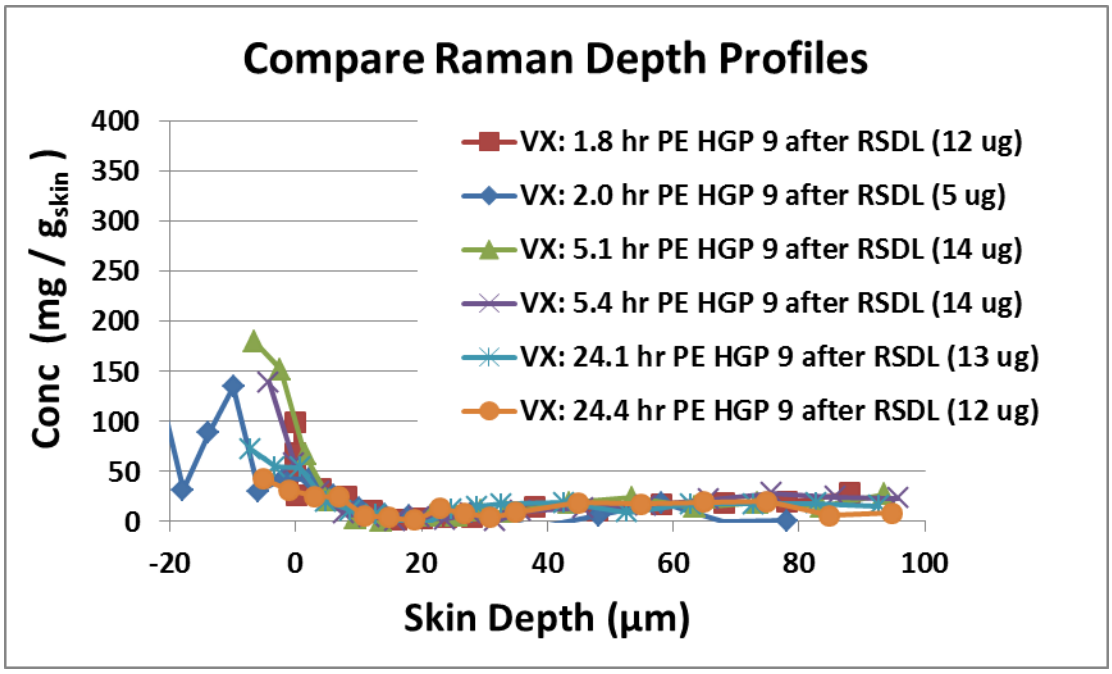
c.



d. Figure 51. Raman depth profiles comparing before and after RSDL decontamination in HGP 9.

6. Compare Raman depth profiles (p3,4,8,9,12,13) after RSDL decontamination; Template (T4) 17 spectra.
   a. No VX peaks observed in any spectra for profiles after RSDL decontamination.

b.



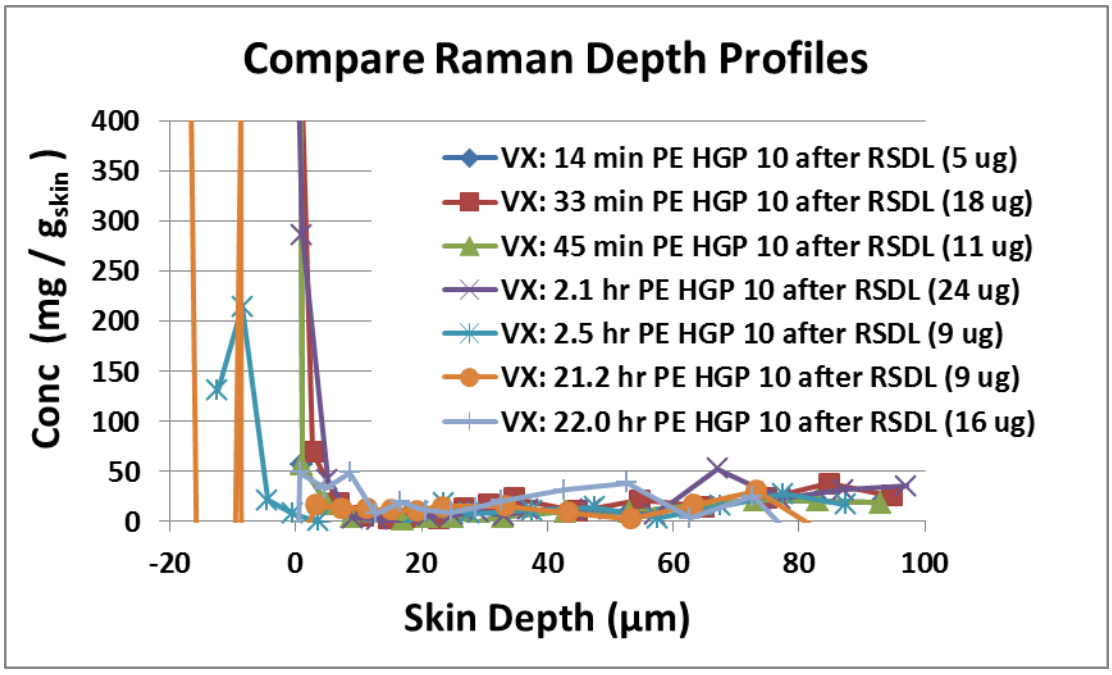
c. Figure 52. Raman depth profiles after RSDL decontamination in HGP 9.
d. Profiles at 1.8, 2.0, 5.1, 5.4, 24.1, and 24.4 hr PE.
e. Several of the profiles indicated high values of VX above the skin surface, but the values were below the LOD calculated by the instrument software. Also, no VX peaks were observed in any of the spectra from these profiles.

HGP 10 Observations 28-29 September 2011: RSDL Decontamination

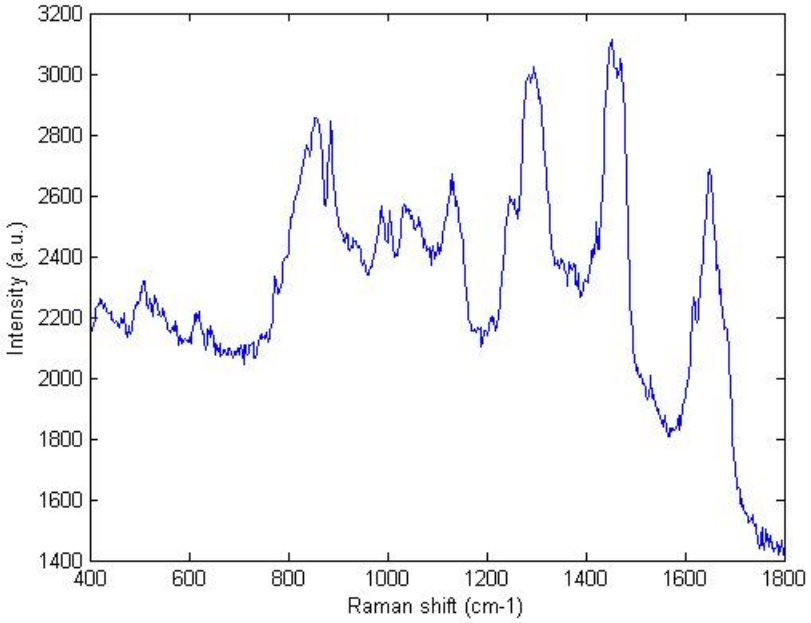
1. No HuBuChE injected (catheter not patent).
   a. No preexposure blank was recorded.
   b. No pre-RSDL Raman profile recorded.
   c. RSDL decontamination 2 min PE.
   d. Animal died overnight. It is possible that VX got under the Teflon template outside the area of the hole and could not be completely decontaminated by using RSDL and wiping the top of the template.

2. Compare Raman depth profiles (p 5, 6, 7, 10, 11, 14, and 16) after RSDL decontamination; Template (T4) 17 spectra.

a.



b. Figure 53. Raman depth profiles after RSDL decontamination in HGP 10.
c. VX not observed in any spectra from Raman depth profiles recorded after RSDL decontamination.
d. Several of the profiles indicated high values of VX above the skin surface, but the values were below the LOD calculated by the instrument software.



e.

f. Figure 54. Raman spectrum 3 for profile 5 for HGP 10 after RSDL.

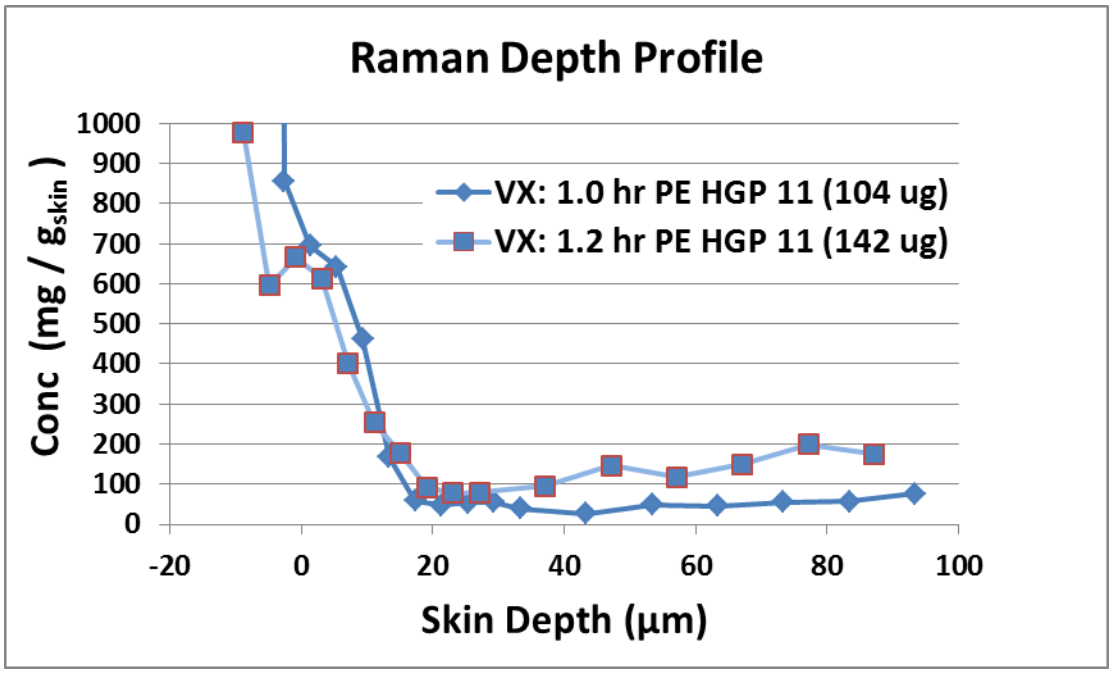
g. Observed no VX peak at 730 cm^{-1} just below the skin surface.

HGP 11 Exposure on 19 October 2011: RSDL Decontamination

1. No Blank depth profiles were recorded.

2. HGP 11 was kept overnight, but useful Raman depth profiles could not be obtained the next day because a bubble formed in the Raman recording window.

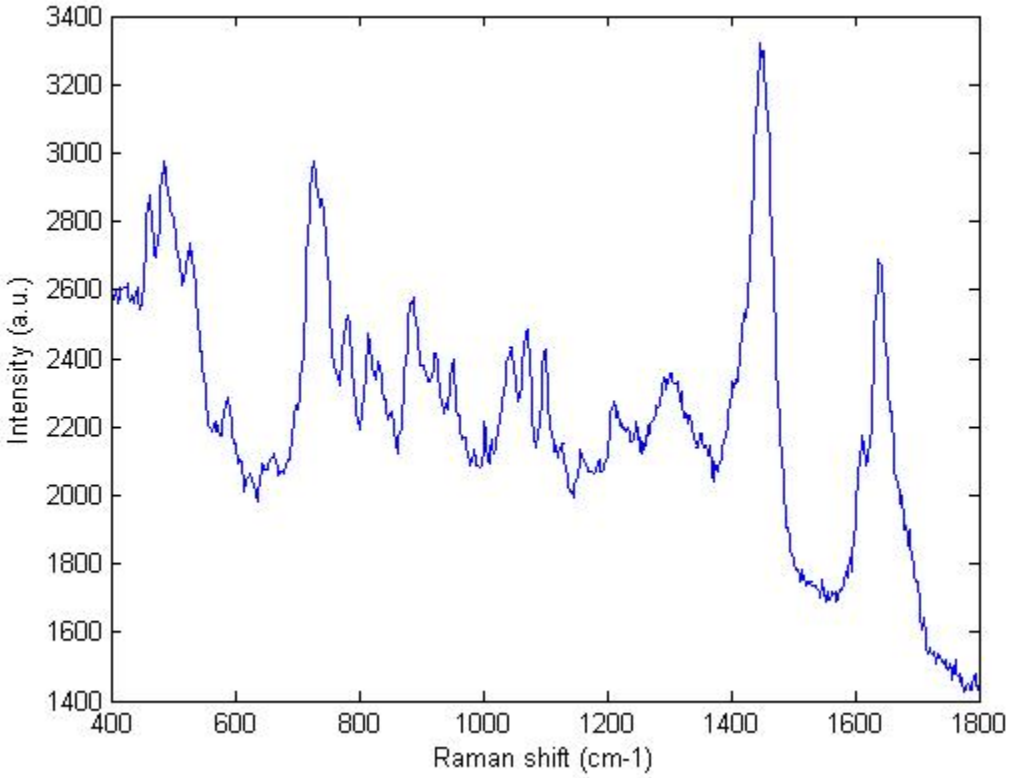
3. (p1 and 2) 1.0 and 1.2 hr PE: Template (T4) 17 spectra.

a. 

b. Figure 55. Raman depth profiles before RSDL decontamination in HGP 11.

c. Cumulative constant mass of VX using all peaks for profiles 1 and 2 = 104 and 142 µg, respectively.

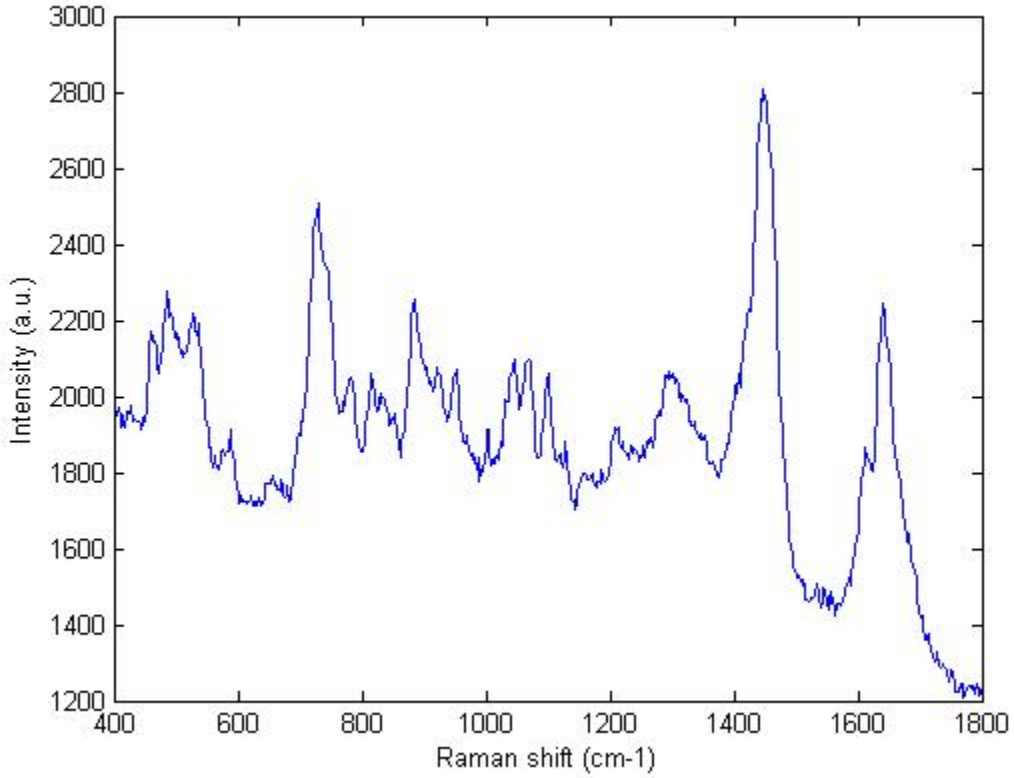
d. Large VX peaks observed in spectra from profiles above and in the skin.



e.

f. Figure 56. Raman spectrum 3 for profile 1 in HGP 11 before RSDL decontamination.

g. Observed large VX peak at 730 cm^{-1} about 5 µm deep in skin.

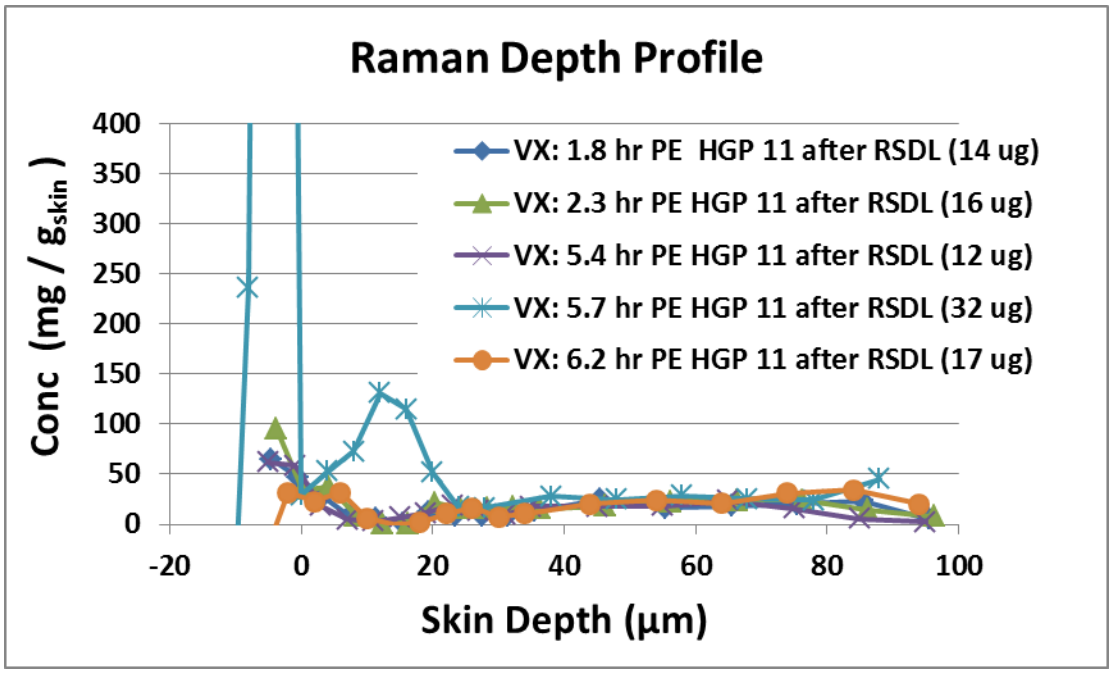


h.

i. Figure 57. Raman spectrum #5 for profile 2 in HGP 11 before RSDL decontamination.

j. Observed large VX peak at 730 cm^{-1} about 5 µm deep in skin.

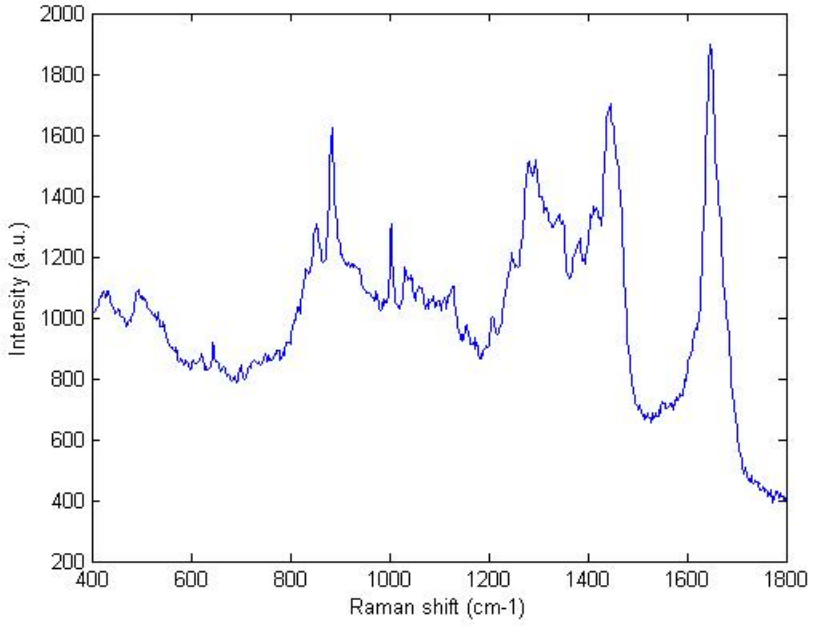
4. (p3, 5, 11, 12, 13,) 1.8, 2.3, 5.4, 5.7, and 6.2 hr PE; Template (T4); 17 spectra.
    a. RSDL decontamination at 1.4 hr PE: 10 wipes, new pad 10 min; dry gauze wipes.



b.

c. Figure 58. Raman depth profiles in HGP 11 after RSDL decontamination.

d. Observed no VX peaks in any spectra from profiles except for profile 12 at 5.7 hr PE.



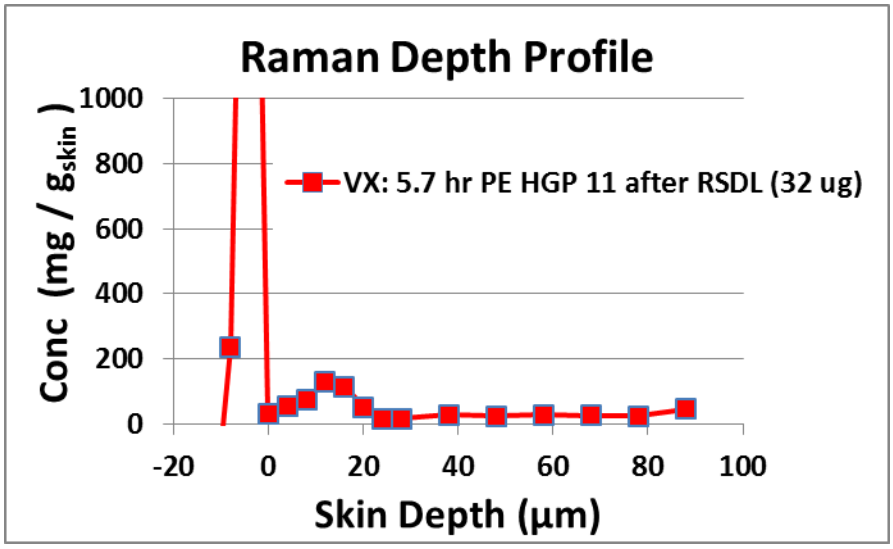
e.

f. Figure 59. Raman spectrum 3 for profile 3 in HGP 11 about 5 µm into skin.

g. Observed no VX peak at about 730 cm^{-1}.

5. (p12) at 5.7 hr PE after RSDL decontamination.
   a. Small VX peaks possible in spectra 6-8.

b. Spectra 1 and 3 off-scale, but no VX peaks observed.



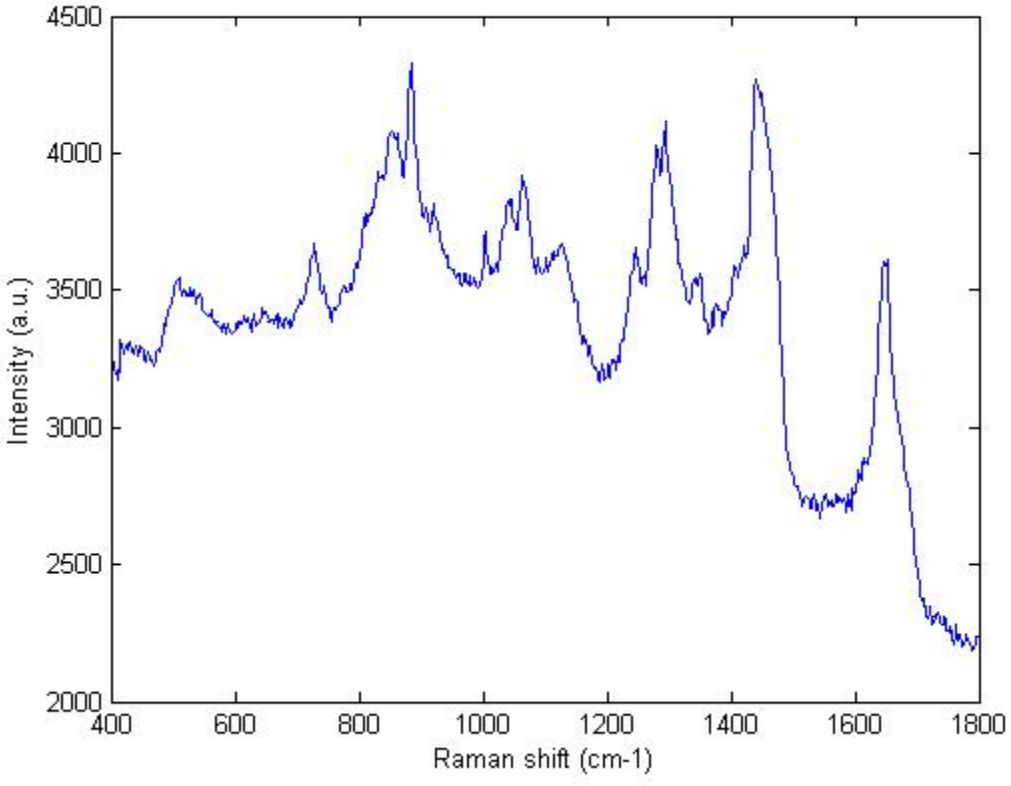
c.

d. Figure 60. Raman depth profile 12 in HGP 11 after RSDL decontamination.

e. Cumulative constant mass of VX using all peaks = 32 µg.

f. Cumulative constant mass of VX using only peaks with VX = 11 µg.

g. Spectrum 7 (small VX peak at 730 cm^{-1}) about 12 µm deep in skin.

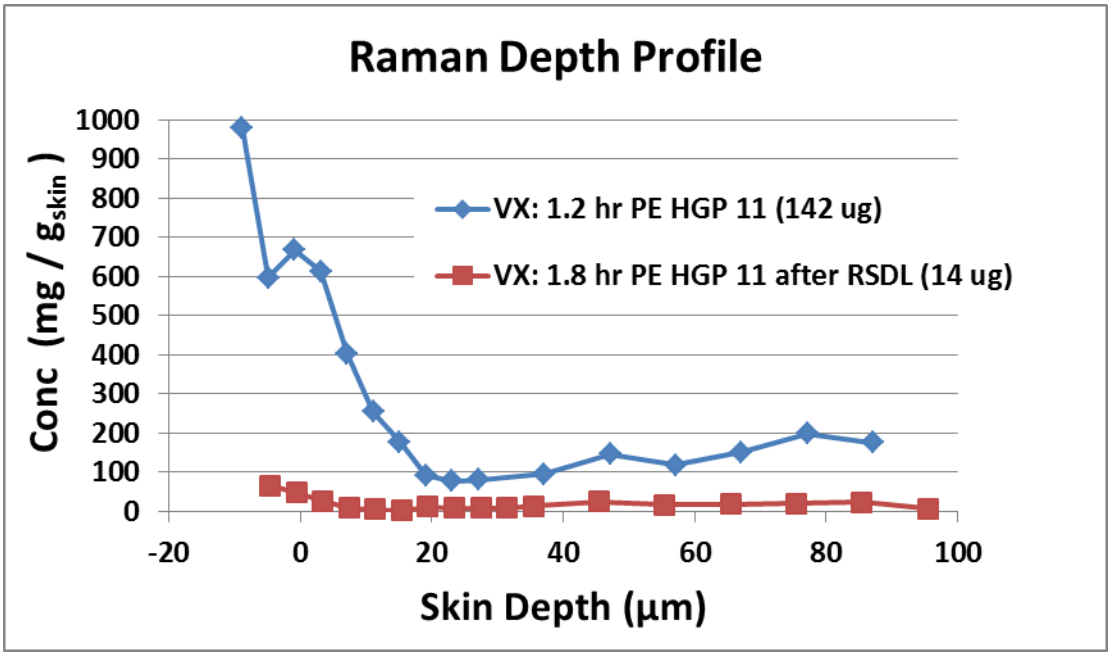


h.

i. Figure 61. Raman spectrum 7 from profile 12 in HGP 11 after RSDL decontamination.

j. Observed VX peak at 730 cm^{-1} about 12 µm deep in skin.
k. It is a rare observation to find VX in skin decontaminated with RSDL. No VX was found in the other five Raman profiles collected from this animal after RSDL decontamination.
l. Note: Spectra for RSDL were observed to only be in the very top layers of the skin about 5 µm deep.

6. Comparative Raman profiles for VX before and after RSDL in HGP 11.
   a. Raman profiles 2 and 3 at 73 and 110 min PE.
   b.



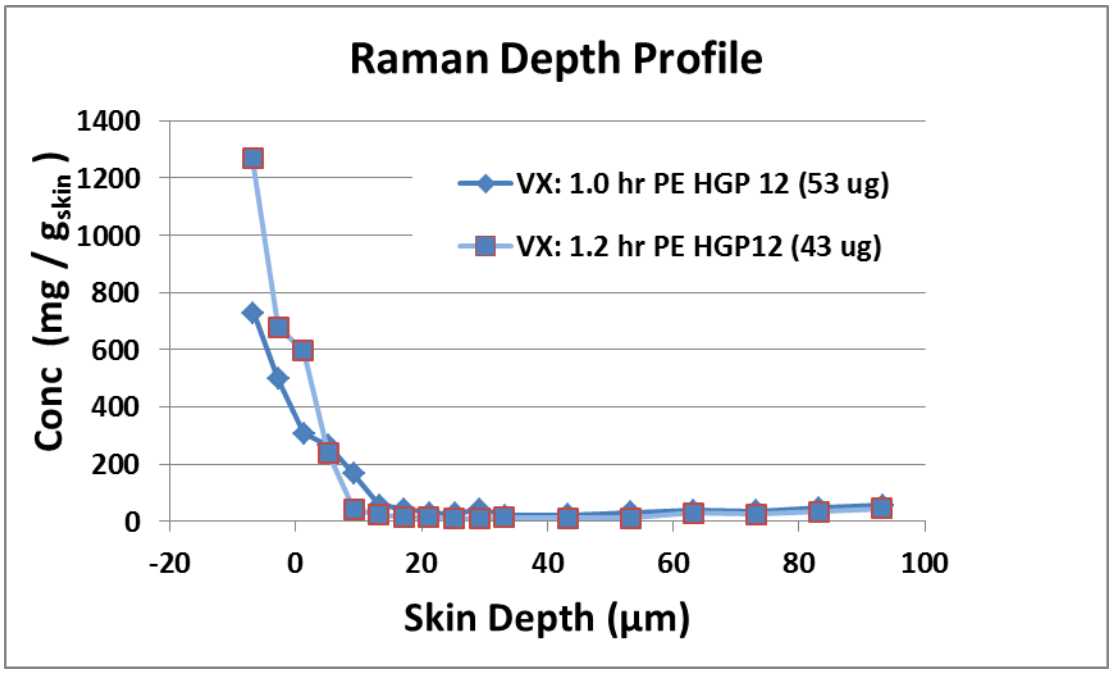
   c. Figure 62. Raman profiles before and after RSDL in HGP 11.
   d. Large VX peaks were observed before RSDL decontamination, but no VX observed after RSDL decontamination.

HGP 12 Exposure on 19 October 2011: RSDL Decontamination

1. No Blank recorded.

2. HGP 12 was kept overnight, but useful Raman depth profiles could not be obtained the next day because a bubble formed in the Raman recording window.

3. (p6 and 7) 1.0 and 1.2 hr PE: Template (T4) 17 spectra.

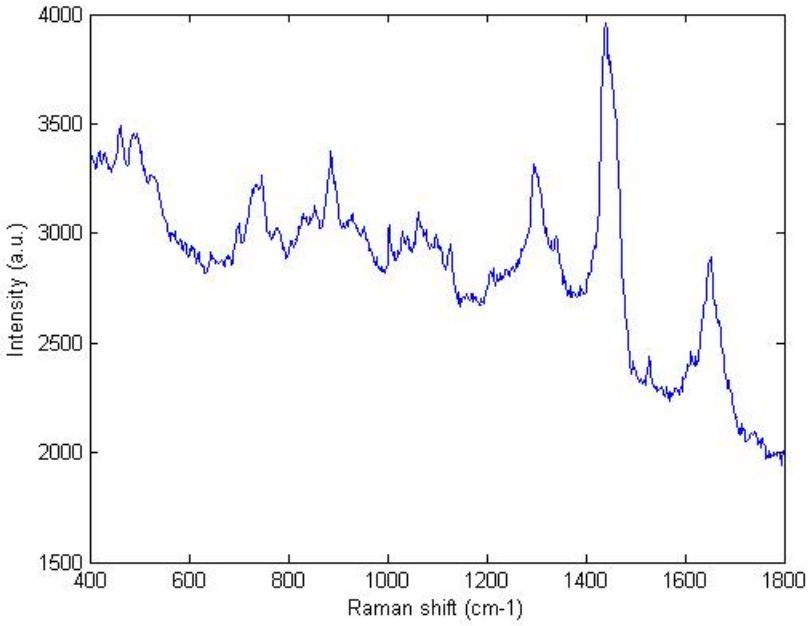
a. 

b. Figure 63. Raman depth profiles for HGP 12 before RSDL decontamination.

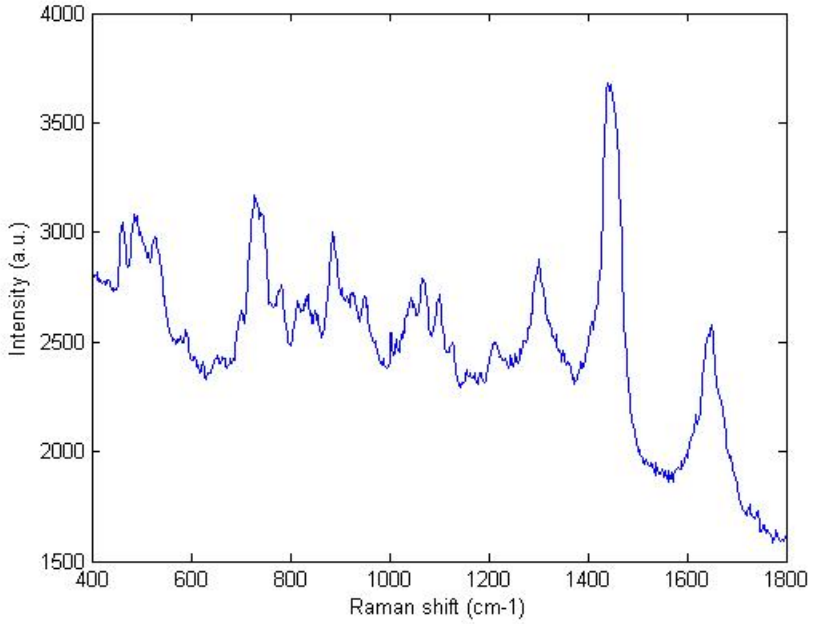
c. Cumulative constant mass of VX for profiles 6 and 7 using all peaks = 53 and 43 µg, respectively.

d. In profile 6 (1.0 hr PE) observed large VX peaks at 730 cm^{-1} in spectra 3-6.

e. In profile 7 (1.2 hr PE) observed large VX peaks at 730 cm^{-1} in spectra 2-4.

f. 

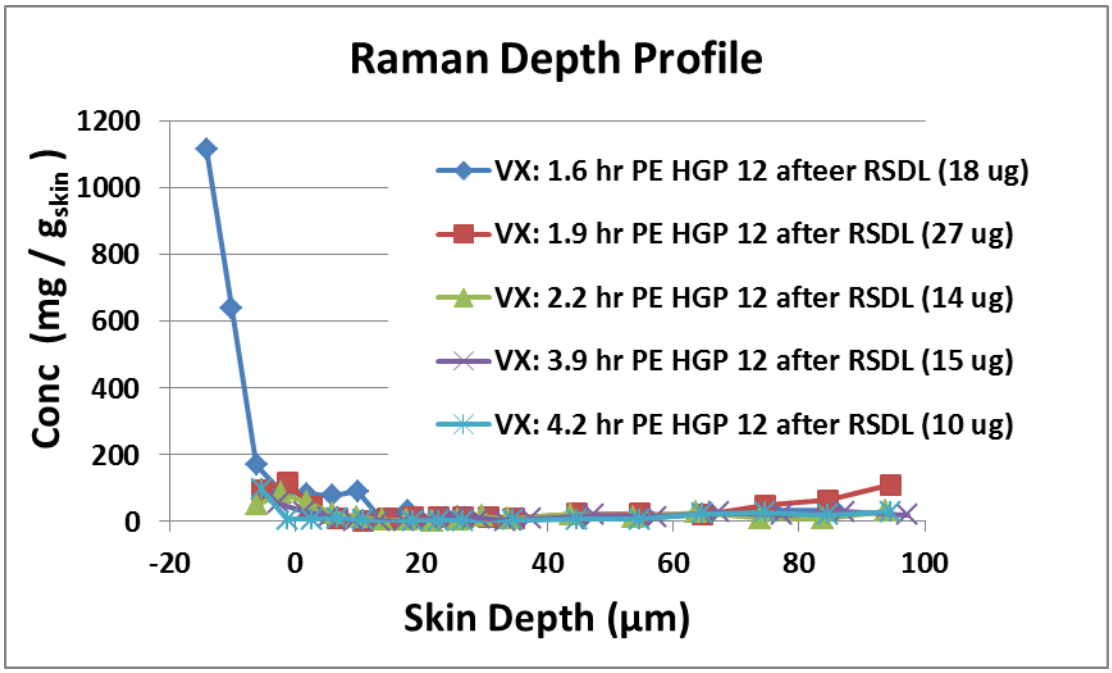
g. Figure 64. Raman spectrum 3 for profile 6 in HGP 12 before RSDL decontamination.
h. Observed large VX peak at 730 cm^{-1} about 5 µm deep in skin.



i.
j. Figure 65. Raman spectrum 3 for profile 7 in HGP 12 before RSDL decontamination.
k. Observed large VX peak at 730 cm^{-1} about 5 µm deep in skin.

4. (p8, 9, 10, 14, and 15) at 1.6, 1.9, 2.2, 3.9, and 4.2 hr PE; Template (T4); 17 spectra.
    a. RSDL decontamination at 1.4 hr PE: 10 wipes, new pad 10 min; dry gauze wipes.

b.

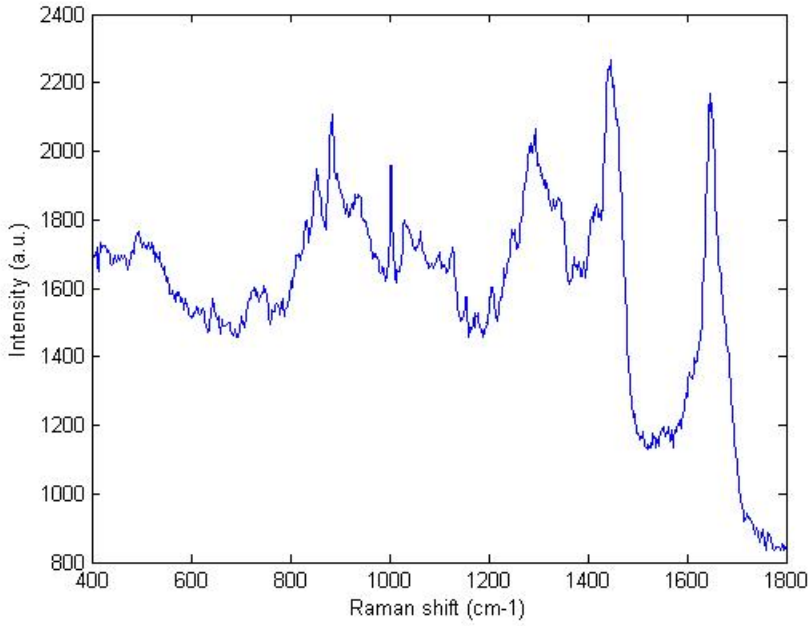


c. Figure 66. Raman depth profiles in HGP 12 after RSDL decontamination.

d. Raman profile 8 at 1.6 hr PE consisted of very poor quality spectra at all depths. The large measured VX concentrations observed above the skin surface (spectra 1-4) were an anomaly resulting from the poor quality spectra. The values were below the LOD calculated by the instrument software. VX peaks were not observed at 730 cm^{-1} in these spectra.

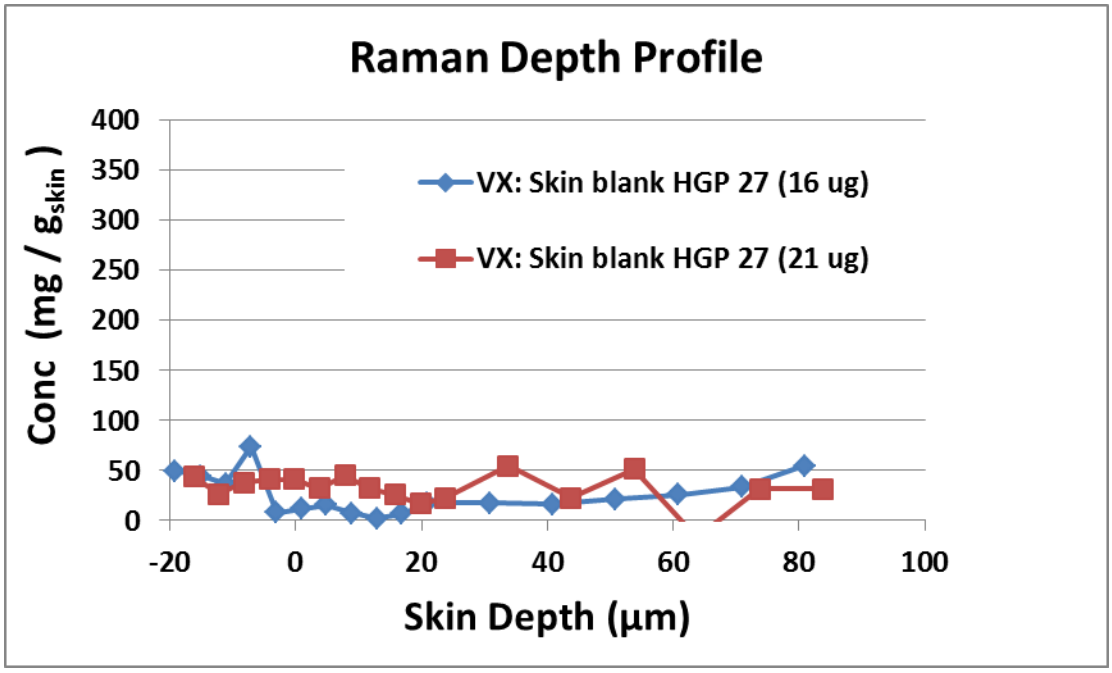
e. No VX peaks were observed in spectra from any of the Raman profiles except for profile 9 at 1.9 hr PE.

i.

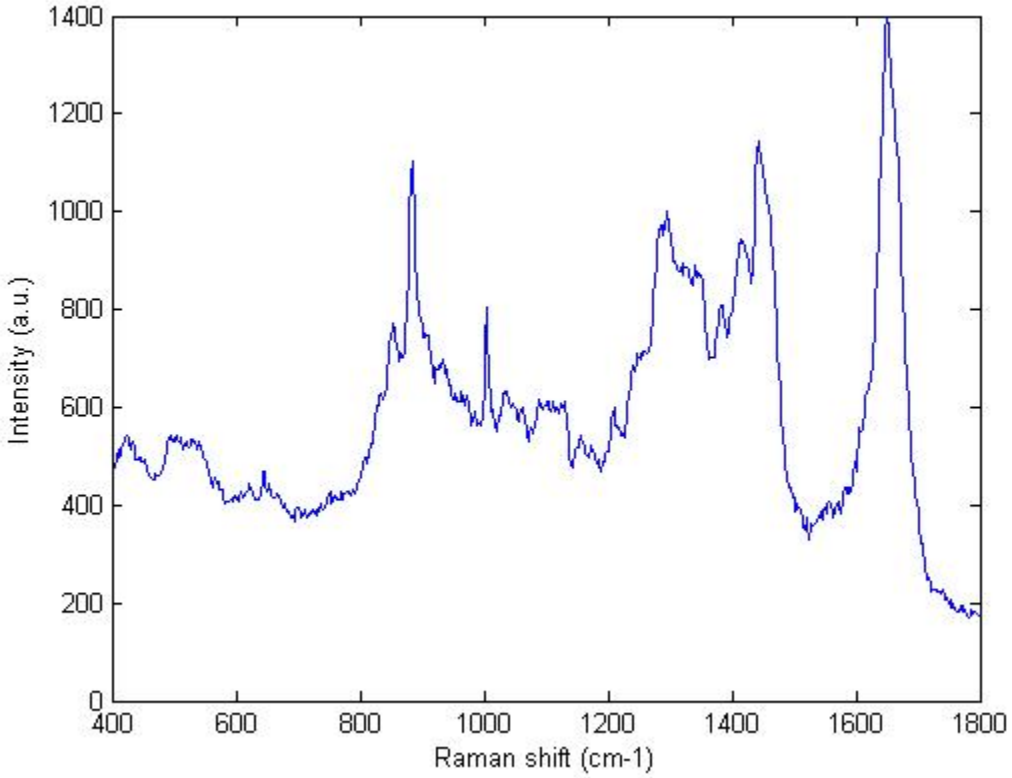


ii. Figure 67. Raman spectrum 3 in profile 9 in HGP 12 after RSDL decontamination.
iii. The small peak observed at 730 cm^{-1} could represent VX.

HGP 27 Exposure on 29 February 2012: Total Mass of VX in skin

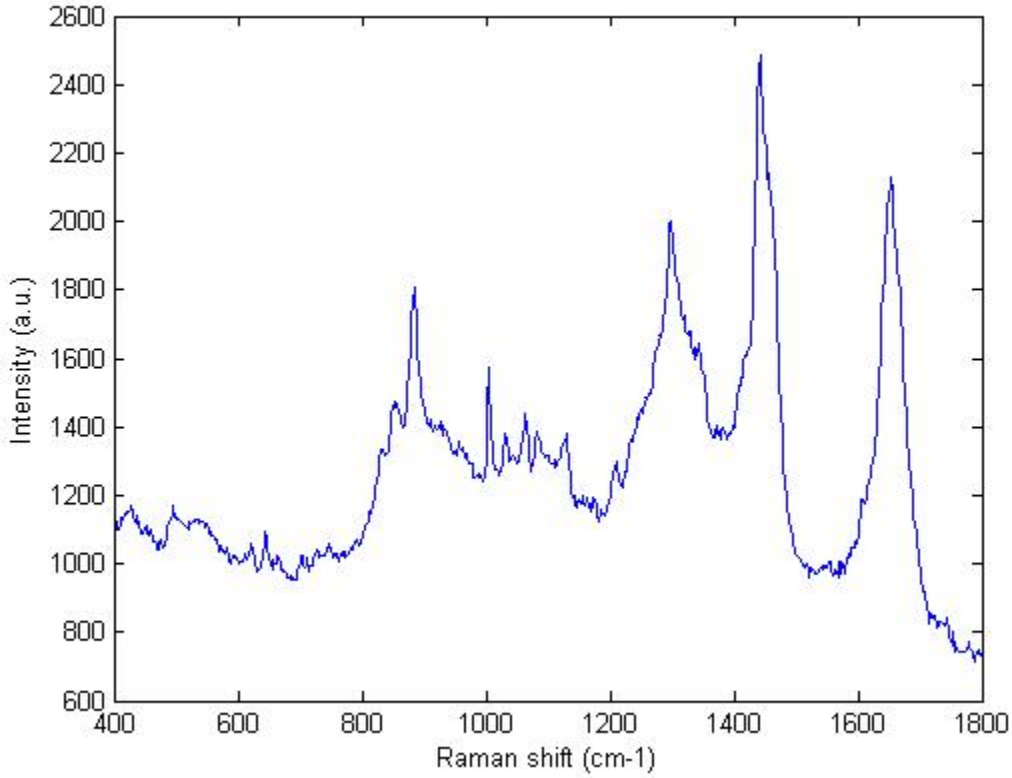
1. Comparison of Raman profiles 1 and 3 skin blanks before VX exposure.
    a. Template T5, 2 steps with 17 spectra.
    b. Both profiles were recorded on the exact same site by not moving the animal between collections.
    c. 

    d. Figure 68. Raman depth profiles for HGP 27 before VX exposure.
    e. Cumulative constant mass of VX for HGP 27 (p1,3) using all peaks = 16 and 21 µg, respectively (mean = 18.5 µg; SD = 3.5).
    f. Cumulative constant mass of VX for HGP 27 (p1,3) using only peaks with VX = 0 µg.



g.

h. Figure 69. Raman spectrum 7 for profile 1 (skin blank) in HGP 27 about 5 µm into skin.

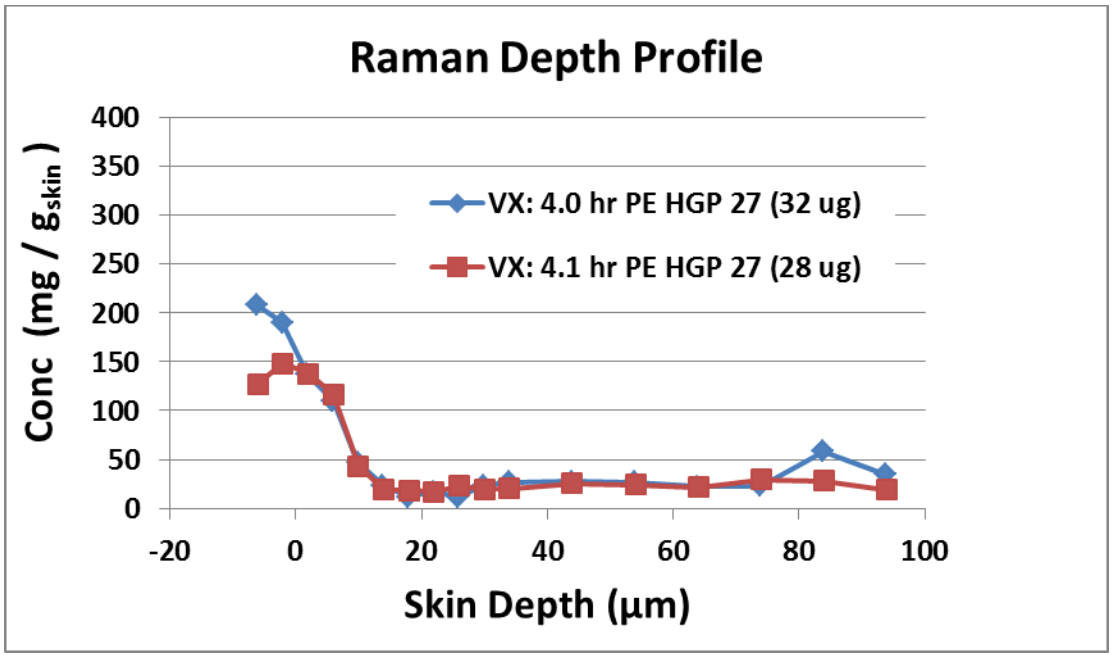


i.

j. Figure 70. Raman spectrum 6 for profile 3 (skin blank) in HGP 27 about 5 µm into skin.

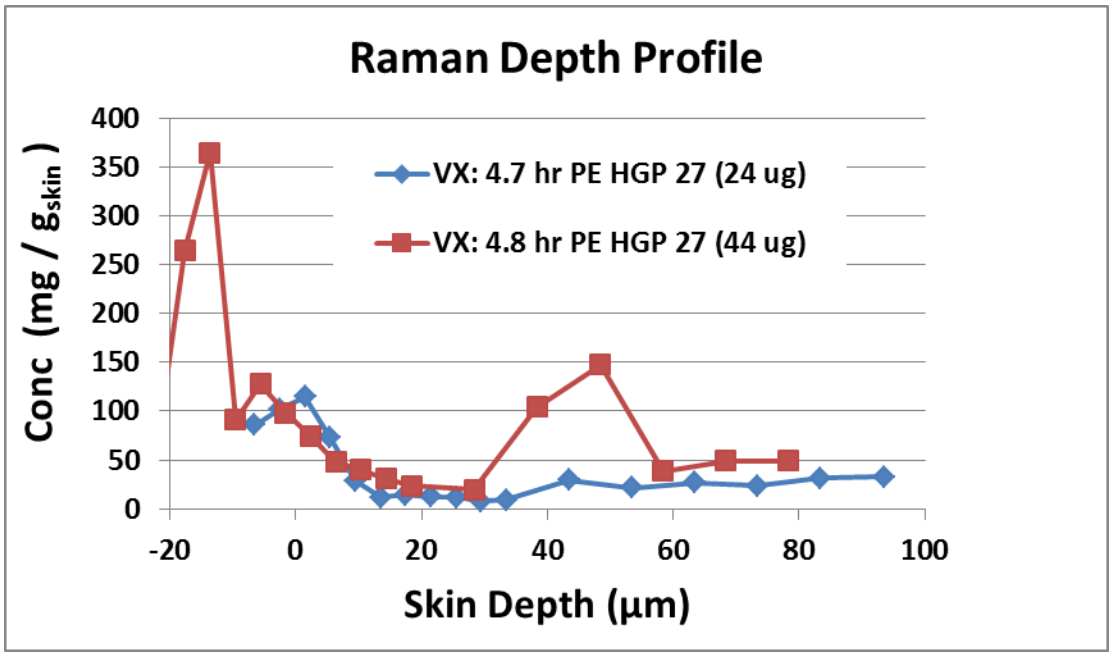
k. VX peaks were not observed in any spectra from either profile.

2. Comparison of Raman profiles 6 and 7 at 4.0 and 4.1 hr PE.
    a. Template T5, 2 steps with 17 spectra.
    b. Both profiles were recorded on the exact same site by not moving the animal between collections.

c. 

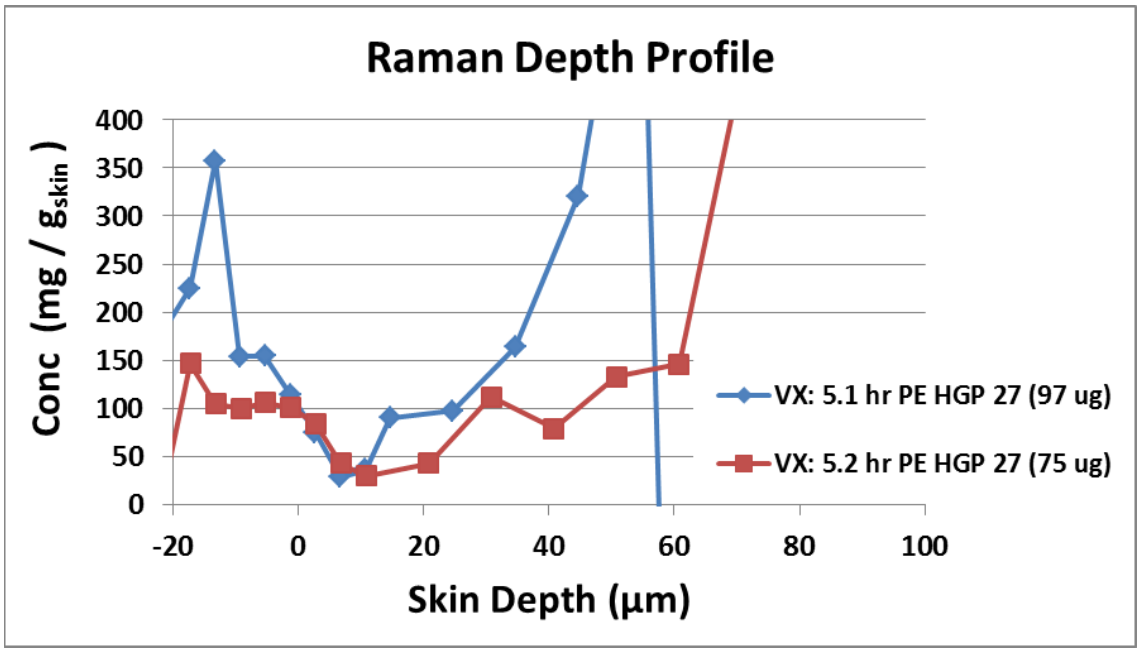
d. Figure 71. Raman depth profiles 6 and 7 in HGP 27 at 4.0 and 4.1 hr PE.
e. Cumulative constant mass of VX (p6,7) all peaks = 32 and 28 µg, respectively (mean = 30 µg, SD = 2.8).
f. Cumulative constant mass of VX (p6,7), only peaks with VX = 9.5 and 6.9 µg, respectively (mean = 8.2 µg, SD = 1.8).
g. In profile 6 (4.0 hr PE) VX peaks observed in spectra 3-5.
h. In profile 7 (4.1 hr PE) VX peaks observed in spectra 3-4.
i. Profiles are virtually identical when recorded on exactly the same site.

3. Comparison of Raman profiles 8 and 9 at 4.7 and 4.8 hr PE.
    a. Template T5, 2 steps with 17 spectra.
    b. Both profiles were recorded on the exact same site by not moving the animal between collections.

c. 

d. Figure 72. Raman depth profiles 8 and 9 in HGP 27 at 4.7 and 4.8 hr PE.
e. Cumulative constant mass of VX (p8,9), all peaks = 24 and 44 µg, respectively (mean = 34 µg, SD = 14).
f. Cumulative constant mass of VX (p8,9), only peaks with VX = 4.8 and 5.3 µg, respectively (mean = 5.0 µg, SD = 0.35).
g. Profile 9 (4.8 hr PE) had two issues. First, the skin surface was not detected until spectrum 7, which is much later than typically observed. Second, the high values for spectra 13 and 14 (at around 40-50 µm into the skin) were caused by very poor quality spectra. VX peaks were not observed in these spectra, and the VX values were below the LOD line generated by the instrument software.
h. If one omits the data in profile 9 from points above the skin surface and spectra 13 and 14, both profiles are virtually identical.

4. Comparison of Raman profiles 10 and 11 at 5.1 and 5.2 hr PE.
   a. Template T5, 2 steps with 17 spectra.
   b. Both profiles were recorded on the exact same site by not moving the animal between collections.

c. 

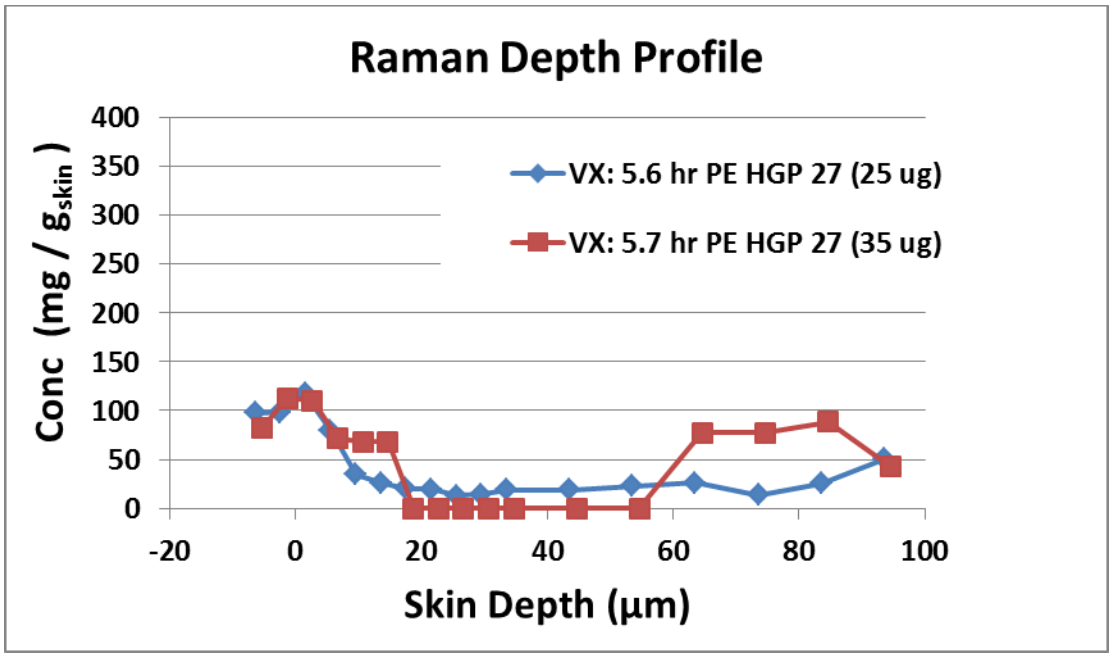
d. Figure 73. Raman depth profiles 10 and 11 in HGP 27 at 5.1 and 5.2 hr PE.

e. Cumulative constant mass of VX (p10,11), all peaks = 97 and 75 µg, respectively (mean = 86 µg, SD = 16).

f. Cumulative constant mass of VX (p10,11), only peaks with VX = 3.5 and 4.2 µg, respectively (mean = 3.8 µg, SD = 0.49).

g. These two Raman depth profiles were very similar in the top layers of the skin. At a depth of about 40 µm they started to diverge, corresponding to a decrease in the quality of the Raman spectra as one goes deeper into the skin. As a general trend, the quality of Raman spectra decreases with increasing depth into the skin.

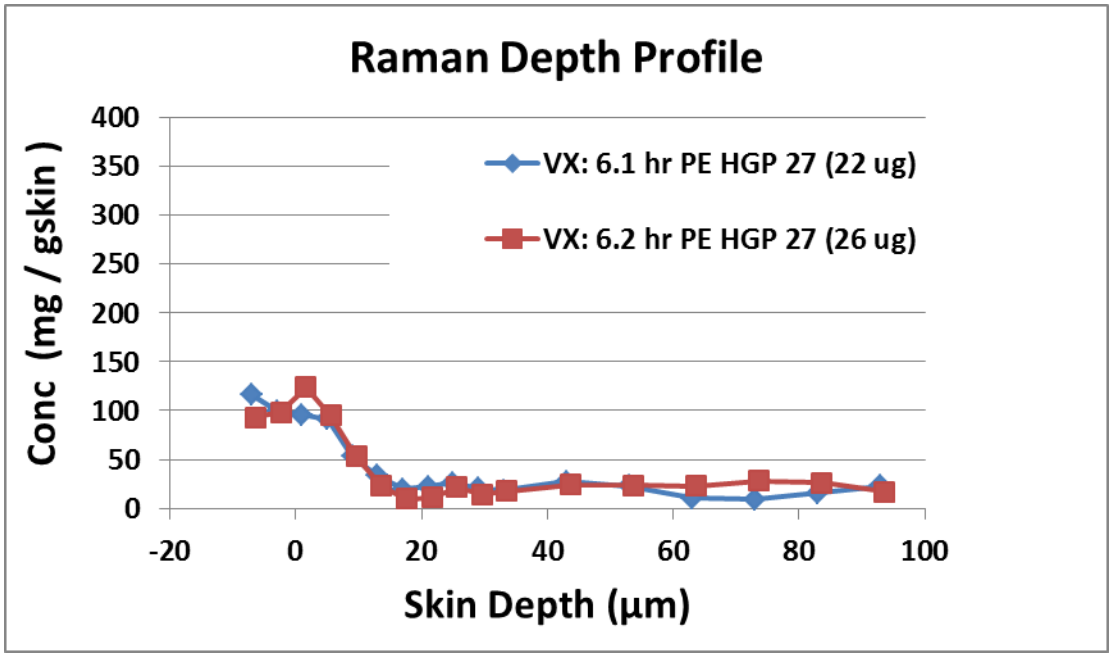
5. Comparison of Raman profiles 12 and 13 at 5.6 and 5.7 hr PE.
   a. Template T5, 2 steps with 17 spectra.
   b. Both profiles were recorded on the exact same site by not moving the animal between collections.

c. 

d. Figure 74. Raman depth profiles 12 and 13 in HGP 27 at 5.6 and 5.7 hr PE.
e. Cumulative constant mass of VX (p12,13), all peaks = 25 and 35 µg, respectively (mean = 30 µg, SD = 7.1).
f. Cumulative constant mass of VX (p12,13), only peaks with VX = 7.2 and 5.7 µg, respectively (mean = 6.4 µg, SD = 1.1).
g. Both profiles were again very similar. There were slight deviations at the deeper skin depths.

6. Comparison of Raman profiles 14 and 15 at 6.1 and 6.2 hr PE.
    a. Template T5, 2 steps with 17 spectra.
    b. Both profiles were recorded on the exact same site by not moving the animal between collections.

c.



d. Figure 75. Raman depth profiles 14 and 15 in HGP 27 at 6.1 and 6.2 hr PE.

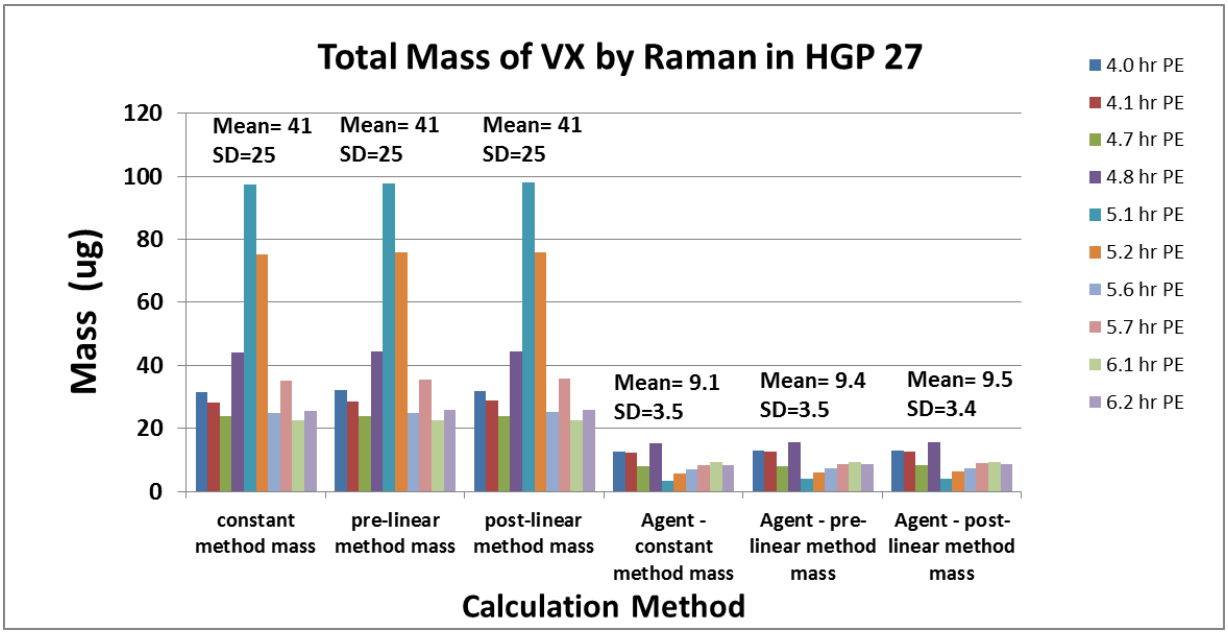
e. Cumulative constant mass of VX (p14,15), all peaks = 22 and 26 µg, respectively (mean = 24 µg, SD = 2.8).

f. Cumulative constant mass of VX (p14,15), only peaks with VX = 6.1 and 8.4 µg, respectively (mean = 7.2 µg, SD = 1.6).

g. Profiles 14 and 15 were virtually identical.

7. Total mass of VX by Raman estimates.

a.

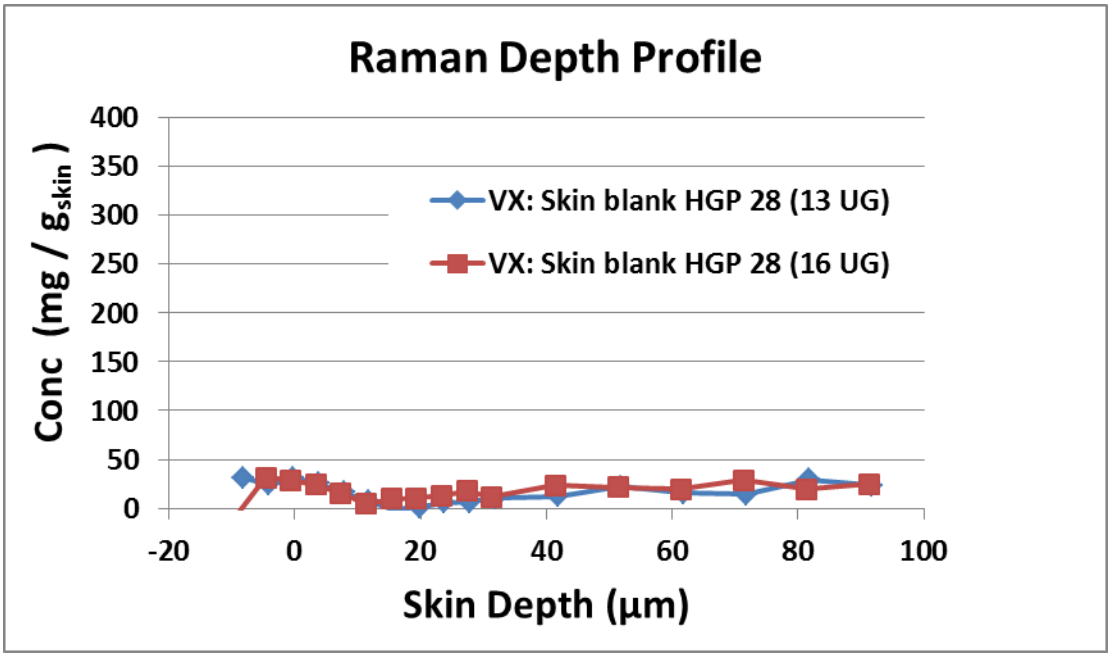


b. Figure 76. Total mass of VX by Raman in HGP 27. Left side of figure estimated the total mass of VX using all spectra in profile. Right side of figure estimated the total mass of VX using only spectra that contained an observable VX peak at 730 cm-1. Constant, pre-linear, and post-linear were three different methods used to estimate the total mass of VX.
c. Total mass of VX applied = 284 µg (0.30 µl of neat agent).
d. Total mass of VX (free and bound) from analysis of exposure skin punches = 42.6 µg.
e. Total mass of free VX estimated from analysis of skin punches = 21.3 µg.
f. Mean total mass of VX by Raman (all peaks) = 41 µg (SD = 0).
g. Mean total mass of VX observed in Raman blank profiles = 18 µg.
h. Mean mass of VX estimated by Raman using all spectra: (mean VX profiles – mean blank profiles) = 23 µg.
i. Mean total mass of VX by Raman (only VX peaks) = 9.3 µg (SD = 0.21).

8. ECBC values for skin punches for HGP 27.
    a. Control skin blank: total VX = 4.9 ng (2.4 ng free VX).
    b. Exposure skin site: total VX = 42.6 µg (21.3 µg free VX).

HGP 28 Exposure on 29 February 2012: Total Mass of VX in skin

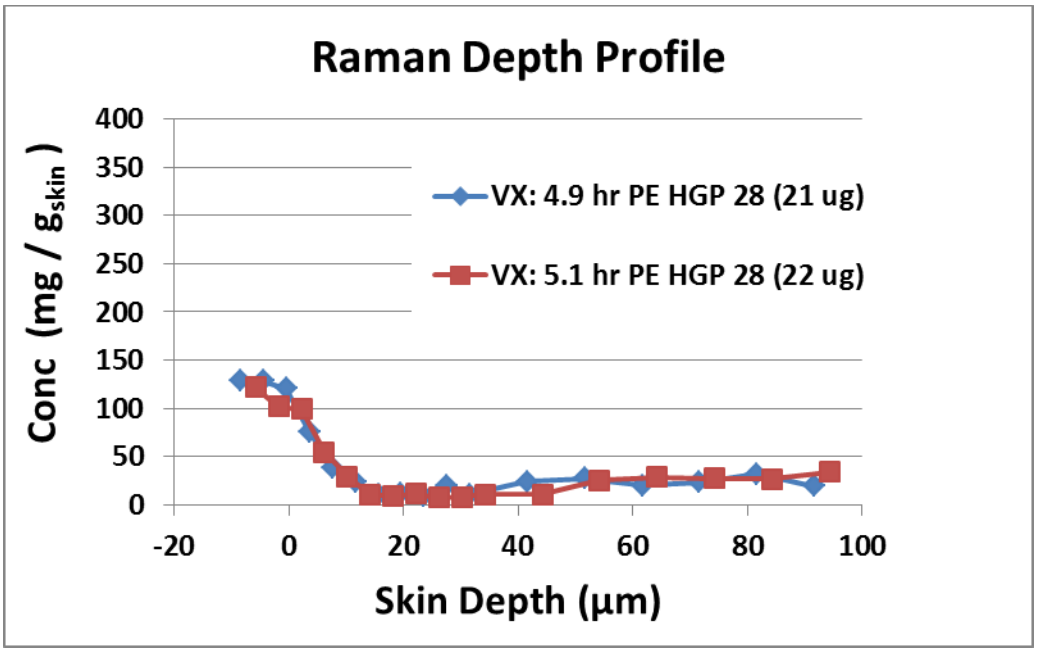
1. Comparison of Raman profiles 4 and 5; skin blanks before VX exposure.
    a. Template T5, 2 steps with 17 spectra.
    2. Both profiles were recorded on the exact same site by not moving the animal between collections.

a. 

b. Figure 77. Raman depth profiles in HGP 28 before VX exposure.
c. Cumulative constant mass of VX for HGP 28 (p4,5) using all peaks = 13 and 16µg, respectively (mean = 14.5 µg; SD = 2.1).
d. Cumulative constant mass of VX for HGP 28 (p4,5) using only peaks with VX = 0 µg.
e. VX peaks were not observed in any spectra from either profile.
f. Profiles were virtually identical when recorded on exactly the same site.

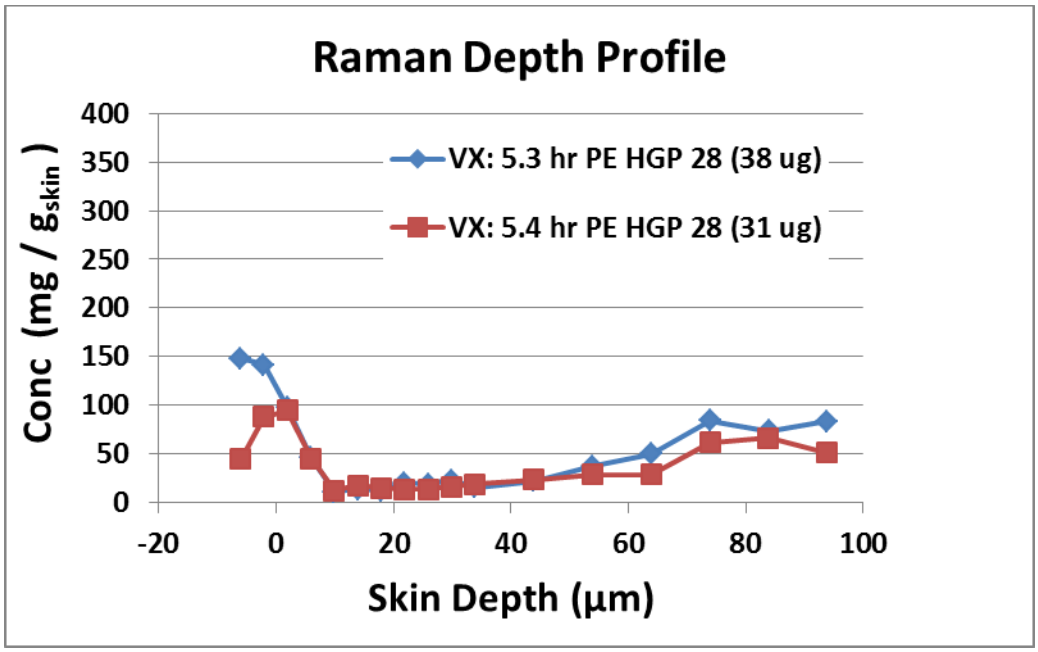
3. Comparison of Raman profiles 16 and 17 at 4.9 and 5.1 hr PE.
   a. Template T5, 2 steps with 17 spectra.
   b. Both profiles were recorded on the exact same site by not moving the animal between collections.

c.



d. Figure 78. Raman depth profiles 16 and 17 in HGP 28 at 4.9 and 5.1 hr PE.
e. Cumulative constant mass of VX (p16,17), all peaks = 21 and 22 µg, respectively (mean = 21.5 µg; SD = 0.71).
f. Cumulative constant mass of VX (p16,17), only peaks with VX = 2.2 and 4.6 µg, respectively (mean = 3.4 µg; SD = 1.7).
g. Profiles were virtually identical when recorded on exactly the same site.

4. Comparison of Raman profiles 18 and 19 at 5.3 and 5.4 hr PE
   a. Template T5, 2 steps with 17 spectra.
   b. Both profiles were recorded on the exact same site by not moving the animal between collections.



c.

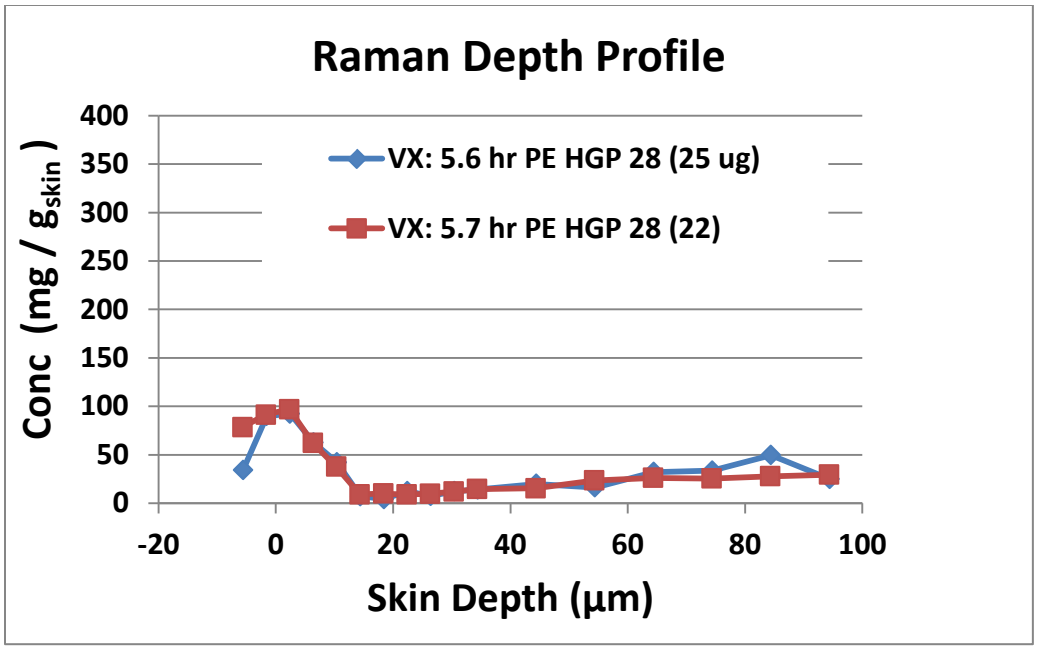
d. Figure 79. Raman depth profiles 18 and 19 in HGP 28 at 5.3 and 5.4 hr PE.

e. Cumulative constant mass of VX (p18,19), all peaks = 38 and 31 µg, respectively (mean = 34.5 µg; SD = 4.9).

f. Cumulative constant mass of VX (p18,19), only peaks with VX = 1.5 and 1.4 µg, respectively (mean = 1.4 µg; SD = 0.071).

g. Profiles were virtually identical when recorded on exactly the same site.

5. Comparison of Raman profiles 20 and 21 at 5.6 and 5.7 hr PE.
   a. Template T5, 2 steps with 17 spectra.
   b. Both profiles were recorded on the exact same site by not moving the animal between collections.



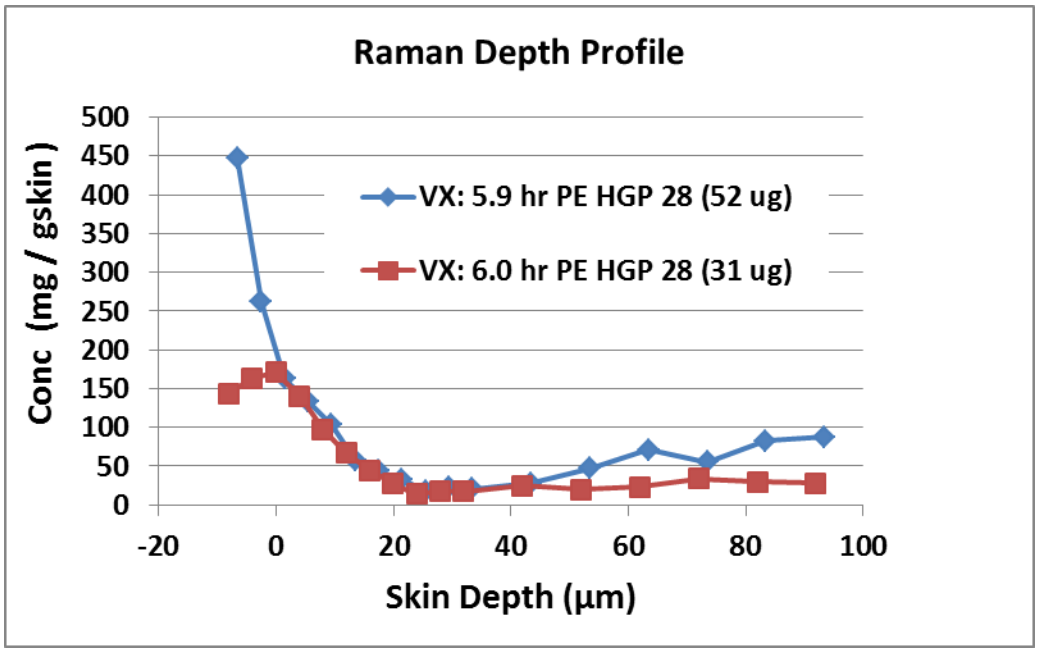
c.

d. Figure 80. Raman depth profiles 20 and 21 in HGP 28 at 5.6 and 5.7 h PE.

e. Cumulative constant mass of VX (p20,21), all peaks = 25 and 22 µg, respectively (mean = 23.5 µg; SD = 2.1).

f. Cumulative constant mass of VX (p20, 21), only peaks with VX = 4.7 and 4.8 µg, respectively (mean = 4.75 µg; SD = 0.071).

6. Comparison of Raman profiles 22 and 23 at 5.9 and 6.0 h PE.
   a. Template T5, 2-steps with 17 spectra.
   b. Both profiles were recorded on the exact same site by not moving the animal between collections.

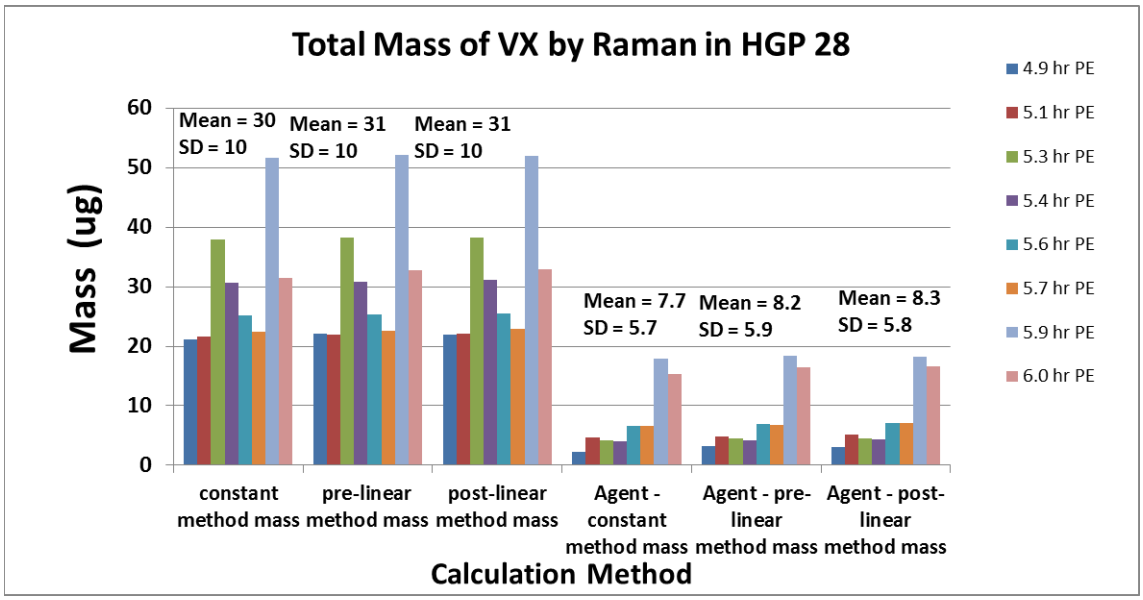


c.

d. Figure 81. Raman depth profiles 22 and 23 for HGP 28 at 5.9 and 6.0 hr PE.
e. Cumulative constant mass of VX (p22,23), all peaks = 52 and 31 µg, respectively (mean = 41.5 µg; SD = 14.8).
f. Cumulative constant mass of VX (p22,23), only peaks with VX = 15 and 12 µg, respectively (mean = 13.5 µg; SD = 2.1).

7. Total mass of VX by Raman estimates

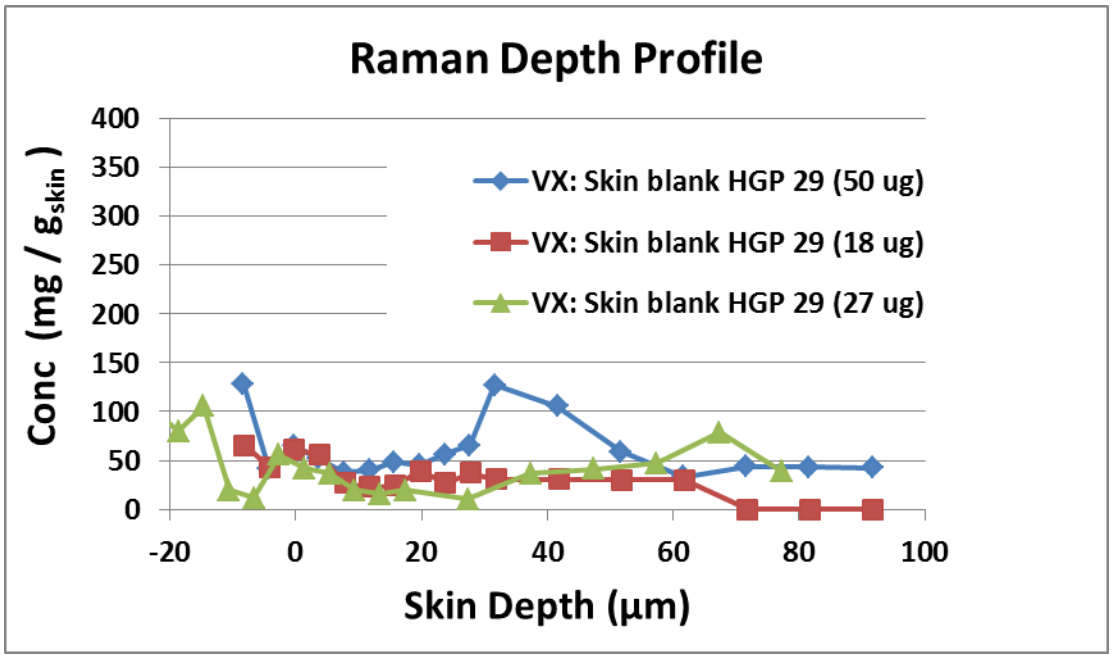
a.



b. Figure 82. Total mass of VX by Raman in HGP 28. Left side of figure estimated the total mass of VX using all spectra in profile. Right side of figure estimated the total mass of VX using only spectra that contained an observable VX peak at 730 cm-1. Constant, pre-linear, and post-linear were three different methods used to estimate the total mass of VX.
c. Total mass of VX applied = 284 µg (0.30 µl of neat agent).
d. Total mass of VX (free and bound) from analysis of exposure skin punches = 52.8 µg.
e. Total mass of free VX estimated from analysis of skin punches = 26.4 µg.
f. Mean total mass of VX by Raman (all peaks) = 30.7 µg (SD = 0.58).
g. Mean total mass of VX observed in Raman blank profiles = 14.5 µg (SD = 2.1).
h. Mean mass of VX estimated by Raman using all spectra: (mean VX profiles – mean blank profiles) = 16 µg.
i. Mean total mass of VX by Raman (only VX peaks) = 8.1 µg (SD = 0.32).

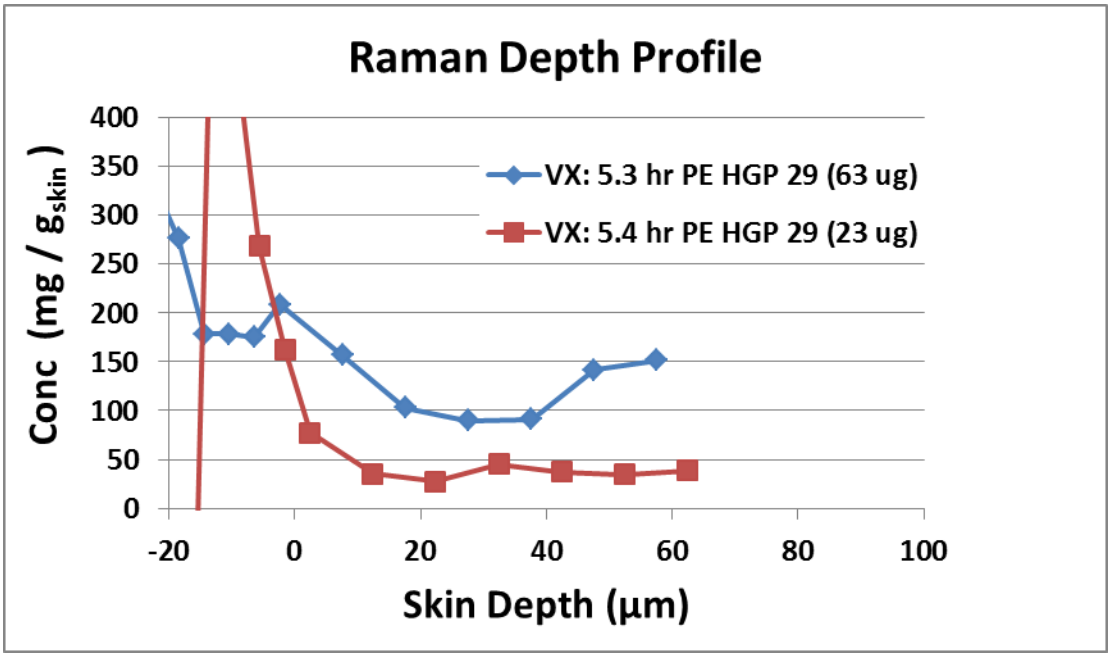
8. ECBC values for skin punches for HGP 28.
   a. Control skin blank: total VX = 38.8 ng (free VX = 19.4 ng).
   b. Exposure skin site: total VX = 52.8 µg (free VX = 26.4 µg).

HGP 29 Exposure on 6 March 2012: Total Mass of VX in skin

1. Comparison of Raman profiles 1, 2 and 4 skin blanks before VX exposure.
    a. Template T5, 2 steps with 17 spectra.
    b. 

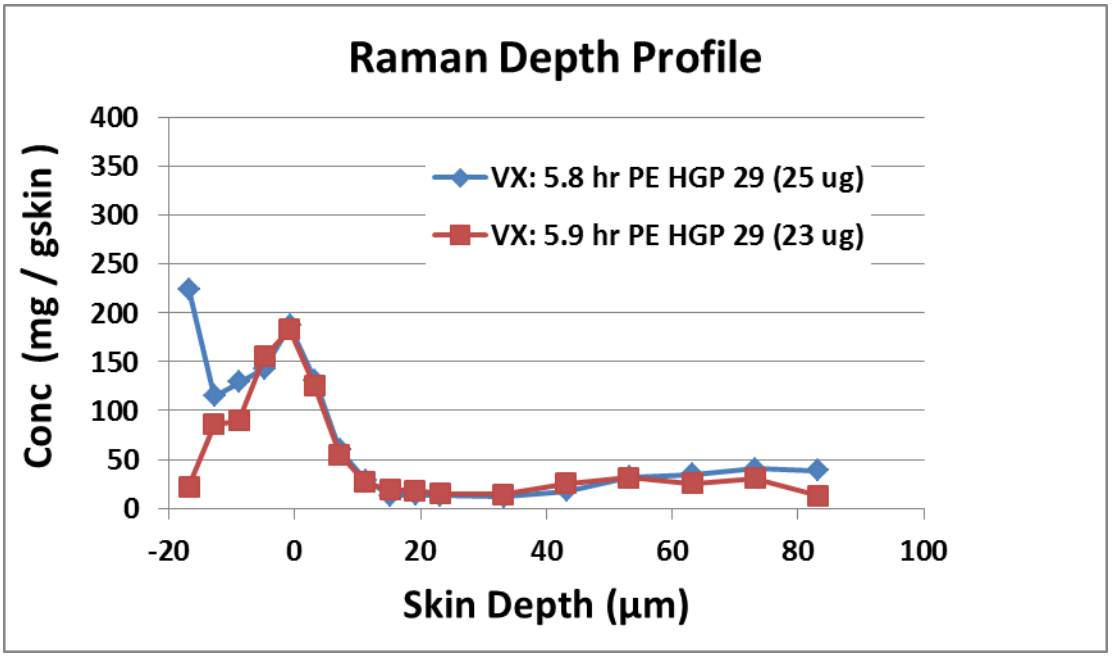
    c. Figure 83. Raman depth profiles in HGP 29 before VX exposure.
    d. Cumulative constant mass of VX (p1,2,4), all peaks = 50,18, and 27 µg, respectively (mean = 31.7 µg; SD = 16.5).
    e. Cumulative constant mass of VX (p1,2,4), only peaks with VX = 0,0, and 0 µg, respectively (mean = 0 µg; SD = 0).
    f. VX peaks were not observed in any spectra from these profiles.

1. Comparison of Raman profiles 17 and 18 at 5.3 and 5.4 hr PE.

a. 

c. Figure 84. Raman depth profiles 17 and 18 in HGP 29 at 5.3 and 5.4 hr PE.
d. Cumulative constant mass of VX (p17,18), all peaks = 63 and 23 µg, respectively (mean = 43 µg; SD = 28).
e. Cumulative constant mass of VX (p17,18), only peaks with VX = 39 and 1.5 µg, respectively (mean = 20 µg; SD = 26).
f. The quality of profiles 17 and 18 was not good. Skin surface very late (spectra 12 and 11).
g. In profile 17 (5.3 hr PE) spectra 1-6 have VX values below the instrument LOD, and no VX peaks were observed. Also, there were no VX peaks observed in spectra 16 and 17 (small bump at 45-60 µm skin depth).
h. In profile 18 (5.4 hr PE) spectra 1-6 have VX values below the instrument LOD, and no VX peaks were observed.

2. Comparison of Raman profiles 19 and 20 at 5.8 and 5.9 hr PE.

a. 

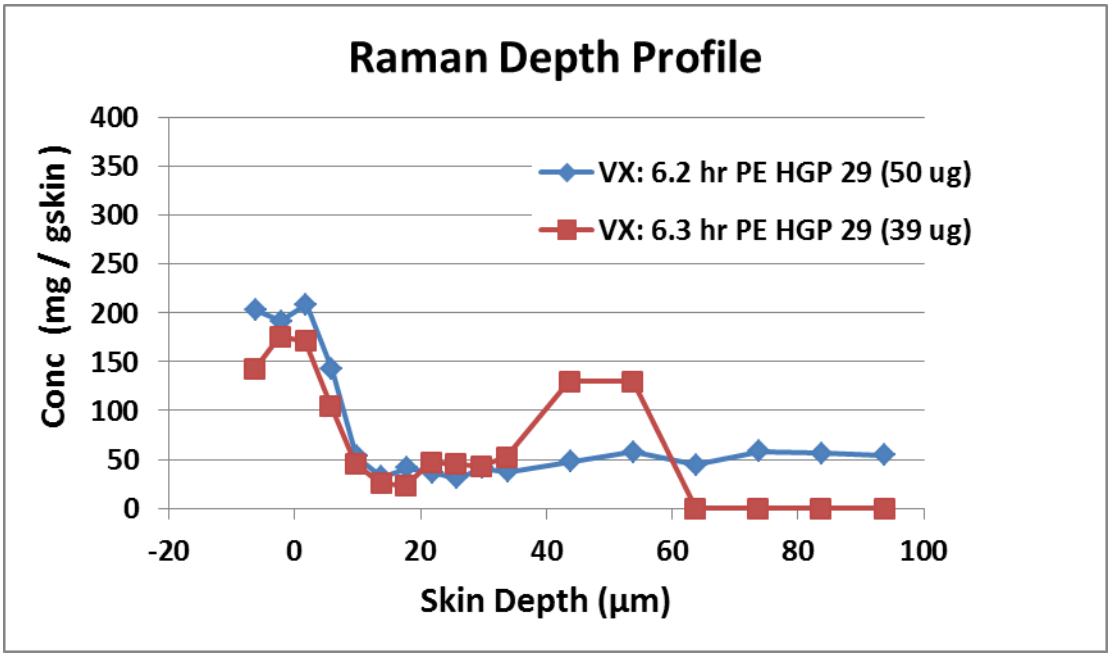
b. Figure 85. Raman depth profiles 19 and 20 in HGP 29 at 5.8 and 5.9 hr PE.

c. Collection repeated without moving animal.

d. Cumulative constant mass of VX (p19,20), all peaks = 25 and 23 µg, respectively (mean = 24 µg; SD = 1.4).

e. Cumulative constant mass of VX (p19,20), only peaks with VX = 8.5 and 8.9 µg, respectively (mean = 8.7 µg; SD = 0.28).

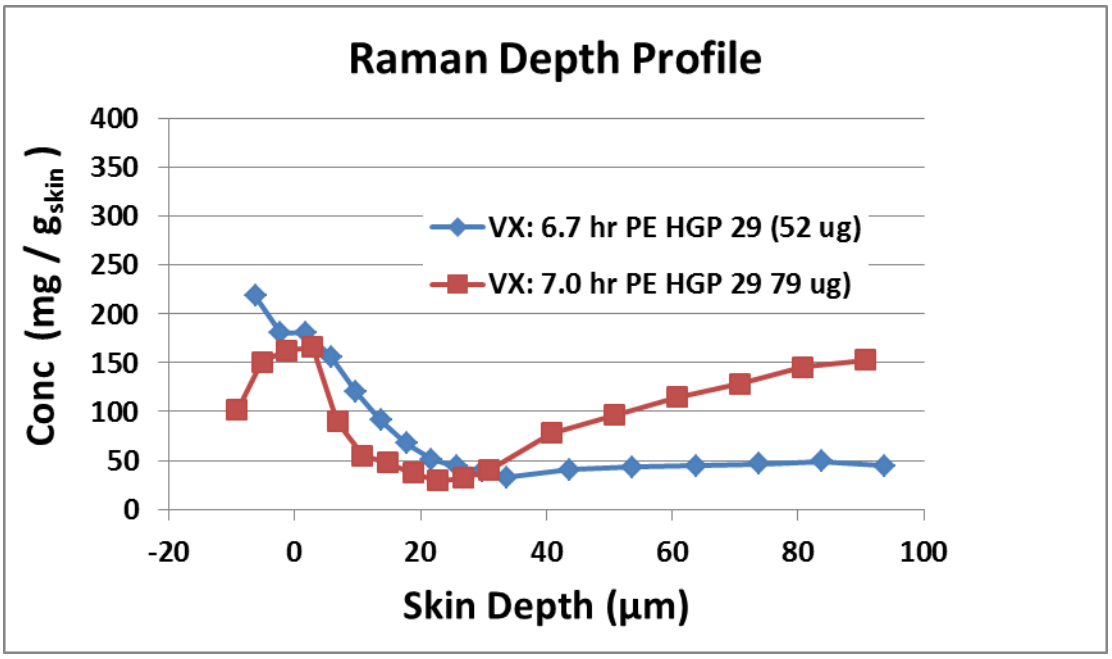
3. Comparison of Raman profiles 21 and 22 at 6.2 and 6.3 hr PE.

a. 

b. Figure 86. Raman depth profiles 21 and 22 for HGP 29 at 6.2 and 6.3 hr PE.
c. Collection repeated without moving animal.
d. Cumulative constant mass of VX (p21, 22), all peaks = 50 and 39 µg, respectively (mean = 44 µg; SD = 7.8).
e. Cumulative constant mass of VX (p21, 22), only peaks with VX = 13 and 11 µg, respectively (mean = 12 µg; SD = 1.4).
f. In profile 22 (6.3 hr PE) spectra 13-17 were very poor, and no useful data can be obtained from them.

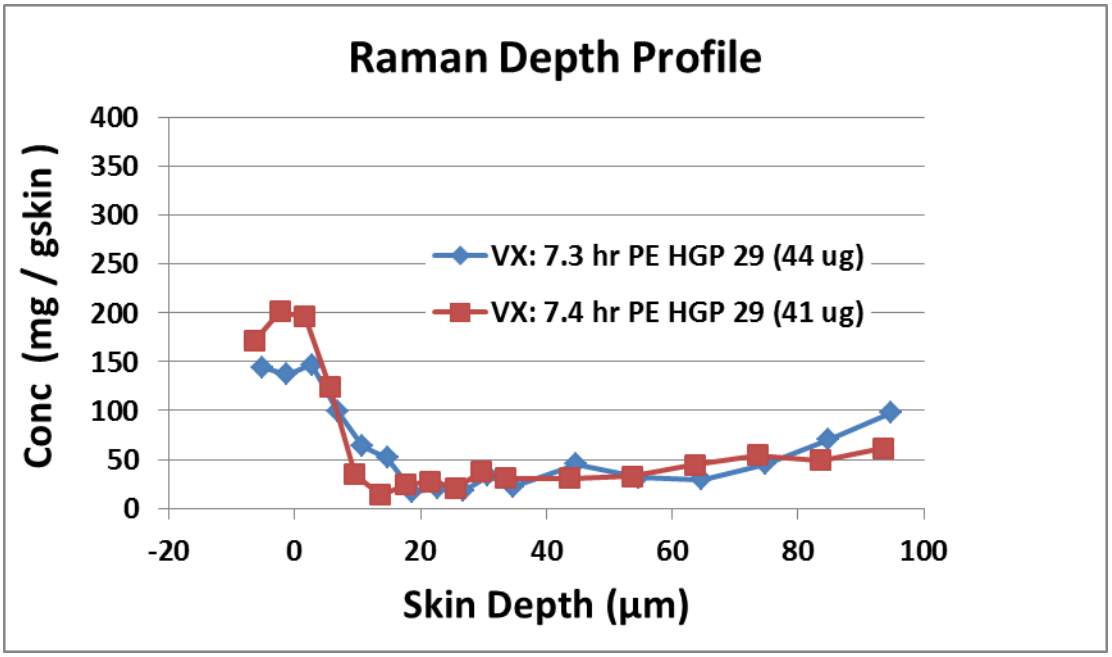
4. Comparison of Raman profiles 23 and 25 at 6.2 and 6.3 hr PE.

a.



b. Figure 87. Raman depth profiles 23 and 25 in HGP 29 at 6.7 and 7.0 hr PE.
c. Collection repeated after moving animal.
d. Cumulative constant mass of VX (p23, 25), all peaks = 52 and 79 µg, respectively (mean = 66 µg; SD = 19).
e. Cumulative constant mass of VX (p23, 25), only peaks with VX = 21 and 14 µg, respectively (mean = 18 µg; SD = 4.9).
f. The quality of spectra 12-17 in Profile 25 (7.0 hr PE) was very poor, and no useful data could be obtained from them. The VX values in these spectra were below the instrument LOD, and no VX peaks were observed.
g. Profiles 24 and 26 were aborted because of lost skin contact with the Raman window. While recording profile 26, the animal stopped breathing and was observed to be dead.

5. Comparison of Raman profiles 27 and 28 at 7.3 and 7.4 hr PE

a. 

b. Figure 88. Raman depth profiles 27 and 28 in HGP 29 at 7.3 and 7.4 hr PE.

c. Animals are dead before recording profiles.

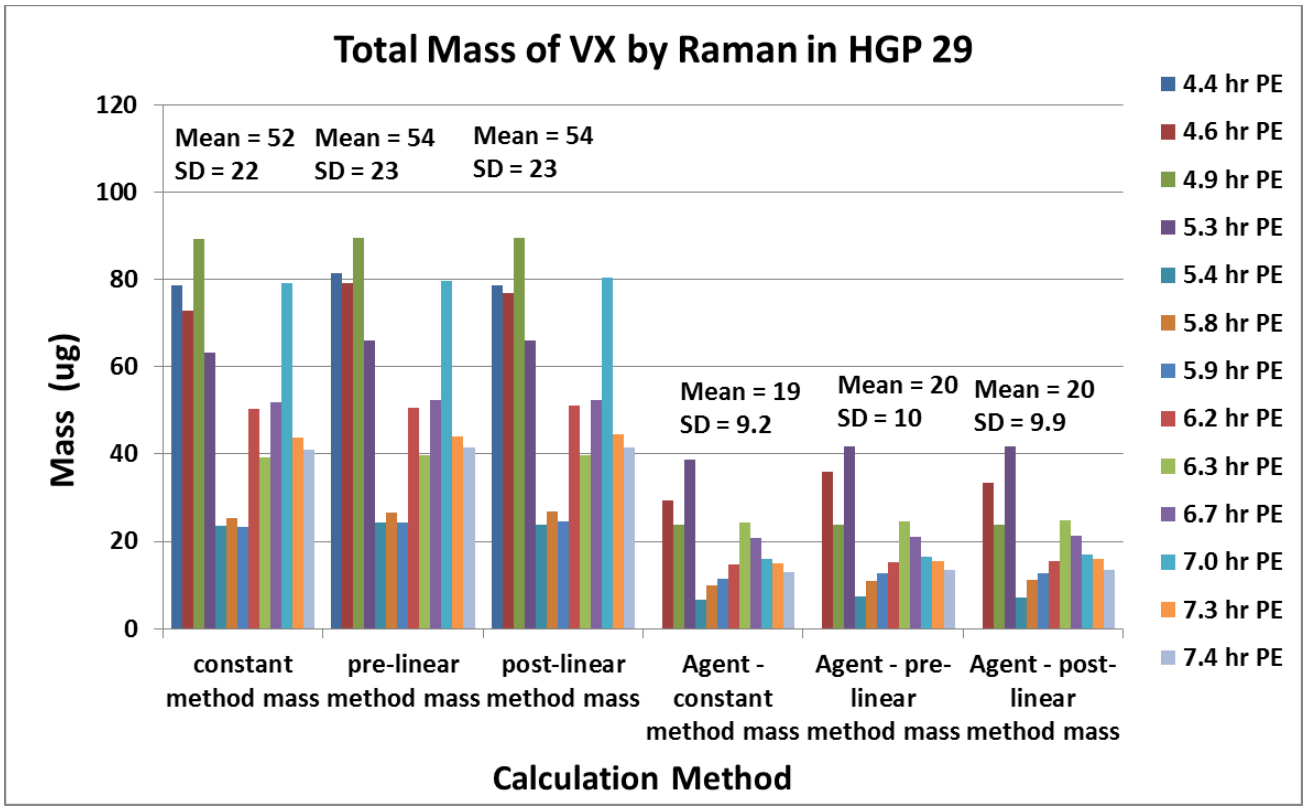
d. Collection repeated without moving animal.

e. Cumulative constant mass of VX (p27, 28), all peaks = 44 and 41 µg, respectively (mean = 42 µg; SD = 2.1).

f. Cumulative constant mass of VX (p27, 28), only peaks with VX = 7.8 and 11µg, respectively (mean = 9.4 µg; SD = 2.3).

6. Total mass of VX by Raman estimates.

a.



b. Figure 89. Total mass of VX by Raman in HGP 29. Left side of figure estimated the total mass of VX using all spectra in profile. Right side of figure estimated the total mass of VX using only spectra that contained an observable VX peak at 730 cm-1. Constant, pre-linear, and post-linear were three different methods used to estimate the total mass of VX.

c. Total mass of VX applied = 284 µg (0.30 µl of neat agent).

d. Total mass of VX (free and bound) from analysis of exposure skin punches = 42.8 µg.

e. Total mass of free VX estimated from analysis of skin punches = 21.4 µg.

f. Mean total mass of VX by Raman (all peaks) = 53.3 µg (SD = 1.2).

g. Mean total mass of VX observed in Raman blank profiles = 31.7 µg (SD = 16.5).

h. Mean mass of VX estimated by Raman using all spectra: (mean VX profiles – mean blank profiles) = 21.6 µg.

i. Mean total mass of VX by Raman (only VX peaks) = 19.7 µg (SD = 0.58).

7. VX amounts measured by ECBC for skin punches for HGP 29.
   a. Control skin blank: total VX = 44.3 ng (free VX = 22.2 ng).
   b. Exposure skin site: total VX = 42.8 µg (free VX = 21.4 µg).

8. Summary of Total Mass of VX in Skin Experiments with HGPs 27, 28, and 29.
   a.

HGP #	Skin Punch Analysis Free VX µg	Raman All peaks with Blank Subtraction VX µg	Raman VX only peaks VX µg	Mass VX % Difference Analysis vs. Raman all peaks	Mass VX % Difference Analysis vs. Raman VX only Peaks
27	21.3	23	9.3	+ 8.0 %	- 56 %
28	26.4	17	8.1	- 36 %	- 69 %
29	21.4	21	20	- 2.0 %	- 4.8 %

Table 2. Comparison of VX total mass in skin by chemical analysis vs. Raman spectroscopy in HGPs 27, 28, and 29.

b. The skin punch analysis VX mass values were estimated from the total VX (bound and free) observed in the ECBC fluoride regeneration method. This estimate was based on the ECBC validation experiments that showed for the fluoride regeneration method that free VX represents about 50% of the total VX calculated in the analysis.
c. The Raman blank subtraction method used all Raman spectra that were recorded in the skin during the depth profile measurements. The mass value was the mean of the three estimation methods (constant, pre-linear, and post-linear). The blank VX value subtracted was the mean VX mass calculated from all the control blank depth profiles that were recorded for each animal. The Raman VX only peak method used only the spectra in which visual observation confirmed the presence of VX peaks in each spectrum. There was no blank subtraction.

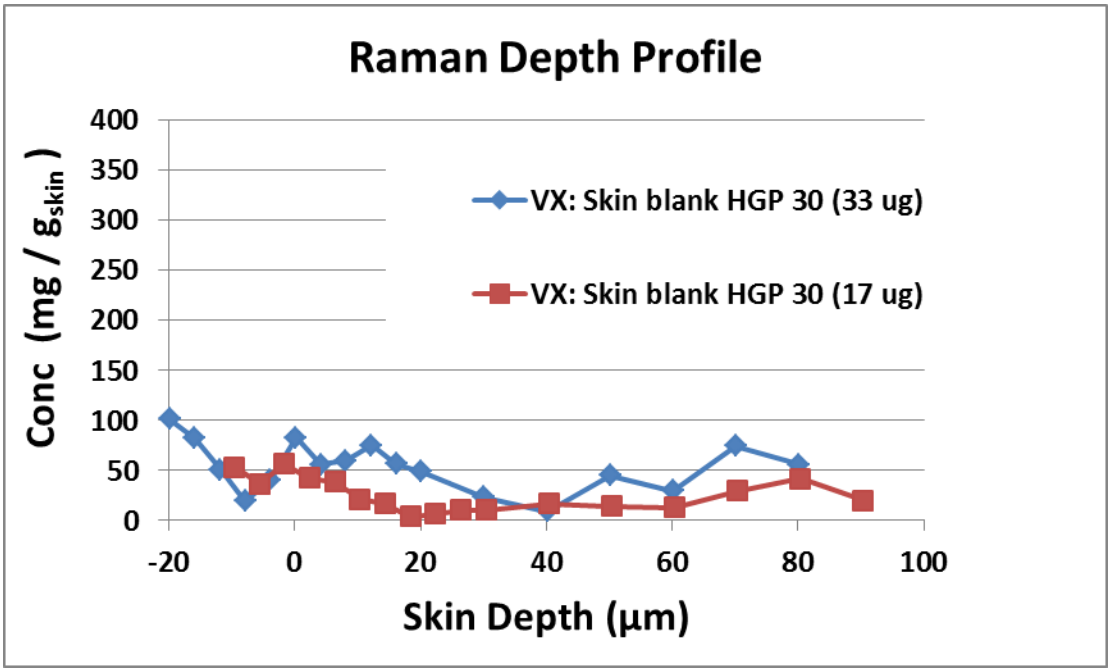
HGP 30 Exposure on 6 March 2012: RSDL model development

1. This animal was used to develop a procedure for removing the dosing template prior to RSDL decontamination, performing standard RSDL decontamination, and replacing a new dosing template for Raman analysis.
   a. HGP 30 did not get HuBuChE protective enzyme because the catheter was not patent. Thus, the animal was used for a different purpose than planned.
   b. Although most of the Raman depth profiles demonstrated that VX peaks were not observed following RSDL decontamination, a few depth profiles did show traces of VX. A possible explanation for this is the observation that VX migrated under the edges of the Teflon disc in the dosing template around the borders of the 3 mm hole. The VX under the Teflon disc would

be missed using our RSDL decontamination technique that wipes the top surface of the template including the 3 mm hole.

c. This animal was used to develop a procedure that allowed the RSDL pad to directly wipe the skin around the dosing area and to place a new dosing pad onto the skin in the exact position of the original template. To achieve the repositioning of the dosing template after decontamination, we marked the hole position with a pen, performed the standard decontamination (10 wipes, 10 additional min holding a new RSDL pad, and dry wiping with gauze), and placed the new template on the skin. We noticed that the original dosing template left an impression of the hole on the skin when it was removed. This impression lasted throughout the decontamination process. Marking the hole position on the skin with a pen was probably not necessary for accurate replacement.

2. (p5,6) skin blank before VX exposure (2 replicates) Template T5 (2 stages, 17 spectra).

a. 

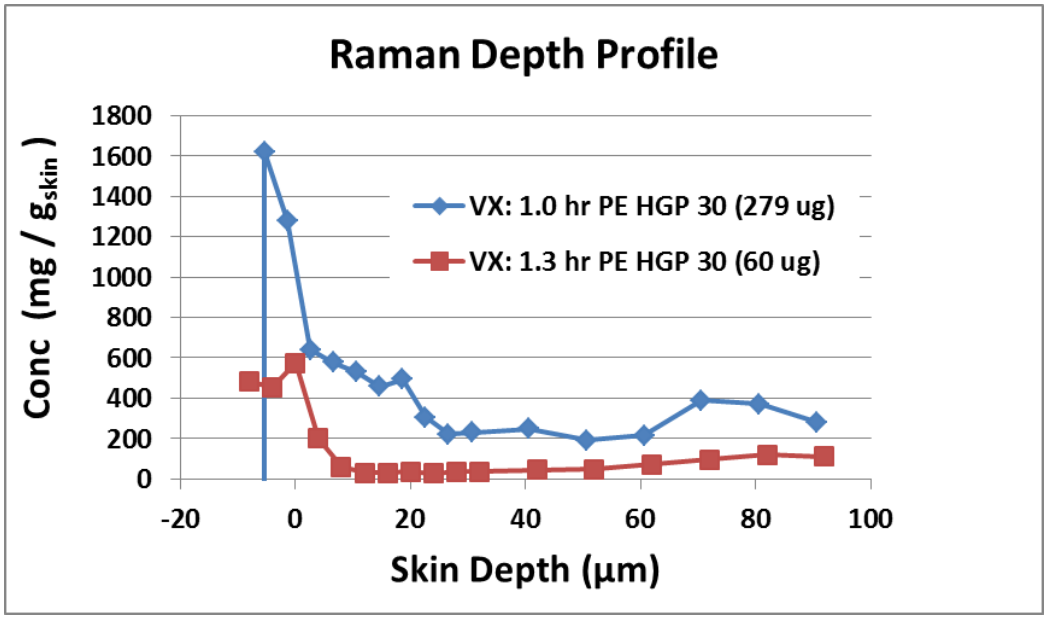
b. Figure 90. Raman depth profiles in HGP 30 before VX exposure.

c. Cumulative constant mass of VX (p5,6), all peaks = 33 and 17 µg, respectively (mean = 25 µg; SD = 11).

d. Cumulative constant mass of VX (p5,6), only peaks with VX = 0,0, and 0 µg, respectively (mean = 0 µg; SD = 0). Normal skin blank profiles.

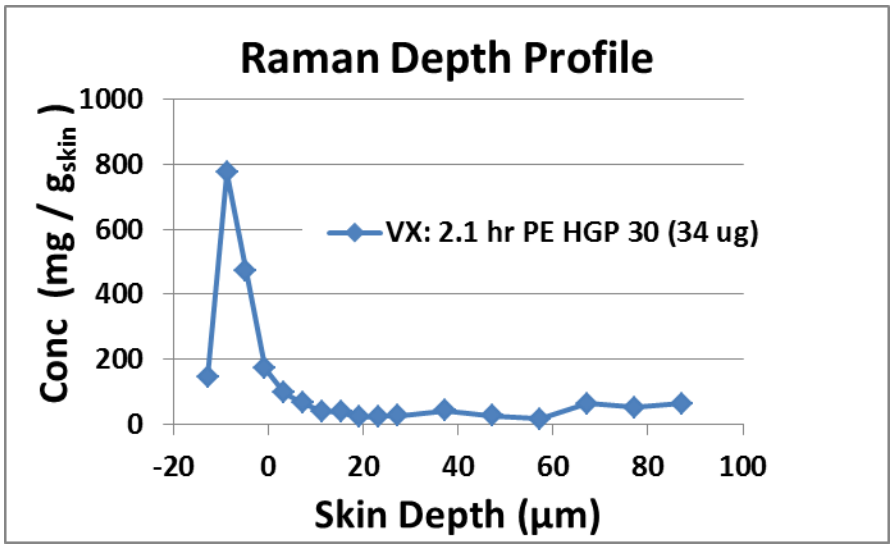
e. No VX peaks observed in spectra.

3. (p7,8) 1.0 and 1.3 hr PE Template T5

a. 

b. Figure 91. Raman depth profiles 7 and 8 in HGP 30 at 1.0 and 1.3 hr PE.
c. Animals repositioned between profiles.
d. Cumulative constant mass of VX (p7,8), all peaks = 279 and 60 µg, respectively (mean = 170 µg; SD = 155).
e. Cumulative constant mass of VX (p7,8), only peaks with VX = 168 and 17 µg, respectively (mean = 92.5 µg; SD = 107).
f. P7 at 1.0 hr: very large VX peaks in spectra 3-13.
g. After profile 7, the exposure site was wiped with a 2 X 2 inch gauze to remove VX from the skin surface because of the very large VX peaks observed.
h. P8 at 1.3 hr PE: VX peaks in spectra 2-5 and 14.
i. During profile 8 the animal showed signs of nerve agent toxicity with shallow breathing.
j. After profile 8 the dosing template was coming loose, so it was removed and replaced with a new template in the exact position of the original template.
k. HGP 30 died at 2.0 hr PE.

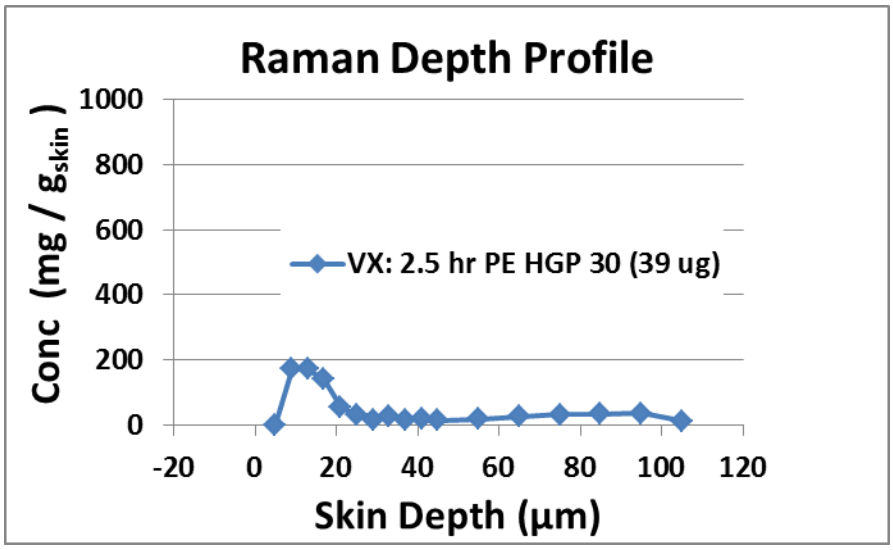
4. (p11) 2.1 hr PE Template T5.
   a. Animal was dead.

b. 

c. Figure 92. Raman depth profile 11 in HGP 30 at 2.1 hr PE.
d. VX peaks were observed in spectra 4 and 5.
e. Cumulative constant mass of VX (p11), all peaks = 34 µg.
f. Cumulative constant mass of VX (p11), only peaks with VX = 2.5 µg.

5. New RSDL decontamination procedure with removing and replacing dosing template.

6. (p12) 2.5 hr PE; Mechanical depth; Template T5.

a. 

b. Figure 93. Raman depth profile 12 in HGP 30 at 2.5 hr PE and after RSDL decontamination.
c. No VX peaks observed in any spectra.
d. Skin surface estimated at spectrum 3 (about 12 µm on X-axis).
e. Cumulative constant mass of VX (p12), all peaks = 39 µg.
f. Cumulative constant mass of VX (p12), only peaks with VX = 0 µg.

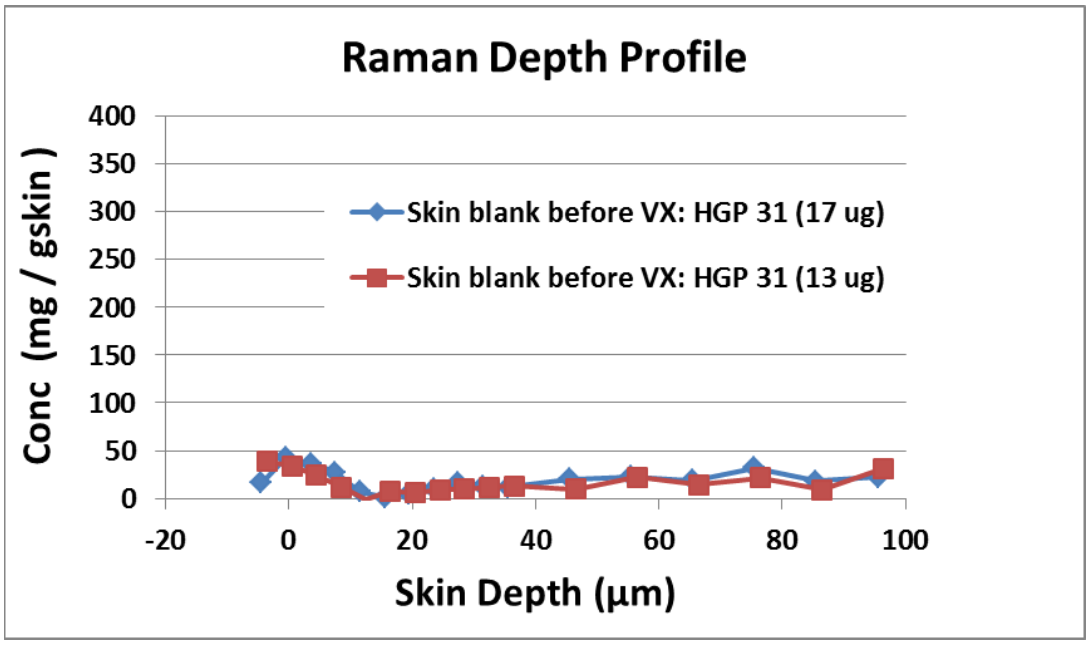
7. ECBC analysis.
    a. Blank negative control skin: 275 ng free un-bound VX.
    b. Exposure site: 17 µg free un-bound VX.
    c. ECBC found 17 µg in the exposure site sample after RSDL decontamination. No VX peaks were observed by Raman in the upper SC layers. A possible explanation is that the VX that normally had penetrated deeper into the skin had not distributed into circulating blood because the animal was already dead.

HGP 31 Exposure on 15 May 2012: Mass Balance Experiment with RSDL Decontamination

1. HGP 31 was not catheterized, and no HuBuChE was given. We tried to use this animal for a mass balance experiment with RSDL. Unfortunately the animal died about 80 min PE while we were taking the Raman profiles prior to RSDL decontamination. We continued the experiment anyway on the dead animal.

2. Procedure summary.
    a. Recorded skin blank Raman profiles before exposure.
    b. Applied 0.30 µl VX using standard dosing template.
    c. About 1 hr PE wiped dosing site with 3 Q-tips (saved for analysis).
    d. Recorded Raman depth profiles (at least 4 replicates) at different sites.
    e. Wiped Raman window (saved wipes for analysis).
    f. Marked hole and removed dosing template (saved for analysis).
    g. The exposure area was decontaminated with RSDL: 10 wipes (1/4 pad), wait 10 min, wipe site with at least 3 dry gauze pads (saved all wipes for analysis).
    h. Applied new dosing template.
    i. Recorded Raman profiles.
    j. Wiped Raman window (saved wipes for analysis).
    k. Euthanized animal and collected 8 mm skin punches for analysis.

3. (p1,2) skin blank before VX exposure (2 replicates); Template T5 (2 stages, 17 spectra).



a.

b. Figure 94. Raman depth profiles in HGP 31 before VX exposure.

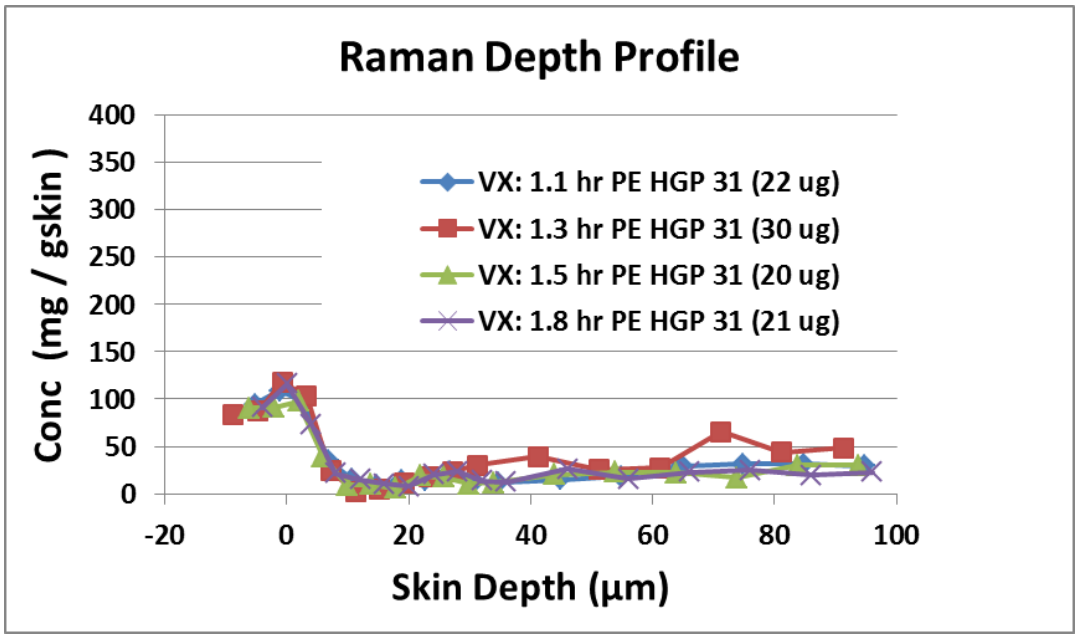
c. Normal skin blank profiles.

d. No VX peaks observed in spectra.

e. Cumulative constant mass of VX (p1,2), all peaks = 17 and 13 µg, respectively (mean = 15 µg; SD = 2.8).

f. Cumulative constant mass of VX (p1,2), only peaks with VX = 0 and 0 µg, respectively (mean = 0 µg; SD = 0).

4. (p3,4,5,6) 1.1, 1.3, 1.5, and 1.8 hr PE; Template T5.

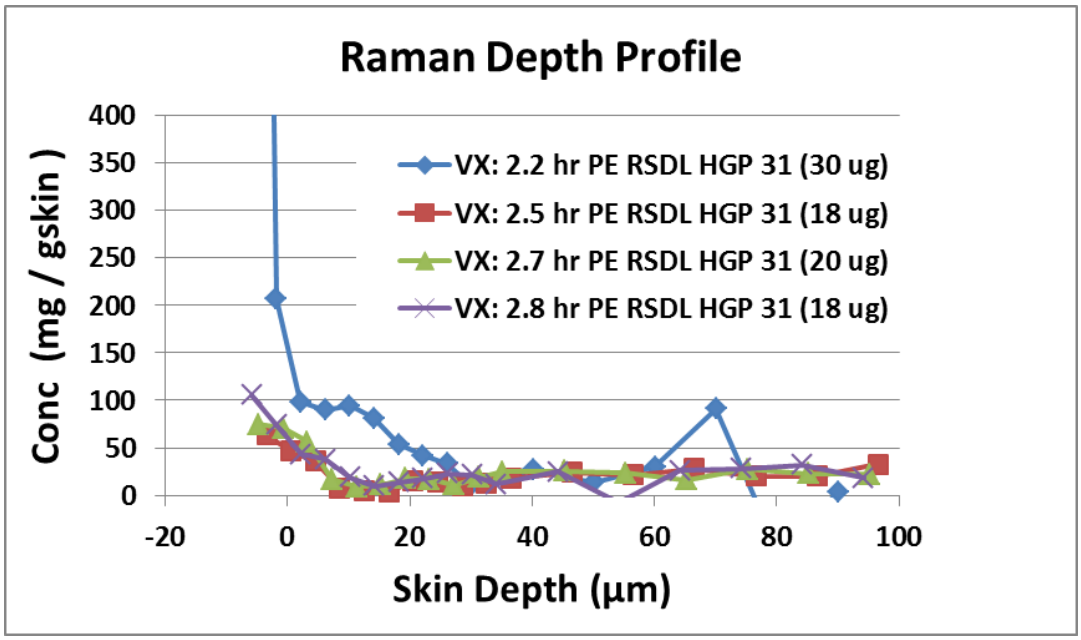


a.

b. Figure 95. Raman depth profiles (p3,4,5,6), in HGP 31 after VX exposure.

c. Cumulative constant mass of VX (p3,4,5,6), all peaks = 22, 30, 20,and 21 µg, respectively (mean = 15 µg; SD = 2.8).
d. Cumulative constant mass of VX (p3,4,5,6), only peaks with VX = 4.7,2.7,1.5, and 3.5 µg, respectively (mean = 3.1 µg; SD = 1.3).

5. (p7,8,9,10) 2.2, 2.5, 2.7, and 2.8 hr PE; After RSDL decontamination; Template T5

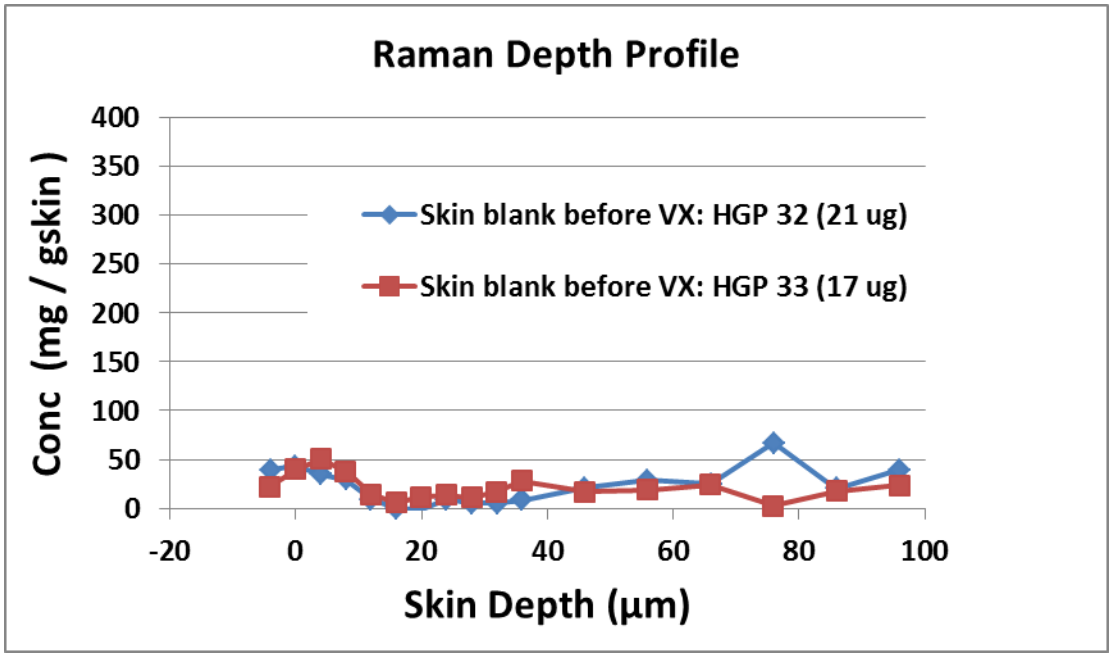
a. 

b. Figure 96. Raman depth profiles (p=7,8,9,10) in HGP 31 after RSDL, decontamination at 2.2, 2.5, 2.7 and 2.8 hr PE.
c. No VX peaks were observed in any spectra.
d. Cumulative constant mass of VX (p7,8,9,10), all peaks = 30, 18, 20, 18 µg, respectively (mean = 22 µg, SD = 5.7).
e. Cumulative constant mass of VX (p7,8,9,10), only peaks with VX = 0,0,0,0 µg (mean = 0 µg).
f. The high VX values at the beginning of profile 7 (2.2 hr PE) were below the instrument LOD and did not represent VX.
g. Total mass of VX in skin estimated from Raman profiles (all peaks – blank) after RSDL decontamination was (22-15) 7 µg.
h. Analysis of exposure site skin punch collected after euthanasia and RSDL decontamination estimated 2.8 µg free un-bound VX.
i. Analysis of non-exposed skin punch collected after euthanasia and RSDL decontamination estimated 0.72 µg free un-bound VX.

HGP 32 Exposure on 15 May 2012: Mass Balance Experiment with RSDL Decontamination

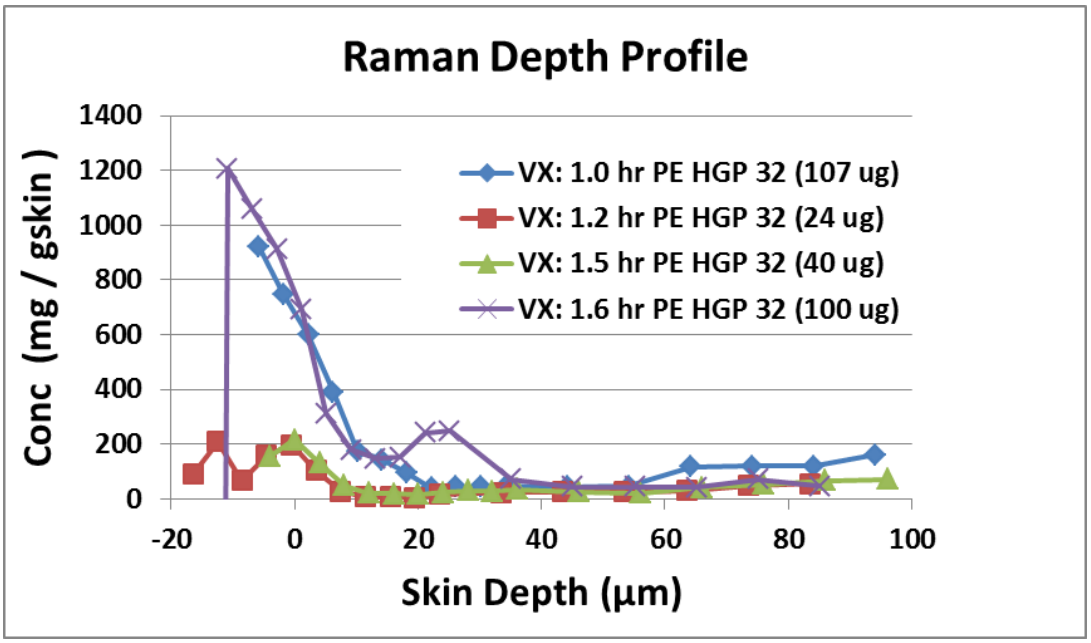
1. HGP 32 was a repeat of the experiment with HGP 31. HGP 32 was not catheterized, and no HuBuChE was given. Unfortunately it also died about 86 min PE while we were taking the Raman profiles prior to RSDL decontamination. We continued the experiment anyway on the dead animal. We followed the same procedure used with HGP 31, except we were not able to obtain usable Raman depth profiles after RSDL decontamination.

2. (p11,12) skin blank before VX exposure (2 replicates); Template T5 (2 stages, 17 spectra).



a.
b. Figure 97. Raman depth profiles in HGP 32 before VX exposure.
c. Normal skin blank profiles.
d. No VX peaks observed in spectra.
e. Cumulative constant mass of VX (p11,12), all peaks = 21 and 17 µg, respectively (mean = 19 µg; SD = 2.8).
f. Cumulative constant mass of VX (p11,12), only peaks with VX = 0 and 0 µg, respectively (mean = 0 µg; SD = 0).

3. (p13,14,15,16) 1.0, 1.2, 1.5, and 1.6 hr PE; Template T5.

a. 

b. Figure 98. Raman depth profiles (p13,14,15,16) in HGP 32 after VX exposure.
c. Cumulative constant mass of VX (p13,14,15,16), all peaks = 107, 24, 40, and 100 µg. (mean = 68 µg SD = 42).
d. Cumulative constant mass of VX (p13,14,15,16), only peaks with VX = 45, 0, 6.6, and 76 µg (mean = 32 SD = 35).
e. The animal died between profiles 14 and 15.
f. In profile 16 at 1.6 hr PE, spectrum 1 was poor quality, demonstrated no peaks, and was off the negative scale.

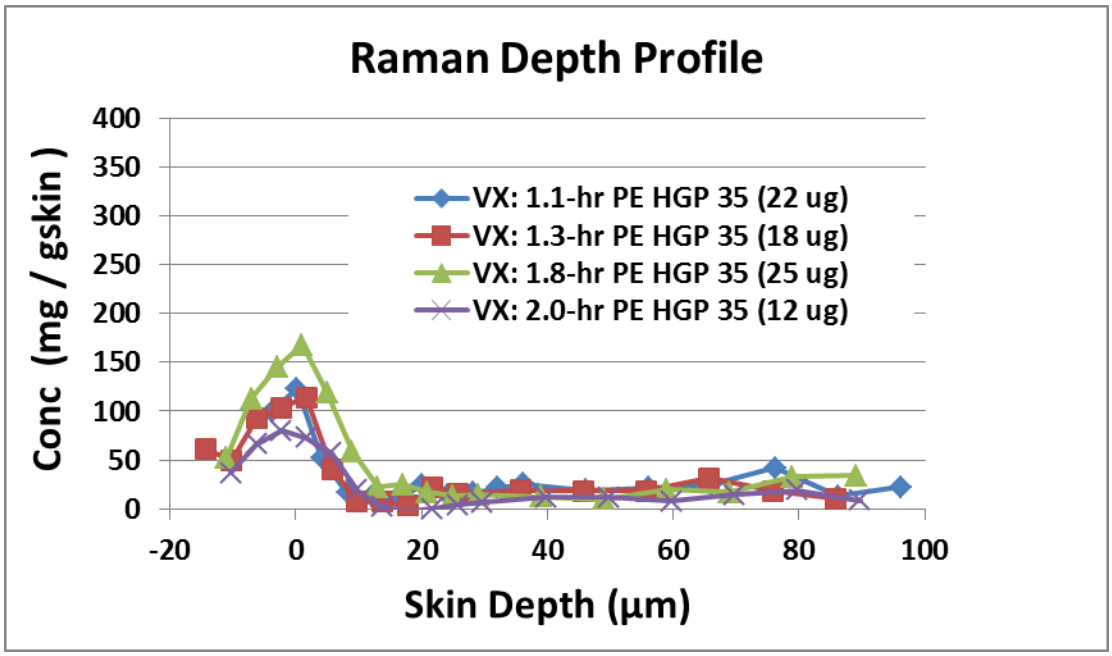
4. We could not obtain usable Raman profiles after RSDL decontamination. Skin punches and wipes were collected for ECBC to analyze.
    a. Analysis of exposure site skin punch collected after euthanasia and RSDL decontamination estimated 11 µg free un-bound VX.
    b. Analysis of non-exposed skin punch collected after euthanasia and RSDL decontamination estimated 0.039 µg free un-bound VX.

HGP 35 Exposure on 14 August 2012: Mass Balance Experiment with RSDL Decontamination

1. Procedure summary (same procedure used with HGPs 31 and 32 on 15 May 2012 except we did not remove the dosing template before RSDL decontamination).
    a. The catheter on HGP 35 was not initially patent, so we exposed HGP 36 first.

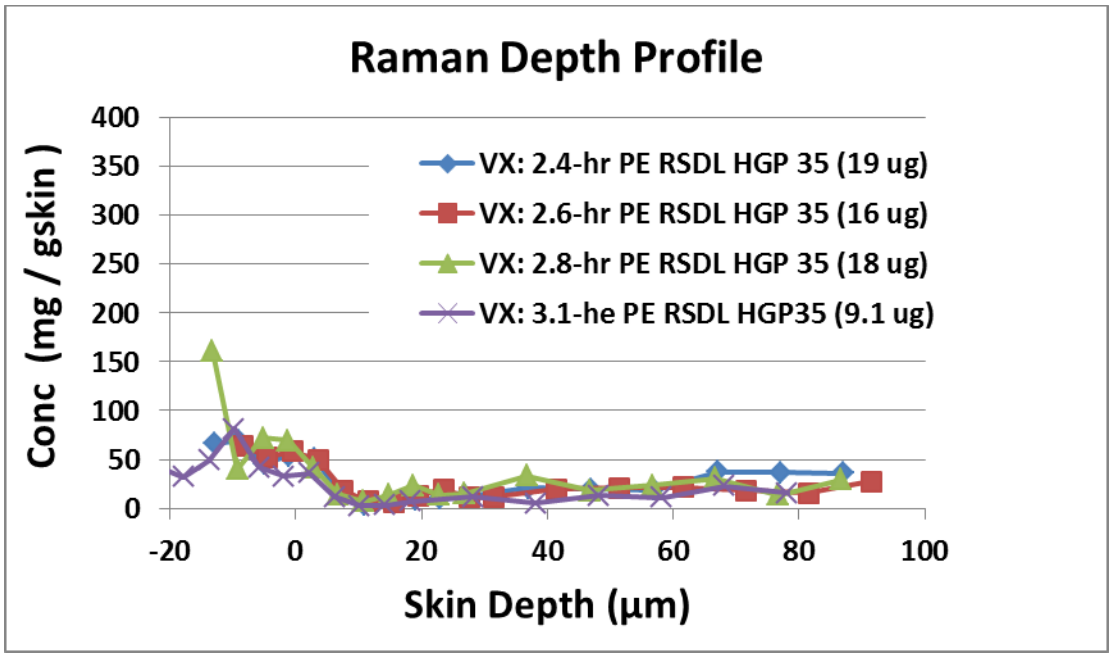
b. We finally were able to inject bioscavenger with help from the on-call veterinarian.

2. Raman depth profiles were not recorded for control skin blanks on this animal because we needed time to complete VX dosing before the end-of-day cutoff for agent operations.

3. (p10,11,13,14) 1.1, 1.3, 1.8, and 2.0 hr PE; Template T5.
   a. Observed VX peaks in spectra from all four Raman profiles after VX exposure.
   b. 

   c. Figure 99. Raman depth profiles (p10,11,13,14) for HGP 35 after VX exposure.
   d. Cumulative constant mass of VX (p10,11,13,14), all peaks = 22, 18, 25, and 12 µg (mean = 19 µg SD = 5.6; n=4).
   e. Cumulative constant mass of VX (p10,11,13,14), only peaks with VX = 3.4, 4.4, 11, and 3.4 µg (mean = 5.6 SD = 3.7; n=4).
   f. Large VX peaks observed in spectra for all four profiles.

4. (p15,16,17,18) 2.4, 2.6, 2.8, and 3.1 hr PE; after RSDL decontamination; Template T5.

a. 

b. Figure 100. Raman depth profiles (p=15,16,17,18) after RSDL decontamination for HGP 35 at 2.4, 2.6, 2.8 and 3.1 hr PE.

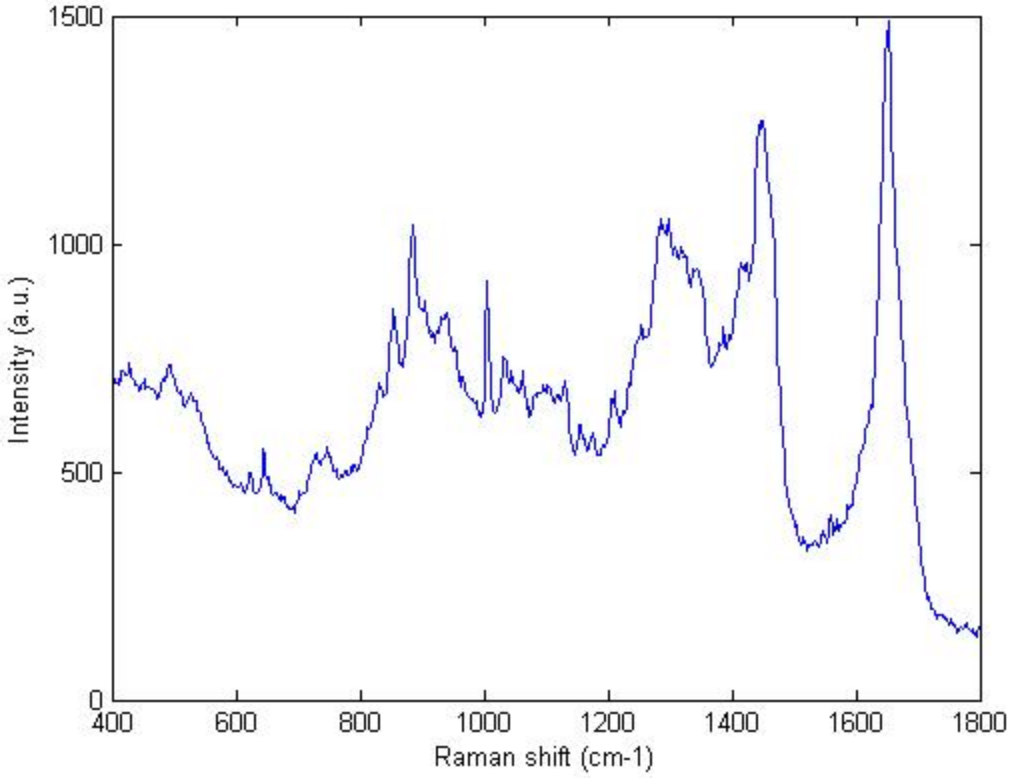
c. Cumulative constant mass of VX (p15,16,17,18), all peaks = 19,16,18, and 9.1 µg. (mean = 16 µg SD = 4.5).

d. Cumulative constant mass of VX (p15,16,17,18), only peaks with VX = 0,1.4,0,and 0-µg (mean = 0.35 µg; SD = 0.7; n=4).

e. Total mass of VX in skin estimated from Raman profiles (all peaks blank) after RSDL decontamination could not be calculated because there were no blank profile values, but the VX determined without blank subtraction was 16 µg, which was typical for blank profiles.

f. Analysis of exposure site skin punch collected after euthanasia and RSDL decontamination estimated 9.4 µg free un-bound VX.

g. Analysis of non-exposed skin punch collected after euthanasia and RSDL decontamination estimated 0.002 µg free un-bound VX.



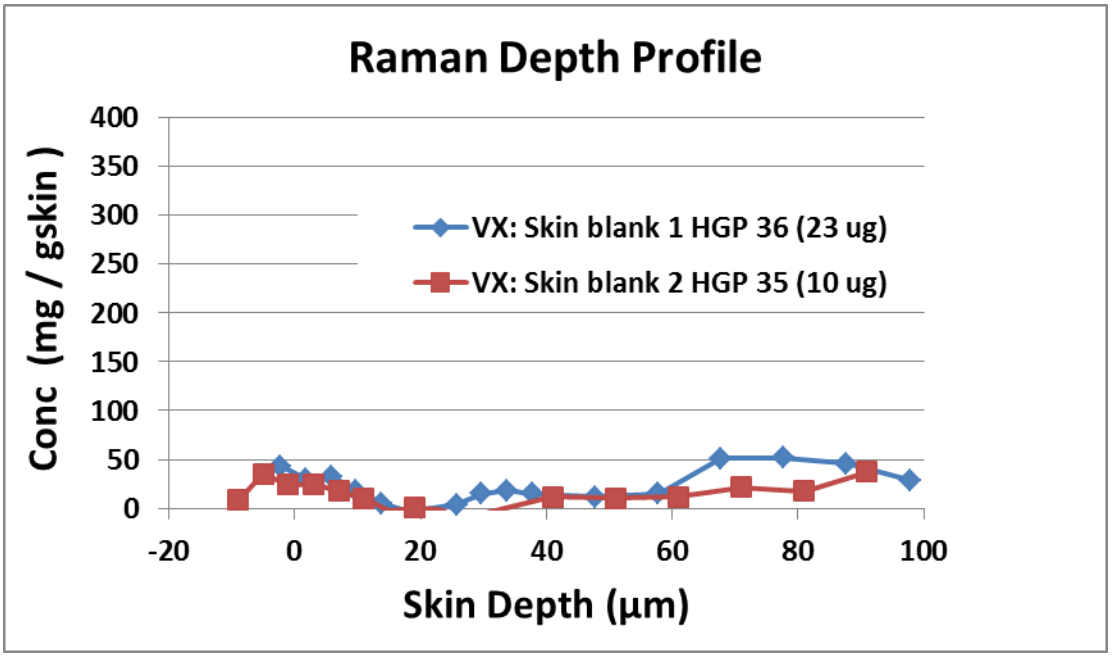
h. Figure 101. Raman spectrum 4 for profile 16 in HGP 35 (VX peak at 730 cm^{-1}) about 4 µm deep in skin.
i. In the 4 Raman profiles recorded, observed only one very small VX peak in spectrum 4 in profile 16 at 2.6 hr PE.

HGP 36 Exposure on 14 August 2012: Mass Balance Experiment with RSDL Decontamination:

1. Same experiment as HGP 35, except no trouble with patency and skin blank Raman profiles were recorded.

2. (p1,2) skin blank before VX exposure (2 replicates); Template T5.
   a. Normal skin blank profiles.
   b. No VX peaks observed in spectra.

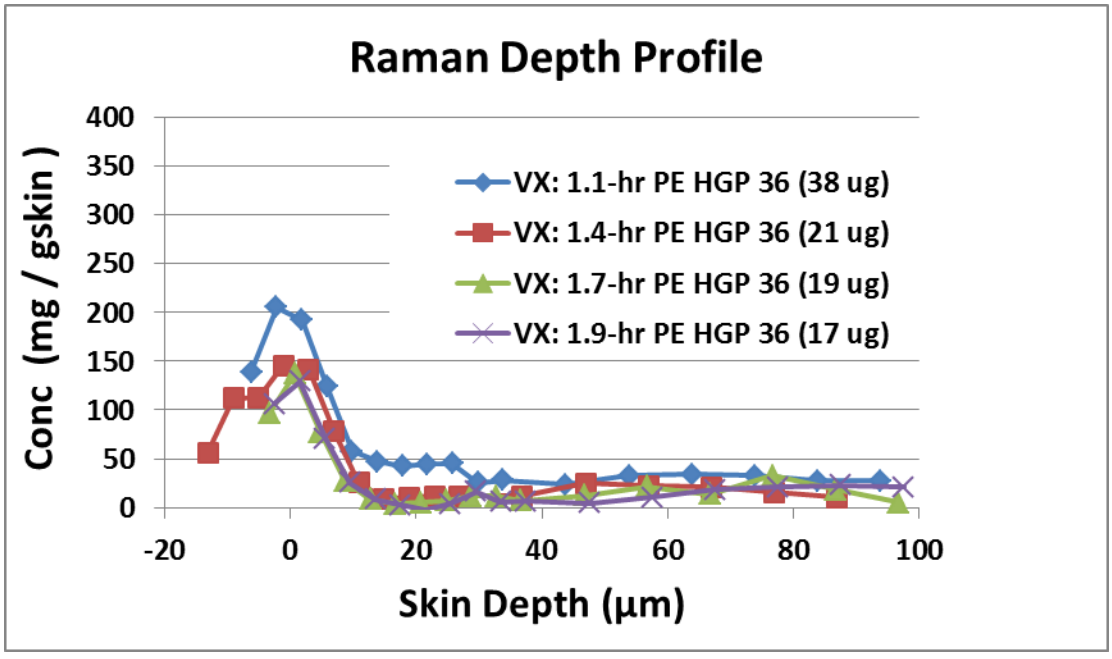
c.



d. Figure 102. Raman depth profiles for HGP 36 before VX exposure.
e. Normal skin blank profiles.
f. No VX peaks observed in spectra.
g. Cumulative constant mass of VX (p1,2), all peaks = 23 and 10 µg (mean = 16 µg; SD = 9.2).
h. Cumulative constant mass of VX (p1,2), only peaks with VX = 0 and 0 µg (mean = 0).

3. (p3,4,5, and 6): 1.1, 1.4, 1.7, and 1.9 hr PE; Template T5.

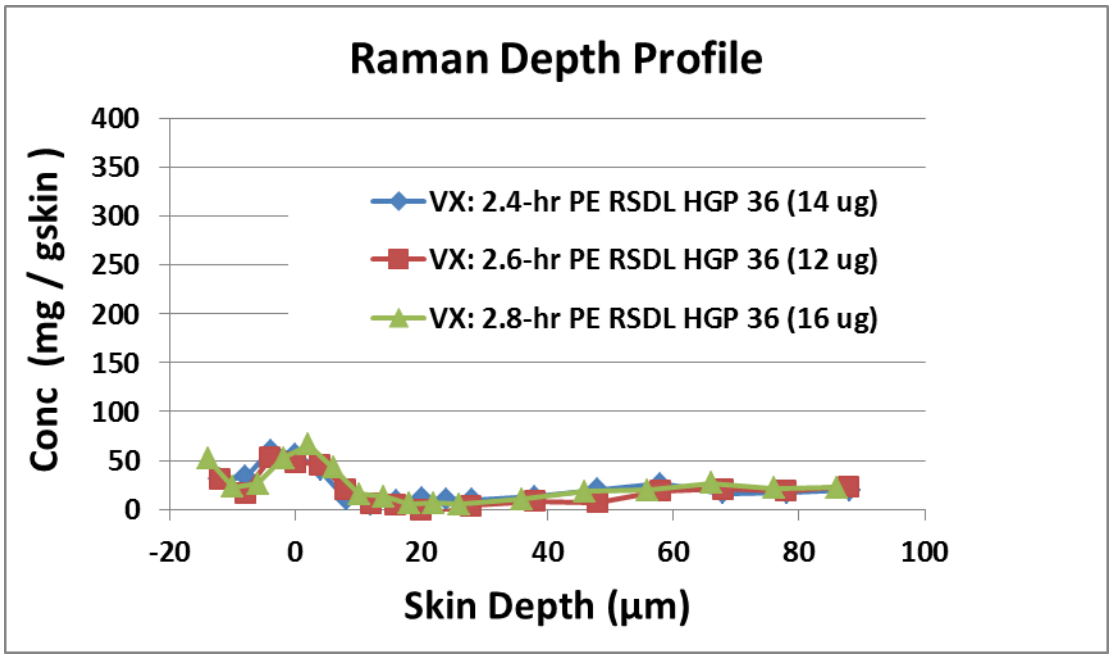
a.



b. Figure 103. Raman depth profiles (p3,4,5,6) for HGP 36 after VX exposure.
c. Observed VX peaks in spectra from all 4 Raman profiles after VX exposure.
d. Cumulative constant mass of VX (p3,4,5,6), all peaks = 38, 21, 19, and 17 µg. (mean = 24 µg; SD = 9.6).
e. Cumulative constant mass of VX (p3,4,5,6), only peaks with VX = 8.6, 7.4, 4.7, and 7.0 µg (mean = 6.9 µg; SD = 1.6).

4. (p 7,8,and 9) 2.4, 2.6, and 2.8 hr PE; after RSDL decontamination; Template T5.

a.



b. Figure 104. Raman depth profiles (p=7,8,9) after RSDL decontamination for HGP 36 at 2.4, 2.6, 2.8 hr PE.
c. No VX peaks observed in any spectra after RSDL decontamination.
d. Cumulative constant mass of VX (p7,8,and 9), all peaks = 14, 12, and 16 µg. (mean = 14 µg; SD = 2.0).
e. Cumulative constant mass of VX (p7,8,and 9), only peaks with VX = 0, 0, and 0 µg (mean = 0 µg).
f. Total mass of VX in skin estimated from Raman profiles (all peaks blank) after RSDL decontamination was (14-16) a negative value.
g. Analysis of exposure site skin punch collected after euthanasia and RSDL decontamination estimated 6.8 µg free un-bound VX.
h. Analysis of non-exposed skin punch collected after euthanasia and RSDL decontamination estimated 0.00023 µg free un-bound VX.

Mass balance Summary

SAMPLE	HGP 31 VX µg	HGP 32 VX µg	HGP 35 VX µg	HGP 36 VX µg
Swab 1	13.8	18.7	13.2	1.8
Swab 1	4.2	2.1	3.1	0.62
Swab 1	2.6	1.6	0.75	0.40
Template 1	9.3	62.4	0.34	0.52
RSDL pad 1	0.10	0.1	0.415	0.0097
RSDL pad 2	none	none	0.030	0.050
Template 2	4.3	3.8	none	none
Gauze	1.0	0.4	none	none
Skin Punch PC	5.5	22.1	18.9	13.6
Skin Punch NC	1.4	0.078	0.0032	0.00046
Totals	42.2	111	17.8	17.0

Table 3. Mass balance summary for HGPs 31, 32, 35, and 36.

DISCUSSION

Model Development Experiments

The use of clipped haired guinea pigs was initially planned in this project. Using training animals from the Veterinary Medicine and Surgery Branch (VMSB) of the USAMRICD, we prepared several skin areas which were close-clipped with The Dander Free Clipper System (Hazard Technology, Millersville, MD 21108) using Oster Brand clippers (model: Golden A5) with a number 40 CryogenX blade. With this preparation, however, we were not able to establish good skin contact with the Raman recording window, and the recorded depth profiles were poor in quality. We believe this resulted from the short hair stubble that remained after clipping. With this observation, we switched from using haired to hairless guinea pigs.

Prior to VX exposure, several model development animals were used to determine a variety or experimental parameters such as the suitability of using non-anesthetized animals, the design of the exposure template, determining the minimum volume needed to cover the 3.2 mm diameter hole in the dosing template, methodology for using the isoflurane anesthesia system, instrumental parameters for recording Raman depth profiles, and determining the best skin location for VX exposure.

Our first approach was to use un-anesthetized animals because anesthesia may affect the toxicity and LD_{50} values of agents applied to the skin (E. Clarkson, USAMRICD, personal communication). Observations showed that anesthesia affects skin temperature and shunts blood away from the skin. This result may change the rate of VX penetration through the skin. Unfortunately, we were not able to obtain usable Raman spectra using un-anesthetized animals. Quality Raman depth profiles required continuous good skin contact with the instrument recording window for 3-5 minutes. Un-anesthetized animals would not remain still for this time; thus, we had to use anesthetized animals.

After VX exposure, several model development animals were used to determine the amount of bioscavenger necessary to protect animals against the 0.30 µl VX challenge and to identify the best RSDL decontamination method.

Semi-Quantitative Estimate of VX from Raman Depth Profiles

The output from the Raman depth profiles included concentration values from slices examined moving progressively deeper into the skin. From these concentration values the mass of the identified compound was estimated by using an “area under the curve” method of calculation (Figure 105). A precondition set for these calculations was that they only include the concentration values for which the Raman instrument indicated that the skin had been penetrated by the beam (represented by a positive

depth value). The mass calculations also included an assumption that agent distributed uniformly across the exposed area. A standard circular exposure site of diameter 3.2 mm was selected, and all subsequent volume calculations were based on the corresponding circular area being applied over the incremental depth difference between profiles (typically 4 or 10 µm). A further restriction was that incremental mass sums were not included whenever the agent concentration for a particular depth was reported to be negative, since negative concentrations were not possible. If a spectrum was not usable (poor quality or cosmic ray) the value for that point was estimated by using the average of the points just before and after the excluded spectrum.

Three mass calculation methods were employed when evaluating the Raman depth file data. The critical difference between each method was their approach to determining the initial point at which measurements represented VX measurement in skin. The initial point posed a challenge in terms of estimating the mass of VX because the precise slope of the profile line was unknown for the few microns immediately at the surface of the skin and above the level of the first scan into the skin. Each of the three methods treated all subsequent incremental skin measurements by calculating the mass based on a trapezoidal approach to area under the curve, averaging the concentration value at the specified depth with the preceding concentration value. Using each of the three methods, a total mass calculation for the full depth of the scan was obtained by summing the incremental masses.

The first method was called the “constant mass” method. With this method the first incremental skin measurement was estimated with the assumption that the agent was at a constant concentration to the depth of the first scan. The first increment thus assumed a rectangular shape. The next method was called the “pre-linear” method. With this method, the VX concentration in the first skin increment was estimated assuming the concentration of agent to be linear between the first scan into the skin and the final scan outside of the skin (nearest to the skin surface). The shape of the VX skin distribution in the first increment was assumed to be trapezoidal (unless the two concentration values were exactly equal), but the volume was determined using skin depth only. The third method was called the “post-linear” method. With this method, the slope of the first increment into the skin was assumed to be the same as the slope of the second increment into the skin. Thus, the concentration values of the first two scans into the skin were used to estimate the skin surface concentration, employing a trapezoidal distribution shape from the mass measured in the first skin increment.

Among these three methods the “pre-linear” assumed the most risk because it included the concentration value from a point outside of the skin, which may not be reliable. Much of the difference between calculation methods for each scan was related to the depth of the initial scan into the skin. When the first scan penetrated less than 1 µm into the skin, all three methods were expected to produce highly similar estimations. As the initial depth increased, however, up to the early incremental value of 4 microns, the differences between the methods were observed to grow. The least risk was probably assumed using the “constant mass” method as it assumed that skin surface agent amount never exceeded that found in the first skin scan.

The Raman Skin Analyzer software automatically calculated the agent concentration in depth profiles. These calculations were based on calibration curves using known concentrations of aqueous solutions of VX and bovine serum albumin (BSA). We observed, however, that the automatic software for depth profiles calculated small VX concentrations in normal control skin that was not exposed to VX. To estimate the total mass of VX in skin using Raman depth profile data, we assumed that it would be necessary to subtract out the VX mass determined on un-exposed blank skin. The mass calculation estimation procedure we used made several critical assumptions that may not always be true: 1) during dosing, VX was uniformly spread over the entire skin surface exposed by the 3.2 mm diameter hole in the dosing template, 2) VX distributed uniformly through the skin following dosing, and 3) VX did not distribute radially beyond the margins of the 3.2 mm diameter hole in the dosing template as it distributed axially through the skin.

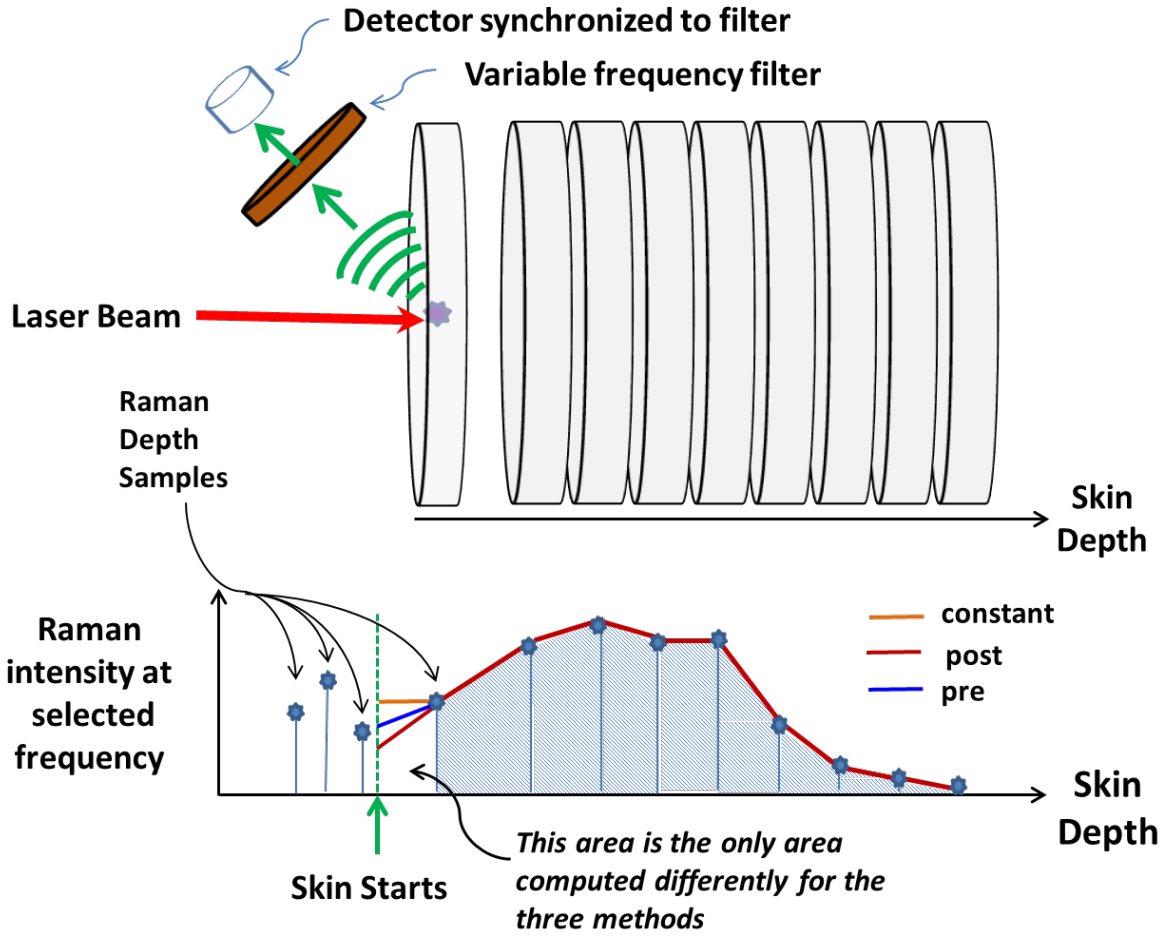


Figure 105. Mathematical modeling program to estimate the amount of VX in the skin.

When an animal was not moved between Raman depth profile collections (HGPs 27, 28, and 29) corresponding depth profile results were very similar. However, when

animals were repositioned between depth profile collections, different amounts of VX were measured in corresponding depth profiles. This is a direct consequence of the heterogeneity of the skin and the very small detection volume (about 3.9 μm^3) of the Raman sampling technique. Thus, to obtain reasonable estimates of the mass of VX (or other compounds being analyzed) in skin, multiple depth profiles were collected and averaged to normalize for skin heterogeneity.

In the experiments that evaluated the VX estimation model (HGPs 27, 28, and 29) there was no difference between the measured VX mass values across the three methods of calculation: constant, pre-linear, and post-linear (Figures 76, 82, and 89).

Another method that we evaluated to estimate the mass of VX in skin used only spectra that had visible VX peaks. This method, however, gave VX mass values that were generally much lower than the analysis values from skin punches. The blank subtraction method, on the other hand, gave VX mass values that were similar to the analysis values from skin punches. Therefore, based on these data, we concluded that the best method to estimate the mass of VX in skin samples was to use all spectra and subtract out the VX mass calculated from Raman profiles on control blank skin recorded prior to exposure.

Considering the assumptions that were made in our VX estimation model, this method at best can only give a semi-quantitative estimate of the mass of VX in skin following percutaneous exposure. Taking the mean of several Raman depth profiles from different locations will make the estimation more reliable. Tape stripping is another method that could be used to obtain the mass of a chemical in skin, but this method is invasive and only applicable to the top skin layers. Taking biopsy skin punches and analyzing for the compound of interest remains the most accurate method for determining the amount of a chemical in all layers of skin.

Analytical Procedure for Determining VX in Skin Punches

ECBC analyzed tissue for the G-analogue of VX. Following the fluoride reactivation, the VX that was bound to the receptor, as well as any VX that was unbound, is converted to G analog of VX, VX-G, a methylphosphonofluoridate. This assay measured what was originally bound as well as any unbound VX. A validation experiment at ECBC, using hairless guinea pig skin, demonstrated that free VX represents about 50% of the total VX measured by the fluoride reactivation method (Byers et al. 2012).

Occasionally, ECBC analyzed other samples such as swabs, gauze, templates, etc. For these samples, the analysis was a simple extraction of free VX. To compare the VX and the VX-G numbers one must consider the molar equivalents. The molecular weight (MW) of VX-G is 126 g/mole and the MW of VX is 267 g/mole. Thus, using mole equivalency, 1.00 g of VX-G is equivalent to 2.12 g of VX.

CONCLUSIONS

1. Confocal Raman microspectroscopy was an effective tool for obtaining real-time, non-invasive VX concentrations in the upper skin layers of living animals. Recording useful Raman spectra required animals to be under anesthesia so that they remained completely immobile. Useable Raman spectra required good skin contact with the Raman recording window; thus haired animals required depilation.

2. When dosing with small volumes of liquid, recording spectra from the site of exposure required the use of a specially designed dosing template that allowed the Raman beam to hit VX-dosed skin.

3. VX was observed to distribute into the outer 0-20 µm of the stratum corneum, over the initial 10-20 minutes postexposure with skin, in effect, forming a skin depot over this time period. Later, VX concentrations decreased, but VX was still detectable at 48 hr postexposure.

4. Following percutaneous exposure, RSDL effectively removed VX from the skin surface and from the depot formed in the upper skin layers. The lack of peaks at 730 cm^{-1}, the characteristic frequency for VX in Raman spectra, confirmed that VX was not present in the skin.

5. Semi-quantitative total mass estimates for VX in the skin could be obtained using our procedure. The most reliable total mass estimate for VX in the skin was obtained by using all spectra in the profiles (constant mass method) and subtracting the mass from the non-exposed skin blanks. Because of skin heterogeneity, it was necessary to use the mean mass values of several depth profiles recorded at slightly different skin locations.

6. The Raman depth profile is a collection of many spectra. To accurately measure concentration, the validity of each spectrum in the profile should be evaluated for the presence of typical skin peaks, characteristic peaks of the compound of interest, and the presence of random cosmic ray interference.

REFERENCES

Bide, RW, Burczyk, AF, and Risk, DJ, Comparison of skin decontaminants for HD: Canadian Reactive Skin Decontaminant Lotion, Canadian Decontaminating Mitt and US Skin Decontaminating Kit, *Proceedings of the 1996 Medical Defense Bioscience Review*, U.S. Army Medical Research Institute of Chemical Defense. Vol No. III, pp 1218-1227, 1996. AD A321842.

Bide, RW and Risk, DJ, Decontamination of GF in vivo by the Reactive Skin Decontamination Lotion (RSDL), Technical Memorandum, Defence R&D Canada (DRDC), Suffield, 2002, TM 2002-046.

Bjamason, S, Mikler, J, Hill, I, Tenn, C, Garrett, M, Caddy, N, and Sawyer, TW, Comparison of selected skin decontaminant products and regimens against VX in domestic swine. Hum Exp Toxicol, 27 (3): 253-61, 2008.

Braue, EH Jr, Hanssen, KA, Doxzon, BF, Lumpkin, HL, an Clarkson, ED, Evaluation of RSDL, M291 SDK, 0.5% Bleach, 1% Soapy Water, and SERPACWA; Part 1: Challenge with VX, USAMRICD-TR-09-01. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, June 2009.

Braue, EH Jr, Hanssen, KA, Doxzon, BF, Lumpkin, HL, and Clarkson, ED, Efficacy Studies of RSDL, M291 SDK, 0.5% Bleach, 1% Soapy Water and SERPACWA, Part 1: Guinea Pigs Challenged with VX. Cutan Ocul Toxicol, 30 (1): 15-28, 2011.

Byers, CE, Whalley, CE, Lumley, LA, Clarkson, ED, and Jakubowsky, EM, Absorption characteristics of VX following percutaneous exposure of hairless guinea pigs, Poster No. 1348 presented at the 2012 SOT meeting in San Francisco, CA. http://www.toxicology.org/AI/Pub/Tox/2012Tox.pdf.

Caspers PJ, Lucassen GW, Carter EA, Bruining HA, Puppels GJ, In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. J Invest Dermatol,116(3): 434-42, 2001.

Joint Requirements Office for Chemical, Biological, Radiological and Nuclear Defense (2004). Joint Service Personnel/Skin Decontamination System (JSPDS). J.R. Office, ed. (Washington, DC).