Part 3: Solid phase extraction of Russian VX and its chemical attribution signatures in food matrices and their detection by GC-MS and LC-MS

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ABSTRACT

Chemical attribution signatures indicative of *O*-isobutyl S-(2-diethylaminoethyl) methylphosphonothioate (Russian VX) synthetic routes were investigated in spiked food samples. Attribution signatures were identified using a multifaceted approach: Russian VX was synthesized using six synthetic routes and the chemical attribution signatures identified by GC-MS and LC-MS. Three synthetic routes were then down selected and spiked into complex matrices: bottled water, baby food, milk, liquid eggs, and hot dogs. Sampling and extraction methodologies were developed for these materials and used to isolate the attribution signatures and Russian VX from each matrix. Recoveries greater than 60% were achieved for most signatures in all matrices; some signatures provided recoveries greater than 100%, indicating some degradation during sample preparation. A chemometric model was then developed and validated with the concatenated data from GC-MS and LC-MS analyses of the signatures; the classification results of the model were > 75% for all samples. This work is part three of a three-part series in this issue of the United States-Sweden collaborative efforts towards the understanding of the chemical attribution signatures of Russian VX in crude materials and in food matrices.

1. Introduction

In the case of a chemical attack, priority lies in rapidly and efficiently detecting and neutralizing the chemical agent to minimize exposure. However, forensic investigation into the source of the agent is perhaps equally as important. Significant information about the synthetic origin of a chemical agent can be obtained from its chemical attribution signatures (CAS), which consist of byproducts, impurities, and unreacted starting materials present or absent in the sample. The concentration and stability of these CAS can vary significantly from sample to sample, and can be further complicated when the chemical agent and its associated CAS are present in a complex matrix. Food is one group of such matrices and is considered a high-value matrix of interest attested by the fact that Homeland Security Presidential Directive (HSPD) 9 has developed important policy to protect and defend the country's food supply against terrorist attacks [1].

Organophosphorus-based nerve agents are a type of chemical warfare agent (CWA) and rank among the most highly toxic compounds ever made. The mechanism of action of nerve agents involves inhibition of cholinesterase enzymes. This inhibitory event leads to build up of the

neurotransmitter acetylcholine in synaptic gaps causing overstimulation of the nerves leading to incapacitation or death in a matter of minutes [2]. Russian VX (O-isobutyl S-(2-diethylaminoethyl) methylphosphonothioate) is a third generation, V-type chemical warfare agent. As such, it is listed as a schedule I compound by the Chemical Weapons Convention, prohibiting its synthesis and subsequent use [2,3]. Exhibiting a vapor pressure of 0.00062 mm Hg at 25 °C [4], Russian VX has a low volatility and high viscosity that allow it to persist for several weeks under most climatic conditions [2] making it a highrisk agent for food contamination. As an Organisation for the Prohibition of Chemical Weapons (OPCW) accredited laboratory, the Forensic Science Center (FSC) at Lawrence Livermore National Laboratory (LLNL) can synthesize small amounts of chemical agents, including Russian VX, for research purposes. The following work describes our collaborative research effort with the Swedish Defence Research Agency (FOI), itself an OPCW-accredited laboratory in Sweden.

In order to increase the level of preparedness, methods for the sampling and extraction of a chemical agent as well as its chemical attribution signatures from food matrices are needed. Through the US-Sweden collaboration, we have developed complimentary methods to

approach the analysis of food samples based on the understanding that the equipment and infrastructure at laboratories using these methods may vary. Additionally, a sample collected for forensic analysis may require different sample preparation methods based on the complexity of the food matrix. One of our methods, concurrently presented in this issue, relies on an acetonitrile extraction of the food samples followed by LC-MS [5]. The other method is presented in this work, where we describe a solid phase extraction (SPE) protocol for the extraction of Russian VX along with its CAS and its subsequent testing using five food matrices. Russian VX, synthesized in a crude manner using several different pathways (synthesis presented in this issue) [6], was spiked into food, and the CAS components were studied in each food matrix using a combination of GC-MS and LC-MS. Note that while this study discusses the results of the analysis of Russian VX in food samples, these methods have also been applied to other chemical agents and will be presented in future publications.

CAS studies previously performed by our group have focused on employing statistical tools to identify the chemical route by which a compound may have been synthesized. Many different data processing techniques have been used to describe these complex data sets [5,7–9]. Supervised chemometric analytical methods have been quite useful as they can be used to describe the variance between known classes of materials and help prioritize the signatures most useful in describing the variance [9,10]. In this work, partial least squares-discriminant analysis (PLS-DA) and a machine learning algorithm well-suited for application to CAS data were used to identify and rank compounds of importance to accomplish route discrimination. The technique also generated a model in principle capable of identifying the synthetic pathway used to generate the Russian VX from samples taken from a variety of food materials. This work is part three of a three-part series in this issue of the United States-Sweden collaborative efforts towards the understanding of the chemical attribution signatures of Russian VX in crude materials and in food matrices.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all reagents and solvents were ACS grade or higher, obtained from commercial suppliers, and used as received. Anhydrous magnesium sulfate (> 99.5%) was procured from Sigma-Aldrich (St. Louis, MO). Bottled water,), baby food (pureed peas), milk, liquid eggs (Ingredients: egg whites (99%), less than 1%: natural flavor, color (includes beta carotene), spices, guar gum, xanthan gum, salt, onion powder), and hot dogs (Ingredients: contains less than 22% of salt, potassium lactate, sodium phosphate, flavor(s), sodium diacetate, ascorbic acid, sodium nitrate (nitrite), paprika extractives) were purchased from a local supermarket.

2.2. Spiking solutions

Six different synthetic methods were selected to produce Russian VX, and after initial GC-MS analysis described in the concurrent manuscript by Holmgren *et al.* (this issue) [4], samples from three routes were down selected for use in the initial studies described herein due to agent usage considerations; these routes were selected due to the number of attribution signatures identified in these routes and are shown in Fig. 1. Two involve the use of methylphosphonic dichloride (dichlor) as the starting material (Routes 1 and 3) whereas Route 4 makes use of dichloromethylphosphine as the starting material.

A 1000 ppm standard of the product of a single replicate of each Russian VX synthetic route was prepared in methanol for use as the spiking solution. ^{31}P-NMR was used to determine each reaction yield (based on phosphorus intensities only) and each crude sample was corrected for purity. Food matrices were spiked with each Russian VX sample to a concentration of 50 ppm (μg/mL for bottled water and milk,
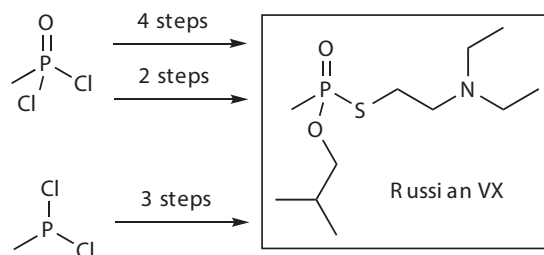

Fig. 1. Chemical routes for Russian VX synthesis employed in this work. These methods were based on those previously described in the literature [4] and represent Route 1, 3, and 4 from Holmgren *et al.*

μg/g for baby food, hot dogs, and liquid eggs). Note that this study was designed to determine the variability in the CAS of Russian VX that are introduced by the matrices. For further information on the variation in the CAS of each Russian VX route, please see Holmgren et al. [6]

2.3. Extraction method

A Solid Phase Extraction (SPE) method using Strata-X SPE Cartridges (Phenomenex, 33 μm, polymeric reverse phase, 30 mg/mL, Torrance, CA) was developed for all matrices, though each matrix required different processing before loading on the cartridge.

2.3.1. Bottled water preparation
The bottled water samples (2 mL) were loaded directly onto the SPE cartridge.

2.3.2. Baby food preparation
A 2 g baby food sample was extracted 2 × 2 mL in methanol, sonicated, and centrifuged (1500 RPM) for 5 min. The resulting supernatant was reduced to near dryness at 40 °C, and reconstituted in 2 mL of DI water, which was then loaded onto the SPE cartridge.

2.3.3. Milk preparation
Milk (2 mL) was diluted 1:1 with deionized (DI) water in a glass vial, which was capped, shaken, and loaded onto the SPE cartridge.

2.3.4. Liquid eggs preparation
Liquid eggs (5 g) were processed via a modified QuEChERS method [11]. Thus, 10 mL of DI water, 10 mL of acetonitrile, 1 mL of chloroform, and 4 g of MgSO$_4$ were added, and the organic later isolated after mixing. 10 μL of triethylamine was added, and the sample was reduced to near dryness at 40 °C, and reconstituted in 3 mL of DI water, which was subsequently loaded onto the SPE cartridge.

2.3.5. Hot dog preparation
Hot dogs were homogenized in a commercial blender by adding half the hot dog's weight of DI water to the solid hot dog and blending in 10 s increments for 2 min, creating a hot dog paste. 5 g of hot dog paste were processed via a modified QuEChERS method. 10 mL of DI, 10 mL of acetonitrile, 1 mL of chloroform, and 4 g of MgSO$_4$ were added, and the organic later isolated after mixing. 10 μL of triethylamine was added, and the sample was reduced to near dryness at 40 °C, and reconstituted in 3 mL of DI water, which was subsequently loaded onto the SPE cartridge.

For all matrices, each cartridge was conditioned with 2 mL of methanol followed by 2 mL of DI water (MilliQ, 18 Ω). An aliquot of each processed matrix (2 mL bottled water and baby food, 4 mL milk, 3 mL liquid eggs and hot dogs) was then loaded onto the SPE cartridge and allowed to drip through by gravity. The cartridge was dried by applying positive pressure using a 5-mL plastic syringe that was fitted with a latex rubber gasket to form a good seal between the syringe and the flanges of the cartridge. Elution was performed with 0.5 mL of DI water,