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In This Issue:

International intrigue Part II: Novichok nerve agent use in attempted murder of ex-Russian spy

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Introduction- On March 4, 2018, a former convicted Russian double agent, Sergei Skripal and his 33-year-old daughter, Yulia, were found comatose on a shopping center park bench in Salisbury, England. Nearly dead, they were rapidly transported to a nearby regional hospital and received extensive medical treatments that likely saved their lives presumably from exposure to an unknown toxic poison. A first responder policeman was also severely injured requiring hospitalization and 21 other potentially exposed civilians received medical treatment. Yulia was discharged in early April, while her father did not fully recover for another month. On March 12, 2018, United Kingdom (U.K.) officials publicly claimed that this mysterious mass poisoning was due to a relatively unknown Novichok nerve agent that had been covertly developed by the Russians at the end of the Cold War. Tragically, this incident repeated itself three months later when a British middle-aged couple became severely ill in Amesbury, just 7 miles from where the Skripal's were found. They were rushed to the same hospital that treated the Skripals where Dawn Sturgess never regained consciousness and Charlie Rowley eventually recovered.

The Novichoks are classified as a group of organophosphorus (OP) nerve agents that include sarin, soman and VX. These chemical weapons represent some of the most toxic compounds known to man and are lethal at extremely low doses. This short article will primarily focus on describing what is currently published about these clandestine agents and the scientific evidence behind this mysterious event. The evidence will allow us to determine if this incident was plausibly an accident or a deliberate crime targeting a Russian dissident living in England. For those seeking to learn more about nerve agents please consult the February 2016 issue of *Therapeutics and Toxins News* for a timely review on the topic (1). An April 2017 article discussed the use of VX in the recent assassination of Kim Jong Nam, the half-brother of North Korean dictator Kim Jong Un (2). For those interested in learning more broadly about chemical weapons please access the new Lab Tests Online entry on Chemical Terrorism Agents (3).

Background- Novichok loosely translates to "newcomer" in Russian and they represent a new 4th generation chemical weapon. Their development was preceded by 1st generation blister agents used extensively in World War I, 2nd generation nerve agents developed by Nazi Germany in the 1940s and 3rd generation V series nerve agents developed early during the Cold War. The presumed Novichok chemical structure includes the traditional OP backbone with an attached dihaloformaldoxime group (Figure 1). From the 1970s to the dismantling of the Soviet Union in the early 1990s, the Russians were committed to discovering and developing more potent weapons of mass destruction (WMD). The

Soviets had an extensive program centered on a series of top secret laboratories designed to develop and test biological and chemical warfare agents (4).

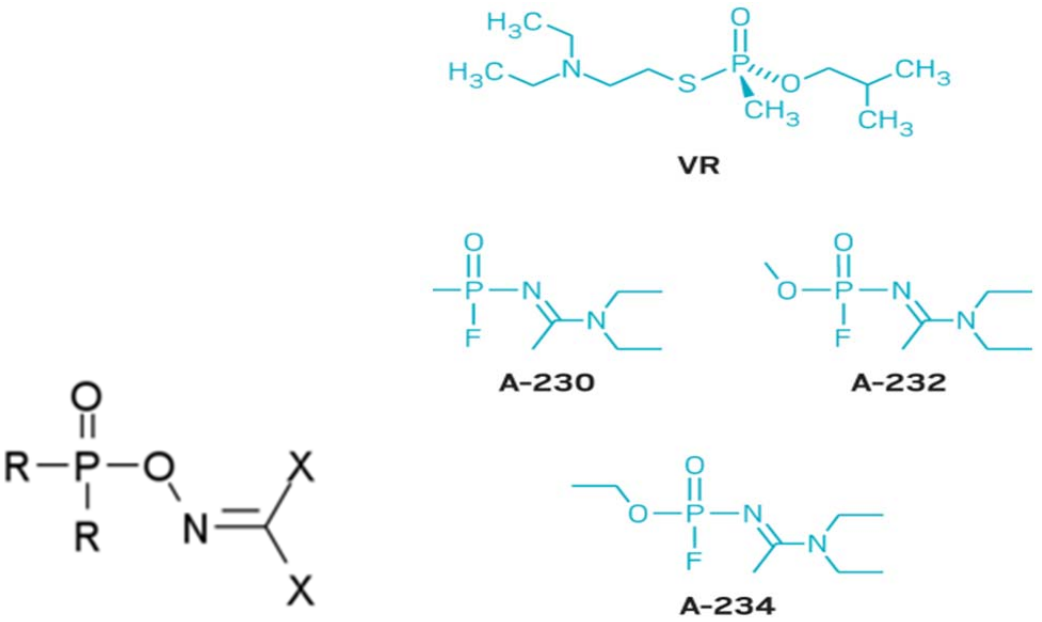

Figure 1. Left- the presumed generic chemical structure of Novichok nerve agents. R= alkyl, alkoxy, alkylamino or fluorine and X is a halogen (F, Cl, Br) or a pseudohalogen like a nitrile group. Right- proposed chemical structures for some Novichok agents (7).

One such program was entitled Foilant and its mission was to secretly create the Novichoks (4). These nerve agents were engineered to achieve four main objectives; to be undetectable to conventional chemical detection tests, to breach NATO military chemical protective gear, to be safer to handle and last, to circumvent Chemical Weapons Convention (CWC) guidelines that ban chemical agents. If Foilant proved successful, then these weapons could potentially be used with impunity against Russia's enemies.

The Novichoks and all other nerve agents specifically target the life sustaining enzyme acetylcholinesterase (AChE). They rapidly bind critical amino acids in the active site thereby preventing AChE from degrading the neurotransmitter acetylcholine in neuronal synapses (5). The accumulation of acetylcholine results in an array of clinical manifestations collectively called the cholinergic toxidrome. If left untreated, this can lead to death often due to the extended contraction of the diaphragm muscle and asphyxiation. It is unknown how long it takes the Novichoks to age (i.e. to irreversibly bind) AChE. The decontamination procedure and treatments for Novichok nerve agent poisoning is similar to that for all other nerve agents (5). It is worth noting that some Novichoks may age AChE as rapidly as does soman, possibly within minutes (1), so oxime treatments should be administered as soon as possible.

Most of the limited information publicly disclosed about the Novichoks comes from a few Russian scientists who supported the Cold War mission. Their existence was first reported by chemist Dr. Vil Mirzayanov in a 1992 *Moscow News* article. He described in some detail the secret Russian Foilant program in which he was assigned. Shortly after this publication, he was arrested for divulging state secrets and imprisoned. The Russian government has always radically denied conducting any Novichok research, however, their existence was admitted by Russian military authorities when bringing charges against Dr. Mirzayanov. The court testimonies of three government scientists stated Novichok agents had been covertly produced so his public disclosure represented high treason. In 1995 these charges were dropped and Dr. Mirzayanov immigrated to the U.S.

As with most nerve agents, the Novichoks reportedly can be delivered as a liquid, aerosol or gas. They can also allegedly be dispersed as an ultra-fine powder by adsorbing the liquid onto a carrier like silica, pumice or talc (6). Unlike previous nerve agents where the end product is unitarily manufactured, many Novichoks can be made as a binary agent. In effect, these weapons are formed by mixing two relatively benign precursor chemicals prior to use which makes them much safer to handle and likely undetectable. When mixed, they produce the active nerve agent. The Novichoks are reported to be the most toxic chemical weapons ever made.

It is generally assumed that seven Novichoks have been adopted as weapons by the Russian military (6). According to Jonathan Tucker, the first Novichok developed in the Foilant program was Substance 33, also known as VR (4). This compound is very similar to VX, differing only in the alkyl substituents on its nitrogen and oxygen atoms. It is estimated that the Russians made ~15 tons of Substance 33 and also developed a binary analog (4). The program then developed a range of other compounds to include a molecule dubbed A-230 that is allegedly 5-8 times more potent than VX, the most toxic known nerve agent (6). Another agent made was A-232 which had similar toxicity to Substance 33 but was significantly more volatile, and its ethoxy analogue, A-234. The binary analog of A-232 was designated Novichok-5 and for A-234 as Novichok-7. The binary components of these agents are reported to be acetonitrile and a low potency OP pesticide so that they can be ostensibly manufactured for a legitimate industrial use. The USSR carried out open-air tests of experimental batches in a remote area of Uzbekistan prior to weaponizing the best performing Novichok agents. The right panel of Figure 1 shows the proposed chemical structures of the Novichoks designated as VR (i.e. Substance 33), A-230, A-232 and A-234 (7).

There are no documented incidents of Novichok agents being used on the battlefield. Allegedly, in 1995, Substance 33, was used to murder Ivan Kivelidi, a powerful Russian banker and his secretary (8). The Russian government analyzed the substance used and reported that it was a “phosphorus-based military grade nerve agent whose formula was strictly classified.” (8) To date, Russia is the only country known to have developed Novichoks.

Since the end of the Cold War there has been collective global concern over the development and use of chemical weapons. This led to the 1997 Chemical Weapons Convention (CWC) which represents an international agreement that effectively bans chemical weapons production and mandates scheduled destruction of existing declared stockpiles (9). To date, there are 193

state signatories that include the U.S. and Russia. The Organization for the Prohibition of Chemical Weapons (OPCW) provides an unbiased 3rd party lab confirmation testing and serves as the CWC's enforcement arm. In 2017, the OPCW confirmed that VX nerve agent had been used to brazenly assassinate Kim Jong Nam; the half-brother of North Korean dictator Kim Jong Un; at the Kuala Lumpur Airport in Malaysia (2). Does the current evidence in the Skripal incident eerily parallel this sensational international event?

International intrigue: what we currently know- Sergei Skripal served as a military intelligence officer and former Russian Colonel. During much of his career he traveled across Europe assigned to various foreign embassies. In 2006, he was convicted of leaking state secrets to the U.K. and sentenced to 13 years in prison for high treason. In 2010, Sergei was released from prison in a swap for Russian spies that had been arrested in the U.S. After being pardoned by then Russian President Medvedev, he resettled in England. In "retirement" Sergei seems to have remained active in espionage. Working with several NATO countries, his actions allegedly led to the discovery of multiple secret Russian operatives across Europe. These actions may have again made him an enemy of the Russian state. Yulia was visiting from Russia in March 2018 when they were poisoned, likely by contamination of his front door.

Shortly after the Skripals were admitted to the hospital, biological samples taken from them were sent to the U.K. Porton Down Defense Laboratory. Their analysis revealed that the chemical used was a Novichok nerve agent. On March 12th, U.K. Prime Minister Theresa May stated that "it is now clear that Mr. Skripal and his daughter were poisoned with a military-grade nerve agent of a type developed by Russia." In April 2018, the OPCW confirmed these results and identified the same material from samples taken at Sergei's house. The Skripals likely survived due to their rapid transport to a nearby hospital followed by quick and accurate diagnosis that led to appropriate aggressive medical treatments.

A major breakthrough in the investigation occurred during a police search of the 2nd poisoning victim's house on July 11th where a small counterfeit perfume bottle was recovered. Scientists at Porton Down and the OPCW confirmed its contents to be the same Novichok agent that was used to poison the Skripals three months previously. Charlie Rowley had found this discarded bottle and given it to Dawn Sturgess as a gift. It is thought that she sprayed the Novichok on her wrists believing it was perfume, and within 15 minutes became severely ill. He later became ill after coming into contact with her. Dawn died ~8 days after the exposure, while Charlie survived likely because he received a significantly smaller dose of the poison.

There is no plausible explanation that this 2018 incident was accidental but instead clearly appears to have involved deliberate targeting. The only reason for a Novichok agent's presence is to kill silently and until now, undetectably. The OPCW report stated the chemical had an "almost complete absence of impurities" implying a very skilled manufacturing process. Since samples of the parent compound were found in the bottle several months after the Skripal incident, the Novichok used seems to be extraordinarily stable. The limited Novichok literature implies that some were designed to be persistent agents like VX. In addition, the presence of Novichok on the victims' hands and wrists suggests that it may be readily absorbed through the skin.

In mid-March 2018, the British requested an emergency meeting of the United Nations Security Council to disclose their findings. This event led to a major international row; based on the evidence presented most Western countries were convinced of Russian involvement. Ultimately this led to the expulsion of 23 Russian envoys from England followed in kind by several of Britain's main allies. The Russians loudly denied any involvement and responded in the same political manner.

It is noteworthy that the Novichok agents are not specifically listed in the schedules of the CWC as they only became public after the treaty had been signed. This is also true of the presumed precursor chemicals used to make the binary agents. However, these facts shouldn't amount to a legal loophole that allows their production and use because the CWC clearly places a blanketed prohibition on the manufacture of any toxic chemical intended to be a weapon. This is true whether it be from the past, present or future. Prime Minister May has stated that there are only two plausible explanations for the Skripal incident. "Either this was a direct act by the Russian State against our country, or the Russian government lost control of this potentially catastrophically damaging nerve agent and allowed it to get into the hands of others (8)." Either situation would strongly suggest that Russia is in breach of the CWC.

As England has become a home for many Russian emigrants, an alarming number of residing Kremlin critics have died under unusual circumstances. The Skripal case seems reminiscent of the 2006 assassination of Alexander Litvinenko, a former KGB operative and a renown antagonist of the Kremlin. While living in exile in London, he bizarrely died of radiation poisoning after drinking tea that had been laced with polonium-210. The subsequent British investigation indicated that this was a high profile assassination likely approved by the most senior Russian officials. After a thorough crime scene investigation of this March 2018 incident, the U.K. has charged two Russian military officers for the attempted assassination of the Skripals. Shortly after the attack, both flew back to their homeland and by current Russian law can't be extradited back to England.

Conclusion- We appear to be living in troubled times where brazen chemical weapon attacks against innocent civilians and covert nerve agent assassinations are becoming more common. Just as an evidential smoking gun led back to North Korea's role in the 2017 Kim Jong Nam assassination with VX, so too does the Novichok presence lead to Russia's involvement in this attempted assassination in England. Given these increasing global incidents attributed to chemical weapons, it is in the best interest of the toxicology community to become more aware of their poisoning symptomology and lifesaving therapies. As scientists, we should condemn the use of any chemical weapon confirmed by the OPCW and advocate for criminal punishment of the perpetrators.

References.

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Editor's Corner:

National Meeting in Anaheim, CA, USA (August 4-8)

Dear Readers,

Division Events:

1. Division will have a Table at the **AACC Opening Mixer & Division Networking Event** (Sunday Aug 4, 6.45-8PM) *Anaheim Convention Center, Grand Plaza (outside the entrance doors).* There will be many fun activities!
2. **Division Annual Meeting and Luncheon**, Monday Aug 5, 2019 from 12:00 – 2:00 pm at the Marriott (Platinum 7).
3. **Division ePoster session:** Tuesday, August 6 from 4:15-5:00 PM in the poster theater in Hall A of the Anaheim Convention Center.

Educational Sessions:

Monday: 32220 Opioids and Beyond: The Clinical Laboratory's Role in the Opioid Epidemic

Tuesday 33108 Impact of Hormones on Drug Testing: From the Bench to the Bedside

Tuesday *33219* Interactive Pain Management Case Studies: Clinician and Laboratory Perspectives

Wednesday 44130 or 54230 Supporting Opioid Addiction Programs With Unexpected Testing—Ethanol Metabolite Test Development in an Appalachian Laboratory

Wednesday 34109 Moving Beyond Immunoassays for the Poisoned Patient: Analytical Approaches and Interactive Case Studies

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-Pradip Datta, Editor.

AACC TDM TOX Web Resources:

https://www.aacc.org/community/divisions/tdm-and-toxicology/

Editorial Board

The editorial board invites ideas and article contributions for this newsletter. Please contact Dr. Pradip Datta at pradip.datta@siemens.com.