Theoretical study of the molecular aspect of the suspected novichok agent A234 of the Skripal poisoning

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Novichoks are the suspected nerve agents in the March 2018 Skripal poisoning. In this context, the novichok agent A234 (chemical structure proposed by Mirzayanov) was studied using computational methods to shed light on its molecular, electronic, spectroscopic, thermodynamic and toxicity parameters as well as on potential thermal and hydrolysis degradation pathways. The poisoning action and antidote of A234 were also investigated. Some of these parameters were compared to three common G- and V-series nerve agents, namely GB, VR and VX. The research findings should be useful towards the detection, development of antidotes and destruction of A234.

1. Introduction

Nerve agents, organophosphate containing chemical warfare agents, are among the most toxic chemicals known to mankind [1]. They can inactivate acetylcholinesterase (AChE) which is a key central nervous system (CNS) enzyme responsible for the breakdown of the neurotransmitter acetylcholine; thus, leading to rapid and severe adverse effects on the environment, human and animals [2,3]. Several nerve agents of the G- [tabun (GA), sarin (GB), chlorosarin (GC) and soman (GD)] and V-series (VE, VR, VS and VX) have been deployed not only in warfare but also in acts of terrorism and high-profile assassinations [4,5]. For instance, GB was used in chemical attacks in the Syrian civil war between 2013 and 2017, while VX was employed to murder the half-brother of the North Korean leader Kim Jong-un at Kuala Lumpur Airport in February 2017. Such events are indicative that
these nerve agents are still a threat to the international community, despite the fact that their use is being regulated by the Organisation for the Prohibition of Chemical Weapons under the Chemical Weapons Convention (CWC) [6].

A novel class of nerve agents, the novichoks or the A-series, has recently come into the limelight following the March 2018 assassination attempt on the former Russian spy, Sergei Skripal and his daughter Yulia in Salisbury, UK [7–9]. Almost 30 years have passed since these ‘fourth generation’ nerve agents were developed by the Soviet Union in a Cold War-era weapons programme [10]. However, information on novichoks is still guarded as ‘top secret’ and exact reliable data are missing. The Skripal poisoning has catalysed the pursuit of detailed information on the history, chemical structure, synthesis, toxicity, deployment, detection and destruction of novichoks. This yielded some reports which are insightful although incomplete at molecular level [4,7–9,11–13]. One of the concerns is related to their chemical structure. For example, there is still a debate as to whether a novichok agent denoted as A234 (scheme 1) corresponds to either structure (a) as proposed by Mirzayanov [14] or structure (b) as proposed by Hoenig [15] and Ellison [16].

To the best of our knowledge, there is only one scientific publication that provides experimental data for a chemical (alkyl-N-[bis(dimethylamino)methylidene]-P-methylphosphonamidates) that is specifically identified as a novichok and this compound is included in the Schedule 2.B.04 of the CWC [17]. We are reporting a theoretical study on novichok and in particular on the novichok agent A234 (structure (a) of scheme 1; N’-[ethoxy(fluoro)phosphoryl]-N,N-diethylethanimidamide) because some open sources [18–20] speculated the use of A234 in the absence of official communications on the exact chemical identified from the UK incident. Henceforth, A234 will correspond to structure (a) of scheme 1 throughout this paper. It is noteworthy that A234 has not yet been scheduled under the CWC and at the revision stage of this manuscript, two reports on novichoks pertaining to QSAR [21] and molecular docking approaches [22] appeared.

This study aims at providing the molecular, electronic, spectroscopic, thermodynamic and toxicity parameters of A234 together with plausible thermal and hydrolysis degradation pathways. The poisoning action and antidote of A234 were also investigated. Some of these parameters are compared to three common G- and V-series nerve agents, namely GB (2-[fluoro(methyl)phosphoryl]oxypropane), VR (N,N-diethyl-2-[methyl(2-methylpropoxy)phosphoryl]sulfanylthalamine) and VX (N-[2-[ethoxy(methyl)phosphoryl]sulfanylthethyl]-N-propan-2-ylpropan-2-amine).

2. Methodology

Conformational analysis for A234, VR and VX was carried out using the MMFF94s force field as implemented in the CONFLEX software [23–25]. The conformational search was limited to 5 kcal mol$^{-1}$ and this led to 115, 8061 and 2473 conformers of A234, VR and VX, respectively. The optimization of the 115 conformers of A234 using the B3LYP/6-311++G(d,p) [26–28] method converged to 26 conformers; their optimized structures together with their relative energies are provided in electronic supplementary material, figure S1. The first 125 conformers of VR and VX were also optimized using the B3LYP/6–311++G(d,p) and the relative energies and Cartesian coordinates of their resulting first 15 minimum energy structures are provided in the electronic supplementary material. Previously reported [29] minimum energy structures of GB were herein revisited using the B3LYP/6-311++G(d,p) method. The B3LYP lowest minimum energy structures of A234, GB, VR and VX were also optimized using the M06-2X/6-311++G(d,p) [30] and MP2/6-311++G(d,p) [31] methods. The B3LYP [29,32,33] and M06-2X [34,35] DFT functionals and the MP2 [29,36] method in
conjunction with different Pople basis sets have been used to study nerve agents and related organophosphorus species.

Geometry optimization was followed by analytic Hessian computation using the same methods. The absence of negative Hessian eigenvalues confirmed the stationary points as minima on the potential energy hypersurfaces. Zero-point energy correction was included in the relative energies (ΔEs). Reported energies are given at 298.15 K and 1 atm. Natural bond orbital (NBO) analysis was also carried out using the M06-2X/6-311++G(d,p) method [37]. Computations were performed by means of resources provided (Gaussian 16 [38] package) by SEAGrid [39–42].

Geometry optimization was also conducted in solvents (water and n-octanol) using the M06-2X/6-311++G(d,p) method in view to calculate the lipophilicity of A234, GB, VR and VX. Solvent effect was taken into account based on the polarizable continuum model [43]. The classical descriptor for lipophilicity is the log $P_{o/w}$ (partition coefficient between n-octanol and water) [44]. The log $P_{o/w}$ values were obtained using the following equation:

$$\log P_{o/w} = \frac{-\Delta G}{2.303RT}$$

where R is the universal gas constant (8.314 J K$^{-1}$ mol$^{-1}$), $T$ is the system temperature (298.15 K) and $\Delta G$ (in J mol$^{-1}$) is the difference between the solvation absolute Gibbs free energies in water ($G_{water}$) and in n-octanol ($G_{n-octanol}$). The log $P_{o/w}$ values for the four nerve agents were also calculated via the SwissADME [44] and the ALOGPS 2.1 program [45].

The optimized structures in water were used for computing nuclear magnetic resonance (NMR) chemical shifts with the gauge-including atomic orbital method [46] using shieldings of trimethylsilane (for 1H NMR and 13C NMR), nitromethane (for 15N NMR), phosphoric acid (for 31P NMR) and trichlorofluoromethane (for 19F NMR) computed using the M06-2X/6-311G(2d,d,p) method (also denoted as cbsb7). The CBS-QB3 model is known to perform well and predict accurate energies for organophosphorus compounds [48,49]. The Δ$H_{298}$ of A234 was further employed to determine its bond dissociation energies (BDEs) as per the following process: $RR' \rightarrow R \cdot + R' \cdot$ (where $RR'$ and $R \cdot / R' \cdot$ represent A234 and the individual radical fragments, respectively) to provide an insight into favourable thermal decomposition pathways [48]. The BDE corresponds to the enthalpy of formation ($\Delta H_{298}$) of the thermal dissociation process, where

$$\text{BDE} = \Delta H_{298} = [\Delta H_{298}(R \cdot) + \Delta H_{298}(R' \cdot)] - \Delta H_{298}(RR').$$

The Δ$H_{298}$ of each radical was determined using the CBS-QB3 method.

### 3. Results and discussion

Section 3.1 consists of the minimum energy structures of A234, GB, VR and VX. Their spectroscopic parameters, conceptual DFT-based reactivity descriptors, molecular electrostatic potential (MEP) and ADME (absorption, distribution, metabolism, excretion) properties are discussed in §§3.2–3.5, respectively. Their poisoning action and possible antidotes based on model reactions are reported in §3.6. The hydrolysis and thermal degradation of A234 are discussed in §§3.7 and 3.8, respectively.

#### 3.1. Structural parameters

Selected bond lengths of the gas-phase optimized structures of A234, GB, VR and VX are provided in figure 1 and additional details are collected in electronic supplementary material, table S1. The rotational constants are tabulated in electronic supplementary material, table S2. The root-mean-square deviation (RMSD) between the atomic positions of the M06-2X and B3LYP optimized structures are 0.1390 and that of the M06-2X and MP2 optimized structures are 0.7747. The high RMSD value arises due to a rotation about the N–P bond in the MP2 optimized structure. A234 has structural difference with GB, VR and VX. The P=O centre of A234 is attached to an acetoamidine group (which consists of an active –N=CR–N< framework) along with –OR and –F.

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3.2. Spectroscopic analysis

The IR and Raman spectra of GB, VR and VX were revisited and those of A234 are reported based on the M06-2X/6-311++G(d,p) method in the gas phase (figure 2 and table 1). The IR and Raman spectra of the nerve agents consist of two distinct regions, notably a low-wavenumber region at 200–1700 cm⁻¹ and a high-wavenumber region at 2950–3200 cm⁻¹. The high-wavenumber region consists of C–H stretching vibrations which are weakly IR active (A234: 3052–3173 cm⁻¹; GB: 3053–3176 cm⁻¹; VR: 2965–3179 cm⁻¹; VX: 3006–3172 cm⁻¹). This particular region is highly Raman active with broad absorption bands. The low-wavenumber region (fingerprint region comprising several vibrational modes) of each nerve agent differs from each other. The low-wavenumber region features weakly to non-Raman active absorption bands. A sharp and distinct peak due to C=N stretching at 1670 cm⁻¹ is noticeable in the IR spectra of A234. This peak is characteristic for substituted amidines which absorb strongly at 1600–1700 cm⁻¹ [53]. The C=N stretching of an N-phosphorylated alkylisourea of the type (EtO)₂P(O)NC(OEt)N(CH₂CH=CH₂)₂ has been reported at 1640 cm⁻¹ and this correlates with that of A234 [50]. The C–N stretching of the –N=CR–N< acetoamidine unit of A234 is of weaker intensity and appears at a lower wavenumber (1560 cm⁻¹) than that of C=N stretching. Literature values indicate that the C–N bond of amide derivatives vibrates at lower frequencies within a range of 1230–1412 cm⁻¹ [54–56]. The peak of highest intensity within the low-wavenumber region corresponds to the O–C stretching for A234, VR and VX (1113, 1091 and 1107 cm⁻¹, respectively) and P–O–C asymmetric stretching for GB (1045 cm⁻¹).

The $^1$H, $^{13}$C, $^{15}$N, $^{31}$P and $^{19}$F NMR chemical shifts of A234, GB, VR and VX in water are provided in electronic supplementary material, table S3 and the atom labelling of A234 are given in figure 3.

3.2.1. $^{13}$C NMR

The tertiary carbon atom (C2) of the acetoamidine unit of A234 resonates in a weak field with the highest chemical shift of 190.71 ppm compared to the other carbon atoms present in either A234 or GB, VR and VX. This is characteristic of amide/guanidine-containing molecules [57,58]. The C2 atom is in an electron-poor environment, being bonded to two electronegative nitrogen atoms, and hence, less shielded as opposed to the other carbon atoms. Further, the carbon atom (C26) bonded to oxygen in
A234 resonates at a higher chemical shift of 65.45 ppm while those bonded to the less electronegative nitrogen resonate at 48.30 ppm (C8) and 47.28 ppm (C15). The shielded carbon atoms (C9, C16, C22 and C27) have upfield chemical shifts within 12.43–20.94 ppm.

**Figure 2.** Simulated IR and Raman spectra of the nerve agents.

**Table 1.** Assignment of selected vibrational modes of the nerve agents.

| vibrational modes | wavenumber (cm⁻¹) | A234 | GB | VR | VX | literature (exp.) |
|-------------------|-------------------|------|----|----|----|-------------------|
| ν(P=O)            | 1266, 1319, 1352 | 1294 | 1269 | 1269 | 1255 [50], 1278 [51], 1227 [51], 1317 [52] |
| ν(0–C)            | 113, 1148, 1091 | 117 | 1045 | 983 | 1015 [51], 1031 [51], 1000–1050 [52] |
| ν₁(P–O–C)         | 1117, 1045, 1117 | 854 | 745, 786 | 842 | 762, 795 |
| ν₂(P–O–C)         | 1251, 1269, 1255 | 990 | 950 | 928, 972 | 1015 [51], 1031 [51], 1000–1050 [52] |
| ν(P–F)            | 873, 843         | 873 | 840 | 838 [51] |
| δ(P–O–C)          | 594, 521, 473, 473 | 594 | 521 | 473 | 461 | 778 [51] |
3.2.2. ¹H NMR

Hydrogen atoms (e.g., H10 and H11 of A234) associated with the electronegative N1 atom have downfield chemical shifts ($\delta_{H10} = 4.04$ ppm and $\delta_{H11} = 2.85$ ppm). Hydrogen atoms (H28 and H29) associated with the more electronegative O7 atom have slightly larger chemical shift ($\delta_{H28} = 4.14$ ppm and $\delta_{H29} = 3.92$ ppm). The presence of weak hydrogen bonding in A234 (figure 3; C22–H25$\cdots$O5; 2.198 Å) is also evident from the chemical shift of the H25 atom (3.53 ppm). The other two hydrogen atoms (H23 and H24) of the methyl group are at upfield chemical shifts ($\delta_{H23} = 1.87$ ppm and $\delta_{H24} = 1.82$ ppm).

3.2.3. ³¹P NMR

The ³¹P NMR spectroscopy is particularly useful in distinguishing between the nerve agents. The chemical shift of A234 (3.89 ppm) is upfield with respect to that of GB (39.94 ppm), VR (63.45 ppm) and VX (62.72 ppm). The chemical shifts (referenced to 85% H₃PO₄ in C₆D₆ solution) of N¹-phosphoryl-N¹,N²-dimethylamidines of the type (EtO)₂P(O)N(Me)C(R)¼NMe (where R¼Ph, CHCl₂) were reported within $\pm 1.4$ to $\pm 4.4$ ppm [59], close to that of A234. In this context, it is worth highlighting that the chemical shifts are dependent on the solvent used. For instance, the chemical shift of VX lies between 60 and 63 ppm in water compared to 57.0 ppm in CDCl₃ [60].

3.2.4. ¹⁵N NMR

The N1 atom ($\sim$295.91 ppm) of the N1–CR=NR3 unit of A234 has an upfield chemical shift in contrast to N3 ($\sim$269.40 ppm). This compares well with the chemical shifts of the N¹ atom of amidines of the type R¹N²=CR¹–N¹R²R³ (where R¹–R⁴ are alkyl groups) which lie from $\sim$269.2 to $\sim$310.5 ppm (converted to the MeNO₂ scale) [61]. The chemical shift of the tertiary amino N1 atom of A234 is downfield compared to that of VR ($\sim$396.54 ppm) and VX ($\sim$394.96 ppm).

3.2.5. ¹⁹F NMR

The chemical shift of the F6 atom of A234 appears at $\sim$86.22 ppm while that of GB is at $\sim$63.69 ppm.

3.3. Conceptual DFT-based reactivity descriptors

The HOMO and LUMO plots of A234, GB, VR and VX obtained using the M06-2X/6-311++G(d,p) method are illustrated in figure 4. The HOMO of A234 is mainly localized on the N atoms and the ethyl groups of the acetoamidine unit. A small contribution from the orbitals of the electronegative O and F atoms is also observed in HOMO. Its LUMO is mainly centred on the alkyl groups of the acetoamidine unit. Some similarities are also reflected from the HOMO and LUMO of GB, VR and VX. In GB, the HOMO is mainly localized on its alkyl groups as well as its O atom, while in VR/VX, the HOMO is centred on their tertiary amino unit. Further, LUMO is mainly localized on the alkyl groups of GB, VR and VX. A close analysis of the MO coefficients indicates that the orbitals of the P atom contribute to some extent to the HOMO and LUMO of A234, GB, VR and VX. Population analysis shows that the active orbitals associated with acetoamidine unit dominate over that of the
fluorophosphate unit within the low-lying HOMOs and higher-lying LUMOs (electronic supplementary material, table S4 and figure S3).

DFT-based reactivity descriptors derived from the HOMO and LUMO energies of the nerve agents are gathered in table 2. The HOMO–LUMO energy gap ($\Delta E$) or global hardness ($h$) indicates the overall stability of the molecules. The energy values ($\Delta E$ or $h$) increase in the order of VX (6.92 eV), VR (7.35 eV), A234 (7.99 eV), GB (10.06 eV). Further, table 2 indicates that both global softness and polarizability increase in the order of GB, A234, VR/C25 VX. Thus, VR and VX are more soft, polarizable and reactive than GB and A234.

A large HOMO–LUMO energy gap has been associated with a large dipole moment [74]. VX has both the lowest energy gap and the lowest dipole moment. A234 has the largest dipole moment (which points towards the N3–P4 bond) and hence, is more polar. The dipole moment decreases on going from A234 $\rightarrow$ GB $\rightarrow$ VR $\rightarrow$ VX.

The electrophilicity index increases from VX $\rightarrow$ VR $\rightarrow$ A234 $\rightarrow$ GB. All the nerve agents are highly electrophilic. They are prone to accept electron density from incoming nucleophiles, bearing oxygen, nitrogen and sulfur donor atoms, preferentially at the electropositive centre/s of the nerve agents. NBO analysis of A234 indicates that the carbon atom (0.578e) of the &N1–CR=N3– acetoamidine unit and the phosphorus atom (2.498e) are both positively charged. All other carbon atoms are

Table 2. Conceptual DFT-based reactivity descriptors for the nerve agents.

| parameters         | A234 | GB   | VR   | VX   | derivation                      |
|--------------------|------|------|------|------|---------------------------------|
| dipole moment ($\mu$) | 5.72 | 3.11 | 1.60 | 1.24 |                                |
| polarizability ($\alpha$) ($\text{Å}^3$) | 20.52 | 10.80 | 28.21 | 27.94 | $\alpha = (\alpha_{xx} + \alpha_{yy} + \alpha_{zz})/3$ [62] |
| ionization potential ($I$) (eV) | 8.36 | 10.26 | 7.49 | 7.09 | $I = -E_{\text{HOMO}}$ [63] |
| electron affinity ($A$) (eV) | 0.37 | 0.20 | 0.14 | 0.17 | $A = -E_{\text{LUMO}}$ [63] |
| chemical potential ($\mu$) (eV) | -4.36 | -5.23 | -3.82 | -3.63 | $\mu = -(I + A)/2$ [64] |
| electronegativity ($\chi$) (eV) | 4.36 | 5.23 | 3.82 | 3.63 | $\chi = -\mu$ [65,66] |
| global hardness ($\eta$) (eV) | 7.99 | 10.06 | 7.35 | 6.92 | $\eta = I - A$ [64,67–69] |
| global softness ($S$) (eV$^{-1}$) | 0.13 | 0.10 | 0.14 | 0.14 | $S = 1/\eta$ [62] |
| electrophilicity index ($\omega$) (eV) | 1.19 | 1.36 | 0.99 | 0.95 | $\omega = \mu^2/2\eta$ [70–73] |
negatively charged within a range of \(-0.033e\) to \(-0.598e\) (see electronic supplementary material, table S5). Thus, these two particular centres (C2 and P4) will be the prime target of nucleophiles. By contrast, GB, VR and VX have only one electropositive centre where nucleophilic attack can take place. Their phosphorus centres (\(P_{GB} = 2.365e\), \(P_{VR} = 1.974e\), \(P_{VX} = 1.971e\)) are less positively charged than that of A234. This suggests that a nucleophile may attack the phosphorus centre of A234 more readily than that of GB, VR and VX.

3.4. Molecular electrostatic potential

The MEP surfaces of A234, GB, VR and VX, obtained using the M06-2X/6-311++G(d,p) method, are shown in figure 5 and their back surfaces are also provided in electronic supplementary material, figure S4. Some common features are observed in the MEP surfaces of the four nerve agents. The negative charge (red region) is mainly localized on the oxygen atom of the \(P=O\) unit and this will favour strong hydrogen bonding, for example, with the amino acid residue of the AChE active site. The regions governing the electron-rich O (coordinated to alkyl group), S and F atoms are weakly negative (yellow in colour) and these may form weak hydrogen bonding with the \(-NH_2\) moiety of the amino acid of AChE. The region at the nitrogen atom of the tertiary amino unit of VR and VX are also weakly negative (see electronic supplementary material, figure S4); however, the same observation is not reciprocated by A234. The surfaces around the nitrogen atom (N1) together with the coordinated ethyl groups of A234 are weakly positive (pale blue in colour). The \(-NEt_2\) unit is electron-deficient most probably due to the significant charge transfer from the lone pair of N1 atom to the anti-bonding orbital of C2=N3 bond (as observed from the second-order perturbation theory analysis within the NBO framework). The observation made from the MEP analysis also correlates with the natural charges of the N atoms of the nerve agents. The N1 atom of A234 (\(-0.493e\)) is less negatively charged than that of VR (\(-0.597e\)) and VX (\(-0.598e\)). On the other hand, the N3 atom of A234 is more negative (\(-1.005e\)). Electrophiles will approach the N3 centre more readily than the N1 centre.
3.5. ADME parameters

The ADME parameters, namely lipophilicity (log \( P_{o/w} \)), solubility (log \( S \)), topological polar surface area (TPSA) and skin permeability (log \( K_p \)) of the nerve agents, are summarized in table 3. The nerve agents are lipophilic in nature (the log \( P_{o/w} \) values are positive). The lipophilicity generally increases in the order of VX ≈ VR > A234 > GB. Data derived from SwissADME indicate a good correlation among the molecular weight, TPSA, lipophilicity, solubility, human gastrointestinal absorption, blood–brain barrier permeability and skin permeability of the nerve agents A234, GB, VR and VX (see electronic supplementary material, figures S5a–d). Lipophilicity increases as the molecular weight and TPSA increase. This can be correlated with an increase in the penetration of the nerve agent in the CNS [76]. All the nerve agents are soluble in water and solubility is in the order of GB > A234 > VR ≈ VX. It is known that the solubility of the compound can influence the absorption and blood–brain barrier permeability [77]. The solubility of the nerve agents can be associated with their high human gastrointestinal absorption as well as good blood–brain barrier and skin permeability. SwissADME predicts that the skin permeability of A234 and GB are comparable and that their more negative log \( K_p \) values indicate that they are marginally less skin permeant than VR and VX [44].

3.6. Nerve agent poisoning and antidotes

Nerve agent poisoning is caused from the inhibition of the AChE enzyme activity; thereby, forming a covalent bond between the phosphorus atom of the nerve agent and the alcoholic oxygen of the serine residue of the active site. Modelling the reaction between AChE and nerve agents is complex and time-consuming due to the large size of the protein and the requirement of high-accuracy computations. This reaction can alternatively be studied with model species. The simplest model for the active serine site of AChE is methanol [78]. Thus, the phosphorylation reactions of A234 with both the deprotonated \( CH_3O^- \) anion and the neutral \( CH_3OH \) molecule were investigated using the M06-2X/6-311++G(d,p) method (scheme 2). The corresponding reactions for GB, VR and VX are provided in electronic supplementary material, scheme S1. The central phosphorus atom of A234 is attached to three potential leaving groups –F, –OEt or –N. The substitution of –F by –OMe are comparable for A234 (see electronic supplementary material, scheme S2). Thus, this suggests that the A234-inhibited AChE model will preferentially be induced by hydroxylamine than by the formoximate anion. Reaction with the formoximate anion is highly endergonic for all studied nerve agents (scheme 3; electronic supplementary material, scheme S2). Thus, this suggests that the A234-inhibited AChE model obtained from the displacement of the –F atom can be successfully reactivated using the hydroxylamine anion.

3.7. Hydrolysis

Nerve agents commonly undergo nucleophilic attack by the water molecule at the electropositive phosphorus centre via either an \( S_2 \)1 or \( S_2 \)2 hydrolysis reaction [81]. Thus, hydrolysis reaction at the P4 centre of A234 was investigated using the M06-2X/6-311++G(d,p) method. This is illustrated by reactions 5a–c (scheme 4) which involve the displacement of the leaving groups –F, –OEt and –N=C(Me)NEt2 respectively. The free energy values indicate that these processes are endergonic. The carbon atom (C2) of the acetoamidine unit of A234 is also electropositive. Hydrolysis at the acetoamidine unit can take place via two different pathways as highlighted by Wu et al. [82].
Table 3. ADME properties of the nerve agents.

|        | log $P$ | consensus $^b$ | Alog $P^c$ | lit. $^d$ | log $S^b$ | TPSA $^b$ (Å$^2$) | log $K_p/(cm s^{-1})^b$ |
|--------|---------|----------------|------------|-----------|-----------|-------------------|--------------------------|
|        | computed$^a$ |      |            |           |           |                   |                          |
| A234   | 1.33    | 1.87           | 1.64       | —         | —         | 1.49              | —                        |
| GB     | 0.75    | 1.08           | 0.54       | 0.30      | 0.77      | 0.62              | 1.10                     |
| VR     | 2.12    | 2.47           | 2.15       | —         | 2.29      | 3.08              | 2.72                     |
| VX     | 1.16    | 2.55           | 2.41       | 2.09      | 2.28      | 3.18              | 3.10                     |

$^a$log $P$ obtained using the M06-2X/6-311++G(d,p) method.
$^b$Consensus log $P$, log $S$, TPSA and log $K_p$ obtained from SwissADME [44].
$^c$log $P$ obtained from VCCLab [45].
$^d$Literature values [75].
Scheme 2. Phosphonylation reaction between A234 and AChE models. The enthalpy ($\Delta H$) and free energy change ($\Delta G$) are in kcal mol$^{-1}$.

Scheme 3. Reactivation of A234-inhibited AChE model induced by formoximate and hydroxylamine anions. The enthalpy ($\Delta H$) and free energy change ($\Delta G$) are in kcal mol$^{-1}$.
Nucleophilic attack by the water molecule at the C2 centre is illustrated by reactions 6a,b (scheme 4) which involve bond breaking at N3=C2 and C2–N1, respectively. Reaction 6a is exergonic ($\Delta G = -9.2$ kcal mol$^{-1}$). Based on the reaction energetics and in the absence of information on A234, it can be deduced that its hydrolysis may potentially take place via reaction 6a to yield N,N-diethylacetamide and ethyl phosphoramidofluoridate.

### 3.8. Heat of formation and bond dissociation energy

The $\Delta H_{298}$ was calculated using the CBS-QB3 method for the nerve agents. The formation of the nerve agents is exothermic with $\Delta H_{298}$ of $-238.13$ (A234), $-240.49$ (GB), $-179.54$ (VR) and $-179.51$ (VX) kcal mol$^{-1}$. The $\Delta H_{298}$ values were estimated as in [48] and details are provided in the electronic supplementary material. GB is marginally more stable than A234. GB and A234 have greater thermal stability than VR and VX ($\Delta H_{25}$ $= 60$ kcal mol$^{-1}$).

Figure 6 illustrates the $\Delta H_{298}$ values for the dissociation of various bonds in A234 and these were compared with those of GA and GB [48]. The $\Delta H_{298}$ for the dissociation of the P–F bond to form the radicals •F and •P=O(OEt)(N=C(CH$_3$)$_2$(NEt$_2$)) was calculated to be 144.5 kcal mol$^{-1}$. The P–F bond is the strongest and this is comparable to that of GB (143.2 kcal mol$^{-1}$) [48]. The C–C bond breakings of the acetoamidine unit correlate to the lowest BDEs (82.5 and 85.1 kcal mol$^{-1}$) and these dissociations will predominate in unimolecular initiation reactions. These C–C BDEs are significantly lower than that of the –OEt unit of A234 (91.7 kcal mol$^{-1}$) and GA (93.8 kcal mol$^{-1}$) [48]. Significant amount of electron density transfer from the lone pair of N1 atom to the anti-bonding orbital of the C2=N3 bond has a destabilizing effect on the ethyl groups attached to the N1 atom. There is also a slight difference between the C–C bond distances of the acetoamidine (C8–C9 = 1.530 Å and C15–C16 = 1.532 Å) and –OEt (C26–C27 = 1.515 Å) units of the B3LYP/6-311G(2d,p) optimized structure of A234. The next favourable unimolecular initiation will occur at the O–C bond of A234.

### 4. Summary

Theoretical methods were employed to study (i) the molecular, spectroscopic, electronic and toxicity properties, (ii) poisoning action and antidotes based on model reactions, and (iii) hydrolysis and thermal degradation of A234. Some of these parameters were compared with GB, VR and VX. Distinct
features in the spectra of A234 are observed, namely (i) a sharp peak due to C=\text{N} stretching at 1670 cm\(^{-1}\) in the IR spectra, (ii) a large \(^{13}\text{C}\) NMR chemical shift of 190.71 ppm due to the \(-\text{N}=\text{CR}–\text{N}–\) acetoamidine carbon atom, and (iii) a relatively small \(^{31}\text{P}\) NMR chemical shift of 3.89 ppm. NBO analysis indicates that A234 may have diverse chemistry to other nerve agents due to the presence of two active electronegative centres, namely the carbon atom of the \(-\text{N}=\text{CR}–\text{N}–\) acetoamidine group and the phosphorus atom. The energetics for (i) the reactions of the nerve agents with AChE model nucleophiles, (ii) the reactions of nerve agents-inhibited AChE with model antidotes, and (iii) hydrolysis and thermal degradation of A234 will serve as foundations for future computations. Detailed mechanistic studies are currently being carried out in our laboratory for an in-depth understanding of the hydrolysis of A234. Overall, this study suggests that VX and VR are potentially more reactive than A234 and GB further to (i) their lowest HOMO–LUMO energy gaps, (ii) marginally high skin permeability, and (iii) highly negative \(\Delta G\) values associated with their reaction with MeO\(^2\) and MeOH (for the cleavage of the P–S bond). The current theoretical work could not authenticate the claim made by Mirzayanov on A234 being more potent than VX and this is in accordance with the recent study carried out by Carlsen [21]. The findings from this research work should provide incentives towards efficient detection, development of antidotes and destruction of A234.

Data accessibility. Cartesian coordinates for the optimized geometries are available as electronic supplementary material. Authors’ contributions. H.B. performed all the computations, analysis of the data and writing up of the manuscript for this project, with inputs from L.R. and P.R. All authors gave final approval for publication.

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References

1. Marrs TC, Maynard RL, Sidell FR (eds). 2007 Chemical warfare agents: toxicology and treatment, 2nd edn. New York, NY: Wiley.
2. Schwenk M. 2018 Chemical warfare agents: classes and targets. Toxicol. Lett. 293, 253–263. (doi:10.1016/j.toxlet.2017.11.040)
3. Vucinic S et al. 2017 Environmental exposure to organophosphorus nerve agents. Environ. Toxicol. Pharmacol. 56, 163–171. (doi:10.1016/j.etap.2017.09.004)
4. Costanzi S, Machado J-H, Mitchell M. 2018 Nerve agents: what they are, how they work, how to counter them. ACS Chem. Neurosci. 9, 873–885. (doi:10.1021/acschemneuro.8b00148)
5. Naughton SX, Terry Jr MW. 2018 Neurotoxicity in acute and repeated organophosphate exposure. Toxicology 408, 101–112. (doi:10.1016/j.tox.2018.08.011)
6. Crowley M, Dando M, Shang L. (eds) 2018 Preventing chemical weapons: arms control and disarmament as the sciences converge. Croydon, UK: Royal Society of Chemistry. (doi:10.1039/9781788010092)
7. Stone R. 2018 U.K. attack puts nerve agent in the spotlight. Science 359, 1314–1315. (doi:10.1126/science.359.6382.1314)
8. Vale JA, Marrs TC, Maynard RL. 2018 Novichok: a murderous nerve agent attack in the UK. Clin. Toxicol. 56, 1093–1097. (doi:10.1080/15563650.2018.1469759)
9. Nepovimova E, Kuca K. 2019 The history of poisoning: from ancient times until modern era, Arch. Toxicol. 93, 11–24. (doi:10.1007/s00204-018-2290-0)
10. Hoenig SL. 2007 Compendium of chemical warfare agents, p. 79. New York, NY: Springer.
11. Gussow L. 2018 Toxicology rounds: the mysteries of novichok and brodifacoum. Emerg. Med. News 40, 30. (doi:10.1097/01.EMN.0000535030.80353.31)
12. Chai PR, Hayes BD, Erickson TB, Bauer EW. 2018 Novichok agents: a historical, current, and toxicological perspective. Toxicol. Commun. 2, 45–48. (doi:10.1080/24734306.2018.1475151)
13. Nepovimova E, Kuca K. 2018 Chemical warfare agent novichok—mini-review of available data. Food Chem. Toxicol. 121, 343–350. (doi:10.1016/j.fct.2018.09.015)
60. Creasy WR, McSaveney DJ, Brevett CAS. 2013 The chemistry of amidines and imidates, vol. 2. p. 258. New York, NY: Wiley.

59. Patai S, Rappoport Z. (eds). 1991 The chemistry of amidines and imidates, vol. 2. p. 266. New York, NY: Wiley.

58. Heldebrant DJ, Koech PK, Ang MTC, Liang C, Khoo J, Yen YC. 1998 The chemistry of amidines and imidates, vol. 2. pp. 155–162. Boca Raton, FL: CRC Press.

57. Patai S, Rappoport Z. (eds). 1991 The chemistry of amidines and imidates, vol. 2. p. 275. New York, NY: Wiley.

56. Gupta K, Ghanty TK, Ghosh SK. 2012 The chemistry of amidines and imidates, vol. 2. p. 276. New York, NY: Wiley.

55. Creasy WR, McSaveney DJ, Brevett CAS. 2013 Speciation of VX in aqueous solution. J. Phys. Chem. C 117, 22 677–22 682. (doi:10.1021/ jp409671y)

54. Gupta K, Ghanty TK, Ghosh SK. 2012 The chemistry of amidines and imidates, vol. 2. p. 279. New York, NY: Wiley.

53. Gupta K, Ghanty TK, Ghosh SK. 2012 Polarizability, ionization potential, and softness of water and methanol clusters: an intermolecular potential energy surface. J. Chem. Phys. A 116, 6831–6836. (doi:10.1021/jp1048357)

52. Koopman T. 1934 About the assignment of wave functions and eigenvalues to the individual electrons of an atom. Physica 1, 104–113. (doi:10.1016/S0031-8914(34)90011-2)

51. Parr RG, Pearson RG. 1983 Absolute hardness: definition and electronegativity. J. Am. Chem. Soc. 105, 7512–7516. (doi:10.1021/ja00364a005)

50. Parr RG, Donnelly RA, Malke W. 1978 Electronegativity: the density functional viewpoint. J. Chem. Phys. 68, 3801–3807. (doi:10.1063/1.436185)

49. Mulliken RS. 1934 A new electroaffinity scale: together with data on states and an ionization potential and electron affinities. J. Chem. Phys. 2, 782–793. (doi:10.1063/1.1749394)

48. Ayers PW. 2007 The physical basis of the hard/soft acid-base principle. Faraday Discuss. 135, 161–190. (doi:10.1039/b606670d)

47. Pearson RG. 2009 The hardness of closed systems. In Chemical reactivity theory: a density functional view (ed. PK Chattaraj), pp. 153–162. Boca Raton, FL: CRC Press.

46. Pearson RG, Ghanty TK, Ghosh SK. 2012 The chemistry of amidines and imidates, vol. 2. p. 280. New York, NY: Wiley.

45. Parr RG, Szentpaly LV, Liu S. 1999 Electrophilicity index. J. Am. Chem. Soc. 121, 1922–1924. (doi:10.1021/ja983494h)

44. Chattaraj PK, Sarkar U, Roy DR. 2006 Functional view of amidines and imidates together with data on states and an ionization potential and electron affinities. J. Phys. Chem. A 113, 10 335–10 342. (doi:10.1021/ ja060370v)

43. Chattaraj PK, Giri S, Duley S. 2011 Update 2 of: Chemical reactivity theory: a density functional view. Faraday Discuss. 151, 135–161. (doi:10.1039/B606877D)

42. Chattaraj PK, Roy DR. 2007 Update 1 of: Chemical reactivity theory: a density functional view. J. Chem. Phys. A 116, 7031–7041. (doi:10.1021/ ja060370v)

41. Parr RG, Pearson RG. 1983 Absolute hardness: Weinheim, Germany: Wiley-VCH.

40. Parr RG, Szentpaly LV, Liu S. 1999 Electrophilicity index. J. Am. Chem. Soc. 121, 1922–1924. (doi:10.1021/ja983494h)

39. Perrin RG, Donnelly RA, Malke W. 1978 Electronegativity: the density functional viewpoint. J. Chem. Phys. 68, 3801–3807. (doi:10.1063/1.436185)

38. Mulliken RS. 1934 A new electroaffinity scale: together with data on states and an ionization potential and electron affinities. J. Chem. Phys. 2, 782–793. (doi:10.1063/1.1749394)

37. Ayers PW. 2007 The physical basis of the hard/soft acid-base principle. Faraday Discuss. 135, 161–190. (doi:10.1039/b606670d)

36. Pearson RG. 2009 The hardness of closed systems. In Chemical reactivity theory: a density functional view (ed. PK Chattaraj), pp. 153–162. Boca Raton, FL: CRC Press.

35. Chattaraj PK, Sarkar U, Roy DR. 2006 Functional view of amidines and imidates together with data on states and an ionization potential and electron affinities. J. Phys. Chem. A 113, 10 335–10 342. (doi:10.1021/ ja060370v)

34. Chattaraj PK, Giri S, Duley S. 2011 Update 2 of: Chemical reactivity theory: a density functional view. Faraday Discuss. 151, 135–161. (doi:10.1039/B606877D)

33. Chattaraj PK, Roy DR. 2007 Update 1 of: Chemical reactivity theory: a density functional view. J. Chem. Phys. A 116, 7031–7041. (doi:10.1021/ ja060370v)

32. Parr RG, Pearson RG. 1983 Absolute hardness: Weinheim, Germany: Wiley-VCH.

31. Parr RG, Szentpaly LV, Liu S. 1999 Electrophilicity index. J. Am. Chem. Soc. 121, 1922–1924. (doi:10.1021/ja983494h)

30. Perrin RG, Donnelly RA, Malke W. 1978 Electronegativity: the density functional viewpoint. J. Chem. Phys. 68, 3801–3807. (doi:10.1063/1.436185)

29. Mulliken RS. 1934 A new electroaffinity scale: together with data on states and an ionization potential and electron affinities. J. Chem. Phys. 2, 782–793. (doi:10.1063/1.1749394)

28. Ayers PW. 2007 The physical basis of the hard/soft acid-base principle. Faraday Discuss. 135, 161–190. (doi:10.1039/b606670d)

27. Pearson RG. 2009 The hardness of closed systems. In Chemical reactivity theory: a density functional view (ed. PK Chattaraj), pp. 153–162. Boca Raton, FL: CRC Press.

26. Chattaraj PK, Sarkar U, Roy DR. 2006 Functional view of amidines and imidates together with data on states and an ionization potential and electron affinities. J. Phys. Chem. A 113, 10 335–10 342. (doi:10.1021/ ja060370v)

25. Perrin RG, Donnelly RA, Malke W. 1978 Electronegativity: the density functional viewpoint. J. Chem. Phys. 68, 3801–3807. (doi:10.1063/1.436185)

24. Mulliken RS. 1934 A new electroaffinity scale: together with data on states and an ionization potential and electron affinities. J. Chem. Phys. 2, 782–793. (doi:10.1063/1.1749394)

23. Ayers PW. 2007 The physical basis of the hard/soft acid-base principle. Faraday Discuss. 135, 161–190. (doi:10.1039/b606670d)

22. Pearson RG. 2009 The hardness of closed systems. In Chemical reactivity theory: a density functional view (ed. PK Chattaraj), pp. 153–162. Boca Raton, FL: CRC Press.

21. Perrin RG, Donnelly RA, Malke W. 1978 Electronegativity: the density functional viewpoint. J. Chem. Phys. 68, 3801–3807. (doi:10.1063/1.436185)

20. Mulliken RS. 1934 A new electroaffinity scale: together with data on states and an ionization potential and electron affinities. J. Chem. Phys. 2, 782–793. (doi:10.1063/1.1749394)