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Review Article

A-series ("Novichok") nerve agents: Chemistry, toxicology, detection, and medical countermeasures

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Abstract

Available evidence indicates that A-series ("Novichok") nerve agents are highly toxic organophosphorus compounds whose primary mechanism of action involves potent inhibition of acetylcholinesterase, resulting in severe cholinergic toxicity and potentially prolonged neurological consequences. Recent analytical advances, including high-resolution mass spectrometry and protein adduct analysis, have substantially improved exposure verification and forensic attribution. Current data support the use of established nerve agent treatment principles, including atropine, oximes, benzodiazepines, and intensive supportive care, although evidence regarding oxime responsiveness and aging kinetics remains limited for several compounds. Existing knowledge of environmental persistence, toxicokinetics, and long-term health effects is constrained by the scarcity of authenticated reference materials and experimental studies. Computational toxicology and machine-learning approaches have provided valuable mechanistic insights but require further experimental validation. Continued multidisciplinary integration of toxicology, analytical chemistry, computational modeling, medicinal chemistry, and public health preparedness will therefore remain essential for improving scientific understanding and response capabilities related to emerging organophosphorus threats.

Keywords

A-series nerve agents, acetylcholinesterase (AChE), Novichok, organophosphorus compounds (OPCs), oxime reactivators

Introduction

A-series ("Novichok") nerve agents (NAs) represent a highly toxic group of organophosphorus compounds (OPCs) that have gained substantial international attention because of their alleged use in several poisoning incidents, including the Salisbury and Navalny cases (OPCW 2018, 2020b). These agents are generally considered part of a broader class of cholinesterase inhibitors structurally related to classical organophosphorus NAs, such as sarin, soman, VX, and related compounds (Bajgar 2004; Marrs et al. 2007). However, compared with traditional G-series and V-series agents, A-series compounds remain considerably less characterized experimentally because of re-

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stricted accessibility, biosafety concerns, and longstanding uncertainty regarding their exact chemical structures.

Much of the early public-domain information regarding A-series compounds originated from disclosures associated with the former Soviet chemical weapons program (Mirzayanov 2008). Subsequent analytical and synthetic investigations have provided increasing support for the proposed structures of several agents, including A-230, A-232, and A-234, although complete and independently verified structural characterization remains unavailable for a number of compounds attributed to the A-series (Costanzi and Koblentz 2019; Mirbabaei et al. 2022). Compared with classical G-series and V-series agents, A-series compounds are proposed to possess increased structural complexity and steric shielding around the phosphorus center, potentially influencing hydrolytic stability, acetylcholinesterase (AChE) inhibition, and responsiveness to oxime reactivators, although the magnitude of these effects remains insufficiently characterized experimentally (Bhakhoa et al. 2019).

The primary toxicological mechanism of A-series agents involves irreversible inhibition of AChE, resulting in excessive accumulation of acetylcholine (ACh) and severe cholinergic toxicity (Taylor and Radić 1994; Quinn 1987). Similar to other organophosphorus NAs, intoxication may lead to life-threatening respiratory failure, seizures, autonomic dysfunction, and prolonged neurological impairment (Thiermann et al. 2013; Worek et al. 2016). However, substantial uncertainty persists regarding the aging behavior, toxicokinetics, environmental persistence, and oxime susceptibility of individual A-series compounds because many currently available conclusions rely on computational predictions, surrogate systems, or limited experimental datasets.

Recent advances in analytical toxicology, mass spectrometry, biomarker identification, computational toxicology, and bioscavenger research have improved scientific understanding of these compounds despite restricted experimental access (Wang et al. 2021; de Bruin-Hoegée et al. 2023). At the same time, important challenges remain in forensic verification, medical countermeasure development, environmental decontamination, and public health preparedness.

The present review critically summarizes current knowledge regarding the chemistry, toxicology, analytical detection, environmental fate, and medical management of A-series ("Novichok") NAs. Particular emphasis is placed on distinguishing experimentally validated findings from conclusions derived primarily from computational modeling or indirect evidence while highlighting major knowledge gaps and future research priorities.

Chemical structures and classification

A-series ("Novichok") NAs comprise a structurally heterogeneous group of OPCs believed to have been developed during late Cold War chemical weapons research programs in the former Soviet Union (Hoenig 2007; Ellison 2008; Mirzayanov 2008). Despite increasing scientific and regulatory attention, the exact chemical identities of several proposed A-series agents remain incompletely resolved because authenticated reference materials and experimental data are limited (Kloske and Witkiewicz 2019).

Current evidence suggests that many A-series compounds belong to the phosphoramidate class of organophosphorus agents and contain structurally distinctive phosphorus–nitrogen linkages with amidine- or guanidine-like substituents (Fig. 1) (Bhakhoa et al. 2019). Among the most frequently discussed representatives are A-230, A-232, and A-234 (Fig. 2), which have been associated with recent forensic investigations and regulatory actions (Costanzi and Koblentz 2019). Analytical studies employing high-resolution mass spectrometry

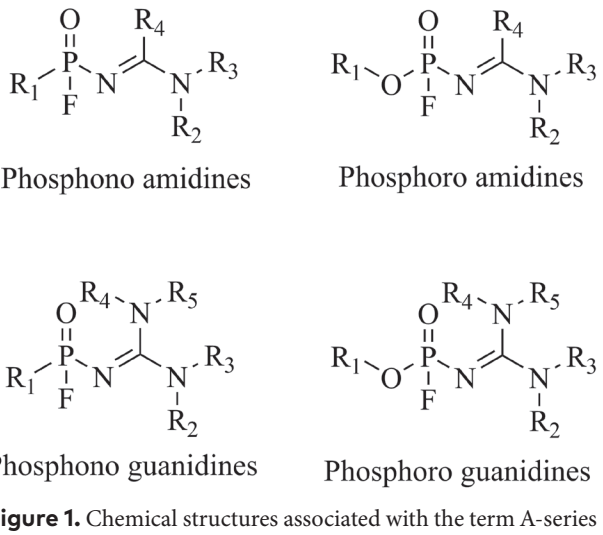

Figure 1. Chemical structures associated with the term A-series "Novichok" agents.

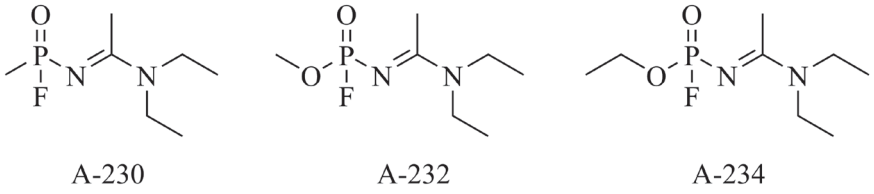

Figure 2. Comparison of the basic structures of A-series "Novichok" agents proposed by Mirzayanov (A-230 = *N*-[1-(diethylamino)ethylidene]-*P*-methylphosphonamidic fluoride or *N,N*-diethyl-*N'*-[fluoro(methyl)phosphoryl]ethanimidamide [IUPAC name]; A-232 = methyl *N*-[(1*E*)-1-(diethylamino)ethylidene]phosphoramidofluoridate or *N,N*-diethyl-*N*'-[fluoro(methoxy)phosphoryl] ethanimidamide [IUPAC name]; A-234 = ethyl *N*-[1-(diethylamino)ethylidene]phosphoramidofluoridate or *N*'-[ethoxy(fluoro) phosphoryl]-*N,N*-diethylethanimidamide [IUPAC name]).

(HRMS), degradation product characterization, and synthetic reference standards have provided increasing support for the proposed structures of several compounds, although uncertainties remain regarding the broader diversity of the A-series group (Mirbabaei et al. 2022; Otsuka et al. 2023a).

Compared with classical G-series agents, such as sarin and soman, A-series compounds are generally proposed to possess greater steric shielding around the phosphorus center and increased structural complexity (Bhakhoa et al. 2019). These features may influence hydrolytic stability, environmental persistence, interactions with AChE, and susceptibility to oxime-mediated reactivation. Notably, many structure–activity relationships remain insufficiently characterized experimentally because most available data derive from computational modeling, surrogate compounds, or limited *in vitro* investigations.

Importantly, structural uncertainty continues to represent a major limitation in the interpretation of toxicological and pharmacological findings related to A-series agents. Several historically proposed structures were reconstructed indirectly from secondary reports, computational analyses, or degradation products rather than through comprehensive characterization of authenticated compounds (Costanzi and Koblentz 2019). Consequently, toxicological conclusions should be interpreted cautiously, particularly when generalized across the entire A-series group.

The regulatory significance of these compounds increased substantially following amendments to Schedule 1 of the Chemical Weapons Convention (CWC), adopted by the Organization for the Prohibition of Chemical Weapons (OPCW) in 2019 and implemented in 2020 (OPCW 2020a; Kelle 2022). These amendments expanded international control measures to include several classes of A-series-related compounds and precursors, reflecting growing concern regarding emerging organophosphorus threats.

Primary molecular target—acetylcholinesterase

The principal molecular target of A-series ("Novichok") NAs is AChE (EC 3.1.1.7), a serine hydrolase responsible for the rapid hydrolysis of the neurotransmitter ACh at cholinergic synapses and neuromuscular junctions (Taylor and Radić 1994; Quinn 1987). Physiologically, AChE plays a critical role in terminating cholinergic neurotransmission and maintaining normal neuromuscular, autonomic, and central nervous system function. Inhibition of AChE by OPCs results in the pathological accumulation of ACh, causing sustained stimulation of muscarinic, nicotinic, and central cholinergic receptors (Bajgar 2004; Marrs et al. 2007).

Structurally, AChE contains a narrow aromatic gorge that guides ACh toward the catalytic active center located near the base of the enzyme cavity (Quinn 1987; Taylor and Radić 1994). The catalytic machinery consists primarily of the conserved catalytic triad Ser203–His447–Glu334 (human AChE numbering), together with the oxyanion hole and peripheral anionic site, both of which contribute to substrate orientation and catalytic efficiency. Organophosphorus NAs inhibit AChE through covalent phosphylation of the catalytic serine hydroxyl group (Fig. 3), generating a highly stable enzyme–adduct complex that markedly impairs the enzymatic hydrolysis of ACh (Worek et al. 2020a).

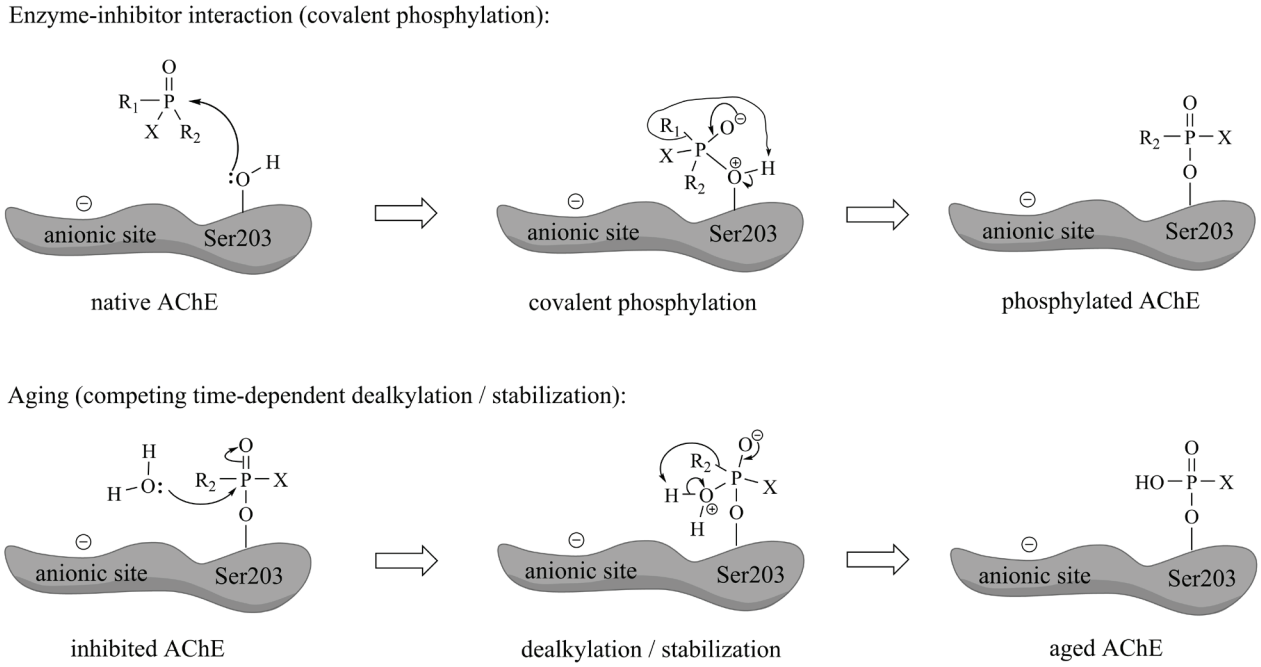

Figure 3. Schematic mechanism of AChE inhibition by OPCs. OPCs inhibit AChE through covalent phosphylation of the catalytic Ser203 residue, resulting in impaired ACh hydrolysis and cholinergic overstimulation. The subsequent process involves an aging-related stabilization pathway.

Current evidence suggests that A-series agents interact with AChE through mechanisms broadly analogous to those established for classical G-series and V-series NAs (Bhakhoa et al. 2019; Jeong and Choi 2019). Computational docking studies and limited experimental investigations indicate a strong affinity of several proposed A-series structures for the catalytic gorge of human AChE, consistent with their expected extreme toxicity (Bhakhoa et al. 2019; Wang et al. 2021). However, because authenticated A-series compounds remain difficult to access experimentally, much of the currently available mechanistic information derives from theoretical modeling, surrogate compounds, recombinant enzyme systems, or indirect biochemical studies rather than comprehensive *in vivo* toxicological investigations.

Following phosphylation, the inhibited AChE complex may undergo a secondary process commonly referred to as "aging" (Fig. 3), in which dealkylation or related structural rearrangements stabilize the phosphylated enzyme and reduce susceptibility to nucleophilic reactivation by oximes (Worek et al. 2016, 2020a). Aging is a well-established phenomenon for several classical NAs, particularly soman, for which rapid stabilization of the inhibited complex severely limits the therapeutic window for oxime-mediated reactivation (Marrs et al. 2007). In the case of A-series agents, however, currently available evidence remains substantially more limited. Several computational studies have predicted that at least some A-series compounds may undergo rapid or structurally favored aging reactions (Bhakhoa et al. 2019; Jeong and Choi 2019). Nevertheless, direct experimental confirmation of aging kinetics for authentic A-series agents remains scarce, and conclusions regarding the rate or extent of aging should therefore be interpreted cautiously.

Similarly, the susceptibility of A-series-inhibited AChE to oxime-mediated reactivation (Fig. 4) remains incompletely resolved. Experimental *in vitro* studies using recombinant human AChE and selected A-series analogues suggest that currently available oximes may demonstrate variable and, in some cases, limited reactivation efficacy (Santos et al. 2022; Opravil et al. 2026). Proposed explanations include steric shielding around the phosphylated active site, altered orientation of the phosphoramidate adduct, and restricted accessibility of nucleophilic oximes to the phosphorus center (Worek et al. 2020a). However, differences in experimental models, surrogate compounds, enzyme preparations, and reaction conditions complicate direct comparisons among studies.

Beyond catalytic inhibition, growing evidence suggests that interactions involving the peripheral anionic site and noncatalytic regions of AChE may contribute to differences in binding behavior among structurally diverse organophosphorus agents (Taylor and Radić 1994). Such interactions may potentially influence inhibition kinetics, the conformational stability of the inhibited enzyme, and the accessibility of therapeutic reactivators. Despite these advances, the relevance of these mechanisms specifically to A-series agents remains insufficiently characterized experimentally.

In addition to AChE, butyrylcholinesterase (BChE) has attracted considerable attention as both a biomarker of exposure and a potential bioscavenger for organophosphorus poisoning (Masson and Lockridge 2010; Masson and Nachon 2017). BChE can bind and sequester OPCs before they reach neuronal AChE, thereby reducing the toxic burden. Although bioscavenger-based strategies represent a promising direction for future countermeasure development, their efficacy specifically against A-series agents remains incompletely established because of the limited experimental availability of these compounds.

Overall, the available evidence suggests that A-series agents primarily act by potently inhibiting cholinesterases, particularly AChE, resulting in severe cholinergic toxicity. However, significant uncertainties remain concerning the precise kinetics of enzyme inhibition, aging behavior, the structural determinants of oxime resistance, and the relative contribution of catalytic vs. noncatalytic interactions.

Toxicodynamics and clinical manifestations

The toxicological effects of A-series ("Novichok") NAs are primarily mediated through inhibition of AChE, resulting in excessive accumulation of ACh at cholinergic synapses throughout the central and peripheral nervous systems. The consequent overstimulation of muscarinic, nicotinic, and central cholinergic receptors produces the characteristic cholinergic toxidrome observed following exposure to organophosphorus NAs (Bajgar 2004; Marrs et al. 2007).

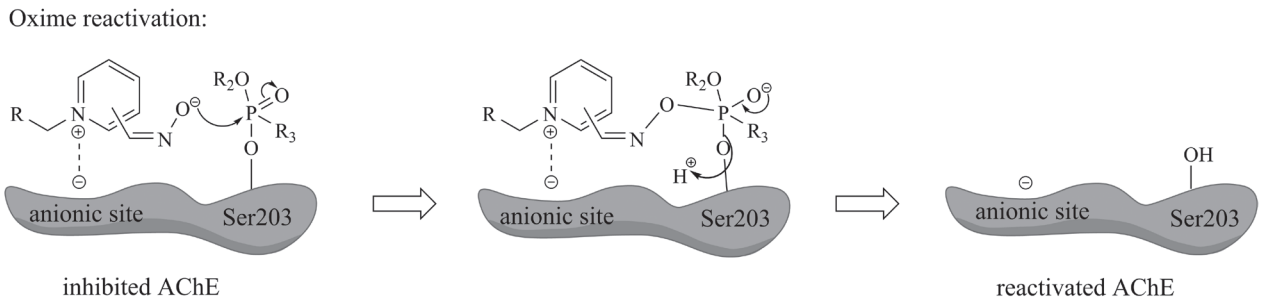

Figure 4. Oxime reactivation pathway following OPC-induced AChE inhibition. Oxime-mediated reactivation of phosphylated AChE occurs through nucleophilic attack on the phosphorus center.

Cholinergic toxidrome

The clinical manifestations of A-series poisoning are expected to resemble those observed following exposure to classical NAs (Table 1), although systematic human data remain extremely limited. Muscarinic effects include miosis, excessive salivation, lacrimation, bronchorrhea, bronchospasm, bradycardia, gastrointestinal hyperactivity, and increased secretory activity. Nicotinic manifestations typically involve skeletal muscle fasciculations, progressive weakness, and paralysis. Central nervous system (CNS) involvement may result in anxiety, confusion, seizures, coma, and respiratory depression (Marrs et al. 2007; Worek et al. 2016).

Table 1. Principal toxicodynamic effects of organophosphorus NAs and underlying mechanisms.

Clinical effect	Primary mechanism	Major consequences
Miosis, bronchorrhea, and bradycardia	Muscarinic receptor overstimulation	Respiratory compromise and autonomic dysfunction
Fasciculations and paralysis	Nicotinic receptor overstimulation	Neuromuscular failure
Seizures and coma	Central cholinergic excess	CNS injury and neurological sequelae
Respiratory failure	Combined central, airway, and neuromuscular mechanisms	Major cause of mortality

Evidence from the Salisbury and Navalny incidents, together with experimental studies involving structurally related compounds, suggests that severe poisoning can develop rapidly and may require prolonged intensive care management (OPCW 2018, 2020b). From a practical perspective, detailed toxicokinetic and dose–response data for authentic A-series agents remain unavailable in the open scientific literature.

Respiratory failure as the major cause of mortality

Respiratory failure represents the principal life-threatening consequence of NA intoxication and is typically multifactorial in origin (Eddleston et al. 2008; Worek et al. 2016). Three major mechanisms contribute simultaneously:

1. Central respiratory depression, resulting from excessive cholinergic stimulation within brainstem respiratory centers;
2. Airway obstruction and impaired gas exchange, caused by bronchoconstriction, bronchorrhea, and excessive respiratory secretions; and
3. Neuromuscular respiratory failure, resulting from sustained depolarization and subsequent paralysis of the diaphragm and accessory respiratory muscles.

The relative contribution of these mechanisms likely varies according to the specific agent, exposure route, absorbed dose, and timing of medical intervention. Because experimental studies involving authentic A-series compounds remain scarce, current understanding is largely extrapolated from established organophosphorus toxicology and the limited available case information.

Seizures and neurotoxicity

Seizures are among the most severe neurological manifestations of NA poisoning and may occur following substantial cholinergic overstimulation within the CNS. Prolonged seizure activity can contribute to excitotoxic neuronal injury, neuroinflammation, and long-term neurological sequelae (McDonough Jr and Shih 1997; Collombet 2011).

Potential long-term consequences reported after severe organophosphorus intoxication include cognitive impairment, mood disturbances, memory deficits, and persistent neurological dysfunction. However, whether A-series agents possess unique neurotoxic properties beyond those associated with classical NAs remains unclear. Current evidence is insufficient to determine whether reported long-term effects reflect agent-specific toxicity, prolonged hypoxia, seizure-related injury, or a combination of these factors.

Toxicokinetic considerations

Information regarding the absorption, distribution, metabolism, and elimination of A-series compounds remains limited. Available computational analyses and indirect experimental observations suggest that some A-series agents may exhibit physicochemical properties favorable for rapid systemic absorption and efficient penetration into target tissues (Bhakhoa et al. 2019; Wang et al. 2021). To date, however, no comprehensive toxicokinetic studies using authenticated compounds have been published.

Similarly, uncertainty remains regarding environmental degradation rates, biological persistence, and the formation of toxicologically relevant metabolites. Consequently, many currently proposed toxicokinetic characteristics should be regarded as provisional rather than definitively established.

Medical countermeasures and emerging therapeutic strategies

Medical management of A-series ("Novichok") NA poisoning follows the general principles established for organophosphorus NA intoxication (Table 2) and is based on the rapid administration of antimuscarinic agents, oxime reactivators, anticonvulsants, and intensive supportive care (Marrs et al. 2007; Worek et al. 2016). However, uncertainties regarding the aging behavior and oxime susceptibility of A-series compounds have raised concerns regarding the effectiveness of currently available antidotal

Table 2. Medical countermeasures for organophosphorus NA poisoning: mechanisms, benefits, and limitations.

Countermeasure	Primary mechanism	Major benefits	Major limitations
Atropine	Muscarinic receptor antagonism	Rapid symptom control	No AChE reactivation
Oximes	AChE reactivation	Restores enzyme activity	Variable efficacy; limited CNS penetration
Benzodiazepines	Anticonvulsant action	Seizure control and neuroprotection	No effect on cholinesterase inhibition
Bioscavengers	Agent sequestration/ detoxification	Potential prophylactic and therapeutic value	Experimental status

regimens. Because direct experimental evidence remains limited, therapeutic recommendations continue to rely largely on established NA treatment paradigms and extrapolation from surrogate models.

Atropine

Atropine remains the cornerstone of antidotal therapy for organophosphorus NA poisoning. As a competitive muscarinic receptor antagonist, it counteracts excessive cholinergic stimulation and alleviates bronchorrhea, bronchospasm, bradycardia, and other life-threatening muscarinic manifestations (Eddleston et al. 2008; Worek et al. 2020b).

Importantly, atropine does not reactivate inhibited AChE and therefore does not reverse neuromuscular paralysis or restore enzymatic activity. Treatment endpoints should focus on clinical improvement rather than fixed dosing schedules, with repeated administration often required in severe poisoning cases. Reports from recent high-profile poisoning incidents indicate that prolonged atropinization may be necessary following significant exposure to highly potent organophosphorus agents (OPCW 2018, 2020b).

Oxime reactivators

Oximes represent the only clinically available pharmacological approach capable of restoring cholinesterase activity through nucleophilic cleavage of the phosphyl–enzyme bond (Fig. 4). Currently used oximes include pralidoxime, obidoxime, trimedoxime, and HI-6, although substantial differences exist in their efficacy against individual NAs (Kovarik et al. 2010; Worek et al. 2020b).

The effectiveness of oxime therapy depends on several factors, including the structure of the inhibiting agent, the stability of the phosphylated enzyme complex, the timing of treatment, and the ability of the oxime to access the active site of inhibited AChE. Experimental studies involving A-series-related compounds have reported variable reactivation efficiencies, suggesting that steric hindrance near the phosphorus center may reduce oxime effectiveness in some cases (Santos et al. 2022; Opravil et al. 2026).

An additional limitation is the generally poor penetration of currently available oximes across the blood–brain barrier. Consequently, peripheral cholinesterase reactivation may occur while significant central cholinergic toxicity persists (Worek et al. 2016). This limitation has stimulated ongoing efforts to develop next-generation oximes with improved CNS bioavailability and broader activity against structurally diverse organophosphorus agents.

Importantly, although computational studies have proposed rapid aging processes for certain A-series compounds, direct experimental confirmation remains limited. Therefore, definitive conclusions regarding universal oxime resistance across the A-series group are not currently justified.

Benzodiazepines and neuroprotection

Benzodiazepines are essential components of NA medical management because they suppress seizure activity and reduce the risk of secondary neuronal injury. Diazepam remains the most widely used anticonvulsant in both civilian and military treatment protocols, although midazolam has gained increasing importance because of its rapid onset of action and ease of administration (McDonough Jr and Shih 1997; Shih et al. 1999).

Early seizure control is particularly important because prolonged status epilepticus contributes substantially to excitotoxic neuronal damage, neuroinflammation, and long-term neurological sequelae. Experimental studies have consistently demonstrated that therapeutic efficacy decreases as treatment delays increase, emphasizing the importance of rapid intervention.

Bioscavengers and emerging therapeutic approaches

Growing interest has focused on bioscavenger-based countermeasures capable of neutralizing organophosphorus agents before they interact with neuronal targets. Human BChE represents the most extensively investigated stoichiometric bioscavenger and has demonstrated protective efficacy in multiple experimental models involving classical NAs (Masson and Lockridge 2010; Masson and Nachon 2017).

Additional approaches under investigation include engineered catalytic bioscavengers, recombinant cholinesterases, phosphotriesterases, and nanotechnology-based detoxification systems. Although these strategies remain largely experimental, they may offer advantages over conventional antidotes by directly eliminating toxic agents rather than solely mitigating their physiological consequences.

Several novel oxime derivatives, centrally acting reactivators, and multifunctional compounds combining reactivation and neuroprotective properties are also under development. Nevertheless, validation against authentic A-series agents remains extremely limited, and their clinical applicability remains uncertain.

Biomarkers and analytical detection strategies

The analytical identification of A-series ("Novichok") NAs represents one of the most challenging areas of contemporary forensic and toxicological science. Unlike classical NAs, for which extensive analytical reference data are available, investigations involving A-series compounds are complicated by limited access to authenticated standards, structural uncertainty, rapid biotransformation, and strict regulatory restrictions (Kloske and Witkiewicz 2019; Noort et al. 2021).

Consequently, modern analytical strategies increasingly focus not only on the detection of the parent compound but also on the identification of characteristic degradation products, protein adducts, and long-lived biomarkers that can provide evidence of exposure after the original agent has disappeared.

Environmental and chemical detection

Direct detection of intact NAs may be possible shortly after contamination using advanced analytical techniques (Table 3), such as gas chromatography–mass spectrometry (GC–MS), liquid chromatography–tandem mass spectrometry (LC–MS/MS), and HRMS (John et al. 2018; Otsuka et al. 2023a).

However, environmental degradation, hydrolysis, and decontamination processes can quickly reduce the concentration of the parent agent. Consequently, analytical investigations often rely on detecting characteristic hydrolysis and transformation products that remain detectable for longer periods.

Recent advances in HRMS have significantly improved the ability to identify trace levels of organophosphorus degradation products in complex environmental and biological matrices. Such approaches have played a critical role in forensic investigations associated with recent high-profile poisoning incidents.

Protein adduct biomarkers

Protein adduct analysis has become one of the most important approaches for the retrospective verification of NA exposure. Following intoxication, OPCs react covalently with nucleophilic residues in biological proteins, generating stable biomarkers that may persist for days or weeks after exposure (Noort et al. 2021; de Koning et al. 2022).

Among these biomarkers, BChE adducts are currently regarded as one of the most reliable approaches for the retrospective confirmation of exposure because of their relative stability, specificity, and analytical accessibility. Albumin adducts and adducts involving other plasma proteins may provide complementary information and extend the temporal detection window.

The identification of phosphylated protein biomarkers using LC–MS/MS has become an essential component of modern forensic toxicology and has been successfully applied in investigations involving suspected exposure to highly toxic organophosphorus agents.

Advances in mass spectrometry

Recent developments in HRMS, Orbitrap-based platforms, and hybrid analytical workflows have substantially improved sensitivity, selectivity, and confidence in compound identification (Otsuka et al. 2023a, 2023b). These technologies enable accurate mass determination, structural elucidation, and nontargeted screening approaches that are particularly valuable when dealing with compounds for which authenticated reference standards may be unavailable.

In addition, isotope-pattern analysis, fragmentation profiling, and retrospective data mining have expanded the ability of laboratories to identify previously unrecognized organophosphorus-related species. Such capabilities are especially relevant to emerging chemical threats and structurally diverse A-series compounds.

Table 3. Major analytical approaches for the detection and verification of organophosphorus NA exposure.

Analytical approach	Target analyte	Main application	Advantages	Limitations
GC–MS	Parent compounds and degradation products	Environmental analysis	Established methodology	Limited detection window
LC–MS/MS	Biomarkers, metabolites, and protein adducts	Clinical and forensic investigations	High sensitivity and specificity	Requires specialized instrumentation
HRMS/Orbitrap	Unknown compounds and nontargeted screening	Forensic identification	Structural elucidation capability	Complex data interpretation
BChE adduct analysis	Phosphylated cholinesterase	Retrospective exposure verification	Long detection window	Specialized analytical expertise
Albumin adduct analysis	Protein biomarkers	Complementary exposure assessment	Persistent biomarkers	Lower standardization

Biomedical and forensic challenges

Despite major analytical advances, several limitations remain. The scarcity of authenticated standards complicates method validation and interlaboratory comparison. Structural uncertainty surrounding some proposed A-series compounds further complicates the interpretation of analytical findings and may affect confidence in compound assignment.

Another important challenge involves distinguishing direct exposure from secondary contamination or environmental residues. Reliable forensic attribution therefore requires the integration of analytical chemistry, biomarker analysis, clinical information, and contextual investigative evidence.

International laboratory networks coordinated through the OPCW continue to play a critical role in standardization, proficiency testing, and verification of analytical findings involving scheduled chemical warfare agents (OPCW 2020a).

Environmental fate, persistence, and decontamination

Understanding the environmental behavior of A-series ("Novichok") NAs is essential for risk assessment, forensic investigations, environmental remediation, and emergency preparedness. Currently, compared with classical NAs such as sarin, soman, and VX, limited experimental data are available regarding the environmental fate of authentic A-series compounds. Consequently, current knowledge is derived from a combination of analytical investigations, degradation studies, physicochemical modeling, and extrapolation from structurally related OPCs (Bhakhoa et al. 2019; Kloske and Witkiewicz 2019).

Environmental persistence

The persistence of a chemical warfare agent depends on multiple factors, including molecular structure, temperature, humidity, pH, surface characteristics, and sunlight exposure. Available evidence suggests that some A-series compounds may exhibit physicochemical properties associated with potentially increased environmental persistence relative to some classical NAs (Bhakhoa et al. 2019). Proposed explanations include steric shielding around the phosphorus center and reduced susceptibility to spontaneous hydrolysis.

Nevertheless, substantial uncertainty remains regarding the persistence of individual A-series agents under realistic environmental conditions. Published studies are limited in number, often rely on surrogate compounds, and may not accurately reflect the behavior of authentic agents. Consequently, generalized claims regarding exceptional environmental stability should be interpreted cautiously.

Hydrolysis and degradation pathways

Hydrolysis represents one of the principal degradation mechanisms for organophosphorus NAs. The rate and extent of hydrolytic degradation are influenced by environmental conditions and molecular structure. For A-series compounds, available experimental and computational studies indicate that hydrolysis may generate a variety of phosphorus-containing degradation products that can remain analytically detectable even after the disappearance of the parent compound (Mirbabaei et al. 2022; Otsuka et al. 2023a).

These degradation products are of particular forensic importance because they may provide evidence of prior exposure when direct detection of the parent agent is no longer possible. In some cases, degradation products may also contribute to environmental monitoring and attribution efforts.

Despite increasing analytical characterization of hydrolysis products, important uncertainties remain regarding degradation kinetics, environmental transformation pathways, and the toxicological significance of certain secondary products.

Surface contamination and secondary exposure

Following release, NAs may contaminate environmental surfaces, equipment, clothing, and other materials, creating the potential for secondary exposure. The extent and duration of contamination depend on both the properties of the agent and environmental conditions.

Available information from documented poisoning investigations suggests that contamination control and decontamination procedures remain critical components of emergency response. At the same time, systematic studies evaluating the surface persistence and transfer dynamics of authentic A-series agents remain scarce. As a result, risk assessments frequently rely on established principles derived from experience with other organophosphorus chemical warfare agents.

Decontamination strategies

Effective decontamination aims to rapidly remove, neutralize, or degrade toxic agents before significant absorption or secondary exposure occurs. Standard decontamination approaches include physical removal, adsorption, alkaline hydrolysis, oxidative degradation, and the use of specialized decontamination formulations (Munro et al. 1999; Dorandeu et al. 2007).

For exposed individuals, prompt removal of contaminated clothing and thorough washing with soap and water remain among the most important initial interventions. For environmental decontamination, a combination of physical and chemical methods is often required, depending on the contaminated surface and operational circumstances.

Although current decontamination protocols are expected to be broadly effective against A-series compounds, relatively few published studies have systematically evaluated their efficacy using authenticated agents. Additional validation under realistic operational conditions remains desirable.

Computational toxicology and machine-learning approaches

Computational toxicology has become an increasingly important tool for investigating A-series ("Novichok") NAs because experimental studies involving authentic compounds are restricted by safety, regulatory, and ethical considerations. As a result, molecular modeling, quantitative structure–activity relationship (QSAR) analysis, molecular docking, molecular dynamics simulations, density functional theory (DFT) calculations, and machine-learning (ML) approaches have been widely employed to predict physicochemical properties, toxicological behavior, and interactions with biological targets (Bhakhoa et al. 2019; Wang et al. 2021).

Although these approaches provide valuable mechanistic insights, their conclusions must be interpreted cautiously because many predictions remain insufficiently validated by experimental evidence.

Molecular modeling and mechanistic predictions

Molecular docking and molecular dynamics simulations have been extensively used to investigate interactions between A-series compounds and AChE. These studies generally support the hypothesis that A-series agents can efficiently access the catalytic gorge of AChE and form stable phosphylated enzyme complexes (Bhakhoa et al. 2019; Wang et al. 2021).

DFT-based investigations have further explored reaction energetics, phosphylation mechanisms, and potential aging pathways. Several computational studies have proposed structural features that may contribute to high inhibitory potency and reduced susceptibility to oxime-mediated reactivation.

However, because most of these findings are derived from theoretical calculations rather than direct biochemical measurements, they should be regarded as mechanistic hypotheses rather than experimentally established facts. This distinction is particularly important when interpreting predictions related to aging kinetics, enzyme reactivation, and the long-term stability of phosphylated complexes.

QSAR and toxicity prediction models

QSAR methodologies have been applied to estimate the acute toxicity, physicochemical properties, environmental behavior, and biological activity of OPCs. Such models typically use molecular descriptors related to lipophilicity, steric properties, electronic structure, and reactivity to predict toxicological outcomes (Bhakhoa et al. 2019; Franca et al. 2019; Wang et al. 2021).

For A-series compounds, QSAR analyses have generally predicted high biological activity and potent cholinesterase inhibition, consistent with available experimental observations. Nevertheless, prediction accuracy remains dependent on the quality and representativeness of the training datasets used for model development.

Because relatively few authenticated A-series compounds have been experimentally characterized, model extrapolation beyond the available chemical space remains a significant limitation.

Machine learning applications

Recent advances in ML have expanded the ability to analyze large chemical datasets and identify patterns associated with toxicity, enzyme inhibition, and environmental behavior. Algorithms including random forests, support vector machines, gradient-boosting methods, and artificial neural networks have been used to predict toxicological endpoints for OPCs (Cherkasov et al. 2014; Noga et al. 2024).

Potential applications relevant to A-series agents include the following:

- Prediction of acute toxicity;
- Identification of structural determinants of AChE inhibition;
- Prioritization of candidate oxime reactivators;
- Prediction of physicochemical and environmental properties;
- Biomarker discovery and analytical classification.

ML approaches may also assist in the interpretation of complex mass spectrometric datasets and forensic analytical workflows, although their performance remains highly dependent on the quality and diversity of available training data.

Conclusions and future perspectives

A-series ("Novichok") NAs remain among the most scientifically challenging and politically significant OPCs investigated in recent decades. Although substantial progress has been made in understanding their chemistry, toxicological mechanisms, analytical detection, and potential medical management, many aspects of their biological behavior remain incompletely characterized. A recurring theme throughout the available literature is the marked imbalance between public attention and experimentally verified knowledge.

Current evidence indicates that the principal toxic mechanism of A-series agents involves the inhibition of AChE, resulting in profound cholinergic overstimulation and a clinical syndrome broadly consistent with that observed following exposure to other NAs. However,

several characteristics frequently attributed to these compounds—including exceptional environmental persistence, rapid aging of phosphylated AChE, and reduced susceptibility to oxime-mediated reactivation—remain supported largely by computational modeling, indirect observations, or studies involving surrogate compounds rather than authenticated agents. Consequently, such findings should be interpreted cautiously until validated through rigorous experimental investigation.

Recent advances in analytical toxicology, particularly HRMS and protein adduct analysis, have substantially improved the ability to detect and verify exposure to A-series agents. These developments have strengthened forensic attribution capabilities and enhanced preparedness for chemical incidents involving highly toxic OPCs. At the same time, growing interest in bioscavengers, next-generation oximes, catalytic detoxification systems, and computationally assisted antidote discovery has expanded the range of potential medical countermeasures available for future evaluation.

Despite these advances, major knowledge gaps persist. The scarcity of authenticated reference materials, strict regulatory restrictions, and the inherent hazards associated with research on highly toxic compounds have limited the generation of robust experimental data. As a result, important questions concerning toxicokinetics, environmental fate, aging kinetics, biomarker persistence, treatment efficacy, and long-term neurological outcomes remain unresolved.

Future research should prioritize the integration of advanced analytical chemistry, mechanistic toxicology, computational modeling, and translational biomedical research. Particular emphasis should be placed on the experimental validation of computational predictions, development of broadly effective medical countermeasures, identification of reliable long-term biomarkers, and refinement of forensic detection methodologies. International collaboration among toxicologists, analytical chemists, clinicians, regulatory authorities, and public health agencies will remain essential for addressing these challenges.

In conclusion, the available evidence supports the classification of A-series agents as highly hazardous organophosphorus NAs of continuing toxicological, forensic, medical, and regulatory importance. However, many widely cited assumptions regarding their unique properties remain incompletely validated by direct experimental evidence. Progress in the field will depend on maintaining a clear distinction between experimentally demonstrated findings and plausible but as-yet-unconfirmed hypotheses. Such an evidence-based approach is essential for improving scientific understanding, strengthening preparedness for chemical threats, and supporting the development of effective medical and public health responses.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Data availability

All of the data that support the findings of this study are available in the main text.