Exposure Pathways
Dermal and oral contact are the most common exposure pathways.

Toxicokinetics
Choline is metabolized to trimethylamine, which is excreted in skin, lungs, and kidney.

Mechanism of Toxicity
Choline is a cholinergic agonist; therefore, it exerts toxicity by directly hyperstimulating the postganglionic cholinergic receptors. This may lead to stimulation of gastrointestinal, urinary, uterine, bronchial, cardiac, and vascular receptors.

Human Toxicity
The estimated oral lethal dose for humans is 200–400 g. Oral doses of 10 g produce no obvious effect. Vital signs may include bradycardia, hypotension, hypothermia, miosis, salivation and lacrimation, ocular pain, blurred vision, bronchospasm, muscle cramps, fasiculations, weakness, nausea, vomiting, diarrhea, and involuntary urination. No study has reported chronic toxicity of choline.

Clinical Management
Atropine sulfate is the drug of choice. Epinephrin may assist in overcoming severe cardiovascular or bronchoconstriction. Diazepam, phenytoin, and phenobarbital may be given in cases of seizures. Induction of emesis is not necessary due to spontaneous vomiting. Activated charcoal slurry with or without saline cathartic may be used. Sorbitol should not be used because it may contribute to the nausea and diarrhea. Skin decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min.

Animal Toxicity
Animals are known to show similar hyperstimulating effects of the cholinergic system as humans.

—Sushmita M. Chanda

Related Topics

Anticholinergics
Cholinesterase Inhibition
Neurotoxicology: Central and Peripheral

Cholinesterase Inhibition

Introduction and History

Cholinesterases (ChEs) are a ubiquitous groups of enzymes that hydrolyze esters of choline. A well known example is acetylcholinesterase (AChE, acetyl choline hydrolase, EC 3.1.1.7), the enzyme responsible for hydrolyzing the important neurotransmitter acetylcholine (ACh). Another ChE is butyrylcholinesterase (BuChE, acylcholine acylhydrolase, EC 3.1.1.8), also known as nonspecific cholinesterase. The preferred substrate for AChEs is ACh; BuChEs prefer to hydrolyze esters like butyrylcholine and propionylcholine. Both AChE and BuChE are inhibited by some organophosphate (OP) and carbamate (CB) esters, and also by other chemicals.

Many ChE inhibitors act at the catalytic site of the enzyme, forming enzyme-inhibitor complexes that are slow to hydrolyze. The use of ChE inhibitors as insecticides and as chemical warfare agents, their toxicity to humans, and their impact on wildlife have made them important to toxicology researchers and, public health and environmental health officials.

This entry focuses on ChE inhibitions by OPs and CBs. Other chemicals, such as tacrine, cocaine, and succinylcholine, are briefly discussed.

One of the first ChE inhibitors to be studied was a CB, physostigmine (eserine), an alkaloid from the calabar bean (*Physostigma venenosum*) used in a "trial by ordeal" in West Africa. The accused were forced to eat the poisonous beans; survivors were proclaimed innocent. The drug has been used as a treatment for glaucoma since 1877. Englehart and Loewi showed it blocked ChE activity in 1931. Shortly after, neostigmine, an analog, was shown to be effective in the symptomatic treatment of myasthenia gravis.

OPs with high toxicity were synthesized as chemical warfare agents in the late 1930s and early 1940s. During this period Schrader discovered the insecticidal properties of OPs resulting in the synthesis of tetraethyl pyrophosphate in 1941 and of parathion in 1944. Synthetic CBs developed as pesticides have been in commercial use since the 1950s. Some OPs and CBs exhibit toxicities in addition to their direct inhibitions of ChEs. These include long-term and short-term damage to nerves and muscles, mutagenicity, and effects on reproduction.

Acetylcholinesterase, Butyrylcholinesterase, and Other Esterases

AChEs and BuChEs are specialized carboxylic ester hydrolases that preferentially hydrolyze choline esters. They are classed among the B-esterases, enzymes that are inhibited by OPs. Another B-esterase is neuropathy target esterase (NTE), an enzyme implicated in organophosphate–induced delayed neuropathy (OPIDN; see Neurotoxicity, Delayed). Enzymes that actively hydrolyze OPs are known as A-esterases. They provide an important route of detoxification. Examples are paraoxonase and DFPase (Table C-14). Recently, the tertiary structure and amino acid sequences of several AChEs and BuChEs have been elucidated.

ChEs are widley distributed in the body. AChEs regulate excitation at cholinergic synapses by destroying the neurotransmitter ACh. The enzyme is one of the most active known, cycling within a few milliseconds. AChEs are found in excitable tissues at synapses, neuromuscular junctions, myotendinous junctions, central nervous system (CNS) neuron cell bodies, axons, and muscles (Table C-15). AChEs are also found in the erythrocytes [red blood cells (RBCs)] of mammals, in the serum of some birds and mammals, and in the blood platelets of rodents (rats and mice) and ruminants (sheep). (For example, the serum ChE activity of the American Kestrel, a small falcon, consists almost entirely of AChE and the serum ChE of the laboratory rat is high in both AChE and BuChE activities.) The AChE activity of human blood is localized to its RBCs. AChE activity occurs in the serum of developing mammals and birds and in precursors of formed blood elements in some species; it decreases to adult levels after birth.

BuChEs are also widely distributed. They are found at synapses, motor endplates, and muscles fibers together with AChE. BuChE activity in blood is restricted to serum.

Substrate preferences of AChE and BuChE enzymes vary with species. For example, although both mammals and bird AChEs rapidly hydrolyze ACh and its thiocholine analog acetylthiocholine (AcTh), avian AChEs also readily hydrolyze acetyl β-methylcholine and acetyl β-methylthiocholine, while mammal AChEs do not. AChEs and BuChEs respond differently to increasing substrate concentration. AChEs are inhibited

TABLE C-14
Esterase Classes

A esterases
Hydrolyze OPs to inactive products
Found in liver and HDL in plasma
High activity in mammals
Lower activity in birds
Examples: Paraoxonase and DFPase
B esterases
Widely distributed in cells and tissues
Inhibited by OPs and CBs
Slow hydrolysis of OP–enzyme complex
Relatively rapid hydrolysis of CN–enzyme complex
Examples: AChE, BuChE, CaE, and NTE

Note. Abbreviations used: OP, organophosphate ester; HDL, high-density lipoprotein; CB, carbamate; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; CaE, carboxylesterase; NTE, neuropathy target estarase.

TABLE C-15
Cholinesterase Properties

All
Hydrolyze ACh and other choline esters
AChE
Prefers ACh, is inhibited by excess substrates
Found at neural junctions and in mammal RBCs and plasma and platelets of some vertebrates
BuChE
Prefers butyrylcholine, propionylcholine
Widely distributed in vertebrate tissues and plasma

by excess substrate (often above 2 m*M*); BuChEs are less sensitive.

AChEs and BuChEs have multiple molecular forms and complicated life histories (Figs. C-18 and C-19). Some of the forms move from site to site within cells, others are secreted into body fluids. AChEs consist of asymmetric and globular forms. The asymmetric forms tend to be localized at synapses and motor end-plates; they have glycosylated heads joined together by sulfhydryl groups containing the active sites, and collagen tails that attach the enzymes to cell surfaces. The globular forms lack collagen tails; they are made up of the catalytic subunits.

AChE and BuChE subunits are synthesized within cells (e.g., nerve, muscle, liver, and some megakaryocytes), glycosylated within the Golgi apparatus, and secreted. Collagen-tailed forms become attached to the cell surface at specific binding sites. Globular forms are released into body fluids or bind to cell surfaces by ionic bonds. Antibodies have been prepared to several purified AChEs and BuChEs, and protein and nucleic acid sequences have been determined.

The three-dimensional structure of AChE from the electric organ of *Torpedo californica* has recently been established. One interesting feature is that the active site is embedded in a "gorge" of about 20 Å that reaches halfway into the protein. The postulated "anionic site," theoretically invoked to bind the quaternary ammonium ion of ACh, appears to be represented by aromatic amino acids in the gorge itself; these and charges in the active center are believed to stabilize the choline group. In addition, some inhibitions, such as that due to excess substrate, are believed to be due to a "peripheral site." Elucidation of the structure of ChE molecules opens the way to a new generation of "designer" anti-ChE agents with improved specificities of action.

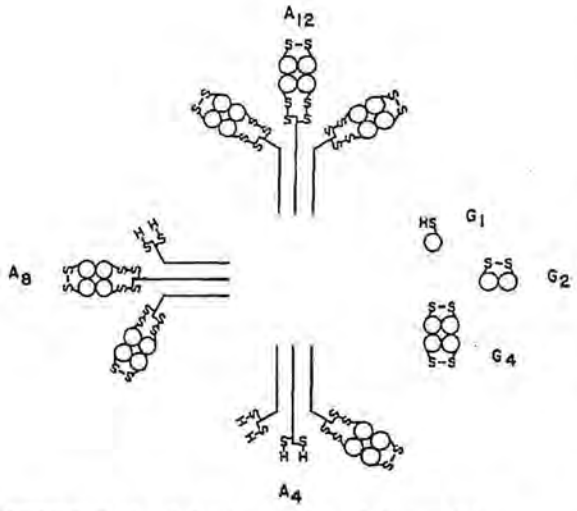


FIGURE C-18. Subunit structure of the multiple molecular forms of ChEs. G, globular forms; A, asymmetric forms with collagen-like tails. Each circle is a catalytic subunit; disulfide bridges indicated by S–S as found in the electric organ of the electric eel (modified from Brimijoin, U.S. EPA Workshop on Cholinesterase Methodologies, 1992).

Functions of Cholinesterases

$$E + AX \underset{k_{-1}}{\overset{k_{+1}}{\longleftrightarrow}} EAX \xrightarrow{k_2} EA \xrightarrow{k_3} E + A$$

where E is the enzyme, AX is the substrate (ACh) or inhibitor, EAX is the reversible enzyme complex, and k's are reaction rate constants.

A hundred years of research has established that a major function of AChE is to hydrolyze the ACh released by cholinergic neurons, regulating the course of neural transmission at synapses, motor end plates, and other effector sites. The reaction is multistep: First is the formation of a reversible enzyme–substrate complex (EAX); second is the acetylation of the catalytic site of the enzyme (EA); and third is the hydrolysis of the enzyme–substrate complex yielding acetic acid, choline, and the regenerated enzyme ($E + A$). The generally accepted mechanism has been (a) an electrostatic attraction between the positive charge on the quaternary nitrogen atom of ACh and the negative charge on the so-called "anionic site" on the enzyme forms the enzyme substrate complex, (b) a basic imidazole moiety (histidine) and an acidic moiety (tyrosine hydroxyl) at the active site catalyze the acetylation of a serine hydroxyl, followed by (c) a rapid deacetylation restoring the enzyme and cleaving acetylcholine into acetate and choline. A similar reaction scheme is believed to apply to BuChEs. It is safe to say that the new information on the conformation of these molecules will soon result in a greater understanding of

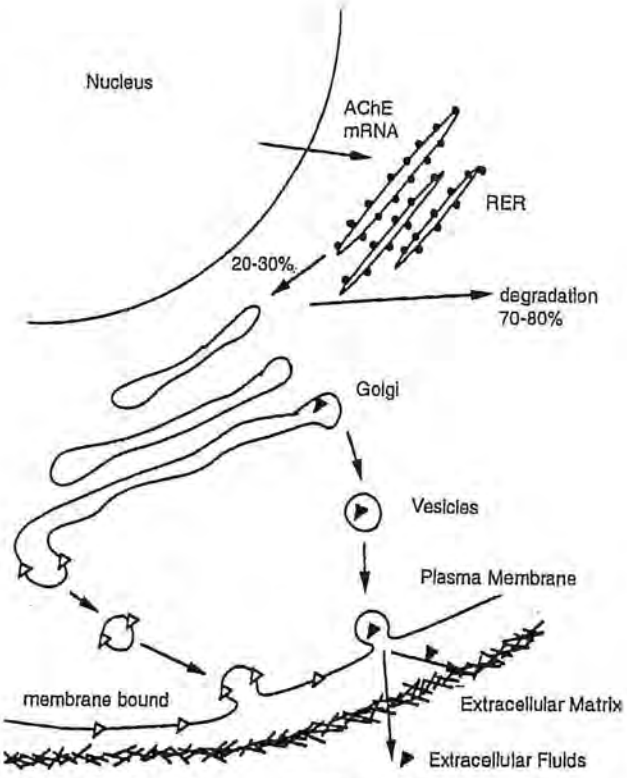


FIGURE C-19. Life cycle of ChEs. AChE is synthesized as a monomer globular form (G_1). Up to 80% is degraded by intracellular processes. Secretory forms are separated from membrane-bound forms, collagen tails are added to asymmetric forms, and the enzyme is glycosylated and becomes enzymatically active. After secretion, globular forms may escape into the body fluids, while asymmetric forms are bound to the synaptic basal lamina (modified from Brimijoin, U.S. EPA Workshop on Cholinesterase Methodologies, 1992).

the biophysical mechanisms underlying their catalytic actions.

In contrast to the functional information available for the roles of ACh and AChE, the function or functions of RBC and serum ChEs are still matters for speculation. One idea is that they protect the body from natural anti-ChE agents (e.g., phyosostigmine) encountered during the evolution of the species; another idea is that they have specific but still unknown roles in tissues. For example, there are recent reports that inhibition of BuChE activity blocks adhesion of neurites from nerve cells in culture and that AChE promotes outgrowth of neurites as if the enzymes had roles in cell adhesion and differentiation.

Toxicities

The toxicities of OPs and CBs often roughly parallel their effectiveness as inhibitors of brain AChE. For example, Fig. C-20 shows the relationship between the toxicity *in vivo* of directly acting OPs and their inhibition of AChE *in vitro*, plotting intraperitoneal LD_{50} versus the P_{50} in mice. (The LD_{50} is the dose resulting in 50% mortality; the P_{50} is the negative logarithm of the concentration of toxicant resulting in 50% inhibition of the enzyme.) Only two of the chemicals tested did not "fit" the curve.

In general, many of the physiological effects of anti-ChEs are those attributable to excess ACh at junctions

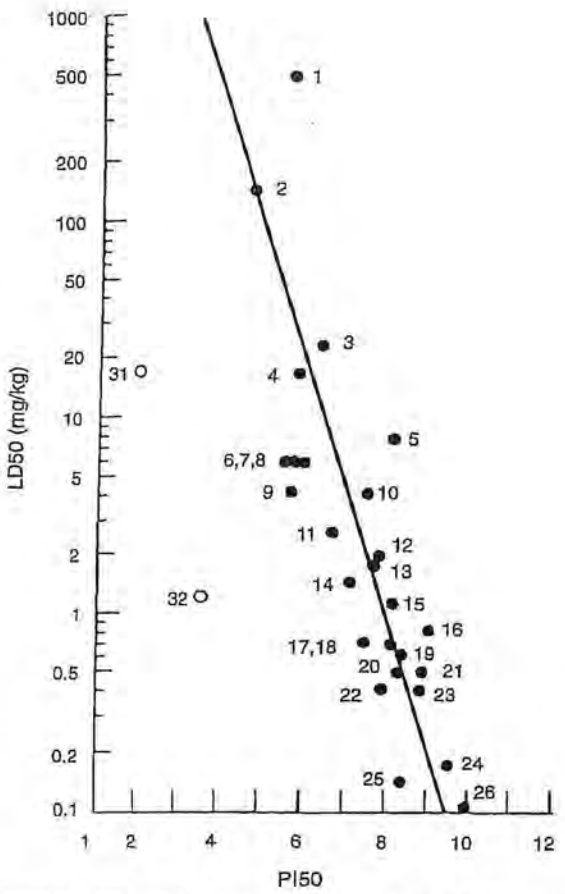


FIGURE C-20. Relationship of the toxicity in vivo *(LD_{50}) of directly acting OPs to AChE inhibition* in vivo *(PI_{50}). 1, dipterex; 2, O,O-diethyl-4-chlorophenyl-phosphate; 3, O,O-diethyl-bis-dimethylpyrophos-phoramide(sym); 4, TIPP; 5, O,O-diethylphosphostigmine; 6, isodemeton sulfoxide; 7, isodemeton; 8, isodemeton sulfone; 9, DFP; 10, diethylamidoethoxy-phosphoryl cyanide; 11, O,O-dimethyl-O,O-diisopropyl pyrophosphate (asym); 12, diethylamidomethoxyphosphoryl cyanide; 13, tetramethyl pyrophosphate; 14, O,O-diethylo phosphorocyanidate; 15, O,O-dimethyl-O,O-diethyl pyrophosphate (asym); 16. soman; 17, TEPP; 18, O-isopropyl-ethylphosphonofluoridate; 19, tabun; 20, amiton; 21, diethylamido ispropoxyphosphoryl cyanide; 22, O,O-diethyl-S-(2-diethylaminoethyl)phosphorothioate; 23, sarin; 24, O,O-diethyl-S-(2-triethylammoniumethyl) thiophosphate iodide; 25, echothiophate; 26, methylfluorophosphorylcholine iodide; 27, methylfluorophosphoryl-B-methylcholine iodide; 28, O-ethyl-methylphosphorylthiocholine iodide; 29, methylfluorophosphoryl-homo-choline iodide; 31, schradan; 32, dimefox. (27–30, LD_{50}s 0.03–0.07) (adapted from Gallo, L., Organophosphorus insecticides, in Handbook of Pesticide Toxicology (W. J. Hayes, Jr., and E. R. Laws, Jr., Eds.,. Vol. 2, p. 932, 1991).*

in the nervous system. The precise symptoms and the time course of ChE inhibition depend on the chemicals and the localization of the receptors affected. The properties of some cholinergic receptors are listed in Table C-16. Cholinergic junctions are classified into several categories based on their pharmacological sensitivities to nicotine, muscarine, atropine, and curare. Early symptoms of cholinergic poisoning represent stimulation of neuroeffectors of the parasympathetic system. These effects are termed muscarinic—stimulated by muscarine and blocked by atropine. Effects include slowing of the heart (bradycardia), constriction of the puplic of the eye, diarrhea, urination, lacrimation, and salivation. Actions at skeletal neuromuscular junctions (motor end-plates) are termed nicotinic—stimulated by nicotine, blocked by curare, but not by atropine. Overstimulation results in muscle fasciculation (disorganized twitching), and, at higher doses, muscle paralysis. A third site of action of anti-ChEs is the cholinergic junctions of the sympathetic and parasympathetic autonomic ganglia. These junctions are also nicotinic—stimulated by nicotine but not muscarine, atropine, or curare, except at high concentrations. Their actions affect the eye, bladder, heart, and salivary glands, with one set often antagonizing the actions of another. Finally, there are the junctions of the CNS: Some are stimulated by nicotine, and some are affected by atropine. They are not responsive to muscarine or curare. CNS symptoms include hypothermia, tremors, head-

TABLE C-16
Properties of Cholinergic Receptors

Muscarinic peripheral NS
Parasympathetic nervous system
Muscarine stimulates
Atropine blocks
Nicotinic peripheral NS
Skeletal muscle motor endplates
Nicotine stimulates
Curare blocks
Atropine has no effect
Nicotinic CNS
Autonomic NS antagonist
Sympathetic and parasympathetic NS
Nicotine stimulates respiratory center

Note. Abbreviations used; NS, nervous system; CNS, central nervous system.

ache, anxiety, convulsions, and coma. Death generally occurs when the agents extensively affect the respiratory centers in the brain. Whether or not there are consistent behavioral effects at low dose levels of OPs and CBs, such as deficits in learning and memory, is a matter of current research, especially on drugs that are under development to treat Alzheimer's disorder.

The excess ACh produced at the motor end-plate brings about a transient myopathy in experimental animals. Experiments *in vivo* and *in vitro* of Dettbarn, Wecker, Salpeter, and others using cholinergic drugs and ACh receptor blockers indicate that excess ACh leads to an influx of Ca^{++} ions and other cations into the post-synaptic cell, resulting in regions of necrosis in the muscle fiber around the motor end-plates. From 10 to 30% of the fibers may be damaged and recovery may take several weeks or more. A disorder known as Intermediate Syndrome (see Organophosphate Poisoning, Intermediate Syndrome) in humans involves prolonged muscle weakness and some muscle damage lasting several weeks or longer after exposure to high levels of some OPs, including methyl parathion, fenthion, and dimethoate.

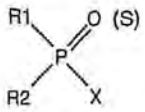
Although most of the effects of OPs and CBs are considered to be caused by AChE inhibition, there is increasing evidence that anti-ChEs directly affect ACh receptors in the CNS and that anti-AChE pesticides depress the immune system in experimental animals.

A few OPs, such as tri-ortho cresyl phosphate (TOCP), leptophos, mipafox, methamidophos, isofenphos, and chlorpyrifos cause OPIDN, a neuropathy that results in death of motor neurons in humans and experimental animals (see Neurotoxicity, Delayed). Some, such as chlorpyrifos and isofenphos, require very high dose levels to be neuropathic—higher levels than could occur if the chemicals were used as directed. TOCP, an industrial chemical, has been responsible for the paralysis of thousands of people since the turn of the century. Inhibition of approximately 70% or more of the carboxylesterase NTE often is associated with the disorder. It is known as a "delayed" neuropathy because onset of the disorder is usually 10 days to several weeks after exposure. Discussion of this neuropathy is beyond the scope of this article, except to note that neuropathic chemicals that are the most dangerous often are those that are better NTE inhibitors than AChE inhibitors, permitting a higher dose of the chemical to be reached before cholinergic symptoms or death occurs. Agricultural chemicals are routinely screened for OPIDN using hens, since chickens are sensitive to the disorder.

The action of many toxicants, including anticholinergic compounds, often involve specific sites on molecules and cells. Such finely-tuned molecular events suggest the possibility of discovering "genocopies," genetic abnormalities that mimic chemically induced disorders. For example, patients have been reported with smaller than normal motor end-plates, defective in AChE, and suffering from muscle weakness. There are no reported AChE-less mutants; it is likely that such a genetic disaster would be lethal. There are humans with inherited differences in their serum BuChEs with decreased activity of the enzyme in their blood. Possessors of these genotypes usually are symptomless, unless they are given succinylcholine (or a similar drug) during surgery to bring about muscle relaxation. Lack of sufficient blood BuChE to speedily destroy the drug intensifies and prolongs the activity of succinylcholine, sometimes with fatal consequences. BuChEs also may play a detoxifying role in cocaine intoxication by hydrolyzing the drug. Several studies on experimental animals indicate that depressing ChEs with anti-ChEs intensifies the toxic effect of cocaine.

Organophosphorus Cholinesterase Inhibitors

OP inhibitors are substituted phosphoric acids of the form



where R_1 and R_2 are usually alkyl or aryl groups linked either directly, or via-O-or-S-groups to the P atom. According to one classification, *X*, termed the leaving group, may be (1) a quaternary nitrogen; (2) a fluoride; (3) a CN, OCN, SCN, or a halogen other than F; or (4) other groups. (see Fig. C-21 for representative organophosphorus cholinesterase inhibitors.)

1. OPs containing quaternary nitrogen (phosphorylcholines) are strong inhibitors of ChEs and directly acting cholinergics. One, ecothiophate iodide, is used in the treatment of glaucoma.

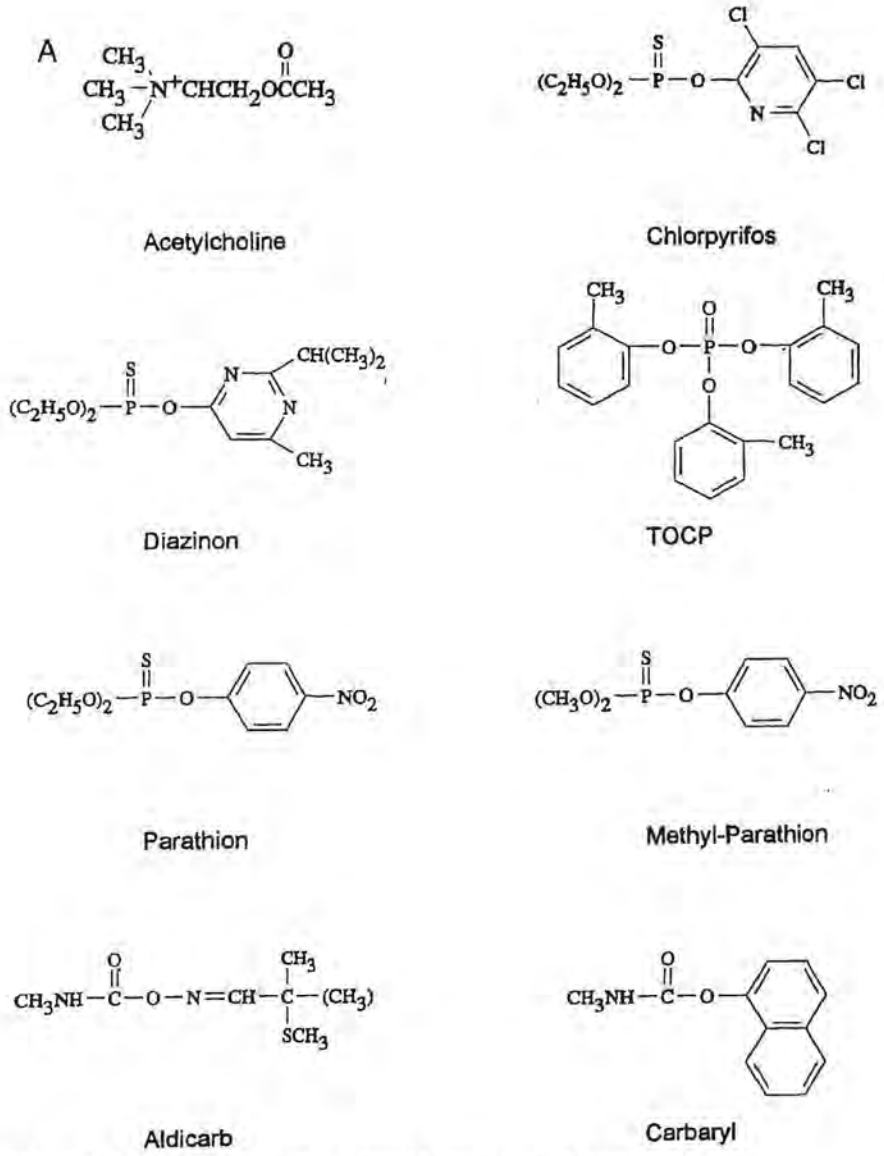
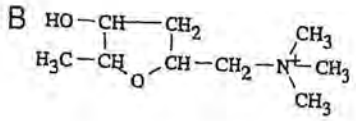


FIGURE C-21. Representative organophosphorus and organocarbamate cholinesterase inhibitors.

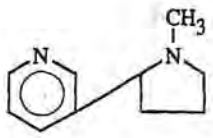
2. Fluorophosphates are also highly toxic and relatively volatile. Sarin and soman are chemical warfare agents. Diisopropyl fluorophosphate (DFP) is often used by biochemists of study serine-active enzymes. Mipafox and DFP cause OPIDN in humans and experimental animals.

3. An example of a CN containing nerve gas is Tabun.

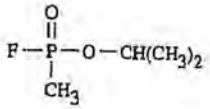
4. Most OP pesticides are in the fourth and largest category. Many are dimethoxy or diethoxy compounds. OPs used in agriculture tend to be manufactured in the relatively stable P=S form. They are less toxic than OPs with the P=O (oxon) group. (Phosphates lack a sulfur atom, phosphorothioates have a single sulfur atom, and phosphorodithioates have two sulfur atoms.) Many pesticides, such as parathion, methyl parathion,



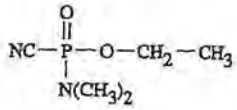
Muscarine



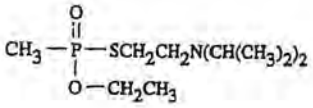
Nicotine



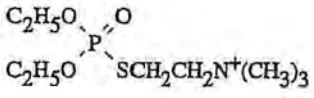
Sarin



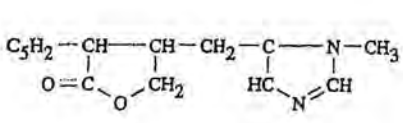
Tabun



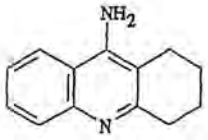
VX



Ecothiophate



Pilocarpine



Tacrine

FIGURE C-21 (continued)

diazinon, and chlorpyrifos, are phosphorothionates.

Three important chemical reactions that underlie ChE inhibitions are hydrolysis, desulfuration, and alkylation.

Hydrolysis: The rate of hydrolysis is a function of the acid and alcohol groups, pH, and temperature. It usually increases with increasing pH, temperature, and UV light.

Desulfuration: An important oxidation is the conversion of the P=S group of phosphorothionates to P=O, the oxon form, increasing the intensity of ChE inhibition.

Alkylation: Alkyl substituents, especially methoxy groups, may act as alkylating agents. They are capable of altering nucleic acids, leading some to be concerned about OPs as mutagens.

Carbamate Cholinesterase Inhibitors

The CBs used as pesticides are N-substituted esters of carbamic acid. CBs developed in the 1950s as insect

repellents were found to have insecticidal activity, leading to the development of the napthyl CBs with high anti-ChE activity and selective toxicity against insects. One example is carbaryl; it is widely used because of its low toxicity to mammals and its degradability. Aldicarb, a plant systemic, is more toxic than carbaryl. Recently, aldicarb was associated with a July 4th holiday incident when West Coast residents complained of anticholinergic symptoms after eating watermelons that may have become contaminated with aldicarb.

Most *N*-methyl and *N,N* dimethyl carbamates are better AChE inhibitors than BuChE inhibitors. However, *N*-carbamylated AChE spontaneously reactivates faster than *N*-carbamylated BuChE. AChE activity may recover as rapidly as 30 min following exposure—much faster than after exposure to OPs.

Although phosphorylation of AChE by OPs is heavily influenced by the electron withdrawing power of the leaving group, carbamylation by methyl carbamates is also greatly dependent on molecular complementarity with the conformation of the enzyme as well as reactivity of the molecule. In general, phenolic and oxime moieties are more reactive than benzyl alcohol groups.

N-methyl carbamates do not need activation to inhibit ChEs. However, at least in the case of aldicarb, inhibition increases with metabolism. Aldicarb is rapidly oxidized to the relatively stable aldicarb sulfoxide, which in turn is more slowly metabolized to aldicarb sulfone, a stronger AChE inhibitor. These products are then detoxified by conversion to oximes and nitriles, which in turn are degraded to aldehydes, acids, and alcohols. Procarbamate derivatives were developed to reduce the toxicity of CBs to mammals; the hydrogen atom on the carbamate nitrogen is replaced by a wide variety of nucleophiles—many with a sulfur atom—causing reduction in anti-ChE activity. The bond is rapidly broken in insects, restoring the activity and toxicity of the parent compound.

The rapid spontaneous reactivation of carbamates can be a problem in determining ChE activity. For example, some testing routines require that animals be put on a control diet for 24 hr before sampling. With CBs, the inhibitions may have disappeared by the time the assays are performed. In addition, the dilutions specified in some assays may reduce the inhibition and high concentrations of substrate may compete with the carbamate to further reactivate the enzyme.

Chemical Warfare Anticholinesterase Agents

Anticholinesterase chemical warfare agents have been available and stockpiled since their development immediately before and during World War II. Many countries have active research programs into their toxicity and control. P=O groups confer potent anti-ChE inhibition properties. (However, the toxicity of agents such as soman and VX may be due in part to their actions on receptor and perhaps other proteins as well as to their inhibition of AChE.) The toxicity of the nerve agents is greater than that of agricultural chemicals. For example, the dermal LD_{50} of agent VX is estimated to be 0.04–0.14 mg/kg for humans, which is at least an order of magnitude more toxic than most pesticides.

LD_{50}s for representative agricultural OPs and CBs are shown in Table C-17.

Assay Techniques

An early assay for ChE activity was a manometric method in which the change in pH due to ACh hydrolysis released CO_2 from a reaction buffer. A common technique (that of Michel) directly determines ACh hydrolysis by changes in pH. Another assay, that of Hestrin, utilizes the reaction of ACh with hydroxyl amine and ferric chloride, producing a reddish-purple complex. A test developed by Okabe and colleagues oxidizes the choline released from ACh hydrolysis and determines the H_2O_2 produced. Several assays use radioactive ACh; one method counts the acetate produced by the reaction by separating it into an organic phase, leaving the unhydrolyzed ACh behind in an aqueous phase. Another common approach utilizes thioanalogs of ACh and other esters. In the assay developed by Ellman and colleagues in 1961, hydrolysis of thiocholines such as acetylthiocholine (AcTh) is measured at 410 nm with the color reagent dithionitrobenzoate. Although assays that rely on pH or radioactivity of ACh have the advantage of using a natural substrate, assays utilizing thiocholine esters are inexpensive, readily automated, and do not require expensive disposal of radioactive wastes. Negative features are the possibility of interference of hemoglobin (Hb) in RBC samples and a nonliner reaction of the reduced glutathion in some RBCs with the color reagent. Some of

TABLE C-17
Representative Acute LD50s of Selected Organophosphates and Carbamates

Compound	*LD50 (mg/kg)*	
	Oral	*Dermal*
Organophosphates		
Dimethoxy compounds		
Azinphosmethyl (*O,O*-dimethyl-*S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl]phosphorodithioate)	13	220
Malathion (*O,O*-dimethyl-*S*-(1,2-dicarbethoxyethyl)phosphorodithioate)	1375	>4000
Methyl parathion (*O,O*-dimethyl-*O*-(*p*-nitrophenyl)phosphorothioate)	14	67
Diethoxy compounds		
Parathion (*O,O*-diethy-*O*-(4-nitorphenyl)phosphorothioate)	13	21
Diazinon (*O,O*-diethyl-*O*-(2-isopropyl-6-methyl-4-pyrimidnyl)phosphorothioate)	108	200
Carbamates		
Aldicarb (2-methyl-2-(methylthio)propylideneamino-*N*-methylcarbamate)	0.8	3.0
Carbaryl (1-naphthyl-*N*-methylcarbamate)	850	>4000

Note. Adapted from Gaines, *Toxicol. Appl. Pharmacol.* 14, 515–534, 1969.

the methods have been adapted for field use. A new generation of techniques utilize amperometric methods in which enzymes bound to electrode-like probes permit assay for the presence of anti-ChE chemicals. Whatever the assay, it is important that its conditions be validated for the species, tissues, and chemicals under study.

Biochemistry of Cholinesterase Inhibition

The inhibition of the activity of ChEs by OPs and CBs proceeds in a manner similar to the action of the enzymes on ACh. However, instead of forming a rapidly hydrolyzed acetyl–enzyme complex, the OPs and CBs respectively phosphorylate and carbamylate the catalytic sites of the enzymes. The major biochemical features of the inhibition of ChEs by OPs and CBs involve (a) activation of the inhibitors; (b) detoxification; (c) reaction of the inhibitor with the serine active site of the enzyme and loss of a "leaving group"; (d) hydrolysis of the complex and spontaneous reactivation of the enzyme; (e) loss of a second group, known as aging; and (f) recovery by synthesis of new enzyme.

One way to visualize the biochemical mechanisms underlying the toxicity of OPs and CBs is to trace the fate of an OP such as parathion from its entry into the body. Mixed function oxidases (MFO) in the liver (or in other tissues) convert parathion, a thionophosphate, to its oxygen analog, paraoxon, increasing its anti-ChE potential by orders of magnitude. The paraoxon may exert its toxic action by inhibiting AChE, or be inactivated by conjugation with glutathione, reaction with glutathione transferases, further oxidation by MFO, or hydrolysis by A-esterases, in this case paraoxonase. Such reactions may lead to a loss in toxicity of either parathion or paraoxon. Paraoxon may also be inactivated by binding and reacting with B-esterases other than AChE, such as BuChE and carboxylesterases.

The reaction of an OP with AChE, BuChE, or other B-esterases is similar to the reaction of AChE with ACh, except that the hydrolysis step is much slower; or, in some cases, may not occur at all. Its basis is a phosphorylation of the enzyme via a nucleophilic attack. The electronegative serine hydroxyl at the catalytic site reacts with the electropositive phosphorus atom of the inhibitor to form an OP–ChE complex and loss of a side group on the phosphorus atom, known as the leaving group (X). The phosphorylated enzyme may, in time, reactivate by rehydrolysis. A similar set of reaction leads to carbamylation, except that the spontaneous reactivation tends to be more rapid than that for an OP. Spontaneous reactivation of an OP may take hours to days, whereas CBs may reactivate as soon as 30 min. In addition, OPs undergo a further reaction known as "aging" in which a second group (often an alkyl group) is lost from the phosphate, stabilizing the OP–ChE complex.

Structure/Activity

Some general rules for OPs based on their structures include the following:

1. The P=O group is more toxic than the P=S group because it is more reactive. It is more reactive due to its higher electronegativity, which causes a more electropositive P atom, facilitating its reaction with the serine hydroxyl at the active site.

2. The electron-withdrawing ability of the leaving group *X* is predicted by the strength of its acid. For example, fluoride is a more powerful leaving group than nitrophenol since HF is a strong acid.

3. Reactivity of the R groups is in the order methoxy ≧ ethoxy ≧ propoxy ≧ isopropoxy ≧ amino groups. The more difficult a compound is to hydrolyze, the weaker is likely to be its ChE inhibition.

4. Steric effects are also important. The longer and more branched a compound, the more reduced is its rate of inhibition, probably because of the conformation of the proteins around the catalytic site.

The terms "reversible" and "irreversible" are often misused in describing Che inhibitions. For example, statements such as "OPs are irreversible inhibitors and CBs are reversible inhibitors" are useful insofar as they refer to the stability of the aged OP enzyme and to the more rapid hydrolysis of the CB enzyme compared to that of the unaged OP enzyme. Technically, one could argue that the term reversible should be reserved for cases in which there is an equilibrium between the substrate and the enzyme–substrate complex.

Spontaneous Reactivation of Organophosphates

Table C-18 lists the half-lives of recovery for some OP-inhibited AChEs. In general, OP–AChE complexes from dimethoxy-substituted OPs (e.g., malathion) spontaneously dephosphorylate faster than diethoxy (e.g., parathion) or diisopropoxy (e.g., DFP) complexes. Eto pointed out in 1974 that the stability of a phosphorylated AChE may be predicted from the stability of the specific OP inhibitor itself. One possibility is that methyl groups have less steric hindrance and greater electronegativity than ethyl or isopropyl groups.

Chemical Reactivation of Organophosphates

It has been almost 40 years since I. B. Wilson and colleagues observed that nucleophiles, oximes like hydroxamic acid, reactivated OP-inhibited AChE above and beyond that occurring from spontaneous reactivation, opening the way to a treatment for OP poisoning. The oxime registered for use in the United States is 2-PAM Cl (Protopam); its methanesulfonate salt (P2S) is used in Europe. Oxime therapy should be recommended with caution for carbamate poisonings. Although it has been reported to have been beneficial in the case of aldicarb, there is evidence that 2-PAM treatment increases the toxicity of carbaryl.

The mechanism of action of oxime reactivation involves transfer of the substituted phosphate or phosphonate residue from the catalytic site of the enzyme to the oxime. In addition, 2-PAM may react directly with the free OP molecule itself. Other oximes, such as TMB-4, obidoxime, and Hl-6, are reported to be superior to 2-PAM as reactivators and antidotes to chemical warfare agents. Oxime therapy should not be used in the absence of ChE inhibition since 2-PAM itself is a weak ChE inhibitor. In addition to the reactions discussed previously, direct effects of these compounds on muscle contraction and nicotinic receptors led Albuquerque and colleagues to propose that oximes also act directly on cholinergic receptors.

Aging

Research on oximes revealed an important phenomenon: The extent of reactivation of an OP–AChE complex decreased with time and depended on the OP used. This "aging" prevents both spontaneous and chemical reactivation. Evidence indicates aging is due to the loss of a second group from the phosphorus atom. Harris and colleagues in 1966 demonstrated the loss of an alkyl group from a soman–AChE complex, and showed that the percentage of enzyme losing an alkyl group was correlated to the percentage of enzyme resistant to oxime activation. In general, OP–ChE complexes that spontaneously reactivate slowly tend to age

TABLE C-18
Spontaneous Reactivation and Aging of Selected Organophosphates

Compound	*Tissue*	*Spontaneous (hr)*	*Aging (hr)*
Malathion	Human RBC	0.85	3.9
Methamidophos	Bovine RBC	0.13	0.54
Chlorpyrifos	Bovine RBC/mouse brain	58	36
Diazinon	Human RBC	58	41
Parathion	Rat brain/bovine RBC	103	58
Tabun	Human RBC	ND	13
Sarin	Human RBC	ND	3.0
DFP	Human RBC	ND	4.6
Soman	Human RBC	ND	0.02

Note. Adapted from Wilson *et al.*, In *Organophosphates, Chemistry, Fate, Effects* (J. E. Chambers and P. E. Levi, Eds.), Academic Press, New York, 1992.

rapidly. Exceptions are dimethoxy-phosphorylated AChEs, which both rapidly age and spontaneously reactivate. In general, agricultural chemicals (e.g., malathion, parathion, and diazinon) have half-lives of aging of hours and longer, while chemical warfare agents age rapidly (e.g., 10 min for soman).

Treatment for Anticholinesterase Poisoning

The information included here is educational; it should not be construed as specific recommendations for treatment of patients.

Inhibitions of ChEs by OPs and CBs are one of the few toxicities for which there are antidotes. The usual treatment for OP poisoning is atropine (Table C-19). The presence of atropine reduces the effectiveness of the ACh receptors, counterbalancing the excess ACh present. The recommended doses for humans are 1 g PAM Cl (intramuscular or intravenous) two or three times a day, and 2 mg atropine (intravenous) at 15- to 30-min intervals as needed. Higher doses may be used, depending on the extent of the OP intoxication. Environmental Health Criteria No. 63 describes the case of a patient who drank a large amount of dicrotophos while inebriated. Treatments were progressively increased up to 6 mg atropine intravenously every 15 min with continuous infusion of 2-PAM Cl at 0.5 g/hr. All told, 92 g of 2-PAM and 3912 mg of atropine were given to the patient, who was discharged after 33 days.

TABLE C-19
Treatments for Anticholinesterase Poisoning

Atropine
2 mg intravenously, at 15- to 30-min intervals as needed to suppress symptoms
2-Pralidoxime
1 g either intramuscularly or intravenously two or three times per day or to suppress symptoms
Diazepam
10 mg subcutaneously or intravenously, repeated as required

Note. Adapted from Environmental Health Criteria 63, 1986.

Much of the research on treatments of ChE inhibitions has concerned chemical warfare agents providing little direct information for the treatment of agricultural chemicals.

Considerable attention has been given to prophylactic treatments to protect military units and civilian populations in the event of either accidental or deliberate release of nerve gas agents. One kit contains a combination of atropine, 2-PAM, and diazepam. Another contains pyridostigmine, a carbamate with actions similar to physostigmine. Diazepam is included to lessen CNS symptoms. The use of pyridostigmine is based on the idea that if a readily rehydrolyzable carbamate will compete for AChE catalytic sites with the high-affinity binding nerve gas agents, it may reduce the percentage of AChE that becomes irreversibly inhibited. Using these agents is not without risk, since they are themselves toxic. Issuance of atropine kits to the general population of Israel during the Persian Gulf crisis led to the accidential injection of more than 200 children;

some had systemic effects but fortunately there were no fatal consequences.

The discovery of methods to isolate relatively large amounts of ChE enzymes in essentially pure form has led to a unique method of treating OP intoxication—that of adding ChEs to the blood. Several experiments indicate enough of the OPs bind to the ChEs to reduce their toxicity in experimental animals. One issue is that of possible immune responses to what might be recognized by the body as a foreign protein, but to date there is no evidence suggesting this to be a problem.

Treatments with Anticholinesterase Agents

Several anticholinesterase agents have been used to treat human disorders.

Alzheimer's Disease and Tacrine

The findings that senile dementia of the Alzheimer's type was accompanied by a loss of AChE activity (as well as other neurochemical markers for cholinergic neurons) in parts of the brain has stimulated study of cholinergic nerve activity, learning and memory, and the use of anti-ChE compounds in the treatment of Alzheimer's disease. The strategy is to increase the effective level of ACh by reducing the activity of the AChE present. One drug under test is physostigmine; others are Tacrine and its analogs. Tacrine is a weakly binding anti-ChE agent, recently approved for treatment by the U.S. FDA. The dose of Tacrine recommended (100 mg/day) was chosen on the basis of the side effects the drug has on liver function rather than on unequivocal demonstration of its effectiveness. (In some trials, up to a third of the patients were removed from the studies due to side effects of the drug.)

Glaucoma

Glaucoma is a disorder of vision accompanied by an increase in ocular pressure. Although mostly replaced by other drugs (e.g., beta-blockers, and pilocarpine), anti-ChE drugs such as ecothiopate and pyridostigmine are still used in the treatment of these common disorders.

Myasthenia Gravis

Myasthenia gravis is a progressive disorder characterized by muscle weakness; eye muscles are often the first affected. Research has shown it to be an autoimmune disease in which the victim forms antibodies to his or her nicotinic acetylcholine receptors at motor endplates. It is characterized by fatigability and weakness of skeletal muscles, especially those of the eyes. Approximately 90% of the patients have droopy eyelids and double vision. Treatments include corticosteroids and thymectomy to reduce the actions of the immune system and anti-ChE agents such as pyridostigmine to improve the effectiveness of the receptors that remain.

Wildlife and Domestic Animal Exposures

The recognition that chlorinated hydrocarbons are a persistent danger to wildlife led to a decrease in their use as agricultural chemicals and to an increase in the use of OPs and CBs. In general, OPs and CBs do not bioaccumulate as do chlorinated hydrocarbons and they are relatively biodegradable. However, they are more acutely toxic than chlorinated hydrocarbons to humans and wildlife. A thorough discussion of the comparative toxicology of OPs and CBs is outside the scope of this entry. ChE inhibitions are generally the same, regardless of the animal; differences between species are often in the overall pharmacokinetics and metabolism. For example, although birds have higher brain AChE activities than mammals, they also have less hepatic MFOs to activate OPs and less A-esterases to hydrolyze them. Much research has been done on the toxicology of OPs to wild birds from sparrows to hawks and eagles. For example, E. P. Hill and colleagues of the U.S. Fish and Wildlife Service studied the toxicity of 19 OPs and 8 CBs to 35 species of birds. In general, such studies showed that over 50% of OPs and 90% of CBs have LD_{50}s of less than 40 mg/kg for most birds.

Route of exposure may have much to do with the recovery from OPs. When pigeons were treated orally with an OP, inhibition of blood ChE was rapid, and recovery of activity occurred within a few days. However, when the treatment was conducted dermally, putting the OP on the feet, recovery of activity took several

weeks, implying the presence of a depot for OPs and the possibility that birds can accumulate OPs by flying from site to site. The possibility of bioaccumulation of OPs in a food chain (usually considered to be a characteristic of chlorinated hydrocarbons) was demonstrated by the report of an eagle poisoned by an OP (Warbex) in magpies that, in turn, had obtained the OP by ingesting hair from a steer that had been treated with it for internal parasites.

Beef cattle, horses (more than sheep), goats, and swine are treated several times each year with OPs to control parasites and are fed tetrachlorvinphos to prevent fly larvae hatching in their feces. Carbaryl is commonly used for flea and tick control. Oehme states that insecticides are a common cause of poisoning of domestic animals and that "the majority of insecticide problems in domestic animals result from ignorance or mismanagement." Indeed, there is some epidemiological evidence that animal technicians in pet grooming and veterinary hospitals are exposed to the OP and CB chemicals used to control fleas and ticks while washing the animals.

Exposures in the Workplace

Worldwide, estimates of the number of humans requiring treatment due to anti-ChE chemicals run into the many thousands annually. Concern for those who manufacture and use agricultural chemicals has resulted in studies of pesticide residues, protective clothing, urinary metabolites, and blood ChE levels of farmworkers, greenhouse workers, and spray applicators. In general, the rule has been to consider decreases of blood cholinesterases of 30% or more as meaningful, signifying the worker should be removed from contact with the agent. In the United States, California requires workers to be monitored; however, even there, there is no single standard method to determine ChE activities.

Chemical Warfare and Terrorism

The use of chemical weapons, nerve gases, mustard gases, and blistering agents is banned by international treaty. Nerve agents are known to have been released from storage sites during the Persian Gulf conflict. At the time this entry was written, the role that nerve agents may have played, whether alone or in company with other chemicals, in a baffling set of symptoms known as the Gulf War syndrome is under investigation.

Millions of pounds of chemical warfare agents are stored throughout the world. Their destruction by incineration at high temperatures, up to 2500°F (1480°C) is planned or under way in several countries. These include eight sites in the United States, such as the Tooele Army Depot in Utah and Johnston Atoll in the Pacific, which is 750 miles from Hawaii. Some of the ordinance has been stored since World Wars I and II. Complaints have been lodged by citizens groups concerned about possible risks to residents during the destruction of the chemicals.

Recently, General Schwartzkopf warned of the danger that chemical warfare weapons pose in future conflicts. Two recent episodes in which sarin was used by terrorists in Japan cast a cloud over attempts to control the use of these weapons. Sarin was released in a residential area of the city of Matsumoto on June 27, 1994, and in a crowded Tokyo subway less than a year later, on March 20, 1995. In Matsumoto, about 600 residents and rescuers were affected and 7 died. More than 5500 people were poisoned and 12 died in the Tokyo incident. Many more might have perished if it were not for the quick action and bravery of firemen, police, and others and the availability of antidotes in Japanese hospitals. (Two subway attendants died removing containers of sarin from subway cars.)

Significance of Blood Cholinesterase Levels

There has been a continuing discussion of the significance of monitoring blood ChEs of humans and wildlife. The setting of no-observable-adverse-effect levels (NOAELs) is an example. (NOAELs are the highest dose levels at which no important effect of a drug is observed). Determining NOAELS is an important step in assigning risks and safe levels for the use of a toxic chemical. Some propose that batteries of behavioral tests performed under controlled laboratory conditions provide the best data for setting safe levels of exposure. Under field conditions others propose that measurements of residues on skin and clothing, urinary metabolites of agricultural workers, and fecal metabolites of wild animals provide evidence of exposure to chemicals without invasive procedures. Proponents of the use of ChE levels point out that they represent standardized,

relatively inexpensive measurements that directly demonstrate a biochemical effect of an exposure to a toxic chemical rather than merely providing evidence of the exposure itself. Recent technology permits determinations of enzyme activities on 100 μl or less of blood, obtainable by a finger prick.

Regardless, as long as millions of pounds of OPs and CBs are used annually, ChE measurements will be a useful tool in the protection of humans, domestic animals, and wildlife from overexposure to these toxic agents.

Further Reading

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—Barry W. Wilson

Related Topics

A-esterase
Anticholinergics
Carbamate Pesticides
Carboxylesterases
Nerve Gases
Neurotoxicity, Delayed
Neurotoxicology: Central and Peripheral
Organophosphate Poisoning, Intermediate Syndrome
Organophosphates
Pesticides
Veterinary Toxicology

Chromium (Cr)

- CAS: 7440-47-3
- SELECTED COMPOUNDS: Chromous chloride, $CrCl_2$ (CAS: 10049-05-5); chromous sulfate, $CrSO_4$ (CAS: 13825-86-0); chromic oxide, Cr_2O_3 (CAS: 1308-38-9); chromic sulfate, $Cr_2(SO_4)_3$ (CAS: 10101-53-8)
- CHEMICAL CLASS: Metals

Uses

Chromic oxide (chromium III) is an essential element. Commercially, chromium is used extensively in metal plating to obtain a shiny surface. It is a major ingredient for alloying with iron to produce special stainless steels. By treating the ore followed by electrolysis, the pure metal is obtained. Roasting the ore with lime or soda ash produces the chromate and dichromate, which have extensive commercial uses in pigments, electroplating, catalysts, corrosion inhibition, leather tanning, and wood preservation.

Exposure Pathways

Most human exposure to chromium is by ingestion, although inhalation and dermal contact are possible exposure pathways. Low levels of chromium III are found in some meats and vegetables. This biologically essential form is also found in yeasts and liver. Chromium intake in food is low, with daily human intakes less than 100 μg per day; most of this chromium intake is due to ingestion of food with minimal contribution by water supplies and ambient air.

In air and drinking water, chromium is predominately found in its hexavalent form (chromium VI), although most publications report total chromium, which includes chromium present in all valence states. The main sources of chromium VI in the ambient air are ore refining, fossil fuel combustion, cement production, and industrial operations producing fly ash.

Both chromium VI and chromium III are found in nature, but the latter predominates. Chromous (chromium II), a biologically inactive valence state, exists, but once exposed to air it rapidly oxidizes to chromium III, a biologically active form of chromium. Although