CLIN. CHEM. 41/12, 1814–1818 (1995) • **European Beckman Conference**

Cholinesterase Inhibition: Complexities in Interpretation

Marcello Lotti

Cholinesterases are measured to assess exposures to or effects of organophosphorus esters and carbamates. Plasma butyrylcholinesterase is usually most sensitive to inhibitors, but it has no known physiological function(s); its inhibition reflects exposure. The physiological function of erythrocyte acetylcholinesterase (AChE) also is not known, but the enzyme is the same as that involved in synaptic transmission and its measurement is used to mirror effects on the nervous system. Erythrocyte AChE has large inter- and intraindividual variation, and small changes are detectable by comparison with preexposure values. The relation between inhibition of erythrocytes and nervous tissue AChE depends on the pharmacokinetics of inhibitors. Usually, erythrocyte AChE inhibition overestimates that in the nervous system. Pharmacodynamic factors such as spontaneous reactivation and aging of inhibited enzyme should also be considered in assessing AChE inhibition. Other factors, such as timing of measurement, add complexity because erythrocyte AChE inhibition persists longer than that in the nervous tissues. Cholinergic transmission might also be impaired because of direct effects of organophosphorus esters and carbamates on receptors.

Indexing Terms: *organophosphorus esters/carbamates/toxicology/pesticides*

Organophosphorus esters (OP) and carbamates form a large family of chemicals widely used as pesticides. For most of them, pesticidal action is the result of inhibition of acetylcholinesterases (AChE) at nerve endings (*1*).

Humans are exposed to cholinesterase inhibitors at the workplace, from food and drinking water, and in the environment. Several studies on workers who spray and apply insecticides assessed exposure to a variety of OPs and have previously been summarized (*2*). Exposure of the general population through food contamination is generally low (*3*), as are exposures from drinking water and environmental air, unless accidental. Biomarkers for these exposures have been developed and include measurements of AChE in erythrocytes and butyrylcholinesterase in plasma (*4*, *5*).

Methods for measuring blood cholinesterases are validated and have been widely used (*5*). Nevertheless, given the substantial reduction of exposures that is observed now, particularly at the workplace, and the subsequent need to assess small changes in cholinesterase activity, a better appreciation of the complexity in interpreting such tests is useful. Besides dose and persistence of compounds, some key factors influencing the degree of cholinesterase inhibition are discussed.

Istituto di Medicina del Lavoro, Università degli Studi di Padova, Via Facciolati 71, 35127 Padova, Italy. Fax +39 49 8216644; e-mail lottitox@ipdunidx.unipd.it.

Received June 12, 1995; accepted July 28, 1995.

Inhibitor–Cholinesterase Interactions

AChE at nerve endings represents the molecular target of OP and carbamate toxicity. When the enzyme is blocked, it can no longer participate in the hydrolysis of acetylcholine. Thus, the neurotransmitter accumulates; its action is enhanced; and, given the widespread distribution of cholinergic transmission, toxic effects involve the parasympathetic, sympathetic, motor, and central nervous systems.

The three-dimensional atomic structure of AChE has only recently been determined (*6*), and probably our knowledge of the molecular interactions between AChE and inhibitors will be revised. For instance, the anionic subsite of AChE, thought to attract the quaternary nitrogen of substrate, may be misnamed because it contains, at most, one negative charge. Instead, the quaternary moiety of acetylcholine is proposed to bind chiefly through interactions with aromatic residues that line the walls and floor of the active-site gorge.

Both substrate and inhibitors react covalently with the esterase in essentially the same manner because acetylation of the serine residue at the AChE catalytic center is analogous to phosphorylation and carbamylation (*7*) (Fig. 1). In contrast to the acetylated enzyme, which rapidly yields acetic acid and restores the catalytic center, the phosphorylated enzyme is stable. Carbamylated AChE restores its catalytic activity more slowly than acetylated AChE but more rapidly than the phosphorylated enzyme. However, inhibited AChE might also reactivate spontaneously when inhibited by OPs. Reactivation requires several hours, as with dimethoxyphosphorylated AChE, or does not occur at all, as with AChE phosphorylated by OPs with secondary or tertiary alkyl groups. For these reasons, carbamates and dimethyl organophosphates are considered reversible inhibitors, whereas other OPs are irreversible. The loss of one alkyl group, through a nonenzymatic process called aging, further enhances the stability of the phosphorylated enzyme.

Figure 2 shows some examples of these reactions between AChE and inhibitors. The degree of AChE inhibition and its duration in vivo largely depend on these rates (*7*, *8*).

Extrapolation from Inhibition in Blood to That in the Nervous System

AChE is involved in synaptic transmission but is also present in the outer membrane of erythrocytes and to a

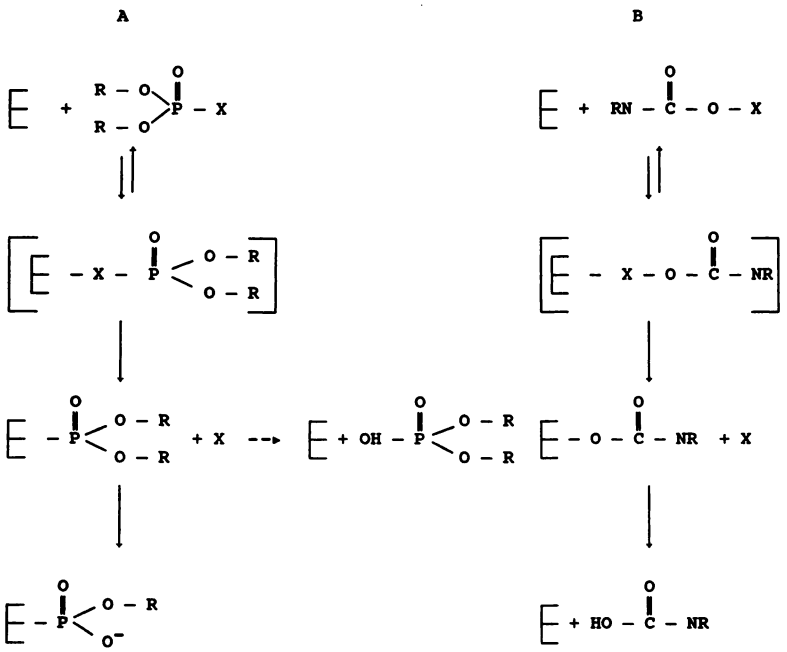


Fig. 1. Interactions of esterases (E) with OP (*A*) and carbamates (*B*).

When inhibitors reach the catalytic center, they either phosphorylate or carbamylate the enzyme via the formation of a Michaelis complex. The major difference between OPs and carbamates is that reactivation is quick with the latter, whereas spontaneous reactivation is slow with the former. Phosphorylated enzyme might also undergo the aging reaction, which involves the loss of an R group, leading to a negatively charged phosphoryl–enzyme complex. Aging further enhances the stability of phosphorylated enzyme.

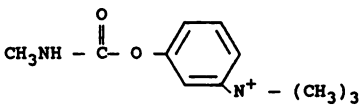
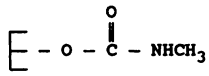
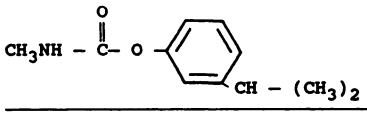
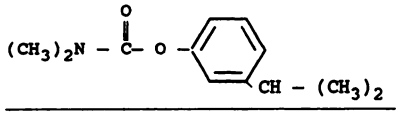
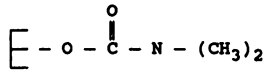
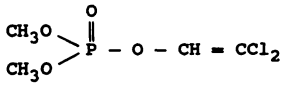
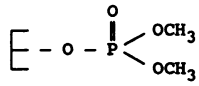
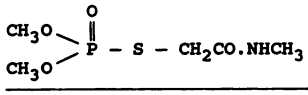
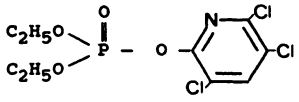
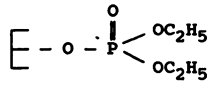
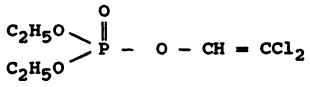
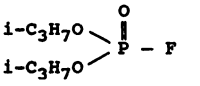
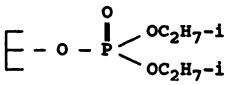
	Chemical	Inhibition[a] (AChE I_{50}, M)	Inhibited AChE	Spontaneous reactivation (t½ hours)	Aging (t½, hours)
1		1.2×10^{-11}		0.5	NO
2		6.4×10^{-8}			
3		1.1×10^{-5}		0.9	NO
4		9.5×10^{-7}		0.85	3.9
5		1.4×10^{-4}			
6		7×10^{-9}		58	41
7		4.1×10^{-7}			
8		8.3×10^{-7}		no reactivation at 6	4.6

Fig. 2. Interactions of inhibitors with AChE.

[a]Concentration to inhibit 50% of AChE at comparable conditions. Bovine erythrocytes (*7*) were used in carbamate experiments, and human brain or erythrocytes were used in OP experiments (*9–11*). Compounds are as follows: *1*, (3-hydroxyphenyl)trimethylammonium *N*-methylcarbamate (analog of neostigmine); *2*, 3-isopropylphenyl *N*-methylcarbamate (*m*-cumenylmethylcarbamate); *3*, 3-isopropylphenyl *N*-dimethylcarbamate (analog of compound *2*); *4*, dimethyldichlorovinyl phosphate (dichlorvos); *5*, dimethyl *S*-methylcarbamoylmethylphosphorothioate (omethoate); *6*, diethyl *O*-(3,5,6-trichloro-2-pyridinyl)phosphorothioate (chlorpyrifos); *7*, diethyldichlorovinyl phosphate (methyldichlorvos); *8*, diisopropylfluorophosphate.

lesser extent in plasma. The physiological function(s) of AChE in blood is unknown. Plasma cholinesterase (or plasma pseudocholinesterase) hydrolyzes butyrylcholine and is different from AChE. Thus, although plasma butyrylcholinesterase is often inhibited by OPs and carbamates more effectively than AChE is, its inhibition has no relation to that of AChE in either erythrocytes or brain. Plasma cholinesterase has no recognized function(s); consequently, inhibition of plasma cholinesterase is interpreted as a biomarker of exposure and not of toxicity.

Only the inhibition of erythrocyte AChE reflects somewhat the inhibition at synapses, causing accumulation of acetylcholine and toxicity. Therefore, erythrocyte AChE inhibition represents a biomarker of toxicity. However, for a given inhibitor, it is difficult to know how closely AChE inhibition in erythrocytes reflects that in the nervous system. Because access to blood is always easier than access to the brain and no evidence exists that OPs and carbamates accumulate in the nervous system, the inhibition of erythrocyte AChE usually overestimates that in the brain (Fig. 3).

When AChE is irreversibly inhibited by OPs in erythrocytes, the recovery toward normal values depends on new cells entering the bloodstream, and has been calculated for most OPs to correspond to 1%/day (*8*). The half-life of AChE resynthesis in the nervous system has been estimated from animal data to be 5–7 days (*13*). Therefore, the enzyme is restored in brain more rapidly than in erythrocytes. When AChE is inhibited by carbamates, its recovery is faster because of spontaneous reactivation in the nervous system and in erythrocytes.

In conclusion, the correlation between AChE inhibition in erythrocytes and that in nervous system depends on the pharmacokinetics of the compound, i.e., how effectively it crosses the blood–brain barrier, as well as on how long after exposure the inhibition is measured.

Toxicological Significance of Erythrocyte AChE Inhibition

Usually, a good correlation exists between the levels of erythrocyte AChE inhibition and the severity of symptoms in OP and carbamate poisoning (Table 1), although difficulties arise when assessing lower levels of erythrocyte AChE inhibition in asymptomatic subjects. For instance, given the large inter- and intraindividual variation of erythrocyte AChE activity, a marginal inhibition is impossible to assess unless preexposure values have been determined on each subject. The intraindividual CV of erythrocyte AChE is ~10%, whereas the interindividual CV is 10–40% (*8*). Gallo and Lawryk (*8*) reported that one measurement of erythrocyte AChE before exposure must detect a minimum of 15% inhibition to be statistically significant.

Substantial inhibition of erythrocyte AChE may not be correlated with symptomatology. In such circumstances, a postsynaptic effect of OPs and carbamates could be inferred. Animals in which chronic exposures downregulated muscarinic receptors because of excessive and prolonged cholinergic stimulation developed tolerance toward cholinergic effects (*15*). Tolerance is unlikely to develop in humans, given the degree of AChE inhibition and the excessive cholinergic stimulation needed to downregulate receptors. Eldefrawi et al. (*16*) and Shaw et al. (*17*) proposed that OPs and carbamates may interact with cholinergic receptors directly, thereby acting as either agonists or antagonists, independently from inhibition of AChE. In most cases, concentrations used in vitro to affect receptors directly were higher than those inhibiting AChE; therefore, the toxicological significance of these direct interactions is not understood.

Nevertheless, recent evidence suggests that downregulation of receptors may also be possible in humans after repeated exposures, causing few signs of cholinergic toxicity and little erythrocyte AChE inhibition (*18*).

OP exposures are also assessed by measuring blood or urinary concentrations of the compound to which subjects have been exposed, or more often by measuring a urinary metabolite. Such measurements are difficult in poisoned patients because of the usual rapid decrease in the rate of OP excretion; here, erythrocyte

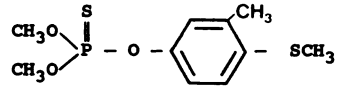
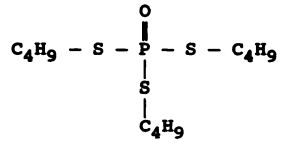
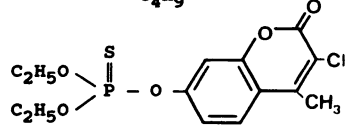
	OP	Dietary level for 50% inhibition	
		Brain	Serum
1		33	27
2		130	52
3		80	25

Fig. 3. Dietary concentrations (mg/kg) of OPs that produce 50% inhibition of AChE in brain and serum of rats (*12*).

1, dimethyl *O*[-4-(methylthio)-*m*-tolyl]phosphorothioate (fenthion); *2*, tributylphosphorotrithioite (Folex); *3*, diethyl *O*-3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-ylphosphorothioate (coumaphos).

Table 1. Signs and symptoms of OP poisoning and their correlation with erythrocyte AChE activity (*14*).

Severity of poisoning (% erythrocyte AChE)	Symptoms and signs		
	Muscarinic	Nicotinic	Central nervous system
Mild, >40%	Nausea, vomiting, diarrhea, salivation/lacrimation, bronchoconstriction, increased bronchial secretions, bradycardia		Headache, dizziness
Moderate, >20%, <40%	Same as above plus miosis (unreactive to light); urinary/fecal incontinence	Muscle fasciculation (fine muscles)	Same as above plus dysarthia, ataxia
Severe, <20%		Same as above plus muscle fasciculation (diaphragm and respiratory muscles)	Same as above plus coma, convulsions

AChE inhibition is more indicative of the severity of poisoning.

In occupational exposures, concentrations of the urinary metabolite of parathion, *p*-nitrophenol, correlated with erythrocyte AChE and plasma cholinesterase activity; *p*-nitrophenol was the most sensitive indicator of exposure (*19*). Given the high sensitivity of current techniques in analytical chemistry, one may postulate that measurements of any metabolite of any cholinesterase inhibitor would be more sensitive than measurements of cholinesterase inhibition. However, the toxicological significance of a given concentration of metabolite, in the absence of cholinesterase inhibition, remains to be ascertained. For instance, urinary dialkyl- and dialkylthiophosphates are widely used to assess exposures to a variety of OP pesticides that share these moieties in their molecule (i.e., methylparathion and methylchlorpyrifos) (*20*). However, measurement of these metabolites might be interpreted only when the parent compound is known and if exposure was restricted to that compound only. One can then determine how far the concentration of urinary dimethylphosphate is from the one associated with cholinesterase inhibition. In fact, although certain exposures to either methylparathion or to methylchlorpyrifos caused a comparable excretion of dimethyl- and dimethylthiophosphates, their toxicological assessment would be different because the two compounds display a quite different toxicity (median oral lethal dose, 6 and >3000 mg/kg, respectively) (*21*).

In conclusion, the toxicological significance of a given inhibition of erythrocyte AChE may be difficult to assess, although such measurements represent the best way to evaluate toxic effects of OPs and carbamates. Moreover, some practical indications should be given as a guide and not as a rule for interpreting the significance of erythrocyte AChE measurements (Table 2).

Table 2. Biomonitoring of occupational exposures to cholinesterase inhibitors (*22*).

Erythrocyte AChE (% inhibition)[a]	Significance	Action
20–30	Evidence of exposure	Check hygienic conditions
30–50	Hazard	As above plus remove from exposure
50–60	Poisoning	As above plus hospitalization

[a] Calculated on the basis of preexposure values.

References

1. Chambers JE, Levi PE, eds. Organophosphates: chemistry, fate, and effects. San Diego, CA: Academic Press, 1992:443pp.
2. International Agency for Research on Cancer. IARC Monographs on the evaluation of carcinogenic risks to humans. Vol. 53: Occupational exposures in insecticide application and some pesticides. Lyon: IARC, 1991:612pp.
3. FAO/WHO. Monographs on pesticide residues in food. Rome: Food and Agriculture Organization of the United Nations, 1962–1995.
4. Coye MJ, Lowe JA, Maddy KT. Biological monitoring of agricultural workers exposed to pesticides: I. Cholinesterase activity determinations. J Occup Med 1986;28:619–27.
5. Ballantyne B, Marrs TC, eds. Clinical, experimental toxicology of organophosphates and carbamates. Oxford, UK: Butterworth-Heinemann, 1992:641pp.
6. Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, et al. Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. Science 1991;253:872–9.
7. Aldridge WN, Reiner E. Enzyme inhibitors as substrates. Amsterdam: North Holland, 1972:328pp.
8. Gallo MA, Lawryk NJ. Organic phosphorus pesticides. In: Hayes WJ Jr, Laws ER Jr, eds. Handbook of pesticide toxicology. San Diego, CA: Academic Press, 1991:917–1123.
9. Capodicasa E, Scapellato ML, Moretto A, Caroldi S, Lotti M. Chlorpyrifos-induced delayed polyneuropathy. Arch Toxicol 1991; 65:150–5.
10. Lotti M, Johnson MK. Neurotoxicity of organophosphorus pesticides: predictions can be based on in vitro studies with hen and human enzymes. Arch Toxicol 1978;41:215–21.
11. Wilson BW, Hooper MJ, Hansen ME, Nieberg PS. Reactivation of organophosphorus inhibited AChE with oximes. In: Chambers JE, Levi PE, eds. Organophosphates: chemistry, fate, and effects. San Diego, CA: Academic Press, 1992:107–37.
12. Su MQ, Kinoshita FK, Frawley JP, DuBois KP. Comparative inhibition of aliesterases and cholinesterase in rats fed eighteen organophosphorus insecticides. Toxicol Appl Pharmacol 1971;20: 241–9.
13. Lotti M. Central neurotoxicity and behavioural effects of anticholinesterases. In: Ballantyne B, Marrs TC, eds. Clinical and experimental toxicology of organophosphates and carbamates. Oxford, UK: Butterworth-Heinemann, 1992:75–83.
14. Lotti M. Treatment of acute organophosphate poisoning. Med J Aust 1991;154:51–5.
15. Costa LG, Murphy SD. [^{3}H]Nicotinic binding in rat brain: alteration after chronic acetylcholinesterase inhibition. J Pharmacol Exp Ther 1983;226:392–7.
16. Eldefrawi A, Manssour NA, Eldefrawi ME. Insecticides af-

fecting acetylcholine receptor interactions. Pharmacol Ther 1982; 16:45–65.
17. Shaw KP, Aracava C, Akaike A, Daly JW, Rickett DL, Albuquerque EX. The reversible cholinesterase inhibitor physostigmine has channel-blocking and agonist effects on the acetylcholine receptor–ion channel complex. Mol Pharmacol 1985;28: 527–38.
18. Good JL, Khurana RK, Mayer RF, Cintra WM, Albuquerque EX. Pathophysiological studies of neuromuscular function in subacute organophosphate poisoning induced by phosmet. J Neurol Neurosurg Psychiatry 1993;56:290–4.
19. Arterberry JD, Durham WF, Elliot JW, Wolfe HR. Exposure to parathion: measurement by blood cholinesterase level and urinary *p*-nitrophenol excretion. Arch Environ Health 1961;3: 476–85.
20. Jeyaratnam J, Maroni M. Organophosphorus compounds. Toxicology 1994;91:15–27.
21. Moretto A, Capodicasa E, Bertolazzi M, De Paris P, Saia BO, Lotti M. Biological monitoring of occupational exposures to organophosphorus insecticides. In: McDuffie HH, Dosman JA, Semchuk KM, Olenchock SA, Senthilselvan A, eds. Agriculture health and safety: workplace, environment, sustainability. Boca Raton, FL: CRC Press, Lewis Publishers, 1995:217–21.
22. WHO. Organophosphorus insecticides: a general introduction. Environmental health criteria 63. Geneva: World Health Organization, 1986:181pp.