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Cresylbenzodioxaphosphorin oxide pretreatment alters soman-induced toxicity and inhibition of tissue cholinesterase activity of the rat

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SUMMARY

The toxicity of soman was investigated in the rat with and without pretreatment with cresylbenzodioxaphosphorin oxide (CBDP). Without pretreatment, the 24-h LD_{50} for soman was 118.2 μg/kg s.c., and soman inhibited carboxylesterase (CaE) activity in plasma (ED_{50} of 55 μg/kg) and cholinesterase (ChE) activity in brain regions (ED_{50} values of 65–105 μg/kg) in a dose-related manner. With pretreatment, the 24-h LD_{50} for soman was reduced by approximately 6-fold and 8-fold (by 1.0 mg/kg and 16.0 mg/kg of CBDP, respectively), and the ED_{50} values for soman-induced inhibition of ChE activity in brain regions were reduced by approximately 10-fold (by 1.0 mg/kg of CBDP). The dose-dependent severity of soman intoxication varied widely in rats treated with soman alone but not in CBDP-pretreated rats, and the ED_{50} for the occurrence of signs of soman intoxication was reduced approximately 7-fold following CBDP (1.0 mg/kg) pretreatment. These data support the hypothesis that CBDP pretreatment effectively blocks tissue CaE sites which serve to detoxify soman, thus potentiating both the soman-induced inhibition of ChE in the CNS and the lethality of soman.

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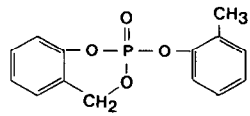
Abbreviations: AChE, acetylcholinesterase; CBDP, 2-(*o*-cresyl)-4*H*-1:3:2-benzodioxaphosphorin-2-oxide; CaE, carboxylesterase; ChE, cholinesterase; CNS, central nervous system; OP, organophosphorus compound; PNS, peripheral nervous system; SAL, saline; VEH, vehicle.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals' (NIH 85-23) of the Institute of Laboratory Animal Resources, National Research Council, U.S.A.

INTRODUCTION

Irreversible inhibition of acetylcholinesterase (AChE) in the peripheral (PNS) and central (CNS) nervous systems is believed to cause, in large part, the toxicity of organophosphorus compounds (OPs), such as soman [1–3]. The lethality of soman, as well as of other toxic OPs, can be potentiated in mice [4–6], rats [7–9] and other species [9, 10] by pretreating these animals with 2-(*o*-cresyl)-4*H*-1:3:2-benzodioxaphosphorin-2-oxide (CBDP; Fig. 1) [11], a metabolite of tri-*o*-cresylphosphate. Potentiation of OP toxicity by CBDP is the result, presumably, of CBDP's irreversible binding to, and preferential inhibition of, carboxylesterase (CaE) [4–6,12,13], especially in plasma and lung, which are important sites of OP detoxification [5, 9, 14]. CaE, whose only known role is in the metabolism of certain xenobiotic esters [15], offers an important means of OP detoxification, in that a percentage of OP binds irreversibly to CaE sites [16]. This binding to CaE by the OP, which otherwise would be non-consequential [5, 17], limits or prevents the association of the OP with its toxic site(s) of action, e.g., the cholinesterases (ChE). When CBDP is administered, CaE sites become occupied by CBDP and, thus, are not available to bind with subsequently administered toxic OPs [4–6, 12]. Presumably, this blockade of CaE sites by CBDP allows a greater proportion of the unbound OP to reach and inhibit critical regulatory enzymes, such as neuronal AChE [4, 6, 12].

Previous studies [4, 5, 7–9, 13] of the role of CBDP in the potentiation of soman toxicity have used doses of CBDP (up to 50 mg/kg s.c. in mice, and 16 mg/kg s.c. in rats, guinea-pigs or rabbits), that themselves inhibit ChE activity substantially in brain and peripheral tissues. Thus, potentiation of soman toxicity by CBDP in these cases may not be attributable solely to increased availability of soman at neuronal AChE sites. Therefore, in the present study we chose a dose (1.0 mg/kg) of CBDP which inhibited CaE activity maximally in plasma and lung but inhibited tissue ChE activity minimally [18, 19]. This CBDP pretreatment was used to determine whether CBDP potentiates soman-induced inhibition of ChE in the CNS of the rat. Additionally, we chose a higher dose (16.0 mg/kg) of CBDP (which inhibited tissue ChE activity substantially) as a pretreatment to determine whether CBDP-induced ChE inhibition in the brain contributes to CBDP's potentiation of soman lethality.



2-(o-CRESYL)-4H-1:3:2-BENZODIOXAPHOSPHORIN-2-OXIDE

Fig. 1. Structure of CBDP. The LD_{50} for CBDP in the rat is greater than 564 mg/kg s.c. [9].

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Crl:CD[SD]BR) weighing 250–300 g were obtained from Charles River Labs (Wilmington, MA), housed 3 per plastic cage in temperature/humidity-controlled quarters, and maintained on a 12-h light-dark cycle with lights on at 0600. Food and water were freely available. Experiments were performed between 0900 and 1200.

Materials

CBDP (99.5% pure) was obtained from Starks Associates Inc. (Buffalo, NY). Soman (97% pure) was obtained from the Chemical Research, Development and Engineering Center (Aberdeen Proving Ground, MD). Purity of both compounds was determined by ^{31}P-NMR spectroscopy. CBDP was prepared in a vehicle (VEH) consisting of 10% ethanol in propylene glycol, USP. Soman was diluted in ice-cold 0.9% saline (SAL). All injections were made subcutaneously (s.c.) and the volume of injection for VEH, SAL, CBDP and soman was 0.5 ml/kg body weight.

Experimental procedures

LD_{50} determination Rats were randomly assigned to one of 3 pretreatment groups: one group received VEH; the other groups received 1.0 or 16.0 mg/kg of CBDP. One hour following pretreatment, different doses of soman (as fractions of a logarithmic dose increment) were administered, and 24 h later an LD_{50} was determined for VEH- and CBDP-pretreated groups respectively.

Cholinesterase study Rats were randomly selected as controls or assigned to several treatment groups. Each group ($n=6$) received a pretreatment (SAL, VEH or CBDP) followed 60 min later by either SAL or soman. Controls received SAL followed by SAL. One experimental group received VEH followed by SAL; another received VEH followed by selected doses of soman (subgroups received 11.8, 35.4, 59.0, 70.8, 82.6 or 106.2 μg/kg). The third experimental group received 1.0 mg/kg of CBDP followed by SAL; the fourth received 16.0 mg/kg of CBDP followed by SAL; the fifth received 1.0 mg/kg of CBDP followed by selected doses of soman (subgroups received 2.1, 6.2, 10.3, 12.3, 14.4 or 18.5 μg/kg). Soman doses were selected based on fractions (0.1, 0.3, 0.5, 0.6, 0.7, 0.9) of the respective LD_{50} values determined as listed above. Rats were scored (by observers unaware of treatment) for signs of OP toxicity based on a modification of the behavioral code developed by Jovic [21]. Scores were 0 (sign-free), 1 (ataxia, licking/chewing behavior and/or muscle fasciculation), 2 (tremors, salivation, limb weakness with hindlimb splaying, respiratory distress and/or convulsions) and 3 (moribund with loss of righting reflex).

Thirty minutes following SAL or soman treatment (i.e., 90 min after pretreatment) rats were killed by decapitation. Trunk blood was collected into heparin-treated tubes and centrifuged, and the plasma was retained for CaE analysis. Brain and spi-

nal cord were removed and the brain was hemisected sagittally along the interhemispheric fissure. One hemisphere was used for 'whole brain' analysis; the other hemisphere was dissected into the following regions: brainstem, cortex, hippocampus, midbrain, cerebellum and striatum. These regions were selected since the study of specific brain regions or nuclei which subserve specific functions or behaviors and where toxic sites of action may be located provides more useful data than similar studies conducted in whole brain. All CNS specimens were homogenized using a Potter-Elvehjem homogenizer in 1% Triton X-100 in 0.9% saline (40 ml/g tissue for striatum; 15 ml/g tissue for all other tissues). Homogenates were centrifuged at 4°C for 10 min at 15000 × g, and the supernatant was retained for ChE analysis.

Enzymatic analysis

CaE activity was determined in plasma by a titrimetric method which measured the hydrolysis of tributyrin (glyceryl tributyrate) [22]. ChE activity and protein concentrations were determined in tissue homogenate supernatants by the automated spectrophotometric method of Groff et al. [23].

Data analysis

Both CaE and ChE activity are expressed as a percentage (± standard error of the mean) of enzyme activity in control animals (i.e. SAL-pretreated/SAL-treated rats). The 24-h LD_{50} for soman after VEH or CBDP pretreatment, as well as the ED_{50} values for the ChE dose–response data, was calculated using log-probit analysis [24].

RESULTS

Effect of CBDP on soman lethality

The 24-h LD_{50} values for soman were 118.3 μg/kg for VEH-pretreated rats, 20.5 and 15.7 μg/kg for CBDP-pretreated (1.0 and 16.0 mg/kg) rats. The respective 95% confidence intervals around these values were 98.9–152.4, 17.3–27.3 and 13.4–19.2 for VEH and CBDP pretreatment, respectively. CBDP pretreatment significantly potentiated the lethality of soman at both pretreatment doses tested. There was a slight, though insignificant, reduction of the LD_{50} values for soman associated with the increase in CBDP pretreatment dose.

Effect of CBDP and soman on plasma CaE activity

CBDP alone inhibited plasma CaE activity maximally (>99%) 90 min after administration of either 1.0 or 16.0 mg/kg. Plasma CaE activity in rats treated with soman (2.1–18.5 μg/kg) subsequent to pretreatment with 1.0 mg/kg of CBDP was not detectable (Fig. 2).

In VEH-pretreated animals, soman (11.8–106.2 μg/kg) caused a dose-dependent inhibition of plasma CaE activity 30 min after administration (Fig. 2). Inhibition ranged from 0 to 99% over the dose range examined. The ED_{50} for soman-induced CaE inhibition in the plasma was determined to be 55 μg/kg.

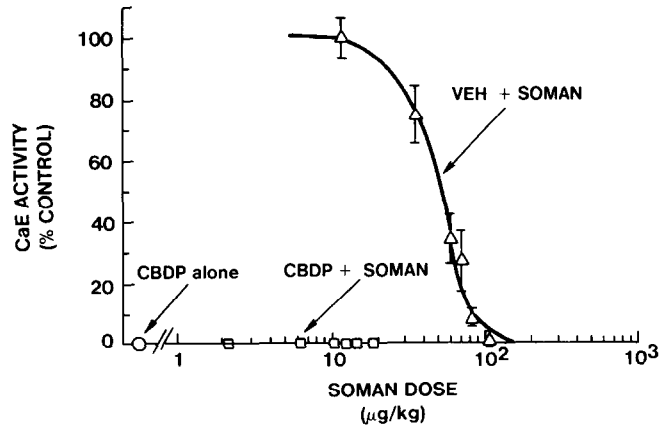


Fig. 2. Effect of CBDP pretreatment on soman-induced CaE inhibition in the plasma. Rats were treated as in Table II. Data shown represent mean ± SEM ($n=6$) of control enzyme activity 30 min after soman treatment (90 min after pretreatment). Data were fitted linearly using log-probit analysis [24]. ○ CBDP alone; □ CBDP + soman; △ VEH + soman.

Effect of CBDP alone and with soman on tissue ChE activity

CBDP alone inhibited ChE activity minimally (< 15%) after 1.0 mg/kg and substantially (27–50%) after 16.0 mg/kg 90 min after administration (Table I). Following pretreatment with either VEH or CBDP (1.0 mg/kg), soman inhibited ChE activity

TABLE I

EFFECT OF CBDP ON CHOLINESTERASE ACTIVITY IN THE RAT CENTRAL NERVOUS SYSTEM[a]

Region	(% control ChE activity)	
CBDP dose (mg/kg):	1.0	16.0
Brainstem	90.4 ± 3.2[b]	59.3 ± 6.0[b]
Cortex	94.5 ± 4.7	67.2 ± 0.4
Hippocampus	89.0 ± 2.7	53.8 ± 7.1
Midbrain	86.7 ± 2.1	62.2 ± 1.6
Cerebellum	89.5 ± 3.3	50.7 ± 3.6
Striatum	85.3 ± 2.3	70.2 ± 2.2
Spinal cord	86.3 ± 1.8	72.6 ± 4.0

[a]Rats were pretreated with VEH or with 1.0 or 16.0 mg/kg s.c. of CBDP followed 60 min later by SAL. CNS tissues were analyzed for ChE activity 30 min after SAL administration.

[b]Values are the mean ± SEM ($n=6$) of the percentage of ChE activity in control (VEH-treated) animals.

in a dose-related manner for all CNS tissues examined. With CBDP pretreatment, however, inhibition occurred at doses of soman approximately one order of magnitude lower than with VEH pretreatment. The ED_{50} values for ChE inhibition fell into the range of 65–105 μg/kg after VEH pretreatment, and 5.5–11.5 μg/kg after CBDP pretreatment (Table II). Fig. 3 shows the effect of soman on ChE activity in the hippocampus following VEH and CBDP pretreatment. Data from this brain region are representative of those obtained from whole brain, spinal cord, and the other brain regions examined. The ED_{50} values for soman-induced ChE inhibition in the hippocampus were 68.4 μg/kg for VEH, and 6.1 μg/kg for CBDP pretreatment. There was no difference in tissue ChE activity between the SAL-pretreated/SAL-treated control group and the VEH-pretreated/SAL-treated group.

Effect of CBDP on signs of soman toxicity

Rats showed typical signs of soman intoxication (including ataxia, salivation, tremor, hindlimb splaying, jerking, respiratory distress and/or convulsions) at soman doses exceeding 70.8 μg/kg after VEH-pretreatment and 10.3 μg/kg after CBDP-pretreatment (1.0 mg/kg). The ED_{50} values for the soman-related occurrence of toxic signs following VEH and CBDP pretreatment were 76.7 and 10.3 μg/kg, respectively (Table III).

TABLE II

EFFECT OF CBDP PRETREATMENT OF SOMAN-INDUCED CHOLINESTERASE INHIBITION IN THE RAT CENTRAL NERVOUS SYSTEM[a]

Region	ED_{50}[b] (μg/kg)		Ratio (VEH/CBDP)
Pretreatment:	VEH	CBDP	
Whole brain	78.7	7.4	10.6
Brainstem	74.6	6.7	11.1
Cortex	73.9	5.5	14.3
Hippocampus	68.4	6.1	11.2
Midbrain	73.6	7.1	10.4
Cerebellum	70.0	5.7	12.3
Striatum	104.0	11.6	9.0
Spinal cord	85.0	9.0	9.4

[a]Rats were pretreated with VEH or 1.0 mg/kg s.c. of CBDP followed 60 min later by doses of soman. CNS tissues were analyzed for ChE activity 30 min after soman administration.

[b]The ED_{50} values were calculated using log-linear analysis [24].

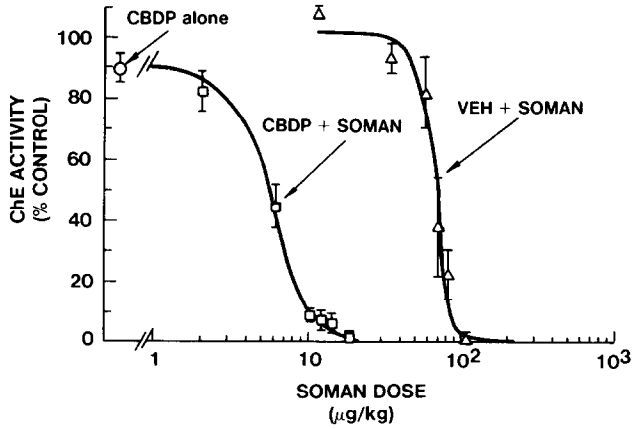


Fig. 3. Effect of CBDP pretreatment on soman-induced ChE inhibition in rat hippocampus. Rats were treated as in Table II. Data are represented and were analyzed as in Fig. 2. ○ CBDP alone; □ CBDP + soman; △ VEH + soman.

DISCUSSION

In this study, we have demonstrated that pretreatment with a low dose (1.0 mg/kg) of CBDP potentiates soman toxicity in the rat. As we have reported [19], this dose of CBDP causes selective (>99%) inhibition of CaE activity in plasma and lung, two tissues which have been reported to be major sites of irreversible binding and detoxification of soman [5, 13, 20]; inhibition of ChE activity in brain regions is minimal (5–15%) after this dose. The non-availability of CaE 'detoxification' sites following the administration of CBDP presumably increases the concentration of 'free' soman which can then bind with and inhibit more critical regulatory enzymes [4, 6, 12]. The result might be, for example, an increase in the degree of ChE inhibition and in the apparent toxicity of soman. Although several authors have demonstrated that CBDP potentiates some toxicity in several mammalian species [4–7, 9], these studies used CBDP doses (up to 50 mg/kg in mice and 16 mg/kg in rats, guinea-pigs or rabbits) which caused substantial inhibition of tissue ChE activities as well as the desired effect, i.e. selective inhibition of tissue CaE activities.

Since the severity of soman toxicity is thought to be related to the degree of brain regional ChE inhibition [25], a substantial contribution to this inhibition by CBDP might be expected to increase the apparent toxicity of soman. Several investigators have reported an apparent relationship between the pretreatment dose of CBDP and the resultant LD_{50} for soman in mice but not in rats. For example, McKay et al. [6] have shown in mice that the 24-h intravenous LD_{50} for soman was decreased significantly by increasing the pretreatment doses (from 1.0 to 35.0 mg/kg s.c.) of CBDP. Likewise, Clement [5] has shown in mice the same decrease in both subcuta-

TABLE III

OCCURRENCE OF SIGNS OF SOMAN INTOXICATION IN VEHICLE- AND CBDP-PRETREATED RATS[a]

	Vehicle		CBDP	
	Soman dose (μg/kg s.c.)	% Occurrence	Soman dose (μg/kg s.c.)	% Occurrence
	11.8	0	2.1	0
	35.4	0	6.2	0
	59.0	0	10.3	67
	70.8	33	12.3	67
	82.6	67	14.4	83
	106.2	100	18.5	100
ED_{50}[b]	76.7 (68.2–89.5)		10.3 (7.5–12.2)	

[a]Rats were treated as in Table II and were scored for toxic signs based on our modification of the behavioral code developed by Jovic [21].

[b]The ED_{50} values for occurrence of signs of intoxication were calculated using log-probit analysis [24]. Numbers in parentheses are the 95% confidence interval.

neous and intraperitoneal LD_{50} values for soman following increasing intravenous doses (0.1 to 5.0 mg/kg) of CBDP. However, in rats, Maxwell et al. [9] have reported a slight, if non-significant, decrease in the 24-h LD_{50} for soman (from 15.6 to 13.3 μg/kg s.c.) when the CBDP pretreatment dose was increased from 2.0 to 16.0 mg/kg s.c. In the present study, our data showed that in rats increasing the dose of CBDP from 1.0 to 16.0 mg/kg did not significantly decrease the LD_{50} of soman (20.5 to 15.7 μg/kg s.c.). The reason for this apparent species difference is not clear but may reflect differences in dispositional factors related to route of administration and CBDP dose.

It has been reported in rats that sublethal toxicity following acute soman exposure is dose-related [25, 26]. Additionally, a dose-related spectrum of observable signs of intoxication has been reported at soman doses between 55 and 100 μg/kg s.c. (approx. 0.5 and 0.9 LD_{50}, respectively) [25, 26]. In these studies, rats either were sign-free or showed signs of intoxication ranging from increased sniffing and grooming behavior to convulsions, prostration and death. We have made similar observations in VEH-pretreated animals in the present study. Moreover, with CBDP pretreatment (1.0 mg/kg) we have observed that, at each dose of soman tested between 6.2 and 18.5 μg/kg s.c. (0.3 and 0.9 LD_{50}, respectively), there was less variation in the severity of intoxication. In fact, severe signs of intoxication were observed in virtually all animals receiving soman doses exceeding 12.3 μg/kg s.c. (approx. 0.6 LD_{50}). However,

a 'basement' effect may have occurred. That is, with the blockade of soman 'detoxification' sites on CaE by CBDP, subsequently administered soman may be more effectively targeted to its toxic site(s) of action, such as neuronal AChE. The apparent reduction in animal to animal variability in soman toxicity, then, may reflect this dispositional effect.

To assess the relative toxicity of soman following CBDP pretreatment, we used the ED_{50} dose ratio for the occurrence of toxic signs (Table III) to calculate that, after CBDP pretreatment, approximately 87% less soman was necessary to elicit observable signs of intoxication. In addition to reducing the effective dose of soman necessary to elicit observable toxic signs in the rat, we have shown that CBDP pretreatment reduces the amount of soman necessary to inhibit ChE activities in various regions of the CNS. For example, based on the ED_{50} values for inhibition of ChE activity in brain regions, soman was 9 (striatum) to 14 (cortex) times more potent after CBDP pretreatment (Table II). We used the ED_{50} dose ratio from each individual CNS region to calculate that, after CBDP pretreatment, an average of about 90% less soman was necessary to achieve equivalent ChE inhibition in these brain regions. Thus, CBDP pretreatment caused a similar reduction in the effective dose of soman necessary to elicit inhibition of brain regional ChE activity and occurrence of toxic signs.

Besides inhibition of ChE activity, another effect of soman is inhibition of plasma CaE activity. This occurs in a dose-related manner with an ED_{50} of 55 μg/kg s.c. in VEH-pretreated rats. Interestingly, we have previously reported this dose as the threshold for the appearance of signs of soman toxicity in naive animals [25]. In CBDP-pretreated animals, a dose of 1.0 mg/kg of CBDP was sufficient to completely inhibit plasma CaE activity, confirming our observations in other studies [8, 18, 19]. Since maximal inhibition of CaE activity by CBDP occurred, further inhibition of CaE activity following soman administration could not be demonstrated.

In conclusion, the data obtained in this study have shown that, using a dose of CBDP selected for its maximal inhibition of plasma and lung CaE activity but minimal inhibition of CNS ChE activity in the rat, there was a significant reduction of the dose of soman necessary to produce an equivalent degree of effect on ChE inhibition, occurrence of toxic signs and lethality. These data support the hypothesis that CBDP pretreatment effectively blocks tissue CaE sites which serve to detoxify soman, thus potentiating both the inhibition of ChE sites in the CNS and the lethality of soman.

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REFERENCES

1 Karczmar, A.G. (1970) History of the research with anticholinesterase agents. In: A.G. Karczmar (Ed.), Anticholinesterase Agents, Vol. 1, International Encyclopedia of Pharmacology and Therapeutics, Sect. 13. Pergamon, Oxford, pp. 1–44.

2 Koelle, G.B. (1963) Handbuch der Experimentellen Pharmakologie, Vol. 15, Cholinesterase Agents. Springer-Verlag, Berlin.

3 Taylor, P. (1985) Anticholinesterase agents. In: A.G. Gilman, L.S. Goodman, T.W. Rall and F. Murad (Eds.), The Pharmacological Basis of Therapeutics, Macmillan, New York, pp. 110–129.

4 Boskovic, B. (1979) The influence of 2-(*o*-cresyl)-4*H*-1:3:2-benzodioxaphosphorin-2-oxide (CBDP) on organophosphate poisoning and its therapy. Arch. Toxicol. 42, 207–216.

5 Clement, J.G. (1984) Role of aliesterase in organophosphate poisoning. Fund. Appl. Toxicol. 4, S96–S105.

6 McKay, D.H., Jardine, R.V. and Adie, P.A. (1971) The synergistic action of 2-(*o*-cresyl-4*H*-1:3:2-benzodioxaphosphorin-2-oxide with soman and physostigmine. Toxicol. Appl. Pharmacol. 20, 474–479.

7 Jimmerson, V.R., Shih, T.-M., Maxwell, D.M., Koviak, T., Harris, S., Henry, K. and Hodge, Y. (1986) Effects of *o*-cresylbenzodioxaphosphorin oxide (CBDP) pretreatment on soman-induced cholinesterase inhibition in the rat central nervous system. Soc. Neurosci. Abstr. 12, 891.

8 Jimmerson, V.R., Shih, T.-M. and Maxwell, D.M. (1987) Cresylbenzodioxaphosphorin oxide (CBDP) pretreatment of the rat alters soman toxicity and cholinesterase (ChE) inhibition in blood and brain regions. Toxicologist 7, 132.

9 Maxwell, D.M., Brecht, K.M. and O'Neill, B.L. (1987) The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. Toxicol. Lett. 39, 35–42.

10 Sterri, S.H. (1981) Factors modifying the toxicity of organophosphorus compounds including dichlorvos. Acta Pharmacol. Toxicol. 49, 67–71.

11 Casida, J.E., Eto, M. and Baron, R.L. (1961) Biological activity of a tri-*o*-cresyl phosphate metabolite. Nature 191, 1396–1397.

12 Cohen, S.D. (1981) Carboxylesterase inhibition and potentiation of soman toxicity. Arch. Toxicol. 49, 105–106.

13 Maxwell, D.M., Lenz, D.E., Groff, W.A., Kaminskis, A. and Froelich, H. (1987) The effects of blood flow and detoxification on in vivo cholinesterase inhibition by soman in rats. Toxicol. Appl. Pharmacol. 88, 66–76.

14 Sterri, S.H. and Fonnum, F. (1984) Detoxification of organophosphorus compounds, In: M. Brzin, E.A. Barnard and D. Sket (Eds.), Cholinesterase: Fundamental and Applied Aspects. Walter de Gruyter, Berlin, pp. 389–400.

15 Heymann, E. (1980) Carboxylesterases and amidases. In W.B. Jakoby (Ed.), Enzymatic Basis of Detoxification, Vol. 1. Academic Press, New York, pp. 291–323.

16 Fonnum, F. and Sterri, S.H. (1981) Factors modifying the toxicity of organophosphorus compounds including soman and sarin. Fund. Appl. Toxicol. 1, 143–147.

17 Junge, W. and Krisch, K. (1975) The carboxylesterases/amidases of mammalian liver and their possible significance. CRC Crit. Rev. Toxicol. 3, 371–434.

18 Jimmerson, V.R., Maxwell, D.M., Hodge, Y., Brecht, K. and Shih, T.-M. (1987) Effects of cresylbenzodioxaphosphorin oxide (CBDP) on tissue carboxylesterase (CaE) and cholinesterase (ChE) activities in the rat. Toxicologist 7, 133.

19 Jimmerson, V.R., Shih, T.-M., Maxwell, D.M. and Mailman, R.B. (1989) The effect of 2-(*o*-cresyl-4*H*-1:3:2-benzodioxaphosphorin-2-oxide (CBDP) on tissue cholinesterase and carboxylesterase activities in the rat. Toxicol. Appl. Pharmacol. (submitted).

20 Sterri, S.H., Lyngaas, S. and Fonnum, F. (1983) Cholinesterase and carboxylesterase activities in soman poisoned rats treated with bispyridinium mono-oximes HI-6 and HS-6. Biochem. Pharmacol. 32, 1646–1649.

21 Jovic, R.C. (1974) Correlation between signs of toxicity and some biochemical changes in rats poisoned with soman. Eur. J. Pharmacol. 25, 159–164.
22 Clement, J.G. (1982) Plasma aliesterase – a possible depot for soman (pinacolyl methylphosphonofluoridate) in the mouse. Biochem. Pharmacol. 31, 4085–4088.
23 Groff, W.A., Kaminskis, A. and Ellin, R.I. (1976) Interconversion of cholinesterase enzyme activity units by the manual pH method and a recommended automated method. Clin. Toxicol. 9, 353–358.
24 Tallarida, R.J. and Jacob, L.S. (1979). The Dose–Response Relation in Pharmacology. Springer-Verlag, New York, pp. 14–19.
25 Jimmerson, V.R., Shih, T.-M., Koviak, T., Smith, O., Pannella, M. and Mailman, R. (1985) Signs of soman toxicity and brain regional cholinesterase activity. Soc. Neurosci. Abstr. 11, 1240.
26 Shih, T.-M. (1983) Effects of soman on blood and regional brain cholinesterase activities. Trans. Am. Soc. Neurochem. 14, 148.