TABLE 4-39.
(Continued)

Animal No.	GD Dose (µg/kg) (Targeted)	GD Dose (µg/kg) (Measured)	Group[a]	Survival[b]	Inhibition, of RBC AChE[c]	Body Wt. (kg)
46R	20.2	20.0	3		N/A	2.7
4E9	20.0	19.9	3		N/A	3.4
DES5	20.0	20.2	3		N/A	5.4
333D	20.0	20.1	3		N/A	4.4
827C	22.0	21.8	3		N/A	4.3
405D	22.0	21.9	3		N/A	3.5
873T	22.0	21.9	3	DIED	N/A	4.5
2GY	22.0	21.9	3		N/A	3.2
47W	22.0	22.5	3		N/A	4.2
105T	22.2	22.8	3	DIED	N/A	4.3
3KX	24.2	23.6	3	DIED	N/A	4.0
215R	24.2	23.9	3	DIED	N/A	5.0
4E1	24.2	24.0	3		N/A	3.1
D278	24.2	24.1	3	DIED	N/A	4.9
399D	26.6	24.3	3	DIED	N/A	4.3
027D	24.2	24.4	3	DIED	N/A	4.4
4GV	24.2	24.5	3		N/A	3.2
315D	24.2	25.4	3		N/A	3.8
C603	26.6	25.9	3	DIED	N/A	4.6
D238	26.6	26.2	3		N/A	3.8
45S	26.6	26.2	3		N/A	2.7
003D	26.6	26.4	3		N/A	4.6
E42	26.6	26.5	3		N/A	3.1
2JG	29.2	28.4	3	DIED	N/A	3.2
904C	29.2	29.2	3	DIED	N/A	3.6
070D	29.2	30.0	3		N/A	4.0
120D	29.2	30.0	3	DIED	N/A	4.7
830T	35.4	34.7	3	DIED	N/A	3.5
				MEAN (N = 28)	--	3.9
				SD	--	0.7
				HIGH	--	5.4
				LOW	--	2.7

TABLE 4-39.
(Continued)

Animal No.	GD Dose (µg/kg) (Targeted)	GD Dose (µg/kg) (Measured)	Group[a]	Survival[b]	Inhibition of RBC AChE[c]	Body Wt. (kg)
246D	4.35	4.60	4		N/A	3.7
2YW	5.26	5.40	4		N/A	4.2
966C	6.36	6.50	4		N/A	4.2
D274	7.70	7.40	4		N/A	4.9
C390	7.70	7.60	4		N/A	5.8
041D	9.32	9.30	4		N/A	4.3
4GA	9.32	9.70	4		N/A	2.8
4EA	11.3	11.2	4		N/A	3.9
2VF	11.3	11.3	4		N/A	4.4
D583	12.4	12.7	4		N/A	5.1
366D	13.6	13.3	4	DIED	N/A	4.6
3L4	13.6	13.3	4		N/A	3.6
3LG	13.6	13.4	4	DIED	N/A	4.0
3LS	13.6	13.6	4		N/A	3.9
47L	13.6	14.2	4		N/A	2.6
925T	15.0	14.6	4		N/A	3.8
4GB	15.0	14.7	4		N/A	3.2
188D	15.0	14.7	4		N/A	3.4
E196	15.0	14.8	4	DIED	N/A	3.7
E248	15.0	15.4	4	DIED	N/A	3.8
417D	18.2	15.9	4	DIED	N/A	3.7
339D	16.5	16.2	4	DIED	N/A	3.9
936C	22.0	16.4	4	DIED	N/A	3.9
3LI	16.5	16.4	4	DIED	N/A	4.3
822C	16.5	16.5	4	DIED	N/A	5.1
087D	16.5	16.7	4		N/A	4.3
137D	16.5	17.1	4		N/A	4.0
44L	18.2	17.2	4	DIED	N/A	2.8
858C	18.2	17.2	4	DIED	N/A	5.5
143D	18.2	18.7	4	DIED	N/A	4.5
H25	20.0	19.2	4	DIED	N/A	4.5
3LB	20.0	20.0	4		N/A	4.0
3XD	20.0	20.1	4	DIED	N/A	3.2
3EW	20.0	20.3	4	DIED	N/A	3.1
4HL	26.6	26.4	4	DIED	N/A	3.2
986C	32.2	32.4	4	DIED	N/A	5.8

TABLE 4-39.
(Continued)

Animal No.	GD Dose (µg/kg) (Targeted)	GD Dose (µg/kg) (Measured)	Group[a]	Survival[b]	Inhibition of RBC AChE[c]	Body Wt. (kg)
				MEAN (N = 36)	--	4.0
				SD	--	0.8
				HIGH	--	5.8
				LOW	--	2.6

[a]Group 1 = lower pyridostigmine dose plus atropine/2-PAM; Group 2 = higher pyridostigmine dose plus atropine/2-PAM; Group 3 = atropine/2-PAM; Group 4 = no pretreatment or therapy.
[b]"DIED" indicates those animals that died within 48 hr of GD dosing. All others survived for at least 48 hr.
[c]Percent inhibition of RBC AChE at the time of GD dosing.

FIGURE 4-16. PROBIT VERSUS Ln OF GD DOSE (FULL SCALE)

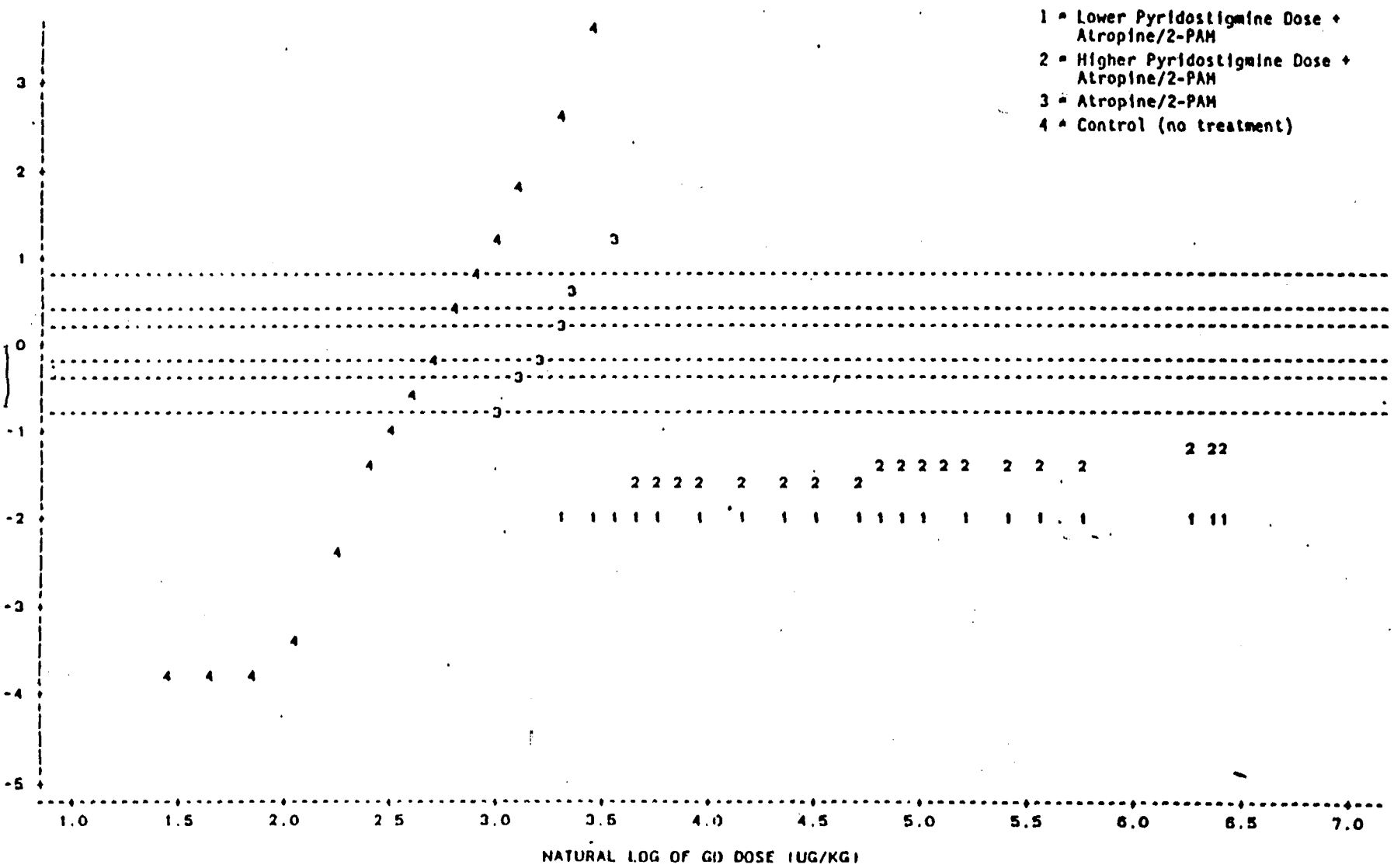

TABLE 4-44. CALCULATED LD_{50} VALUES, PROTECTIVE RATIOS, AND 95 PERCENT CONFIDENCE LIMITS (CL) FOR PHASE 2 EXPERIMENTS[a]

Group	GD LD_{50} (μg/kg) and [95% CL]	Protective Ratios and [95% CL]
Control	15.3 [13.7, 17.1]	--
Atropine/2-PAM	25.1 [22.0, 28.8]	1.64 [1.38, 1.95][b]
Lower Pyridostigmine Dose plus Atropine/2-PAM	>617	Indeterminate, but greater than 40[c] and greater than 25[d]
Higher Pyridostigmine Dose plus Atropine/2-PAM	>617	Indeterminate, but greater than 40[c] and greater than 25[d]

[a]Based on a separate slopes model.
[b](LD_{50} for Atropine/2-PAM) ÷ (LD_{50} for Control).
[c](LD_{50} for Pyridostigmine/Atropine/2-PAM) ÷ (LD_{50} for Control)
[d](LD_{50} for Pyridostigmine/Atropine/2-PAM) ÷ (LD_{50} for Atropine/2-PAM)

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TABLE 4-42. SUMMARY OF CLINICAL OBSERVATIONS BY PRETREATMENT AND THERAPY GROUP AND BY GD DOSE RANGE

GD Dose Range (μg/kg)	Group (Treatment)[a]	Observation[b]	Frequency[c]	Approximate Time to Reversal (hr)[d]
4.7-11.3 (non-lethal)	4 (none)	tremors/fasciculations[e]	3/9	2-6
		convulsions	2/9	1-2
		prostration	3/9	4-8
		unconsciousness	0/9	--
		death	0/9	--
12.7-14.8 (low-lethal)	4 (none)	tremors/fasciculations	10/10	1-8
		convulsions	5/10	1-8
		prostration	9/10	12-36
		unconsciousness	2/9	1-4
		death	3/10	--
15.4-20.3 (high-lethal)	4 (none)	tremors/fasciculations	14/14	not generally reversed
		convulsions	10/14	1-24
		prostration	14/14	not generally reversed
		unconsciousness	5/14	0.5-2
		death	12/15	--
15.4-20.3 (non-lethal)	3 (A/2-PAM)	tremors/fasciculations	4/4	1-6
		convulsions	1/4	1
		prostration	4/4	1-8
		unconsciousness	1/4	1
		death	0/4	--
21.8-26.5 (mid-lethal)	3 (A/2-PAM)	tremors/fasciculations	19/19	2-36
		convulsions	12/19	0.5-4
		prostration	18/19	2-24
		unconsciousness	8/19	1-2
		death	9/19	--
27.0-52.2[f] (lethal)	4 (none)	tremors/fasciculations	1/1	died within 30 min
		convulsions	1/1	
		prostration	0/1	
		unconsciousness	0/1	
		death	1/1	

TABLE 4.42.
(Continued)

GD Dose Range (μg/kg)	Group (Treatment)[a]	Observation[b]	Frequency[c]	Approximate Time to Reversal (hr)[d]
27.0-52.2 (high-lethal)	3 (A/2-PAM)	tremors/fasciculations	5/5	not generally reversed
		convulsions	4/5	2-4
		prostration	5/5	not generally reversed
		unconsciousness	2/5	0.5-1
		death	4/5	--
27.0-52.2 (non-lethal)	2 (HiP+A/2-PAM)	tremors/fasciculations[d]	4/4	1-6
		convulsions	3/4	2-4
		prostration	4/4	2-12
		unconsciousness	1/4	1
		death	0/4	--
27.0-52.2 (non-lethal)	1 (LoP+A/2-PAM)	tremors/fasciculations	7/7	1-8
		convulsions	3/7	0.5-4
		prostration	5/7	1-8
		unconsciousness	0/7	--
		death	0/7	--
55.0-312	2 (HiP+A/2-PAM)	tremors/fasciculations	15/15	1-4
		convulsions	8/14	0.5-2
		prostration	14/14	2-8
		unconsciousness	1/14	0.5
		death	1/14	--
55.0-312	1 (LoP+A/2-PAM)	tremors/fasciculations	11/11	1-48
		convulsions	5/11	0.5-1
		prostration	11/11	2-24
		unconsciousness	1/11	0.5
		death	1/11	--
501-617	2 (HiP+A/2-PAM)	tremors/fasciculations	18/18	2-48
		convulsions	9/18	0.5-2
		prostration	18/18	2-48
		unconsciousness	10/18	0.5-1
		death	2/18	--
501-617	1 (LoP+A/2-PAM)	tremors/fasciculations	18/18	4-48
		convulsions	7/18	1-4
		prostration	17/18	2-36
		unconsciousness	12/18	0.5-1
		death	0/18	--

TABLE 4-42.
(Continued)

should be reversed

[a]Animals in group 4 received no pretreatment or therapy; those in group 3 received atropine and 2-PAM therapy (A/2-PAM); those in group ② received the lower dose of pyridostigmine pretreatment and atropine and 2-PAM therapy (LoP+A/2-PAM); those in group ① received the higher dose of pyridostigmine pretreatment plus atropine and 2-PAM therapy (HiP+A/2-PAM)

[b]Uncoordinated movements are not included in this summary table because of the lack of a clear pattern. Hypersalivation, also not shown, occurred with nearly the same frequency as fasciculations and tremors.

[c]Number of monkeys exhibiting the sign over the total number of (surviving) monkeys in this GD dose range.

[d]These times are generalizations, based on an objective review of the data, and should not be misconstrued as absolute, calculated parameters.

[e]Because of an inability to distinguish objectively between these two clinical signs in some cases, they were grouped for tabular presentation.

[f]The general absence of observations other than tremors/fasciculations and convulsions was probably due to the rapid death (between 15 and 30 min after GD injection).

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Table 5.1.2.1.1.2.D: **Symptomology in Rhesus Monkeys after Challenge with Soman Untreated or Treated with Atropine/Pralidoxime and No Pyridistogmine Pretreatment (Joiner and Kluwe, 1988)**

Animal I.D.	G.D. Dose (μg/kg)	Treatment	48-Hour Observation	Fate [Up to 9 days after GD]
D583	12.7	None	Prostrate, uncoordinated	Sac'd at 3 days, not eating, tremors, uncoordinated
366D	13.5	None	Died after 1.5 hours	Died after 1.5 hours
3L4	13.3	None	Uncoordinated, prostrate, lethargic, weak	Sac'd at 3 days, very weak, unresponsive, not eating, dehydrated
3LG	13.4	None	Died after 30 minutes	Died after 30 minutes
47L	14.2	None	Lethargic, uncoordinated, not eating	Sac'd at 3 days, lethargic, tremors, not eating
925T	14.6	None	Uncoordinated, lethargic, tremors	Sac'd at 3 days, lethargic, uncoordinated
188D	14.7	None	Uncoordinated, prostrate, labored breathing	Sac'd at 3 days, prostrate, labored breathing
4GB	14.7	None	Normal	Sac'd at 3 days, not eating but alert
E196	14.8	None	Died after 34 hours	Died after 34 hours
E248	15.4	None	Died after 1 hour	Died after 1 hour
417D	15.9	None	Died after 25 minutes	Died after 25 minutes
339D	16.2	None	Died after 30 minutes	Died after 30 minutes
3LI	16.4	None	Died after 2.5 hours	Died after 2.5 hours
936C	16.4	None	Died after 15 minutes	Died after 15 minutes
822C	16.5	None	Died after 40 minutes	Died after 40 minutes
087D	16.7	None	Lethargic, weak, not eating well	Sac'd 3 days, weak, lethargic, disoriented
44L	17.2	None	Died after 26 hours	Died after 26 hours
143D	18.7	None	Died after 40 minutes	Died after 40 minutes
858C	18.7	None	Died after 20 minutes	Died after 20 minutes
H25	19.2	None	Died after 30 minutes	Died after 30 minutes

Table 5.1.2.1.1.2.D: **Symptomology in Rhesus Monkeys after Challenge with Soman Untreated or Treated with Atropine/Pralidoxime and No Pyridistogmine Pretreatment (continued) (Joiner and Kluwe, 1988)**

Animal I.D.	G.D. Dose (µg/kg)	Treatment	48-Hour Observation	Fate [Up to 9 days after GD]
3LB	20	None	Fasciculations, prostrate, excess salivation	Sac'd at 2 days, prostrate
3EW	20.3	None	Died after 30 minutes	Died after 30 minutes
4HL	26.4	None	Died after 15 minutes	Died after 15 minutes
986C	32.4	None	Died after 15 minutes	Died after 15 minutes
4E9	19.9	A/P	Tremors, uncoordinated, lethargic, miosis	Slightly uncoordinated at 8 days
46R	20	A/P	Normal	Diarrhea at 1 week
333D	20.1	A/P	Lethargic	Normal at 9 days
DE863	20.2	A/P	Lethargic, depressed, not eating	Normal at 9 days
837C	21.8	A/P	Uncoordinated, lethargic, ptosis	Fasciculations, slight lethargy, eating well at 8 days
2GY	21.9	A/P	Died after 1 hour	Died after 1 hour
405D	21.9	A/P	Normal	Normal at 9 days
873D	21.9	A/P	Died after 37 minutes	Died after 37 minutes
47W	23.5	A/P	Normal	Normal at 9 days
105T	22.8	A/P	Died after 3.5 hours	Died after 3.5 hours
3KX	23.6	A/P	Died after 30 minutes	Died after 30 minutes
215R	23.9	A/P	Died after 1 hour	Died after 1 hour
4E1	24	A/P	Fasciculations, lethargic	Depressed, upper body tremors at 1 week

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Animal ID	Pretreatment	Measured GD Dose (mg/kg)	GD Dose Date	Treatment	Convulsions*	Prostration*	Condition at 48 hr	Disposition	Date	Comments
D37S	NA	24.1	09-Sep-96	A/P	none	3		Died	09-Sep-96	3 hrs
399D	NA	24.3	14-Aug-96	A/P	0.5	3.5		Died	14-Aug-96	3.5 hrs
821D	NA	24.4	25-Sep-96	A/P	1	1.25		Died	25-Sep-96	15 min
4GY	NA	24.5	12-Aug-96	A/P	none	36	uncoordinated, not eating well	WRAIR	31-Aug-96	diarrhea, slight tremors, uncoordinated at shipment
315D	NA	25.4	09-Sep-96	A/P	1	36	lethargic, uncoordinated	WRAIR	18-Sep-96	normal at shipment
C09S	NA	25.9	12-Aug-96	A/P	0.5	2		Died	12-Aug-96	2.5 hrs
45S	NA	26.2	25-Sep-96	A/P	0.5	8	uncoordinated, lethargic, fasciculations	WRAIR	01-Oct-96	tremors and uncoordinated at shipment
D23S	NA	26.3	23-Sep-96	A/P	1	8	uncoordinated, lethargic, weak	Sac'd	29-Sep-96	extremely lethargic and uncoordinated
003D	NA	26.4	11-Sep-96	A/P	none	24	fasciculations, lethargic	WRAIR	18-Sep-96	normal at shipment
E43	NA	26.5	09-Sep-96	A/P	0.5	36	lethargic, uncoordinated, tremors	WRAIR	18-Sep-96	very slight tremors at shipment
2JG	NA	28.4	11-Sep-96	A/P	0.5	1		Died	11-Sep-96	1 hr
984C	NA	29.2	12-Aug-96	A/P	none	4		Died	12-Aug-96	6 hrs
070D	NA	30	25-Sep-96	A/P	1	24	fasciculations, lethargic, uncoordinated	WRAIR	01-Oct-96	lethargic at shipment
130D	NA	30	14-Aug-96	A/P	0.5	12		Died	15-Aug-96	12 hrs
830T	NA	34.7	12-Aug-96	A/P	0.5	4		Died	12-Aug-96	3.5 hrs
4AT	lo	27	12-Aug-96	A/P	2	8	normal	WRAIR	31-Aug-96	normal at shipment
3TG	lo	29	12-Aug-96	A/P	none	none	normal, not eating well	WRAIR	31-Aug-96	diarrhea at shipment
917T	lo	29.8	12-Aug-96	A/P	4	2	uncoordinated, lethargic	WRAIR	31-Aug-96	normal at shipment

*First time frame (in hours) at which clinical sign was no longer observed in 48-hr period.

(This page from Vol 2.82; data apparently omitted from these tables inadvertently)

Table 5.1.2.1.1.2.E: **Symptomology in Rhesus Monkeys Surviving One Week or Longer after Challenge with Soman and Treatment with Various Antidotal Combinations (Joiner and Kluwe, 1988)**

Animal I.D.	G.D. Dose (μg/kg)	Treatment/ Pretreatment*	48-Hour Observation	Condition at One Week or Longer
396D	34.2	A/P PB Low	Normal, slightly depressed	Normal at 9 days
398D	43.5	A/P PB Low	Normal	Diarrhea at 9 days
41I	51.6	A/P PB Low	Normal	Normal at 7 days
2YZ	52.2	A/P PB Low	Diarrhea, not eating well	Normal at 9 days
2Z2	62.1	A/P PB Low	Lethargic	Normal at 7 days
968C	75.8	A/P PB Low	Fasciculations, depressed	Normal at 7 days
D429	87.9	A/P PB Low	Fasciculations, uncoordinated, lethargic	Diarrhea at 7 days
3L8	108.9	A/P PB Low	Fasciculations, lethargic	Diarrhea at 7 days
3HD	126.7	A/P PB Low	Normal	Normal at 9 days
161D	141.2	A/P PB Low	Fasciculations, lethargic	Normal at 7 days
196D	148.1	A/P PB Low	Dead	Dead 50 minutes
942C	177.9	A/P PB Low	Lethargic, uncoordinated, diarrhea	Normal at 9 days
969C	210.2	A/P PB Low	Ptosis, lethargic, periodic prostration, uncoordinated	Normal at 9 days
3N7	261.3	A/P PB Low	Lethargic, weak, fasciculations	Normal at 9 days
907T	307.8	A/P PB Low	Fasciculations, lethargic, uncoordinated	Normal at 9 days
320D	502.9	A/P PB Low	Fasciculations, lethargic, not eating well	Tremors at 7 days
276D	508.1	A/P PB Low	Lethargic, miosis, not eating well	Tremors at 7 days
47H	550.9	A/P PB Low	Fasciculations, lethargic, miosis, tremors, not eating	Tremors at 7 days
3I8	563.6	A/P PB Low	Lethargic, miosis, not eating well	Normal at 7 days
328D	600.4	A/P PB Low	Fasciculations, lethargic, miosis, not eating	Normal
893T	605.9	A/P PB Low	Prostrate, not eating	Prostrate, not eating at 4 days ***

* Low Dose PB 1.2 mg/kg q8h for 6 doses
High Dose PB 1.2 mg/kg, 1.8 mg/kg 2nd dose, 2.4 mg/kg 3rd-6th dose

Table 5.1.2.1.1.2.E: **Symptomology in Rhesus Monkeys Surviving One Week or Longer after Challenge with Soman and Treatment with Various Antidotal Combinations (continued) (Joiner and Kluwe, 1988)**

Animal I.D.	G.D. Dose (µg/kg)	Treatment/ Pretreatment*	48-Hour Observation	Condition at One Week or Longer
363D	606.1	A/P PB Low	Tremors, uncoordinated, some prostration	Fasciculations at 8 days
996C	607.1	A/P PB Low	Fasciculations, uncoordinated, lethargic, tremors	Miosis, not eating well at 8 days
48W	607.2	A/P PB Low	Lethargic, weak	Normal at 8 days
E723	607.7	A/P PB Low	Uncoordinated, ptosis, lethargic, weak	Fasciculations at 8 days
306D	609.6	A/P PB Low	Uncoordinated, lethargic, some prostration	Slightly lethargic, diarrhea, at 8 days
3L9	609.8	A/P PB Low	Prostrate, fasciculations, tremors, not eating	Died after 4 days
025D	609.9	A/P PB Low	Convulsions, lethargic, uncoordinated, miosis	Uncoordinated, miosis at 8 days
819C	611.7	A/P PB Low	Fasciculations, ptosis, lethargy, miosis, weak	Diarrhea at 8 days
190D	612.7	A/P PB Low	Uncoordinated, ptosis, lethargy, weak	Fasciculations, lethargic at 8 days
4LJ	612.7	A/P PB Low	Uncoordinated	Tremors at 8 days
937C	616.4	A/P PB Low	Uncoordinated, lethargic, vomiting, weak	Fasciculations at 8 days
E510	617.2	A/P PB Low	Lethargic	Diarrhea at 8 days
153D	38.4	A/P PB High	Normal	Normal
230D	41.5	A/P PB High	Lethargic	Normal
210D	45.0	A/P PB High	Normal	Normal
981C	49.6	A/P PB High	Lethargic, uncoordinated, not eating well	Diarrhea at 9 days
48E	55.0	A/P PB High	Normal, not eating well	Diarrhea at 9 days
4HP	63.7	A/P PB High	Normal	Diarrhea at 7 days
3J8	71.6	A/P PB High	Normal	Normal
904T	77.6	A/P PB High	Normal	Normal

* Low Dose PB 1.2 mg/kg q8h for 6 doses
High Dose PB 1.2 mg/kg, 1.8 mg/kg 2nd dose, 2.4 mg/kg 3rd-6th dose

7 W

Table 5.1.2.1.1.2.E: **Symptomology in Rhesus Monkeys Surviving One Week or Longer after Challenge with Soman and Treatment with Various Antidotal Combinations (continued) (Joiner and Kluwe, 1988)**

Animal I.D.	G.D. Dose (μg/kg)	Treatment/ Pretreatment*	48-Hour Observation	Condition at One Week or Longer
3MU	93.5	A/P PB High	Prostrate 1.75 hours	Died within 1.75 hours
314D	109.8	A/P PB High	Lethargic	Normal at 1 week
159D	132.0	A/P PB High	Lethargic	Normal at 9 days
410D	135.1	A/P PB High	Lethargic	Normal at 1 week
48V	145.2	A/P PB High	Lethargic, uncoordinated, diarrhea, not eating	Diarrhea at 9 days
3JT	160.9	A/P PB High	Lethargic	Diarrhea at 7 days
C766	180.9	A/P PB High	Lethargic, diarrhea, not eating well	Normal at 9 days
989C	215.6	A/P PB High	Ptosis, lethargic, diarrhea	Normal at 9 days
48P	258.4	A/P PB High	Lethargic, tremors, uncoordinated, diarrhea	Normal at 9 days
3JH	302.9	A/P PB High	Lethargic, diarrhea, not eating well	Not eating well at 7 days
D384	312.0	A/P PB High	Slight tremors, diarrhea, lethargic	Fasciculations, diarrhea at 9 days
D675	506.3	A/P PB High	Slight fasciculations, l- argic	Ptosis at 7 days
4G1	530.0	A/P PB High	Prostrate at 1.5 hours	Died within 1.5 hours
902T	556.0	A/P PB High	Prostrate at 10 minutes	Died within 10 minutes
C380	596.7	A/P PB High	Fasciculations, lethargic, miosis, not eating	Tremors, not eating well at 7 days
4BR	599.4	A/P PB High	Uncoordinated, miosis, lethargic	Ptosis, tremors at 6 days
927C	602.5	A/P PB High	Uncoordinated, ptosis, lethargic, weak	Normal at 8 days
46S	602.9	A/P PB High	Slight fasciculations, lethargic	Slight tremors at 1 week
47B	606.0	A/P PB High	Lethargic	Diarrhea at 8 days
47A	607.0	A/P PB High	Lethargic, diarrhea, weak	Diarrhea at 8 days

* Low Dose PB 1.2 mg/kg q8h for 6 doses
High Dose PB 1.2 mg/kg, 1.8 mg/kg 2nd dose, 2.4 mg/kg 3rd-6th dose

Table 5.1.2.1.1.2.E: **Symptomology in Rhesus Monkeys Surviving One Week or Longer after Challenge with Soman and Treatment with Various Antidotal Combinations (continued) (Joiner and Kluwe, 1988)**

Animal I.D.	G.D. Dose (μg/kg)	Treatment/ Pretreatment*	48-Hour Observation	Condition at One Week or Longer
932T	608.1	A/P PB High	Prostrate, miosis, not eating	Prostrate, not eating, miosis at 4 days **
809C	608.4	A/P PB High	Uncoordinated, miosis, lethargic, salivating, diarrhea	Diarrhea, fasciculations at 6 days
3M1	609.3	A/P PB High	Uncoordinated, not eating well	Normal at 6 days
279D	612.4	A/P PB High	Prostrate, uncoordinated, not eating, weak, lethargic	Prostrate, lethargic, not eating well at 6 days **
5TD	614.6	A/P PB High	Convulsions, uncoordinated, tremors, prostrate	Died at 3 days
015D	614.8	A/P PB High	Uncoordinated, lethargic, diarrhea	Normal at 8 days
931C	616.7	A/P PB High	Fasciculations, miosis, lethargic, weak	Diarrhea, miosis, fasciculations at 8 days
31T	617.3	A/P PB High	Diarrhea, uncoordinated	Tremors at 6 days

** Animal was sacrificed, according to data in vol. 2.82

* Low Dose PB 1.2 mg/kg q8h for 6 doses
High Dose PB 1.2 mg/kg, 1.8 mg/kg 2nd dose, 2.4 mg/kg 3rd-6th dose

Selected animals from this study which were exposed to lethal doses of GD survived for considerably longer periods of time and were healthy enough to use in later studies. Twenty of these monkeys were shipped to Walter Reed Army Institute of Research (WRAIR) upon completion of the Joiner and Kluwe study and their health condition monitored during subsequent studies. Table 5.1.2.1.1.2.F provides a summary of the condition of these monkeys in 1995, more than 10 years after the Joiner and Kluwe study. Examination of this table reveals that 15 of these 20 monkeys not only survived for 10 years but returned to functionality and signs of good health (Olson, 1995).

Table 5.1.2.1.1.2.F History of Selected Monkeys Surviving Soman Poisoning

Animal I.D.	GD Dose (μg/kg)	Treatment/ Pretreatment*	Date Received WRAIR	Date/ Results of Last Physical	Date of Death
016D	9.1	A/P	7AUG86	13FEB95/HEALTHY	N/A
44I	10.1	A/P	7AUG86	14NOV94/SCOLIOSIS	N/A
3LT	18.2	A/P	7AUG86	JUN95/NO PROBLEMS NOTED	N/A
3WN	19.3	A/P	7AUG86	30JAN95/HEALTHY	N/A
47W	22.5	A/P	21AUG86	30JAN95/NO PROBLEMS NOTED	N/A
4AT	27	A/P PB Low	21AUG86	30JAN95/HEALTHY	N/A
41I	51.6	A/P PB Low	21AUG86	26FEB91/NO PROBLEMS NOTED	28JAN92 EUTHANIZED
3N7	261.3	A/P PB Low	18SEP86	JAN91/NO PROBLEMS NOTED	25JAN93 EUTHANIZED
907T	307.8	A/P PB Low	15SEP86	14NOV94/ENLARGED PROSTATE	N/A
47H	560.9	A/P PB Low	18SEP86	30JAN95/NO PROBLEMS NOTED	N/A
48W	607.2	A/P PB Low	2OCT86	14NOV94/ NORMAL	N/A
937C	616.4	A/P PB Low	2OCT86	FEB91/HEALTHY	OCT93 EUTHANIZED

Table 5.1.2.1.1.2.F History of Selected Monkeys Surviving Soman Poisoning (continued)

Animal I.D.	GD Dose (μg/kg)	Treatment/ Pretreatment*	Date Received WRAIR	Date/ Results of Last Physical	Date of Death
47U	21.8	A/P PB High	7AUG86	30JAN95/NO PROBLEMS NOTED	N/A
159D	132	A/P PB High	18SEP86	14NOV94/GOOD	N/A
3JT	160.9	A/P PB High	21AUG86	10NOV94/HEALTHY	N/A
48P	258.4	A/P PB High	18SEP86	HEALTHY	NOV92 EUTHANIZED
47R	606	A/P PB High	20OCT86	JUN 95/NO PROBLEMS NOTED	N/A
47A	607	A/P PB High	20OCT86	JAN95/NO PROBLEMS NOTED	24MAY95 (died)
3M1	609.3	A/P PB High	2OCT86	30JAN95/NO SIGNIFICANT PROBLEMS	N/A
931C	616.7	A/P PB High	2OCT86	13FEB95/MILD HEART MURMUR	N/A

* Low Dose PB 1.2 mg/kg q8h for 5 doses
High Dose PB 1.2 mg/kg, 1.8 mg/kg 2nd dose, 2.4 mg/kg 3rd-6th dose
N/A Not Applicable

Study A-2

An additional study was done in male rhesus monkeys which evaluated the efficacy of doses of P producing lower degrees of RBC AchE inhibition than that produced in the above study. It was performed by the same lab as the above study, and was stated to conform to the GLP regulations. It is stated to be a draft report. The study is in volume 2.10 (sponsor's #2.83) of the NDA.

The study was divided into several phases; those phases bearing on the efficacy of P on the relationship between the efficacy of P and AchE inhibition are as follows:

a) Phase III

Monkeys were given either 0, 4.0, 8.4, or 24 ug/kg P i.m. 45 minutes prior to Soman challenge. (45 minutes was the predicated Tmax for AchE inhibition as determined in preliminary studies). A single dose level of Soman was used, 32.5 ug/kg i.m., which was 5x the previously determined LD 50.) (A vehicle control for P was apparently not used.) Atropine (0.4 mg/kg) and 2-PAM (25.7 mg/kg) were given to all groups i.m. at 1 min. post-Soman. (These doses of atropine and 2-PAM were the same as those used in the first monkey study). The results at 48 hr. post-Soman (Table 4) indicate that survival was 0/4, 8/10, 9/10, and 7/10 in the control, low dose, medium dose, and high dose groups, respectively. (Observation was continued to 10 days post-Soman, during which time additional deaths occurred in 1 low dose and 2 high dose animals. At 10 days animals either appeared normal or exhibited various cholinergic signs). There were no differences in the incidence of clinical signs between the three P dosage groups, or between P-treated and non-P-treated groups.

The mean % RBC AchE inhibition at the time of Soman challenge are shown in table 4, and were 3%, 7%, 12%, and 29% in the control, low dose, medium dose, and high dose groups, resp. (AchE inhibition was evaluated by comparing enzyme activity just before the time of Soman challenge to a baseline value obtained 24 hr. before Soman challenge in each group). It is noted that survival was not correlated with mean AchE inhibition. (In addition, examination of the individual values for enzyme inhibition [Table D-4] shows that among P-treated animals, animals which died did not have enzyme inhibition which differed from that seen in other animals in the same group). It is also noted that the mean AchE inhibition in the low dose group (7%) was said to be not statistically significantly different from that in controls (3%), implying that the efficacy of P can be demonstrated in the absence of measurable drug-related enzyme

inhibition. (An examination of the individual values for enzyme inhibition [Table D-4], however, does seem to indicate a possible slight effect at the low dose as there was almost no overlap of values.)

b) Phase IV

In this phase 10 monkeys received P by intragastric intubation at 40 ug/kg and were challenged with 5 LD 50 Soman (dose as in phase III) 150 min. later (i.e., the predicted Tmax for AchE inhibition as determined in preliminary studies) and treated with atropine/PAM (doses as in phase III) 1 min. post-Soman. Results were compared to those obtained in phase III, above. As shown in table D-7, survival at 48 hrs. post-Soman was 8/10, i.e. comparable to that seen in phase III. (A table giving observations through 10 days post-Soman showed 2 additional deaths; also similar to phase III results). It was stated that there were no "statistical differences" in the incidence or duration of clinical signs between these animals and the P-treated animals in phase III.

The RBC AchE inhibition (enzyme activity just before Soman challenge compared to activity just before P administration) was 3.5%. This was not different from the 3% obtained in the non-P-treated control group in phase III, again suggesting that the efficacy of P can occur in the absence of measurable drug-related enzyme inhibition. Combined with the results of phase III these results reinforce the lack of a correlation between RBC AchE inhibition and the efficacy of P in these studies. (See figure 6). (Examination of individual animal values for enzyme inhibition [Table D-7] shows, paradoxically, that the 2 monkeys which died by 48 hours had the highest pre-Soman enzyme inhibition).

c) Phase V

In this phase protective ratios ("PR"; i.e. ratio of 48 hour Soman i.m. LD 50 in the presence of various pre- and post-Soman treatments to that in the absence of such treatments) were determined using a variety of groups as shown in table "7". (Briefly, comparing atropine/PAM treatment, atropine/PAM + diazepam treatment, and P-pre-treatment in

combination with each of the above. Dose of P = 4 ug/kg i.m., given 45 min. before Soman [i.e. the same as in phase III]; dose of diazepam ["DZM" in table] = 0.1 mg/kg i.m. given at the time of atropine/PAM. Doses and timing of atropine and PAM same as for earlier phases).

As shown in table 7, atropine/PAM alone had a slight, non-statistically significant effect (PR = 1.35). Adding P-pretreatment to this regimen gave a PR of 27.8. Diazepam did not affect the PR of atropine/PAM but apparently lowered the PR of the P/atropine/PAM regimen to 14.5 (said to be statistically different from the PR with P/atropine/PAM alone). (Note that the LD 50 for Soman alone was taken from previous studies, thus possibly calling into question the accuracy of PRs involving this group. At any rate, P pretreatment clearly raised the 48 hour PR of atropine/PAM [with or without diazepam] [although, as shown in the individual animal data in table D-8, a majority of the P-treated animals which were alive at 48 hours post-Soman had died by 10 days post-Soman, suggesting the benefit of adding P to atropine/PAM is not as great as suggested by the 48 hour PR comparison in this study. (Note that there is a discrepancy between the deaths shown in table D-8 and those in Table D-19, which did not show 10 day deaths in animals #H398, 6TY, 73C, 7C6, and 7D6. The sponsor is being requested to clarify this)]).*

The incidence of some clinical signs (salivation, miosis, prostration) was greater is the P-treated groups than in the groups given only atropine/PAM ± diazepam.

The mean RBC AchE inhibition (comparison of enzyme activity just before Soman administration to activity obtained 24 hr. before Soman) in P-treated groups was 5-7%. Inhibition in the groups not receiving P averaged about 0%. A quantitative correlation between efficacy and enzyme inhibition obviously cannot be made in this phase since only a single dose of P was used. Examination of the individual animal data (table D-8) shows no obvious correlation.

*Note added in proof: In the submission of 7/18/96 it was stated that these animals were sacrificed in moribund condition between 48 hr. and 10 days post-Soman. Furthermore, 2 additional animals listed in table D-8 as "alive" at 10 days had been similarly sacrificed. Thus, 19 of the 20 P-treated animals did not survive to 10 days.

TABLE 4. SUMMARY STATISTICS FOR PHASE III RESULTS BY PYRIDOSTIGMINE DOSE

Pyridostigmine Dose (μg/kg)	Number Tested	Number Surviving	Percent Surviving	Mean (S.D.) of % AChE-I	
0.0	4	0	0.0	3.00	(0.47)
4.0	10	8	80.0	6.92	(2.25)
8.4	10	9	90.0	12.12	(3.48)
24.0	10	7	70.0	28.74	(3.28)

TABLE D-4. DATA LISTING OF PHASE III RESULTS

Animal	Body Weight (kg)	Date	GD* Dose (μg/kg)	PYR Dose (μg/kg)	Baseline AChE Activity (U/mL)	Percent AChE Inhibition	48-Hour Results
75G	6.6	04/20/93	31.9	0.0	10.2	3.7	Died
78J	7.6	04/20/93	32.1	0.0	10.7	2.8	Died
7CA	7.3	05/25/93	30.9	0.0	9.4	2.7	Died
H355	7.9	05/25/93	32.2	0.0	8.7	2.8	Died
6RA	7.2	04/20/93	31.6	4.0	13.2	10.7	Lived
79Y	7.4	04/20/93	32.0	4.0	9.4	6.4	Lived
G654	8.4	04/27/93	32.6	4.0	9.5	5.5	Lived
H831	7.9	04/27/93	32.5	4.0	9.2	6.9	Died
78X	7.6	05/04/93	32.2	4.0	11.7	5.2	Lived
H818	7.4	05/04/93	31.4	4.0	8.0	7.9	Lived
6WB	8.1	05/18/93	33.3	4.0	10.2	3.1	Lived
75F	7.8	05/18/93	32.5	4.0	9.7	5.6	Lived
76P	7.3	05/25/93	32.1	4.0	10.4	9.5	Died
7AH	7.3	06/08/93	32.6	4.0	9.9	8.4	Lived
77K	7.8	04/06/93	31.4	8.4	9.0	13.9	Lived
G244	7.4	04/06/93	32.2	8.4	11.5	6.0	Lived
6Z1	7.9	04/13/93	32.5	8.4	9.5	12.9	Lived
78Y	8.0	04/13/93	32.5	8.4	10.0	14.9	Lived
6PJ	7.3	04/27/93	32.2	8.4	10.1	18.6	Lived
H436	7.7	04/27/93	30.6	8.4	11.8	9.1	Lived
6W2	7.6	05/18/93	32.0	8.4	11.9	13.0	Lived
H602	7.9	05/18/93	32.9	8.4	8.0	10.7	Lived
H432	7.8	05/25/93	31.9	8.4	9.3	12.4	Died
6XR	7.4	06/08/93	31.3	8.4	8.8	9.7	Lived
77V	6.3	04/06/93	32.9	24.0	11.6	28.8	Died
H632	7.5	04/06/93	32.6	24.0	10.8	32.7	Lived
6XC	7.4	04/13/93	33.0	24.0	8.6	23.8	Died
H227	7.4	04/13/93	33.0	24.0	8.5	23.2	Lived
71D	7.6	05/04/93	32.0	24.0	8.9	28.9	Lived
H789	6.9	05/04/93	32.0	24.0	8.4	29.8	Lived
78S	7.4	05/18/93	33.3	24.0	11.3	29.1	Lived
H282	8.1	05/18/93	32.6	24.0	8.9	33.4	Lived
H816	7.2	05/25/93	32.7	24.0	7.4	30.1	Died
H483	7.5	06/08/93	31.8	24.0	9.0	27.6	Lived

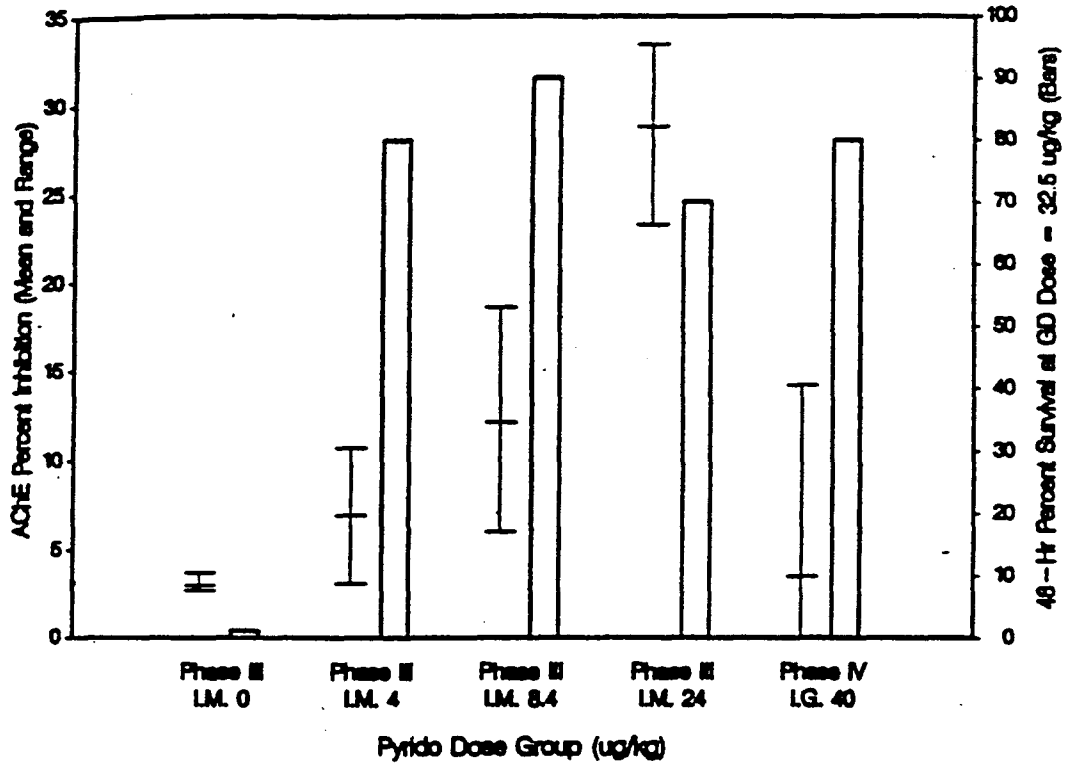
Targeted GD dose was 32.5 μg/kg. GD doses in this column are based on weight losses of syringes and initial chemical concentration analysis of the dosing solution.

TABLE D-7. AChE INHIBITION AND LETHALITY RESULTS FOR PHASE IV EXPERIMENTS: 40 μg/kg PYR i.g.; 5 X 48-hr GD LD50 150 MIN FOLLOWING PYR; ATR/2-PAM TREATMENT

Date	Animal	Body Weight (kg)	Baseline AChE Activity -10 min (U/mL)	Baseline AChE Activity -5 min U/mL	AChE-I -10 min (%)*	AChE-I -5 min (%)	Calculated* PYR Dose (μg/kg)	Calculated* GD Dose (μg/kg)	48-Hour Results
8/03/93	75H	6.1	10.1	9.8	-1.6	2.1	40	32.9	Alive
8/03/93	H167	6.4	7.9	7.7	0.3	-2.0	40	33.2	Alive
8/09/93	6TR	7.3	10.0	10.0	2.8	-1.8	40	32.2	Alive
8/09/93	5U3	7.3	10.0	10.1	9.4	12.4	40	32.1	Dead
8/10/93	H843	7.3	10.1	9.9	12.5	9.4	40	31.9	Alive
8/10/93	G923	7.3	11.3	11.2	4.2	3.1	40	31.8	Alive
8/23/93	H237	6.9	11.4	11.2	2.0	2.2	40	31.5	Alive
8/23/93	7CU	7.2	8.9	9.0	-3.0	-2.3	39	31.2	Alive
8/24/93	7BK	7.3	9.9	10.3	15.7	14.2	39	32.1	Dead
8/24/93	6XM	7.2	13.1	13.1	-5.6	-2.3	40	32.3	Alive

* Doses calculated from weight losses of syringes and concentrations of dosing solutions based on chemical analyses.

FIGURE 6. COMPARISON OF PERCENT ACHE-I AT 5 MIN PRIOR TO GD INJECTION AND SURVIVAL RATES IN PHASES III AND IV



$\overline{\overline{I}}$ = AchEI (mean and range)

$\prod$ = % survival (48 hr.)

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TABLE 7. SUMMARY OF RESULTS OF PROBIT MODELS: LD_{50} VALUES AND PROTECTIVE RATIOS

Treatment Group	N	LD_{50}	LD_{50} (95% Confidence Limits)	Protective Ratio	Protective Ratio (95% Confidence Limits)
Untreated[a]	25	6.53	(5.60,7.75)	1.00	(-)
ATR/2-PAM (Phase I)	10	20.6	(16.0,26.9)	3.16	(2.38,4.18)[b]
ATR/2-PAM/DZM	10	11.1	(7.94,15.3)	1.70	(1.22,2.39)[b]
ATR/2-PAM (Phase V)	8	8.81	(6.56,11.9)	1.35	(0.99,1.84)
PYR/ATR/2-PAM	10	182	(50.5,385)	27.8	(19.2,40.4)[b]
PYR/ATR/2-PAM/DZM	10	94.5	(29.1,252)	14.5	(9.67,21.7)[b]

[a]Data for untreated groups from Tasks 89-08, 89-12, and 92-30 are combined.
[b]Protective ratio is significantly greater than one ($p \leq 0.05$) since the lower confidence limit is greater than one.

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TABLE D-8. PHASE V DATA LISTING

Treatment Group	Date	Animal	Target QD Dose (µg/kg)	Calculated QD Dose (µg/kg)	Body Weight (kg)	Percent AChE Inhibition	48-Hour Results	10-Day Results
ATR/2-PAM/DZM	10/19/93	7CK	7.3	5.8	6.6	-2.3	Alive	Alive
ATR/2-PAM/DZM	10/12/93	71G	7.5	6.6	7.9	-1.3	Alive	Alive
ATR/2-PAM/DZM	09/21/93	78V	10.0	7.3	5.1	-0.2	Alive	Alive
ATR/2-PAM/DZM	09/28/93	6S4	10.5	10.9	8.2	3.2	Died	Died
ATR/2-PAM/DZM	09/24/93	75U	11.0	11.6	8.3	-0.1	Died	Died
ATR/2-PAM/DZM	09/14/93	7AU	12.0	12.3	7.7	1.0	Died	Died
ATR/2-PAM/DZM	09/10/93	6W6	15.0	15.3	6.9	1.6	Died	Died
ATR/2-PAM/DZM	09/03/93	7CC	18.0	17.9	6.5	-1.3	Alive	Died
ATR/2-PAM/DZM	09/07/93	H453	20.5	19.4	7.1	-2.2	Died	Died
ATR/2-PAM/DZM	08/31/93	H264	25.0	28.5	7.4	4.9	Died	Died
ATR/2-PAM	10/12/93	H482	5.5	5.7	8.4	-6.6	Alive	Alive
ATR/2-PAM	10/12/93	66P	7.0	6.9	7.3	0.4	Alive	Alive
ATR/2-PAM	10/19/93	75P	8.5	8.5	8.0	-2.3	Alive	Alive
ATR/2-PAM	09/28/93	7AC	8.0	7.9	8.0	0.8	Died	Died
ATR/2-PAM	09/24/93	74A	8.0	8.5	6.9	-2.8	Alive	Alive
ATR/2-PAM	09/21/93	6YZ	10.0	10.0	6.9	1.3	Died	Died
ATR/2-PAM	10/19/93	H258	13.0	13.2	7.0	-1.1	Died	Died
ATR/2-PAM	09/28/93	6RB	14.0	13.3	5.7	3.0	Died	Died

TABLE D-8.
(Continued)

Treatment Group	Date	Animal	Target GD Dose (µg/kg)	Calculated* GD Dose (µg/kg)	Body Weight (kg)	Percent AChE Inhibition	48-Hour Results	10-Day Results
PYR/ATR/2-PAM	08/31/93	H398	80.0	79.4	5.8	2.2	Alive	Died
PYR/ATR/2-PAM	09/14/93	6WG	130.0	129.4	6.0	9.8	Alive	Alive
PYR/ATR/2-PAM	09/03/93	6TY	160.0	158.3	6.9	7.8	Alive	Died
PYR/ATR/2-PAM	09/21/93	73C	175.0	173.8	7.0	7.0	Alive	Died
PYR/ATR/2-PAM	10/12/93	75Z	210.0	109.4	8.0	8.6	Alive	Alive
PYR/ATR/2-PAM	10/19/93	H525	215.0	212.3	7.4	9.5	Died	Died
PYR/ATR/2-PAM	09/10/93	6T4	200.0	199.7	7.5	7.9	Died	Died
PYR/ATR/2-PAM	09/28/93	73P	210.0	208.3	6.6	9.5	Died	Died
PYR/ATR/2-PAM	09/24/93	H074	210.0	208.4	8.0	7.5	Died	Died
PYR/ATR/2-PAM	09/07/93	H585	260.0	257.8	7.0	3.3	Died	Died
PYR/ATR/2-PAM/DZM	10/19/93	79P	60.0	60.3	7.8	5.4	Alive	Alive
PYR/ATR/2-PAM/DZM	10/12/93	H472	75.0	76.1	7.7	1.4	Died	Died
PYR/ATR/2-PAM/DZM	09/28/93	7C6	75.0	75.4	7.3	1.3	Alive	Died
PYR/ATR/2-PAM/DZM	08/31/93	5R2	80.0	78.1	5.3	4.5	Alive	Died
PYR/ATR/2-PAM/DZM	09/24/93	7C4	95.0	96.4	7.9	5.7	Died	Died
PYR/ATR/2-PAM/DZM	09/21/93	H309	110.0	108.8	7.7	8.4	Died	Died
PYR/ATR/2-PAM/DZM	09/14/93	H300	130.0	127.9	7.0	6.6	Died	Died
PYR/ATR/2-PAM/DZM	09/03/93	7D6	160.0	158.6	7.5	4.7	Alive	Died
PYR/ATR/2-PAM/DZM	09/10/93	H612	200.0	198.7	7.5	5.7	Died	Died
PYR/ATR/2-PAM/DZM	09/07/93	7AL	260.0	258.9	6.9	6.7	Died	Died

* GD doses calculated using weight losses of syringes and chemical analysis of dosing solution.

B) GUINEA PIG (5 studies)

Study B-1 Study of Capacio, et.al.
(Located in volume 1.5, page 1+, and, in slightly altered form, volume 2.9 [sponsor's # 2.82] of the NDA)

Dose of P = 0.94 mg/kg p.o., given 45 min. prior to Soman (s.c.). Atropine (16 mg/kg) and 2-PAM (25 mg/kg) were given i.m. 1 minute post-Soman. Strain of animals = Crl : (HA) ——— (males).

Results shown in attached tables 4 and 5. (Each table represents a separate study; these studies were done in part to evaluate the effects of administering either ondansetron ["OND" in table 4] or granisetron ["GRN" in table 5] in combination with P. Diazepam ["DZ" in tables] treatment [1 mg/kg i.m. given at the same time as atropine/PAM] was evaluated in some groups). Results are expressed as a protective ratio ("PR"), i.e. ratio of the 24 hour Soman LD 50 in the presence of treatment to that in untreated controls. The PRs for atropine + PAM alone were 1.9 and 1.7 in the two studies (i.e. group # 2 in tables 4 and 5, resp.). Pretreatment with P (followed by post-Soman atropine/PAM) gave PRs of 4.9 and 4.1 resp.(i.e. group #3 in tables 4 and 5, resp.). (Note that it is not clear if a statistical analysis was performed on the PR values, although note that there was no overlap between the 95% CLs between groups 1, 2, and 3. Results as presented were not broken down by Soman dose.)

AchE activity was determined in separate groups of animals (i.e. different animals from those used in the "efficacy " study.). Whole blood was used; the substrate was acetyl-β-methylcholine which the report states specifically measures RBC AchE activity. Results are shown in figure 3B. (Note that the legibility of this figure is limited; some of the following is based on statements in the text). P alone caused an inhibition of about 40% at 45 min. post-dose (i.e. the time of Soman challenge in the "efficacy" study).

Only a single dose level of P was used in this study. However, some information concerning the relationship between AchE inhibition and efficacy can be obtained for the results with ondanseron, which was given at the same time as P in some groups in the efficacy study. In that study, ondansetron at various doses (0.5 - 30 mg/kg p.o.) did not alter the efficacy of P. (Table 4, groups 4-7). However, ondansetron at doses of 10 and 20 mg/kg in combination with P were shown to cause an inhibition of AchE greater than that with P alone (figure 3B), e.g., at 45 min. post-dosing (i.e. time of Soman exposure), inhibition was approx. 50-55% compared with approx. 40% with P alone. Although this difference is relatively small, it does not support an association of AchE inhibition with protection from Soman-induced lethality.

STUDY B-1

Table 4: Interaction of Ondansetron (OND) on the 24-Hour Efficacy of Pyridostigmine (PYR), Atropine (AT), 2-PAM and Diazepam (DZ) Therapy of Soman Poisoning in Guinea Pigs.

Grp ID	Pretreatment[a] (mg/kg)		Treatment[b] (mg/kg)			N	LD50 soman[c] (μg/kg)	95% CI[c]	PR[d]
	OND	PYR	AT	2-PAM	DZ				
1	-	-	-	-	-	39	30.4	28.2-33.2	1.0
2	-	-	16	25	-	37	58.9	50.8-70.7	1.9
3	-	0.94	16	25	-	34	150.1	98.6-314.9	4.9
4	0.5	0.94	16	25	-	40	139.4	114.8-173.4	4.7[e]
5	10.0	0.94	16	25	-	40	190.9	144.5-276.7	6.3
6	20.0	0.94	16	25	-	39	152.3	129.1-188.3	5.0
7	30.0	0.94	16	25	-	37	155.1	128.8-204.0	5.1
8	-	0.94	16	25	1.0	36	234.9	181.2-331.0	7.7
9	0.5	0.94	16	25	1.0	40	232.8	167.3-321.9	7.9[e]
10	10.0	0.94	16	25	1.0	37	297.1	228.0-446.8	9.8
11	20.0	0.94	16	25	1.0	37	149.1	83.9-353.0	4.9
12	30.0	0.94	16	25	1.0	39	263.1	208.6-353.1	8.7

a. Pretreatment was admixed and administered orally by gavage 45 min before soman.
b. Treatment was administered im 1 min post-soman. Atropine and 2-PAM were admixed, diazepam was administered as a separate injection.
c. LD50s and 95% Confidence Intervals were calculated by Probit Analysis using SAS NLIN based on 24-hr mortality.
d. PR = treated GD LD50/untreated GD LD50.
e. PR based on untreated GD L50 of 29.3 μg/kg from Granisetron study.

Table 5: Interaction of Granisetron (GRN) on the 24-Hour Efficacy of Pyridostigmine (PYR), Atropine (AT), 2-PAM and Diazepam (DZ) Therapy of Soman Poisoning in Guinea Pigs.

Grp ID	Pretreatment[a] (mg/kg)		Treatment[b] (mg/kg)			N	LD50 soman[c] (μg/kg)	95% CI[c]	PR[d]
	GRN	PYR	AT	2-PAM	DZ				
1	-	-	-	-	-	40	29.3	26.8-31.7	1.0
2	-	-	16	25	-	40	49.5	41.7-57.6	1.7
3	-	0.94	16	25	-	40	120.4	94.4-152.5	4.1
4	0.05	0.94	16	25	-	40	129.9	84.6-203.2	4.4
5	0.5	0.94	16	25	-	40	145.9	111.4-188.5	5.0
6	5.0	0.94	16	25	-	40	160.0	117.4-223.8	5.5
7	-	0.94	16	25	1.0	40	203.4	147.6-276.3	7.0
8	0.05	0.94	16	25	1.0	40	230.6	150.3-362.9	7.9
9	0.5	0.94	16	25	1.0	40	262.0	195.0-351.2	8.9
10	5.0	0.94	16	25	1.0	40	215.8	158.7-289.6	7.4

a. Pretreatment was admixed and administered orally by gavage 45 min before soman.
b. Treatment was administered im 1 min post-soman. Atropine and 2-PAM were admixed, diazepam was administered as a separate injection.
c. LD50s and 95% Confidence Intervals were calculated by Probit Analysis using SAS NLIN based on 24-hr mortality.
d. PR = treated GD LD50/untreated GD LD50.

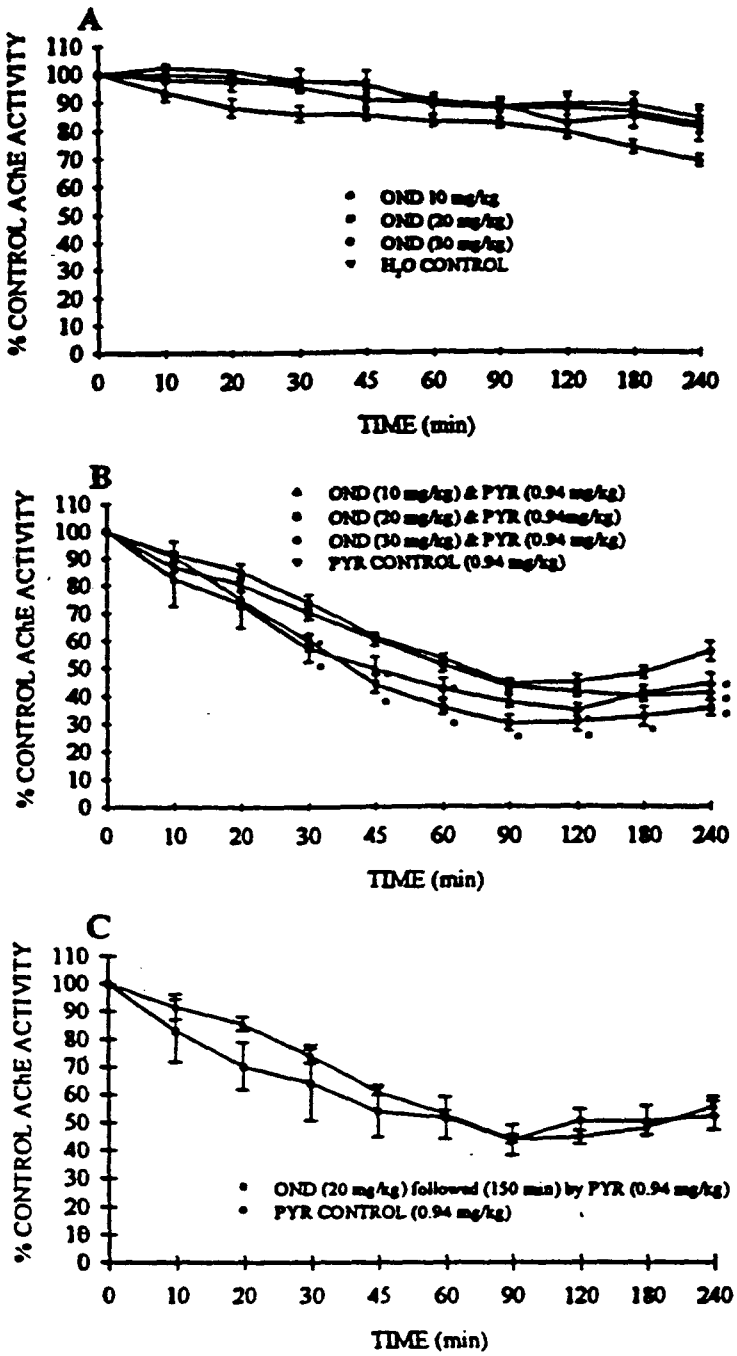

Figure 3. A - Time-course effects of water and OND (10, 20 and 30 mg/kg, po)(n=6) on AChE activity. B - Time-course effects of PYR control (0.94 mg/kg, po) and PYR (0.94 mg/kg, plus OND 10, 20 or 30 mg/kg, po) on AChE activity(n=8). *indicates significant difference between PYR control and OND plus PYR. C - Time-course effects of PYR control (0.94 mg/kg, po)(n=8) and OND (20 mg/kg, po)(n=7) on blood AChE activity. All data are mean ± SEM.

Study B-2 Study of Lennox, et-al.

(Located in vol 1.17, p.39+, of NDA. This is a published paper, a copy of which is attached).

Various doses of P were given i.m. 30 min. prior to Soman (given s.c.); actual doses of P were not stated but were based on a preliminary study done to determine dose causing 10, 20, 30, 45, and 70% inhibition of cholinesterase at 30 min. post-dose. (The dose range of P in the preliminary study was said to be 0.005 - 0.20 mg/kg). Atropine (16 mg/kg i.m.) and 2-PAM (50 mg/kg i.m.) were given 1 minute post-Soman. Strain of animals = [AMRI : (HA)] BR (males and females).

Results are shown in table III of the paper. (Note that physostigmine was also studied). Results are expressed as a protective ratio ("PR"), i.e. ratio of the 24 hour Soman LD 50 in the presence of treatment to that in untreated controls. Atropine + PAM ("therapy control" in the table) gave a PR of 2.0. Only the highest dose of P (i.e. that predicted to cause AchE inhibition of 70% at time of Soman administration) caused a statistically significant increase in PR (7.1) over atropine/PAM alone. (The next highest dose of P used was predicted to cause a 45% inhibition of AchE.). (Results as presented were not broken down by Soman dose or animal gender).

As indicated in table III, the calculated correlation between efficacy and AchE inhibition was said to be statistically significant. The relationship between these 2 variables is shown graphically in figure 1. (open triangles). (Note that the degrees of enzyme inhibition were not determined in the animals used in the efficacy study, but were predicted values based on a preliminary study. Also note that whole blood was used for the enzyme assay; substrate was acetyl-β-methylcholine.).

Study B-3 Study of Jones, et.al.

(Located in vol. 1.16, p. 173+, of NDA). This is a published paper, a copy of which is attached).

Doses of P ranged from 0.06 to 15.0 mg/kg p.o., given 1 hour prior to Soman (given s.c.). It was not stated if a vehicle control for P was used. Atropine (64 mg/kg) and 2-PAM (100 mg/kg) were given i.m. at 1 min. after Soman administration. Strain of animals = Duncan Hartley Albino, from Charles River. ("Mixed sex").

Results are shown in table 1 and figure 2. Results are expressed as a protective ratio ("PR"), i.e. ratio of 24 hour Soman LD 50 in the presence of treatment to that in untreated controls. Atropine/PAM alone gave a PR of approx. 3.4 (estimating from figure 2); it was not stated if this was statistically significant. Doses of P of 0.23 mg/kg and above, in combination with atropine/PAM therapy, caused statistically significant increases in PR (PRs ~ 5-6.4) compared to atropine/PAM alone. (Also note, as indicated in figure 2, that P alone, or in combination with 2-PAM therapy only, had no effect.) (Note that the results as presented were not broken down by Soman dose or animal gender. [We requested more detailed data from the sponsor at the meeting of 4/6/95; volume 2.9 (sponsors # 2.82) contains some data which is partly unintelligible and does not appear to address these points]).

As indicated in table 1, the minimally effective dose of P, 0.23 mg/kg, caused 16.4% inhibition of AchE at 1 hour post-dosing (i.e. the time of Soman administration, although note that the AchE data were obtained in different animals from those used in the efficacy study). (The next highest dose of P used was 0.12 mg/kg, which caused 5.4% enzyme inhibition; the PR at this dose was said to be not statistically significantly different from that with atropine/PAM alone, although note that numerically the PR was actually greater than that seen with the 0.23 mg/kg dose). As also indicated in table 1, although the degree of AchE inhibition was highly correlated with the dose of P, the PR was not statistically significantly correlated with either the dose of P or with the degree of P-induced AchE inhibition. (As discussed in the article, modeling of the data by "Response Surface Modeling," which uses predicted "optimal" doses for atropine and PAM, results in a correlation between P-induced AchE inhibition and efficacy). Note that the assay for "AchE" in this study used whole blood, using a method cited in the literature but not described.

Study B-4 Study of Inns and Leadbeater

(Located in vol. 1.7, p. 258+, of the NDA. This is a published paper, a copy of which is attached).

Dose of P was 0.32 u mol/kg given i.m. 30 min. prior to Soman (given s.c.). (It was not stated if a vehicle control for P was used). Atropine (50 u mol/kg) and P2S (mesylate salt of 2-PAM) (130 u mol/kg) were given i.m. one minute (or at time of signs of poisoning if these occurred sooner) after Soman. (Diazepam, 25 u mol/kg i.m., was given immediately after the atropine/P2S in some groups). Strain of animals = Porton strain (Dunkin-Hartley derived) (females).

Results are shown in table 3. (Note that most of this paper involves evaluating the effects of a series of oxime derivatives which does not involve evaluating the effect of P per se). Results are expressed as a protective ratio ("PR"), i.e. the ratio of the 24 hour Soman LD 50 in the presence of treatment to that in untreated controls. As seen in table 3, the PR with P/atropine/P2S was 6.8 vs 1.7 for atropine/P2S alone. When diazepam was included, the PR with P/atropine/P2S/diazepam was 14 vs 2.5 for atropine/P2S/diazepam alone. (Note that no statistics were presented, although note that the 95% CL values for P-treated and non-P-treated groups did not overlap. The results as presented were not broken down by Soman dose. The numbers of animals used were not clear). The results in table 3 also indicate that adding P2S had little or no effect on the efficacy of P/atropine/± diazepam.

Other nerve agents besides Soman were studied in this study (Sarin, VX, and Tabun); however there were no valid evaluations of the efficacy of P on these compounds (since when P was used diazepam was also used, but diazepam was not present in the non-P-treated groups).

AchE inhibition was not measured in this study.

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Study B-5 Study of Koplovitz, et.al.

(Located in vol. 1.16, p. 226+ of the NDA. This is a published paper, a copy of which is attached).

Dose of P = 0.47 or 1.9 mg/kg p.o. given 60 min. prior to Sarin, VX or Tabun (given s.c.). Atropine (32 mg/kg) and 2-PAM (25 mg/kg) were given i.m. at one minute after nerve agent administration. Strain of animals = Hartley albino, Charles River Labs. (Males and females).

Results are shown in table 1. Results are expressed as PRs as defined above. (Based on 24 hr. LD 50s for nerve agents.) Atropine/PAM alone gave large PRs against Sarin and VX and a relatively lower PR (4.4) against Tabun. Addition of P further increased the PR against Tabun to 7.8 and 12.1 at P doses of 0.47 and 1.9 mg/kg, resp. (It was not stated if these 2 PRs were statistically significantly differed from each other; table 1 indicates significant overlap in the 95% CL values). Against Sarin and VX, the addition of P tended to slightly decrease the PRs of atropine/PAM alone, but this was not statistically significant. (Results as presented were not broken down by dose of nerve agent or animal gender).

AchE inhibition was not measured in this study; based on data cited in the literature it was estimated that the low and high doses of P used would cause approx. 30 and 60% inhibition, resp. (Contradictory statements are made in the paper whether this refers to whole blood or RBC enzyme).

C) RABBIT (3 studies)

Study C-1 Study of [redacted]

Dose of P was 0.08 mg/kg i.m., given 2 hours prior to Soman or Tabun given i.m. (It was not stated if a vehicle control for P was used). Atropine (2 mg/kg) and 2-PAM (25 mg/kg) were given i.m. 2 min. (or at the first signs of of general intoxication) after nerve agent. Strain of rabbits = New Zealand White (males).

Results were expressed as PRs (based on 48 hour LD 50s) as defined earlier. Results were as follows (PR with 95% CL):

	SOMAN	TABUN
atropine/PAM	1.36 (1.04, 1.77)	2.44 (1.73, 3.44)
P/atropine/PAM	1.46 (1.02, 2.09)	3.85 (2.18, 6.80)

It was concluded that the effect of atropine + PAM was statistically significant (i.e., the CL did not bracket 1.0) for both nerve agents. A statistical comparison of the atropine/PAM group with the P/atropine/PAM group was apparently not performed, but it appears that adding P caused little or no added protection. (Results were not broken down by nerve agent dose. Ns were as follows: atropine/PAM = 117 and 100 for Soman and Tabun, resp.; P/atropine/PAM = 30 with either Soman or Tabun).

. The dose of P used was estimated (based on data in other groups of rabbits) to cause approx. 20-40% RBC AchE inhibition (substrate: acetylthiocholine) at the time of nerve agent administration.

Study C-2 Study of [redacted]

Dose of P = 0.32 mg/kg i.m. given 30 min. before Soman (i.m.). (It was not stated if a vehicle control for P was used). Atropine (5 mg/kg) and 2-PAM (10 mg/kg) were given i.m. at the onset of the first sign of intoxication (usually tremors) after Soman administration. Strain of rabbits = New Zealand white, both genders.

Results, expressed as PRs as defined earlier (based on 24 hour Soman LD 50), are shown in attached tables 4 (no P prophylaxis) and 7 (P prophylaxis). The PR (with 95% CL) for atropine/PAM alone was 2.2 (1.9, 2.7) and for P/atropine/PAM was 3.1 (2.2, 4.2). The report stated that P "significantly" enhanced the efficacy of the other treatments; however it is not clear that the effects of P were tested statistically; also note that the overlap in the 95% CL for the PRs noted above was considerable. (It is also noted that atropine alone and atropine/PAM alone [i.e., without P prophylaxis] caused apparently statistically significant increases in the LD 50 of Soman [PR = 1.9 and 2.2, resp.; 95% CL did not bracket 1, see Table 4]. [However, note that the value for the LD 50 of Soman alone was apparently based on historical data, making the accuracy of these latter comparisons questionable]).

Results broken down by Soman dosage are shown in tables 3 (no P prophylaxis) and 6 (P prophylaxis). Results were not broken down by gender.

It was stated that P reduces "whole blood cholinesterase" activity by approx. 30-70% at the dose of P used in this study; this was based on values cited in a literature report, i.e. enzyme activity was not measured in the present study.

Study C-2

Table 3. Data for Groups of 10 Rabbits Treated Intramuscularly at First Sign of Intoxication After Challenge with GD, Also Given Intramuscularly

Treatment	Challenge (mg/kg)	Median Time to Death in Minutes	Median Quality of Life of Survivors	No. of Survivors at 24 hours
GD Control*	0.00787	-	Not Observed	6/6
	0.00984	77	" "	3/6
	0.0128	123	" "	2/6
	0.0157	111	3	2/6
	0.0197	188	-	0.6
Atropine	0.00938	-	1	10
	0.0149	65	1	9
	0.0226	O.N.**	1-2	5
	0.0358	8	-	0
PAM/Atropine	0.0127	-	1	10
	0.0201	O.N.	1	8
	0.0319	20	2	2
	0.0506	10	-	0
TMB-4/Atropine	0.0127	100	1	8
	0.0201	153	1	5
	0.0319	24	-	0
	0.0506	19	-	0
TMB-4 Atropine/ Benactyzine	0.0170	-	1	10
	0.0269	O.N.	1	7
	0.0428	27	2	2
	0.0678	13	-	0

* Data from previous experiment for comparative purposes; LD50 is 0.0115 (0.00913 - 0.0140) mg/kg. Agent potency on experimental days was assessed using mice, and found to be within the expected range.

** O.N. = Overnight

Table 4. Protective Ratios Found With Various Therapies. All Injections Were Intramuscular. Treatment Administered 10 Seconds After Challenge in Mice, At First Sign of Intoxication in Rabbits.

Treatment	Species	LD50 Multiples Treated with 95% Confidence Limits and Slope Value of Fitted Line*
GD Control	Mouse	1.0
Atropine · SO_4	Mouse	1.4 (1.3-1.6); Slope Value = -9.24
PAM · Cl Atropine · SO_4	Mouse	1.1 (0.94-1.3); Slope Value = -6.94
TMB-4 Atropine · SO_4	Mouse	2.1 (1.9-2.3); Slope Value = -13.7
TMB-4 Atropine · SO_4 Benactyzine · HCl	Mouse	2.0 (1.8-2.3); Slope Value = -11.8
GD Control	Rabbit	1.0
Atropine · SO_4	Rabbit	1.9 (1.6-2.3); Slope Value = -8.55
PAM · Cl Atropine · SO_4	Rabbit	2.2 (1.9-2.7); Slope Value = -9.04
TMB-4 Atropine · SO_4	Rabbit	1.6 (1.3-2.0); Slope Value = -6.90
TMB-4 Atropine · SO_4 Benactyzine · HCl	Rabbit	2.9 (2.4-3.5); Slope Value = -8.07

* An LD50 value of 0.115 mg/kg was used for mouse calculations, and a value of 0.0115 mg/kg for rabbit calculations. Agent potency was checked, using mice on each experimental day. The LD50 values varied from 0.0831 to 0.131 mg/kg, with a mean value of 0.102 mg/kg.

Table 6. Data for Groups of 10 Rabbits* Administered Pyridostigmine Prophylaxis 30 Minutes Before Challenge, Challenged with GD, and Treated Intramuscularly at First Sign of Intoxication

Treatment	Challenge (mg/kg)	Median Time to Death in Minutes	Median Activity Level of Survivors	No. of Survivors at 24 hrs.
GD Control**	0.00787	-	Not observed	6/6
	0.00984	77	" "	3/6
	0.0128	123	" "	2/6
	0.0157	111	3	2/6
	0.0197	188	-	0/6
None*** (Pyridostigmine prophylaxis only)	0.0187	>312	-	0/6
	0.0236	>177	2	2/12
Atropine Sulfate	0.0201	>207	1	8
	0.0319	86	1	5
	0.0402	70	1	5
	0.0506	8 1/2	1	3
PAM/Atropine	0.0143	-	1	10
	0.0226	105	1	6
	0.0358	>119	1	3
	0.0567	8	1	5
	0.0890	5 1/2	1-2	1
	0.140	6 1/2	-	0
TMB-4/Atropine	0.0179	>262	1	8
	0.0358	>249	1	4
	0.0714	12	1	4
	0.143	5 1/2	1	1
Atropine/TMB-4/ Benactyzine	0.0358	>340	1	9
	0.0899	12	1	6
	0.111	6 1/2	1-2	3
	0.282	7	-	0

* Except for GD Control.

** Data from previous experiment for comparative purposes; LD50 is 0.0115 (0.00913-0.0140) mg/kg. Agent potency on experimental days was assessed using mice, and found to be within the expected range.

*** Data from previous experiments.

Table 7. Protective Ratios Found With Pyridostigmine Prophylaxis Alone or Combined With Various Therapies. All Injections Were Intramuscular, With Prophylaxis Administered 7.5 Minutes Before Challenge to Mice and 30 Minutes Before Challenge to Rabbits. Treatment Given 10 Seconds After Challenge to Mice, At First Sign of Intoxication to Rabbits.

Treatment	Species	LD50 Multiples Treated With 95% Confidence Limits and Slope Value of Fitted Line
GD Control	Mouse	1.0
Pyridostigmine • Br	Mouse	0.88 (0.59-1.3); Slope Value = -13.1
Atropine • SO_4	Mouse	1.9 (1.7-2.2); Slope Value = -6.87
PAM • Cl Atropine • SO_4	Mouse	2.7 (1.4-4.6); Slope Value = -5.0
TMB-4 Atropine • SO_4	Mouse	3.3 (2.9-3.8); Slope Value = -7.16
TMB-4 Atropine • SO_4 Benactyzine • HCl	Mouse	4.1 (3.5-4.8); Slope Value = -5.96
GD Control	Rabbit	1.0
Pyridostigmine • Br	Rabbit	<1.6
Atropine • SO_4	Rabbit	3.2 (1.9-7.3); Slope Value = -3.18
PAM • Cl Atropine • SO_4	Rabbit	3.1 (2.2-4.2); Slope Value = -3.16
TMB-4 Atropine • SO_4	Rabbit	3.4 (1.5-5.9); Slope Value = -2.03
TMB-4 Atropine • SO_4 Benactyzine • HCl	Rabbit	7.7 (5.3-11.0); Slope Value = -3.97

Dose of P = 0.08 mg/kg i.m., given 2 hours prior to i.m. administration of Soman, Tabun, Sarin, or VX. Atropine (13 mg/kg) and 2-PAM (25 mg/kg) were given i.m. at the onset of clinical signs (fasciculations or tremors), or at 2 minutes (whichever came first) after nerve agent administration. Strain of rabbits = New Zealand white (males). Rabbits were "atropinesterase free".

Results for Soman and Tabun are shown in Table 6. The 24 hour Soman LD 50 in animals treated with atropine/PAM was 28.4 ug/kg compared with 42.0 ug/kg when P was also given. It is not clear from the paper if the difference between these LD 50 values was considered to be statistically significant. Examination of the results broken down by Soman dosage (comparison of Table 1 [for atropine/PAM] and table 2 [for P/atropine/PAM]) do not allow a firm conclusion regarding the efficacy of P.

The 24 hour Tabun LD 50 in animals treated with atropine/PAM was 987 ug/kg compared with > 2000 ug/kg when P was also given (Table 6). Again it is not indicated if this was considered to be statistically significant; examination of the limited data broken down by Tabun dose (compare tables 4 and 5) does not allow a firm conclusion regarding the efficacy of P (particularly in view of the variability of the data in the P/atropine/PAM group [table 5]).

Note that in table 6 there appears to be a slight efficacy of atropine + PAM alone (i.e. without P) against Soman and a more substantial effect against Tabun; however note that the LD 50 values for nerve agents alone were taken from previous studies.

. For studies using Sarin and VX, a single dose level of each agent, 10 times the LD 50, was given, and survival determined 24 hours later. Results shown in table 7. There was a suggestion of a beneficial effect of P against Sarin (1/6 deaths vs. 4/8 deaths with atropine/PAM alone) although the data are rather limited. All animals given VX and treated with atropine/PAM survived whether or not P was also given.

AchE inhibition was not measured in this study; it was stated, based on referenced data, that the dose of P used was reported to cause 20-40% inhibition of RBC AchE.

STUDY C-3

TABLE 1

EFFECT OF ATROPINE AND OXIME TREATMENT AGAINST SOMAN POISONING IN THE RABBIT

Atr/2-PAM			Atr/HI6		
Soman ug/kg	No. Responding	No. Sampled	Soman ug/kg	No. Responding	No. Sampled
13.2	0	1	21.0	0	1
16.7	0	2	26.4	1*	2
18.6	0	2	33.2	0	2
19.8	0	1	41.9	0	2
21.0	0	2	47.5	0	2
22.2	0	2	52.7	1	1
26.4	2	5	53.3	0	2
31.1	1	2	56.5	1	2
35.2	2	2	67.1	0	2
37.3	1	1	71.1	1	1
			80.0	0	2
			95.0	1	2
			120.0	3	4
LD50	28.4 ug/kg		LD50	98.6 ug/kg	(89.8)*
95% CI	24.0 -37.9		95% CI	-	(64.6-354.8)*
Slope	16.6		Slope	2.2	(4.3)*
N	20		N	25	(25)*

*This animal's response was responsible for the inability of the maximum likelihood procedure to calculate a 95% Confidence Interval (95%CI) for the LD50 estimate. When the animal's response was changed from dead to alive, the values shown in parentheses were obtained. The LD50 estimate was changed by only 10% and 95% CI were determined. Comparison of this LD50 estimate and 95% CI with that of 2-PAM shows a significant difference ($p<0.05$) between the efficacy of HI6 and 2-PAM against soman.

APPEARS THIS WAY
ON ORIGINAL