3. Although the authors stress the role of various drug combinations in producing the effects seen, it cannot be ruled that the same effects would not also have been seen with higher doses of the individual drugs given singly. Thus it might be possible that the effects in the veterans, assuming they are related at all to these compounds, could be due not to any particular combination but to one of the compounds by itself if exposure were high enough. Both DEET and chlorpyrifos have produced significant neurotoxicity in humans (references cited in present article and in my previous review).

This new study does not change any of the conclusions reached in my review of 7/24/96.

/S/

Barry N. Rosloff, Ph. D.

NDA
Original IND 20-414
Div. File HFD-120/
/BRosloff/RKatz/RTresley/
/CSO/RNighswander/
/PLeber/
Supv. Init. /GFitzgerald/
N:\TUBBS\I20414.LET

Nighswander

Barry N. Rosloff, Ph.D.
10/28/96

Pharmacologist Review of NDA 20-414
Submissions of 09/20/96 and 09/24/96

SPONSOR: Office of the Surgeon General
Department of Army
Fort Detrick
Frederick, Maryland

DRUG: pyridostigmine bromide

CATEGORY: Cholinesterase inhibitor for use as a prophylactic treatment for organophosphorus nerve agent - induced lethality

PREVIOUS PHARMACOLOGIST REVIEW: Original Summary of 07/24/96

CONTENTS OF PRESENT SUBMISSIONS:

A) **09/20/96** - Amended final report for "Task 85-18," i.e., the primary monkey study submitted in support of the efficacy of pyridostigmine for the presently proposed indication (study # "A-1" in my review of 07/24/96).

B) **09/24/96** - Four new genotoxicity studies. All were performed by [redacted], using pyridostigmine bromide "Bottle Number" BM03894.

ABBREVIATION: Pyridostigmine will be abbreviated as "P" throughout this review.

A) **Submission of 09/20/96**

This submission consists of a long list of changes/corrections to the report of the previously submitted and reviewed monkey efficacy study noted above. This amended report was apparently prompted by the numerous inconsistencies found during an FDA audit of this study. The changes are generally minor, and do not impact on the interpretation and conclusions of the study, with one exception as follows. The report originally stated that "Because of the potential for rapid reversal of enzyme inhibition, all AChE analyses were completed within 3 minutes of sample collection." The amendment states that "Not all analyses were performed within 3 minutes of sample collection, but were analyzed as rapidly as possible." No further details are given. See "Evaluation" section for further discussion.

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B) **Submission of 09/24/96**

1) Ames Test

Plate incorporation method used. P was negative up to the highest concentration used (5000 μg per plate, determined to cause no toxicity in a preliminary assay), both with and without the presence of an Aroclor - induced rat liver S9 preparation. Summary of results, showing bacterial strains used, is attached.

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Salmonella/E.coli Mutagenicity Assay
Summary of Results

Table 16

Test Article Id : Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894
Study Number : G96AP65.502 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	18 ± 3	139 ± 12	10 ± 2	7 ± 2	23 ± 3
10	15 ± 1	137 ± 4	13 ± 2	3 ± 2	25 ± 3
33	21 ± 1	136 ± 4	7 ± 3	6 ± 1	22 ± 2
100	22 ± 2	143 ± 8	7 ± 2	4 ± 2	22 ± 3
333	19 ± 2	135 ± 18	4 ± 1	7 ± 2	21 ± 3
1000	20 ± 6	134 ± 13	9 ± 6	4 ± 3	21 ± 2
5000	17 ± 1	146 ± 3	8 ± 3	5 ± 2	18 ± 3
Pos	168 ± 27	551 ± 36	416 ± 24	180 ± 70	160 ± 9

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	34 ± 2	155 ± 9	8 ± 3	9 ± 3	17 ± 3
10	31 ± 6	158 ± 16	10 ± 2	7 ± 1	22 ± 3
33	29 ± 2	161 ± 17	9 ± 4	9 ± 5	18 ± 1
100	26 ± 2	144 ± 28	9 ± 3	8 ± 2	21 ± 4
333	22 ± 1	122 ± 11	8 ± 2	5 ± 1	20 ± 8
1000	26 ± 6	138 ± 15	12 ± 2	9 ± 5	24 ± 5
5000	25 ± 4	150 ± 26	10 ± 2	6 ± 2	19 ± 5
Pos	994 ± 295	657 ± 94	169 ± 7	175 ± 14	180 ± 38

0.0 = Vehicle plating aliquot of 50 μl
Pos = Positive Control concentrations as specified in Materials and Methods section.

2) Mouse Lymphoma L5178Y/TK Assay

Results of preliminary toxicity assay shown in attached table 1. At the highest concentration of P used (5000 μg/ml) P reduced cell suspension growth to 56 and 23% of control in the absence and presence, respectively, of an Aroclor - induced rat liver S9 preparation. It was stated (p. 11) that based on these results concentrations of 100 - 5000 μg/ml were used for the mutagenesis assay, although the results presented show concentrations of 1000 - 5000 μg/ml as being used.

Results of the mutagenesis assay are shown in attached tables 2 and 3 (mutation frequency and cytotoxicity results, resp., in absence of metabolic activation), tables 4 and 5 (mutation frequency and cytotoxicity results, resp., in presence of metabolic activation), and figure 2 (colony size distribution in presence of metabolic activation; also attached are figures 1 and 3 showing colony size distribution for positive controls). P was considered positive in the presence of S9. As shown in table 4, all concentrations of P increased the mutant frequency above solvent control, although according to the stated criteria only 3000 μg/ml and above were clearly positive (since induced mutant frequencies of < 55 were considered to be negative and induced mutant frequencies of 55-99 were considered to be equivocal). The increase was dose-related. The data on colony size distribution (figure 2) "showed an increase in the frequency of small, medium, and large colonies." It was stated that the increase in small colonies "is consistent with damage to multiple loci on chromosome 11 in addition to loss of the TK locus", and an increase in large colonies "is consistent with very localized damage, possibly in the form of a point mutation or small deletion within the TK locus."

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TABLE 1

PRELIMINARY TOXICITY ASSAY USING Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894

Test Article Concentration (µg/ml)	Cell Concentration (X 10^6) Day 1	Day 2	Suspension Growth[a] Total[b]	% of Control[c]
WITHOUT ACTIVATION				
Water 1	1.589	1.502	26.5	
2	1.594	1.466	26.0	
Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894				
0.5	1.551	1.479	25.5	97
1.0	1.641	1.415	25.8	98
5.0	1.556	1.437	24.9	95
10	1.489	1.517	25.1	96
50	1.549	1.586	27.3	104
100	1.625	1.516	27.4	104
500	1.518	1.460	24.6	94
1000	1.381	1.490	22.9	87
5000	1.003	1.318	14.7	56
WITH S9 ACTIVATION				
Water 1	1.107	1.452	17.9	
2	1.239	1.340	18.4	
Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894				
0.5	1.180	1.494	19.6	108
1.0	1.155	1.380	17.7	98
5.0	1.075	1.494	17.9	98
10	1.118	1.431	17.8	98
50	1.118	1.467	18.2	100
100	1.130	1.326	16.7	92
500	1.035	1.367	15.7	87
1000	0.919	1.311	13.4	74
5000	0.379	0.995	4.2	23

[a] - Cultures containing <$0.3x10^6$ cells/ml on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / $0.3x10^6$ cells/ml) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

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TABLE 2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/ml)		TFT Plates 1	2	3	Avg.	Stand. Dev	V.C. Plates 1	2	3	Avg.	Stand. Dev	Mutant Frequency[a]	Induced Mutant Frequency[b]	%Total Growth[c]
Water	1	53	27	59	46	±14	214	221	205	213	±7	43		
	2	66	54	45	55	±9	191	209	179	193	±12	57		
Mean Water Mutant Frequency= 50														
Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894:														
1000	A	54	37	48	46	±7	195	195	204	198	±4	47	-3	88
	B	44	38	34	39	±4	205	224	219	216	±8	36	-14	100
2000	A	34	45	+	40	±4	181	184	192	186	±5	43	-8	73
	B	55	53	45	51	±4	256	304	263	274	±21	37	-13	86
3000	A	48	53	46	49	±3	173	175	148	165	±12	59	9	66
	B	41	46	42	43	±2	192	191	197	193	±3	44	-6	68
4000	A	46	51	45	47	±3	184	190	149	174	±18	54	4	70
	B				+		166	168	170	168	±2	0	0	58
5000	A	42	65	40	49	±11	195	192	197	195	±2	50	0	72
	B	48	52	48	49	±2	195	155	221	190	±27	52	2	67
Positive Control - Methyl Methanesulfonate (µg/ml)														
10		160	160	189	170	±14	159	149	148	152	±5	223	173	53
20		151	133	151	145	±8	50	50	53	51	±1	569	518	12

+ - Culture lost

[a] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

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TABLE 3

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH Pyridostigmine Bromide (WR25071080) Bottle No. BM03894
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/ml)	Cell Concentration (X 10^6) Day 1	Day 2	Suspension Growth[a] Total[b]	%Cntl[c]	Cloning Growth Ave VC	%Cntl[d]	%Total Growth[e]
Water 1	1.241	1.505	20.8		213		
2	1.111	1.590	19.6		193		
Pyridostigmine Bromide (WR25071080) Bottle No. BM03894:							
1000 A	1.141	1.435	18.2	90	198	97	88
B	1.066	1.597	18.9	94	216	106	100
2000 A	0.903	1.597	16.0	79	186	91	73
B	1.085	1.067	12.9	64	274	135	86
3000 A	0.977	1.502	16.3	81	165	81	66
B	0.942	1.388	14.5	72	193	95	68
4000 A	0.951	1.564	16.5	82	174	86	70
B	0.907	1.414	14.2	71	168	83	58
5000 A	0.955	1.435	15.2	75	195	96	72
B	0.887	1.457	14.4	71	190	94	67
Positive Control - Methyl Methanesulfonate (µg/ml)							
10	0.961	1.329	14.2	70	152	75	53
20	0.871	0.999	9.7	48	51	25	12

[a] - Cultures containing <0.3x10^6 cells/ml on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3x10^6 cells/ml) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

[d] - % control cloning growth = (average V.C. of treated culture / average V.C. of solvent control) x 100

[e] - % total growth = (% suspension growth x % cloning growth) / 100

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TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/ml)		TFT Plates 1	2	3	Avg.	Stand. Dev	V.C. Plates 1	2	3	Avg.	Stand. Dev	Mutant Frequency[a]	Induced Mutant Frequency[b]	%Total Growth[c]
Water	1	87	90	58	78	±14	204	164	184	184	±16	85		
	2	90	76	86	84	±6	192	201	194	196	±4	86		
Mean Water Mutant Frequency= 86														
Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894:														
1000	A	104	92	100	99	±5	157	178	171	169	±9	117	31	71
	B	96	87	98	94	±5	176	156	159	164	±9	114	29	65
2000	A	127	107	116	117	±8	184	156	157	166	±13	141	55	62
	B	127	132	138	132	±4	169	171	159	166	±5	159	74	57
3000	A	135	114	129	126	±9	122	119	111	117	±5	215	129	28
	B	136	135	133	135	±1	151	171	175	166	±10	163	77	41
4000	A	159	139	162	153	±10	163	144	165	157	±9	195	109	24
	B	143	151	170	155	±11	155	169	159	161	±6	192	107	20
5000	A	155	156	162	158	±3	151	136	142	143	±6	221	135	18
	B	+	+	146	146	±0	126	131	126	128	±2	229	143	13
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/ml)														
2.5		204	210	241	218	±16	124	136	145	135	±9	323	238	41
4.0		223	203	212	213	±8	86	89	77	84	±5	506	421	12

+ - Culture lost

[a] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

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TABLE 5

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/ml)	Cell Concentration (X 10^6) Day 1	Day 2	Suspension Growth[a] Total[b]	%Cntl[c]	Cloning Growth Ave VC	%Cntl[d]	%Total Growth[e]
Water 1	0.913	1.547	15.7		184		
2	0.990	1.607	17.7		196		
Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894:							
1000 A	0.727	1.647	13.3	80	169	89	71
B	0.753	1.505	12.6	76	164	86	65
2000 A	0.686	1.554	11.8	71	166	87	62
B	0.651	1.505	10.9	65	166	88	57
3000 A	0.478	1.415	7.5	45	117	62	28
B	0.514	1.363	7.8	47	166	87	41
4000 A	0.398	1.088	4.8	29	157	83	24
B	0.349	1.019	3.9	24	161	85	20
5000 A	0.340	1.040	3.9	24	143	75	18
B	0.310	0.907	3.1	19	128	67	13
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/ml)							
2.5	0.672	1.289	9.6	58	135	71	41
4.0	0.508	0.830	4.7	28	84	44	12

[a] - Cultures containing <0.3x10^6 cells/ml on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3x10^6 cells/ml) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

[d] - % control cloning growth = (average V.C. of treated culture / average V.C. of solvent control) x 100

[e] - % total growth = (% suspension growth x % cloning growth) / 100

Figure 2

pyridostigmine

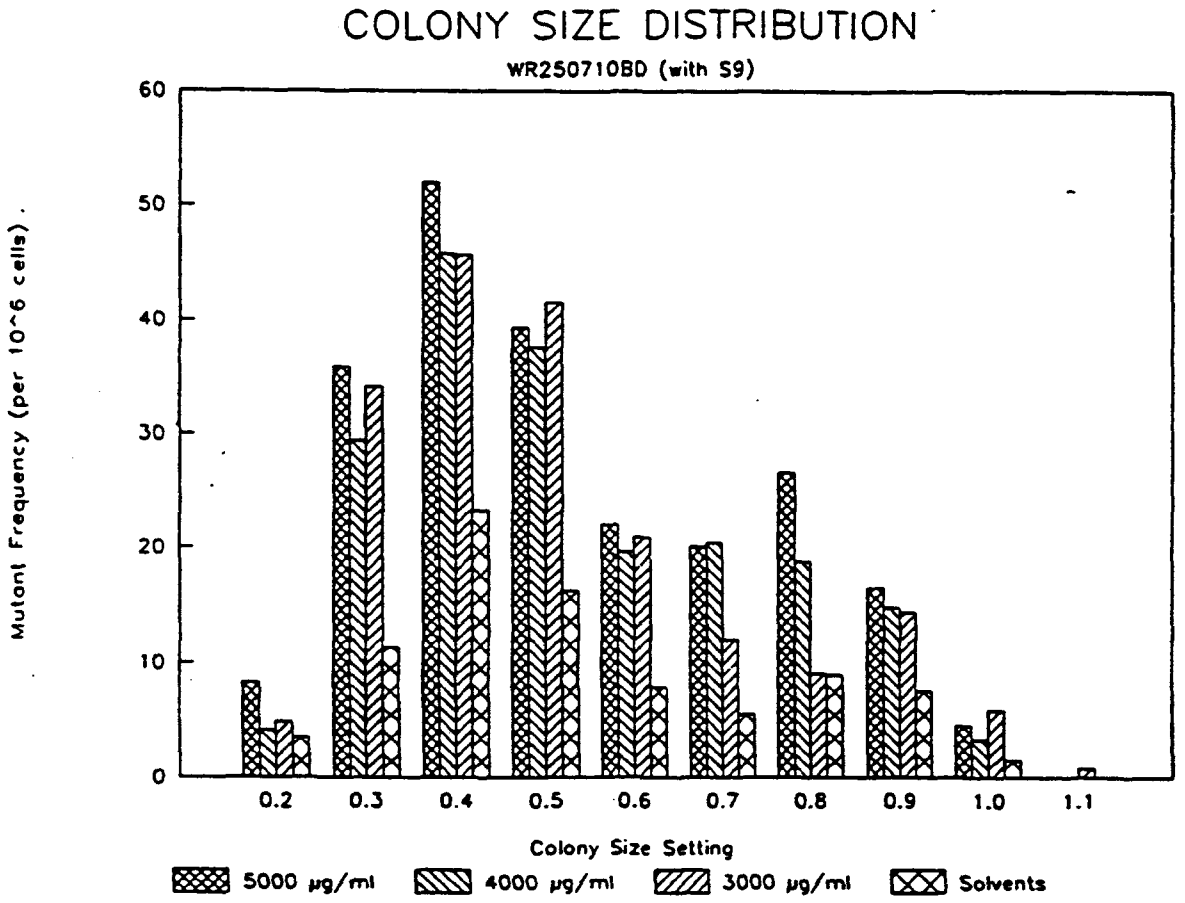

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Figure 1

- positive control
- without S9

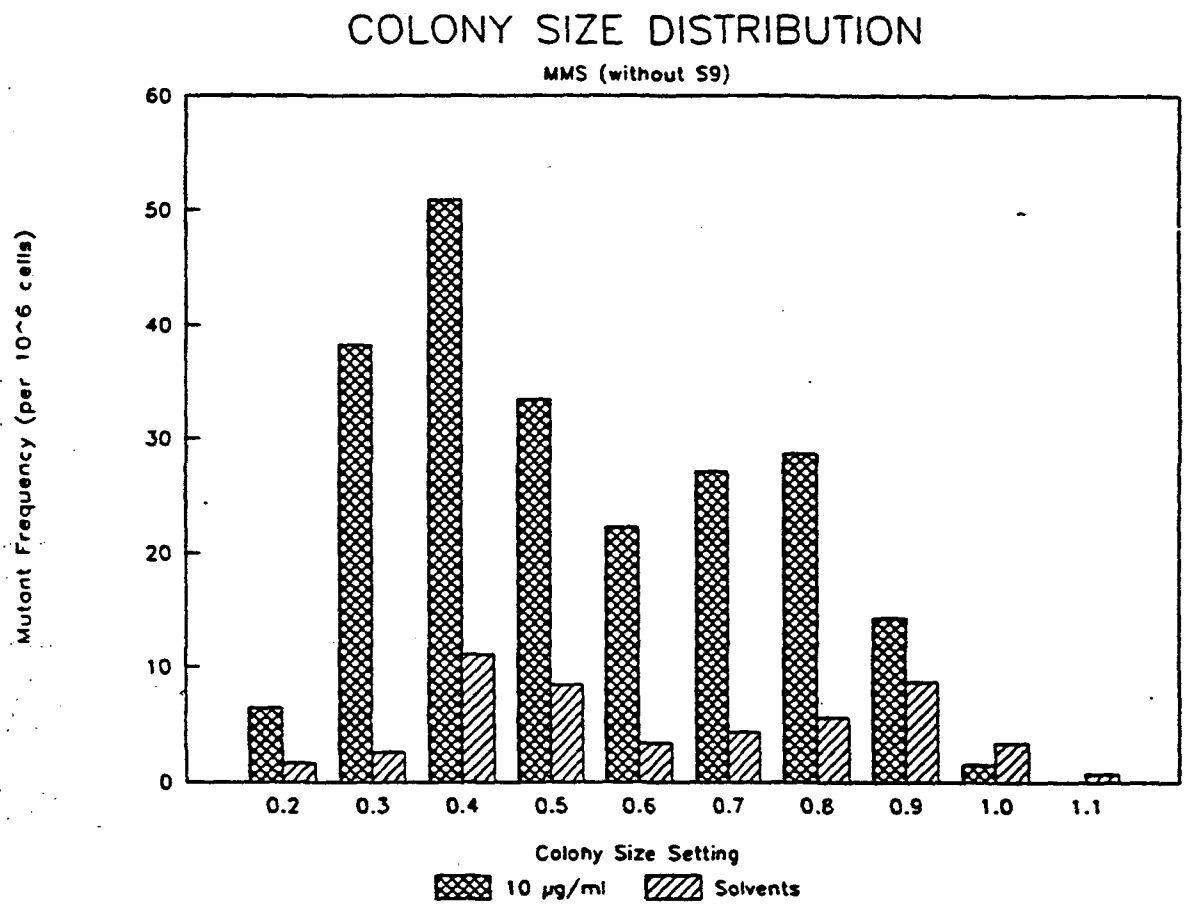

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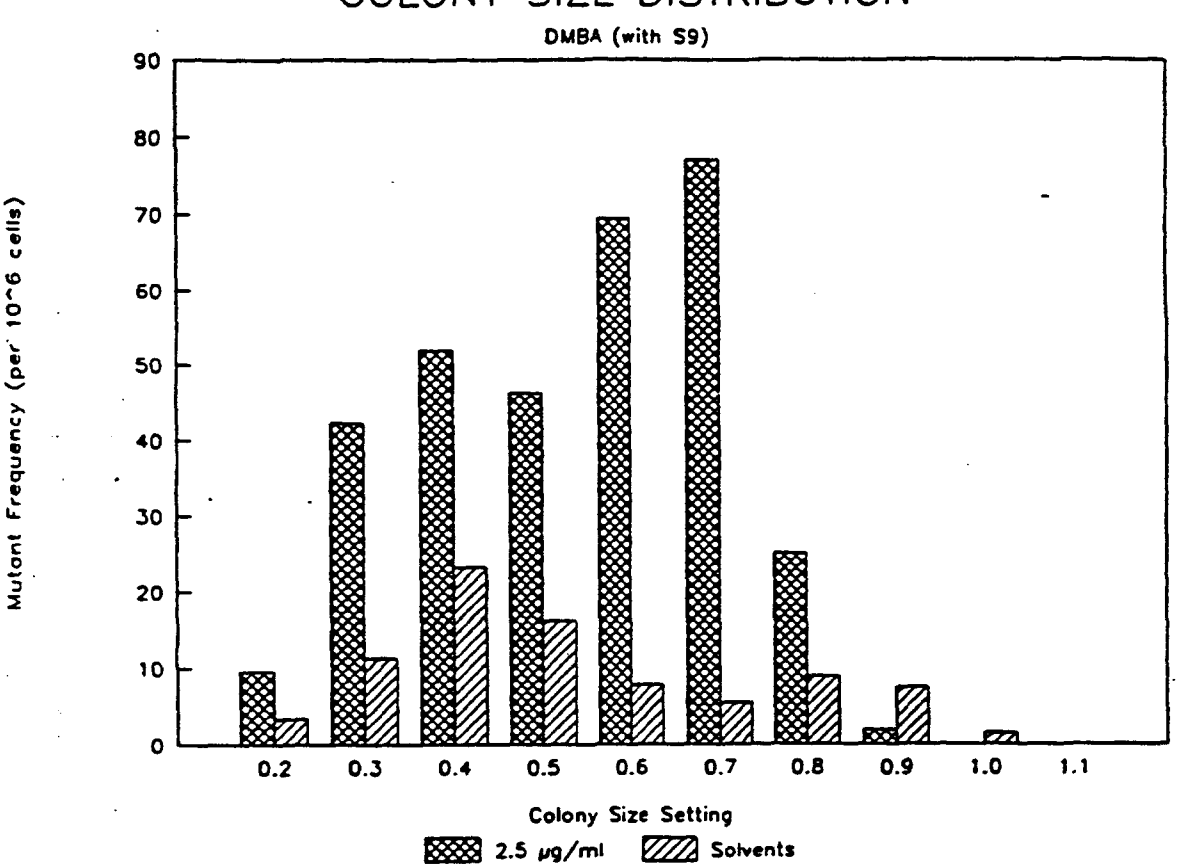
Figure 3

- positive control
- with S9



3) Chromosomal Aberrations in CHO Cells

P was negative, both with and without metabolic activation (Aroclor - induced rat liver S9), up to the highest concentration tested (5000 μg/ml, which was shown to cause no significant inhibition of cell growth in preliminary toxicity studies, although an increase in AGT [average generation time] was seen, leading to the use of a cell harvest time of 18 hours). Results are attached.

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TABLE 1

PRELIMINARY TOXICITY TEST USING PYRIDOSTIGMINE BROMIDE (WR25071080) BOTTLE NO. BM03894 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

6 HOUR TREATMENT, 20 HOUR RECOVERY PERIOD

Treatment[1]	Cell Count ($x10^6$)	Cell Viability[2] (%)	Viable Cells per Flask[3] ($x10^6$)	Cell Growth Index[4] (%)	Cell Growth Inhibition[5] (%)
Water	2.53	98%	2.48	100%	N/C
Pyridostigmine Bromide (WR25071080) Bottle No. BM03894					
0.5 µg/ml	2.37	95%	2.25	91%	9%
1.5 µg/ml	2.84	98%	2.78	112%	-12%
5 µg/ml	2.36	93%	2.19	88%	12%
15 µg/ml	2.78	91%	2.53	102%	-2%
50 µg/ml	2.34	98%	2.30	93%	7%
150 µg/ml	2.36	94%	2.22	90%	10%
500 µg/ml	2.16	94%	2.03	82%	18%
1500 µg/ml	2.41	94%	2.26	91%	9%
5000 µg/ml	2.09	97%	2.03	82%	18%

[1]CHO cells were treated in the absence of an exogenous source of metabolic activation for 6 hours at 37±1°C.
[2]Viability determined by trypan blue dye exclusion.
[3]Viable cells/flask = cell count x % viable cells
[4]Growth index = (cells per flask treated group/cells per flask control group), expressed as a percentage.
[5]Cell growth inhibition = 100% - % cell growth index.

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TABLE 2
PRELIMINARY TOXICITY TEST USING PYRIDOSTIGMINE BROMIDE (WR25071080) BOTTLE NO. BM03894 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 22 HOUR RECOVERY PERIOD

Treatment[1]	Cell Count ($x10^6$)	Cell Viability[2] (%)	Viable Cells per Flask[3] ($x10^6$)	Cell Growth Index[4] (%)	Cell Growth Inhibition[5] (%)
Water	1.61	93%	1.49	100%	N/C
Pyridostigmine Bromide (WR25071080) Bottle No. BM03894					
0.5 µg/ml	1.78	90%	1.60	107%	-7%
1.5 µg/ml	1.80	93%	1.67	112%	-12%
5 µg/ml	1.80	90%	1.62	109%	-9%
15 µg/ml	1.63	86%	1.40	94%	6%
50 µg/ml	2.17	92%	2.00	134%	-34%
150 µg/ml	1.39	96%	1.33	89%	11%
500 µg/ml	1.63	89%	1.45	97%	3%
1500 µg/ml	1.79	91%	1.63	109%	-9%
5000 µg/ml	1.57	94%	1.47	99%	1%

[1]CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.
[2]Viability determined by trypan blue dye exclusion.
[3]Viable cells/flask = cell count x % viable cells
[4]Growth index = (cells per flask treated group/cells per flask control group), expressed as a percentage.
[5]Cell growth inhibition = 100% - % cell growth index.

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TABLE 3

PRELIMINARY TOXICITY TEST USING PYRIDOSTIGMINE BROMIDE (WR250710BD) BOTTLE NO. BM03894 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Cell Cycle Kinetics Percentage of cells in M_1	M_2	M_3	Average Generation Time[2] (AGT)
Water	1	99	0	12.1
Pyridostigmine Bromide (WR250718D) Bottle No. BM03894				
0.5 µg/ml	1	99	0	12.1
1.5 µg/ml	3	97	0	12.2
5 µg/ml	6	94	0	12.4
15 µg/ml	8	92	0	12.5
50 µg/ml	6	94	0	12.4
150 µg/ml	11	89	0	12.7
500 µg/ml	14	86	0	12.9
1500 µg/ml	14	86	0	12.9
5000 µg/ml	29	71	0	14.0

[1]CHO cells were treated in the absence of an exogenous source of metabolic activation for 6 hours at 37±1°C. Metaphase cells were collected following a 24 hour growth period in BrdU.

[2]Average Generation Time:

$$\frac{24 \text{ hours} \times 100}{[(\text{number of M1 cells} \times 1) + (\text{number of M2 cells} \times 2) + (\text{number of M3 cells} \times 3)]}$$

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TABLE 4

PRELIMINARY TOXICITY TEST USING PYRIDOSTIGMINE BROMIDE (WR25071BD) BOTTLE NO. BM03894 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Cell Cycle Kinetics Percentage of cells in M_1	M_2	M_3	Average Generation Time[2] (AGT)
Water	8	92	0	12.5
Pyridostigmine Bromide (WR25071BD) Bottle No. BM03894				
0.5 µg/ml	10	90	0	12.6
1.5 µg/ml	12	88	0	12.8
5 µg/ml	9	91	0	12.6
15 µg/ml	11	89	0	12.7
50 µg/ml	10	90	0	12.6
150 µg/ml	5	95	0	12.3
500 µg/ml	14	86	0	12.9
1500 µg/ml	33	67	0	14.4
5000 µg/ml	58	42	0	16.9

[1]CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C. Metaphase cells were collected following a 24 hour growth period in BrdU.

[2]Average Generation Time:

$$\frac{24 \text{ hours} \times 100}{[(\text{number of M1 cells} \times 1) + (\text{number of M2 cells} \times 2) + (\text{number of M3 cells} \times 3)]}$$

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TABLE 5

CONCURRENT TOXICITY TEST USING PYRIDOSTIGMINE BROMIDE (WR250710BD) IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION BOTTLE NO. BM03894

Treatment[1]	Replicate Flask	Cell Count ($x10^6$)	Cell Viability[2] (%)	Mean Viable Cells per Flask[3] ($x10^6$)	Cell Growth Index[4] (%)	Cell Growth Inhibition[5] (%)
Untreated	A	2.21	99%			
	B	2.22	100%	2.21	N/C	N/C
Water	A	1.82	97%			
	B	2.23	100%	2.00	100%	0%
Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894						
313 µg/ml	A	2.28	99%			
	B	2.31	99%	2.27	114%	-14%
625 µg/ml	A	1.90	99%			
	B	2.18	100%	2.03	101%	-1%
1250 µg/ml	A	1.92	99%			
	B	1.98	100%	1.94	97%	3%
2500 µg/ml	A	2.01	99%			
	B	1.84	100%	1.92	96%	4%
5000 µg/ml	A	1.61	98%			
	B	1.97	100%	1.78	89%	11%
MMC, 0.08 µg/ml	A	1.85	99%			
	B	1.67	98%	1.73	87%	13%
MMC, 0.15 µg/ml	A	1.67	100%			
	B	1.67	99%	1.66	83%	17%

[1]CHO cells were treated in the absence of an exogenous source of metabolic activation for 18 hours at 37±1°C.
[2]Viability determined by trypan blue dye exclusion.
[3]Viable cells/flask = cell count x % viable cells, reported as mean of Flasks A and B.
[4]Growth index = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage. (Test article concentrations and positive control were compared to solvent control)
[5]Cell growth inhibition = 100% - % cell growth index.

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TABLE 6

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH PYRIDOSTIGMINE BROMIDE (WR250710BD) BOTTLE NO. BM03894 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

18 Hour Harvest

Treatment[1,7]	Flask	Mitotic Index	Cells Scored	Aberrant Cells[2] (%)	Total Number of Structural Aberrations: Chromatid-type[3] Gaps	Breaks	Exch	Chromosome-type[4] Breaks	Dic	Ring	Severely Damaged Cells[5]	Average Aberrations Per Cell[6]
Untreated cells	A	3.8	100	0	0	0	0	0	0	0	0	0.000
	B	4.0	100	1	2	0	0	0	1	0	0	0.010
Water	A	4.8	100	0	3	0	0	0	0	0	0	0.000
	B	5.0	100	1	0	0	0	0	1	0	0	0.010
Pyridostigmine Bromide (WR250710BD) Bottle No. BM03894												
625 µg/ml	A	5.2	100	1	0	1	0	0	0	0	0	0.010
	B	6.2	100	3	2	3	1	0	0	0	0	0.040
1250 µg/ml	A	6.2	100	1	1	2	0	0	0	0	0	0.020
	B	4.4	100	1	0	0	0	0	1	0	0	0.010
2500 µg/ml	A	4.2	100	0	0	0	0	0	0	0	0	0.000
	B	4.6	100	1	1	0	1	0	0	0	0	0.010
5000 µg/ml	A	5.0	100	1	1	1	0	0	0	0	0	0.010
	B	4.8	100	1	0	0	0	0	1	0	0	0.010
MMC	A	6.2	100	17	8	17	5	1	0	0	0	0.230
0.08 µg/ml	B	4.4	100	23	8	16	10	0	1	1	0	0.280

[1]CHO cells were treated for 18 hours at 37±1°C in the absence of an exogenous source of metabolic activation.
[2]Excluding cells with only gaps.
[3]Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures include quadriradials, triradials and complex rearrangements.
[4]Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.
[5]Severely damaged cells include cells with one or more pulverized chromosomes and cells with 10 or more aberrations.
[6]Severely damaged cells and pulverizations were counted as 10 aberrations.
[7]313 µg/ml was dosed but not needed for evaluation.

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TABLE 7

CONCURRENT TOXICITY TEST USING PYRIDOSTIGMINE BROMIDE (WR250710BD) BOTTLE NO. BM03894 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Replicate Flask	Cell Count ($x10^6$)	Cell Viability[2] (%)	Mean Viable Cells per Flask[3] ($x10^6$)	Cell Growth Index[4] (%)	Cell Growth Inhibition[5] (%)
Untreated	A	1.77	98%			
	B	1.49	97%	1.59	N/C	N/C
Water	A	1.49	95%			
	B	1.45	97%	1.41	100%	0%
Pyridostigmine Bromide (WR250710BD) Bottle no. BM03894						
313 µg/ml	A	1.72	97%			
	B	1.69	95%	1.64	116%	-16%
625 µg/ml	A	1.44	94%			
	B	1.63	98%	1.48	105%	-5%
1250 µg/ml	A	1.64	99%			
	B	1.84	96%	1.69	120%	-20%
2500 µg/ml	A	1.60	100%			
	B	1.75	98%	1.66	118%	-18%
5000 µg/ml	A	1.40	98%			
	B	1.68	98%	1.51	107%	-7%
CP, 10 µg/ml	A	1.05	97%			
	B	1.17	94%	1.06	75%	25%
CP, 20 µg/ml	A	1.11	95%			
	B	0.92	98%	0.98	69%	31%

[1]CHO cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.
[2]Viability determined by trypan blue dye exclusion.
[3]Viable cells/flask = cell count x % viable cells, reported as mean of Flasks A and B.
[4]Growth index = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage. (Test article concentrations and positive control were compared to solvent control)
[5]Cell growth inhibition = 100% - % cell growth index.

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TABLE 8

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH PYRIDOSTIGMINE BROMIDE (WR25071080) BOTTLE NO. BM03894 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

18 Hour Harvest

Treatment[1,7]	Flask	Mitotic Index	Cells Scored	Aberrant Cells[2] (%)	Total Number of Structural Aberrations — Chromatid-type[3] Gaps	Breaks	Exch	Chromosome-type[4] Breaks	Dic	Ring	Severely Damaged Cells[5]	Average Aberrations Per Cell[2,6]
Untreated cells	A	5.8	100	3	1	2	0	0	1	0	0	0.030
	B	6.4	100	1	1	0	0	0	2	0	0	0.020
Water	A	6.2	100	2	2	2	0	0	0	0	0	0.020
	B	7.0	100	3	0	2	0	1	0	0	0	0.030
Pyridostigmine Bromide (WR25071080) Bottle no. BM03894												
625 µg/ml	A	2.8	100	4	0	3	0	1	0	0	0	0.040
	B	4.8	100	3	0	3	0	0	0	0	0	0.030
1250 µg/ml	A	5.0	100	0	0	0	0	0	0	0	0	0.000
	B	6.0	100	1	0	1	0	0	0	0	0	0.010
2500 µg/ml	A	3.4	100	3	0	4	0	0	0	0	0	0.040
	B	4.4	100	1	0	0	0	0	1	0	0	0.010
5000 µg/ml	A	4.0	100	1	0	1	0	1	0	0	0	0.020
	B	4.8	100	2	0	2	0	0	0	0	0	0.020
CP	A	1.6	100	33	0	38	5	17	0	0	1	0.700
10 µg/ml	B	1.8	63	38	0	26	8	5	0	0	2	0.937

[1]CHO cells were treated for 4 hours at 37±1°C in the presence of an exogenous source of metabolic activation.
[2]Excluding cells with only gaps.
[3]Chromatid breaks include chromatid and isochromatid breaks and fragments; chromatid exchange figures include quadriradials, triradials and complex rearrangements.
[4]Chromosome breaks include breaks and acentric fragments; dic, dicentric chromosome.
[5]Severely damaged cells include cells with one or more pulverized chromosomes and cells with 10 or more aberrations.
[6]Severely damaged cells and pulverizations were counted as 10 aberrations.
[7]313 µg/ml was dosed but not needed for evaluation.

TABLE 9

SUMMARY

Treatment	S9 Activation	Harvest Time (hrs)	Mitotic Index	Cells Scored	Aberrations Per Cell[1] (Mean ± SD)	Cells With Aberrations[2] (%)
Untreated	-	18	3.9	200	0.005 ± 0.071	0.5
Water	-	18	4.9	200	0.005 ± 0.071	0.5
Pyridostigmine Bromide (WR2507108D)						
625 µg/ml	-	18	5.7	200	0.025 ± 0.186	2.0
1250 µg/ml	-	18	5.3	200	0.015 ± 0.158	1.0
2500 µg/ml	-	18	4.4	200	0.005 ± 0.071	0.5
5000 µg/ml	-	18	4.9	200	0.010 ± 0.100	1.0
MMC 0.08 µg/ml	-	18	5.3	200	0.255 ± 0.549	20.0**
Untreated	+	18	6.1	200	0.025 ± 0.186	2.0
Water	+	18	6.6	200	0.025 ± 0.157	2.5
Pyridostigmine Bromide (WR2507108D)						
625 µg/ml	+	18	3.8	200	0.035 ± 0.184	3.5
1250 µg/ml	+	18	5.5	200	0.005 ± 0.071	0.5
2500 µg/ml	+	18	3.9	200	0.025 ± 0.186	2.0
5000 µg/ml	+	18	4.4	200	0.020 ± 0.172	1.5
CP 10 µg/ml	+	18	1.7	163	0.791 ± 1.608	35.5**

[1]Severely damaged cells were counted as 10 aberrations.
[2]*, p≤0.05, **, p≤0.01; Fisher's exact test.

4) Mouse Micronucleus Assay

Doses of P were 0.5, 1.0, and 2.0 mg/kg, i.p.; 5/sex were evaluated at 24, 48, and 72 hours post-dose. (Doses based on preliminary range finding study showing lethality at higher doses; in main study lethargy and tremors were seen at 1 and 2 mg/kg and 1/20 M at 2 mg/kg died). Strain of mouse = ICR - Sprague Dawley. P was negative in this assay. A summary of the results is attached.

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TABLE 3

SUMMARY OF BONE MARROW MICRONUCLEUS STUDY USING PYRIDOSTIGMINE BROMIDE (WR250710BD) BOTTLE NO. BM03894

TREATMENT	SEX	TIME (HR)	NUMBER OF MICE	PCE/TOTAL ERYTHROCYTES (MEAN +/- SD)	CHANGE FROM CONTROL (%)	MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES NUMBER PER 1000 PCES (MEAN +/- SD)	ERYTHROCYTES NUMBER PER PCES SCORED
Distilled water							
10 ml/kg	M	24	5	0.60 ± 0.04	---	1.4 ± 0.89	7 / 5000
	F	24	5	0.52 ± 0.07	---	1.6 ± 1.14	8 / 5000
Pyridostigmine Bromide (WR250710BD) Bottle NO. BM03894							
0.5 mg/kg	M	24	5	0.58 ± 0.09	-3	1.0 ± 1.41	5 / 5000
	F	24	5	0.60 ± 0.06	15	1.0 ± 0.71	5 / 5000
1.0 mg/kg	M	24	5	0.62 ± 0.03	3	1.8 ± 0.84	9 / 5000
	F	24	5	0.57 ± 0.10	10	1.4 ± 0.55	7 / 5000
2.0 mg/kg	M	24	5	0.61 ± 0.07	2	1.0 ± 0.71	5 / 5000
	F	24	5	0.54 ± 0.10	4	1.8 ± 0.84	9 / 5000
CP,							
60 mg/kg	M	24	5	0.53 ± 0.03	-12	34.4 ± 8.76	172 / 5000*
	F	24	5	0.50 ± 0.05	-4	32.4 ±10.16	162 / 5000*
Distilled water							
10 ml/kg	M	48	5	0.57 ± 0.03	---	0.6 ± 0.55	3 / 5000
	F	48	5	0.57 ± 0.03	---	1.6 ± 1.82	8 / 5000
Pyridostigmine Bromide (WR250710BD) Bottle NO. BM03894							
0.5 mg/kg	M	48	5	0.56 ± 0.09	-2	0.8 ± 0.45	4 / 5000
	F	48	5	0.62 ± 0.04	9	0.6 ± 0.55	3 / 5000
1.0 mg/kg	M	48	5	0.57 ± 0.04	0	1.0 ± 0.71	5 / 5000
	F	48	5	0.58 ± 0.05	2	0.8 ± 0.45	4 / 5000
2.0 mg/kg	M	48	5	0.59 ± 0.04	4	0.8 ± 0.84	4 / 5000
	F	48	5	0.55 ± 0.05	-4	0.8 ± 1.30	4 / 5000
Distilled water							
10 ml/kg	M	72	5	0.53 ± 0.04	---	1.4 ± 1.14	7 / 5000
	F	72	5	0.53 ± 0.03	---	1.6 ± 1.14	8 / 5000
Pyridostigmine Bromide (WR250710BD) Bottle NO. BM03894							
0.5 mg/kg	M	72	5	0.54 ± 0.06	2	0.4 ± 0.55	2 / 5000
	F	72	5	0.51 ± 0.04	-4	1.2 ± 1.30	6 / 5000
1.0 mg/kg	M	72	5	0.60 ± 0.02	13	1.0 ± 0.71	5 / 5000
	F	72	5	0.61 ± 0.05	15	0.8 ± 0.84	4 / 5000
2.0 mg/kg	M	72	5	0.57 ± 0.05	8	0.6 ± 0.55	3 / 5000
	F	72	5	0.54 ± 0.04	2	1.0 ± 1.22	5 / 5000

*, p≤0.05 (ANOVA followed by Dunnett's t-test)

EVALUATION:

A) Regarding the amended final report of the pivotal monkey efficacy study, the only change of significance regarding the interpretation and conclusions of the study, i.e., that the time between blood sampling and assay for P-induced AChE inhibition was longer than the originally - specified 3 minutes, was already known to us as the result of a DSI audit (see my review of 07/24/96). Although the present submission does not give any further specifics regarding the actual times between sampling and assay, the DSI inspection report dated 07/07/94 states that the time between sample collection and analysis ranged from 3.1 to 40.63 minutes and the FDA inspector (Mr. McClure) told me that most of the times were at the upper end of this range.

This issue is of considerable importance in view of the proposed use of the degree of P-induced inhibition of RBC AChE as a "surrogate marker" for efficacy. As indicated in my review of 07/24/96, we had previously noted the problem to the sponsor at a meeting on 04/06/95, where we indicated that what is needed is a knowledge of how much the measured enzyme inhibition decreased over the 3-40 minute period between sampling and assay, as well as a knowledge of what the delay times and the effect of these delay times were in other species and studies (including humans) to which the monkey data are being compared. The sponsor has not as yet responded to this issue.

B) As indicated in my review of 07/24/96 and communicated to the sponsor by phone on 12/11/95, the genotoxixcity battery previously submitted for P was suboptimal, consisting of an Ames Test (but not using an A-T-detecting strain) and rat micronucleus test (lacking in adequate detail). The present submission contains draft reports for 4 genotoxicity studies. P was negative in an Ames Test, chromosomal aberration assay in CHO cells in vitro, and mouse micronucleus assay. It was positive in the mouse lymphoma L5178Y/TK assay, in the presence of metabolic activation only; increases in small, medium, and large cell colonies were seen indicative of chromosomal damage in addition to a mutational effect. The sponsor states that as a consequence of this finding "we are currently conducting a CHO/HGPRT [assay] to investigate further the potential of [P] to induce gene mutations." It is also stated that "In the event that the HGPRT assay is found to be positive, further investigation into the in vivo mutagenic potential would be recommended using the Big Blue mouse or rat mutation assay."

RECOMMENDATIONS:

None at this time, aside from pointing out the following discrepancy to the sponsor: On page 11 of the mouse lymphoma assay it is stated that the lowest concentration chosen for the assay was 100 μg/ml, whereas the results showed the lowest concentration to be 1000 μg/ml.

/S/

Barry N. Rosloff, Ph.D.

cc:Original NDA 20-414
HFD-120 file
\GFitzgerald
\BRosloff
\RNighswander
dt:10/31/96/gt/11/12/96/gt
doc:n:nda20414.prs

Barry N. Rosloff, Ph.D.
7/24/96

Pharmacologist Review of NDA 20-414
Original Summary

SPONSOR: Office of the Surgeon General
Department of the Army
Fort Detrick
Frederick, MD 21702-5012

DRUG: pyridostigmine bromide

(see attached page for chemical name and structure)

CATEGORY: cholinesterase inhibitor for use as a prophylactic treatment for organophosphorus nerve agent-induced lethality

RELATED IND/NDA:

1) IND —— (companion to present NDA)

2) NDAs 9829, 9830, 11665, 15193, 17398 (NDAs for various marketed formulations of pyridostigmine, which is indicated for use in myasthenia gravis and as an antagonist to nondepolarizing muscle relaxants).

ABBREVIATIONS: The following abbreviations are used throughout this review:

P = pyridostigmine
AchE = acetylcholinesterase

D. Chemistry, Manufacturing, and Controls Summary

1. Drug Substance

Generic Name:

Pyridostigmine bromide

Other Code Designations:

WR 270,170

RO 1-5130

Proprietary Names:

Mestinon®

Regonol®

Chemical Name(s):

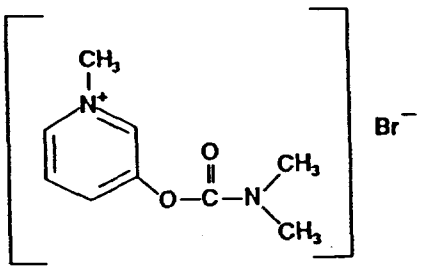
a. 3-{[(dimethylamino) carbonyl] oxy}-1-methylpyridinium bromide

b. 3-hydroxy-1-methylpyridinium bromide dimethyl carbamate

Empirical Formula:

$C_9H_{13}BrN_2O_2$

Structural Formula:



Manufacturer:

TABLE OF CONTENTS

"EFFICACY" STUDIES IN ANIMALS

The following studies were identified by the sponsor as "well-controlled," i.e. where the effect of pyridostigmine (P) pretreatment on nerve agent lethality could be distinguished from that of post-nerve agent treatment (primarily atropine and 2-PAM, which are the antidotes proposed for use in conjunction with P pretreatment in humans). The primary nerve agent studied was Soman; Tabun, Sarin, and VX were also used in some studies. There are 2 studies in rhesus monkeys, 5 in guinea pigs, 3 in rabbits, 3 in mice, and 2 in rats.

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A) MONKEY (2 studies)

Study A-1

A study was done in male rhesus monkeys (2.5 - 5.5 kg) to determine the efficacy of pyridostigmine (P), given as an oral pretreatment (syrup formulation given by gavage), in the prevention of GD (Soman)-induced lethality. Preliminary pharmacokinetic studies were performed to help determine appropriate doses for the efficacy study as well as to help in assessing the validity of extrapolating the efficacy results to humans. The studies were performed by _____ and were said to conform to GLP regulations. They are located in volume 1.8 - 1.14 of the NDA.

a. Pharmacokinetic Studies

Plasma levels of P after single p.o. doses of 0.286, 0.571, and 1.14 mg/kg are shown in attached figure "4-9". (N = 12/dose). Calculated pharmacokinetic parameters are shown in tables "4-23" (individual values) and "4-24" (means). Mean Tmax was about 1 hour. Plasma levels (Cmax and AUC) were proportional to dose. It was stated that T1/2 values were uncalculable due to erratic multiphasic rates of decline (thought to be possibly due to low, prolonged, or erratic absorption, although no data were presented on this point). Intersubject variation in Tmax and plasma levels was relatively great. Cmax values from human data are shown in table "4-27", where it can be seen that dose-normalized values were similar between humans and the "smaller" monkeys (which were used in these studies).

The pharmacokinetics of the inhibition of RBC acetylcholinesterase (AchE) activity was also studied. (Activity measured from the same plasma samples drawn for measurement of P levels, above). Mean values for percent inhibition vs time after dosing are shown in attached figure "4-13". Pharmacokinetic parameters are shown in tables "4-29" (individual values) and "4-30" (means). Mean Tmax was about 80 minutes. Maximum inhibition ("INH MAX" in table) and AUC increased less than proportionally with increasing dose. The T1/2 values for AchE recovery ("slow T1/2" in table) were 162, 185, and 228 minutes at the low, medium and high dose, respectively; the report states that T1/2 thus appears to increase with dose, although the magnitude of this effect seems rather slight. As was the case with P levels, there was a relatively large intersubject variation in AchE inhibition (although it is interesting to note that a rough eyeballing indicates some correlation between P level and AchE inhibition in individual animals).

For the "efficacy" study the sponsor wished to use doses which, when given repeatedly to reach steady state, would cause AchE inhibition of 15 and 30% (in a low and high dose group, respectively) at 5 hours after the last dose (i.e., the time at which Soman is administered). Based on the observed degrees of enzyme inhibition and reactivation T1/2 values determined above after acute dosing, and on predications of inhibition which would occur with repeat dosing, the low and medium doses used in the pharmacokinetic study (i.e. 0.286 and 0.571 mg/kg) were originally chosen as being likely to meet this objective. (The high dose of 1.14 mg/kg was predicted to cause degrees of inhibition which might be associated with signs of intoxication). However, pilot studies using repeat dosing showed that the degree of enzyme inhibition was much less than expected (e.g. see attached table "4-37"); the reason for this is not known. It was ultimately decided to use the following regimens for the efficacy study: (1) 1.2 mg/kg q. 8 hours with a total of 6 doses, and (2) 1.2 mg/kg followed after 8 hours by 1.8 mg/kg followed after 8 hours by 4 doses of 2.4 mg/kg given q. 8 hours (It was not stated if these dosage regimens caused signs of intoxication). As determined in association with the efficacy study, mean AchE inhibition at the time of Soman administration (i.e., 5 hours after the last dose of P) was 23 and 37% respectively, with these 2 regimens, thus approximating the targets of 15 and 30% inhibition, respectively. (Degrees of inhibition at other times during the dosing interval were obtained but not presented in the report).

b) "Efficacy" Study:

Soman was given at various doses i.m. (See results in attached table "4-39" for actual doses). Four therapeutic groups were used:

1) No treatment.

2) Atropine (0.4 mg/kg i.m.) + 2-PAM (25.7 mg/kg i.m.); these were given as divided doses (2/3 at 1 minute after Soman; 1/3 10 minutes later or at the onset of signs of AchE inhibition).

3) Pyridostigmine low dose (1.2 mg/kg p.o. q. 8 hours x6); Soman given 5 hours after the last dose.

   Atropine + 2-PAM as above.

4) Pyridostigmine high dose (1.2 mg/kg p.o. x1 followed by 1.8 mg/kg x1 followed by 2.4 mg/kg x4; all doses 8 hours apart); Soman given 5 hours after the last dose.

Atropine + 2-PAM as above.

Thus, the primary test is to see if prophylactic treatment with P (at two dose levels) has an effect on Soman lethality over and above that produced by the "standard" treatment atropine + 2-PAM. (It does not appear that vehicle or dosing procedure controls were employed, although this likely would have little or no effect on the interpretation of the study which used lethality as the primary endpoint). The animals were observed for 48 hours after Soman dosing, the primary endpoints being toxic signs and numbers of deaths; less systematic observations were made beyond 48 hours as noted below.

Results are summarized in attached Table "4-39", which shows the numbers of animals given each treatment regimen and the numbers of deaths. (Note that the treatment group numbers 1, 2, 3, and 4 in the table correspond to the low dose P, high dose P, atropine + PAM alone, and untreated control groups, respectively, as indicated in the legend). It can be seen that very few deaths occurred in the P-treated groups (1 at the lower P dose and 3 at the higher), compared with a large number of deaths (at lower Soman doses) in groups 3 and 4. Most deaths across all groups occurred within 3 hours post-Soman (not shown in the table). The results are shown graphically in figure "4-16", and calculated "protective ratios" based on Soman LD 50 values are shown in table "4-44". It can be seen that treatment 3 (atropine + 2-PAM alone) produced a slight parallel shift of the Soman mortality curve to the right, resulting in a small but statistically significant protective ratio of 1.64. Protective ratios for the P-treated groups could not be calculated since the LD 50 for Soman in these groups was obviously not reached. However, these ratios are large (Soman LD 50 in P-treated groups was > 617 ug/kg, compared with 15.3 and 25.1 in the control and atropine + 2-PAM alone groups, respectively). Thus the results show a protective effect of the complete regimen (i.e., P + atropine + 2-PAM) compared to untreated control, as well as a contributory effect of P. (Note 2 caveats in estimating the magnitude of the effect of P. First, at least some monkeys used for the pharmacokinectic studies were re-used for the efficacy study; the details of prior drug exposure and time elapsed prior to re-use were not given. While it is difficult to imagine this significantly affected the large drug effect noted, there may be implications for the estimations of the protective ratios. Second, there was no data on the bioavailability of the high Soman doses used in the P-treated groups. If bioavailability decreased with increasing dose of Soman, the values estimated for the protective ratios for P would be too high.).

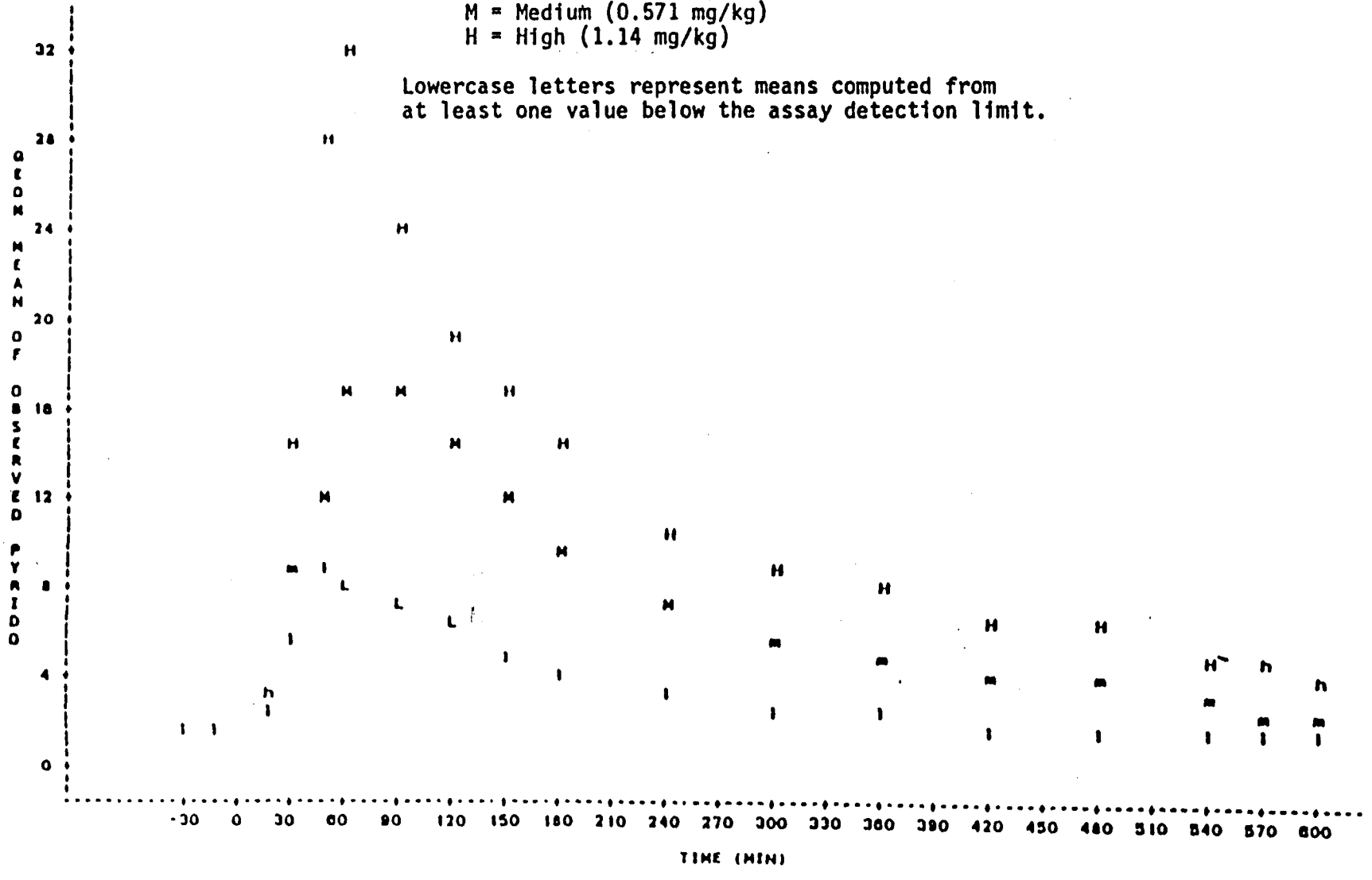
Toxic signs seen in the study (through 48 hours post-Soman) are summarized in table "4-42". (Hypersalivation not listed, said to occur with nearly same frequency as fasciculations and tremors. It was also stated that P-treated animals receiving over 200 ug/kg Soman developed severe miosis within a few minutes of Soman administration, which reversed within 15-30 minutes to an "apparent" mydriasis). Toxic signs in the P-treated groups were at least as great as those in the non-P-treated groups.

The above study, as originally submitted, only contained results (i.e. lethality and toxic signs) through 48 hours post-Soman. At our meeting with the sponsor on 4/6/95 we requested that any available data on longer term outcome be submitted. These results are shown in the attached tables "5.1.2.1.1.2.D-E". As shown, only a small number of monkeys died or were sacrificed up to the final time point reported (approximately 1 week post-Soman), with the remaining animals appearing normal or showing various toxic signs. In addition, as shown in table "5.1.2.1.1.2.F," selected monkeys were kept for further study and most have survived in good health for several years.

c) Correlation between "efficacy" and RBC AChE inhibition

No correlation was seen; i.e. equivalent efficacy was seen at doses of P which produced either 23 or 37% AchE inhibition at the time of Soman challenge. A determination of the correlation across individual animals is difficult since so few animals pre-treated with P died; in the low-dose P group the 1 animal that died had the lowest degree of AchE inhibition in this group, but the AchE inhibition in the 3 animals which died in the high-dose P group was similar to that of the overall mean for this group (Table 4-39).

FIGURE 4-9. PLOTS OF GEOMETRIC MEANS OF OBSERVED PLASMA PYRIDOSTIGMINE CONCENTRATIONS (ng/ml) VERSUS TIME



NOTE: 5 DAYS HIDDEN

TABLE 4-23. PHARMACOKINETIC PARAMETERS FOR PYRIDOSTIGMINE PLASMA CONCENTRATION IN INDIVIDUAL ANIMALS

DOSEGRP=A(L)

OBS	SEQ	ANIMAL	WEEK	DOSE	TMAX	CPMAX	AUCL	AUCU	CL_AUCL	CL_AUCU	AUCL_D	AUCU_D
1	73	M245D	1	0.3264	30	12.00	2011.38	2139.18	162.28	152.582	6162.3	6553.9
2	74	M410D	1	0.2944	45	6.72	1047.30	1430.70	281.10	205.773	3557.4	4859.7
3	78	M404D	1	0.2940	30	37.80	3426.93	3809.33	85.82	77.178	11652.8	12956.9
4	80	M931C	1	0.3120	60	5.44	929.93	1419.04	335.51	219.868	2980.5	4548.2
5	86	M827C	2	0.3250	45	18.60	3349.18	3455.68	97.04	94.048	10305.2	10632.9
6	87	M333D	2	0.2970	45	24.00	1819.12	2202.52	163.27	134.848	6125.0	7415.8
7	92	ME42	2	0.3096	30	7.52	519.45	1179.58	596.01	262.465	1677.8	3810.0
8	93	M143D	2	0.2923	45	6.67	906.53	1398.43	322.44	209.320	3101.4	4777.4
9	99	M937C	3	0.3010	60	18.00	4389.20	4546.30	68.42	66.179	14615.3	15110.6
10	100	M070D	3	0.2940	120	15.50	1844.53	2228.10	159.39	131.951	6273.9	7578.8
11	106	M087D	3	0.3001	45	6.96	1030.50	1350.00	291.22	222.296	3433 9	4498.5
12	108	M986C	3	0.2963	120	10.20	2587.20	2578.50	115.87	114.912	8630.4	8702.3

DOSEGRP=B(M)

OBS	SEQ	ANIMAL	WEEK	DOSE	TMAX	CPMAX	AUCL	AUCU	CL_AUCL	CL_AUCU	AUCL_D	AUCU_D
13	75	M847C	1	0.6123	90 00	36.90	5022.7	5044.0	121.91	121.391	8203.0	8237.8
14	76	M366D	1	0.6342	60.00	27.40	3411.7	3496.9	185.88	181.360	5379.5	5513.9
15	81	ME723	1	0 5940	120 00	49.60	13472.1	13472.1	44.09	44.091	22880.3	22880.3
16	83	M810T	1	0.6080	45.00	19.80	3845.9	3845.9	158.09	158.088	6525.6	6525.6
17	88	M320D	2	0.6010	30.00	7.22	1048.4	1538.3	573.28	390.693	1744.3	2559.6
18	89	M893T	2	0.6120	90 01	38.30	7568.9	7590.2	80.86	80.631	12367.5	12402.3
19	94	M353D	2	0.6140	45.00	26.00	3893.3	4021.1	157.71	152.694	6340.8	6549.1
20	96	M4000	2	0.6024	45 00	23.90	5521.2	5542.5	109.11	108.888	9165.3	9200.6
21	101	M894C	3	0.5999	45.00	26 40	4501.0	4501.0	133.28	133 283	7502.9	7502 9
22	102	M925T	3	0.6130	45.00	13.60	3152.7	3174.0	194.44	193.132	5143.1	5177.8
23	107	M889C	3	0.5820	90 00	26.60	6955.9	6998.5	83.67	83.161	11951.7	12024.9
24	108	M328D	3	0.5796	120.00	23.90	4218.1	4237.5	137.47	136.780	7274.2	7311.0

DOSEGRP=C(H)

OBS	SEQ	ANIMAL	WEEK	DOSE	TMAX	CPMAX	AUCL	AUCU	CL_AUCL	CL_AUCU	AUCL_D	AUCU_D
25	77	M246D	1	1.1707	60.06	21.7	4244.0	4244.0	275.85	275.846	3625.2	3625.2
26	78	M803C	1	1.1857	90.00	22.8	6386.8	6407.8	185.65	185.038	5386.3	5404.3
27	84	M373D	1	1.1700	45.00	73.6	7889.1	8020.4	148.27	145.878	8838.8	8855.0
28	85	M310D	1	1.1800	120.00	18.3	4170.4	4181.7	282.85	281.510	3534.2	3552.3
29	90	M025D	2	1.1880	30.00	44.4	7921.1	7921.1	149.98	149.980	6667.6	6667.6
30	91	M405D	2	1.1860	30.00	24.4	4718.3	4718.3	251.36	251.363	3978.3	3978.3
31	97	M6942	2	1.1830	45.00	47.0	7242.3	7242.3	163.35	163.346	6122.0	6122.0
32	98	M390D	2	1.1852	60.00	73.2	11424.7	11424.7	103.74	103,740	9639.4	9639.4
33	103	M843C	3	1.1840	90.00	54.8	14292.8	14313.9	82.84	82.717	12071.5	12089.5
34	104	M819C	3	1 1879	45 00	58.1	7798.0	7798.0	149 77	149 769	6877.0	6877.0
35	109	M315D	3	1.1760	150 00	24 4	5798 2	5798 2	202.82	202 820	4930.5	4930 5
36	110	M2000	3	1.1750	60 00	77.3	9881.8	9967.0	118 80	117.888	8410.1	8482.8

TABLE 4-24. GROUP MEAN VALUES FOR THE CALCULATED PHARMACOKINETIC PARAMETERS FOR PYRIDOSTIGMINE IN PLASMA

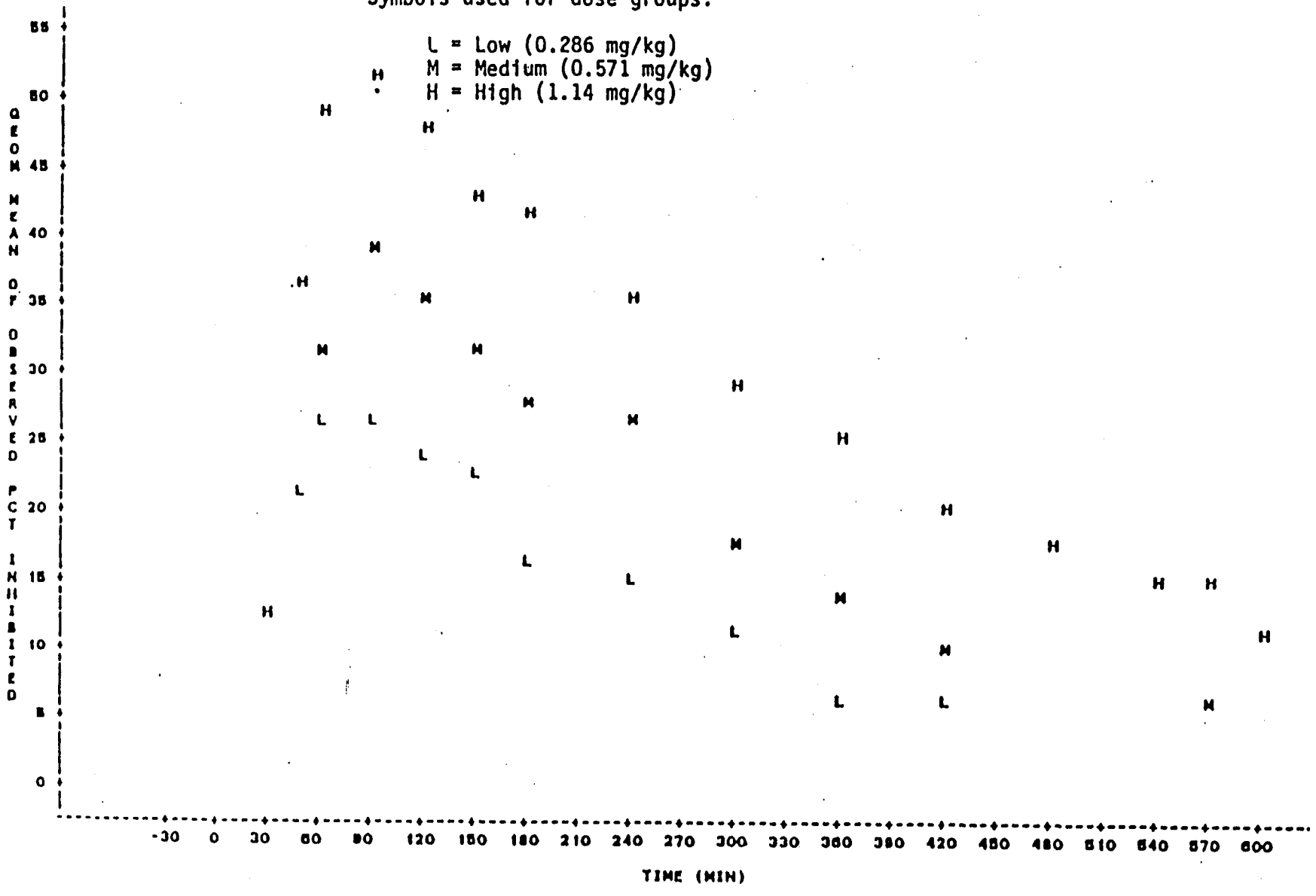
VARIABLE	LABEL	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN	MINIMUM VALUE	MAXIMUM VALUE	C.V.
DOSEGRP=A(L)								
TMAX	TIME TO MAXIMUM CONC	12	56.25000000	31.4154710	9.0688853			55.850
CPMAX	MAXIMUM CONCENTRATION	12	14.12583333	9.5366417	2.7529913			67.512
AUCL	LOWER BOUND OF A.U.C.	12	1986.68750000	1219.3667930	352.0008731			61.377
AUCU	UPPER BOUND OF A.U.C.	12	2311.44750000	1099.2198385	317.3174348			47.555
CL_AUCL	CL * DOSE/AUCL * 1,000,000	12	223.19750000	151.0777328	43.6123848			67.688
CL_AUCU	CL * DOSE/AUCU * 1,000,000	12	187.61817500	84.6267042	18.6561225			41.002
AUCL_D	AUCL / DOSE	12	8542.89262140	4010.9166918	1157.8518159			61.301
AUCU_D	AUCU / DOSE	12	7620.40837226	3635.8868467	1049.5801248			47.712
DOSEGRP=B(M)								
TMAX	TIME TO MAXIMUM CONC	12	68.75083333	31.6323699	9.1314788			46.010
CPMAX	MAXIMUM CONCENTRATION	12	26.63500000	11.1941937	3.2314854			42.028
AUCL	LOWER BOUND OF A.U.C.	12	5217.48166667	3117.0519896	899.8154028			59.742
AUCU	UPPER BOUND OF A.U.C.	12	5288.49083333	3052.1834939	881.0884809			57.714
CL_AUCL	CL * DOSE/AUCL * 1,000,000	12	184.98333333	135.8991568	39.2307074			82.371
CL_AUCU	CL * DOSE/AUCU * 1,000,000	12	148.66805000	87.5442302	25.2718424			58.886
AUCL_D	AUCL / DOSE	12	8673.17683883	5278.9299141	1524.1844786			60.877
AUCU_D	AUCU / DOSE	12	8790.46431006	5173.9250072	1493.5834978			58.858
DOSEGRP=C(H)								
TMAX	TIME TO MAXIMUM CONC	12	68.75500000	36.9953355	10.67983344			53.807
CPMAX	MAXIMUM CONCENTRATION	12	44.80833333	22.6422958	6.53626780			50.531
AUCL	LOWER BOUND OF A.U.C.	12	7656.42833333	3031.1348354	875.01325657			39.589
AUCU	UPPER BOUND OF A.U.C.	12	7670.82833333	3038.3327761	877.09112309			39.610
CL_AUCL	CL * DOSE/AUCL * 1,000,000	12	176.12333333	65.6918757	18.96355333			37.298
CL_AUCU	CL * DOSE/AUCU * 1,000,000	12	175.82462500	65.5845292	18.93262281			37.301
AUCL_D	AUCL / DOSE	12	6489.90797817	2558.1132070	738.48367433			39.417
AUCU_D	AUCU / DOSE	12	6501.98802208	2564.3315248	740.25874806			39.438

TABLE 4-27. COMPARISON OF PHARMACOKINETIC DATA FOR PYRIDOSTIGMINE FROM THREE DIFFERENT STUDIES

Parameter	Source: Humans (MRDC)[a]	Source: Larger Rhesus Monkeys (LAIR)[b]	Source: Smaller Rhesus Monkeys (Battelle)[c]
Doses (mg/kg)			
lower	0.37	1.99	0.286
middle	0.52	3.01	0.571
higher	0.67	3.99	1.14
highest	0.83	--	--
Cl/F (ml/min/kg)	--[d]	252[e]	
lower limit[f]			161
upper limit[f]			188
C_{max}			
lower dose	15.3	25.07	14.13
middle dose	19.9	69.70	26.64
higher dose	23.6	42.77	44.81
highest dose	30.5	--	--
C_{max}/Dose			
lower dose	41.4	12.6	49.4
middle dose	38.3	23.2	46.7
higher dose	35.2	10.7	39.3
highest dose	36.7	--	--

[a]Data obtained from the USAMRDC, Fort Detrick, Frederick, Maryland, as reported in the study protocol (Appendix A).
[b]Data obtained from ——, as reported in the study protocol (Appendix A).
[c]Data from this report.
[d]Data not yet available from USAMRDC.
[e]Calculated from background data in the protocol, assuming a mean of 10 kg body weight for the larger Rhesus monkeys and equal numbers of subjects per dose group.
[f]Based on upper and lower limits for AUC (see text).

FIGURE 4-13. PLOTS OF GEOMETRIC MEANS OF OBSERVED RBC AChE INHIBITION VERSUS TIME AFTER PYRIDOSTIGMINE ADMINISTRATION



NOTE: 18 OBS HAD MISSING VALUES OR WERE OUT OF RANGE.

TABLE 4-29. INDIVIDUAL ANIMAL PHARMACOKINETIC PARAMETERS FOR RBC AChE

DOSEGRP=A(L)

OBS	SEQ	ANID	WEEK	DOSE	BASELINE	A	K1	K2	LAGTIME	FASTT12	SLOWT12	AUC	TMAX	FMIN	INHMAX	AUCD	FMIND	INHMAXD
1	73	245D	1	0.3264	11.190	4.452	0.05385	0.004568	21.33	12.850	151.70	892.0	71.33	7.945	28.98	2703	24.07	87.84
2	74	410D	1	0.2944	11.240	2.895	0.04339	0.003590	26.76	15.970	193.10	765.3	89.37	9.043	19.53	2638	31.18	87.34
3	78	404D	1	0.2940	10.810	7.087	0.07398	0.006348	14.87	9.369	109.20	1022.0	51.28	5.662	47.65	3526	18.82	184.30
4	86	827C	2	0.3250	10.460	5.578	0.05111	0.004427	22.89	13.560	156.60	1151.0	75.10	8.421	38.51	3596	20.06	120.70
5	87	333D	2	0.2870	9.512	4.202	0.12270	0.007043	23.83	5.647	98.41	562.4	48.64	8.184	34.66	1875	20.81	116.60
6	92	E42	2	0.3098	11.560	2.645	0.04707	0.003557	15.01	14.730	194.80	687.3	74.37	9.585	17.12	2217	30.92	55.22
7	99	937C	3	0.3010	12.330	7.698	0.03278	0.004640	27.06	21.150	149.40	1424.0	96.54	7.540	38.83	4748	25.13	129.40
8	105	087D	3	0.3001	11.570	4.178	0.05882	0.008028	27.73	12.200	86.34	448.7	67.84	8.268	21.90	1489	30.89	73.00
9	106	986C	3	0.2963	12.010	3.470	0.03734	0.002185	31.81	18.560	320.10	1510.0	112.80	8.287	22.84	5032	30.89	76.14

DOSEGRP=B(M)

OBS	SEQ	ANID	WEEK	DOSE	BASELINE	A	K1	K2	LAGTIME	FASTT12	SLOWT12	AUC	TMAX	FMIN	INHMAX	AUCD	FMIND	INHMAXD
10	75	847C	1	0.6123	12.54	10.250	0.05200	0.005706	43.30	13.330	121.5	1599.0	91.03	5.592	55.41	2622.0	8.188	90.84
11	76	366D	1	0.6342	11.46	6.063	0.09811	0.003725	22.81	7.212	186.1	1565.0	58.10	6.368	44.53	2484.0	10.110	70.68
12	81	E723	1	0.5940	10.56	9.500	0.03518	0.002334	11.50	19.690	296.8	3798.0	94.07	3.241	69.30	6440.0	5.483	117.50
13	83	810T	1	0.6080	12.30	4.991	0.09334	0.005447	26.04	7.426	127.2	862.8	68.37	8.355	32.05	1414.0	13.700	52.54
14	88	320D	2	0.6010	13.80	5.772	0.02048	0.006903	13.50	33.880	100.4	554.0	93.65	11.600	15.93	923.3	18.340	26.58
15	89	893T	2	0.6120	10.72	12.010	0.02464	0.005357	26.34	28.130	128.4	1755.0	105.50	4.562	57.42	2877.0	7.478	84.14
16	94	353D	2	0.6140	10.66	5.695	0.07210	0.003264	12.73	9.614	212.3	1666.0	57.68	5.958	43.95	2731.0	8.817	72.04
17	96	400D	2	0.6024	12.18	8.646	0.04825	0.002360	25.76	14.070	283.8	2682.0	90.56	8.758	44.55	4468.0	11.260	74.26
18	101	894C	3	0.5998	11.83	8.015	0.04819	0.005874	23.77	15.010	118.0	1181.0	74.93	6.852	43.78	1988.0	11.080	72.88
19	102	926T	3	0.6130	11.21	4.161	0.05432	0.003823	27.35	12.760	186.8	1105.0	81.21	7.893	28.71	1811.0	13.100	47.08
20	107	889C	3	0.5820	.	.	.	0.002499	.	.	277.4	.	.	.	.	.	.	.
21	108	328D	3	0.5796	10.96	7.918	0.02626	0.004492	56.14	26.400	184.3	1461.0	137.30	6.398	41.62	2520.0	11.030	71.76

DOSEGRP=C(H)

OBS	SEQ	ANID	WEEK	DOSE	BASELINE	A	K1	K2	LAGTIME	FASTT12	SLOWT12	AUC	TMAX	FMIN	INHMAX	AUCD	FMIND	INHMAXD
22	77	246D	1	1.171	11.02	6.088	0.04636	0.002677	25.22	14.950	256.8	2143	90.50	6.202	43.72	1832	5.301	37.37
23	78	803C	1	1.186	11.48	9.846	0.01633	0.003285	26.33	42.440	211.0	2394	149.20	6.228	45.76	2012	5.233	38.45
24	84	373D	1	1.170	11.36	8.265	0.18620	0.004119	24.09	3.832	166.3	1964	44.20	3.908	65.88	1678	3.341	56.06
25	85	3100	1	1.180	10.58	7.375	0.02503	0.004004	30.38	27.700	173.1	1547	117.80	8.187	41.38	1311	8.243	35.08
26	90	025D	2	1.188	11.84	7.206	0.18810	0.002584	24.12	3.686	268.2	2750	47.24	5.143	56.55	2311	4.322	47.52
27	91	405D	2	1.186	11.12	8.122	0.08478	0.003924	11.21	8.176	176.6	1488	49.21	6.088	45.24	1250	5.116	38.02
28	97	8942	2	1.183	13.39	8.610	0.07163	0.002605	13.50	9.676	266.1	3185	61.82	5.065	54.69	2700	5.140	46.35
29	98	3900	2	1.186	12.46	9.319	0.10380	0.002438	26.05	6.673	284.2	3731	63.04	4.144	66.74	3136	3.482	56.08
30	103	843C	3	1.184	12.41	9.858	0.06031	0.002187	37.04	11.490	317.0	4345	94.11	4.028	67.55	3682	3.414	57.25
31	104	819C	3	1.188	10.10	6.644	0.12280	0.003250	26.52	5.638	213.3	1990	56.87	4.238	58.03	1701	3.822	48.60
32	109	315D	3	1.178	11.29	8.901	0.03100	0.004155	31.98	22.360	166.8	1855	106.80	5.642	50.03	1572	4.781	42.39
33	110	2000	3	1.178	11.49	9.809	0.05194	0.002819	26.62	13.350	237.5	3172	85.35	3.894	67.86	2711	3.167	58.00

TABLE 4-30. GROUP MEAN VALUES OF PYRIDOSTIGMINE BROMIDE PHARMACOKINETIC PARAMETERS

VARIABLE	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN	MINIMUM VALUE	MAXIMUM VALUE	C.V.
DOSEGRP=A(L)							
BASELINE	9	11.22022222	0.87024160	0.29008053			7.756
A	9	4.70122222	1.75812434	0.58604145			37.387
K1	9	0.05768222	0.02716068	0.00905355			47.087
K2	9	0.00462833	0.00166820	0.00062207			37.859
LAGTIME	9	23.46666556	5.70695214	1.90231738			24.321
FASTT12	9	13.78177778	4.83513999	1.54504666			33.632
SLOWT12	9	162.19444444	70.81788848	23.59322950			43.539
AUC	9	840.07777778	369.62870823	123.20980308			35.318
TMAX	9	78.36333333	20.84661595	6.88220532			27.041
FMIN	9	7.87944444	1.50641753	0.50213918			19.116
IN=MAX	9	30.06111111	10.49947075	3.49982358			34.939
AUCD	9	3081.66666667	1230.81226332	410.30408777			39.914
FMIND	9	25.91888889	5.11873828	1.70624543			19.749
IN=MAXD	9	88.94888889	35.72647073	11.90882358			38.106
DOSEGRP=B(M)							
BASELINE	11	11.66090909	0.99258707	0.29927626			8.512
A	11	7.36563636	2.42130321	0.73006039			32.873
K1	11	0.05180545	0.02606424	0.00785866			50.312
K2	12	0.00428033	0.00155442	0.00044872			36.231
LAGTIME	11	26.30363636	13.28836579	4.00859304			50.519
FASTT12	11	17.04745455	8.89040800	2.68055887			52.151
SLOWT12	12	184.50833333	71.87815843	20.74872802			38.957
AUC	11	1658.16363636	898.21832848	270.82241322			54.169
TMAX	11	85.67363636	23.81025463	7.17906189			27.792
FMIN	11	6.68227273	2.16554677	0.65283782			32.407
IN=MAX	11	43.38636364	14.65006067	4.38700838			33.536
AUCD	11	2752.39090909	1526.96440722	460.39706154			55.478
FMIND	11	11.05336364	3.58962748	1.08532652			32.566
IN=MAXD	11	71.85000000	24.47125048	7.37838884			34.069
DOSEGRP=C(H)							
BASELINE	12	11.64333333	0.85920121	0.25689028			7.703
A	12	8.17041667	1.44033028	0.41578754			17.628
K1	12	0.08320667	0.05881218	0.01726628			71.884
K2	12	0.00317800	0.00071488	0.00020838			22.480
LAGTIME	12	25.25583333	7.08556052	2.04831768			28.065
FASTT12	12	14.13925000	11.64433020	3.33256108			81.847
SLOWT12	12	228.41666667	51.04423808	14.73520230			22.347
AUC	12	2547.00000000	900.03282567	259.81703936			35.337
TMAX	12	80.47000000	32.64545687	9.42393166			40.568
FMIN	12	5.13058333	1.04724656	0.30231404			20.412
IN=MAX	12	55.26083333	10.00681900	2.88871882			18.106
AUCD	12	2158.08333333	759.87565252	219.38607378			35.215
FMIND	12	4.34600000	0.87799265	0.25345465			20.202
IN=MAXD	12	46.84666667	8.56738247	2.47318029			18.288

TABLE 4-37. INHIBITION OF RBC AChE AFTER FOUR CONSECUTIVE PYRIDOSTIGMINE ADMINISTRATIONS AT 0.286 OR 0.571 mg/kg

Dose	No.	Time After Administration (hr):	Percent Inhibition of RBC AChE[a]		
			3.5	4.5	6.0
0.286 mg/kg	6		8 ± 1	9 ± 3	7 ± 1
0.571 mg/kg	6		12 ± 1	11 ± 1	7 ± 1

[a]Mean ± SD, relative to a pre-pyridostigmine baseline level of enzyme activity.

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TABLE 4-39. SUMMARY OF GD DOSE, RBC AChE INHIBITION, BODY WEIGHT, AND SURVIVAL FOR PHASE 2 STUDIES

Animal No.	GD Dose (µg/kg) (Targeted)	GD Dose (µg/kg) (Measured)	Group[a]	Survival[b]	Inhibition of RBC AChE[c]	Body Wt. (kg)
24AT	26.6	27.0	1		24.8	2.8
3TG	32.2	29.0	1		22.1	3.4
917T	38.9	29.8	1		28.7	4.2
396D	35.4	34.2	1		22.1	3.8
390D	42.8	43.5	1		29.7	4.7
2YZ	51.8	52.2	1		29.9	3.7
41I	51.8	51.6	1		26.5	3.0
2Z2	62.7	62.1	1		22.6	3.6
968C	75.8	75.8	1		24.7	3.6
D429	91.8	87.9	1		14.2	5.1
3LP	111	109	1		30.8	3.6
161D	134	141	1		28.9	4.2
311D	122	127	1		16.8	3.2
196D	148	148	1	DIED	12.0	4.2
942C	179	178	1		14.6	4.6
969C	216	210	1		25.5	4.6
3N7	262	261	1		18.4	2.8
907T	317	308	1		26.3	3.8
320D	510	503	1		19.9	4.3
276D	510	508	1		18.6	4.0
47H	561	551	1		26.3	2.9
318	561	564	1		17.1	3.1
328D	617	600	1		22.5	4.3
363D	617	606	1		28.8	4.8
893T	617	606	1		26.7	4.6
48W	617	607	1		23.8	2.6
996C	617	608	1		18.7	4.1
E723	617	608	1		15.9	3.2
3L9	617	610	1		28.9	3.9
306D	617	610	1		27.2	5.0
025D	617	610	1		19.0	3.9
819C	617	612	1		24.8	4.5
190D	617	613	1		22.4	4.0
4IJ	617	613	1		23.3	3.4
937C	617	616	1		27.7	3.9
E510	617	617	1		22.0	3.6
				MEAN (N = 36)	23.12	3.9
				SD	5.0	0.7
				HIGH	30.8	5.1
				LOW	12.0	2.6

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)
153D	38.9	38.4	2		34.4	4.6
230D	42.8	41.5	2		44.3	4.1
210D	47.1	45.0	2		35.2	4.3
981C	51.8	49.6	2		37.8	3.6
48E	62.7	55.0	2		33.9	2.9
4HP	62.7	63.7	2		49.7	4.0
3J8	75.8	71.6	2		50.8	3.6
904T	75.8	77.6	2		31.3	4.4
3MU	91.8	93.5	2	DIED	34.5	3.6
314D	111	110	2		35.2	3.9
159D	122	132	2		29.3	.4
410D	134	135	2		36.9	4.0
48V	148	145	2		39.7	3.4
3JT	163	161	2		41.2	3.7
C766	179	181	2		45.7	4.9
989C	216	216	2		23.0	4.6
48P	262	258	2		36.2	3.3
D384	317	312	2		31.4	4.6
3JH	510	503	2		32.0	3.7
D675	510	506	2		37.1	4.1
4G1	561	530	2	DIED	32.2	3.1
902T	561	556	2	DIED	38.2	3.8
C380	617	599	2		34.9	4.9
4BR	617	599	2		24.4	3.7
927C	617	603	2		50.9	4.6
46S	617	603	2		29.6	2.9
47R	617	606	2		46.7	3.2
47A	617	607	2		35.3	2.6
932T	617	608	2		36.6	4.3
809C	617	608	2		40.3	4.7
3M1	617	609	2		36.2	3.9
279D	617	612	2		44.1	4.4
3TD	617	615	2		39.1	3.9
015D	617	615	2		42.9	4.0
931C	617	617	2		32.7	3.5
3IT	617	617	2		36.0	3.6
				MEAN (N = 36)	37.2	3.9
				SD	6.6	0.6
				HIGH	50.9	4.9
				LOW	23.0	2.6