CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-414

PHARMACOLOGY REVIEW

Barry N. Rosloff, Ph. D.
4/27/98

PHARMACOLOGIST REVIEW OF NDA 20-414
SUBMISSION OF 3/20/98 (APPEAL OF NOT-APPROVABLE ACTION)

INTRODUCTION:

The sponsor has submitted a "Position Paper" which is intended as an appeal of our not-approvable action. This paper contains no new data; it contains several arguments in response to our not-approvable letter of 5/27/97. These arguments may be divided into those concerning (1) general criteria for what may be regarded ·as a valid surrogate marker, (2) the association of the proposed surrogate marker (RBC acetylcholinesterase inhibition, abbreviated hereafter as RBC-CI) with "efficacy" (protection against Soman-induced lethality in animals), and (3) the efficacy of·pyridostigmine across animal species. The following discussion will address the latter 2 points.

(The position paper also notes that our not-approvable letter requested that the sponsor address several other preclinical, chemistry, and EA issues; it is stated that "these issues will be resolved once the fundamental issues of the surrogate endpoint acceptability, and effectiveness are resolved.")

SPONSOR'S ARGUMENTS:

1) The sponsor is arguing (p. 5) that we are requiring evidence of an "unfailing linear proportional relationship between RBC-CI and survival after exposure to soman." It is stated that although the not-approvable letter "appears" to set such a standard, the sponsor does not "understand nor know of a precedent for this proportionality requirement." Rather, the sponsor is proposing that approval be based on the fact that the surrogate marker was "correlative in a yes/no manner," i.e. whenever pyridostigmine produced an RBC-CI of 5% or above, efficacy was seen.

Although the not-approvable letter does not mention the need for an "unfailing linear proportional relationship" it does convey the idea that for a surrogate marker to be considered

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"reasonably likely" to predict clinical benefit, some reliable, quantitative relationship to the degree of efficacy should be shown, and that this was not shown in the majority of the animal studies, in particular the pivotal monkey studies. The reason for wanting to see such a relationship is to help determine that the marker is truly related to and predictive of (animal) efficacy, rather than the results merely being an instance of two independent drug effects being coincidentally present together. (It may even be argued that even if a quantitative relationship were shown it would not necessarily mean the the effects were related inasmuch as many drug effects are dose-related and will therefore be quantitatively related to each other only coincidentally.)

The need for demonstration of a quantitative relationship aside, the "yes/no" correlation claimed by the sponsor was not seen; as discussed in my review of 7/24/96 and noted in the not-approvable letter, efficacy was seen in monkeys at a dose of pyridostigmine which caused no demonstrable RBC-CI (and the degree of efficacy was similar to that seen with higher doses causing up to 26% RBC-CI.) (Note that the sponsor's appeal, in describing the results of this study ["Task 92-30", p. 6 of the appeal], states that "a lower limit of 5% RBC-CI was predictive of improved PB efficacy...", without providing any explanation for the difference between this statement and the finding of a lack of a lower limit as indicated in the not-approvable letter. Attached is a table taken from this monkey study showing that a dose of pyridostigmine [40 ug/kg i.g.] which was effective against Soman did not cause RBC-CI which was statistically significantly different from control.)

(The fact that pyridostigmine had little or no efficacy in rats, rabbits, and mice at doses causing up to 70% cholinesterase inhibition also appears to be evidence against a yes/no correlation, although note that in the past the sponsor has postulated various reasons why humans will respond more like monkeys, and is now apparently claiming that pyridostigmine is effective in these other species [see below].)

The following additional points concerning the relationship between pyridostigmine-induced RBC-CI and efficacy were made in the sponsor's appeal:

a) In our not-approvable letter we noted that in the pivotal monkey studies there was no clear correlation between RBC-CI and efficacy within individual animals, and in fact there

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were some instances where the results were the opposite of those predicted (e.g. in one study the only 2 animals that died had the highest degree of pyridostigmine-induced RBC-CI.) The sponsor states that under the type of experimental design used, "analysis of individual animal responses within the statistical population is not meaningful" (p. 5). At any rate, the mean values in these studies did not show any correlation between RBC-CI and efficacy.

b) The sponsor cites literature references which support the relationship between RBC-CI and inhibition of acetylcholinesterase at the neuromuscular junction (NMJ), and states that "Since inhibtion of RBC-CI by [pyridostigmine] is an indicator of [acetylcholinesterase] activity at the synapse, it is predictive of a therapeutic outcome, therefore, we believe it fully meets the definition of a surrogate endpoint" (p. 7). The relationship between enzyme inhibition in RBC and NMJ was not mentioned in the not-approvable letter. Although perhaps of theoretical interest (e.g. regarding the plausibility of the proposed mechanism of action of pyridostigmine) and of importance when attempting to quantitatively extrapolate the animal findings to humans (e.g. does the RBC enzyme inhibition quantitatively reflect NMJ enzyme inhibition in animals to the same degree that it does in humans?), this question was not a pivotal reason for non-approval, which rather was the empirical lack of correlation between RBC-CI and efficacy. (As indicated in my review of 7/24/96, there are also literature data which show a lack of association of enzyme activity between RBC and the nervous system. In addition, the relative amount of drug-induced enzyme inhibtion in different regions will depend in part on the distribution of the drug; e.g. a drug which doesn't leave the blood compartment might greatly inhibit RBC enzyme but cause no nervous system effects.)

c) After stating that the not-approvable letter "appears to set a standard for unfailing linear proportional relationship [between RBC-CI and efficacy]", it is stated that "This, in essence, sets an approval standard that is not consistent with that used for other drug products. For example, attainment of 'therapeutic' blood levels of anticonvulsants such as phenytoin and carbamazepine, do not guarantee absence of seizures in all epileptic patients." I do not understand the relevance of this example.

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TABLE 6. ANALYSIS OF VARIANCE COMPARISON OF ACHE-I FOR PHASE III (I.M.) AND PHASE IV (I.G.) EXPERIMENTS

Phase	PYR Dose (ug/kg), Route	N	Mean	(S.D.)	Tukey Grouping [a]	
III	24 i.m.	10	28.74	(3.28)		A
III	8.4 i.m.	10	12.12	(3.48)		B
III	4 i.m.	10	6.92	(2.25)	C	B
IV	40 i.g.	10	3.50	(6.31)	C	
III	0 i.m.	4	3.00	(0.47)	C	

[a]Tukey's Studentized Range (HSD) Test for AChE-I conducted at the 95 percent level. Means with the same letter are not significantly different.

The above table is taken from "Task 92-30", NDA volume 2.10, p. 46. It shows the degree of pyridostigmine-induced RBC-CI at the time of Soman challenge in groups of monkeys given various doses of pyridostigmine. (Degree of enzyme inhibition was calculated in each animal using samples obtained pre- and post-pyridostigmine administration.) All doses produced approximately equal efficacy despite the different degrees of RBC-CI. As shown in the table, the dose of 40 ug/kg i.g. produced a degree of RBC-CI (3.5%) which was not statistically significantly different from control (3.0%). (The table also indicates, as does the text of this report, that the degree of RBC-CI produced by a dose of 4 ug/kg i.m. [6.92%] was also not significantly different from control, although examination of the individual animal data does appear to indicate a likely difference.)

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2) The sponsor stresses the point that pyridostigmine was effective against Soman in 5 animal species. (e.g., "we know of no exceptions", page 7). However, as concluded in my review of 7/24/96, in contrast to the efficacy seen in guinea pigs and particularly in monkeys, it appeared that little or no efficacy was seen in rats, mice, and rabbits at doses of pyridostigmine causing substantial RBC-CI. (It might be inferred that, at least previously, the sponsor agreed with this conclusion since a great deal of data and argument had been presented to support their contention that humans will respond to pyridostigmine more like monkeys than like rats, mice, and rabbits.)

It is noted that the question of trans-species efficacy was not pivotal the Division's not-approvable decision: As indicated in the not-approvable letter, the decision was based on a lack of association of the proposed surrogate marker and efficacy in the species in which efficacy was more clearly shown, i.e. monkeys and guinea pigs. (Our letter did note that the sponsor was nominating RBC-CI as a surrogate marker associated with efficacy in "some" animal species; this appears to be what the sponsor is responding to [p. 7 of appeal]).

The appeal contains "Table 1" (attached) in support of the contention that pyridostigmine was efficacious in all species studied. The results appear to be selectively chosen from the studies previously submitted to the NDA (and not always to the sponsor's advantage, e.g. of the 2 rat studies performed, data from only the one showing no effect of pyridostgmine appears in the table.) A detailed description of the results of studies in mice, rats, and rabbits is present in my review of 7/24/96. Briefly, the findings were as follows (Study identification numbers as used in my review):

a) Mice

Two studies were done. The data in the table presumably refers to study D-1, although the protective ratio (PR) for the PB/atropine/PAM regimen should be 2.7 rather than 2.5. The dose of pyridostigmine used in this study was estimated (from other studies) to cause 30-70% inhibition of "whole blood cholinesterase". The other study in mice (D-3) did not use a PR paradigm but appeared to also show a slight effect of pyridostigmine.

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b) Rats

Two studies were done. The data in the table refer to study E-1, which showed no effect of pyridostigmine at a dose estimated to cause 67% inhibition of whole blood acetylcholinesterase. The other study, E-2, which used mecamylamine instead of atropine, showed a slight effect (PR for complete regimen = 2.4, vs 1.3 for mecamylamine/PAM alone. This was seen at a dose of pyridostigmine estimated to cause 70% inhibition of whole blood acetylcholinesterase; the next lowest dose, estimated to cause 45% inhibition, was ineffective.)

c) Rabbits

Three studies were done. The data in the table are presumably a composite of the results of studies C-2 and C-3. As indicated in my review, it was not clear if these small effects of pyridostigmine were statistically significant. The dose of pyridostigmine used in these studies was estimated (from other studies) to cause 30-70% inhibition of "whole blood cholinesterase" (study C-2) or 20-40% RBC-CI (study C-3.)

The other study in rabbits (C-1), not included in the table, showed no effect of pyridostigmine (at a dose estimated from other studies to cause 20-40% RBC-CI.)

Table taken from sponsor's appeal (p. 9)

Table 1

Enhancement of Protective Ratios after PB pretreatment for Nerve Agents in Various Species:

Agent	Species	Protective Ratio	
		Atropine/PAM	
		PB/Atropine/PAM	
Soman	Mouse	1.1	2.5
(GD)	Rat	1.2	1.4
	Guinea Pig	1.5 - 2.0	2.7 - 6.8
	Rabbit	2	2.8 - 3.1
	Monkey	1.6	25 - >40

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CONCLUSION:

No new data or arguments were presented which would lead me
to alter my conclusion that NDA 20-414 should not be approved.

/S/

Barry N. Rosloff, Ph. D.

cc: NDA 20414 division file
    Rosloff
    Fitzgerald
    Tresley
    Leber
    Katz
    Nighswander
    Temple

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Barry Rosloff
4/26/99

SUBJECT: Interspecies differences in efficacy of pyridostigmine

I feel that I was somewhat inarticulate in explaining why I think the sponsor has not made a persuasive case for concluding that humans will respond like monkeys, and not like rats and mice, to the beneficial effects of pyridostigmine (P) in reducing Soman lethality.

The sponsor has postulated that the presence or absence of the enzyme carboxylesterase (CaE) in blood is a determinant of the efficacy of P and the basis for the species differences observed; i.e. it is present in species in which P is not effective (rats and mice) and absent in monkeys, in which P is effective. Since humans, like monkeys, have no CaE, the sponsor concludes that P will be effective in humans. (However, note that no data showing these species differences in CaE have been submitted; this was requested at the meeting of 4/22).

In support of the role of CaE as a determinant of the efficacy of P, the sponsor has performed studies from which they conclude that an inhibitor of CaE, CBDP, makes P effective in rats and mice. However, the design of these studies did not allow the effects of P to be separated from those of atropine and/or 2-PAM. (In another study submitted in the NDA [published article by Boscovic, NDA vol. 1.4, p.83+], it was in fact concluded that CBDP potentiated the protective effects of atropine and AchE reactivators against Soman in mice [P was not tested]). In addition, for the sponsor's CBDP studies to validly support their argument, it would have to be shown that CBDP was working only by inhibiting CaE. Although the sponsor's study did conclude that CBDP inhibited CaE and not AchE, the above-mentioned Boscovic study showed the opposite, and concluded that inhibition of AchE was the basis of the potentiation of Soman by CBDP. Thus it is not clear that the mechanism by which CBDP allows rats and mice to respond to the protective effect of P has been clearly established. Finally, even if CBDP is working by inhibiting CaE, there is no obvious explanation for why this would make P effective. As stated by the sponsor, the effect of CaE is to "detoxify" Soman, which presumably lowers the effective blood levels of Soman. (It is not clear to me how CaE is supposed to do this, e.g. by metabolizing Soman, binding Soman, or some other mechanism). Thus the postulated interaction between CaE and Soman is merely a pharmacokinetic one; i.e. the more CaE present, the higher the dose of Soman needed to achieve lethal blood levels, but the Soman blood levels needed to produce lethality should not be affected. When an inhibitor of CaE is given to rats or mice, less Soman has to be given to achieve lethal blood levels, but if these levels are the same as those in the absence of the inhibitor, it is not clear why this should make P more effective; i.e. the levels of Soman that P "sees" are the same in either case and thus there is no obvious reason why P should not compete with Soman for AchE equally well, and raise the LD 50 for Soman by the same multiples, in both cases. The following diagram expresses this reasoning. (Doses and concentrations arbitrarily chosen for illustrative purposes):

	SOMAN LD 50	SOMAN BLOOD LEVEL AT LD 50
NORMAL RAT/MOUSE	130 μg/kg	100 μg/ml
CaE - INHIBITED RAT/MOUSE	13 μg/kg	100 μg/ml

AChE ← P

    Aside from studies using a CaE inhibitor, there is no obvious explanation for why species differences in CaE activity would translate into species differences in sensitivity to P, using the above reasoning. Although higher CaE activity should result in higher LD 50 values for Soman (as is seen in rats and mice as compared to monkey) it is not clear, all else being equal* and assuming that the effect of CaE on Soman is only a pharmacokinetic one, why P should not be able to raise the LD 50 of Soman by the same multiples independent of CaE activity. (At the 4/22 meeting the sponsor stated that the higher LD 50 values in rats and mice represented "noise," and that the Soman LD could be raised by a similar absolute amount in all species. As I stated at the meeting, it is not clear why a pharmacokinetically-related higher LD 50 represented noise, why an increase in absolute LD 50 values was meaningful if no significant increase in LD 50 multiples was seen [were these absolute differences even statistically significant?], and that it was not clear if the absolute increases shown were indeed due to P [I stated that there were some studies in the NDA that showed that P had no effect on the Soman LD 50 on either an absolute or multiple basis]).

    Thus I do not believe that a persuasive case has been made showing that CaE activity is a determinant of the efficacy of P and the basis for the species differences observed. It is not enough to point to species differences in a given trait and show that this is correlated with differences in another trait; e. g. the presence of claws (rats and mice) may correlate with lack of efficacy of P, and the presence of fingernails (monkeys) with efficacy, but we would not want to conclude that type of nail was a determinant of efficacy. There should be more empirical data and theoretical plausibility which, in the case of CaE activity, I believe is lacking.

    *(It is of course possible that there are species differences in blood levels of Soman needed to produce lethality, but such differences are not being posited by the sponsor as a determinant of the species differences in the efficacy of P).

cc: G. Fitzgerald, R. Katz., R. Temple

Barry N. Rosloff, Ph.D.
12/23/02

NDA 20-414—MEMO TO FILE

PREFACE:

The purpose of this memo is to re-evaluate the animal efficacy data for pyridostigmine for the prophylactic treatment of Soman poisoning in view of the recent establishment of Subpart I (the "animal rule") of the new drug regulations. This NDA had been previously considered under Subpart H by virtue of the sponsor's attempt to establish a surrogate marker for efficacy; it was felt that such a marker had not been established and the NDA was considered not approvable. Under Subpart I the primary issues for approval center around the relevance of the animal data to humans and the ability to predict an efficacious dose for humans. A particular concern which will be discussed is the apparent species differences in the efficacy of pyridostigmine.

Most of the issues to be discussed in the present memo have been discussed in more detail in my previous reviews and memos and in communications with the sponsor; the most comprehensive review is my NDA review of 7/24/96. The reader is referred to these previous reviews for details not repeated in the present memo. References and literature citations given in my previous reviews will generally not be repeated here.

The following abbreviations will be used:

P = pyridostigmine
AChE = acetylcholinesterase
CaE = carboxylesterase
PR = protective ratio (ratio of Soman LD 50 with *vs* without a given treatment)

PROPOSED MECHANISM/RELEVANCE OF THE ANIMAL MODEL:

Subpart I specifies that the mechanism of toxicity of the toxin and its amelioration by the treatment be reasonably well-understood and that the endpoint in animals be clearly related to the desired benefit in humans. Of course such mechanisms are never conclusively known; however it can be said that there is a fair degree of consensus in the scientific community that Soman toxicity is due to its inhibition of AChE at neuromuscular junctions in the nervous system with consequent excessive buildup of synaptic acetylcholine which leads to the various physiologic consequences typical of Soman poisoning.

The mechanism of P in decreasing the lethality of Soman is less clear. The theory is that P reversibly carbamylates a fraction of the same active site on AChE which Soman irreversibly phosphorylates; when the AChE is subsequently decarbamylated there is a small residual

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amount of AChE activity which is adequate to sustain life (provided atropine/2-PAM are subsequently administered). While it has been shown that P can protect AChE in this way, it is less clear that this is the mechanism by which it improved survival. As reviewed in detail in my previous reviews, there has been virtually no quantitative correlation established between P-induced AChE inhibition (in RBC or whole blood) and prevention of Soman-induced lethality in animals (including a finding, in monkeys, of such prevention at a dose of P which caused no measurable inhibition of RBC AChE). Various possible reasons for such a lack of correlation were discussed in my review of 7/24/96, p. 43-44, including the possibility that the degree of P-induced AChE inhibition in blood does not correlate with that at critical sites in the nervous system, that P-induced AChE is only the first step in a long chain of events (e.g. protection of AChE, reduction in acetylcholine levels, changes in degree of receptor activation and subsequent desensitization, changes in end organ function) each with its own quantitative relationship to the preceding step in the chain, and that P has several other cholinergic (and non-cholinergic) actions, aside from AChE inhibition, which might account for its efficacy. The sponsor is currently planning and performing studies to determine if an alternative parameter, i.e. the degree of P induced *protection* of AChE (i.e. the amount of residual AChE after exposure to Soman) is better correlated with protection against lethality and loss of neuromuscular function induced by Soman; such a correlation would provide some evidence for the proposed mechanism of action of P.

Subpart I mentions the need for the animal study endpoint to be related to the desired benefit in humans; this is clearly the case here. However, there are some potential caveats to consider in extrapolating the animal studies as performed to the anticipated human situation; for example (1) the routes of Soman administration in animals (various parenteral routes) were different from those expected in humans (dermal/inhalation); such differences are known to cause different patterns of intoxication; (2) in the animal studies Soman was administered at a fixed time after administration of P; in the pivotal monkey studies this time was optimized for showing efficacy, i.e. at or after the Tmax for P-induced AChE inhibition (when, according to theory, such inhibition should be declining and releasing critical AChE sites); it is not clear how well the time interval between P and Soman exposure can be controlled in humans, but it might be predicted that P would not be effective if Soman exposure occurred relatively soon after dosing, i.e. at a time when P-induced AChE inhibition was increasing or not declining significantly; also the animal studies did not consider possible prolonged or re-exposure to Soman.

ANIMAL EFFICACY DATA/SPECIES DIFFERENCES:

The primary endpoint in the animal studies was prevention of Soman-induced lethality, measured by either an increase in the LD 50 of Soman or, in a smaller number of studies, a reduction in the percent of animals dying from a fixed lethal dose of Soman. P had a large effect in rhesus monkeys, increasing the Soman LD 50 by as much as 25 fold compared to the effect of atropine/2-PAM, a smaller but consistent effect in guinea pigs (2-4 fold), but little or no effect in several studies in rats, mice, and rabbits. There are many possible reasons for such species differences (noted later). The primary reason posited by the sponsor is based on species differences in the amounts of the enzyme carboxylesterase (CaE); specifically the species in which P did not work (or did not work well) have high amounts of CaE which serves as a "sink"

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for Soman, thus resulting in relatively high LD 50 values for Soman and creating a situation of too much "noise" to be able to show an effect of P. The sponsor claimed that P caused a similar *absolute increment* in the Soman LD 50 in all species studied, and that this measure was more relevant than fold-increases in the LD 50 for purposes of interspecies comparison. It was further stated that monkeys and humans have little or no CaE and therefore (if the amount of CaE is indeed an important determinant of the efficacy of P) P, as it is in monkeys, should be efficacious in humans (and cause a clinically significant degree of protection as defined by the sponsor as a several fold increase in the Soman LD 50). In support of this argument, the sponsor submitted a paper (Maxwell, et. al., JPET 246:986-991, 1988) purporting to show that an inhibitor of CaE (CBDP) given to rats, rabbits, and guinea pigs allowed (by reducing the "noise") the beneficial effect of P, as indicated by a several fold increase in the Soman LD 50, to become manifest. (A similar result was reported in mice in a separate paper [Maxwell et. al., JPET 264:1085-1089, 1993]; however there were no groups of mice not given CBDP for comparison; the conclusion of a role for CaE was based on historical data showing little or no effect of P in mice not given CBDP).

We have found several problems with the above hypothesis concerning species differences; most of these have been previously communicated to the sponsor (e.g. letter of 6/23/00). They are as follows:

1. The postulated species differences in CaE content have not been well-documented. There are some scattered data in the literature, but no study I am aware of which has compared all of the different species head-to-head. Comparison across studies is difficult because of problems with enzyme nomenclature and the lack of substrate specificity for the various plasma esterases. The sponsor is intending to do a study comparing CaE content in monkeys, rats and guinea pigs using labelled Soman; binding of labelled Soman will apparently be measured in the presence and absence of CBDP to control for non-specific binding. It will be difficult to conclude that the results will accurately reflect the content of the single enzyme "CaE" without knowing the specificity of both Soman and CBDP for this enzyme; in the absence of such knowledge all that can be said is that what is being measured are CBDP- sensitive Soman binding sites. However, this may be a relevant parameter since such sites have been shown to have toxicological relevance by the observation that CBDP reduces the LD 50 of Soman (Maxwell, 1988 ibid.; Maxwell, et.al., Toxicol. Lett. 39:35-42, 1987). (Conversely, it may be difficult to relate CaE activity measured with other substrates to the *in vivo* studies with Soman and CBDP without knowing the relative specificities of the various substrates for the various plasma esterases).

2. As noted above, the sponsor claims that the data show that, as measured by an increase in the absolute value of the Soman LD 50, P actually works equally well in all species tested. The tables on the following page (Tables "1" and "4") were submitted to IND . (7/29/99) in support of this argument. It was stated that Table 1 indicates that the magnitude of the increases in Soman LD 50s were "quite consistent...across species". Note that only those 3 studies with the superscript "e" studied the contribution of P. (Note that for the monkey study the stated values for the Soman LD 50 [40.4] and increase in Soman LD 50 [25.1] for the atropine/PAM treatment are incorrect and should be 25.1 and 9.8,

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Table 1. Studies Demonstrating a Consistent Increase in Soman LD$_{50}$ in PB-pretreated Animals Receiving an Adequate Dose of Atropine

Species	Soman LD$_{50}$ (µg/kg) sc				Increase in Soman LD$_{50}$ (µg/kg)			Dose of Atropine (mg/kg)	Reference
	Control	At/PAM	At/PB	PB/At/PAM	At/PAM[a]	At/PB[b]	PB/At/PAM[c]		
Rhesus[d]	13.0		176			163		4.0	Dirnhuber
Rhesus[e]	15.3	40.4		>617	25.1		>602	0.4	Joiner '88
Rabbit[d]	20		121			101		17.4	Maxwell '88
Guinea Pig[e]	26.6	54.0		190	27.4		163.4	16	Lennox
Guinea Pig[d]	28		146			118		17.4	Maxwell '88
Rat[e]	157	199		374	42		217	16	Lennox
Rat[d]	126		262			136		17.4	Maxwell '88
Mean±S.D.					31.5+9.2	130+27	190+38[f]		

a. (Soman LD$_{50}$ in At/PAM group) – (Soman LD$_{50}$ in Control group).
b. (Soman LD$_{50}$ in At/PB group) – (Soman LD$_{50}$ in Control group).
c. (Soman LD$_{50}$ in PB/At/PAM group) – (Soman LD$_{50}$ in Control group).
d. Studies in which Soman LD$_{50}$ values were only determined in Control groups and At/PB groups.
e. Studies in which Soman LD$_{50}$ values were determined in Control groups, At/PAM groups, and PB/At/PAM groups.
f. Mean does not include the >602 value because it exceeds the Mean by >3 S.D. and, therefore, the >602 value is considered an outlier.

Table 4. Studies Demonstrating Inadequate Protection Against Soman in PB-pretreated Animals Receiving an Inadequate Dose of Atropine

Species	Soman LD$_{50}$ (µg/kg) sc				Increase in Soman LD$_{50}$ (µg/kg)			Dose of Atropine (mg/kg)	Reference
	Control	At/PAM	At/PB	PB/At/PAM	At/PAM[a]	At/PB[b]	PB/At/PAM[c]		
Guinea Pig[d]	25.3			54.6			29.3	8	Jones
Rabbit[e]	5.4		36.5	41.5		21.1	26.1	8	Joiner '89
Rabbit[f]	15.2	28.4		42.0	13.2		26.8	13	Koplovitz '92
Mouse[f]	90.7	159.0		204.4	68.3		113.7	11.2	Koplovitz '84
Mouse[f]	107.4	186.2		238.9	78.8		131.5	11.2	Koplovitz '84

a. (Soman LD$_{50}$ in At/PAM group) – (Soman LD$_{50}$ in Control group).
b. (Soman LD$_{50}$ in At/PB group) – (Soman LD$_{50}$ in Control group).
c. (Soman LD$_{50}$ in PB/At/PAM group) – (Soman LD$_{50}$ in Control group).
d. Study in which Soman LD$_{50}$ values were only determined in Control group and PB/At/PAM group.
e. Study in which Soman LD$_{50}$ values were only determined in Control group, At/PB group, and PB/At/PAM group
f. Studies in which Soman LD$_{50}$ values were determined in Control groups, At/PAM groups, and PB/At/PAM groups.

respectively; the values of 617 and 602 for the entire treatment regimen are correct). The column with the superscript "c" shows the increases in Soman LD 50 caused by the entire treatment regimen (P/atropine/2-PAM) from these 3 studies; to determine the contribution of P one must subtract the increase due to atropine/PAM alone, giving values of ? 592, 136, and 175 in monkey, guinea pig, and rat, respectively. The sponsor excludes the monkey value as an outlier (see footnote "f" to table); therefore the conclusion of consistency across species is based on only 2 species. It is also noted that the rat study (superscript "e"), in contrast to what is indicated in the table, did not use PAM, and used mecamylamine along with the atropine, and therefore comparing this study with the others may be questioned.

Table 4 contains studies said to show inadequate protection by P in studies which supposedly used an inadequate dose of atropine. The definition of an adequate dose of atropine was apparently 16 mg/kg. The basis for this definition is unclear; it seems post-hoc and somewhat circular. At any rate, the effects of P in these studies seem small or nonexistent. (Note that in the first 2 studies in the table the effect of P cannot be isolated. It is not known if the effects in the mouse studies were statistically significant; the reference cited was not in the reference list). Other negative studies were not included in this table. Rat study "E-1" (numbering system used in my review of 7/24/96) showed P increased the Soman LD 50 by only 10 ug/kg (not statistically significant); the dose of atropine was 16 mg/kg thus meeting the sponsor's (post hoc) criterion for an adequate dose. Rabbit studies "C-1" and "C-2" showed increases of 1.5 and 10 ug/kg respectively; the former was not statistically significant, and in the latter the LD 50 of Soman alone was based on historical data and therefore, although the report concluded statistical significance, this conclusion in questionable. (Atropine doses in these studies were 2 and 5 mg/kg, respectively). Mouse study "D-1", also not listed in Table 4, showed a larger effect of P (163 ug/kg); it is not clear if this was statistically significant and at any rate the LD 50 for Soman alone was based on historical data. (Dose of atropine was 11.2 mg/kg).

From the above it may be concluded that P does not produce a similar degree of increase in the Soman LD 50 across species. However, I do not believe that this is necessarily at odds with the "CaE theory" of species differences in the efficacy of P, considering the many other potential factors which could affect the efficacy of P and could differ across species (see later). More troubling are the studies which showed *no* significant effect of P, although it could be argued that because of the high level of noise it is difficult to show such an effect, and that the weight of evidence indicates some effect of P in all species.

3. There are several problems in interpreting the study showing that an inhibitor of CaE, CBDP, potentiated the effects of P (plus atropine), as indicated by several fold increases in the Soman LD 50, in various species (Maxwell, et. al., 1988, ibid.). The experimental design did not distinguish the effects of P from those of atropine. Thus one cannot be certain if the effect of the CBDP was to potentiate P, atropine, or both. Potentiation of the effect of atropine (against Soman) by CBDP has been reported in the literature (Boskovic, Arch. Toxicol. 42: 207-216, 1979), although the dose of CBDP (50 mg/kg s.c.) was much higher than that used by Maxwell et. al. (1988, ibid.) (2 mg/kg s.c.). On the other hand, in a paper by Maxwell and Brecht (Neuroscience and Biobehavioral Reviews 15:135-139, 1991) the effect of atropine did not appear to be potentiated by CBDP (2 mg/kg s.c.) in mice and

guinea pigs, although the timing of dosing with atropine (30-60 sec. *after* Soman) was different than in the study of Maxwell et. al. (1988, ibid.), where the atropine was given 15-30 min. *before* Soman. The doses of atropine were also somewhat different between these studies. (The Maxwell and Brecht paper did show that CBDP potentiated the effect [as measured by fold increases in the Soman LD 50] of PAM in mice [but not guinea pigs] and potentiated the effect of another oxime, HI-6, in both species). In view of these uncertainties, and it view of the pivotal nature of the CBDP study in explaining species differences in the efficacy of P, it would seem prudent to more conclusively show an effect of CBDP on P independent from any effect on atropine, preferably in a study of P which has an atropine-only group.

Interpretation of the results of this study as support for the "CaE theory" assumes that CBDP has no important actions aside from CaE inhibition which might explain the results obtained. One obvious possibility is inhibition of AChE; this was examined in the Maxwell et. al. (1988, ibid) study and found not to occur (although AChE inhibition was reported at a higher dose of CBDP by Boskovic [ibid]; curiously, the latter paper found that CBDP did not inhibit CaE activity). Another possibility is inhibition of the elimination of P by CBDP, although I am not aware of any data on this point.

If CBDP were potentiating P only by inhibiting CaE, it would be predicted that such potentiation should be minimal or absent in species with little or no baseline CaE activity. In the Maxwell et. al. (1988, ibid) study, CBDP potentiated the effects of the P/atropine combination in all 3 species tested: rats (protective ratio increased 4 fold), guinea pigs (2 fold), and rabbits (2 fold). Although as noted earlier it is not clear how well-established the species differences in CaE are, rats are generally considered to have higher levels than the other 2 species, which fits with the greater effect of CBDP in that species; on the other hand, the effect in rats was not that much greater than in the other species; this is especially noteworthy for rabbits where CaE levels have been reported to be very low. A more convincing source of evidence to rule out effects of CBDP aside from inhibition of CaE would be to show that CBDP does not potentiate the efficacy of P in monkeys, a species with putatively little or no CaE.

4. Inasmuch as Subpart I addresses the importance of understanding the mechanisms of toxicity and protection, it would seem important to also understand the mechanism for why a high level of CaE should mask the efficacy of P. If CaE merely serves as a sink which removes a constant fraction of Soman from the blood regardless of dose, it is not clear why, if P "really" works as well in species with high CaE as it does in species with little or no CaE, it wouldn't be able to raise the Soman LD 50 by a similar multiple. For example, if in a species without a CaE sink a Soman dose of X resulted in plasma Soman levels of Y which caused a given degree of lethality, and P were capable of increasing these lethal doses and plasma levels by 10 fold to 10X and 10Y, it is not clear, in a species which had a CaE sink which removed (for example) 50 % of Soman from blood regardless of dose, thus raising the Soman dose needed to cause the same plasma level (Y) and degree of lethality as in the previous species by 2 fold to 2X, why P, if it had the same ability to interfere with Soman at the molecular level as in the previous species, wouldn't be able to raise this Soman dose to 20X (which would give a lethal plasma level of 10Y, the same as in the

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previous species given P), i.e. cause the same fold increase of 10 in the Soman LD 50. Another way of looking at this is that due to the CaE sink which removes 50 % of Soman from blood, a given dose of Soman in a species with such a sink is only half as toxicologically significant as the same dose in a species without such a sink, and therefore any given incremental (as opposed to fold) increase in this dose is only half as toxicologically significant in the former as compared to the latter species.

However, if the CaE sink were relatively large (particularly in relation to the amount of Soman free to interact with AChE) and saturable, it is possible that P would appear to have only a small effect (as measured by fold increases in the Soman LD 50) in a species with such a sink (e.g. rat) even though it "really" has the same effect as in a species without such a sink (e.g. monkey). In such a case, an absolute (as opposed to a fold) increase in the Soman LD 50 would be a meaningful indication of the efficacy of P. A hypothetical example of such a situation is as follows: Assume, for the sake of simplicity, that monkeys have no CaE and that Soman levels are proportional to dose. Say the Soman LD 50 in monkeys is 20 ug/kg, and that P increases this 20 fold to 400 ug/kg. Now say that in rat there is CaE in plasma which sequesters Soman, and that this is a saturable process. Again for the sake of simplicity, say that in the rat all Soman which enters the blood after doses of up to 400 ug/kg is sequestered by CaE, and that at this dose this process becomes saturated such that anything above this dose results in dose-proportional plasma levels (as in the monkey). (In reality, at least some of the Soman entering blood from the initial 400 ug/kg will also be eliminated from blood by other mechanisms, including interacting with AChE). Assuming that at doses above 400 ug/kg in rat any increment in blood Soman level due to increment in dose is the same as that in monkeys, and that rat and monkey are equally sensitive to the lethal effects of a given Soman blood level, the LD 50 of Soman in rats will be 420 ug/kg (i.e. the 400 ug/kg that gave no blood levels + the 20 ug/kg that gave blood levels the same as those at the LD 50 of 20 ug/kg in monkeys). Now if P has the same degree of efficacy against lethal blood levels in both species, it should raise the LD 50 in rats to 800 ug/kg (i.e. the P does nothing to the first 400 ug/kg [which did not result in any blood levels] but, as in monkeys, raises the amount of the next 20 ug/kg, which caused 50 % lethality, by 20 fold, to 400 ug/kg; 400 + 400 = 800 ug/kg). Thus, in this example, P causes equal absolute increases (380 ug/kg) in the Soman LD 50 in monkeys (400-20) and rats (800-420), but causes a much larger fold increase in monkeys (400/20 =20) than in rats (800/420 =1.9).

Support for the above model could come from data showing increases in Soman plasma levels greater than proportional to dose in species in which P doesn't produce significant fold increases in the Soman LD 50; deviations from proportionality should be less or absent in the other species and in species given CBDP to inhibit CaE. I am aware of one paper (Benschop and De Jong, Neuroscience and Biobehavioral Rev 15: 73-77, 1991) which looked at this in 3 species, comparing Soman AUC values at various doses; the text stated that these were linear in marmosets and guinea pigs but not in rats, although the data as presented (one would have liked to have an idea of the inter-animal variability) do not seem to me to show strong deviations from linearity in any case (see Table below). (Note that any plasma level data should take into account the fact that there are 4 stereoisomers of Soman; according to the above paper these have different pharmacokinetics and toxicities).

— TABLE 1

MEAN* TOXICOKINETIC DATA OF C(−)P(−)-SOMAN IN RATS,
GUINEA PIGS AND MARMOSETS

	Rat			Guinea Pig		Marmoset	
Dose of C(±)P(±)-soman (x LD$_{50}$)	6	3	1	6	2	6	2
Dose of C(−)P(−)-soman (μg/kg)	111	55.5	18.5	37	12.3	13.5	4.5
Area under curve (ng·min·ml^{-1})	877	320	76	516	228	191	84
Terminal half life (min)	40	16	5.8	15	16.5	15	9
Acutely toxic levels of C(±)P(−)-soman until (min)	317	95	37	126	104	74	49

*N = 6

It should be noted that the above model does not explain those studies in rodents/rabbits in which *no* effect of P was seen, either on the absolute or relative value of the Soman LD 50.

5. Rabbits are considered to have very low CaE, but do not respond robustly to P. Also, in the paper of Maxwell et. al. (ibid.), CBDP reduced the LD 50 of Soman, and potentiated the effects of P, to a similar extent as that reported in guinea pigs, which have been reported to have substantially higher CaE levels.

DOSING CONSIDERATIONS:

(The following is presented for informational purposes; the question of predicting an effective dose for humans is apparently being addressed by OCPB).

The following are the doses used in the pivotal animal studies; the study numbering system is that used in my review of 7/24/96. Keep in mind that the different studies used various routes of administration and varying study parameters such as the time between dosing with P and administration of Soman. Also note that where efficacy was seen it varied in degree between species (and between studies within a species); in particular, where efficacy was seen in rabbit, mouse or rat studies the protective ratios (ratio of Soman LD 50 with vs without P pre-treatment) were generally 2 or less.

1) Monkey

    A-1: Low dose: 1.2 mg/kg p.o. q 8 hr. (6 total doses)
       High dose: 1.2 mg/kg p.o. followed 8 hr. later by 1.8 mg/kg followed 8 hr. later by four doses of 2.4 mg/kg given 8 hr. apart

    Both doses had equal efficacy.

    A-2:

       Phase III : 4, 8.4, or 24 ug/kg i.m.; all had equal efficacy

       Phase IV : 40 ug/kg intragastric – effective

       Phase V : 4 ug/kg i.m. – effective

2) Guinea pig

    B-1 : 0.94 mg/kg p.o. - effective

    B-2 : Doses not stated (Doses were based on predicted degree of AChE inhibition) – Only the dose predicted to cause 70 % inhibition was effective; the next lowest dose caused 45% inhibition

    B-3 : Dose range 0.06-15 mg/kg p.o. Efficacy seen at 0.23 mg/kg and above but not dose-related; next lowest dose was 0.12 mg/kg

    B-4 : 0.32 u mol/kg i.m. – effective

3) Rabbit

    C-1 : 0.08 mg/kg i.m. – not effective

    C-2 : 0.32 mg/kg i.m. – P increased the Soman LD 50 by 10 ug/kg (but Soman-alone value based on historical data) and by 1.4 fold; not clear if statistically significant

    C-3 : 0.08 mg/kg i.m. – P increased Soman LD 50 by 14 ug/kg and by 1.5 fold; not clear if statistically significant

4) Mouse

    D-1 : 0.13 mg/kg i.m. – P increased Soman LD 50 by 163 ug/kg (but Soman-alone value based on historical data) and by 2.5 fold; not clear if statistically significant

D-3 : 0.20 or 0.82 mg/kg p.o. – reduced lethality of Soman doses up to those stated to be 2x the LD 50; the two doses showed equal efficacy.

Other: Two mouse studies were listed in Table 4 of the submission of 7/29/99 to IND ⸺ (see above); these studies were not included in the pivotal studies described in the NDA but appeared to have evaluated the effect of P; according to the table the increases in Soman LD 50 were 45 and 53 ug/kg; the PRs were 1.3 in both studies. (Doses of P were not stated).

5) Rat

E-1 : 0.13 mg/kg i.m. – not effective

E-2 : Doses not stated (Doses were based on predicted degree of AChE inhibition) – Only the dose predicted to cause 70 % inhibition was effective (Soman LD 50 increased by 175 ug/kg and by 1.9 fold); the next lowest dose caused 45 % inhibition. (Note that in this study, mecamylamine was given with the atropine, and 2-PAM was not used).

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DISCUSSION AND CONCLUSIONS:

1. Proposed mechanism/Relevance of the animal model

Although mechanisms of drug action are never known with certainty, and hypotheses change over time, there is currently fairly wide consensus that Soman toxicity is caused by excessive inhibition of AChE and its sequelae. It is less clear how P causes protection from Soman –induced lethality, although it is reasonable to assume that this mechanism would be similar across species in which P has been shown to be effective.

Some differences in the animal efficacy studies of P and the expected human exposure to Soman were discussed earlier. Some animal studies were designed to optimize the effect of P (e.g. by giving the Soman at or after the Tmax for P-induced AChE inhibition). However, it may be noted that clinical trials of drugs are often designed to optimize drug effects, and the conditions of the trials often do not strictly mimic real-life conditions; it is assumed that efficacy seen in such trials will be seen in at least part of the population under at least some conditions. (However, since, according to the proposed mechanism of action of P it would not be expected to work at a time when P-induced AChE inhibition is increasing, it would be hoped that users of P would be made aware of this).

2. Species differences in the efficacy of P

The sponsor has estimated that a several fold increase in the "protective ratio" (PR) (ratio of Soman LD 50 in the presence of pretreatment with P [with supporting atropine/PAM] to the Soman LD 50 in the absence of such pretreatment) in humans is necessary for P to be considered effective under anticipated levels of exposure to Soman. Increases in the PR of several fold or more were seen in monkeys and guinea pigs; however in several studies in rats, mice, and rabbits there was either no effect of P or a very slight effect (PRs of 2 or less).

An argument has been made that, at least in the case of rats and mice, even though P doesn't increase the PR to a meaningful degree, P does have intrinsic activity, as measured by an *absolute* increase in the Soman LD 50, in these species, and in fact has the same degree of intrinsic activity in all species tested. The lack of significant effect on the PR in rats and mice is due to the presence of high levels of the enzyme CaE, which binds Soman and thus serves as a sink which reduces the effective blood levels of Soman in these species; the consequent high Soman LD 50 values creates a situation where there is too much "noise" to show an effect of P on the PR. Experimental support for the proposed role of CaE comes from a study in which rats were given an inhibitor of CaE (CBDP) to reduce the "noise", and it was found that P, in addition to increasing the absolute Soman LD 50, also increased the PR. (A similar study was also done in mice but there was no concurrent control group not given the CaE inhibitor). It is further argued that humans, as do monkeys, have low CaE, and therefore P should cause a substantial increase in the PR (in addition to an increase in the absolute Soman LD 50) in humans (as it does in monkeys). Another way of stating this argument is that P has the same degree of intrinsic activity across species, and although this is not adequate to provide significant additional protection to that provided by high levels of

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endogenous CaE (i.e. in rats and mice), the same degree of activity (i.e. same increase in the absolute Soman LD 50) would be so adequate when added to the minimal protection provided by CaE in species with low endogenous CaE (i.e. monkeys and humans).

The above argument is plausible, however there are several significant uncertainties which should be addressed:

a) As noted earlier, the magnitude of the absolute increases in the Soman LD 50 was clearly not the same across species; more significantly some studies showed *no* effect of P. (On the other hand, as noted earlier, species differences in magnitude should not necessarily negate the sponsor's CaE theory, and the negative studies might be due to the inability to show a relatively small effect in the presence of noise).

b) As discussed earlier, is not clear if increases in the absolute value of the Soman LD 50 necessarily indicate that P has significant activity relevant to efficacy in rodents. However, a scenario was presented where such a conclusion could be reached, i.e. if the CaE sink were relatively large and saturable. This is presumably testable, and perhaps there are relevant data already available (although the paper cited earlier did not find significant deviations from linearity in the Soman dose-plasma level curve). I feel that there should be some corroboration and better understanding of the proposed mechanism for the lack of effect of P on the PR in rodents to help rule out the conclusion that P has no intrinsic efficacy in these species.

c) Problems with interpreting the study in which rodents given an inhibitor of CaE showed an enhanced response to P (as measured by increase in PR) were discussed earlier. They primarily involve the possibility that the inhibitor has other actions, aside from "noise reduction", which could explain the enhanced effects of P on the PR (and provide an alternative to the conclusion that P has intrinsic activity relevant to efficacy in these species). (Note that it is possible that differences in CaE levels have a role in explaining species differences in the effect of P independent of any effect on "noise". If we believe that P works preferentially in species with little or no CaE, and an inhibitor of CaE makes species with high CaE responsive to P, this would seem to predict that humans, if they have little or no CaE, will respond [all other things being equal-but see later]. However, this would be based purely on empirical observation with no (as yet) underlying rationale for why this should occur. Also, the empirical results are subject to some of the same problems noted above, e.g. possibility of effects of the inhibitor aside from those on CaE).

Also, since this study is important for explaining species differences, I feel that it should be replicated and extended. Replication is an essential part of the scientific enterprise; a role for replication was also noted in Agency responses to comments made regarding Subpart I, i.e. "...the animal studies should be replicated or substantiated in each species to ensure credible results" (response to Comment #5, FR 67, No. 105, p. 37991, 5/31/02); the 5/98 guidance for evidence of effectiveness in clinical trials (the "Evidence Document") also discusses potential problems with reliance on a single site, particularly regarding literature reports. (On the other hand, it could be argued that there is

corroborating evidence for the sponsor's hypothesis outside of this study, although I feel this evidence to be weak. Also, there appears to be some internal inconsistency in this study, i.e. the CaE inhibitor also enhanced the effect of P in rabbits, a species which is said to have very low levels of CaE). In addition to replication per se, the study could be extended to rule out an effect of the inhibitor on atropine (see earlier) or on plasma levels of P, verify the effect in mice (the previous study did not have a proper concurrent control group for this species), and use monkeys (in which, since this species is said to have little or no CaE, the effect of P should not be potentiated; a lack of effect could help rule out other actions of the inhibitor which could have explained the results in the other species). In addition, plasma levels of Soman might be measured to obtain evidence for a saturable sink; it might be expected that in species in which P does not cause a significant increase in the PR, Soman levels increase greater than in proportion to dose, but do not (or do to a lesser degree) in species where P does increase the PR and in species whose CaE has been inhibited.

d) Rabbits are said to have very low levels of CaE, yet they do not respond robustly to P (as measured by increase in PR) and, as noted above, and inhibitor of CaE increased the effect of P in this species.

e) As noted earlier, the postulated species differences in CaE levels have not been well-documented; a study in rats, guinea pigs, and monkeys is being performed by the sponsor. It should be kept in mind that there are a large variety of esterases and carboxylesterases, with overlapping substrate and inhibitor specificities, and thus an assay using one substrate may not necessarily be measuring the same thing as an assay using a different substrate. Also, any given substrate may not be assaying all enzyme activity involved in the elimination of Soman. (The sponsor's study will assay Soman binding to CBDP-sensitive sites).

f) Even if it can be concluded that P has intrinsic activity relevant to efficacy in rodents and rabbits, the magnitude of the effect seen, whether expressed as increases in the PR or in the absolute LD 50 of Soman, was widely different across all species tested. This is not necessarily surprising, considering the many potential sources of species variation (aside from CaE levels), such as differences in the PK of P and Soman, in the rate of decarbamylation of AChE after inhibition by P, in the blood level of Soman needed to produce lethality, possible quantitative pharmacodynamic differences in the various steps along the proposed pathway of protection (e.g. quantitative differences in the blood level of P needed to produce a given degree of AChE inhibition, in the degree of P-induced AChE inhibition needed to protect a given proportion of AChE from Soman, in the degree of protected AChE activity needed to metabolize a given amount of acetylcholine, etc. etc.), and differences in the amount of residual AChE necessary for survival (see my review of 7/24/96 for more details). (The purpose of this paragraph is to point out the uncertainties in predicting the magnitude of efficacy of P in humans even if it is concluded that the animal studies as a whole predict such efficacy; the existence of such uncertainties presumably does not preclude approval under Subpart I).

3. Dosing considerations

The question of predicting an effective dose in humans based on the animal data is apparently being addressed by OCPB. However, the following comments are offered:

a) Choosing a human dose for a prophylactic treatment is more critical than for an antidotal treatment, e.g. atropine, where the dose can be titrated based on observed effects.

b) Pharmacodynamic effects of a drug which could be useful for predicting an effective human dose have not been established for P. For example, increases in heart rate could be used to indicate an antimuscarinic effect of atropine. It had been hypothesized that RBC AChE inhibition was a relevant effect for P, but the data do not support a relationship between this effect and protection from Soman-induced lethality in animals.

RECOMMENDATIONS:

It is recommended that this NDA not be approved, based on the fact that pyridostigmine was shown to be clearly effective in only 2 out of the 5 species in which it was adequately tested. The sponsor has proposed a plausible hypothesis for the species differences, and a rationale for why pyridostigmine should be effective in humans; however I do not believe the data supporting this are adequate. The types of data I believe are needed to more firmly support this hypothesis are discussed above; briefly this should be (1) well-established data demonstrating the stated species differences in carboxylesterase levels and (2) replication of the study of Maxwell et. al., 1988 (ibid), extended to (a) verify the result in mice, (b) rule out the possibility that the effects of the carboxylesterase inhibitor were due to potentiation of atropine, (c) test monkeys to help rule out the possibility that the effects of the carboxylesterase inhibitor were due to effects other than such inhibition, and (d) if possible, measurement of plasma levels of Soman which could help establish the underlying basis (e.g. a saturable sink) for the results obtained. (Note that this study, in addition to potentially providing evidence that the small effects seen in rodents underestimate the degree of efficacy expected in humans, potentially provides evidence that P has *any* activity relevant to efficacy in these species, considering the equivocal results of the pivotal animal efficacy studies). The sponsor should also address the anomaly of the findings in rabbits, i.e. lack of significant effect of pyridostigmine on the protective ratio, and potentiation of this effect by an inhibitor of carboxylesterase, despite the fact that rabbits are considered to have very low carboxylesterase levels.

Barry N. Rosloff, Ph. D.

Barry N. Rosloff, Ph.D.
1/9/03

NDA 20-414----MEMO TO FILE

NOTE: A previous version of this memo was filed in DFS 1/2/03. It is being updated to reflect the fact that a new genotoxicity assay (CHO/HGPRT *in vitro* mutagenicity assay) was submitted 1/3/03. This study was adequate and negative for pyridostigmine, and is included in the proposed labeling. The present memo also corrects a few minor typographical errors in the previous memo.

PREFACE:

The purpose of this memo is to re-evaluate the animal efficacy data for pyridostigmine for the prophylactic treatment of Soman poisoning in view of the recent establishment of Subpart I (the "animal rule") of the new drug regulations. This NDA had been previously considered under Subpart H by virtue of the sponsor's attempt to establish a surrogate marker for efficacy; it was felt that such a marker had not been established and the NDA was considered not approvable. Under Subpart I the primary issues for approval center around the relevance of the animal data to humans and the ability to predict an efficacious dose for humans. A particular concern which will be discussed is the apparent species differences in the efficacy of pyridostigmine.

Most of the issues to be discussed in the present memo have been discussed in more detail in my previous reviews and memos and in communications with the sponsor; the most comprehensive review is my NDA review of 7/24/96. The reader is referred to these previous reviews for details not repeated in the present memo. References and literature citations given in my previous reviews will generally not be repeated here.

The following abbreviations will be used:

P = pyridostigmine
AChE = acetylcholinesterase
CaE = carboxylesterase
PR = protective ratio (ratio of Soman LD 50 with *vs* without a given treatment)

PROPOSED MECHANISM/RELEVANCE OF THE ANIMAL MODEL:

Subpart I specifies that the mechanism of toxicity of the toxin and its amelioration by the treatment be reasonably well-understood and that the endpoint in animals be clearly related to the desired benefit in humans. Of course such mechanisms are never conclusively known; however it can be said that there is a fair degree of consensus in the scientific community that Soman toxicity is due to its inhibition of AChE at neuromuscular junctions in the nervous system with consequent excessive buildup of synaptic acetylcholine which leads to the various physiologic consequences typical of Soman poisoning.

The mechanism of P in decreasing the lethality of Soman is less clear. The theory is that P reversibly carbamylates a fraction of the same active site on AChE which Soman irreversibly phosphorylates; when the AChE is subsequently decarbamylated there is a small residual amount of AChE activity which is adequate to sustain life (provided atropine/2-PAM are subsequently administered). While it has been shown that P can protect AChE in this way, it is less clear that this is the mechanism by which it improved survival. As reviewed in detail in my previous reviews, there has been virtually no quantitative correlation established between P-induced AChE inhibition (in RBC or whole blood) and prevention of Soman-induced lethality in animals (including a finding, in monkeys, of such prevention at a dose of P which caused no measurable inhibition of RBC AChE). Various possible reasons for such a lack of correlation were discussed in my review of 7/24/96, p. 43-44, including the possibility that the degree of P-induced AChE inhibition in blood does not correlate with that at critical sites in the nervous system, that P-induced AChE inhibition is only the first step in a long chain of events (e.g. protection of AChE, reduction in acetylcholine levels, changes in degree of receptor activation and subsequent desensitization, changes in end organ function) each with its own quantitative relationship to the preceding step in the chain, and that P has several other cholinergic (and non-cholinergic) actions, aside from AChE inhibition, which might account for its efficacy. The sponsor is currently planning and performing studies to determine if an alternative parameter, i.e. the degree of P induced *protection* of AChE (i.e. the amount of residual AChE after exposure to Soman) is better correlated with protection against lethality and loss of neuromuscular function induced by Soman; such a correlation would provide some evidence for the proposed mechanism of action of P.

Subpart I mentions the need for the animal study endpoint to be related to the desired benefit in humans; this is clearly the case here. However, there are some potential caveats to consider in extrapolating the animal studies as performed to the anticipated human situation; for example (1) the routes of Soman administration in animals (various parenteral routes) were different from those expected in humans (dermal/inhalation); such differences are known to cause different patterns of intoxication; (2) in the animal studies Soman was administered at a fixed time after administration of P; in the pivotal monkey studies this time was optimized for showing efficacy, i.e. at or after the Tmax for P-induced AChE inhibition (when, according to theory, such inhibition should be declining and releasing critical AChE sites); it is not clear how well the time interval between P and Soman exposure can be controlled in humans, but it might be predicted that P would not be effective if Soman exposure occurred relatively soon after dosing, i.e. at a time when P-induced AChE inhibition was increasing or not declining significantly; also the animal studies did not consider possible prolonged or re-exposure to Soman.

ANIMAL EFFICACY DATA/SPECIES DIFFERENCES:

The primary endpoint in the animal studies was prevention of Soman-induced lethality, measured by either an increase in the LD 50 of Soman or, in a smaller number of studies, a reduction in the percent of animals dying from a fixed lethal dose of Soman. P had a large effect in rhesus monkeys, increasing the Soman LD 50 by as much as 25 fold compared to the effect of atropine/2-PAM, a smaller but consistent effect in guinea pigs (2-4 fold), but little or no

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effect in several studies in rats, mice, and rabbits. There are many possible reasons for such species differences (noted later). The primary reason posited by the sponsor is based on species differences in the amounts of the enzyme carboxylesterase (CaE); specifically the species in which P did not work (or did not work well) have high amounts of CaE which serves as a "sink" for Soman, thus resulting in relatively high LD 50 values for Soman and creating a situation of too much "noise" to be able to show an effect of P. The sponsor claimed that P caused a similar *absolute increment* in the Soman LD 50 in all species studied, and that this measure was more relevant than fold-increases in the LD 50 for purposes of interspecies comparison. It was further stated that monkeys and humans have little or no CaE and therefore (if the amount of CaE is indeed an important determinant of the efficacy of P) P, as it is in monkeys, should be efficacious in humans (and cause a clinically significant degree of protection as defined by the sponsor as a several fold increase in the Soman LD 50). In support of this argument, the sponsor submitted a paper (Maxwell, et. al., JPET 246:986-991, 1988) purporting to show that an inhibitor of CaE (CBDP) given to rats, rabbits, and guinea pigs allowed (by reducing the "noise") the beneficial effect of P, as indicated by a several fold increase in the Soman LD 50, to become manifest. (A similar result was reported in mice in a separate paper [Maxwell et. al., JPET 264:1085-1089, 1993]; however there were no groups of mice not given CBDP for comparison; the conclusion of a role for CaE was based on historical data showing little or no effect of P in mice not given CBDP).

We have found several problems with the above hypothesis concerning species differences; most of these have been previously communicated to the sponsor (e.g. letter of 6/23/00). They are as follows:

1. The postulated species differences in CaE content have not been well-documented. There are some scattered data in the literature, but no study I am aware of which has compared all of the different species head-to-head. Comparison across studies is difficult because of problems with enzyme nomenclature and the lack of substrate specificity for the various plasma esterases. The sponsor is intending to do a study comparing CaE content in monkeys, rats and guinea pigs using labelled Soman; binding of labelled Soman will apparently be measured in the presence and absence of CBDP to control for non-specific binding. It will be difficult to conclude that the results will accurately reflect the content of the single enzyme "CaE" without knowing the specificity of both Soman and CBDP for this enzyme; in the absence of such knowledge all that can be said is that what is being measured are CBDP- sensitive Soman binding sites. However, this may be a relevant parameter since such sites have been shown to have toxicological relevance by the observation that CBDP reduces the LD 50 of Soman (Maxwell, 1988 ibid.; Maxwell, et.al., Toxicol. Lett. 39:35-42, 1987). (Conversely, it may be difficult to relate CaE activity measured with other substrates to the *in vivo* studies with Soman and CBDP without knowing the relative specificities of the various substrates for the various plasma esterases).

2. As noted above, the sponsor claims that the data show that, as measured by an increase in the absolute value of the Soman LD 50, P actually works equally well in all species tested. The tables on the following page (Tables "1" and "4") were submitted to IND . (7/29/99) in support of this argument. It was stated that Table 1 indicates that the magnitude

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Table 1. Studies Demonstrating a Consistent Increase in Soman LD$_{50}$ in PB-pretreated Animals Receiving an Adequate Dose of Atropine

Species	Soman LD$_{50}$ (µg/kg) sc				Increase in Soman LD$_{50}$ (µg/kg)			Dose of Atropine (mg/kg)	Reference
	Control	At/PAM	At/PB	PB/At/PAM	At/PAM[a]	At/PB[b]	PB/At/PAM[c]		
Rhesus[d]	13.0		176			163		4.0	Dimhuber
Rhesus[e]	15.3	40.4		>617	25.1		>602	0.4	Joiner '88
Rabbit[d]	20		121			101		17.4	Maxwell '88
Guinea Pig[f]	26.6	54.0		190	27.4		163.4	16	Lennox
Guinea Pig[d]	28		146			118		17.4	Maxwell '88
Rat[f]	157	199		374	42		217	16	Lennox
Rat[d]	126		262			136		17.4	Maxwell '88
Mean±S.D.					31.5±9.2	130±27	190±38[f]		

a. (Soman LD$_{50}$ in At/PAM group) – (Soman LD$_{50}$ in Control group).
b. (Soman LD$_{50}$ in At/PB group) – (Soman LD$_{50}$ in Control group).
c. (Soman LD$_{50}$ in PB/At/PAM group) – (Soman LD$_{50}$ in Control group).
d. Studies in which Soman LD$_{50}$ values were only determined in Control groups and At/PB groups.
e. Studies in which Soman LD$_{50}$ values were determined in Control groups, At/PAM groups, and PB/At/PAM groups.
f. Mean does not include the >602 value because it exceeds the Mean by >3 S.D. and, therefore, the >602 value is considered an outlier.

Table 4. Studies Demonstrating Inadequate Protection Against Soman in PB-pretreated Animals Receiving an Inadequate Dose of Atropine

Species	Soman LD$_{50}$ (µg/kg) sc				Increase in Soman LD$_{50}$ (µg/kg)			Dose of Atropine (mg/kg)	Reference
	Control	At/PAM	At/PB	PB/At/PAM	At/PAM[a]	At/PB[b]	PB/At/PAM[c]		
Guinea Pig[d]	25.3			54.6			29.3	8	Jones
Rabbit[e]	5.4		36.5	41.5		21.1	26.1	8	Joiner '89
Rabbit[f]	15.2	28.4		42.0	13.2		26.8	13	Koplovitz '92
Mouse[f]	90.7	159.0		204.4	68.3		113.7	11.2	Koplovitz '84
Mouse[f]	107.4	186.2		238.9	78.8		131.5	11.2	Koplovitz '84

a. (Soman LD$_{50}$ in At/PAM group) – (Soman LD$_{50}$ in Control group).
b. (Soman LD$_{50}$ in At/PB group) – (Soman LD$_{50}$ in Control group).
c. (Soman LD$_{50}$ in PB/At/PAM group) – (Soman LD$_{50}$ in Control group).
d. Study in which Soman LD$_{50}$ values were only determined in Control group and PB/At/PAM group.
e. Study in which Soman LD$_{50}$ values were only determined in Control group, At/PB group, and PB/At/PAM group
f. Studies in which Soman LD$_{50}$ values were determined in Control groups, At/PAM groups, and PB/At/PAM groups.

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of the increases in Soman LD 50s were "quite consistent...across species". Note that only those 3 studies with the superscript "e" studied the contribution of P. (Note that for the monkey study the stated values for the Soman LD 50 [40.4] and increase in Soman LD 50 [25.1] for the atropine/PAM treatment are incorrect and should be 25.1 and 9.8, respectively; the values of 617 and 602 for the entire treatment regimen are correct). The column with the superscript "c" shows the increases in Soman LD 50 caused by the entire treatment regimen (P/atropine/2-PAM) from these 3 studies; to determine the contribution of P one must subtract the increase due to atropine/PAM alone, giving values of ? 592, 136, and 175 in monkey, guinea pig, and rat, respectively. The sponsor excludes the monkey value as an outlier (see footnote "f" to table); therefore the conclusion of consistency across species is based on only 2 species. It is also noted that the rat study (superscript "e"), in contrast to what is indicated in the table, did not use PAM, and used mecamylamine along with the atropine, and therefore comparing this study with the others may be questioned.

Table 4 contains studies said to show inadequate protection by P in studies which supposedly used an inadequate dose of atropine. The definition of an adequate dose of atropine was apparently 16 mg/kg. The basis for this definition is unclear; it seems post-hoc and somewhat circular. At any rate, the effects of P in these studies seem small or nonexistent. (Note that in the first 2 studies in the table the effect of P cannot be isolated. It is not known if the effects in the mouse studies were statistically significant; the reference cited was not in the reference list). Other negative studies were not included in this table. Rat study "E-1" (numbering system used in my review of 7/24/96) showed P increased the Soman LD 50 by only 10 ug/kg (not statistically significant); the dose of atropine was 16 mg/kg thus meeting the sponsor's (post hoc) criterion for an adequate dose. Rabbit studies "C-1" and "C-2" showed increases of 1.5 and 10 ug/kg respectively; the former was not statistically significant, and in the latter the LD 50 of Soman alone was based on historical data and therefore, although the report concluded statistical significance, this conclusion in questionable. (Atropine doses in these studies were 2 and 5 mg/kg, respectively). Mouse study "D-1", also not listed in Table 4, showed a larger effect of P (163 ug/kg); it is not clear if this was statistically significant and at any rate the LD 50 for Soman alone was based on historical data. (Dose of atropine was 11.2 mg/kg).

From the above it may be concluded that P does not produce a similar degree of increase in the Soman LD 50 across species. However, I do not believe that this is necessarily at odds with the "CaE theory" of species differences in the efficacy of P, considering the many other potential factors which could affect the efficacy of P and could differ across species (see later). More troubling are the studies which showed *no* significant effect of P, although it could be argued that because of the high level of noise it is difficult to show such an effect, and that the weight of evidence indicates some effect of P in all species.

3. There are several problems in interpreting the study showing that an inhibitor of CaE, CBDP, potentiated the effects of P (plus atropine), as indicated by several fold increases in the Soman LD 50, in various species (Maxwell, et. al., 1988, ibid.). The experimental design did not distinguish the effects of P from those of atropine. Thus one cannot be certain if the effect of the CBDP was to potentiate P, atropine, or both. Potentiation of the effect of atropine (against Soman) by CBDP has been reported in the literature (Boskovic, Arch.

Toxicol. 42: 207-216, 1979), although the dose of CBDP (50 mg/kg s.c.) was much higher than that used by Maxwell et. al. (1988, ibid.) (2 mg/kg s.c.). On the other hand, in a paper by Maxwell and Brecht (Neuroscience and Biobehavioral Reviews 15:135-139, 1991) the effect of atropine did not appear to be potentiated by CBDP (2 mg/kg s.c.) in mice and guinea pigs, although the timing of dosing with atropine (30-60 sec. *after* Soman) was different than in the study of Maxwell et. al. (1988, ibid.), where the atropine was given 15-30 min. *before* Soman. The doses of atropine were also somewhat different between these studies. (The Maxwell and Brecht paper did show that CBDP potentiated the effect [as measured by fold increases in the Soman LD 50] of PAM in mice [but not guinea pigs] and potentiated the effect of another oxime, HI-6, in both species). In view of these uncertainties, and in view of the pivotal nature of the CBDP study in explaining species differences in the efficacy of P, it would seem prudent to more conclusively show an effect of CBDP on P independent from any effect on atropine, preferably in a study of P which has an atropine-only group.

Interpretation of the results of this study as support for the "CaE theory" assumes that CBDP has no important actions aside from CaE inhibition which might explain the results obtained. One obvious possibility is inhibition of AChE; this was examined in the Maxwell et. al. (1988, ibid) study and found not to occur (although AChE inhibition was reported at a higher dose of CBDP by Boskovic [ibid]; curiously, the latter paper found that CBDP did not inhibit CaE activity). Another possibility is inhibition of the elimination of P by CBDP, although I am not aware of any data on this point.

If CBDP were potentiating P only by inhibiting CaE, it would be predicted that such potentiation should be minimal or absent in species with little or no baseline CaE activity. In the Maxwell et. al. (1988, ibid) study, CBDP potentiated the effects of the P/atropine combination in all 3 species tested: rats (protective ratio increased 4 fold), guinea pigs (2 fold), and rabbits (2 fold). Although as noted earlier it is not clear how well-established the species differences in CaE are, rats are generally considered to have higher levels than the other 2 species, which fits with the greater effect of CBDP in that species; on the other hand, the effect in rats was not that much greater than in the other species; this is especially noteworthy for rabbits where CaE levels have been reported to be very low. A more convincing source of evidence to rule out effects of CBDP aside from inhibition of CaE would be to show that CBDP does not potentiate the efficacy of P in monkeys, a species with putatively little or no CaE.

4. Inasmuch as Subpart I addresses the importance of understanding the mechanisms of toxicity and protection, it would seem important to also understand the mechanism for why a high level of CaE should mask the efficacy of P. If CaE merely serves as a sink which removes a constant fraction of Soman from the blood regardless of dose, it is not clear why, if P "really" works as well in species with high CaE as it does in species with little or no CaE, it wouldn't be able to raise the Soman LD 50 by a similar multiple. For example, if in a species without a CaE sink a Soman dose of X resulted in plasma Soman levels of Y which caused a given degree of lethality, and P were capable of increasing these lethal doses and plasma levels by 10 fold to 10X and 10Y, it is not clear, in a species which had a CaE sink which removed (for example) 50 % of Soman from blood regardless of dose, thus

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raising the Soman dose needed to cause the same plasma level (Y) and degree of lethality as in the previous species by 2 fold to 2X, why P, if it had the same ability to interfere with Soman at the molecular level as in the previous species, wouldn't be able to raise this Soman dose to 20X (which would give a lethal plasma level of 10Y, the same as in the previous species given P), i.e. cause the same fold increase of 10 in the Soman LD 50. Another way of looking at this is that due to the CaE sink which removes 50 % of Soman from blood, a given dose of Soman in a species with such a sink is only half as toxicologically significant as the same dose in a species without such a sink, and therefore any given incremental (as opposed to fold) increase in this dose is only half as toxicologically significant in the former as compared to the latter species.

However, if the CaE sink were relatively large (particularly in relation to the amount of Soman free to interact with AChE) and saturable, it is possible that P would appear to have only a small effect (as measured by fold increases in the Soman LD 50) in a species with such a sink (e.g. rat) even though it "really" has the same effect as in a species without such a sink (e.g. monkey). In such a case, an absolute (as opposed to a fold) increase in the Soman LD 50 would be a meaningful indication of the efficacy of P. A hypothetical example of such a situation is as follows: Assume, for the sake of simplicity, that monkeys have no CaE and that Soman levels are proportional to dose. Say the Soman LD 50 in monkeys is 20 ug/kg, and that P increases this 20 fold to 400 ug/kg. Now say that in rat there is CaE in plasma which sequesters Soman, and that this is a saturable process. Again for the sake of simplicity, say that in the rat all Soman which enters the blood after doses of up to 400 ug/kg is sequestered by CaE, and that at this dose this process becomes saturated such that anything above this dose results in dose-proportional plasma levels (as in the monkey). (In reality, at least some of the Soman entering blood from the initial 400 ug/kg will also be eliminated from blood by other mechanisms, including interacting with AChE). Assuming that at doses above 400 ug/kg in rat any increment in blood Soman level due to increment in dose is the same as that in monkeys, and that rat and monkey are equally sensitive to the lethal effects of a given Soman blood level, the LD 50 of Soman in rats will be 420 ug/kg (i.e. the 400 ug/kg that gave no blood levels + the 20 ug/kg that gave blood levels the same as those at the LD 50 of 20 ug/kg in monkeys). Now if P has the same degree of efficacy against lethal blood levels in both species, it should raise the LD 50 in rats to 800 ug/kg (i.e. the P does nothing to the first 400 ug/kg [which did not result in any blood levels] but, as in monkeys, raises the amount of the next 20 ug/kg, which caused 50 % lethality, by 20 fold, to 400 ug/kg; 400 + 400 = 800 ug/kg). Thus, in this example, P causes equal absolute increases (380 ug/kg) in the Soman LD 50 in monkeys (400-20) and rats (800-420), but causes a much larger fold increase in monkeys (400/20 =20) than in rats (800/420 =1.9).

Support for the above model could come from data showing increases in Soman plasma levels greater than proportional to dose in species in which P doesn't produce significant fold increases in the Soman LD 50; deviations from proportionality should be less or absent in the other species and in species given CBDP to inhibit CaE. I am aware of one paper (Benschop and De Jong, Neuroscience and Biobehavioral Rev 15: 73-77, 1991) which looked at this in 3 species, comparing Soman AUC values at various doses; the text stated that these were linear in marmosets and guinea pigs but not in rats, although the data as

presented (one would have liked to have an idea of the inter-animal variability) do not seem to me to show strong deviations from linearity in any case (see Table below). (Note that any plasma level data should take into account the fact that there are 4 stereoisomers of Soman; according to the above paper these have different pharmacokinetics and toxicities).

— TABLE 1

MEAN* TOXICOKINETIC DATA OF C(−)P(−)-SOMAN IN RATS,
GUINEA PIGS AND MARMOSETS

	Rat			Guinea Pig		Marmoset	
Dose of C(±)P(±)-soman (x LD$_{50}$)	6	3	1	6	2	6	2
Dose of C(−)P(−)-soman (μg/kg)	111	55.5	18.5	37	12.3	13.5	4.5
Area under curve (ng·min·ml^{-1})	877	320	76	516	228	191	84
Terminal half life (min)	40	16	5.8	15	16.5	15	9
Acutely toxic levels of C(±)P(−)-soman until (min)	317	95	37	126	104	74	49

*N = 6

It should be noted that the above model does not explain those studies in rodents/rabbits in which *no* effect of P was seen, either on the absolute or relative value of the Soman LD 50.

5. Rabbits are considered to have very low CaE, but do not respond robustly to P. Also, in the paper of Maxwell et. al. (ibid.), CBDP reduced the LD 50 of Soman, and potentiated the effects of P, to a similar extent as that reported in guinea pigs, which have been reported to have substantially higher CaE levels.

DOSING CONSIDERATIONS:

(The following is presented for informational purposes; the question of predicting an effective dose for humans is apparently being addressed by OCPB).

The following are the doses used in the pivotal animal studies; the study numbering system is that used in my review of 7/24/96. Keep in mind that the different studies used various routes of administration and varying study parameters such as the time between dosing with P and administration of Soman. Also note that where efficacy was seen it varied in degree between species (and between studies within a species); in particular, where efficacy was seen in rabbit,

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mouse or rat studies the protective ratios (ratio of Soman LD 50 with vs without P pre-treatment) were generally 2 or less.

1) Monkey

A-1: Low dose: 1.2 mg/kg p.o. q 8 hr. (6 total doses)
High dose: 1.2 mg/kg p.o. followed 8 hr. later by 1.8 mg/kg followed 8 hr. later by four doses of 2.4 mg/kg given 8 hr. apart

Both doses had equal efficacy.

A-2:

Phase III : 4, 8.4, or 24 ug/kg i.m.; all had equal efficacy

Phase IV : 40 ug/kg intragastric – effective

Phase V : 4 ug/kg i.m. – effective

2) Guinea pig

B-1 : 0.94 mg/kg p.o. - effective

B-2 : Doses not stated (Doses were based on predicted degree of AChE inhibition) – Only the dose predicted to cause 70 % inhibition was effective; the next lowest dose caused 45% inhibition

B-3 : Dose range 0.06-15 mg/kg p.o. Efficacy seen at 0.23 mg/kg and above but not dose-related; next lowest dose was 0.12 mg/kg

B-4 : 0.32 u mol/kg i.m. – effective

3) Rabbit

C-1 : 0.08 mg/kg i.m. – not effective

C-2 : 0.32 mg/kg i.m. – P increased the Soman LD 50 by 10 ug/kg (but Soman-alone value based on historical data) and by 1.4 fold; not clear if statistically significant

C-3 : 0.08 mg/kg i.m. – P increased Soman LD 50 by 14 ug/kg and by 1.5 fold; not clear if statistically significant

4) Mouse

D-1 : 0.13 mg/kg i.m. – P increased Soman LD 50 by 163 ug/kg (but Soman-alone value based on historical data) and by 2.5 fold; not clear if statistically significant

D-3 : 0.20 or 0.82 mg/kg p.o. – reduced lethality of Soman doses up to those stated to be 2x the LD 50; the two doses showed equal efficacy.

Other: Two mouse studies were listed in Table 4 of the submission of 7/29/99 to IND ⟶ (see above); these studies were not included in the pivotal studies described in the NDA but appeared to have evaluated the effect of P; according to the table the increases in Soman LD 50 were 45 and 53 ug/kg; the PRs were 1.3 in both studies. (Doses of P were not stated).

5) Rat

E-1 : 0.13 mg/kg i.m. – not effective

E-2 : Doses not stated (Doses were based on predicted degree of AChE inhibition) – Only the dose predicted to cause 70 % inhibition was effective (Soman LD 50 increased by 175 ug/kg and by 1.9 fold); the next lowest dose caused 45 % inhibition. (Note that in this study, mecamylamine was given with the atropine, and 2-PAM was not used).

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DISCUSSION AND CONCLUSIONS:

1. Proposed mechanism/Relevance of the animal model

Although mechanisms of drug action are never known with certainty, and hypotheses change over time, there is currently fairly wide consensus that Soman toxicity is caused by excessive inhibition of AChE and its sequelae. It is less clear how P causes protection from Soman –induced lethality, although it is reasonable to assume that this mechanism would be similar across species in which P has been shown to be effective.

Some differences in the animal efficacy studies of P and the expected human exposure to Soman were discussed earlier. Some animal studies were designed to optimize the effect of P (e.g. by giving the Soman at or after the Tmax for P-induced AChE inhibition). However, it may be noted that clinical trials of drugs are often designed to optimize drug effects, and the conditions of the trials often do not strictly mimic real-life conditions; it is assumed that efficacy seen in such trials will be seen in at least part of the population under at least some conditions. (However, since, according to the proposed mechanism of action of P it would not be expected to work at a time when P-induced AChE inhibition is increasing, it would be hoped that users of P would be made aware of this).

2. Species differences in the efficacy of P

The sponsor has estimated that a several fold increase in the "protective ratio" (PR) (ratio of Soman LD 50 in the presence of pretreatment with P [with supporting atropine/PAM] to the Soman LD 50 in the absence of such pretreatment) in humans is necessary for P to be considered effective under anticipated levels of exposure to Soman. Increases in the PR of several fold or more were seen in monkeys and guinea pigs; however in several studies in rats, mice, and rabbits there was either no effect of P or a very slight effect (PRs of 2 or less).

An argument has been made that, at least in the case of rats and mice, even though P doesn't increase the PR to a meaningful degree, P does have intrinsic activity, as measured by an *absolute* increase in the Soman LD 50 in these species, and in fact has the same degree of intrinsic activity in all species tested. The lack of significant effect on the PR in rats and mice is due to the presence of high levels of the enzyme CaE, which binds Soman and thus serves as a sink which reduces the effective blood levels of Soman in these species; the consequent high Soman LD 50 values creates a situation where there is too much "noise" to show an effect of P on the PR. Experimental support for the proposed role of CaE comes from a study in which rats were given an inhibitor of CaE (CBDP) to reduce the "noise", and it was found that P, in addition to increasing the absolute Soman LD 50, also increased the PR. (A similar study was also done in mice but there was no concurrent control group not given the CaE inhibitor). It is further argued that humans, as do monkeys, have low CaE, and therefore P should cause a substantial increase in the PR (in addition to an increase in the absolute Soman LD 50) in humans (as it does in monkeys). Another way of stating this argument is that P has the same degree of intrinsic activity across species, and although this is not adequate to provide significant additional protection to that provided by high levels of

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endogenous CaE (i.e. in rats and mice), the same degree of activity (i.e. same increase in the absolute Soman LD 50) would be so adequate when added to the minimal protection provided by CaE in species with low endogenous CaE (i.e. monkeys and humans).

The above argument is plausible, however there are several significant uncertainties which should be addressed:

a) As noted earlier, the magnitude of the absolute increases in the Soman LD 50 was clearly not the same across species; more significantly some studies showed *no* effect of P. (On the other hand, as noted earlier, species differences in magnitude should not necessarily negate the sponsor's CaE theory, and the negative studies might be due to the inability to show a relatively small effect in the presence of noise).

b) As discussed earlier, it is not clear if increases in the absolute value of the Soman LD 50 necessarily indicate that P has significant activity relevant to efficacy in rodents. However, a scenario was presented where such a conclusion could be reached, i.e. if the CaE sink were relatively large and saturable. This is presumably testable, and perhaps there are relevant data already available (although the paper cited earlier did not find significant deviations from linearity in the Soman dose-plasma level curve). I feel that there should be some corroboration and better understanding of the proposed mechanism for the lack of effect of P on the PR in rodents to help rule out the conclusion that P has no intrinsic efficacy in these species.

c) Problems with interpreting the study in which rodents given an inhibitor of CaE showed an enhanced response to P (as measured by increase in PR) were discussed earlier. They primarily involve the possibility that the inhibitor has other actions, aside from "noise reduction", which could explain the enhanced effects of P on the PR (and provide an alternative to the conclusion that P has intrinsic activity relevant to efficacy in these species). (Note that it is possible that differences in CaE levels have a role in explaining species differences in the effect of P independent of any effect on "noise". If we believe that P works preferentially in species with little or no CaE, and an inhibitor of CaE makes species with high CaE responsive to P, this would seem to predict that humans, if they have little or no CaE, will respond [all other things being equal-but see later]. However, this would be based purely on empirical observation with no [as yet] underlying rationale for why this should occur. Also, the empirical results are subject to some of the same problems noted above, e.g. possibility of effects of the inhibitor aside from those on CaE).

Also, since this study is important for explaining species differences, I feel that it should be replicated and extended. Replication is an essential part of the scientific enterprise; a role for replication was also noted in Agency responses to comments made regarding Subpart I, i.e. "...the animal studies should be replicated or substantiated in each species to ensure credible results" (response to Comment #5, FR 67, No. 105, p. 37991, 5/31/02); the 5/98 guidance for evidence of effectiveness in clinical trials (the "Evidence Document") also discusses potential problems with reliance on a single site, particularly regarding literature reports. (On the other hand, it could be argued that there is

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corroborating evidence for the sponsor's hypothesis outside of this study, although I feel this evidence to be weak. Also, there appears to be some internal inconsistency in this study, i.e. the CaE inhibitor also enhanced the effect of P in rabbits, a species which is said to have very low levels of CaE). In addition to replication per se, the study could be extended to rule out an effect of the inhibitor on atropine (see earlier) or on plasma levels of P, verify the effect in mice (the previous study did not have a proper concurrent control group for this species), and use monkeys (in which, since this species is said to have little or no CaE, the effect of P should not be potentiated; a lack of effect could help rule out other actions of the inhibitor which could have explained the results in the other species). In addition, plasma levels of Soman might be measured to obtain evidence for a saturable sink; it might be expected that in species in which P does not cause a significant increase in the PR, Soman levels increase greater than in proportion to dose, but do not (or do to a lesser degree) in species where P does increase the PR and in species whose CaE has been inhibited.

d) Rabbits are said to have very low levels of CaE, yet they do not respond robustly to P (as measured by increase in PR) and, as noted above, an inhibitor of CaE increased the effect of P in this species.

e) As noted earlier, the postulated species differences in CaE levels have not been well-documented; a study in rats, guinea pigs, and monkeys is being performed by the sponsor. It should be kept in mind that there are a large variety of esterases and carboxylesterases, with overlapping substrate and inhibitor specificities, and thus an assay using one substrate may not necessarily be measuring the same thing as an assay using a different substrate. Also, any given substrate may not be assaying all enzyme activity involved in the elimination of Soman. (The sponsor's study will assay Soman binding to CBDP-sensitive sites).

f) Even if it can be concluded that P has intrinsic activity relevant to efficacy in rodents and rabbits, the magnitude of the effect seen, whether expressed as increases in the PR or in the absolute LD 50 of Soman, was widely different across all species tested. This is not necessarily surprising, considering the many potential sources of species variation (aside from CaE levels), such as differences in the PK of P and Soman, in the rate of decarbamylation of AChE after inhibition by P, in the blood level of Soman needed to produce lethality, possible quantitative pharmacodynamic differences in the various steps along the proposed pathway of protection (e.g. quantitative differences in the blood level of P needed to produce a given degree of AChE inhibition, in the degree of P-induced AChE inhibition needed to protect a given proportion of AChE from Soman, in the degree of protected AChE activity needed to metabolize a given amount of acetylcholine, etc. etc.), and differences in the amount of residual AChE necessary for survival (see my review of 7/24/96 for more details). (The purpose of this paragraph is to point out the uncertainties in predicting the magnitude of efficacy of P in humans even if it is concluded that the animal studies as a whole predict such efficacy; the existence of such uncertainties presumably does not preclude approval under Subpart I).

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3. Dosing considerations

The question of predicting an effective dose in humans based on the animal data is apparently being addressed by OCPB. However, the following comments are offered:

a) Choosing a human dose for a prophylactic treatment is more critical than for an antidotal treatment, e.g. atropine, where the dose can be titrated based on observed effects.

b) Pharmacodynamic effects of a drug which could be useful for predicting an effective human dose have not been established for P. For example, increases in heart rate could be used to indicate an antimuscarinic effect of atropine. It had been hypothesized that RBC AChE inhibition was a relevant effect for P, but the data do not support a relationship between this effect and protection from Soman-induced lethality in animals.

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RECOMMENDATIONS:

It is recommended that this NDA not be approved, based on the fact that pyridostigmine was shown to be clearly effective in only 2 out of the 5 species in which it was adequately tested. The sponsor has proposed a plausible hypothesis for the species differences, and a rationale for why pyridostigmine should be effective in humans; however I do not believe the data supporting this are adequate. The types of data I believe are needed to more firmly support this hypothesis are discussed above; briefly this should be (1) well-established data demonstrating the stated species differences in carboxylesterase levels and (2) replication of the study of Maxwell et. al., 1988 (ibid), extended to (a) verify the result in mice, (b) rule out the possibility that the effects of the carboxylesterase inhibitor were due to potentiation of atropine, (c) test monkeys to help rule out the possibility that the effects of the carboxylesterase inhibitor were due to effects other than such inhibition, and (d) if possible, measurement of plasma levels of Soman which could help establish the underlying basis (e.g. a saturable sink) for the results obtained. (Note that this study, in addition to potentially providing evidence that the small effects seen in rodents underestimate the degree of efficacy expected in humans, potentially provides evidence that P has *any* activity relevant to efficacy in these species, considering the equivocal results of the pivotal animal efficacy studies). The sponsor should also address the anomaly of the findings in rabbits, i.e. lack of significant effect of pyridostigmine on the protective ratio, and potentiation of this effect by an inhibitor of carboxylesterase, despite the fact that rabbits are considered to have very low carboxylesterase levels.

Barry N. Rosloff, Ph. D.

MEMORANDUM

To: File, NDA 20-414

Through: Robert Temple, M.D., ODE I Office Director
         Russell Katz, M.D., Division Director, Neuropharmacologic Drug Products
         Barry Rosloff, Ph.D., Pharmacology Supervisor, HFD-120
         Robbin Nighswander, Project Manager, HFD-120

From: Jeri El-Hage, Ph.D., ODE I Associate Director for Pharmacology/Toxicology

Subject: NDA 20-414 , Pyridostigmine Bromide, 30 mg tablets
         Tertiary Review of Pharmacology/Toxicology Data

Date: January 20, 2003

Pyridostigmine bromide(PB) , a reversible cholinesterase inhibitor, was initially approved in the late 1950's for the treatment of myasthenia gravis. In this NDA 20-414, it is indicated for prophylaxis against the lethal effects of soman nerve gas poisoning in combination with the administration of atropine and pralidoxime therapy at the first sign of nerve agent poisoning.

The toxicological evaluation conducted for this NDA includes 3 and 6-month oral toxicity studies in rats, a 3-month oral toxicity study in dogs, a complete genotoxicity battery, and a complete reproductive toxicity battery. Pyridostigmine tested negative for genotoxic potential in the Ames assay, chromosomal aberrations assay in Chinese hamster ovary (CHO) cells, CHO/HGPRT in vitro mutagenicity assay, in vivo mouse micronucleus assay, and the mouse lymphoma thymidine kinase assay without metabolic activation(see Pharmacology review of 10/28/96). Pyridostigmine tested positive in the mouse lymphoma assay at doses $\geq$ 3000 mcg/ml in the presence of metabolic activation. Based on a weight of evidence approach, this reviewer concludes there is no significant genotoxic potential associated with pyridostigmine bromide.

There were no drug-related effects on fertility in male or female rats despite the evaluation of high doses producing clinical signs and a low incidence of mortality. No drug-related teratogenic effects were observed in rats. In the rabbit embryofetal toxicity study, increased visceral malformations including hydronephrosis at the high dose of 45 mg/kg/day and variations in the carotid and inominate arteries at 15 and 45 mg/kg/day were observed. It is difficult to determine if the observed developmental effects in rabbits are compound-related or secondary to maternal toxicity since the maternal toxicity findings were not well documented in the review (Dr. Sparenborg's Pharmacology review of 9/18/1995) .

The results of the general toxicity, genotoxicity, and reproductive toxicity studies for PB alone demonstrate that pyridostigmine bromide should be reasonably safe when administered subchronically at the proposed dose of 30 mg, bid. The only concerning non-clinical data are from a publication by Abou-Donia et al. (J. Toxicology and Environ. Health, 48:35, 1996) which described synergistic neurotoxicity associated with PB plus DEET and/or permethrin co-administration in hens compared to the effects of these agents administered alone. The authors concluded that while relatively high doses of PB, DEET, and permethrin appear to have minimal toxicity individually , PB significantly increased the neurotoxic effects of the other agents alone or in combination. The reversibility of the neurotoxic effects in hens was not assessed.

Dr Rosloff's more recent reviews of 12/23/02 and 1/9/03 re-evaluate the adequacy of the animal efficacy data to support approval based on Subpart I (i.e., approval of new drugs when human efficacy studies are not ethical or feasible). Dr Rosloff concludes that the sponsor has not met the criteria defined under Subpart I to permit approval of the PB based on animal efficacy data. Based on a review of the information available in Dr Rosloff's reviews and deferring to his expertise in the area of cholinesterase inhibitors, I agree with Dr Rosloff's conclusions. Since this will be the first application approved under Subpart I the standards applied will be precedent setting. This decision is based on the following understanding of the Subpart I guidance (FR 314.610) and Dr. Rosloff's arguments.

The data appear to support 314.610 (a)(1) that the mechanism of soman toxicity is well understood and (a)(3) that the animal study endpoint of improved survival (or increased soman LD 50), is clearly related to the desired benefit in humans. However, the available data do not conclusively support (a)(2) that the effect is demonstrated in more than one species expected to react with a response predictive for humans. Improved survival in animals pretreated with PB followed by atropine/pralidoxime was clearly demonstrated in monkeys and guinea pigs. However, little or no protective effect of PB pretreatment was observed in mice, rats, and rabbits. In addition, the positive effects in monkeys were observed in studies where soman administration was co-incident with the PB-induced Tmax for AChE inhibition, unlikely to mimic the human exposure situation. Since the efficacy could not be clearly correlated with a surrogate endpoint, such as AChE inhibition in RBC or plasma, it is difficult to conclude that "the monkey is the single animal species that represents a sufficiently well-characterized model for predicting the response in humans" as per 314.610 (a)(2). Absence of pharmacodynamic information for a surrogate endpoint which correlates with improved survival in multiple species or in the species demonstrating a robust survival response (i.e., monkey) makes it impossible to select an effective dose in humans as per 314.610 (a)(4).

While I have not seen the action letter or the recommendations for additional preclinical studies, I agree in concept that the sponsor should do additional studies to establish the mechanism(s) mediating PB-improved survival in responsive species and provide pharmacodynamic information for biochemical markers correlated with that mechanism. This will permit a better assessment of the predictive value of the responsive species to human responsiveness and provide a pharmacodynamic marker to enhance human dose selection.

Labeling comments:
The carcinogenicity , mutagenicity and impairment of fertility sections look acceptable as written. Regarding the pregnancy category, I am assuming it was concluded that none of the findings were clearly drug-related since the first sentence in this section reads " no teratogenic effects in rats... and in rabbits..." .

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/s/

Jeri El Hage
1/23/03 11:33:42 AM
PHARMACOLOGIST

Barry N. Rosloff, Ph. D.
2/18/97

NDA
Pharmacologist Review of NDA 20-414
Addendum to Original Summary of 7/24/96

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

DRUG: pyridostigmine

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

INTRODUCTION:

The purpose of the present review is to summarize and evaluate the published paper of
Abou-Donia et. al., "Increased Neurotoxicity Following Concurrent Exposure to
Pyridostigmine Bromide, DEET and Chlorpyrifos" (Fundamental and Applied Toxicology
34 : 201-222,1996). This paper was not performed or submitted by the sponsor of this
NDA, but is reviewed here in view of the current claims of a "Gulf War Syndrome"
experienced by veterans of the Persian Gulf War, and the claimed role of
pyridostigmine as a possible cause or contributor to this syndrome or to other illnesses
seen in these subjects. The published paper is attached to the present review. (A
similar study was previously performed by Abou-Donia et. al., and is reviewed in my
Original Summary of NDA 20-414 of 7/24/96 [p. 26-27 and 52-53]).

SUMMARY AND EVALUATION:

Hens were given pyridostigmine bromide (5 mg/kg p.o.), DEET (500 mg/kg s.c.), or
chlorpyrifos (10 mg/kg s.c.), either as a single drug or in combinations of 2 or 3, 5 days
per week for 2 months. (In the previous study, permethrin was used instead of
chlorpyrifos). Endpoints included clinical signs observed during treatment (with
emphasis on locomotor dysfunction, e.g. motor activity, leg weakness, gait
disturbances, postural changes, etc.), and histological exam of spinal cord and sciatic
nerve after treatment. (Histopathological effects consisted primarily of enlarged axons).

In general (and as was seen in the previous study), treatment with the 2-drug combos
caused more adverse effect than treatment with the single drugs, and treatment with
the 3-drug combo caused more adverse effect than treatment with the 2-drug combos.

For some parameters, adverse effects were seen with the combos which were not seen at all with the single drugs (e.g. locomotor dysfunction, table 2), implying synergistic (as opposed to simply additive) effects. As in the original paper, the authors consider various pharmacodynamic and pharmacokinetic hypotheses to explain the observed interactions, but provide no data in support of these. (One hypothesis is that the basis for the interaction is cholinesterase inhibition, and that some of the compounds studied may increase the distribution of some of the others to this enzyme in brain or periphery. However, the only data obtained which bears on these points, i.e., measurement of brain and plasma cholinesterase [fig.6], did not show a good quantitative correlation between enzyme inhibition and either clinical signs or histopathological changes).

As in their previous paper, the authors speculate that "the use of [pyridostigimine] concurrently with exposure to pesticides and other chemicals in the Gulf War may be related to some of the complaints of the service personnel." However, as also discussed in my previous review, there are several problems involved when attempting to extrapolate these results in hens to the Gulf War veterans:

1.   The complaints of the veterans involve relatively long-lasting, chronic symptoms, occurring and/or lasting well after the time of exposure to the compounds in question. The hen studies, however, did not include an assessment of reversibility of the findings after drug withdrawal. (The authors did bring up the possibility that the drugs tested might produce "OPIDN" [organophosphorus ester-induced delayed neurotoxicity], a syndrome of long-lasting symptoms known to occur in humans and for which the hen is used as a screening model; however, since the drugs and combinations did not inhibit the enzyme "NTE" ["neurotoxicity target esterase," the inhibition of which is considered to be a marker for OPIDN] to a previously determined threshold level, the authors discounted the possibility that the neurologic deficits and histopathologic lesions seen were related to OPIDN)

2.   The validity of extrapolating the hen data to humans depends on the relative exposure (between hens and humans) to the drugs in question. Although the doses of the individual drugs were chosen to be those that "produced minimum changes in the clinical parameters," the combinations produced pronounced toxic signs, weight loss, and deaths. (This also occurred in the earlier study, although my previous review did not emphasize this adequately). Thus extrapolation to humans might not be valid if the veterans were only exposed to doses which did not produce these pronounced acute effects. It is also noted that the dose of pyridostigmine used in hens caused an 83% inhibition of plasma butyrylcholinesterase (when given alone; even greater inhibition seen when given with the other drugs), whereas the doses used in the veterans were intended to produce an inhibition of RBC acetylcholinesterase of only 20-40%. (The authors raised the possibility that some veterans who may be genetically deficient in their ability to metabolize drugs and pesticides might have reached toxic levels of the drugs in question).