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Computer simulation of the effect of pretreatment with reversible acetylcholinesterase inhibitors on the protection against soman poisoning

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MANAGEMENT-UITTREKSEL

Titel	:	Computer simulatie van het effect van voorbehandeling met reversibele acetylcholinesterase-remmers op de bescherming tegen soman-vergiftiging
Auteur(s)	:	J.P. Langenberg en L.P.A. de Jong
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In het rapport wordt een computermodel beschreven waarmee meer inzicht kan worden verkregen in de factoren die het welslagen van de voorbehandeling tegen somanvergiftiging bepalen. Het model is gebaseerd op de algemeen aanvaarde hypothese dat na voorbehandeling binnen een zekere tijd na zenuwgasvergiftiging een zekere acetylcholinesterase activiteit moet worden hersteld om te kunnen overleven. De diverse relevante kinetische processen zijn verwerkt in het model, waarbij de waarden van de diverse parameters afkomstig zijn uit onderzoek in de cavia of hypothetisch zijn. Met het model wordt het verloop van de fractie vrij acetylcholinesterase in de tijd berekend, waarna aan de hand van arbitraire criteria wordt vastgesteld of de voorbehandeling succesvol is.

De belangrijkste parameters volgens het model zijn de mate van acetylcholinesteraseremming door de voorbehandeling op het moment van vergiftiging met C(±)P(±)-soman, de snelheid van spontane reactivering van dit geremde enzym en de toxicokinetiek van C(±)P(-)-soman, met name de snelheid van eliminatie.

Het model voorspelt een optimale waarde voor de snelheid van spontane reactivering van acetylcholinesterase bij 30-40 % remming door het voorbehandelingsmiddel welke 2 tot 4 maal lager is dan de snelheid die voor pyridostigmine is gemeten, en vrijwel gelijk is aan de gemeten snelheid voor ethyl p-nitrophenyl fosforamidaat. Laatstgenoemde verbinding bleek in eerder onderzoek de cavia aanzienlijk beter te beschermen tegen C(±)P(±)-soman dan pyridostigmine. Adequate bescherming van de cavia tegen C(±)P(±)-soman door pyridostigmine kan volgens het model alleen worden verkregen bij meer dan 50 % acetylcholinesteraseremming, hetgeen in overeenstemming is met literatuurgegevens.

Tevens voorspelt het model dat het gebruik van een scavenger om de eliminatie van C(±)P(-)-soman te versnellen zinvol kan zijn mits dit therapeutisch geschiedt in plaats van profylactisch.

Tot slot worden in het rapport enkele aanbevelingen voor verdere verfijning en uitbreiding van het model gedaan.

Summary

A computerized model has been developed in order to obtain more insight into the factors that determine the efficacy of pretreatment against soman poisoning. The various kinetic processes involved are simulated, i.e., the inhibition of acetylcholinesterase (AChE) by the pretreatment compound, the spontaneous reactivation of this inhibited enzyme, the pharmacokinetics of the pretreatment compound, the inhibition of AChE by C(±)P(-)-soman and the toxicokinetics of C(±)P(-)-soman. The values of the various parameters are either obtained from studies in the guinea pig or hypothetical.

With this model the time course of the fraction (1, 2 or 5 %) of active AChE is calculated subsequent to intoxication with doses corresponding with 2 or 6 LD50 of C(±)P(±)-soman.

The model indicates that the most important parameters are (i) the extent of AChE inhibition by the pretreatment compound at the moment of C(±)P(±)-soman poisoning, (ii) the rate constant for spontaneous reactivation of this inhibited enzyme and (iii) the toxicokinetics of C(±)P(-)-soman, especially the rate constant of elimination.

The pharmacokinetics and the inhibition rate constant of the pretreatment compound hardly influence the restoration of AChE activity.

The consequences of the modelling results for (pre)treatment of soman intoxication are discussed.

Samenvatting

Om meer inzicht te verkrijgen in de factoren die het welslagen van de voorbehandeling tegen somanvergiftiging bepalen, is een computermodel ontwikkeld waarin de diverse relevante kinetische processen zijn verwerkt, namelijk de remming van acetylcholinesterase (AChE) door het voorbehandelingsmiddel, de spontane reactivering van dit geremde enzym, de farmacokinetiek van het voorbehandelingsmiddel, de remming van AChE door C(±)P(-)-soman en de toxicokinetiek van C(±)P(-)-soman. De waarden van de diverse parameters zijn ofwel afkomstig uit onderzoek in de cavia of hypothetisch.

Met het model wordt het verloop van de fractie vrij AChE in de tijd berekend. Op basis hiervan wordt berekend na hoeveel tijd 1, 2 of 5 % AChE activiteit is hersteld na een vergiftiging met een dosis C(±)P(±)-soman die overeenkomt met 2 of 6 LD50.

Uit het model blijkt dat de farmacokinetiek en remmingssnelheidsconstante van het voorbehandelingsmiddel nauwelijks invloed hebben op het herstel van de AChE activiteit. De belangrijkste parameters zijn de mate van AChE remming door de voorbehandeling op het moment van vergiftiging met C(±)P(±)-soman, de snelheid van spontane reactivering van dit geremde enzym en de toxicokinetiek van C(±)P(-)-soman, met name de snelheid van eliminatie.

De consequenties van de resultaten van het model voor de (voor)behandeling van somanvergiftiging worden besproken.

CONTENTS

1 INTRODUCTION

Pretreatment of laboratory animals with the reversible acetylcholinesterase inhibitor pyridostigmine, combined with a therapy consisting of atropine and an oxime, is to a certain extent effective against lethality caused by soman poisoning (Gordon et al. 1978). A problem associated with this pretreatment is the inadequate protection against convulsions caused by the nerve agent leading to brain damage, which can be reduced to some extent by administration of diazepam (Hayward et al. 1988). To overcome the problem of brain damage and incapacitation and to achieve protection against higher doses of C(±)P(±)-soman, a more suitable compound for pretreatment is needed. The development of such a compound requires more insight into the mechanism of the pretreatment process.

Various parameters may influence the efficacy of the pretreatment. The parameters of major importance are presumed to be (i) the toxicokinetics of C(±)P(-)-soman, (ii) the extent of acetylcholinesterase (AChE) inhibition by the pretreatment compound at the moment of C(±)P(±)-soman intoxication, and (iii) the rate of spontaneous reactivation of the enzyme inhibited by the pretreatment compound.

From toxicokinetic studies in various species (Benschop and Jong, de, 1990, 1991) it is known that toxicologically relevant concentrations are present for several hours after intoxication with 2 and 6 LD50 of the agent: there is no hit-and-run kinetics as had been assumed before, which may have important consequences for the qualitative explanation of the antidotal activity of pyridostigmine.

Lennox and co-workers (1985) have shown that the protection against C(±)P(±)-soman increases considerably with increasing percentage of AChE inhibition induced by the pretreatment compound at the moment of nerve agent poisoning.

Up to now the impact of the rate constant of reactivation of AChE, inhibited by the pretreatment compound, has not been studied systematically. However, it can be anticipated that this parameter is important: a very rapid reactivation will regenerate free AChE while the nerve agent is still circulating, thus annulling the profitable effect of the pretreatment. On the other hand, a very slow reactivation will not regenerate sufficient AChE activity in order to maintain life.

A study with a series of p-nitrophenyl phosphoramidates as pretreatment compounds, differing in the rate constant of spontaneous reactivation of the derived inhibited AChEs, has shown that an optimum rate constant may exist (Langenberg et al. 1990, 1993).

In order to obtain more insight into the impact of the three aforementioned parameters on the efficacy of the pretreatment, a large number of compounds and conditions should be tested in animals. As an alternative, a computerized simulation can be performed. This is of course a simplification of the process, but allows variation of a large number of parameters over a wide range in a

relatively short period of time. We describe the development and application of such a simulation program.

2 METHODS

2.1 The model

A certain level of AChE activity is essential for survival. This minimum activity is generally estimated to be 0.1-10 % of the value in healthy individuals (Green 1958). The model is used to calculate the time course of AChE activity after pretreatment and subsequent C(±)P(±)-soman intoxication and, therefore, to analyse the kinetics of these processes. The pharmacokinetics of the pretreatment compound, the rate of inhibition of AChE by the pretreatment compound, its spontaneous reactivation, the toxicokinetics of C(±)P(-)-soman and the rate of inhibition of AChE by the nerve agent have to be combined into a mathematical model.

Similar kinetic analyses have been performed by Green for organophosphate poisoning and its treatment with atropine and oxime (1958) and for carbamate pretreatment against organophosphate poisoning (1983). An obvious drawback in these models was the assumption that the nerve agent is eliminated very rapidly from the body. However, toxicokinetic studies have shown that toxicologically relevant levels of C(±)P(-)-soman persist for circa 5, 2 and 1 h in the rat, guinea pig and marmoset, respectively (Benschop and Jong, de, 1991) after an intravenous dose corresponding with 6 LD50 of C(±)P(±)-soman, and for circa 4 h after subcutaneous administration of 6 LD50 of the agent to the guinea pig (Jong, de, et al. 1991).

Since we perform our *in vivo* pretreatment experiments in guinea pigs, toxicokinetic and other data obtained for this species will be used in our model.

In this model, the guinea pig is considered to be one homogeneous compartment. The kinetic analysis of Green (1958) has shown that semiquantitative deductions are not hampered by this simplified description of the distribution of the enzyme and the organophosphate. In addition, our toxicokinetic studies of the toxic C(±)P(-)-soman stereoisomers, suggest that the decrease in the concentration of these isomers after administration, is the result of a very rapid distribution, and binding to binding sites in a more or less homogeneous compartment (Benschop and Jong, de, 1990, 1991). In other words: the concentration-time course pertains to the time course of binding in an averaged compartment.

The kinetic processes of interest can be expressed in the following equations:

$$E + C_{pt} \xrightarrow{k_1} EC \xrightarrow{k_2} E + C_{pt}' \quad (1)$$

$$E + P_t \xrightarrow{k_3} EP \quad (2)$$

$$E_{tot} = E + EC + EP \quad (3)$$

in which

$$C_{pt} = C_{pt0}.\exp(-\cap^*t) \quad (4)$$

and

$$P_t = A^*\exp(-\alpha^*t) + B^*\exp(-\beta^*t) + C^*\exp(-\gamma^*t) \quad (5)$$

In equations 1-3:

E = concentration of active AChE;

EC = concentration of AChE inhibited by the pretreatment compound;

EP = concentration of AChE inhibited by C(±)P(-)-soman;

C_{pt} = concentration of the pretreatment compound at time t;

C_{pt0} = concentration of the pretreatment compound at time 0;

C_{pt}' = concentration of the residue of the pretreatment compound formed after spontaneous reactivation of EC;

P_t = concentration of C(±)P(-)-soman at time t;

k_1 = rate constant of inhibition of AChE by the pretreatment compound;

k_2 = rate constant of spontaneous reactivation of AChE inhibited by the pretreatment compound;

k_3 = rate constant of inhibition of AChE by C(±)P(-)-soman.

Equation (4) describes the intravenous pharmacokinetics of the pretreatment compound, in which $\cap$ is the elimination rate constant. According to this equation, the pretreatment compound is administered at the same time as C(±)P(±)-soman. In reality the pretreatment compound is of course administered some time before nerve agent intoxication. Since the results are exactly the same for both situations, this time span is not introduced into the model.

Equation (5) describes the time-course of the C(±)P(-)-soman concentration in the homogeneous compartment. This equation is taken from intravenous toxicokinetic studies of C(±)P(±)-soman in the guinea pig: α, β and γ represent the hybrid rate constants for distribution and elimination of C(±)P(-)-soman in the guinea pig.

Since the actual concentrations of AChE in the compartment are not exactly known, the fraction of active enzyme, E/E_{tot}, is used in the model. Equation (3) is transformed into:

$$E/E_{tot} = 1 - EC/E_{tot} - EP/E_{tot} \qquad (6)$$

From equations (1) to (6) rate equation (7) can be derived:

$$d(E/E_{tot})/dt = k_2^* \frac{EC}{E_{tot}} - [k_1^* C_{pt} + k_3^* P_t] = k_2^* EC/E_{tot} - [k_1^* C_{pt0}.\exp(-\cap^* t) + k_3^* \{A^* \exp(-\alpha^* t) + B^* \exp(-\beta^* t) + C^* \exp(-\gamma^* t)\}]^* E/E_{tot} \qquad (7)$$

2.2 Pretreatment compound

The parameters for the pretreatment compound which are relevant for the model are k_1, k_2, C_{pt0} and $\cap$, as well as EC/E_{tot}. However, not all of these parameters are independent In animal experiments, the dose of the pretreatment compound is chosen in such a way, that a desired level of AChE inhibition is reached. Therefore, in our model the value for EC/E_{tot} is chosen. When in addition a value for k_2 is chosen, the value of $k_1^* C_{pt0}$ is fixed. This implies that an increase in k_1 with a certain factor causes a decrease in C_{pt0} with the same factor. Consequently, the independent parameters for which values were chosen are EC/E_{tot}, k_2 and $\cap$.

The elimination rate constant for the pretreatment compound was estimated from experiments with p-nitrophenyl phosphoramidates in guinea pigs. After i.v. administration these compounds disappeared rapidly from the blood: an elimination half-life time of 2 min has been estimated, which means that $\cap = 0.35\ min^{-1}$. For reasons of simplicity we chose to simulate i.v. administration and first-order elimination for the pretreatment compound. Equation (4) can be easily modified, should

the results indicate that the pharmacokinetic profile of the pretreatment compound is a crucial parameter.

The following values were used:

EC/E_{tot} : 0.1-0.7, increasing in steps of 0.1;

k_2 : 0.0011-0.185 min^{-1}, in steps increasing with a factor 2;

γ : 0.0035-3.5 min^{-1}, in steps increasing with a factor 10.

2.3 C(±)P(±)-soman

Considering the marked difference in the toxicity of the C(±)P(±)-soman stereoisomers (Benschop et al. 1984) we chose to use only the toxicokinetics of the 2 orders of magnitude more toxic P(-)-isomers. The sums of the concentrations measured in blood for C(+)P(-)- and C(-)P(-)-soman after i.v. administration of 2 and 6 LD50 to guinea pigs were calculated. Next, the toxicokinetic parameters were determined by means of non-linear regression curve fitting (BMDP-3R). This approach is reasonable, since the toxicokinetics of the two P(-)-isomers differ only slightly (Benschop and Jong, de, 1990, 1991). The results are listed in Table 1.

Table 1 Toxicokinetic parameters for C(±)P(-)-soman, measured after i.v. administration of 2 and 6 LD50 of C(±)P(±)-soman to atropinized guinea pigs

	2 LD50	6 LD50
A (mol/l)	$3.8*10^{-6}$	$4.2*10^{-6}$
B (mol/l)	$1.4*10^{-7}$	$4.2*10^{-7}$
C (mol/l)	$2.0*10^{-8}$	$7.4*10^{-8}$
α (min^{-1})	3.9	4.2
β (min^{-1})	0.20	0.16
γ (min^{-1})	0.039	0.042

The influence of the various phases of the toxicokinetics was tested by comparing the results obtained for the actual kinetics with those obtained after omitting either the α-, β- or γ-phase. Furthermore, the elimination rate constant (γ) was varied from 0.01 to 0.16 min^{-1}, in steps increasing with a factor 2.

The actual k_3 value of C(±)P(-)-soman is circa $1.2*10^8$ $M^{-1}.min^{-1}$ for electric eel acetylcholinesterase at pH 7.7 and 25 °C (Benschop et al. 1984). In this model k_3 was varied from $2.5*10^7$ to $4*10^8$ $M^{-1}.min^{-1}$, in steps increasing with a factor 2.

2.4 Calculations

Since equation (7) cannot be solved analytically, numeric solutions were obtained using the Merson-Runge-Kutta method (Bull 1966). Calculations were performed on an IBM-compatible XT- or AT-computer, equipped with a 8087 processor.

The equations for the toxicokinetic curves of C(±)P(-)-soman were calculated with BMDP statistical software (University of California, Los Angeles, USA).

The figures were drawn with Slide Write Plus (version 4.0, Advanced Graphics Software, Sunnyvale, CA, USA); the three-dimensional plots, however, were made with Boeing Graph (version 4.0, Boeing Co., Seattle, WA, USA).

3 RESULTS AND DISCUSSION

3.1 Data generated by the model

The model allows calculation of approximately 50 data points of the time course of the ratios E/E_{tot}, EC/E_{tot} and EP/E_{tot} from the moment of C(±)P(±)-soman intoxication (t=0) up to 1000 min after intoxication. An example of such a concentration ratio-time course profile is shown in Figure 1. From these data the time periods T_1, T_2 and T_5 are derived, which are needed to restore 1, 2 and 5 % AChE activity (E/E_{tot}= 0.01, 0.02 and 0.05), respectively.

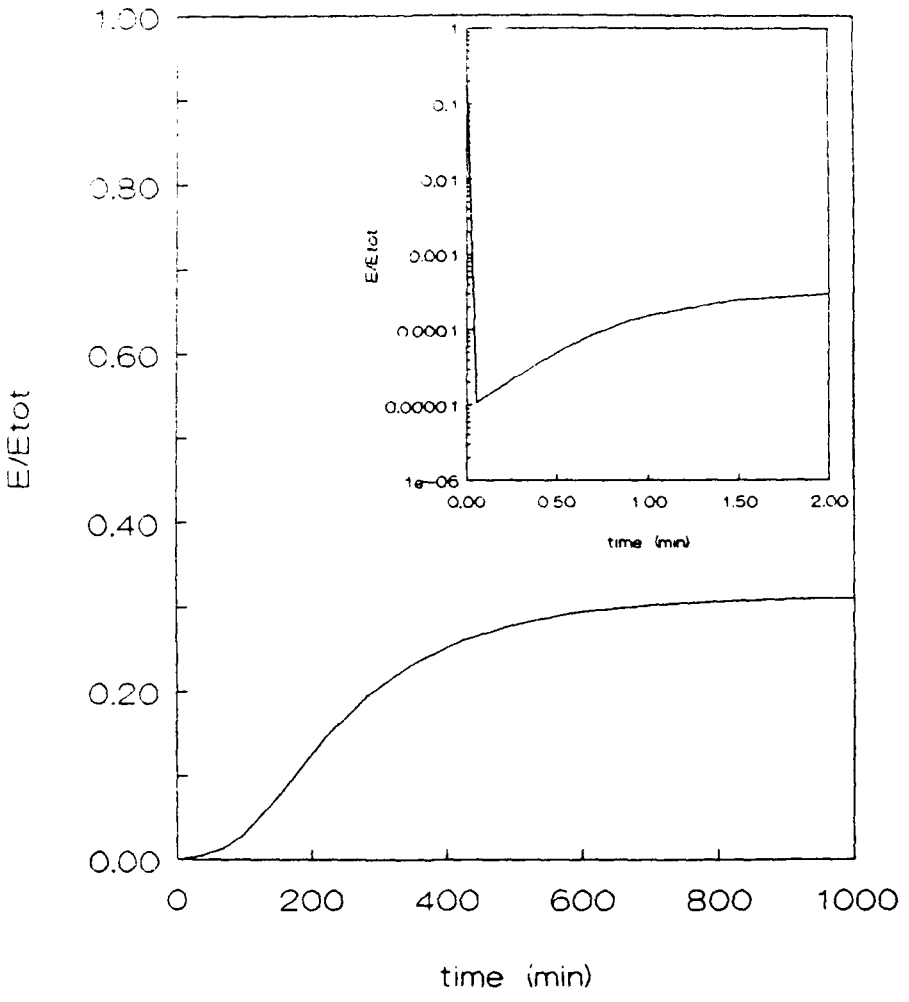

Figure 1 Simulated time course of the fraction of active ACh (E/E_{tot}) after pretreatment (EC/E_{tot} = 0.6, k_2 = 0.0058 min^{-1}, ∩ = 0.35 min^{-1}) followed by intoxication with 2 LD50 of C(±)P(±)-soman (k_3 = 1*10^8 $M^{-1}.min^{-1}$, α = 3.9 min^{-1}, β= 0.20 min^{-1}, γ = 0.039 min^{-1}). The insert shows the time course in the first ? min, on a semi-logarithmic scale

3.2 Criteria for interpretation of the results

Criteria have been chosen with respect to the minimum AChE activity required for survival and the time span in which this activity has to be restored, in order to draw conclusions as to whether a certain set of conditions will provide adequate protection. We have chosen 1-5 % as the minimum AChE activity for survival, which is within a generally accepted range (Green 1958, Hobbiger 1976). The time span in which the minimum activity has to be restored is difficult to assess. In animal experiments, however, we observed that atropinized guinea pigs that were intoxicated with 2 LD50 of C(±)P(±)-soman could survive this challenge for a period of circa 90 min, without artificial respiration. Therefore, it seems reasonable to use this value as a criterion in our model for the time period in which the minimum AChE activity has to be restored.

3.3 The influence of the fraction AChE inhibited by the pretreatment compound (EC/E_{tot}) and of the rate of spontaneous reactivation (k_2)

T_1, T_2 and T_5 were calculated for the various combinations of EC/E_{tot} and k_2, after simulation of intoxication with 2 and 6 LD50 of C(±)P(±)-soman. A survey of the data is presented in Annex 1, Tables 1 and 2.

Figure 2 shows T_1 after intoxication with 2 LD50 of C(±)P(±)-soman. The regeneration of AChE activity is more rapid at higher EC/E_{tot} and intermediate k_2 values. The fastest regeneration of 1 % activity is observed for the combination of $EC/E_{tot} = 0.7$ and $k_2 = 0.023$ min^{-1}. The open spaces in the figure pertain to the combinations of EC/EC_{tot} and k_2 which do not lead to the regeneration of 1 % AChE activity. When at least 1 % AChE activity is necessary for survival, such combinations are unsuitable. Those unfavourable conditions prevail when $k_2 > 0.023$ min^{-1}, as well as with the combination of $EC/E_{tot} = 0.1$, $k_2 = 0.023$ min^{-1}.

Figure 3 shows T_1 after intoxication with 6 LD50 of C(±)P(±)-soman. For all combinations of EC/E_{tot} and k_2, T_1 is higher than after intoxication with 2 LD50. Furthermore, the k_2 value which enables the fastest recovery of activity has decreased to 0.012 min^{-1}, again in combination with $EC/E_{tot} = 0.7$.

Values obtained for T_2 and T_5 are presented in Annex 1, Tables 3-6 and Annex 1, Figures 1-4. The combination of parameters which provides the fastest regeneration is the same as for T_1. As the required activity increases, however, the number of suitable combinations decreases. The combinations which are eliminated are those with a low EC/E_{tot} and a relatively high k_2 value.

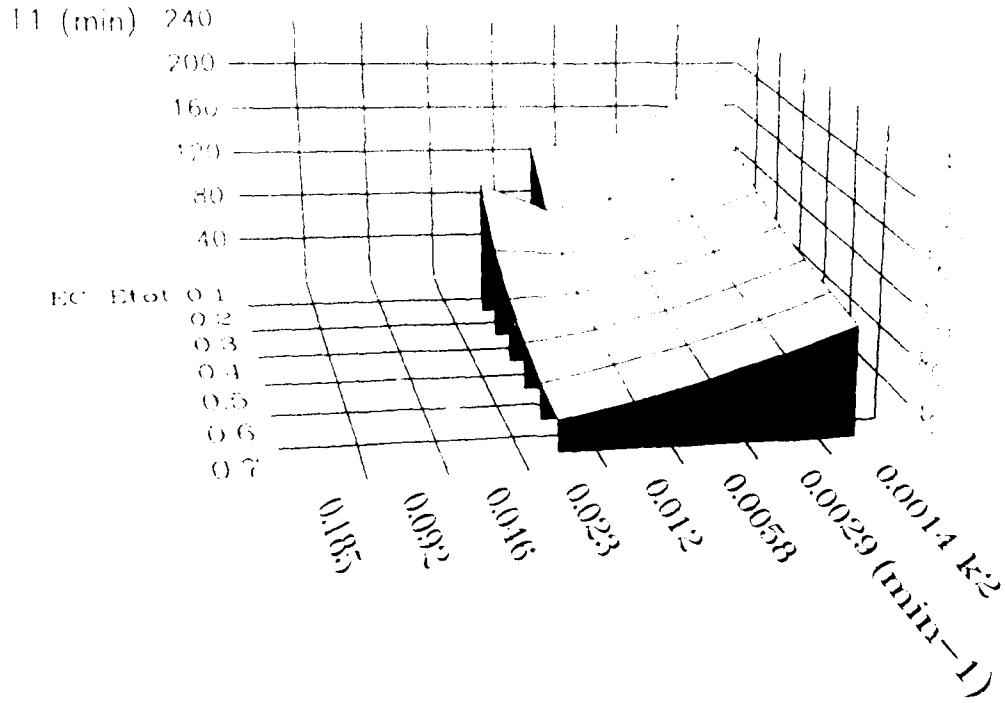

Figure 2 Three-dimensional plot of the influence of the AChE fraction inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme on the period of time required to restore 1 % AChE activity (T_1) after intoxication with 2 LD50 of C(±)P(±)-soman; $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

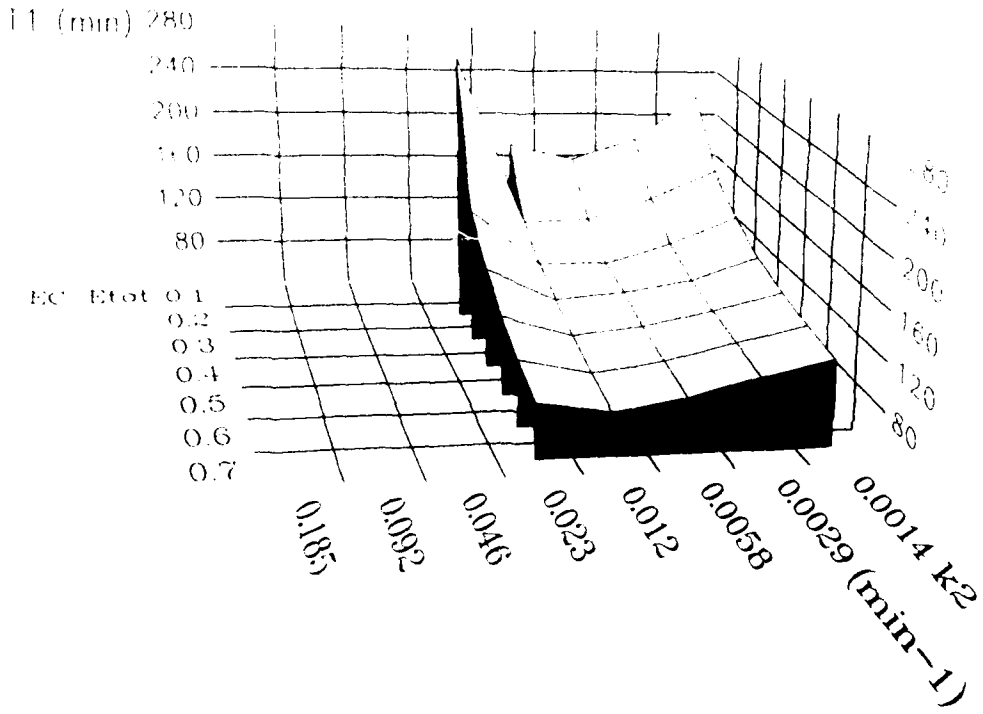

Figure 3 Three-dimensional plot of the influence of the AChE fraction inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme on the period of time required to restore 1 % AChE activity (T_1) after intoxication with 6 LD50 of C(±)P(±)-soman; $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

Three scenarios are studied to evaluate whether T_1, T_2 and T_5 can meet the criteria for adequate protection as mentioned above.

Scenario T_1

It is assumed that the minimum E/E_{tot} = 1 %; 100 % survival is observed after administration of a dose of 2 LD50 and 100 % mortality after administration of 6 LD50 of C(±)P(±)-soman to atropinized guinea pigs. In that case, the protective ratio, which is the ratio of the LD50 of C(±)P(±)-soman in the pretreated animals and the LD50 in the untreated animals, will be circa 4, which is assumed to be an adequate protective ratio (Dunn and Sidell 1989). The shortest time period in which 1 % AChE activity is restored after intoxication with a dose of 6 LD50 is 75 min (EC/E_{tot} = 0.7, k_2 = 0.012 min^{-1}, see Annex 1, Table 2). Since it is assumed in this scenario that mortality is 100 % under these conditions, 1 % AChE activity obviously has to be restored within

75 min. The suitable combinations of EC/E_{tot} and k_2, which restore 1 % AChE activity within 75 min after a dose of 2 LD50 of C(±)P(±)-soman (see Annex 1, Table 1) are:

EC/E_{tot}	k_2 (min^{-1})
0.3	0.012
0.4	0.023-0.0058
0.5	0.023-0.0058
0.6	0.023-0.0058
0.7	0.023-0.0029

Scenario T_2

It is assumed that E/E_{tot} = 2 %; 100 % survival is observed after administration of 2 LD50 and 100 % mortality after administration of 6 LD50 of C(±)P(±)-soman. By analogy with scenario T_1, the conclusion can be drawn that 2 % AChE activity has to be restored within 102 min. Suitable combinations (see Annex 1, Tables 3 and 4) are:

EC/E_{tot}	k_2 (min^{-1})
0.4	0.012-0.0058
0.5	0.023-0.0058
0.6	0.023-0.0029
0.7	0.023-0.0029

Scenario T_5

It is assumed that E/E_{tot} = 5 %; 100 % survival is observed after administration of 2 LD50 and 100 % mortality after administration of 6 LD50 C(±)P(±)-soman. By analogy with scenario T_1, it is concluded that 5 % AChE activity has to be restored within 147 min. Suitable combinations (see Annex 1, Tables 5 and 6) appear to be:

EC/E_{tot}	k_2 (min^{-1})
0.5	0.012-0.0058
0.6	0.012-0.0029
0.7	0.012-0.0029

A survey of the combinations which meet the criteria in the three scenarios is given in Figure 4. Obviously, with increasing minimum AChE activity required for survival, the number of suitable combinations decreases. The required EC/E_{tot} value increases with increasing essential minimum AChE activity. The required k_2 value decreases slightly with increasing demands on the minimum essential AChE activity.

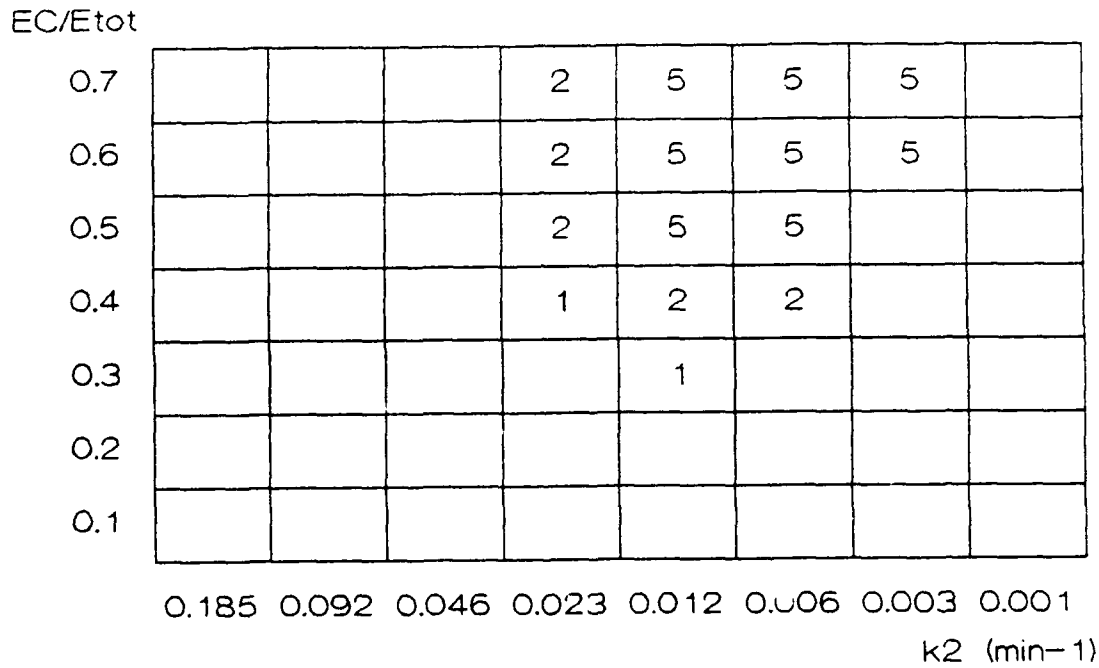

Figure 4 Graphic representation of the evaluation of calculated T_1, T_2 and T_5 values according to scenarios T_1, T_2 and T_5. The open rectangles are the unsuitable combinations of EC/E_{tot} and k_2. "1", "2" and "5" indicate suitable combinations within scenarios T_1, T_2 and T_5, respectively. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

It remains to be assessed whether the criteria that were adopted in the three scenarios are realistic. With the observation in mind that atropinized guinea pigs die about 90 min after administration of 2 LD50 of C(±)P(±)-soman, scenario T_5, in which the minimum AChE activity has to be restored within 147 min, is not very realistic.

In the guinea pig, a protective ratio of 4 can be obtained with pyridostigmine ($k_2 \approx 0.035\ min^{-1}$) if EC/E_{tot} = 0.4-0.5 (Lennox et al. 1985). Approximately the same protective ratio is observed after

pretreatment with 2-fluoroethyl p-nitrophenyl phosphoramidate ($k_2 \approx 0.012$ min^{-1}) when $EC/E_{tot} = 0.3$ (Langenberg et al. 1990). These observations suggest that the conclusions which are drawn from the other two scenarios, concerning the suitable combinations of EC/E_{tot} and k_2, are not unrealistic.

The generally accepted assumption that EC/E_{tot} in blood should not exceed 0.4 in order to avoid incapacitation by the pretreatment compound (Dunn and Sidell 1989), can be used as an additional criterion. This implies that the combinations listed above with $EC/E_{tot} > 0.4$ cannot be used in practical situations. As a consequence, the range of suitable k_2 values is further limited to 0.023 - 0.0058 min^{-1}.

It is almost impossible to perform an assessment of the influence of other parameters for all combinations of EC/E_{tot} and k_2. Based on the evaluation of the results according to the three scenarios, eight combinations have been chosen for the simulation of the variation of other parameters, i.e., EC/Etot = 0.3 and 0.6, each with k_2 = 0.023 and 0.0058 min^{-1}, for doses corresponding with 2 and 6 LD50 of C(±)P(±)-soman.

3.4 The influence of the pharmacokinetics of the pretreatment compound

T_1, T_2 and T_5 were calculated after simulation of the intoxication with 2 and 6 LD50 of C(±)P(±)-soman, with $\cap$ varying from 3.5 to 0.0035 min^{-1}. In Table 2 the T_1 values are listed after intoxication with 2 LD50 of C(±)P(±)-soman subsequent to pretreatment. Similar results are obtained for T_2 and T_5 (Annex 2, Tables 3 and 5). From these data, it is obvious that the pharmacokinetics of the pretreatment compound has a neglible influence on the restoration rate of AChE activity. After administration of 6 LD50 of C(±)P(±)-soman, the influence of $\cap$ on the time necessary to restore the minimum activity, is equally small, as shown in Annex 2, Tables 2, 4 and 6.

Figure 5 shows the time profiles of E/E_{tot} after administration of pretreatment compounds with various elimination rate constants, at a dose which produces 60 % AChE inhibition at t = 0 min, without subsequent administration of C(±)P(±)-soman. Since the profile for $\cap$ = 0.0035 min^{-1} differs considerably from that calculated for $\cap$ = 3.5 min^{-1}, one would expect an effect of $\cap$ on the time needed to restore AChE activity after C(±)P(±)-soman intoxication. This is obviously not the case.

Table 2 Calculated time required to restore 1 % AChE activity (T_1, min) in relation to the elimination rate constant ($\cap$) of the pretreatment compound, after intoxication with 2 LD50 of C(±)P(±)-soman. $k_3 = 1*10^8\ M^{-1}.min^{-1}$

	T_1 (min)			
	$k_2 = 0.023\ min^{-1}$		$k_2 = 0.0058\ min^{-1}$	
	EC/E_{tot}		EC/E_{tot}	
$\cap$ (min^{-1})	0.3	0.6	0.3	0.6
3.5	75	30	82	57
0.35	75	30	82	57
0.035	75	30	82	57
0.0035	76	31	83	58

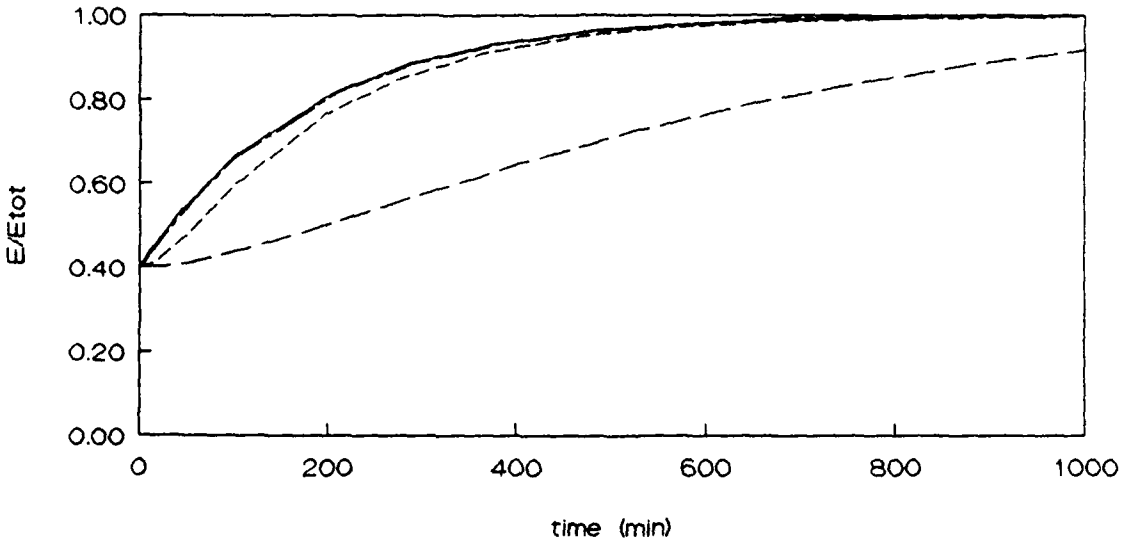

Figure 5 Time profiles of E/E_{tot}, after simulated administration of a pretreatment compound with an elimination rate constant ($\cap$) of 3.5 (—), 0.35 (-- -), 0.035 (- - -) or 0.0035 (-- --) min^{-1}; $k_2 = 0.0058\ min^{-1}$, $EC/E_{tot} = 0.6$, $k_3 = 1*10^8\ M^{-1}.min^{-1}$

Since the effect of $\cap$ on the regeneration of AChE activity is negligible, application of the criteria of scenarios T_1, T_2 and T_5 to the obtained data results in exactly the same suitable combinations of EC/Etot and k_2 as those obtained upon investigation of the influences of EC/E_{tot} and k_2 on the regeneration of AChE activity after pretreatment and subsequent intoxication.

3.5 The influence of the inhibition rate constant of soman

T_1, T_2 and T_5 were calculated for intoxication with doses of 2 and 6 LD50 of C(±)P(±)-soman, with k_3 varying from $2.5*10^7$ to $4*10^8$ $M^{-1}.min^{-1}$.

It is obvious that k_3 will have a profound influence on the efficacy of the pretreatment. The data for T_1 given in Tables 3 and 4 confirm this. If k_3 is lower than the approximate actual value of $1*10^8$ $M^{-1}.min^{-1}$ (Benschop et al. 1984), protection against nerve agent by pretreatment will be much easier. On the other hand, if k_3 is higher than $1*10^8$ $M^{-1}.min^{-1}$, the number of suitable conditions for pretreatment is further limited. Similar results were obtained for T_2 and T_5 (see Annex 3).

Table 3 The influence of k_3 on the time period needed to restore 1 % AChE activity (T_1, min) after intoxication with 2 LD50 of C(±)P(±)-soman. $\cap$ = 0.35 min^{-1}

k_3	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
($M^{-1}.min^{-1}$)	0.3	0.6	0.3	0.6
$2.5*10^7$	16	8	40	23
$5*10^7$	32	15	61	48
$1*10^8$	75	30	82	57
$2*10^8$	132	72	103	78
$4*10^8$	234	120	119	100

Table 4 The influence of k_3 on the time period needed to restore 1 % AChE activity (T_1, min) after intoxication with 6 LD50 of C(±)P(±)-soman. $\cap$ = 0.35 min^{-1}

k_3	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
($M^{-1}.min^{-1}$)	0.3	0.6	0.3	0.6
$2.5*10^7$	57	28	73	50
$5*10^7$	99	49	93	70
$1*10^8$	153	94	106	89
$2*10^8$	266	137	125	105
$4*10^8$	*	195	152	121

* = 1 % AChE activity is not restored under these conditions

Application of the criteria of scenario T_1 to the data obtained for the influence of EC/E_{tot} and k_2 leads to the conclusion that T_1 has to be less than 75 min for survival. Tables 3 and 4 indicate that, if $k_3 = 2.5*10^7$ $M^{-1}.min^{-1}$, all combinations of EC/E_{tot} and k_2 taken into consideration will provide a successful pretreatment against doses of 2 and 6 LD50. With $k_3 = 5*10^7$ $M^{-1}.min^{-1}$, all combinations are effective against 2 LD50, whereas EC/E_{tot} has to be 0.6 in order to survive an intoxication with 6 LD50. With the approximate actual value of k_3, $1*10^8$ $M^{-1}.min^{-1}$, only the combinations with $EC/E_{tot} = 0.6$ are effective, and only against 2 LD50. When $k_3 = 2*10^8$ $M^{-1}.min^{-1}$, only the combination of $EC/E_{tot} = 0.6$ with $k_2 = 0.023$ min^{-1} is suitable, only against 2 LD50. With $k_3 > 2*10^8$ $M^{-1}.min^{-1}$, there is no protection against the nerve agent, under these criteria.

From scenario T_2 the conclusion has been drawn that T_2 should be less than 102 min. Tables 3 and 4 of Annex 3 indicate that the suitable combinations are the same as for scenario T_1, with the exception that none of the conditions is suitable when $k_3 > 1*10^8$ $M^{-1}.min^{-1}$.

According to scenario T_5, T_5 has to be less than 147 min. Tables 5 and 6 of Annex 3 indicate that the number of suitable conditions is further limited. With $k_3 = 2.5*10^7$ $M^{-1}.min^{-1}$ all considered combinations are effective against 2 LD50, whereas EC/E_{tot} has to be 0.6 for protection against 6 LD50. With $k_3 = 5*10^7$ $M^{-1}.min^{-1}$, the combination of $EC/E_{tot} = 0.6$ with $k_2 = 0.023$ min^{-1} is effective against 2 LD50, whereas combination $EC/E_{tot} = 0.6$ / $k_2 = 0.0058$ min^{-1} is effective against both 2 and 6 LD50. When $k_3 = 1*10^8$ or $2*10^8$ $M^{-1}.min^{-1}$, only the combination $EC/E_{tot} = 0.6$ / $k_2 = 0.0058$ min^{-1} is effective, and only against 2 LD50.

If the additional criterion is applied that EC/E_{tot} should not exceed 0.4, the number of suitable conditions is of course further limited.

3.6 The influence of the overall toxicokinetics of C(±)P(-)-soman

T_1, T_2 and T_5 were calculated after simulation of the intoxication with 2 and 6 LD50 of C(±)P(±)-soman, using the actual toxicokinetics for C(±)P(-)-soman or hypothetical kinetics, omitting the α-, β-, or γ-phase. Tables 5 and 6 show the data obtained for T_1. The results for T_2 and T_5, as well as for T_1, are presented in Annex 4. The data indicate that the influence of omitting the α- and β-phase from the toxicokinetics on the recovery of AChE activity is negligible, whereas the influence of omitting the γ-phase is considerable. This may be explained from the ratio between k_2 and the rate constants of the toxicokinetics of C(±)P(-)-soman. The rate constants of the α- and the β-phases are much higher than k_2, whereas the rate constant of the γ-phase is in the same order of magnitude as k_2.

In the scenarios T_1, T_2 and T_5, the omission of the γ-phase makes all of the considered combinations suitable. In fact, all conditions restore either 1, 2 or 5 % within 75 min, with one exception, in which T_5 is 78 min.

Table 5 Influence of the overall toxicokinetics of C(±)P(-)-soman on the time period required to restore 1 % AChE activity (T_1, min) after intoxication with 2 LD50 of C(±)P(±)-soman. αβγ = actual toxicokinetics of C(±)P(-)-soman, αβ= toxicokinetics omitting the γ-phase, αγ = toxicokinetics omitting the β-phase, βγ = toxicokinetics omitting the α-phase. k_3 = 1*10^8 M^{-1}.min^{-1}, ∩ = 0.35 min^{-1}, α = 3.9 min^{-1}, β= 0.20 min^{-1} and γ = 0.039 min^{-1}

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
Toxico-	EC/E_{tot}		EC/E_{tot}	
kinetics	0.3	0.6	0.3	0.6
αβγ	75	30	82	57
αβ	18	14	28	22
αγ	75	30	82	57
βγ	75	30	83	56

Table 6 Influence of the overall toxicokinetics of C(±)P(-)-soman on the time period required to restore 1 % AChE activity (T_1, min) after intoxication with 6 LD50 of C(±)P(±)-soman. αβγ = actual toxicokinetics of C(±)P(-)-soman, αβ= toxicokinetics omitting the γ-phase, αγ = toxicokinetics omitting the β-phase, βγ = toxicokinetics omitting the α phase. k_3 = 1*10^8 M^{-1}.min^{-1}, ∩ = 0.35 min^{-1}, α = 4.2 min^{-1}, β= 0.16 min^{-1} and γ = 0.042 min^{-1}

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
Toxico-	EC/E_{tot}		EC/E_{tot}	
kinetics	0.3	0.6	0.3	0.6
αβγ	135	94	110	89
αβ	26	25	41	34
αγ	135	94	110	89
βγ	135	94	110	89

After omission of the α- or β-phase, the suitable combinations are the same as those for the complete toxicokinetic description for C(±)P)(-)-soman, i.e., including the α-, β- and γ-phase (see the results for the influences of EC/E_{tot} and k_2).

3.7 The influence of the elimination rate constant of C(±)P(-)-soman

T_1, T_2 and T_5 were calculated after simulation of the intoxication with 2 and 6 LD50 of C(±)P(±)-soman, with a varying elimination rate constant (γ) for C(±)P(-)-soman. The results for T_1 are listed in Tables 7 and 8. The results for T_2 and T_5, as well as for T_1, are presented in Annex 5. Benschop and Jong, de, (1990, 1991) have suggested on the basis of calculations, that the concentrations of C(±)P(-)-soman in blood are still toxicologically relevant in the γ phase of the toxicokinetics. This is confirmed by the results of the computer simulation. The data indicate that with decreasing elimination rates the prognosis for pretreatment deteriorates, since AChE which is regenerated from EC will be inhibited by residual C(±)P(-)-soman. On the other hand, an increasing rate of elimination improves the efficacy of the pretreatment.

In scenarios T_1, T_2 and T_5, all conditions considered appear to be suitable, when the elimination rate constant is higher than the actual value of 0.039 min^{-1} or 0.042 min^{-1} for doses of 2 or 6 LD50 of C(±)P(±)-soman, respectively. However, if the elimination rate constant is lower than the actual value, none of the conditions is suitable.

Table 7 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the elimination rate constant (γ, min^{-1}) of C(±)P(-)-soman. $k_3 = 1*10^8$ $M^{-1}.min^{-1}$, $\cap = 0.35$ min^{-1}

	T_1 (min)			
	$k_2 = 0.023$ min^{-1}		$k_2 = 0.0058$ min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
γ (min^{-1})	0.3	0.6	0.3	0.6
0.156	20	15	30	23
0.078	27	19	42	30
0.039*	75	30	82	57
0.020	**	**	195	132
0.010	**	**	882	478

* = actual value

** = 1 % AChE activity is not restored under these conditions

Table 8 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the elimination rate constant (γ, min^{-1}) of C(±)P(-)-soman. $k_3 = 1*10^8$ $M^{-1}.min^{-1}$, $\cap = 0.35$ min^{-1}

	T_1 (min)			
	$k_2 = 0.023$ min^{-1}		$k_2 = 0.0058$ min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
(γ min^{-1})	0.3	0.6	0.3	0.6
0.168	33	26	42	35
0.084	46	35	56	45
0.042*	153	94	110	89
0.021	**	**	266	207
0.010	**	**	**	777

* = actual value
** = 1 % AChE activity is not restored under these conditions

The toxicokinetics of C(±)P(±)-soman are rather invariable for a particular species, administration route and dose of the agent. However, one could try to accelerate the elimination phase by using a scavenger for C(±)P(-)-soman, either prophylactically or therapeutically. The results obtained with this computer model suggest that the therapeutic use of a scavenger can substantially improve the efficacy of pretreatment with a cholinesterase inhibitor.

4 CONCLUSIONS

The developed computer model for the simulation of pretreatment against poisoning with C(±)P(±)-soman appears to be a promising aid in obtaining more insight into the parameters which determine the efficacy of pretreatment. Since the model is a simplified representation of the real situation, by considering the guinea pig to be one homogeneous compartment, by using intravenous toxicokinetics for the nerve agent, and intravenous pharmacokinetics for the pretreatment compound, one has to be cautious when trying to extrapolate the results of the model to the *in vivo* situation. Nevertheless, some conclusions can be drawn. The results obtained with the model indicate that the most crucial factors in the pretreatment process are (i) the extent to which AChE is inhibited by the pretreatment compound at the moment of C(±)P(±)-soman intoxication (E/E_{tot}), (ii) the rate of reactivation of this inhibited enzyme (k_2), (iii) the inhibition rate constant of AChE by C(±)P(-)-soman (k_3), and (iv) the elimination rate constant of C(±)P(-)-soman (γ).

An optimum rate constant of spontaneous reactivation, k_2, appears to exist, in the range of 0.012-0.0029 min^{-1}. This optimum value is lower than k_2 for pyridostigmine-inhibited AChE, which is circa 0.046 (Langenberg et al. 1990). Furthermore, the model indicates that the results improve at higher EC/E_{tot} values. However, in order to avoid incapacitation by the pretreatment compound, EC/E_{tot} should not exceed 0.4. This additional criterion limits the suitable conditions to the range of 0.3-0.4 for EC/E_{tot} and 0.023-0.0058 min^{-1} for k_2.

The model also suggests that therapeutic use of a scavenger for C(±)P(-)-soman, in order to accelerate the decrease of the concentration of the nerve agent in the elimination phase, should improve the efficacy of the pretreatment considerably.

The results of the model are not unrealistic when compared with the findings in animal studies.

5 PROSPECTS FOR FURTHER MODELLING

The model can be modified in various ways to accomplish a higher level of sophistication. The use of the intravenous toxicokinetics of C(±)P(±)-soman in the model might be considered to be a shortcoming, since this administration route is not frequently used in challenge experiments. Therefore, it would be more accurate to use the inhalation or subcutaneous toxicokinetics. Such data were not available when this model was developed.

A further improvement of the model can be obtained when *de novo* synthesis of AChE is taken into account, which appears to proceed at a rate of circa 1.5 % per hour in the rat (Dongen, van, et al. 1988).

The model will be modified with regard to the above-mentioned aspects in order to further improve the practical value.

In addition, some extensions to the model are possible, e.g., the therapeutic use of oximes can be introduced into the model.

The model can also be applied to the simulation of intoxication by other AChE inhibitors, including pesticides.

6 ACKNOWLEDGEMENT

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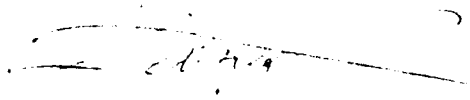
7 AUTHENTICATION



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ANNEX 1 INFLUENCE OF THE FRACTION OF ACHE INHIBITED BY THE PRETREATMENT COMPOUND AND THE RATE OF SPONTANEOUS REACTIVATION OF THIS ENZYME ON THE TIME PERIOD REQUIRED TO RESTORE 1-5 % ACHE ACTIVITY AFTER PRETREATMENT AND SUBSEQUENT INTOXICATION WITH 2-6 LD50 OF C(±)P(±)-SOMAN

Table 1 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the fraction of AChE inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

	T_1 (min)							
	k_2 (min^{-1})							
EC/E_{tot}	0.185	0.092	0.046	0.023	0.012	0.0058	0.0029	0.0014
0.1	*	*	*	*	131	133	158	200
0.2	*	*	*	114	90	99	116	142
0.3	*	*	*	75	70	82	101	121
0.4	*	*	*	54	58	72	89	110
0.5	*	*	*	39	49	63	81	102
0.6	*	*	*	30	42	57	75	96
0.7	*	*	*	25	36	52	70	90

* = 1 % AChE activity is not restored under these conditions

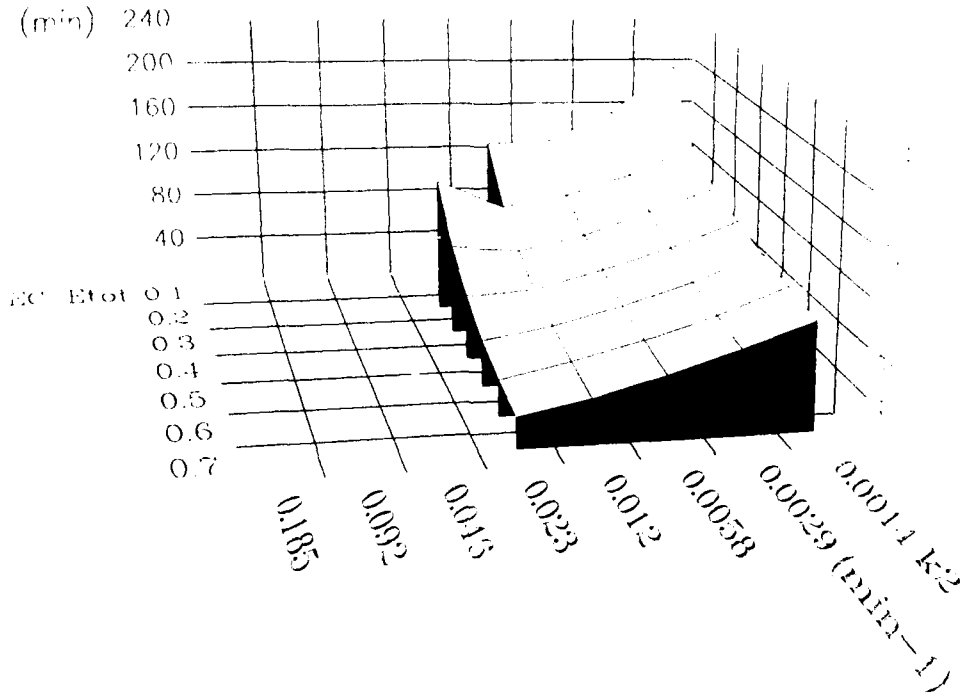

Figure 1 Three-dimensional plot of the influence of the AChE fraction inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme on the time period required to restore 1 % AChE activity (T_1, min) after intoxication with 2 LD50 of C(±)P(±)-soman; $k_3 = 1*10^8$ $M^{-1}.min^{-1}$, $\cap = 0.35$ min^{-1}

Table 2 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the fraction of AChE inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

	T_1 (min)							
	k_2 (min^{-1})							
EC/E_{tot}	0.185	0.092	0.046	0.023	0.012	0.0058	0.0029	0.0014
0.1	*	*	*	*	178	165	184	224
0.2	*	*	*	272	125	126	145	170
0.3	*	*	*	153	107	106	123	145
0.4	*	*	*	123	97	102	111	130
0.5	*	*	*	106	88	95	106	121
0.6	*	*	*	94	81	89	102	115
0.7	*	*	*	84	75	84	98	110

* = 1 % AChE activity is not restored under these conditions

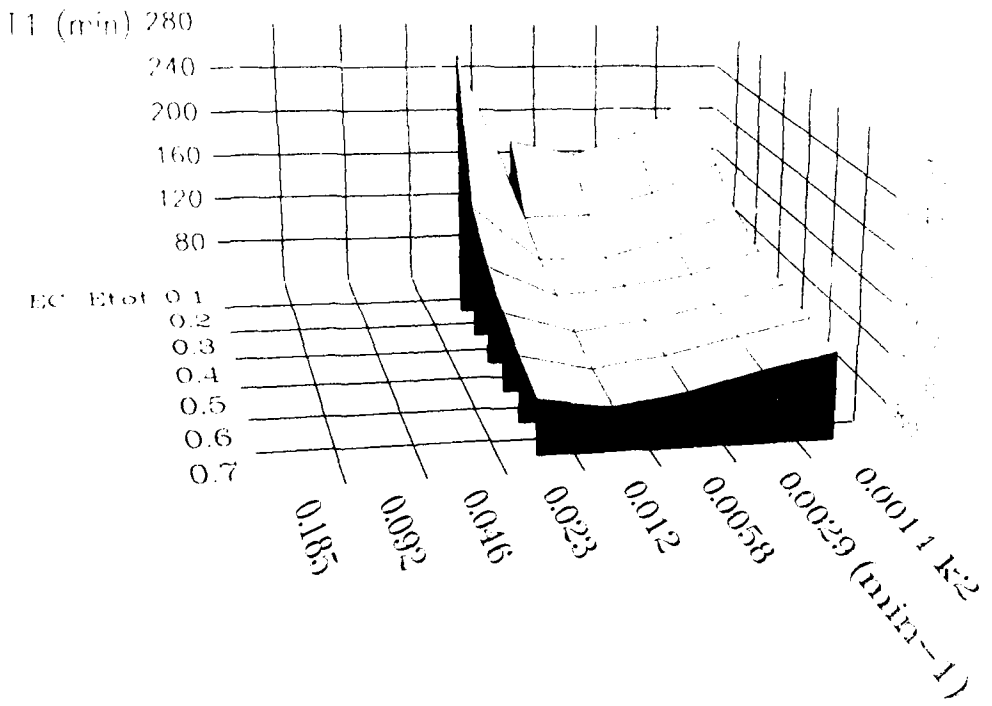

Figure 2 Three-dimensional plot of the influence of the AChE fraction inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme on the time period required to restore 1 % AChE activity (T_1, min) after intoxication with 6 LD50 of C(±)P(±)-soman. $k_3 = 1*10^8$ $M^{-1}.min^{-1}$, $\cap = 0.35$ min^{-1}

Table 3 The time period required to restore 2 % AChE activity (T_2, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the fraction of AChE inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

	T_2 (min)							
	k_2 (min^{-1})							
EC/E_{tot}	0.185	0.092	0.046	0.023	0.012	0.0058	0.0029	0.0014
0.1	*	*	*	*	220	196	227	303
0.2	*	*	*	*	131	134	158	200
0.3	*	*	*	155	105	111	131	164
0.4	*	*	*	114	90	99	114	147
0.5	*	*	*	90	79	90	106	129
0.6	*	*	*	75	71	82	100	119
0.7	*	*	*	63	64	76	94	113

* = 2 % AChE activity is not restored under these conditions

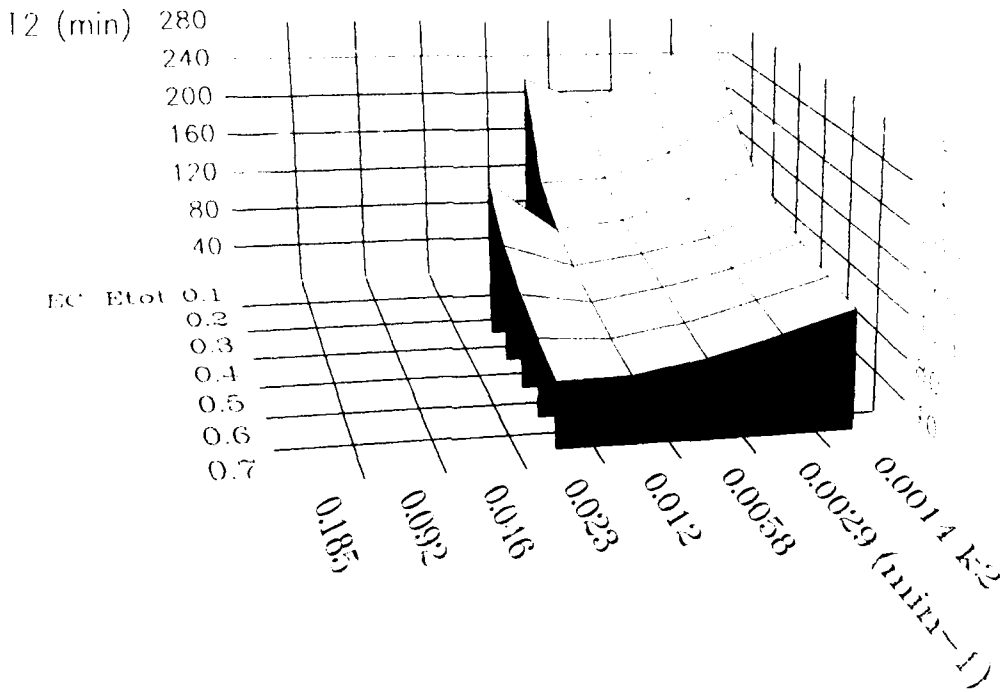

Figure 3 Three-dimensional plot of the influence of the AChE fraction inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme on the time period required to restore 2 % AChE activity (T_2, min) after intoxication with 2 LD50 of C(±)P(±)-soman. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

Table 4 The time period required to restore 2 % AChE activity (T_2, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the fraction of AChE inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

	T_2 (min)							
	k_2 (min^{-1})							
EC/E_{tot}	0.185	0.092	0.046	0.023	0.012	0.0058	0.0029	0.0014
0.1	*	*	*	*	363	235	257	331
0.2	*	*	*	*	178	166	184	224
0.3	*	*	*	*	143	142	158	188
0.4	*	*	*	274	125	126	139	170
0.5	*	*	*	184	114	116	132	158
0.6	*	*	*	152	107	110	120	146
0.7	*	*	*	135	102	105	117	136

* = 2 % AChE activity is not restored under these conditions

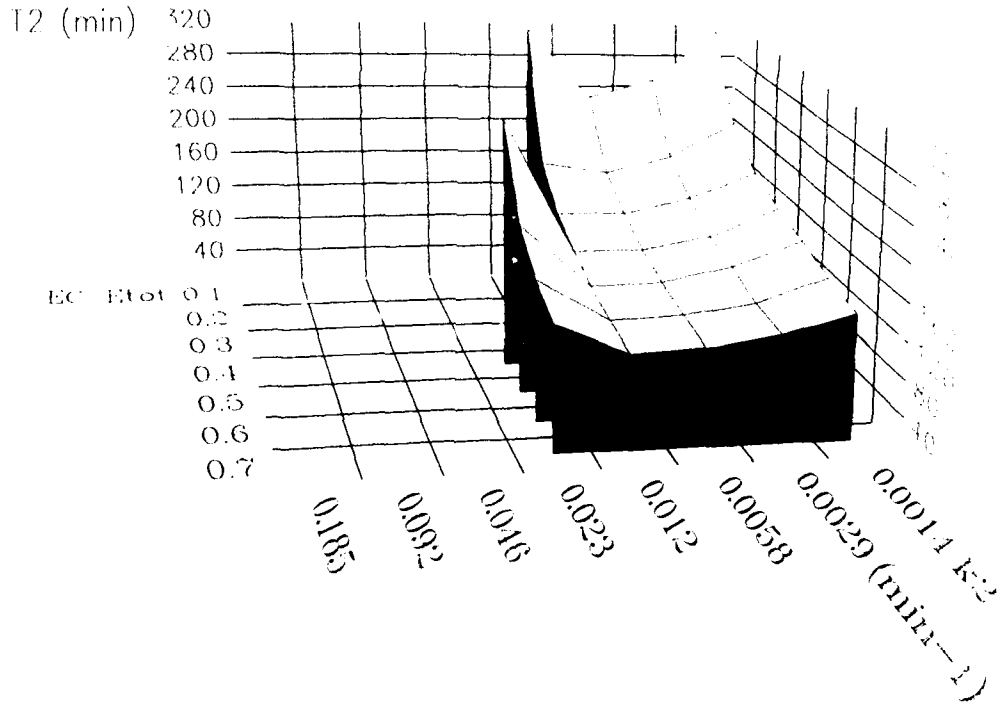

Figure 4 Three-dimensional plot of the influence of the AChE fraction inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme on the time period required to restore 2 % AChE activity (T_2, min) after intoxication with 6 LD50 of C(±)P(±)-soman. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

Table 5 The time period required to restore 5 % AChE activity (T_5, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the fraction of AChE inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

	T_5 (min)							
	k_2 (min^{-1})							
EC/E_{tot}	0.185	0.092	0.046	0.023	0.012	0.0058	0.0029	0.0014
0.1	*	*	*	*	*	690	530	744
0.2	*	*	*	*	326	227	265	357
0.3	*	*	*	*	188	175	204	267
0.4	*	*	*	*	151	151	175	225
0.5	*	*	*	*	131	134	155	200
0.6	*	*	*	265	118	122	146	183
0.7	*	*	*	178	108	114	135	170

* = 5 % AChE activity is not restored under these conditions

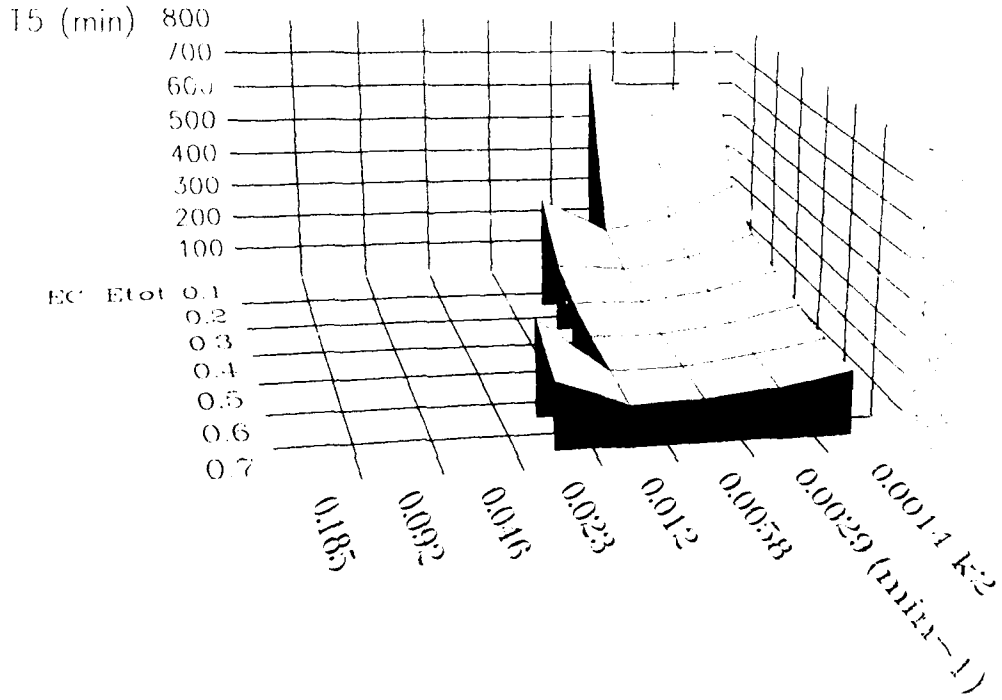

Figure 5 Three-dimensional plot of the influence of the AChE fraction inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme on the time period required to restore 5 % AChE activity (T_5, min) after intoxication with 2 LD50 of C(±)P(±)-soman. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

Table 6 The time period required to restore 5 % AChE activity (T_5, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the fraction of AChE inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

	T_5 (min)							
	k_2 (min^{-1})							
EC/E_{tot}	0.185	0.092	0.046	0.023	0.012	0.0058	0.0029	0.0014
0.1	*	*	*	*	*	*	603	799
0.2	*	*	*	*	*	273	296	389
0.3	*	*	*	*	265	211	234	294
0.4	*	*	*	*	203	182	202	250
0.5	*	*	*	*	178	165	184	224
0.6	*	*	*	*	161	154	172	206
0.7	*	*	*	*	148	147	162	193

* = 5 % AChE activity is not restored under these conditions

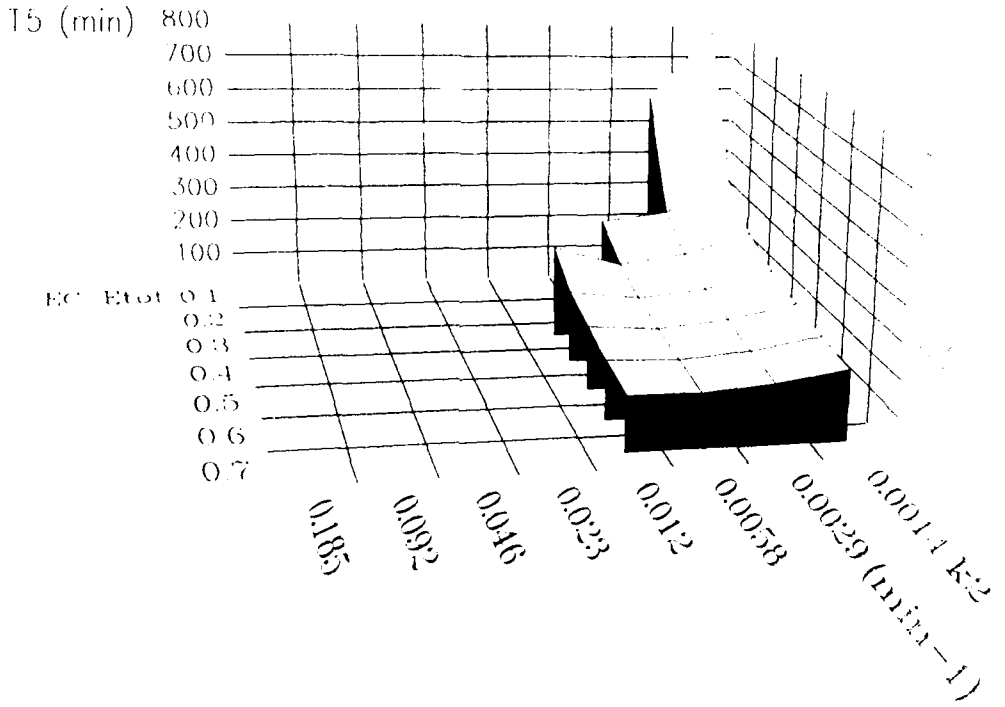

Figure 6 Three-dimensional plot of the influence of the AChE fraction inhibited by the pretreatment compound (EC/E_{tot}) and the rate constant of spontaneous reactivation (k_2) of this enzyme on the time period required to restore 5 % AChE activity (T_5, min) after intoxication with 6 LD50 of C(±)P(±)-soman. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap = 0.35\ min^{-1}$

ANNEX 2 INFLUENCE OF THE PHARMACOKINETICS OF THE PRETREATMENT COMPOUND ON THE TIME PERIOD REQUIRED TO RESTORE 1-5 % ACHE ACTIVITY AFTER PRETREATMENT AND SUBSEQUENT INTOXICATION WITH 2-6 LD50 OF C(±)P(±)-SOMAN

Table 1 Calculated time period required to restore 1 % AChE activity (T_1, min) in relation to the elimination rate constant ($\cap$) of the pretreatment compound, after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman. EC/E_{tot} = 0.3 and 0.6, k_2 = 0.023 and 0.0058 min^{-1}, $k_3 = 1*10^8$ $M^{-1}.min^{-1}$

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
$\cap$ (min^{-1})	0.3	0.6	0.3	0.6
3.5	75	30	82	57
0.35	75	30	82	57
0.035	75	30	82	57
0.0035	76	31	83	58

Table 2 Calculated time period required to restore 1 % AChE activity (T_1, min) in relation to the elimination rate constant ($\cap$) of the pretreatment compound, after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman. EC/E_{tot} = 0.3 and 0.6, k_2 = 0.023 and 0.0058 min^{-1}, $k_3 = 1*10^8$ $M^{-1}.min^{-1}$

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
$\cap$ (min^{-1})	0.3	0.6	0.3	0.6
3.5	153	94	110	89
0.35	153	94	110	89
0.035	153	94	110	89
0.0035	158	94	110	89

Table 3 Calculated time period required to restore 2 % AChE activity (T_2, min) in relation to the elimination rate constant ($\cap$) of the pretreatment compound, after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman. EC/E_{tot} = 0.3 and 0.6, k_2 = 0.023 and 0.0058 min^{-1}, k_3 = $1*10^8$ $M^{-1}.min^{-1}$

	T_2 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
$\cap$ (min^{-1})	0.3	0.6	0.3	0.6
3.5	155	75	111	82
0.35	155	75	111	82
0.035	155	74	111	82
0.0035	172	81	112	84

Table 4 Calculated time period required to restore 2 % AChE activity (T_2, min) in relation to the elimination rate constant ($\cap$) of the pretreatment compound, after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman. EC/E_{tot} = 0.3 and 0.6, k_2 = 0.023 and 0.0058 min^{-1}, k_3 = $1*10^8$ $M^{-1}.min^{-1}$

	T_2 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
$\cap$ (min^{-1})	0.3	0.6	0.3	0.6
3.5	*	152	142	110
0.35	*	152	142	110
0.035	*	151	142	110
0.0035	*	180	143	111

* = 2 % AChE activity is not restored under these conditions

Table 5 Calculated time period required to restore 5 % AChE activity (T_5, min) in relation to the elimination rate constant ($\cap$) of the pretreatment compound, after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman. EC/E_{tot} = 0.3 and 0.6, k_2 = 0.023 and 0.0058 min^{-1}, k_3 = $1*10^8$ $M^{-1}.min^{-1}$

	T_5 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
$\cap$ (min^{-1})	0.3	0.6	0.3	0.6
3.5	*	266	175	122
0.35	*	265	175	122
0.035	*	241	175	122
0.0035	*	389	180	128

* = 5 % AChE activity is not restored under these conditions

Table 6 Calculated time period required to restore 5 % AChE activity (T_5, min) in relation to the elimination rate constant ($\cap$) of the pretreatment compound, after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman. EC/E_{tot} = 0.3 and 0.6, k_2 = 0.023 and 0.0058 min^{-1}, k_3 = $1*10^8$ $M^{-1}.min^{-1}$

	T_5 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
$\cap$ (min^{-1})	0.3	0.6	0.3	0.6
3.5	*	*	211	154
0.35	*	*	211	154
0.035	*	*	211	154
0.0035	*	*	211	154

* = 5 % AChE activity is not restored under these conditions

ANNEX 3 INFLUENCE OF THE INHIBITION RATE CONSTANT (k_3) OF C(±)P(-)-SOMAN ON THE TIME PERIOD REQUIRED TO RESTORE 1-5 % AChE ACTIVITY AFTER PRETREATMENT AND SUBSEQUENT INTOXICATION WITH 2-6 LD50 OF C(±)P(±)-SOMAN

Table 1 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the inhibition rate constant k_3 of C(±)P(-)-soman for AChE. $\cap$ = 0.35 min^{-1}

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
k_3	EC/E_{tot}		EC/E_{tot}	
(M^{-1} . min^{-1})	0.3	0.6	0.3	0.6
$2.5*10^7$	16	8	40	23
$5*10^7$	32	15	61	48
$1*10^8$	75	30	82	57
$2*10^8$	132	72	103	78
$4*10^8$	234	120	119	100

Table 2 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the inhibition rate constant k_3 of C(±)P(-)-soman for AChE. $\cap$ = 0.35 min^{-1}

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
k_3	EC/E_{tot}		EC/E_{tot}	
(M^{-1} . min^{-1})	0.3	0.6	0.3	0.6
$2.5*10^7$	57	28	73	50
$5*10^7$	99	49	93	70
$1*10^8$	153	94	106	89
$2*10^8$	266	137	125	105
$4*10^8$	*	195	152	121

* = 1 % AChE activity is not restored under these conditions

Table 3 The time period required to restore 2 % AChE activity (T_2, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the inhibition rate constant k_3 of C(±)P(-)-soman for AChE. $\cap$ = 0.35 min^{-1}

	T_2 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
k_3	EC/E_{tot}		EC/E_{tot}	
(M^{-1} . min^{-1})	0.3	0.6	0.3	0.6
$2.5*10^7$	35	16	67	40
$5*10^7$	82	32	90	61
$1*10^8$	155	75	111	82
$2*10^8$	*	132	131	103
$4*10^8$	*	234	158	120

* = 2 % AChE activity is not restored under these conditions

Table 4 The time period required to restore 2 % AChE activity (T_2, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the inhibition rate constant k_3 of C(±)P(-)-soman for AChE. $\cap$ = 0.35 min^{-1}

	T_2 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
k_3	EC/E_{tot}		EC/E_{tot}	
(M^{-1} . min^{-1})	0.3	0.6	0.3	0.6
$2.5*10^7$	113	57	104	73
$5*10^7$	200	99	119	93
$1*10^8$	*	152	142	110
$2*10^8$	*	264	164	126
$4*10^8$	*	*	184	153

* = 1 % AChE activity is not restored under these conditions

Table 5 The time period required to restore 5 % AChE activity (T_5, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the inhibition rate constant k_3 of C(±)P(-)-soman for AChE. $\cap$ = 0.35 min^{-1}

	T_5 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
k_3	EC/E_{tot}		EC/E_{tot}	
(M^{-1} . min^{-1})	0.3	0.6	0.3	0.6
$2.5*10^7$	145	49	121	78
$5*10^7$	*	110	147	101
$1*10^8$	*	265	175	122
$2*10^8$	*	*	204	146
$4*10^8$	*	*	235	172

* = 5 % AChE activity is not restored under these conditions

Table 6 The time period required to restore 5 % AChE activity (T_5, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the inhibition rate constant k_3 of C(±)P(-)-soman for AChE. $\cap$ = 0.35 min^{-1}

	T_5 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
k_3	EC/E_{tot}		EC/E_{tot}	
(M^{-1} . min^{-1})	0.3	0.6	0.3	0.6
$2.5*10^7$	*	145	157	110
$5*10^7$	*	*	183	131
$1*10^8$	*	*	211	154
$2*10^8$	*	*	240	176
$4*10^8$	*	*	265	203

* = 5 % AChE activity is not restored under these conditions

ANNEX 4 INFLUENCE OF THE OVERALL TOXICOKINETICS OF C(±)P(-)-SOMAN ON THE TIME PERIOD REQUIRED TO RESTORE 1-5 % ACHE ACTIVITY AFTER PRETREATMENT AND SUBSEQUENT INTOXICATION WITH 2-6 LD50 OF C(±)P(±)-SOMAN

Table 1 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the toxicokinetics of C(±)P(-)-soman. αβγ = actual toxicokinetics of C(±)P(-)-soman, αβ= toxicokinetics omitting γ-phase, αγ = toxicokinetics omitting β-phase, βγ = toxicokinetics omitting α-phase. $k_3 = 1*10^8$ $M^{-1}.min^{-1}$, ∩ = 0.35 min^{-1}, α = 3.9 min^{-1}, β= 0.20 min^{-1} and γ = 0.039 min^{-1}

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
Toxico-	EC/E_{tot}		EC/E_{tot}	
kinetics	0.3	0.6	0.3	0.6
αβγ	75	30	82	57
αβ	18	14	28	22
αγ	75	30	82	57
βγ	75	30	82	56

Table 2 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the toxicokinetics of C(±)P(-)-soman. αβγ = actual toxicokinetics of C(±)P(-)-soman, αβ= toxicokinetics omitting γ-phase, αγ = toxicokinetics omitting β-phase, βγ = toxicokinetics omitting α-phase. $k_3 = 1*10^8$ $M^{-1}.min^{-1}$, ∩ = 0.35 min^{-1}, α = 4.164 min^{-1}, β= 0.16 min^{-1} and γ = 0.042 min^{-1}

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
Toxico-	EC/E_{tot}		EC/E_{tot}	
kinetics	0.3	0.6	0.3	0.6
αβγ	135	94	110	89
αβ	26	25	41	34
αγ	153	94	110	89
βγ	153	94	110	89

Table 3 The time period required to restore 2 % AChE activity (T_2, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the toxicokinetics of C(±)P(-)-soman. αβγ = actual toxicokinetics of C(±)P(-)-soman, αβ= toxicokinetics omitting γ-phase, αγ = toxicokinetics omitting β-phase, βγ = toxicokinetics omitting α-phase. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, ∩ = 0.35 min^{-1}, α = 3.9 min^{-1}, β= 0.20 min^{-1} and γ = 0.039 min^{-1}

	T_2 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
Toxico-	EC/E_{tot}		EC/E_{tot}	
kinetics	0.3	0.6	0.3	0.6
αβγ	155	75	112	82
αβ	25	19	37	28
αγ	155	75	111	82
βγ	155	75	112	82

Table 4 The time period required to restore 2 % AChE activity (T_2, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the toxicokinetics of C(±)P(-)-soman. αβγ = actual toxicokinetics of C(±)P(-)-soman, αβ= toxicokinetics omitting γ-phase, αγ = toxicokinetics omitting β-phase, βγ = toxicokinetics omitting α-phase. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, ∩ = 0.35 min^{-1}, α = 4.2 min^{-1}, β= 0.16 min^{-1} and γ = 0.042 min^{-1}

	T_2 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
Toxico-	EC/E_{tot}		EC/E_{tot}	
kinetics	0.3	0.6	0.3	0.6
αβγ	*	152	142	110
αβ	32	36	52	41
αγ	*	152	142	110
βγ	*	152	142	110

* = 2 % AChE activity is not restored under these conditions

Table 5 The time period required to restore 5 % AChE activity (T_5, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the toxicokinetics of C(±)P(-)-soman. αβγ = actual toxicokinetics of C(±)P(-)-soman, αβ= toxicokinetics omitting γ-phase, αγ = toxicokinetics omitting β-phase, βγ = toxicokinetics omitting α-phase. k_3 = $1*10^8$ $M^{-1}.min^{-1}$, ∩ = 0.35 min^{-1}, α = 3.9 min^{-1}, β= 0.20 min^{-1} and γ = 0.039 min^{-1}

	T_5 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
Toxico-kinetics	EC/E_{tot} 0.3	0.6	EC/E_{tot} 0.3	0.6
αβγ	*	265	175	122
αβ	37	27	61	41
αγ	*	265	175	122
βγ	*	270	178	122

* = 5 % AChE activity is not restored under these conditions

Table 6 The time period required to restore 5 % AChE activity (T_5, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the toxicokinetics of C(±)P(-)-soman. αβγ = actual toxicokinetics of C(±)P(-)-soman, aβ= toxicokinetics omitting γ-phase, αγ = toxicokinetics omitting β-phase, βγ = toxicokinetics omitting α-phase. k_3 = $1*10^8$ $M^{-1}.min^{-1}$, ∩ = 0.35 min^{-1}, α = 4.2 min^{-1}, β= 0.16 min^{-1} and γ = 0.042 min^{-1}

	T_5 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
Toxico-kinetics	EC/E_{tot} 0.3	0.6	EC/E_{tot} 0.3	0.6
αβγ	*	*	211	154
αβ	44	45	78	56
αγ	*	*	211	154
βγ	*	*	211	154

* = 5 % AChE activity is not restored under these conditions

ANNEX 5 INFLUENCE OF THE ELIMINATION RATE CONSTANT OF C(±)P(-)-SOMAN ON THE TIME PERIOD REQUIRED TO RESTORE 1-5 % ACHE ACTIVITY AFTER PRETREATMENT AND SUBSEQUENT INTOXICATION WITH 2-6 LD50 OF C(±)P(±)-SOMAN

Table 1 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the elimination rate constant (γ, min^{-1}) of C(±)P(-)-soman. γ = 0.039 min^{-1} is the actual value. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap$ = 0.35 min^{-1}

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
γ (min^{-1})	0.3	0.6	0.3	0.6
0.156	20	15	30	23
0.078	27	19	42	30
0.039	75	30	82	57
0.020	*	*	195	132
0.010	*	*	882	478

* = 1 % AChE activity is not restored under these conditions

Table 2 The time period required to restore 1 % AChE activity (T_1, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the elimination rate constant (γ, min^{-1}) of C(±)P(-)-soman. γ = 0.042 min^{-1} is the actual value. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap$ = 0.35 min^{-1}

	T_1 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
γ (min^{-1})	0.3	0.6	0.3	0.6
0.168	33	26	42	35
0.084	46	35	56	45
0.042	153	94	110	89
0.021	*	*	266	207
0.010	*	*	*	777

* = 1 % AChE activity is not restored under these conditions

Table 3 The time period required to restore 2 % AChE activity (T_2, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the elimination rate constant (γ, min^{-1}) of C(±)P(-)-soman. γ = 0.039 min^{-1} is the actual value. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap$ = 0.35 min^{-1}

	T_2 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
γ (min^{-1})	0.3	0.6	0.3	0.6
0.156	27	20	39	23
0.078	40	27	57	30
0.039	155	75	111	82
0.020	*	*	270	132
0.010	*	*	*	478

* = 2 % AChE activity is not restored under these conditions

Table 4 The time period required to restore 2 % AChE activity (T_2, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the elimination rate constant (γ, min^{-1}) of C(±)P(-)-soman. γ = 0.042 min^{-1} is the actual value. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap$ = 0.35 min^{-1}

	T_2 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
γ (min^{-1})	0.3	0.6	0.3	0.6
0.168	42	33	52	42
0.084	61	46	71	56
0.042	*	152	142	110
0.021	*	*	349	267
0.010	*	*	*	*

* = 2 % AChE activity is not restored under these conditions

Table 5 The time period required to restore 5 % AChE activity (T_5, min) after pretreatment and subsequent intoxication with 2 LD50 of C(±)P(±)-soman, as a function of the elimination rate constant (γ, min^{-1}) of C(±)P(-)-soman. γ = 0.039 min^{-1} is the actual value. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap$ = 0.35 min^{-1}

	T_5 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
γ (min^{-1})	0.3	0.6	0.3	0.6
0.156	40	29	64	43
0.078	72	46	91	63
0.039	*	265	175	122
0.020	*	*	486	302
0.010	*	*	*	*

* = 5 % AChE activity is not restored under these conditions

Table 6 The time period required to restore 5 % AChE activity (T_5, min) after pretreatment and subsequent intoxication with 6 LD50 of C(±)P(±)-soman, as a function of the elimination rate constant (γ, min^{-1}) of C(±)P(-)-soman. γ = 0.042 min^{-1} is the actual value. $k_3 = 1*10^8\ M^{-1}.min^{-1}$, $\cap$ = 0.35 min^{-1}

	T_5 (min)			
	k_2 = 0.023 min^{-1}		k_2 = 0.0058 min^{-1}	
	EC/E_{tot}		EC/E_{tot}	
γ (min^{-1})	0.3	0.6	0.3	0.6
0.168	60	45	79	57
0.084	102	67	106	77
0.042	*	*	211	154
0.021	*	*	755	387
0.010	*	*	*	*

* = 5 % AChE activity is not restored under these conditions

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Computer simulation of the effect of pretreatment with reversible acetylcholinesterase inhibitors on the protection against soman poisoning
(Computer simulatie van het effect van voorbehandeling met reversibele acetylcholinesterase-remmers op de bescherming tegen soman-vergiftiging)

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15. ABSTRACT (MAXIMUM 200 WORDS (1044 BYTE))

A computerized model has been developed in order to obtain more insight into the factors that determine the efficacy of pretreatment against soman poisoning. The various kinetic processes involved are simulated, i.e., the inhibition of acetylcholinesterase (AChE) by the pretreatment compound, the spontaneous reactivation of this inhibited enzyme, the pharmacokinetics of the pretreatment compound, the inhibition of AChE by C(±)P(-)-soman and the toxico-kinetics of C(±)P(-)-soman. The values of the various parameters are either obtained from studies in the guinea pig or hypothetical.
With this model the time course of the fraction (1, 2 or 5 %) of active AChE is calculated subsequent to intoxication with doses corresponding with 2 or 6 LD50 of C(±)P(±)-soman.
The model indicates that the most important parameters are (i) the extent of AChE inhibition by the pretreatment compound at the moment of C(±)P(±)-soman poisoning, (ii) the rate constant for spontaneous reactivation of this inhibited enzyme and (iii) the toxicokinetics of C(±)P(-)-soman, especially the rate constant of elimination.
The pharmacokinetics and the inhibition rate constant of the pretreatment compound hardly influence the restoration of AChE activity.
The consequences of the modelling results for (pre)treatment of soman intoxication are discussed.

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