
2 Toxicokinetics of Nerve Agents

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I. INTRODUCTION

Toxicokinetic studies of nerve agents deal with the *in vivo* absorption, distribution, and elimination of these agents as a function of animal species, route of administration, dose, and time after administration. Such studies are essential to provide a quantitative basis for the toxicology of nerve agents and, in combination with toxicodynamic studies, are the starting point for development of causal treatment of intoxications with these agents. Toxicodynamic studies of nerve agents have been

the subject of a long and rich tradition of investigations since their introduction as potential agents of chemical warfare during World War II. These studies have led to (e.g., the development of prophylaxis of intoxication based on partial inhibition of cholinesterase activity with carbamates and therapy of intoxication through administration of the muscarinic cholinergic antagonist atropine) reactivation of phosphorylated cholinesterases with oximes, often in combination with the administration of a central nervous depressant in order to suppress convulsions and other central effects.

Toxicokinetic studies of nerve agents were initiated in the last two decennia of the twentieth century. The reasons for this relatively late development were twofold. First, it was often assumed that nerve agents, especially at supralethal doses, act so quickly and are so rapidly degraded *in vivo* that toxicokinetic studies were not relevant for treatment of intoxications, i.e., nerve agents should be regarded as so-called "hit and run poisons." Second, it was intuitively assumed that *in vivo* concentrations of the extremely toxic nerve agents are too low for bioanalysis. However, Wolthuis et al.¹ showed in 1981 that rats initially surviving a challenge with a supralethal dose of soman by immediate treatment with atropine and the oxime HI-6 became fatally re-intoxicated 4–6 h later. Hence, soman appeared to be far more persistent than previously assumed. This suggested that toxicokinetic investigations of nerve agents are toxicologically relevant, especially in view of the refractoriness of intoxication with these agents towards treatment. Moreover, the development of analytical techniques, particularly for gas chromatographic analyses of the rather volatile nerve agents, had evolved to a level that detection limits of a few picograms (10^{-12} g) of these agents became feasible. Finally, the development of chiral gas chromatography opened up the possibility to analyze the separate stereoisomers of nerve agents, which is a *conditio sine qua non* for toxicological interpretation of toxicokinetic studies of nerve agents see (Section II).

II. NERVE AGENT STEREOISOMERS: CHIRAL ANALYSIS, ISOLATION, AND TOXICOLOGY

Interpretation and understanding of the toxicokinetics of nerve agents would not be possible without taking into consideration that these agents consist of mixtures of stereoisomers, which are often extremely different in their toxicokinetic and toxicodynamic properties. A common feature of these agents is the presence of chirality (asymmetry) around the phosphorus atom. Therefore, O-isopropyl methylphosphonofluoridate (sarin) and O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate (VX) consist of equal amounts of stereoisomers, denoted as (+)- and (–)-sarin and (+)- and (–)-VX, respectively. In the case of O-1,2,2-trimethylpropyl methylphosphonofluoridate (soman), an additional chiral center resides in the 1,2,2-methylpropyl (pinacolyl) moiety, leading to the presence of four stereoisomers. Synthetic soman, i.e., a mixture of the four stereoisomers, is denoted as C(±)P(±)-soman, whereas the individual four stereoisomers are denoted as C(+)P(+), C(+)P(–), C(–)P(+), and C(–)P(–), in which C stands for chirality in the pinacolyl moiety and P for chirality around phosphorus. The enantiomeric pairs [C(+)P(+) + C(–)P(–)] and [C(+)P(–) + C(–)P(+)] are present in synthetic

C(\pm)P(\pm)-soman in a ratio of 45:55, with equal amounts of the two enantiomers within each pair.

Separation of the various stereoisomers of the nerve agents for analytical purposes became feasible with the advent of optically active coating materials for columns as used in capillary gas chromatography (GC) and in high performance liquid chromatography (HPLC). The complete separation of the four stereoisomers of soman and of the two stereoisomers of sarin with GC on capillary columns is described in Section III. So far, (+)- and (–)-VX could not be separated by means of capillary gas chromatography, but HPLC on a so-called Chiralcel OD-H column yields complete separation of the two stereoisomers of this agent.²

Using these analytical procedures to monitor progress, the four stereoisomers of soman, as well as (–)-sarin, could be isolated on a mg-scale for toxicological purposes by using judicious combinations of synthetic and enzymatic separation techniques.^{3–5} In the case of C(\pm)P(\pm)-soman, synthetic resolution of the stereoisomers of (\pm)-pinacolyl alcohol and subsequent synthesis of soman from these stereoisomers gave C(+)P(\pm)-soman and C(–)P(\pm)-soman, i.e., two diastereoisomeric mixtures of two soman stereoisomers.⁴ These pairs were separated enzymatically by incubation of C(+)P(\pm)- and C(–)P(\pm)-soman with α -chymotrypsin, which binds the P(–)-stereoisomers of soman. In this way C(+)P(+)- and C(–)P(+)-soman, respectively, could be isolated. Incubation with rabbit plasma hydrolyzes the P(+)-stereoisomers and provides therefore C(+)P(–)- and C(–)P(–)-soman. Similarly, incubation of (\pm)-sarin with α -chymotrypsin gave optically pure (–)-sarin. The two stereoisomers of VX are easily obtained synthetically from optically resolved precursors.⁵ X-ray analysis of the (+)-enantiomer of the O-ethyl analog of VX established the absolute configuration R for this analog and for (+)-VX,^{5,6} whereas the R-configuration for the P(+)-stereoisomers of sarin and soman has been determined beyond reasonable doubt by means of chemical correlation reactions with the above-mentioned precursors of V agents and from interaction of the most actively inhibiting stereoisomer with the active site of acetylcholinesterase (AChE).^{7–9}

With sufficient amounts of the various stereoisomers of the major nerve agents available, it became feasible to investigate the acute lethality of these stereoisomers.^{10–12} *A priori*, it should be expected that the degree of lethality will correlate with the inhibitory potency towards AChE. Therefore, bimolecular rate constants of inhibition of AChE with these stereoisomers were measured, as well as their LD₅₀ values in mice. A summary of the results is given in Table 2.1. Apparently, the P(–)-stereoisomers of soman and sarin inhibit AChE with rate constants which are 3–4 orders of magnitude higher than those of the corresponding P(+)-stereoisomers. At the time of these investigations, only the upper limit for the rate constants of the P(+)-stereoisomers could be determined due to the presence of trace amounts of the P(–)-stereoisomers. Concomitantly, it appeared that the P(–)-stereoisomers of soman are at least two orders of magnitude more acutely lethal than the P(+)-counterparts. For practical purposes the difference in acute lethality is such that the P(+)-stereoisomers should be regarded as a nontoxic impurity in synthetic soman, taking into consideration that the lower limit for the acute lethality of the P(+)-stereoisomers is difficult to determine in view of possible *in vivo* racemization. The same extreme differences will probably

TABLE 2.1

Stereoselectivity in Anticholinesterase Activity and Acute Lethality of Nerve Agent Stereoisomers

Nerve Agent Stereoisomer	Rate Constant for Inhibition of AChE ^a (M ⁻¹ min ⁻¹)	LD ₅₀ Mouse (μg/kg)	Ref.
C(+)-P(-)-soman	2.8×10^8	99 ^b	9
C(-)-P(-)-soman	1.8×10^8	38 ^b	9
C(+)-P(+)-soman	$<5 \times 10^3$	$>5000^b$	9
C(-)-P(+)-soman	$<5 \times 10^3$	$>2000^c$	9
C(±)-P(±)-soman		156 ^b	9
(-)-sarin	1.4×10^7	41 ^c	5, 10
(+)-sarin	$<3 \times 10^{3d}$		10
(±)-sarin		83 ^c	5
(-)-VX	4×10^8	12.6 ^c	5, 11
(+)-VX	2×10^6	165 ^c	5, 11
(±)-VX		20.1 ^c	5

^aElectric eel AChE (pH 7.5, 25°C) for soman stereoisomers; bovine erythrocyte AChE for sarin and VX stereoisomers (pH 7.7, 25°C).

^bSubcutaneous administration.

^cIntravenous administration.

^dEstimated from an experiment with optically enriched sarin (64% enantiomeric excess).

Source: From Benschof, H.P. and De Jong, L.P.A., *Acc. Chem. Res.*, 21, 368, 1988. With permission.

hold for (+)- and (-)-sarin, although this cannot be made explicit since methods to isolate optically pure (+)-sarin are not yet available.

In contrast to soman and sarin, the rate of inhibition of AChE by (+)-VX is only two orders of magnitude less than that of the (-)-stereoisomer. In this case the LD₅₀ of the (+)-stereoisomer could also be determined, revealing that (-)-VX is only 8-fold more acutely lethal than the (+)-stereoisomer.

Very recently, the P(+)-stereoisomers of soman could be exhaustively purified and the rate constants for inhibition of human AChE were determined.⁹ As shown in Table 2.2, the rates of inhibition of the P(-)- and P(+)-stereoisomers differ by 4–5 orders of magnitude, i.e., even one order of magnitude more than estimated previously for electric eel AChE (see Table 2.1). These data in combination with the absolute configuration of the soman stereoisomers, the detailed three-dimensional structure of the active site of human AChE based on X-ray analysis, and molecular modeling were used to create a detailed model of the Michaelis complex for the inhibition of the enzyme by the soman stereoisomers, the stability of which should be regarded as a reflection of the reactivity of the stereoisomer.⁹ Figure 2.1 gives these models for the C(-)P(-)- and C(-)P(+)-stereoisomers of soman. A detailed discussion of the interactions of the stereoisomers with the catalytic system of the active

TABLE 2.2

Rate Constants of Phosphonylation (k_i) of Human AChE by the Stereoisomers of C(\pm)P(\pm)-soman (pH 8.0, 24°C)

k_i ($10^4 \text{ M}^{-1} \text{ min}^{-1}$)			
C(+)-P(-)-soman	C(-)-P(-)-soman	C(+)-P(+)-soman	C(-)-P(+)-soman
$15,000 \pm 3,000$	$8,000 \pm 400$	0.2 ± 0.1	0.2 ± 0.1

Source: Data from Ref. 9.

site is beyond the scope of this book. However, it should be noted that the P = O bond has to be polarized by interactions in the so-called oxyanion hole. These conditions determine the position of the substituents in the pinacolyl moiety of the soman stereoisomers. It appears that the extremely low reactivity of the P(+)-stereoisomers is due to steric constraints which prevent accommodation of the bulky t-butyl group in the pinacolyl moiety and practically exclude it from the acyl pocket.

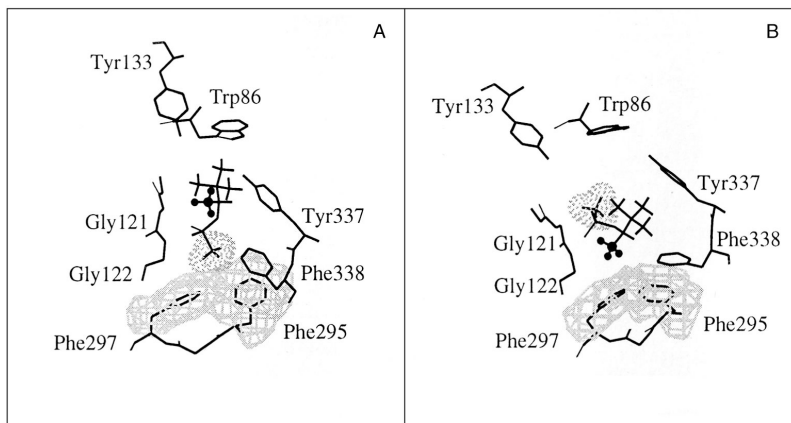


FIGURE 2.1 Michaelis complexes of human AChE with (a) C(-)-P(-) and (b) C(-)P(+) stereoisomers of soman. Only amino acids adjacent to the inhibitor are shown, while hydrogen atoms of the protein are omitted for clarity. The soman C α -methyl substituent is displayed as balls and sticks. Molecular volumes of the phosphorus methyl substituent and of the aromatic moieties of residues Phe295 and Phe297 are shown with dots and grids, respectively. Note that in the C(-)P(+)-soman-AChE complex, the acyl pocket cannot accommodate the bulky tert-butyl portion of the C(-)P(+)-soman alkoxy moiety and it points away from the phenyl groups defining this acyl pocket. (From Ordentlich, A., Barak, D., Kronman, C., Benschop, H.P., De Jong, L.P.A., Ariel, N., Barak, R., Segall, Y., Velan, B., and Shafferman, A., *Biochemistry*, 38, 3055, 1999. With permission.)

III. TRACE ANALYSIS OF NERVE AGENTS IN BIOLOGICAL SAMPLES

Toxicokinetic investigations of nerve agents are only relevant if these agents can be analyzed at minimum levels in blood or tissue samples which are still toxicologically significant. Such relevance in case of anticholinesterases should be related to their capacity to inhibit the enzyme AChE. Since nerve agents inhibit this enzyme with rate constants up to 10^8 – $10^9 \text{ M}^{-1} \text{ min}^{-1}$ (see Section II), it can be derived that blood levels down to a few picograms per milliliter blood (approximately 10^{-10} M) can still cause significant inhibition over a period of time of several hours. Obviously, the need for such extremely low minimum detectable concentrations requires analytical procedures which provide the utmost detection limits and selectivity. Moreover, as elucidated in the previous paragraph, differential analysis of the various stereoisomers of a nerve agent is also required.

Early attempts to investigate the *in vivo* disposition of the nerve agents (\pm)-sarin and C(\pm)P(\pm)-soman, and their metabolites were based on the use of ^3H -labeled agents.^{13–15} Although this approach affords sufficiently low minimum detectable concentrations, the separation of intact nerve agent and metabolites was based solely on liquid extraction schemes, which provide insufficient selectivity. In principle, the approach of Harris et al. based on the measurement of inhibition of bovine AChE added to the sample to be analyzed will provide concentrations of nerve agent stereoisomers having high anticholinesterase activity.¹⁶ This approach was mentioned in a preliminary paper dealing with the toxicokinetics of C(\pm)P(\pm)-soman, (\pm)-VX, and the VX-analog O-cyclopentyl S-diethylaminoethyl methylphosphonothiate in mechanically respiration rabbits and cynomolgus monkeys at very high doses of the nerve agents (10 – 30 LD_{50}).¹⁶

The relatively volatile nature of nerve agents, the extremely low detection limits of modern detectors for gas chromatography, and the recent advances in chiral separation in gas chromatography have led to the extensive use of this technique for toxicokinetic investigations of nerve agents. Primarily, the procedure was developed for analysis of the four stereoisomers of soman, based on separation of these stereoisomers on a capillary column coated with a derivative of L-valine bound to a siloxane backbone (Chirasil-L-Val).^{3,4} As shown in Figure 2.2, this column separates the C(+)P(+)- and C(–)P(+)-stereoisomers of soman perdeuterated in the pinacolyl moiety from the four stereoisomers of soman. Hence, the deuterated stereoisomers are highly useful internal standards for quantitation of soman stereoisomers, without resorting to the use of expensive mass spectrometric detection systems. Instead, highly sensitive alkali flame (NPD) and pulsed flame photometric (PFPD) detectors can be used with absolute detection limits for nerve agents of 1 – 5 pg . This is approximately one order of magnitude higher than can be obtained with single ion detection in tandem mass spectrometric detection, for which a detection limit of 0.1 pg is reported.¹⁷ In order to further increase the selectivity of the analytical procedure, a two-dimensional system was introduced in which a cut containing the analytes is trapped from a precolumn, e.g., a CPSil 8CB column, into a cold trap from which this cut is re-injected onto the chiral column by means of flash heating.

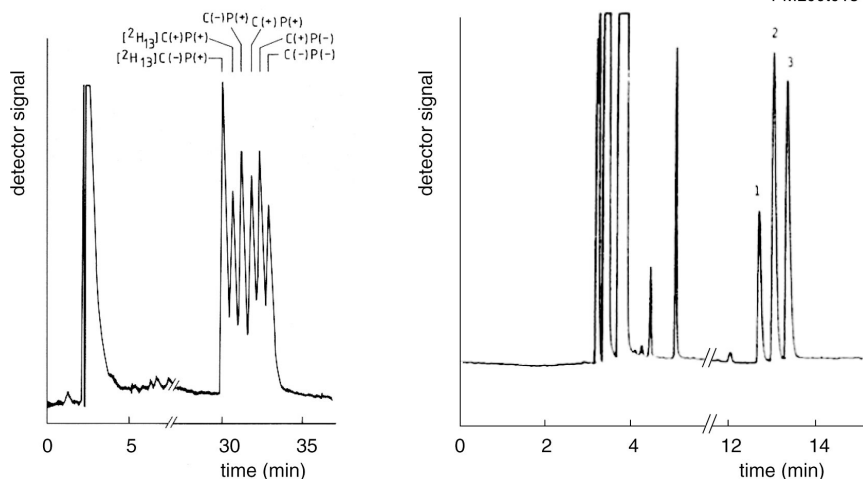


FIGURE 2.2 Gas chromatographic separation (left panel) of the four stereoisomers of soman and two deuterated stereoisomers on a Chirasil-L-Val column and (right panel) of the two stereoisomers of sarin (Peaks 2 and 3) and one deuterated stereoisomer (Peak 1) on a CycloDexB column. The deuterated stereoisomers are used as internal standards for quantitation of the stereoisomers. (Left panel from Benschop, H.P., Bijleveld, E.C., Otto, M.F., Degenhardt, C.E.A.M., Van Helden, H.P.M., and De Jong, L.P.A., *Anal. Biochem.*, 151, 242, 1985. With permission.)

The pronounced volatility of nerve agents, especially of sarin and soman, prevents their concentration into a sufficiently small sample volume of ca. 1–5 μl for injection into the two-dimensional gas chromatograph. Therefore, an on-line large volume injection system was introduced based on the application of the analytes in an organic solvent, up to a volume of 500 μl on Tenax absorption material, from which the solvent is blown off selectively. Next, the analytes are thermally desorbed from the Tenax absorbent into a cold trap for subsequent injection into the two-dimensional system by means of flash heating. A schematic drawing of the complete analytical system is given in [Figure 2.3](#).

For analysis of the stereoisomers of sarin, the optically active Chirasil-L-Val column is replaced by an optically active CyclodexB column coated with β -cyclodextrin.¹⁸ As shown in Figure 2.2, this column separates the two stereoisomers of sarin from the two stereoisomers of sarin which are perdeuterated in the isopropyl moiety. Therefore, these deuterated stereoisomers are convenient internal standards for analysis of sarin stereoisomers in biological samples.

When properly installed, the analytical system involving thermodesorption cold trap injection and two-dimensional chromatography can be used routinely for analysis of the stereoisomers of soman and sarin in blood and tissue samples at minimum detectable concentrations of 1–5 pg of stereoisomer per ml blood or gram tissue. In recent toxicokinetic experiments in pigs,¹⁷ the analytical system comprised chiral gas chromatography on a Chirasil-L-Val column with splitless injection and detection

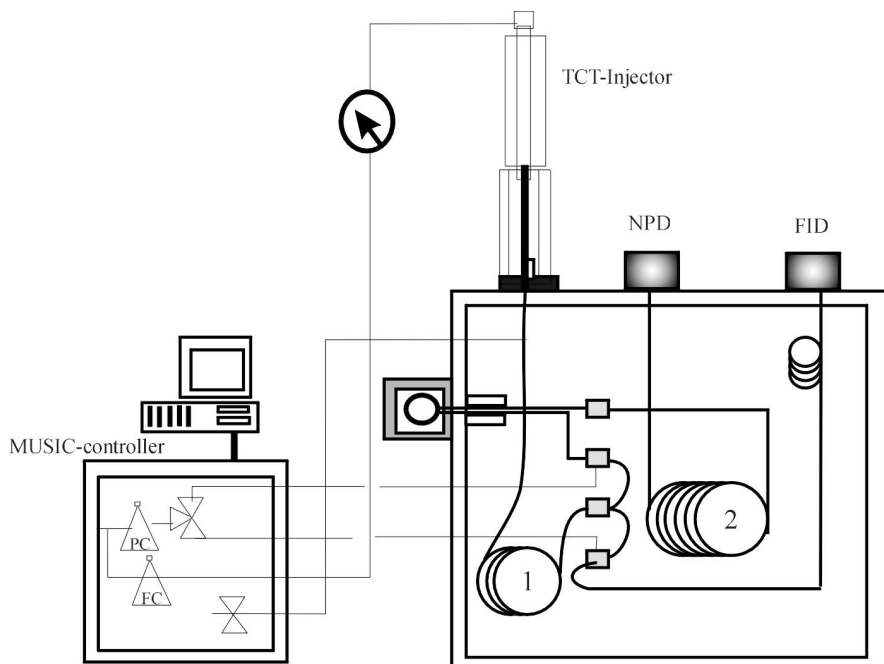


FIGURE 2.3 Scheme for analysis of nerve agent stereoisomers with a two-dimensional (MUSIC) gas chromatographic system with thermodesorption-cold trap (TCT) injection: 1, pre-column; 2, analytical column; PC and FC, constant pressure and constant flow controllers, respectively; NPD and FID, detectors.

with a hybrid tandem mass spectrometer in the single ion mode, yielding approximately the same minimum detectable concentration as the two-dimensional system.

In vivo, the stereoisomers of soman and sarin are subject to rapid processes of elimination, including spontaneous and enzymatically catalyzed hydrolysis and phosphorylation of protein binding sites (see Section VIII). These processes should be “frozen” at the moment that the sample is taken for a period of time that is sufficient for further work-up. Stabilization procedures of nerve agent stereoisomers in biological samples were developed with stringent validation for all separate stereoisomers since these have widely differing rates of degradation.⁴ It appeared that spontaneous and enzymatic hydrolysis of nerve agent stereoisomers can be sufficiently suppressed by immediate acidification of the sample to pH 4 with an acetate buffer. This was validated by adding known amounts of soman to rat blood samples that had been pre-incubated with soman in order to “saturate” irreversible binding sites and from which excess of soman had been removed. However, it then appeared that fluoride ions in the blood, present either from natural sources or from hydrolysis of soman, reactivated soman from phosphorylated binding sites such as carboxylesterases (CaE) which led to substantially higher levels of soman in the samples than added for the purpose of validation. This complication was effectively suppressed by addition of aluminum

sulfate which binds fluoride ions, mostly in the complex $[\text{AlF}_2^+]$. Finally, binding of soman stereoisomers to unoccupied phosphorylation sites, which can be an overriding phenomenon when investigating the toxicokinetics at low level exposures, is blocked effectively by adding a large excess of O-neopentyl methylphosphonofluoridate (neopentyl sarin). This agent saturates the unoccupied binding sites without interfering with the gas chromatographic analysis.⁴

The combined use of acidification to pH 4, addition of aluminum sulfate and of neopentyl sarin, proved to be sufficient to stabilize the stereoisomers of sarin and soman. In subsequent work-up, the analytes and internal standard are extracted from the stabilized blood or tissue sample by means of solid-phase extraction and elution with ethyl acetate, for gas chromatographic analysis. The same work-up and analytical procedure can be used for homogenized brain and diaphragm tissue samples.

IV. INTRAVENOUS TOXICOKINETICS OF SOMAN AND SARIN IN VARIOUS SPECIES

Initial investigations on the toxicokinetics of nerve agents were performed after intravenous (i.v.) administration of doses corresponding with multiple LD_{50} values. This route of administration provides basic toxicokinetic data, which can subsequently be compared with results for more realistic routes of administration, e.g., the subcutaneous (s.c.), percutaneous (p.c.), and respiratory routes. With gradually improving methods of bioanalysis, the administered doses could be lowered. Nevertheless, data obtained at multiple LD_{50} values are highly relevant since these pertain to exposure scenarios where immediate medical treatment of casualties should be applied.

Animal species selected for initial investigations were rats, guinea pigs, and marmosets, with the latter species serving as a primate model for man. In order to perform toxicokinetic measurements at high doses, the anesthetized* animals were provided with a tracheal cannula for artificial respiration and with a carotid cannula. Shortly before administration of nerve agent in the dorsal penis vein, the animals were atropinized intraperitoneally (i.p.) and blood samples were taken from the carotid cannula at various points of time after intoxication for analysis of nerve agent stereoisomers. Blood levels of the individual soman stereoisomers in rats and guinea pigs were measured at each time point randomly in at least six animals, while complete toxicokinetic curves were measured in each individual marmoset.

The LD_{50} values of $\text{C}(\pm)\text{P}(\pm)$ -soman are highly species-dependent, since the amount of CaE in the blood is species-dependent. These enzymes act as scavengers of nerve agents by means of irreversible binding and are present in large amounts in the blood of rats, in significantly smaller amounts in guinea pigs, and are almost absent in the blood of marmosets. Accordingly, the LD_{50} values decrease in the order rat > guinea pig > marmoset.

Blood levels in rats of the relatively nontoxic $\text{C}(-)\text{P}(+)$ -stereoisomer at doses of 6 and 3 LD_{50} of $\text{C}(\pm)\text{P}(\pm)$ -soman are shown in Figure 2.4.¹⁹ It was observed that

*Sodium barbital/sodium hexobarbital (i.p.).

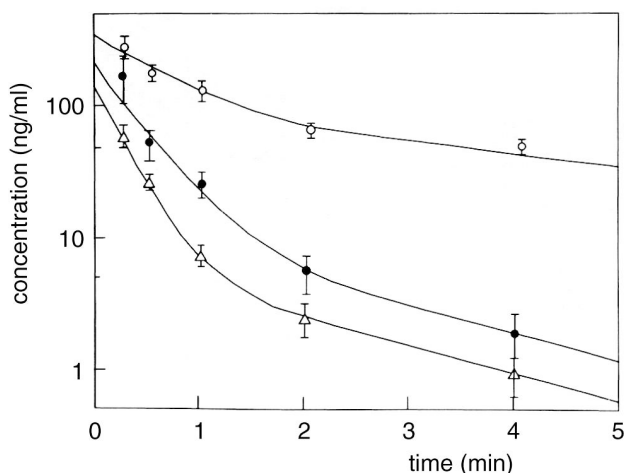


FIGURE 2.4 Semilogarithmic plot of the concentrations in blood (\pm s.e.m; $n = 6$) of C(-)P(+)-soman vs. time after i.v. administration of 3 (Δ , 248 $\mu\text{g/kg}$) and 6 (\bullet) LD_{50} of C(\pm)P(\pm)-soman to anesthetized, atropinized, and mechanically ventilated rats. The lines represent optimal fits of bi-exponential functions to the data. The early data points from the tri-exponential curve of the C(+)-P(-)-stereoisomer (\circ) at a dose of 6 LD_{50} are also given. (From Benschop, H.P. and De Jong, L.P.A., *Neurosci. Biobehav. Rev.*, 15, 73, 1991. With permission.)

the C(+)-P(+)-stereoisomer has completely disappeared from the bloodstream at all doses and in all species at the first time point of analysis, i.e., at 0.3 min after administration. The C(-)P(+)-stereoisomer is somewhat more stable and can be observed for approximately 4 min in rats (see Figure 2.4) and guinea pigs, whereas it disappears completely from the bloodstream of marmosets within 1 min. The rapid decrease of the concentration of the C(\pm)P(+)-stereoisomers is largely due to rapid enzymatic hydrolysis (see Section VIII).

Figures 2.5–2.7 give a survey of the concentrations in blood of the highly toxic C(+)-P(-)- and C(-)P(-)-stereoisomers of C(\pm)P(\pm)-soman at doses varying from 0.8–6 LD_{50} in rats, guinea pigs, and marmosets, respectively.^{19,20} In contrast with the C(\pm)P(+)-stereoisomers, the highly toxic C(+)-P(-)- and C(-)P(-)-stereoisomers of soman can be measured in all species for periods of almost 1 h up to several hours, depending on the species and on the dose, in spite of very steep initial decline of all blood levels (see insets in Figures 2.5–2.7) due to rapid distribution and covalent binding (see Section VIII). A summary of toxicokinetic data for the toxic stereoisomers of soman is given in Table 2.3. All toxicokinetic curves are best described with three-exponential equations, except for those at the lowest dose (1 LD_{50}) in rats and guinea pigs (0.8 LD_{50}), for which the data can be fitted to a two-exponential equation. Areas under the curve (AUC) and terminal half lives have been calculated from these equations.

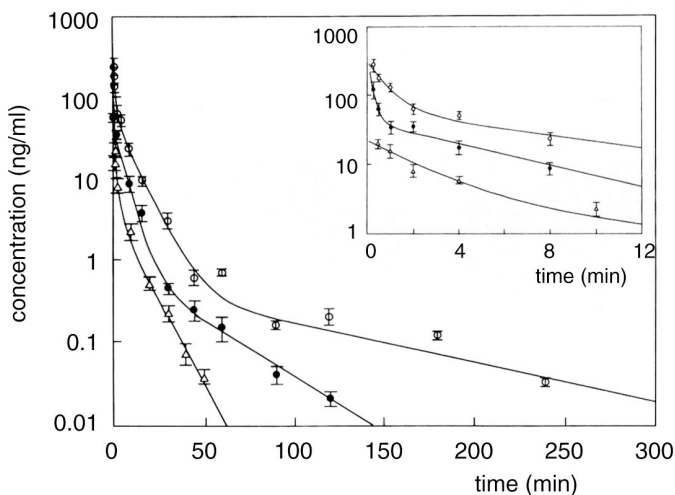


FIGURE 2.5 Semilogarithmic plot of the concentrations in blood (\pm s.e.m; $n = 6$) of C(+)P(–)-soman vs. time after i.v. administration of 1 (Δ , 83 $\mu\text{g/kg}$), 3 (\bullet), and 6 (\circ) LD_{50} of C(\pm)P(\pm)-soman to anesthetized, atropinized, and mechanically ventilated rats. The lines represent optimal fits of a bi-exponential function to the data at a dose of 1 LD_{50} , and of tri-exponential functions at higher doses. The inset shows the data for the first 12 min plotted on an expanded scale. (From Benschop, H.P. and De Jong, L.P.A., *Neurosci. Biobehav. Rev.*, 15, 73, 1991. With permission.)

The derivation of the time period during which acutely toxic levels of (summed) C(\pm)P(–)-soman stereoisomers are present is based, somewhat arbitrarily, on a scenario of intoxication in which an animal resumes spontaneous respiration presumably due to about 5–7% reactivation by oxime (or protection by carbamate) of completely inhibited AChE in diaphragm. Since the concentration of AChE in diaphragm of guinea pigs is approximately 2–2.6 nM, this reactivated fraction corresponds with approximately 150–200 pM AChE. Based on a bimolecular rate constant for inhibition of AChE by C(\pm)P(–)-soman of about $10^8 \text{ M}^{-1}\text{min}^{-1}$, it is calculated that this reactivated fraction of AChE can be re-inhibited by 150 pM (30 $\mu\text{g} \cdot \text{ml}^{-1}$) of C(\pm)P(–)-soman with a half life of about 1 h. An order of magnitude lower concentration of C(\pm)P(–)-soman can only cause toxicologically insignificant re-inhibition. Therefore it is assumed that 150 pM C(\pm)P(–)-soman represents approximately the lowest concentration having toxicological relevance. In a more generalized way, it may be reasoned that an area under the curve (AUC) of $30 \mu\text{g} \cdot \text{ml}^{-1} \times 60 \text{ min} = 1.8 \text{ ng} \cdot \text{min} \cdot \text{ml}^{-1}$ in the last part of the blood level curve is needed for toxicological relevance. The period of time in between intoxication and the point on the time axis at which this area starts can be regarded as the period of time in which toxicologically relevant levels of C(\pm)P(–)-soman are present.

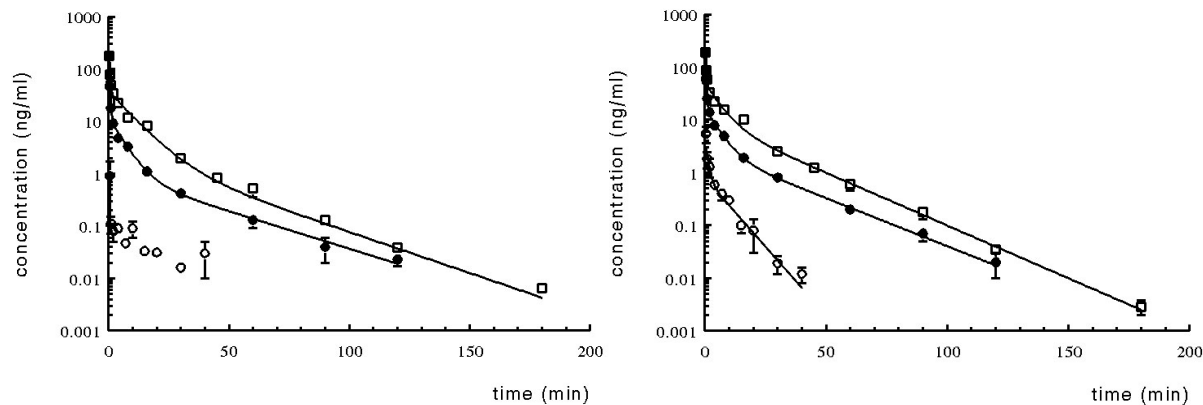


FIGURE 2.6 Semilogarithmic plot of the concentrations in blood (\pm s.e.m; $n = 6$) of (left panel) C(+)P(-)-soman and of (right panel) C(-)P(-)-soman vs. time after i.v. administration of 0.8 (\circ , 22 $\mu\text{g/kg}$), 2 (\bullet), and 6 (\square) LD₅₀ of C(\pm)P(\pm)-soman to anesthetized, atropinized, and mechanically ventilated guinea pigs. The lines represent optimal fits of a bi-exponential function to the data at a dose of 0.8 LD₅₀, and of tri-exponential functions at higher doses.

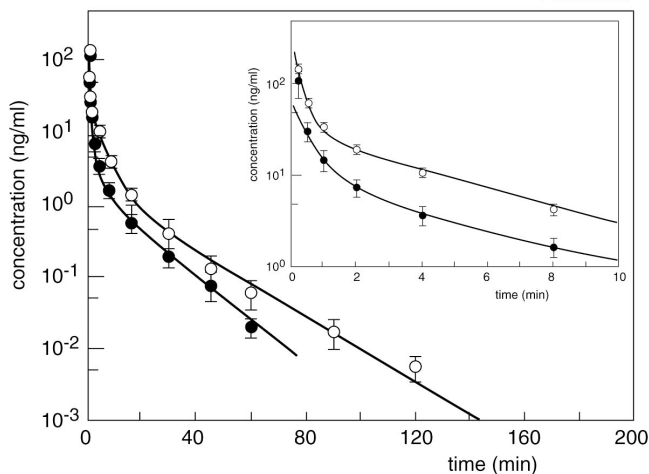


FIGURE 2.7 Semilogarithmic plot of the concentrations in blood (\pm s.e.m; $n = 6$) of C(+)-P(-)-soman vs. time after i.v. administration of 2 (●, 20 $\mu\text{g/kg}$) and 6 LD₅₀ (○) of C(\pm)-P(\pm)-soman to anaesthetized, atropinized, and mechanically ventilated marmosets. The lines represent optimal fits of tri-exponential functions to the data. The inset shows the data for the first 10 min plotted on an expanded scale.

Based on the earlier mentioned ratio of stereoisomers in C(\pm)-P(\pm)-soman and on equal bioavailability of C(+)-P(-)- and C(-)-P(-)-soman, it should be expected that the AUC for C(+)-P(-)-soman should be approximately 20% larger than those of C(-)-P(-)-soman in the same experiment. Instead, it is observed that the AUC for C(+)-P(-)-soman is at most equal to but often smaller than that of the other stereoisomer. This phenomenon could be made plausible by measuring the rate constants of inhibition of CaE by the two stereoisomers. These enzymes are considered as the major covalent binding sites in blood for these two stereoisomers (see Section VIII). It appeared that the C(+)-P(-)-stereoisomer reacts 30-fold faster with CaE in guinea pig blood than the C(-)-P(-)-stereoisomer, which may explain the lesser bioavailability of C(+)-P(-)-soman. The most striking example of this “stereospecific bioavailability” is observed at a dose of 0.8 LD₅₀ in rats. In this case the C(+)-P(-)-stereoisomer is almost instantly scavenged and only blood levels of the C(-)-P(-)-stereoisomer can be measured (see Section VIII).

As mentioned before, the amounts of CaE in blood decrease in the order rat > guinea pig > marmoset. Accordingly, it can be seen from the AUC in Table 2.4 that the intraspecies linearity with dose for the toxicokinetics of the two toxic stereoisomers of soman is reasonable in marmosets and in guinea pigs. Similarly, a reasonable “interspecies linearity” exists between guinea pigs and marmosets as indicated, e.g., by the AUC in guinea pigs at 2 LD₅₀ with that in marmosets at a dose of 6 LD₅₀. In contrast, the AUC pertaining to 6 LD₅₀ in marmosets is 2.5 times larger than that at 1 LD₅₀ in rats, while the absolute dose in marmosets is approximately 30% less.

TABLE 2.3
Survey of Toxicokinetic Parameters^a of C(+)P(–)- and C(–)P(–)-soman in Anesthetized, Atropinized, and Mechanically Ventilated Rats, Guinea Pigs, and Marmosets at Intravenous Doses Corresponding with 0.8 LD₅₀ of C(±)P(±)-soman

Parameter	Rat						Guinea pig						Marmoset			
	6 LD ₅₀		3 LD ₅₀		1 LD ₅₀		6 LD ₅₀		2 LD ₅₀		0.8 LD ₅₀		6 LD ₅₀		2 LD ₅₀	
	C(+)P(–)	C(–)P(–)	C(+)P(–)	C(–)P(–)	C(+)P(–)	C(–)P(–)	C(+)P(–)	C(–)P(–)	C(+)P(–)	C(–)P(–)	C(+)P(–)C(–)P(–)		C(+)P(–)	C(–)P(–)	C(+)P(–)	C(–)P(–)
Dose ^a (μg/kg)	136	111	68	56	22.7	18.6	45.4	37.1	15.1	12.4	6.0	5.0	16.5	13.5	5.5	4.5
A (ng/ml)	253	233	301	259	18	15	339	406	318	354	–	3.8	285	172	61	52
B (ng/ml)	63	61	41	37	3.9	5.9	35	40	11	15	–	0.80	30	22	9.9	9.1
C (ng/ml)	0.55	1.0	0.9	1.1	–	–	2.8	9.9	1.0	1.7	–	–	1.9	1.6	1.8	2.1
a (min ^{–1})	1.3	1.2	5.0	4.7	0.45	0.57	3.8	4.3	3.8	3.9	–	0.95	3.9	3.0	2.2	2.0
b (min ^{–1})	0.11	0.10	0.19	0.15	0.096	0.12	0.12	0.19	0.19	0.21	–	0.12	0.27	0.22	0.35	0.30
c (min ^{–1})	0.011	0.017	0.032	0.042	–	–	0.034	0.046	0.033	0.042	–	–	0.052	0.047	0.073	0.073
Terminal half-life (min)	64	40	22	16	7	6	20	15	21	16.5	–	5.8	13	15	9.5	9.4
Area under curve (ng · min/ml)	806	877	308	320	81	76	458	520	169	228	–	10.6	218	191	81	85
Acutely toxic levels C(±)P(–)-soman until (min) ^b	317		95		37		126		104		–		74		49	

Note: The concentration of each isomer at time t (conc_t) is described by: conc_t = Ae^{–at} + Be^{–bt} + Ce^{–ct}.

^aCalculated on the basis of 55/45 ratio of the [C(+)P(–) + C(–)P(+)]-stereoisomers and the [C(+)P(+) + C(–)P(–)]-stereoisomers.

^bAfter administration of C(±)P(±)-soman. It is assumed that the area under the curve of 1.8 ng.min/ml for C(±)P(–)-soman is the minimum area with toxicological relevance.

TABLE 2.4

Toxicokinetic Parameters of (–)-sarin and C(–)P(–)-soman after i.v. Administration of 0.8 LD₅₀ (19.2 μ/kg) of (±)-sarin and 0.8 LD₅₀ of C(±)P(±)-soman (22 μ/kg) to Anesthetized, Atropinized, and Mechanically Ventilated Guinea Pigs

Parameter	(–)-sarin	C(–)P(–)-soman
Dose (μg/kg)	9.6	4.95
A (ng · ml ⁻¹)	35.9	3.75
B (ng · ml ⁻¹)	0.09	0.80
a (min ⁻¹)	4.6	0.95
b (min ⁻¹)	0.012	0.12
Distribution half-life (min)	0.2	0.7
Terminal half-life (min)	58	5.8
Area under curve (ng · min · ml ⁻¹)	15.3	11

Note: The concentration of each stereoisomer at time *t* (conc_{*t*}) is described by $\text{conc}_t = Ae^{-at} + Be^{-bt}$.

Toxicokinetics in rats are strikingly nonlinear. Evidently, disproportionately large amounts of soman stereoisomers are consumed by CaE at low dose in the rat. Since this is a stoichiometric process, scavenging at higher dose consumes a smaller fraction of the total dose. Of necessity, this phenomenon related to the large amount of CaE in rat blood leads to a period of time of more than 5 h during which toxicologically relevant concentrations of C(±)P(–)-soman are present (see [Figure 2.5](#)). Therefore, it is not surprising that Wolthuis et al.¹ observed fatal re-intoxication in rats challenged at a dose corresponding with 6 LD₅₀ while these animals were initially saved by immediate treatment with atropine and HI-6. Neither is it surprising in view of the data in [Table 2.3](#) that this re-intoxication phenomenon was not observed at lower dose in the rat or at any dose (≤6 LD₅₀) in guinea pigs or in marmosets. In view of the large discrepancies in toxicokinetics between rats on the one hand and guinea pigs and marmosets on the other hand, guinea pigs are considered better model animals for primates than rats in toxicological and therapeutic investigations for nerve-agent intoxication. Pretreatment of rats with the specific CaE inhibitor 2-(*o*-cresyl)-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (CBDP) blocks binding of nerve agents to these enzymes completely in blood and lungs, and partially in kidney and liver.²¹ The LD₅₀ of C(±)P(±)-soman in these pretreated animals is lowered to a value in the same range as that in marmosets. Accordingly, the AUC of C(+)P(–)-soman and C(–)P(–)-soman at a dose of 6 LD₅₀ in the “CBDP-rats” is lowered to the same range as the AUC in marmosets at a dose of 6 LD₅₀.²²

Levels of soman stereoisomers in tissues rather than in blood have been measured to a limited degree in brain and diaphragm, which are considered target organs for central and peripheral toxic effects of nerve agents, respectively.²³ [Figure 2.8](#) shows the levels of C(+)P(–)-soman and of C(–)P(–)-soman decreasing with time in blood, diaphragm, and homogenized brain samples of rats upon i.v. administration of a dose

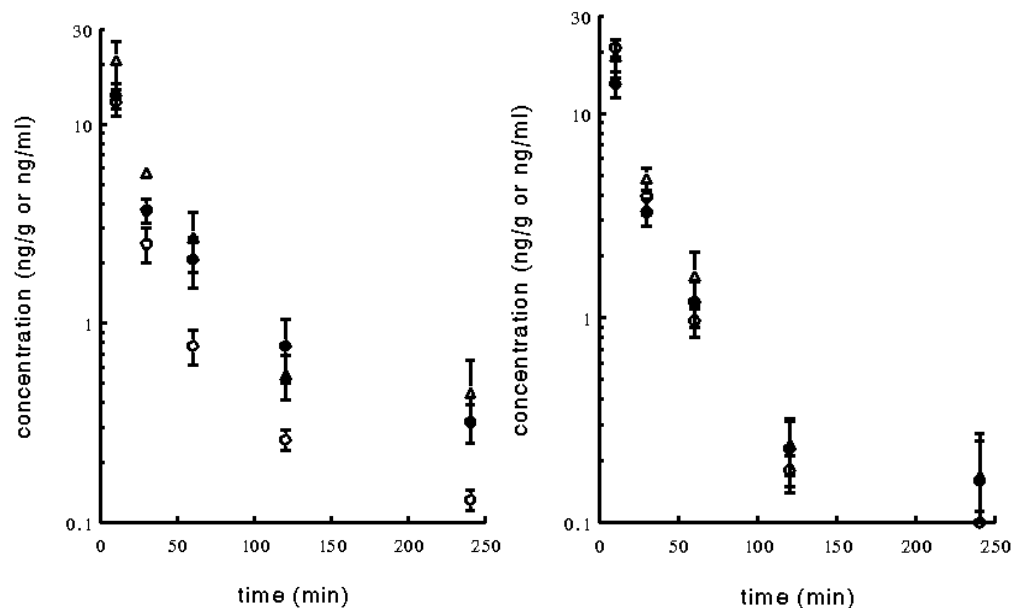


FIGURE 2.8 Semilogarithmic plot of the concentrations of (left panel) C(+)-P(-)-soman and of (right panel) C(-)-P(-)-soman (\pm s.e.m.; $n = 6-8$) in brain (●), diaphragm (○), and blood (Δ) after i.v. administration of 6 LD₅₀ (495 µg/kg) of C(±)-P(±)-soman to anesthetized, atropinized, and mechanically ventilated rats.

corresponding with 6 LD₅₀ of C(±)P(±)-soman. The levels of C(+)P(-)-soman in brain appeared to be significantly (Scheffé F-tests, $p < 0.05$) lower than those in blood, which is not the case for the C(-)P(-)-stereoisomer. Due to experimental restrictions, the first time point is at 10 min after administration of C(±)P(±)-soman. Interestingly, in recent toxicokinetic experiments in pigs at i.v. doses of 0.75–3 LD₅₀, C(+)P(-)- and C(-)P(-)-soman were detected in cerebrospinal fluid (CSF) at > 1 min after administration and were observed to increase for an initial period of 3 min.¹⁷ Evidently, the toxic stereoisomers of soman penetrate rapidly through the blood-brain barrier, which is in accordance with the pronounced central effects upon intoxication with C(±)P(±)-soman.

In comparison with C(±)P(±)-soman, little work has been done so far on the i.v. toxicokinetics of (±)-sarin. In order to obtain reference data for the inhalation toxicokinetics of (±)-sarin (see Section VI), the i.v. toxicokinetics of (±)-sarin was investigated at a sublethal dose of 0.8 LD₅₀ in anesthetized, atropinized, and mechanically ventilated guinea pigs.^{18,24} Blood levels of (-)-sarin vs. time after administration are shown in Figure 2.9, in which the blood concentration-time curve for C(-)P(-)-soman at equitoxic dose (0.8 LD₅₀) is also given for comparison. The toxicokinetic parameters derived from these two curves, as summarized in Table 2.4, show that a striking difference can be noted in the toxicokinetics of the two nerve agents. While the distribution phase of (-)-sarin is nearly an order of magnitude more rapid than that of C(-)P(-)-soman, its elimination phase is approximately an order of magnitude slower. The latter finding is rather surprising since (-)-sarin was not expected to be more persistent than C(-)P(-)-soman. Further studies should

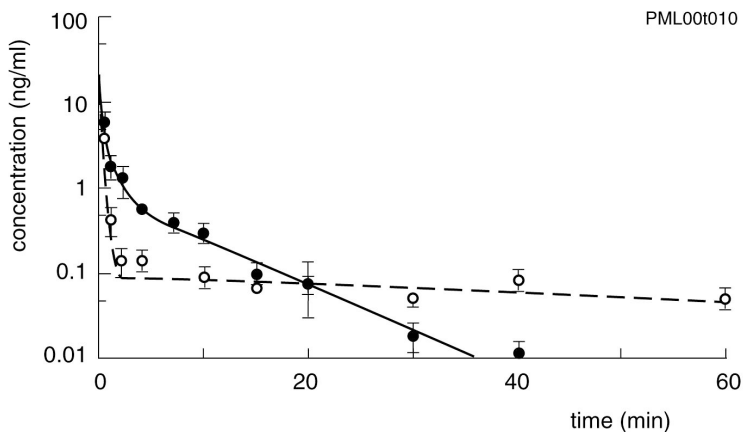


FIGURE 2.9 Semilogarithmic plot of the mean concentrations in blood (\pm s.e.m.; $n = 6$) of (-)-sarin (○) vs. time after i.v. bolus administration of 0.8 LD₅₀ (19.2 $\mu\text{g/kg}$) of (±)-sarin to anaesthetized, atropinized, and mechanically ventilated guinea pigs. For comparison the concentration-time course of C(-)P(-)-soman (●) at an equitoxic dose (i.v. 22 $\mu\text{g/kg}$) of C(±)P(±)-soman is also shown.

reveal whether slower rates of enzymatic hydrolysis and of binding might explain the relative *in vivo* persistence of (–)-sarin. Although (–)-sarin is rather persistent, it can only inhibit blood AChE (rate constant $10^7 \text{ M}^{-1} \text{ min}^{-1}$) up to 40% during the elimination phase, due to the relatively low concentration, i.e., $< 0.09 \text{ ng/ml}$ (see Table 2.4).

V. SUBCUTANEOUS TOXICOKINETICS OF SOMAN

Animal exposure to volatile nerve agents by way of the most realistic route of exposure, i.e., respiratory exposure with concomitant absorption in the respiratory tract, is complex and difficult to analyze experimentally. Intramuscular (i.m.) and s.c. administration of the agent are often considered reasonable substitutes for respiratory exposure in efficacy studies on treatment and pretreatment against nerve agent poisoning. In order to investigate whether the toxicokinetics of nerve agents are reasonably similar upon respiratory exposure (see Section VI) and subcutaneous administration, Due et al.²⁵ investigated the toxicokinetics of C(±)P(±)-soman in anesthetized,* atropinized, and mechanically ventilated guinea pigs after a bolus injection of a dose corresponding to 6 LD₅₀ (148 µg/kg) in the scruff of the neck. Averaged results for the C(+)P(–)- and C(–)P(–)-stereoisomers are shown in Figure 2.10. The toxicokinetics were regarded as a discontinuous process, with a mono-exponential equation for the absorption phase and a bi-exponential equation for the distribution phase. The derived toxicokinetic data are summarized in Table 2.5, in which the bioavailability upon s.c. administration is defined as the ratio of the AUC upon s.c. and i.v. administration.

As evident from Figure 2.10, the blood levels of the C(±)P(–)-stereoisomers have reached clearly measurable levels within 1 min after administration, which shows that these toxic stereoisomers of soman rapidly penetrate the walls of the capillary vessels at the site of injection. In contrast, the C(±)P(+)-stereoisomers of soman never surpassed the minimum detectable concentration (approximately 5 pg/ml) in blood. This example of almost absolute stereospecificity in the absorption phase is readily explained by the ubiquitous presence of phosphoryl phosphatases in blood, skin, and other tissues. These enzymes rapidly hydrolyze the C(±)P(+)-stereoisomers of soman and are presumably available in extra amounts due to tissue damage at the site of injection. Figure 2.10 also shows that the blood levels of the C(±)P(–)-stereoisomers of soman increase during the first 7 min after administration, from which a half-life of absorption of 3.2–3.6 min is derived (see Table 2.5). In comparison with the C(–)P(–)-stereoisomer, the absorption of the C(+)P(–)-stereoisomers is clearly lagging behind. The AUC of the latter stereoisomer is significantly (27%) less than that of the C(–)P(–)-stereoisomer, in spite of the large excess (23%) of the C(+)P(–)-stereoisomer in C(±)P(±)-soman. As in the case of i.v. administration (see Section IV), this decreased bioavailability of C(+)P(–)-soman is explained by its more rapid covalent binding (30-fold in blood) than of the C(–)P(–)-stereoisomer to CaE, which is a major route of elimination for these stereoisomers (see Section VIII). Nevertheless, the

*Hynorm® (im)/Nembutal® (ip)

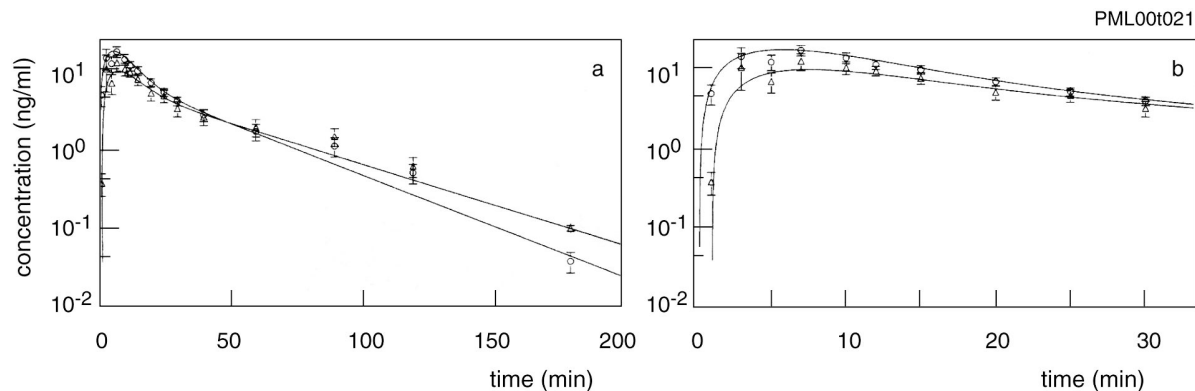


FIGURE 2.10 Semilogarithmic plots of the concentrations (\pm s.e.m.; $n = 6-8$) in blood of $C(+)-P(-)$ -soman (Δ) and of $C(-)-P(-)$ -soman (\odot) vs. time after s.c. bolus administration of $6 LD_{50}$ ($148 \mu\text{g/kg}$) $C(\pm)-P(\pm)$ -soman to anaesthetized, atropinized, and mechanically ventilated guinea pigs. Left panel: all data; right panel: data for the first 35 min plotted on an expanded time scale. (From Due, A.H., Trap, H.C., Langenberg, J.P., and Benschop, H.P., *Arch. Toxicol.*, 68, 60, 1994. With permission.)

TABLE 2.5

Toxicokinetic Parameters of C(+)-P(-)-soman and C(-)-P(-)-soman after s.c. Administration of 6 LD₅₀ (148 µg/kg) of C(±)P(±)-soman to Anesthetized, Atropinized, and Mechanically Ventilated Guinea Pigs

Parameter	C(+)-P(-)-soman	C(-)-P(-)-soman
Dose ^a (µg/kg)	40	33
A (ng · ml ⁻¹)	348	328
B (ng · ml ⁻¹)	337	318
C (ng/ml ⁻¹)	6.2	8.3
a (min ⁻¹)	0.20	0.22
b (min ⁻¹)	0.19	0.20
c (min ⁻¹)	0.023	0.029
Half-life absorption (min)	3.6	3.2
Terminal half-life (min)	30	24
Area under curve ^b (ng · min · ml ⁻¹)	303 (411)	385 (466)
Biological availability ^c (%)	74	83
Acutely toxic levels	228	
C(±)P(-)-soman until ^d (min)		

Note: The concentration of each stereoisomer at time t (conc_{*t*}) is described by $\text{conc}_t = -Ae^{-at} + Be^{-bt} + Ce^{-ct}$, in which the first exponential function describes the absorption phase.

^aCalculated on the basis of 55/45 ratio of the [C(+)-P(-) + C(-)-P(+)]-stereoisomers and the [C(-)-P(-) + C(+)-P(+)]-stereoisomers.

^bThe AUC after i.v. administration of the same dose of C(±)P(±)-soman is given between brackets, assuming linearity with dose upon a dose reduction from 165 to 148 µg/kg.

^cThe biological availability is calculated as the ratio between the AUC after s.c. and i.v. administration $\times 100\%$.

^dIt is assumed that an AUC of 1.8 ng · min · ml⁻¹ for C(±)P(-)-soman is the minimum area with toxicological relevance.

Source: From Due, A.H., Trap, H.C., Langenberg, J.P., and Benschop, H.P., *Arch Toxicol.*, 68, 60, 1994. With permission.

relative bioavailability of the C(+)-P(-)-stereoisomer (74%) is only slightly less than that of the C(-)-P(-)-stereoisomer (83%), since this parameter is calculated relative to the AUC for i.v. administration where the same stereospecific elimination of C(+)-P(-)-soman is encountered.

Consistently higher levels of the (±)P(-)-stereoisomers of soman are present in the terminal elimination phase after s.c. bolus administration than after i.v. bolus administration (see Tables 2.5 and 2.3). Consequently, the time period (228 min) during which the C(±)P(-)-stereoisomers are present at toxicologically relevant concentrations is almost twice as long as after i.v. administration. Tentatively, the more pronounced persistence of C(±)P(-)-soman upon s.c. administration has been explained by gradual absorption of these stereoisomers from the site of s.c. injection.

These results show that s.c. administration can only be regarded as a toxicokinetic model for respiratory exposure in cases where the duration of the latter exposure is in the range of several minutes, which is not often realistic at (supra)lethal doses. The extended period of time in which toxicologically relevant concentrations of C(\pm)P(–)-soman are present after s.c. administration suggests that this route is a more rigorous challenge for prophylaxis and therapy of intoxication than other routes. However, the extremely high blood levels immediately after i.v. bolus administration may also provide a considerable challenge for that route of administration, depending on the pharmacokinetic and pharmacodynamic parameters of the specific treatment.

VI. INHALATION TOXICOKINETICS OF SOMAN AND SARIN

In the case of intoxications with nerve agents under realistic conditions, the primary route of entrance into the body of volatile nerve agents such as sarin, tabun, soman, and GF is the respiratory route. The latter route is almost as effective as parenteral administration, with approximately 70% of an inhaled dose of (\pm)-sarin being retained in guinea pigs, dogs, monkeys, and humans.^{26,27} It was anticipated that the shapes of the toxicokinetic curves for inhalation of nerve agent stereoisomers would differ considerably from those for other routes of exposure, which may have important consequences for the efficacy of pretreatment and therapy of intoxications with these agents. Therefore, Langenberg et al. investigated the inhalation toxicokinetics of C(\pm)P(\pm)-soman and (\pm)-sarin,¹⁸ using an apparatus which they constructed for the continuous generation of nerve agent vapor in air, nose-only exposure of guinea pigs and monitoring of respiratory minute volume and respiratory frequency during exposure.²⁸ Figure 2.11 gives a schematic representation of this apparatus, as well as a short explanation of the functioning of the various elements.

The inhalation and subsequent absorption of nerve agent vapor, largely in the upper part of the respiratory tract, may involve a time period of a few seconds or minutes, up to several hours in the case of low-level exposure (see Section VII).²⁹ Initial investigations of inhalation toxicokinetics involved exposure periods of 4–8 min which was regarded as a compromise between the often shorter exposure time to volatile agents in the case of chemical warfare and the desire to measure in a reasonable time frame the increasing blood levels due to inhalation and absorption. Since it was considered as too involved to use mechanically ventilated animals in inhalation toxicokinetics, the investigations were restricted to sublethal doses in the range of 0.4–0.8 LC_{t50} of C(\pm)P(\pm)-soman and (\pm)-sarin, inhaled by anesthetized* and atropinized guinea pigs.

Concentrations of C(–)P(+)-soman < 13 pg/ml were observed in the blood samples taken during an 8-min exposure to 0.8 LC_{t50} of C(\pm)P(\pm)-soman. In all other cases, this stereoisomer was not observed in detectable concentrations, whereas

*Ketamine hydrochloride (i.m.).

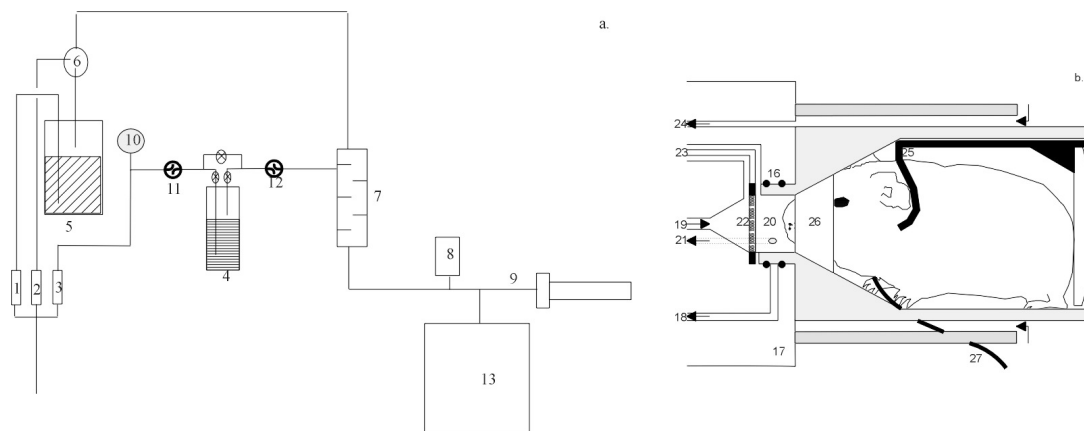


FIGURE 2.11 (a) Apparatus constructed for generation of nerve agent vapor: (1), (2), and (3), mass flow controllers; (4), vial containing the nerve agent; (5), thermostatted water bath; (6) and (7), mixing chambers; (8), temperature/relative humidity meter; (9), towards the exposure modules; (10), overpressure security; (11) and (12), splash heads; and (13), gas chromatograph with gas sampling valve. (b) Guinea pig (14) positioned in the modified Battelle tube (15); (16) O-rings for gastight connection of the tube to the body of the exposure apparatus (17); (18) tubing with a critical orifice, which is connected to an underpressure check for gastight connection of the tube; (19) tubing through which the nerve agent is transported to the exposure chamber; (20) front chamber of the modified Battelle tube, from which the animal breathes; (21) tubing with a critical orifice, which is the outlet of the front chamber; (22) wire mesh resistance; (23) differential pressure measuring device; (24) tubing with a critical orifice, which sucks air from the “underpressure chamber” surrounding the tube; (25) fork for positioning the animal; (26) rubber mask; and (27) carotid artery cannula. Arrows indicate flow directions. (From Langenberg, J.P., Spruit, H.E.T., Van Der Wiel, H.J., Trap, H.C., Helmich, R.B., Bergers, W.W.A., Van Helden, H.P.M., and Benschop, H.P., *Toxicol. Appl. Pharmacol.*, 151, 79, 1998. With permission.)

the C(+)-P(-)-stereoisomer was not observed in any case. The mean concentration-time courses of C(+)-P(-)- and C(-)-P(-)-soman for exposures to 0.8 LCt_{50} in 8 min are presented in Figure 2.12. The effect of inhaled dose on the toxicokinetics is shown in Figure 2.13, where the time course of the concentration of C(-)-P(-)-soman is given for an 8-min exposure to 0.4 and 0.8 LCt_{50} , whereas Figure 2.14 shows the effects on toxicokinetics of the time period of exposure to the same dose, i.e., 0.8 LCt_{50} in a 4 and 8 min period of time.

As in the case of s.c. toxicokinetics, the kinetics of C(+)-P(-)- and C(-)-P(-)-soman were described mathematically as a discontinuous process, with an equation for the exposure period and an equation for the post-exposure period. In view of the limited number of data points during exposure, the absorption phase was described with a mono-exponential function. In order to describe the exposure phase of C(+)-P(-)-soman, lag times of 2 and 4 min were selected for the 8-min exposures to 0.8 and 0.4 LCt_{50} , respectively. These lag times correspond with the earliest time points at which this stereoisomer could be detected. Toxicokinetic parameters derived from the various calculated concentration-time curves are given in Table 2.6. There were no measurable effects of the exposures on the respiratory minute volume (RMV) and respiratory frequency (RF).

The data in Figures 2.12–2.14 suggest that the systemic penetration of C(-)-P(-)-soman during nose-only exposure is very rapid, since this stereoisomer can be measured in blood at 30 s after starting the exposure. Moreover, the

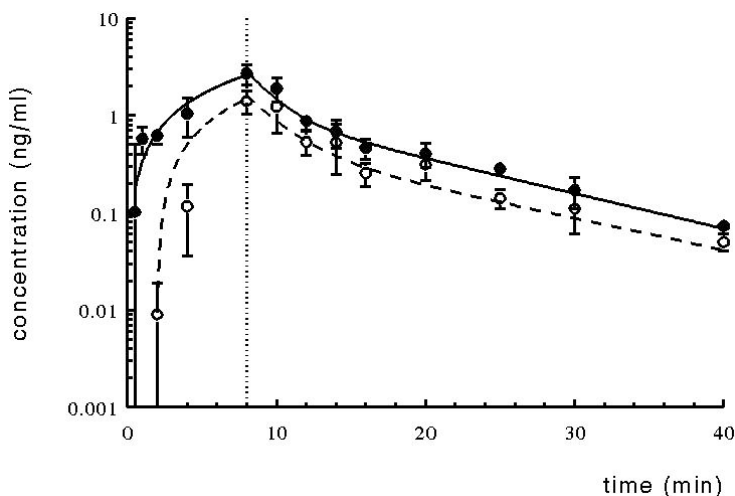


FIGURE 2.12 Semilogarithmic plot of the mean concentrations in blood (\pm s.e.m., $n = 6$) of C(+)-P(-)-soman (\circ) and C(-)-P(-)-soman (\bullet) vs. time during and after nose-only exposure of anesthetized, atropinized, and restrained guinea pigs to $48 \pm 5 \text{ mg} \cdot \text{m}^{-3}$ of C(\pm)-P(\pm)-soman vapor in air for 8 min, which corresponds with 0.8 LCt_{50} . The dotted line marks the end of the exposure period. (From Langenberg, J.P., Spruit, H.E.T., Van Der Wiel, H.J., Trap, H.C., Helmich, R.B., Bergers, W.W.A., Van Helden, H.P.M., and Benschop, H.P., *Toxicol. Appl. Pharmacol.*, 151, 79, 1998. With permission.)

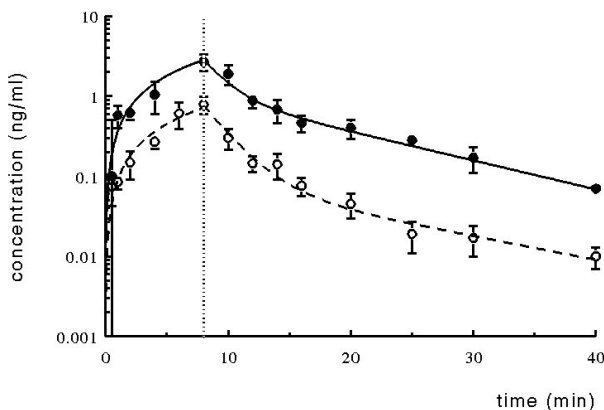


FIGURE 2.13 Semilogarithmic plot of the mean concentrations in blood (\pm s.e.m.; $n = 6$) of C(–)P(–)-soman vs. time during and after nose-only exposure of anesthetized, atropinized, and restrained guinea pigs to C(±)P(±)-soman vapor in air yielding 0.4 (○) and 0.8 (●) LC_{50} in 8 min. The dotted line marks the end of the exposure period. (From Langenberg, J.P., Spruit, H.E.T., Van Der Wiel, H.J., Trap, H.C., Helmich, R.B., Bergers, W.W.A., Van Helden, H.P.M., and Benschop, H.P., *Toxicol. Appl. Pharmacol.*, 151, 79, 1998. With permission.)

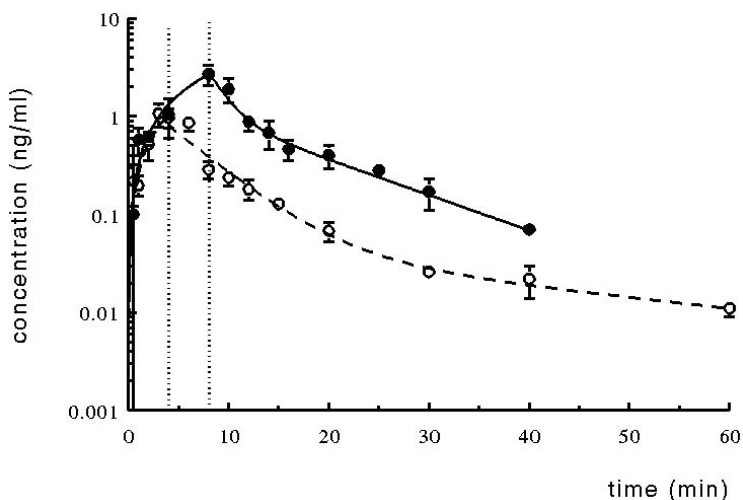


FIGURE 2.14 Semilogarithmic plot of the mean concentrations in blood (\pm s.e.m.; $n = 6$) of C(–)P(–)-soman vs. time during and after nose-only exposure of anesthetized, atropinized, and restrained guinea pigs to C(±)P(±)-soman vapor in air yielding 0.8 LC_{50} in 8 min (●) or 4 min (○). The dotted lines mark the end of the exposure period. (From Langenberg, J.P., Spruit, H.E.T., Van Der Wiel, H.J., Trap, H.C., Helmich, R.B., Bergers, W.W.A., Van Helden, H.P.M., and Benschop, H.P., *Toxicol. Appl. Pharmacol.*, 151, 79, 1998. With permission.)

concentration of this stereoisomer does not increase further after terminating the exposure. In contrast herewith, there is a lag time of several minutes before the C(+)P(-)-stereoisomer can be detected in blood. Furthermore, as observed earlier for i.v. and s.c. administration, the concentrations in blood and the AUC of C(+)P(-)-soman are consistently lower than those of the C(-)P(-)-stereoisomer, despite the 23% excess of the C(+)P(-)-stereoisomer over the C(-)P(-)-stereoisomer in C(±)P(±)-soman. Based on the earlier mentioned 30-fold higher reaction rate of the C(+)P(-)- stereoisomer with guinea pig plasma CaE, this phenomenon can at least partly be explained by preferential binding of the C(+)P(-)-stereoisomer, e.g., to CaE at the absorption site(s) in the respiratory tract and in blood.^{30,31}

The data in Table 2.6 show that the apparent elimination half-life of the C(-)P(-)-stereoisomer after respiratory exposure to 0.8 LC₅₀ in 8 min is somewhat longer than for the equitoxic i.v. dose. Moreover, the maximum concentration in blood of C(+)P(-)-soman in case of the 4-min exposure is reached not earlier than 2 min after cessation of the exposure. This suggests that, despite rapid absorption, some depot formation occurs at the absorption site, from which absorption continues after termination of the exposure. A further argument for some depot formation in the respiratory tract can be gleaned from Figure 2.15, where the concentration-time profiles for C(-)P(-)-soman are compared for the 8-min respiratory exposure and for an 8-min i.v. infusion of an equitoxic dose (0.8 LD₅₀) of C(±)P(±)-soman.²⁴ Evidently, the absorption phase of respiratory absorption is closely mimicked by the i.v. infusion, but blood levels subsequent to the respiratory exposure are distinctly higher than those after the i.v. infusion.

Half-life of elimination appears to increase when the exposure time was shortened from 8 to 4 min, while the exposure concentration is increased two-fold. Apparently, such a phenomenon seems hard to understand. The calculated elimination half-life would probably be appreciably longer if a data point at 60 min would be available.

Rather unexpectedly, the concentrations of C(+)P(-)- and C(-)P(-)-soman decrease over the entire time period of exposure and elimination with a concomitant fourfold lowering of the AUC when the exposure time to 0.8 LC₅₀ is lowered from 8 to 4 min. Intuitively, one would expect a higher maximum concentration but approximately the same AUC upon shortening the exposure time at equitoxic dose. Moreover, the longer terminal half-life after exposure for 4 min seems in contradiction with the lower AUC. Since the RMV and RF are almost the same during the period of exposure, this nonlinearity with exposure time might be due to decreasing retention of soman vapor at higher concentrations of soman vapor in air. Unfortunately, data on the overall retention of soman in experimental animals are not available, and are urgently needed for interpretation and physiologically based modeling of the toxicokinetics of the stereoisomers of soman.

During and after nose-only exposure to 0.4 LC₅₀ of C(±)P(±)-soman in 8 min, the maximum concentration of the C(+)P(-)- and C(-)P(-)-stereoisomers are approximately fourfold lower than the mean maximum concentrations after an 8-min exposure to a dose corresponding to 0.8 LC₅₀. Furthermore, the AUC of the two stereoisomers for the former experiment are about fourfold lower. Assuming that retention of the agent in the respiratory tract is constant, it is likely that the

TABLE 2.6

Toxicokinetic Parameters of C(+)-P(-)-soman and C(-)-P(-)-soman in Anesthetized, Atropinized, and Restrained Guinea Pigs during and after Nose-Only Exposure for 4–8 min to 0.4–0.8 LC₅₀ of C(±)-P(±)-soman. Comparative Parameters for C(-)-P(-)-soman for i.v. Bolus Administration of a Dose Corresponding with 0.8 LD₅₀ Are also Given

Parameter	0.8 LC ₅₀ (8-min exposure)		0.4 LC ₅₀ (8-min exposure)		0.8 LC ₅₀ (4-min exposure)		0.8 LD ₅₀ (i.v., bolus)
	C(+)-P(-) ^a	C(-)-P(-)	C(+)-P(-) ^b	C(-)-P(-)	C(+)-P(-)	C(-)-P(-)	C(-)-P(-) ^c
C _{max} ^d (ng · ml ⁻¹ , n = 6)	1.4 ± 0.4	2.7 ± 0.6	0.2 ± 0.2	0.8 ± 0.2	0.5 ± 0.2 ^e	1.1 ± 0.3	—
A (ng · ml ⁻¹)	-55.4	-35.8	-34.7	-34.7	—	-30	—
B (ng · ml ⁻¹)	32.6	141	1.74	24.8	0.7	1.7	3.8
C (ng · ml ⁻¹)	0.83	1.9	0.014	0.14	0.03	0.05	0.80
D (ng · ml ⁻¹)	55.4	35.8	34.7	34.7	—	30	—
a (min ⁻¹)	0.004	0.009	0.001	0.002	—	0.009	—
b (min ⁻¹)	0.42	0.54	0.28	0.45	0.19	0.20	0.95
c (min ⁻¹)	0.08	0.08	0.005	0.07	0.011	0.026	0.12
Elimination half-life (min)	9.2	8.4	—	10.1	63	27	5.8
AUC ^f (ng · min · ml ⁻¹)							
0 → end exposure	7.2	8.5	1.2	2.5	0.6 ^h	2.1	—
end exposure → ∞	8.8	15.2	3.1	2.7	3.7	5.6	—
Total	16.0	23.7	4.3	5.2	4.3	7.7	10.6
RMV ^g (ng · ml ⁻¹ ; n = 12)	52 ± 3		43 ± 6		37 ± 5		
RF ^h (Hz; n = 12)	0.65 ± 0.05		0.66 ± 0.05		0.80 ± 0.11		

Note: The inhalation results were fitted with discontinuous functions: [nerve agent] = D + A^{-at} for the absorption phase, and [nerve agent] = Be^{-bt} + Ce^{-c} for distribution and elimination.

^aAssuming a lag time of 2 min.

^bAssuming a lag time of 4 min.

^cToxicokinetic parameters could not be obtained from the low and rather erratic concentration of the C(+)-P(-)-isomer.

^dAt end of exposure period, unless noted otherwise.

^eAt t = 6 min (2 min after ending of exposure).

^fAUC measured with the trapezoidal method.

^gRMV, respiratory minute volume. Values are means ± s.e.m.

^hRF, respiratory frequency. Values are means ± s.e.m.

Source: From Langenberg, J.P., Spruit, H.E.T., Van Der Wiel, H.J., Trap, H.C., Helmich, R.B., Bergers, W.W.A., Van Helden, H.P.M., and Benschop, H.P., *Toxicol. Appl. Pharmacol.*, 151, 79, 1998. With permission.

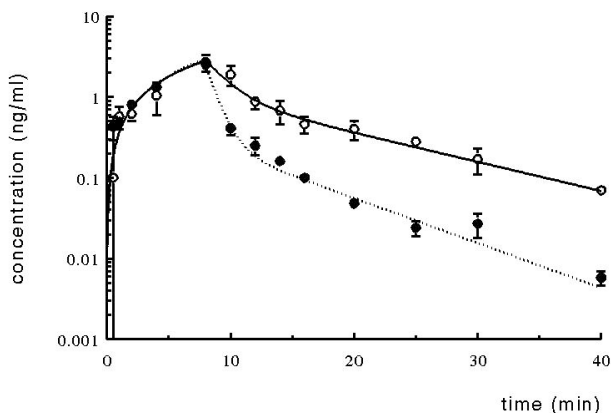


FIGURE 2.15 Semilogarithmic plot of the mean concentrations (\pm s.e.m.; $n = 6$) of C(-)P(-)-soman in blood of anesthetized and atropinized guinea pigs during and after an 8 min nose-only exposure to 0.8 LCt₅₀ (○) and an 8 min i.v. infusion of 0.8 LD₅₀ (●) of C(±)P(±)-soman.

toxicokinetics are nonlinear with dose, as observed for i.v. bolus administration of a sublethal dose.

Experiments on the inhalation toxicokinetics of (±)-sarin have been restricted to 8-min exposures to 0.8 and 0.4 LCt₅₀ of this agent. The concentration-time profile for (-)-sarin at an exposure level of 0.8 LCt₅₀ (38 mg · m⁻³), together with that of C(+)P(-)-soman for an 8-min exposure to an equitoxic dose of C(±)P(±)-soman (exposure level 48 mg · m⁻³) are given in Figure 2.16. The mathematical description of the concentration-time profiles for (-)-sarin was analogous to that for the inhalation experiments of C(±)P(±)-soman. Toxicokinetic parameters derived from the equations are given in Table 2.7. When comparing the measured blood levels in the absorption phase of (-)-sarin with those of C(+)P(-)-soman and C(-)P(-)-soman at equitoxic dose, it appears that the absorption of (-)-sarin resembles that of C(+)P(-)-soman, i.e., featuring a relatively slow build-up of blood levels in comparison with C(-)P(-)-soman. Tentatively, this behavior of (-)-sarin can be ascribed, as in the case of C(+)P(-)-soman, to scavenging by irreversible binding sites prior to and subsequent to entering the bloodstream. Also, the maximum concentration reached by (-)-sarin is rather similar to that of C(+)P(-)-soman. Characteristically for (-)-sarin (see Section IV), the terminal half-life of this stereoisomer at a dose of 0.8 LCt₅₀ is about fourfold longer than for the two C(±)P(-)-stereoisomers of soman (8.4–9.2 min). Due to unrealistically high blood levels of (-)-sarin in the terminal elimination phase after exposure to 0.4 LCt₅₀, it is difficult to judge the (non)linearity with dose relative to exposure to 0.8 LCt₅₀ of (±)-sarin. For the time period 0–120 min, the AUC are rather similar. However, when taking into account that the RMV during exposure to 0.4 LCt₅₀ was about 1.5-fold

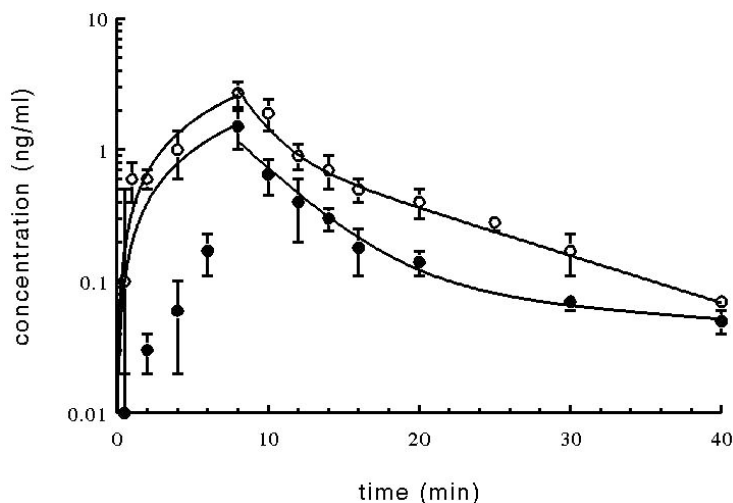


FIGURE 2.16 Mean concentrations in blood (\pm s.e.m.; $n = 6$) of (–)-sarin (●) and of C(–)P(–)-soman (○) vs. time after nose-only exposure of anesthetized and atropinized guinea pigs to 0.8 LC_{50} of (±)-sarin and C(±)P(±)-soman, respectively, in the course of 8 min.

higher than during exposure to 0.8 LC_{50} , nonlinearity with dose seems probable. As in the case of inhalation toxicokinetics of soman stereoisomers, a final interpretation of results will depend on additional data with regard to the retention of sarin vapor in the respiratory system under various conditions.

VII. INHALATION TOXICOKINETICS OF SOMAN UPON LOW-LEVEL EXPOSURE

As evident from the previous paragraphs of this chapter, investigations on the toxicokinetics of nerve agents have centered on lethal and supralethal doses of nerve agent. However, the controversy on the possible relationship between the so-called Gulf War Syndrome and exposure to traces of nerve agent shortly after the Gulf War has emphasized that knowledge on the acute and delayed effects of trace exposure to nerve agents is almost nonexistent.^{32,33} Nevertheless, several situations can be envisaged in which trace exposures becomes realistic. In the case of chemical warfare, small amounts of agent may penetrate into gas masks and protective clothing or into a collective protective shelter. Small amounts of nerve agent may desorb from contaminated skin, clothing, or painted surfaces, posing a risk of long-term, low-level exposure. Miosis, rhinorrhea, dyspnea, and tightness of the chest were observed in rescue workers and medical personnel in hospitals due to secondary exposure to small amounts of agent subsequent to the terrorist attacks with sarin in Matsumoto and in metropolitan Tokyo.^{34–38} Sarin vapor could be detected in houses up to 12 h after the attack with sarin in Matsumoto. Some victims in this city reported their first

TABLE 2.7

Toxicokinetic Parameters of (–)-sarin in Anesthetized, Atropinized, and Restrained Guinea Pigs after Nose-Only Exposure for 8 min to 0.8 and 0.4 LC₅₀ of (±)-sarin and after i.v. Bolus Administration of 0.8 LD₅₀ of (±)-sarin for Comparison.

Parameter	0.8 LC ₅₀ (8 min)	0.4 LC ₅₀ (8 min)	0.8 LD ₅₀ (i.v. bolus)
C _{max} ^a (ng.ml ⁻¹ , ±s.e.m., n = 6)	5	0.3 ± 0.1	–
A (ng.ml ⁻¹)	164	55.6	–
B (ng.ml ⁻¹)	–164	–55.6	–
C (ng.ml ⁻¹)	8.6	0.86	35.9
D (ng.ml ⁻¹)	0.11	0.036	0.09
b (min ⁻¹)	0.0012	0.00085	–
c (min ⁻¹)	0.26	0.18	4.6
d (min ⁻¹)	0.019	0.00063	0.012
Terminal half-life (min)	36	–	58
Area under curve (ng.min.ml ⁻¹)			
0–8 min	6.3	0.8	
8–120 min	8.5	5.5	
Total	14.8	6.3	15.3
RMV ^b (min.ml ⁻¹ , ±s.e.m., n = 12)	31 ± 4	47 ± 6	–
RF ^c (Hz, ±s.e.m., n = 12)	0.70 ± 0.06	0.64 ± 0.04	–

Note: The inhalation results were fitted with a discontinuous function: [(–)-sarin] = A + Be^{–bt} for the absorption phase, and [(–)-sarin] = Ce^{–ct} + De^{–dt} for the distribution and elimination phase. The i.v. results were fitted according to [(–)-sarin] = Ce^{–ct} + De^{–dt}.

^aAt the end of the exposure period (t = 8 min).

^bRMV = respiratory minute volume.

^cRF = respiratory frequency.

symptoms as late as 20 h after the incident, presumably due to the cumulative effect of persistent low-level exposure.³⁹

Systematic investigations on the effects of exposure to small amounts of nerve agents, mostly sarin, on human volunteers pertain invariably to short term (≤ 30 min) exposures, and have led to the definition of so-called “no-effect levels.” In other experiments, volunteers inhaled Cts up to 15 mg · min · m^{–3} of sarin and experienced slight acute phenomena of intoxication.⁴⁰ A subsequent epidemiological study revealed no difference in health status between exposed and non-exposed individuals. Okumura et al.⁴¹ investigated 640 victims of the terrorist attack with sarin in the Tokyo subway. After discharge from the hospital, patients in the severe and moderate exposure categories required follow-up by the hospital’s outpatient system to observe for late effects, especially neurotoxic and behavioral effects, partly due to posttraumatic-stress disorder induced by exposure to sarin.^{42,43} Similarly, long-term effects were observed in the victims of the sarin attack at Matsumoto, 3.5 years after the

event.⁴⁴ It is concluded that exposure to nerve agents at doses leading to acute effects may lead to delayed and persistent adverse effects, mostly of a neuropsychological order. No evidence exists for such effects after single exposure to nerve agents in which acute signs of exposure were absent.

In order to initiate a quantitative basis for the toxicology of low-dose exposure to nerve agents, Benschop et al.⁴⁵ investigated the toxicokinetics of the four stereoisomers of C(±)P(±)-soman upon nose-only exposure of anesthetized, atropinized, and restrained guinea pigs to 20 ppb ($160 \mu\text{g} \cdot \text{m}^{-3}$) of C(±)P(±)-soman over a 5 h exposure period, providing blood levels of the toxic C(±)P(-)-soman stereoisomers at Ct-values accumulating from 0–48 $\text{mg} \cdot \text{min} \cdot \text{m}^{-3}$. Concomitantly, the progressive inhibition of AChE in erythrocytes was measured. The exposures were performed using the apparatus as described in Figure 2.11, with minor adaptations. A bi-exponential equation sufficed to describe the gradually increasing concentrations of the C(+)P(-)- and C(-)P(-)-soman stereoisomers (Figure 2.17), when adopting a lag time of 30 min for the C(+)P(-)-stereoisomer. Table 2.8 summarizes the toxicokinetic data derived from these equations, while Figure 2.17 illustrates the fit of the derived equations to the blood levels of the C(+)P(-)- and C(-)P(-)-soman stereoisomers as measured during exposure.

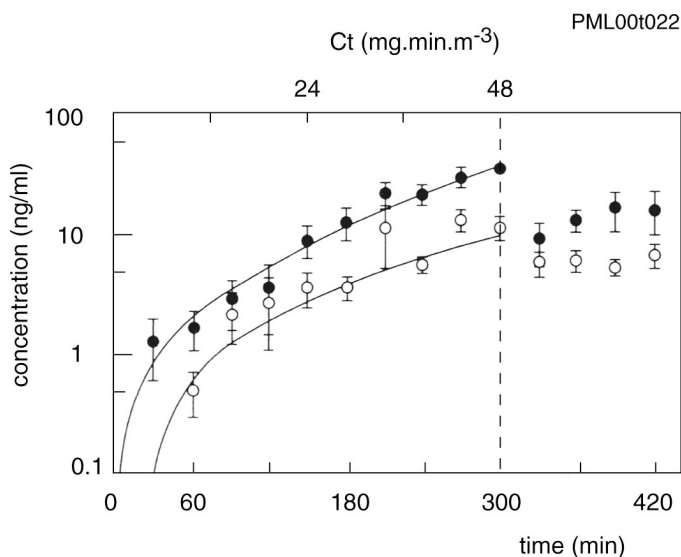


FIGURE 2.17 Semilogarithmic plot of the mean concentrations (\pm s.e.m., $n = 6$) in blood of C(-)P(-)-soman (●) and C(+)P(-)-soman (○) vs. time during nose-only exposure of anesthetized, atropinized, and restrained guinea pigs to $160 \pm 16 \mu\text{g} \cdot \text{m}^{-3}$ of C(±)P(±)-soman for 300 min and up to 120 min after exposure. Accumulated Ct-values are also shown. The solid lines represent optimal fits of bi-exponential functions to the data. The dotted line marks the end of the exposure period. (From Benschop, H.P., Trap, H.C., Spruit, E.T., Van Der Wiel, H.J., Langenberg, J.P., and De Jong, L. P.A., *Toxicol. Appl. Pharmacol.*, 153, 179, 1998. With permission.)

TABLE 2.8

Toxicokinetic Parameters for C(+)P(-)-soman and C(-)P(-)-soman in Anesthetized, Atropinized, and Restrained Guinea Pigs during Nose-Only Exposure to C(±)P(±)-soman at a Concentration of $160 \pm 16 \mu\text{g}\cdot\text{m}^{-3}$ (20 ppb) in Air for 300 min.

Parameter	C(+)P(-)-soman	Parameter	C(-)P(-)-soman
A_+ ($\text{ng}\cdot\text{mL}^{-1}$)	0.00107	A^- ($\text{ng}\cdot\text{mL}^{-1}$)	0.0262
b_+ (min^{-1})	0.00885	b^- (min^{-1})	0.00599
c_+ (min^{-1})	-0.00947	c^- (min^{-1})	0.00509
$\text{AUC}_{0-300 \text{ min}}^a$ ($\text{ng}\cdot\text{min}\cdot\text{mL}^{-1}$)	1.1	$\text{AUC}_{0-300 \text{ min}}$ ($\text{ng}\cdot\text{min}\cdot\text{mL}^{-1}$)	3.5

Note: The data for concentrations in blood determined during exposure were fitted with bi-exponential function: $[\text{C}(+) \text{P}(-)\text{-soman}] = A_+ \{e^{b_+ \cdot (t-30)} - e^{c_+ \cdot (t-30)}\}$ and $[\text{C}(-) \text{P}(-)\text{-soman}] = A_- \{e^{b^- \cdot t} - e^{c^- \cdot t}\}$, in which the subscript + refers to C(+)P(-)-soman and the subscript - to C(-)P(-)-soman.

^aAUC = area under the curve.

Source: From Benshop, H.P., Trap, H.C., Spruit, E.T., Van Der Wiel, H.J., Langenberg, J.P., and De Jong, L.P.A., *Toxicol. Appl. Pharmacol.*, 153, 179, 1998. With permission.

No attempt was made to describe the time-concentration course in the 120 min post-exposure period. The blood levels decrease clearly in the first 90 min post-exposure but remain remarkably constant over the next 90 min period. One intriguing explanation, although needing validation, is the formation of a “depot” of intact soman, for example in the epithelial tissue of the respiratory tract, from which the C(+)P(-)- and C(-)P(-)-stereoisomers diffuse into the bloodstream.

In case of s.c. and short-term respiratory exposure, the C(-)P(-)-soman stereoisomer penetrated almost immediately into the bloodstream, whereas the appearance of the C(+)P(-)-stereoisomer lagged a few minutes behind. In the present case of low-level respiratory exposure, it takes approximately 30 min before even the C(-)P(-)-stereoisomer has penetrated, while it takes another 30 min before the C(+)P(-)-stereoisomer appears in measurable concentrations in the bloodstream. Thus, as should be expected, the preferential scavenging of C(+)P(-)-soman becomes more evident upon lowering the doses of C(±)P(±)-soman. In this extreme case of low-dose exposure, the AUC of the C(+)P(-)-stereoisomer is more than 3-fold lower than that of the C(-)P(-)-stereoisomer (Table 2.8), in spite of the 22% excess of the former stereoisomer in C(±)P(±)-soman. It should also be noted that enzymes such as CaE which scavenge C(+)P(-)-soman preferentially, are abundantly available in the epithelial tissue of the upper respiratory tract where, by analogy with sarin, most of the soman is presumably absorbed.³⁰ Not surprisingly, the C(+)P(+)- and C(-)P(+)-stereoisomers were not detected at any stage of the exposure period.

The progressive inhibition of erythrocyte AChE as well as the concentrations of the C(+)P(-)- and C(-)P(-)-soman stereoisomers in blood were measured

independently. Therefore attempts were made to find out whether the sets of data are consistent, using the measured bimolecular rate constants for inhibition of AChE in erythrocyte cell walls by these two stereoisomers and kinetic equations derived to calculate the progression of AChE inhibition by the two stereoisomers in their concentration-rate profile. As shown in Figure 2.18 the calculated progression of inhibition is only slightly slower than the actually observed values.

At 120 min after ending the 300-min exposure period, AChE activities were measured in brain and diaphragm of the exposed guinea pigs, in order to estimate the relative importance of peripheral and central effects of long-term, low-level exposure to C(\pm)P(\pm)-soman. No inhibition of AChE in these two organs was observed, whereas almost complete inhibition of the activity of AChE in erythrocytes occurs during and after the exposure period. These results corroborate those of Jacobson et al., who observed negligible inhibition of brain AChE in dogs after exposure to Ct values of $10 \text{ mg} \cdot \text{min} \cdot \text{m}^{-3}$ of sarin for a 6-h period daily over 6 months, which yielded 80% inhibition of erythrocyte AChE within a few days.⁴⁶ The results are also in accordance with an efficient detoxification in the blood by covalent binding to CaE and other binding sites which are first exposed to C(\pm)P(\pm)-soman (*vide infra*). The lack of inhibition of two major target organs for intoxication with C(\pm)P(\pm)-soman, i.e., brain and diaphragm, indicates that signs of systemic intoxication, either due to peripheral or central effects, are rather improbable in these long-term, low-level exposures. The same holds true for the occurrence of (delayed) neuropsychological

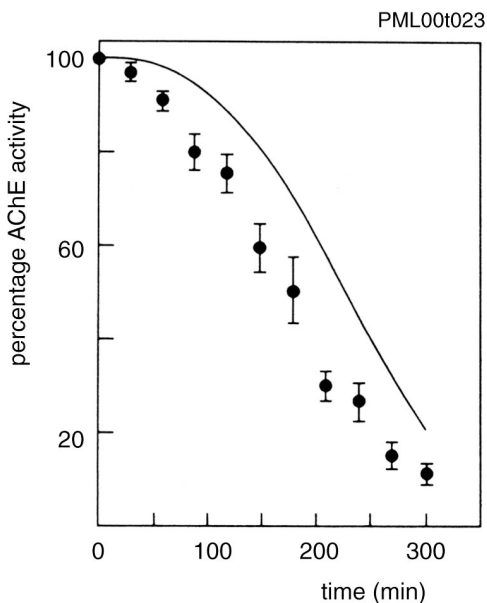


FIGURE 2.18 Mean AChE activity (\pm s.e.m.; $n = 6$) in erythrocytes vs. time during nose-only exposure of anesthetized, atropinized, and restrained guinea pigs to $160 \pm 16 \mu\text{g} \cdot \text{m}^{-3}$ (20 ppb) of C(\pm)P(\pm)-soman for 300 min. The solid line represents AChE activities calculated from the measured concentrations of C(\pm)P(-)-soman in blood.

disorders. During exposure of human volunteers to sarin vapor (< 30 min) at low wind speeds, it has been observed that Ct -values $> 2\text{--}3 \text{ mg} \cdot \text{min} \cdot \text{m}^{-3}$ will cause incapacitating miosis due to direct penetration of the nerve agent into the eye.⁴⁷⁻⁴⁹ This value would even decrease significantly with increasing wind speeds and presumably with replacement of sarin by soman. Therefore, the occurrence of miosis and effects thereof might be a much more probable outcome of the long-term low-level exposure to $C(\pm)P(\pm)$ -soman at the accumulated Ct of $48 \text{ mg} \cdot \text{min} \cdot \text{m}^{-3}$ than systemic anticholinergic effects, albeit that measurements of miosis upon long-term, low-level exposure have not been performed. Moreover, it should be remarked that prolonged exposure of the airways to soman vapor can also be a cause for direct systemic effects, which might be more pronounced in nonatropinized animals.

A further effect that should be taken into account when attempting to extrapolate results of the above-mentioned, low-level exposures to humans is the absence in primates of most of the scavenging effect of CaE as is available in guinea pigs. Therefore, the same challenge levels of $C(\pm)P(\pm)$ -soman vapor might give more pronounced AChE inhibition in primates than in guinea pigs.

It is remarkable that intact $C(-)P(-)$ -soman becomes detectable in blood after a 30-min exposure to $160 \text{ } \mu\text{g} \cdot \text{m}^{-3}$ (20 ppb), i.e., at a total dose of $4.8 \text{ } \mu\text{g} \cdot \text{min} \cdot \text{m}^{-3}$, which is only two orders of magnitude higher than the Ct value allowed by the U.S. Army during an 8-h occupational exposure to sarin.⁵⁰ Inhibition of AChE is still marginal under these conditions, but becomes clearly detectable at 60 min, i.e., at $Ct = 9.6 \text{ } \mu\text{g} \cdot \text{min} \cdot \text{m}^{-3}$. The present investigations are at the limit of possibilities for measuring intact agent in blood upon low-level exposure. A further reduction of the dose can only be investigated on a basis of nerve agent accumulated by internal scavengers like butyrylcholinesterase (BuChE), CaE, and albumin. Recently, methodology for such an approach has been developed in which sarin is released from sarin-inhibited BuChE by means of fluoride ion-induced reactivation and subsequent isolation and analysis of released sarin (see Section VIII). In this way, $\geq 0.01\%$ inhibition of BuChE in blood of primates can be quantified.⁵¹ Preliminary investigations indicate that such a degree of inhibition occurs at or only slightly above the occupational exposure limit.^{50,52}

VIII. ELIMINATION PATHWAYS OF PHOSPHOFLUORIDATES

The elimination routes of $C(\pm)P(\pm)$ -¹⁴C-soman were investigated by Benschop and De Jong in a series of experiments after i.v. administration of doses corresponding to $2\text{--}6 \text{ LD}_{50}$ in anesthetized and mechanically ventilated rats, guinea pigs, and marmosets.¹⁹ O-pinacolyl methylphosphonic acid (PMPA) and soman bound covalently to proteins accounted for more than 80% of the radioactivity 1 h after administration of the agent. Obviously, hydrolysis of the phosphorus-fluorine bond and reaction with binding sites are the major elimination pathways for $C(\pm)P(\pm)$ -soman. Similar results were obtained for $C(\pm)P(\pm)$ -³H-soman and (\pm) -³H-sarin after i.v. administration at sublethal doses to mice.^{13,15} In the latter investigations, the highest

concentrations of both hydrolyzed and bound organophosphate were already achieved 1 min after administration of the agents, indicating that both elimination processes proceed very rapidly. This result is corroborated by the observed rapid decrease of the concentrations in blood of intact $C(\pm)P(\pm)$ -soman and (\pm) -sarin in various toxicokinetic studies (*vide supra*).

A. ELIMINATION BY HYDROLYTIC DEGRADATION

Soman and other phosphofluoridates are degraded by enzymatic as well as by spontaneous hydrolysis. Already in 1946, Mazur⁵³ described that rabbit and human plasma and tissues contain enzymes that accelerate the hydrolysis of phosphorofluoridates yielding O,O-dialkyl phosphoric acid and fluoride ions. These so-called phosphoryl phosphatases were extensively studied by Mounter and co-workers and were also observed to catalyze the hydrolysis of phosphonofluoridates and of the phosphoramidocyanidate tabun.^{54–57} The importance of hydrolysis as an elimination route of phosphofluoridates was illustrated by Cohen and Warringa⁵⁸ who found some protection of rats challenged (s.c.) with supralethal doses of O,O-diisopropyl phosphorofluoridate (DFP) or sarin by means of pretreatment (i.v.) with purified phosphoryl phosphatase from hog kidneys. A similar approach to protection was followed in the attempted conversion of human BuChE into a hydrolytic enzyme for phosphofluoridates by means of site-directed mutagenesis.^{59,60}

Large amounts of O-isopropyl methylphosphonic acid were found in blood and urine of victims of the terrorist attacks with sarin in Matsumoto and Tokyo.^{61,62} Until recently, it was assumed that hydrolysis of phosphofluoridates in plasma and tissues of mammals proceeds exclusively by cleavage of the P–F bond. For example, treatment of $C(\pm)P(\pm)$ -³²P-soman with rat plasma or liver homogenate did not lead to any conversion of PMPA into the secondary hydrolysis product methylphosphonic acid (MPA).⁶³ Ramachandran⁶⁴ observed that the primary hydrolysis product of DF³²P, i.e., O,O-diisopropyl ³²P-phosphoric acid, is not metabolized after s.c. administration to mice. Rather, the product was excreted unchanged into urine. However, Nakajima et al.⁶⁵ reported that MPA was detected (in urine) until the third day after hospitalization of a victim of the terrorist attack with sarin in Matsumoto. This discrepancy needs further investigation.

Stereoselectivity of the enzymatic hydrolysis of the chiral phosphonofluoridates $C(\pm)P(\pm)$ -soman and (\pm) -sarin has been studied extensively.^{3,66–72} In various tissues of several species, (+)-sarin and $C(\pm)P(+)$ -soman are much more rapidly degraded by phosphoryl phosphatases than the more toxic stereoisomers of these nerve agents. The relative order for the rate of hydrolysis of the four stereoisomers of $C(\pm)P(\pm)$ -soman is $C(+)P(+)- \gg C(-)P(+)- \gg C(-)P(-)- \geq C(+)P(-)$ -stereoisomer (Table 2.9).

Acceleration of hydrolysis by means of enzymatic catalysis of the toxic $C(\pm)P(-)$ -stereoisomers of soman and of $(-)$ -sarin in various tissues of rats, guinea pigs, marmosets, and in human plasma is low or even absent.^{67,69,72} Obviously, the overall rates of hydrolysis of $C(\pm)P(-)$ -soman vary with the type of tissue within one species.^{72,73} The rates of hydrolysis are consistently low in target organs of the

TABLE 2.9

First-Order Rate Constants for Hydrolysis of the Four Stereoisomers of C(±)P(±)-soman, Catalyzed by Diluted Liver Homogenate and Plasma from Rat, Guinea Pig, and Marmoset, and by Diluted Human Plasma (pH 7.5, 37°C)

Source		Rate constant (10^{-3} min^{-1})			
		C(+)P(-)	C(-)P(-)	C(-)P(+)	C(+)P(+)
Plasma:	Man	0.7	3.2	480	860
	Marmoset	1.2	5.5	310	1200
	Guinea pig	0.2	0.8	80	220
	Rat	1.5	3.2	1200	3400
Liver:	Marmoset	1.0	1.9	40	90
	Guinea pig	0.9	1.6	270	510
	Rat	2.8	4.4	420	2500

Note: Calculated for hydrolysis by 1 ml 0.6% plasma or 0.15% liver homogenate.

Source: From De Jong, L.P.A., Van Dijk, C., and Benschop, H.P., *Biochem. Pharmacol.*, 37, 2939, 1988. With permission.

three species, such as brain and muscle (see Section IX). In contrast with the phosphofluoridates, the stereoselectivity of phosphoryl phosphatases for hydrolysis of (±)-tabun is less pronounced and appears to be species dependent.^{74,75}

B. ELIMINATION BY COVALENT BINDING

The second major elimination route for phosphofluoridates is covalent reaction with binding sites. It has been demonstrated that reaction with CaE is an important detoxification route for organophosphates *in vivo*.⁷⁶⁻⁷⁸ In addition to CaE, albumin appears to bind covalently to organophosphates.^{79,80} The active site concentrations of AChE and BuChE are only a small percentage of the total concentration of binding sites in rats and guinea pigs.^{73,81} Consequently, this binding has a negligible effect on the toxicokinetics of phosphonofluoridates, albeit that binding to AChE is crucial from a toxicological point of view.

A comparison of the amounts of soman bound in various tissues of rat, guinea pig, and marmoset at 1 h after i.v. administration of a dose corresponding with 6 LD₅₀ of C(±)P(±)-¹⁴C-soman with the amounts bound *in vitro* in plasma and homogenized tissues after incubation with excess of C(±)P(±)-soman reveals that complete occupation of available binding sites has not occurred *in vivo*, even after administration of this relatively high dose of C(±)P(±)-¹⁴C-soman, except for binding in plasma and lung (*vide infra*).^{19,72,73} This outcome is in accordance with the observations of Maxwell et al.,⁸¹ who calculated that the total concentration of CaE in the rat corresponds with more than 14 LD₅₀ of C(±)P(±)-soman.

Pretreatment (s.c.) of mice, rats, and guinea pigs with tri-o-cresyl phosphate (TOCP) or with the CaE-inhibitor CBDP, which is the active metabolite of TOCP,

reduces the LD₅₀ of sarin and soman to approximately the same level as the LD₅₀ value in nonhuman primates, e.g., marmosets.^{21,82,83} Marmosets contain a lower concentration of binding sites in various tissues than rats and guinea pigs,⁷² whereas the concentration of CaE in plasma is also low.⁸⁴ A further indication for the importance of CaE in the detoxification of phosphofluoridates was obtained by Sterri and coworkers.^{78,85,86} They found that repeated s.c. administration of sublethal doses of C(±)P(±)-soman in mouse, rat, and guinea pig and of (±)-sarin to guinea pigs resulted in cumulative LD₅₀ doses which are substantially higher than the acute value. For instance, most of the guinea pigs survived a total exposure to 5–6 times the acute LD₅₀ dose when the animals were challenged every 24 h with a dose corresponding with 0.5 LD₅₀ of C(±)P(±)-soman. One hour after administration of C(±)P(±)-soman, 70% of the CaE activity in plasma was inhibited, but the activity was restored to control values within 24 h. The results of these studies suggest strongly that the lower toxicity of C(±)P(±)-soman and (±)-sarin for mice, rats, and guinea pigs than for marmosets is mainly due to a higher number of covalent binding sites in the former three species. These sites serve as endogenous scavengers for detoxification of the phosphofluoridates.

It should be expected on the basis of these results that pretreatment of nonhuman primates with exogenous scavengers that bind covalently to organophosphates will offer some protection against intoxication with nerve agents. Indeed, very promising results were obtained in studies along this line in rhesus monkeys. For example, Doctor et al. and Maxwell et al. obtained protection against ≤5 LD₅₀ of soman (i.v.) by pretreatment with fetal bovine AChE,^{87,88} whereas Ashani and coworkers protected the animals against ≤4 LD₅₀ of soman (i.v.) and guinea pigs against 4 times the median inhalatory dose of the agent by pretreatment with human plasma BuChE.^{89,90}

Upon increasing the i.v. dose of C(±)P(±)-¹⁴C-soman from 2–3 LD₅₀ to 6 LD₅₀, the concentrations of bound soman in blood and lungs of rats and guinea pigs increased only slightly. In fact, the highest concentrations of bound soman were almost equal to the concentrations of binding sites of soman determined from *in vitro* binding experiments with C(+)P(–)-soman. It does not follow from this almost complete occupancy of binding sites that these sites in blood and lung have the highest intrinsic reactivity. Upon i.v. administration, binding sites in blood are first exposed to soman followed by those in the lungs. Only the fraction of soman surviving passage of the lungs is available for binding in other organs. This description, according to the principle of “first come, first served,” is further supported by investigations in CBDP-pretreated rats and guinea pigs.²¹ Administration of CBDP at a s.c. dose of 2 mg/kg, i.e., a dose which potentiates the lethality of phosphofluoridates considerably (*vide supra*), produced complete inhibition of CaE in plasma and lung of both species and of CaE in rat kidney, but inhibition of CaE in liver of both species and in the kidney of the guinea pig was only marginal. Moreover, only a small further potentiation of the lethality was achieved by pretreatment of the animals with an 8-fold higher dose of CBDP. Apparently, a substantial fraction of a lethal dose of soman is eliminated by the binding sites in blood and lung of guinea pig and rat, first served after an i.v. or s.c. administration, and in kidney of the rat. Binding in other organs plays a less important role in the detoxification of soman. By analogy, a higher uptake of radioactivity in the liver and reduced incorporation into other organs was observed

when the route of administration of DFP to rats was shifted from s.c. or i.v. to i.p. Moreover, the LD_{50} of i.p.-administered DFP was about 2-fold higher than the LD_{50} after i.v. or s.c. administration, stressing the major contribution of the first pass through the liver to the detoxification of DFP.⁹¹

Rate constants of binding for $C(\pm)P(-)^{14}C$ -soman were determined in guinea pig blood and tissue homogenates.⁷³ The determinations were performed in the presence of an equimolar concentration of $C(\pm)P(+)$ -soman in order to account for the possible competition by the less toxic stereoisomers. The relatively high rate constants obtained for part of the binding sites, i.e., approximately 10^6 – 10^7 $M^{-1}min^{-1}$, suggest reaction with highly reactive enzymes such as cholinesterases and CaE. These investigations also show heterogeneity of the binding sites in the various tissues with respect to their reactivity towards $C(\pm)P(-)$ -soman. Heterogeneity may be due to different proteins, e.g., CaE and albumin (*vide supra*), serving as binding sites, but may also be related to heterogeneity within CaE as observed by Sterri and Fonnum.⁷⁸ *Inter alia*, the interspecies nonlinearity found for the toxicokinetics of $C(\pm)P(-)$ -soman in rat vs. guinea pigs and marmosets (see Section IV) may be explained on the basis of such heterogeneity, assuming that some of these binding sites in rats have a very high reactivity. These sites will rapidly bind a fraction of the administered dose, resulting in the sequestration of a larger fraction of $C(\pm)P(-)$ -soman at a low dose than at a high dose.

In summary, the rapid initial decay of $C(\pm)P(\pm)$ -soman in blood after i.v. or respiratory administration is due to three processes, i.e., (i) distribution to various tissues, (ii) spontaneous and enzymatic hydrolysis, and (iii) covalent binding. It has been established that the toxic $C(\pm)P(-)$ -stereoisomers react rapidly with covalent binding sites. Since the less toxic $C(\pm)P(+)$ -stereoisomers are hydrolyzed several orders of magnitude faster than the $C(\pm)P(-)$ -stereoisomers whereas hydrolysis and covalent binding contribute almost to an equal extent (*vide supra*) to the elimination of $C(\pm)P(\pm)$ -soman, it was expected that elimination of $C(\pm)P(+)$ -stereoisomers proceeds almost exclusively by hydrolysis whereas the $C(\pm)P(-)$ -stereoisomers are almost exclusively eliminated by covalent binding. However, experiments performed with an i.v. dose of 6 LD_{50} of $C(\pm)P(\pm)$ -soman reconstituted from $C(\pm)P(-)^{14}C$ -soman and an equimolar amount of unlabeled $C(\pm)P(+)$ -soman and vice versa showed that the differences in elimination of the stereoisomers are not as extreme as expected (Table 2.10).¹⁹ Indeed, there is a clearcut preference for hydrolysis of the $C(\pm)P(+)$ -stereoisomers and for covalent binding of the $C(\pm)P(-)$ -stereoisomers, but binding of the $C(\pm)P(+)$ -stereoisomers and hydrolysis of the $C(\pm)P(-)$ -stereoisomers also take place to a considerable extent. Evidently, while $C(\pm)P(+)$ -soman is also sequestered by covalent binding, the low toxicity of these stereoisomers is primarily due to their rapid hydrolysis by phosphoryl phosphatases (see Table 2.9) and to their low intrinsic reactivity towards AChE.⁹

The high rates of the processes initially taking place indicate that pretreatment can only offer protection against nerve agent intoxication if it provides for very rapid detoxification in the organs that are first passed by the organophosphate, i.e., blood and lung after i.v., s.c., and respiratory exposure. Consequently, scavengers should have a reactivity towards the toxic stereoisomers of organophosphates comparable with that of cholinesterase and CaE. Alternatively, hydrolytic enzymes should

TABLE 2.10

Radiometrically Determined Stereospecificity of *In Vivo* Covalent Binding and Hydrolysis in Guinea Pigs 1 h after i.v. Administration of 6 LD₅₀ of C(±)P(±)-soman Reconstituted from ¹⁴C-C(±)P(–)-soman and Equimolar Unlabeled C(±)P(+)-soman, or *Vice Versa*. Percentages of Total Recovery.

Tissue	Hydrolysis		Covalent binding	
	¹⁴ C-C(±)P(–)	¹⁴ C-C(±)P(+)	¹⁴ C-C(±)P(–)	¹⁴ C-C(±)P(+)
Plasma	23	58	73	39
Lung	54	82	47	16
Liver	10	52	84	45
Brain	31	75	70	25

Note: Mean values from 5 measurements.

Source: From Benschop, H.P. and De Jong, L.P.A., *Neurosci. Biobehav. Rev.*, 15, 73, 1991. With permission.

degrade these stereoisomers at rates comparable with those for hydrolysis of C(±)P(+)-soman in plasma and tissues of rats and guinea pigs. In this connection it is interesting to reconsider the results of Cohen and Warringa⁵⁸ with regard to the protective effect against intoxication with (±)-sarin due to pretreatment with phosphoryl phosphatases. At first, the low capacity, if any, of these enzymes to catalyze the hydrolysis of the toxic stereoisomer of sarin seems difficult to reconcile with such a protective effect. However, it can be speculated that a more rapid decrease of the level of the less toxic (+)-stereoisomer of (±)-sarin due to hydrolysis by this enzyme will lead to less competition of the (+)-stereoisomer with the toxic (–)-stereoisomer for reaction with covalent binding sites and consequently to a more effective scavenging of the latter stereoisomer (*vide infra*).

Whereas the C(±)P(+)-stereoisomers of soman are completely eliminated in the distribution phase of the toxicokinetics, the toxic C(±)P(–)-stereoisomers are still present in the elimination phase. It cannot be derived from the presently available data as to what extent elimination of the C(±)P(–)-stereoisomers in the later phase proceeds either by binding or hydrolysis. The catalytic hydrolysis of C(±)P(–)-soman in various organs participating in central elimination is sufficiently high to account for the terminal half-life of the C(±)P(–)-stereoisomers. Alternatively, elimination may also proceed by binding since a large fraction of the binding sites is still unoccupied. The rate of binding will be much lower than in the initial phase due to the much lower residual concentration of C(±)P(–)-soman, and possibly also due to the heterogeneity of the binding sites.

C. RENAL EXCRETION

As a consequence of the very rapid degradation processes, only small amounts of C(±)P(±)-soman and of related organophosphates are renally excreted. Lenz et al.⁹² found that more than 99% of the renally excreted labeled compounds was hydrolyzed

at 1 h after s.c. administration of $C(\pm)P(\pm)$ - ^{14}C -soman (about 1 LD_{50}) to rats. No unaltered compound was detected by Heilbronn et al.⁹³ in the urine of rats after i.v. administration of tabun. Only after administration of the somewhat less reactive 3H -DFP, 5–10% of the labeled compounds that were renally excreted during the first hour after intoxication of guinea pigs and cats were identified as intact agent.^{94,95} At most, approximately 1% of $C(\pm)P(\pm)$ -soman was renally excreted within 4 h after administration to rats, mainly as $C(\pm)P(-)$ -soman.⁹⁶ Nevertheless, the levels in rats were at least two orders of magnitude higher than those for guinea pigs and marmosets after intoxication with an equitoxic dose of $C(\pm)P(\pm)$ -soman (6 LD_{50}). The levels in rat urine surpass the blood levels which were measured 1 min after i.v. administration, suggesting that most of the $C(\pm)P(-)$ -soman was excreted within the first few minutes after intoxication. This suggestion is supported by the presence of $C(-)P(+)$ -soman in the urine, which disappears from the blood within a few minutes. These results also indicate that phosphoryl phosphatase activity is absent in the urine and in the bladder. Concomitant with the high levels in urine, levels of intact $C(\pm)P(-)$ - ^{14}C -soman were much higher in rat kidney at 1 h after i.v. administration of a dose equivalent to 6 LD_{50} of $C(\pm)P(\pm)$ - ^{14}C -soman than in guinea pig and marmoset kidneys after administration of an equitoxic dose, in spite of the relatively high binding capacity that is also available in rat kidneys. Presumably, the stereoisomers of soman in the kidney are protected against elimination when these are, *de facto*, present in urine that has not yet been transported to the bladder.

The amount of renally excreted $C(\pm)P(-)$ -soman in rats, although very small compared with the administered dose, should be sufficient to be of toxicological relevance (*vide supra*) if the agent can be reabsorbed from the bladder. This process can indeed take place as was deduced from the lethal effect of an intravesical administration of 1.4 LD_{50} of $C(\pm)P(\pm)$ -soman.⁹⁶ On the basis of these results it may be speculated that $C(\pm)P(-)$ -soman excreted in the bladder serves as a “depot” and contributes to the “late death” of rats intoxicated with ≥ 6 LD_{50} of $C(\pm)P(\pm)$ -soman several hours after initial recovery as a result of treatment with oxime. However, survival times of intoxicated rats immediately treated with oxime decreased significantly when accumulation of $C(\pm)P(-)$ -soman in the bladder was prevented by rinsing and drainage of the bladder.⁹⁶ Therefore, eventual reabsorption of $C(\pm)P(-)$ -soman from the bladder does not explain the “late death” in rats, whatever its relevance is for the persistence of the toxic stereoisomers.

D. ELIMINATION PRODUCTS AS TOOLS FOR RETROSPECTIVE DETECTION OF EXPOSURE

Elimination products, i.e., hydrolyzed organophosphate or covalently bound organophosphate, are the biomarkers of choice for detection of exposure, since the persistence of phosphofluoridates, although sufficiently high to interfere with therapeutic measures, is too short for this purpose. The hydrolysis product formed upon intoxication with a phosphofluoridate is rapidly excreted and seems also unsuitable for retrospective detection. However, it has been found that CaE inhibited by organophosphates reactivate spontaneously leading to gradual formation of the hydrolyzed agent which

is consequently excreted over a relatively long period of time.⁷⁸ A procedure for retrospective detection of exposure to sarin, in which its hydrolysis product serves as a marker, was developed by Noort et al.⁶¹ for analysis in blood and urine. By using this method they were able to show hydrolyzed sarin in blood samples obtained from victims of the Tokyo incident in 1995. In addition, bound organophosphate can serve as a biomarker for exposure. Treatment of organophosphate inhibited CaE and BuChE with fluoride ions can inverse the inhibition reaction yielding a phosphofluoridate and restored enzyme.^{51,97} Human serum does not contain CaE but its BuChE concentration is relatively high (70–80 nM).^{70,76} Based on the fluoride-induced reactivation reaction, a method for retrospective detection of exposure to nerve agents has been developed, by which exposure of victims of the Tokyo incident to an organophosphate, probably sarin, could be established from analysis of their blood samples.⁵¹

IX. PHYSIOLOGICALLY-BASED MODELING OF THE TOXICOKINETICS OF SOMAN

Toxicokinetic studies will be more generally applicable, and therefore more valuable, if the results can be described in a physiologically-based model.^{98–101} These models represent the mammalian system in terms of compartments, i.e., specific tissues or groups of tissues, which are connected by arterial and venous blood flow pathways (see Figure 2.19). Processes taking place in the compartments are characterized by physiological parameters, e.g., tissue volumes and blood flow rates and parameters specific for the toxicant under investigation, such as tissue/blood partitioning coefficients and metabolic parameters. The model consists of differential equations describing the mass-balance in the various compartments by which time-dependent toxicokinetic data can be simulated. Physiologically-based toxicokinetic models are especially suitable for studying the effect of changes in physiological or toxicant-specific parameters induced, e.g., by treatment or pretreatment drugs, on the toxicokinetics of a toxicant. Furthermore, models provide a physiologically-based means for interspecies scaling and for extrapolation from animal results to those in human beings, which is an ultimate goal in the toxicology of nerve agents.

The description of the toxicokinetics of C(±)P(±)-soman is complicated by the high *in vivo* reactivity as well as by distinct differences in metabolic properties of the toxic C(±)P(–)-stereoisomers and the less toxic C(±)P(+)-stereoisomers. An early physiologically-based model for C(±)P(±)-soman in the rat was described by Maxwell et al.,¹⁰² whereas Gearhart et al.¹⁰³ developed a similar model for DFP. Both models were validated indirectly, based on the time course of AChE inhibition in blood and tissues. The first model in which the chirality of C(±)P(±)-soman was taken into account has been described by Langenberg et al.⁷³ This model was validated on the basis of toxicokinetic data of the intact stereoisomers obtained after i.v. administration of C(±)P(±)-soman at doses corresponding with 0.8, 2, and 6 LD₅₀. As shown in Figure 2.19, the model follows the general form for modeling of

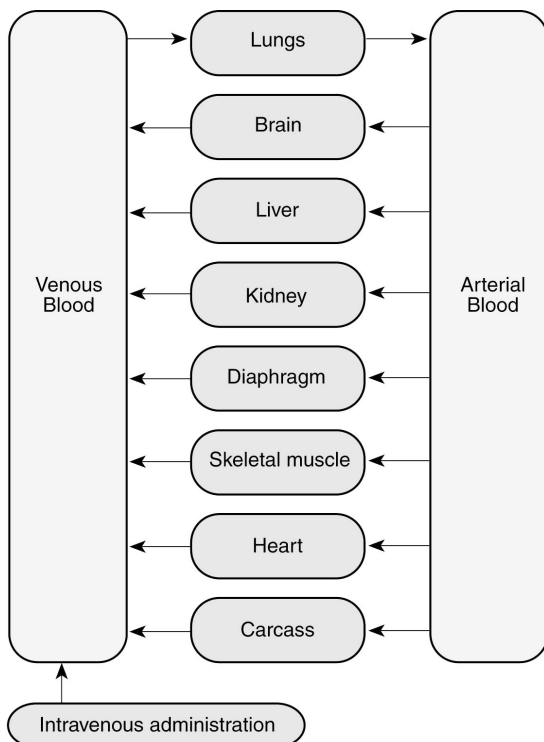


FIGURE 2.19 Schematic outline of the physiologically-based model for the toxicokinetics of C(±)P(±)-soman. (From Langenberg, J.P., Van Dijk, C., Sweeney, R.E., Maxwell, D.M., De Jong, L.P.A., and Benschoop, H.P., *Arch. Toxicol.*, 71, 320, 1997. With permission.)

organophosphorus compounds.^{102,103} Since exhalation as a pathway of elimination can be neglected after i.v. administration of the agent, this pathway was not taken into account.^{94,104} While some of the defined tissues are chosen on the basis of their high metabolic capacity (liver, kidney, lung), other tissues are selected since these are target tissues (brain, diaphragm). The relevant processes in each compartment are (1) partitioning of C(±)P(−)- and C(±)P(+)-soman from blood, (2) elimination defined as reaction with covalent binding sites and (3) elimination by enzymatic and spontaneous hydrolysis. Covalent binding is described as a bimolecular reaction with AChE as well as with rapidly and slowly reacting binding sites, mainly CaE. Combined enzymatic and spontaneous hydrolysis are described as first-order processes. The summed effect of biochemical reaction and mass transfer processes in each compartment is described in differential equations based on mass-balance principles.

A survey of the data used for the parameters in the model is given in [Table 2.11](#). In comparison to values used by Langenberg et al.,⁷³ the following improvements and refinements were recently made.¹⁰⁵

- The partition coefficients were determined from partitioning of $C(\pm)P(-)$ -soman over diluted blood and air, and over tissue homogenates and air after complete inhibition of the enzymatic hydrolysis of the stereoisomers by addition of EDTA, while previously hydrolysis was blocked by acidification to pH 3.3.
- Measured rate constants for inhibition of guinea pig erythrocyte AChE were introduced into the model instead of the values for inhibition of bovine erythrocyte AChE.
- Concentrations of binding sites in blood and lung were derived from results obtained of *in vivo* instead of *in vitro* experiments.
- The rate constants for hydrolysis of $C(\pm)P(-)$ -soman and $C(-)P(+)$ -soman and the concentrations of rapidly and slowly reacting binding sites in plasma were halved since these parameters are used for the blood compartment in the model.
- Finally, the liver blood flow has been corrected in the present model in accordance with literature data, i.e., 10.7 times higher than the hepatic artery flow.¹⁰⁶

The model discriminates between two stereoisomeric pairs, i.e., the $C(\pm)P(-)$ -stereoisomers and the $C(\pm)P(+)$ -stereoisomers. Further refinements were introduced in order to achieve a proper description of i.v. toxicokinetics. Since $C(+)P(+)$ -soman could not be detected in blood even at 0.25 min after administration, it was assumed that this stereoisomer is completely hydrolyzed immediately after administration. Therefore, only the doses of $C(-)P(+)$ -soman and of $C(\pm)P(-)$ -soman were introduced into the model and the parameters for hydrolysis of the $C(\pm)P(+)$ -stereoisomers were adjusted to those of $C(-)P(+)$ -soman. Furthermore, it was assumed that $C(+)P(-)$ -soman is completely bound in blood immediately after administration of a dose corresponding with 0.8 LD_{50} of $C(\pm)P(\pm)$ -soman. This assumption is based on (1) the concentrations of $C(+)P(-)$ -soman in blood which are approximately one order of magnitude lower at this dose than those of the $C(-)P(-)$ -stereoisomer, (2) preferential elimination of $C(+)P(-)$ -soman due to the 30-fold faster inhibition rate of CaE in plasma of guinea pigs by this stereoisomer than by $C(-)P(-)$ -soman, and (3) the excess of rapidly reacting binding sites in blood, being mainly CaE, over the amount of $C(+)P(-)$ -soman at this dose. In view of these considerations, the $C(+)P(-)$ -stereoisomer was omitted from the model at this sublethal dose, with a corresponding decrease of the amount of rapidly reacting binding sites. Furthermore, estimated rate constants for rapid binding of $C(-)P(-)$ -soman were used instead of the overall binding constants for $C(\pm)P(-)$ -soman.

With this refined model, the blood levels of $C(-)P(+)$ -soman and of the $C(\pm)P(-)$ -stereoisomers can be simulated in a satisfactory way for the three i.v.

TABLE 2.11

Values Used for Parameters in the Physiologically-Based Model for the Toxicokinetics of C(±)P(±)-soman after i.v. Administration to Anesthetized, Atropinized, and Mechanically Ventilated Guinea Pigs weighing 500 g.

Tissue	Tissue volume (ml)	Blood flow ^a (ml/min)	Partition coefficient ^b (tissue/blood)	A ^c (g tissue) (nmole/g tissue)	F ^{b,d} (nmole/g tissue)	S ^b (nmole/	f ^{b,e} (M ⁻¹ .min ⁻¹)	s ^{b,e} (M ⁻¹ .min ⁻¹)	h ^f (min ⁻¹)	h' ^{f,g} (min ⁻¹)
Blood	40	80	1.0	0.0097	0.495	0.184	3.0×10^7	8.2×10^4	0.05	3.33
Lung	5	80	0.9	0.0045	0.495	0.278	9.3×10^6	2.1×10^4	0.105	7.0
Brain	2.2	0.9	0.7	0.12	0.065	0.0435	2.3×10^7	2.5×10^4	0.039	2.6
Heart	1.8	4.0	1.4	0.0023	0.031	0.084	1.4×10^7	5.8×10^4	0.044	3.0
Diaphragm	1.5	0.2	1.4	0.00135	0.031	0.084	1.4×10^7	5.8×10^4	0.044	3.0
Liver	19	12.8	5.3	0.0063	20.2	37.6	7.8×10^6	1.5×10^4	0.844	56.0
Kidney	4.4	5.0	2.2	0.0044	5.29	4.69	6.5×10^5	6.5×10^3	0.265	17.6
Muscle	250	37.5	1.4	0.0016	0.031	0.084	1.4×10^7	5.8×10^4	0.044	3.0
Carcass	176.1	19.6	1.4	0.00001	0.031	0.084	1.4×10^7	5.8×10^4	0.0088	0.6

Note: A, F, and S denote the initial concentrations of AChE, rapidly and slowly reacting binding sites, respectively; a, f and s and h and h' denote the rate constants for AChE inhibition, binding to rapidly reacting binding sites and to slowly reacting binding sites by C(±)P(-)-soman and for hydrolysis of C(±)P(-)-soman and C(-)P(+)-soman, respectively.

^aThe blood flow to lung and liver is equal to, and 16% of, the cardiac output, respectively; the blood flow to the carcass is cardiac output minus blood flow to brain, heart, diaphragm, liver, kidney, and muscle.

^bValues for carcass, heart, and diaphragm are chosen equal to that for muscle.

^cThe value for carcass is arbitrarily set at a very low value of 0.01 pmole/g tissue; rate constants for inhibition are 8.25×10^7 and $8.25 \times 10^3 \text{ M}^{-1}\text{min}^{-1}$ for C(±)P(-)-soman and C(±)P(+)-soman, respectively.

^dConcentration of radioactivity bound in blood 1 h after i.v. administration of 6 LD₅₀ of C(±)P(±)¹⁴C-soman is taken as the values for rapidly reacting binding sites in blood and lung.

^eRate constants for C(±)P(+)-soman are chosen 0.01× the corresponding values for C(±)P(-)-soman.

^fValues for heart and diaphragm are chosen equal to that for muscle; the value for carcass is 1/5 of the value for muscle, and the value for blood is half the value for plasma.

^gCalculated as $\{(h_{\text{tissue}}/h_{\text{plasma}}) \times h'_{\text{plasma}}\}$

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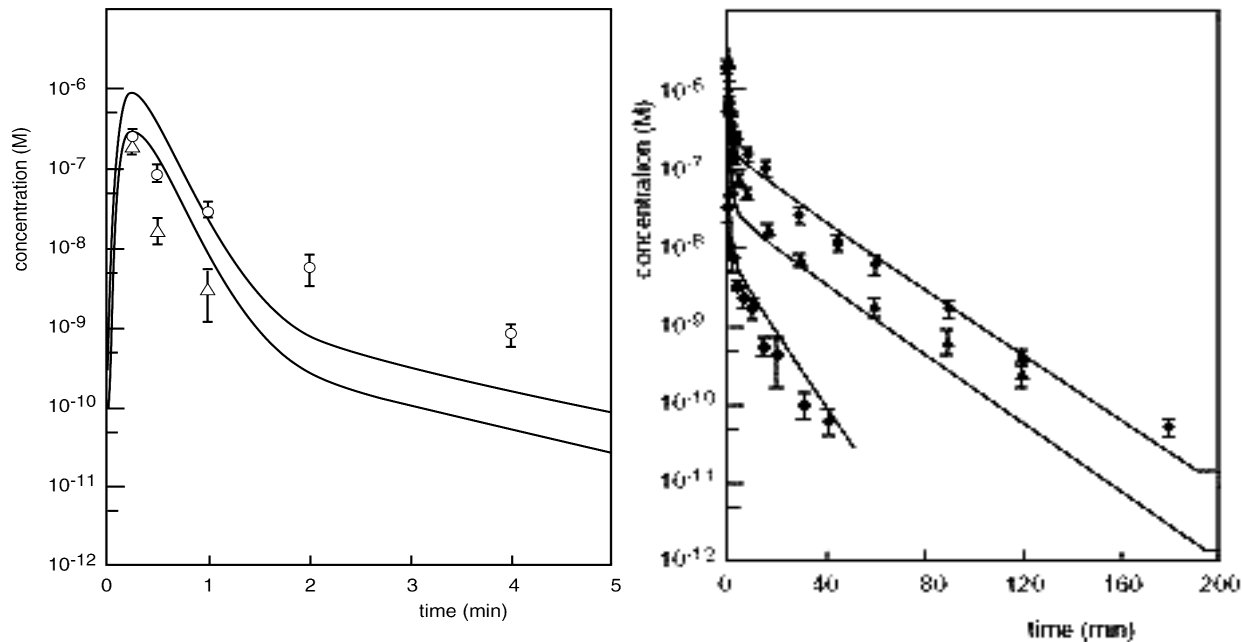


FIGURE 2.20 Physiologically-based modeling of (left panel) the toxicokinetics of $C(-)P(+)$ -soman and (right panel) the $C(\pm)P(-)$ -stereoisomers of soman for i.v. bolus administration of $C(\pm)P(\pm)$ -soman to anesthetized, atropinized, and mechanically ventilated guinea pigs. The solid lines depict the predicted courses of the concentration in blood vs. time. The data points indicate the actually measured concentrations (\pm SE; $n = 6$) of $C(-)P(+)$ -soman (open symbols) and of $C(\pm)P(-)$ -soman (filled symbols) after a dose corresponding with 6 (\circ) and 2 LD_{50} (\triangle) and of $C(-)P(-)$ -soman (\square) after a dose corresponding with 0.8 LD_{50} .

doses of $C(\pm)P(\pm)$ -soman shown in Figure 2.20. Especially for the lowest dose ($0.8 LD_{50}$), the refinements in the model result in an improved simulation relative to earlier published data.⁷³ As should be expected, binding in blood becomes less dominant in overall toxicokinetics when the dose of $C(\pm)P(-)$ -soman exceeds the capacity of the binding sites in blood, e.g., at doses corresponding to $2 LD_{50}$ and especially so at $6 LD_{50}$ of $C(\pm)P(\pm)$ -soman. Accordingly, predictions of the blood levels of $C(\pm)P(-)$ -soman at the latter doses were hardly affected by the changes introduced for modeling of $C(\pm)P(-)$ -soman at a dose of $0.8 LD_{50}$ of $C(\pm)P(\pm)$ -soman.

In a preliminary study, the effect of pretreatment with human plasma BuChE on the toxicokinetics of $C(\pm)P(\pm)$ -soman was simulated with the developed model.¹⁰⁵ The rate constants for reaction of the soman stereoisomers with human BuChE were calculated from the overall rate constant reported by Ashani et al.,¹⁰⁷ and the ratio of the rate constants for inhibition of equine plasma BuChE found by Keijer and Wolring.¹⁰⁸ Since $C(+)P(-)$ -soman has at least a 10-fold higher anti-BuChE activity than the other stereoisomers, it was assumed that this stereoisomer reacts preferentially and instantaneously after i.v. administration of human BuChE. Furthermore, it is assumed that any residual $C(+)P(-)$ -soman will subsequently be eliminated by instantaneous binding to endogenous rapidly reacting binding sites in blood up to complete saturation of these binding sites. This is similar to the assumption made for modeling of the toxicokinetics of $C(\pm)P(-)$ -soman after i.v. administration of a dose corresponding to $0.8 LD_{50}$ of $C(\pm)P(\pm)$ -soman (*vide supra*). In order to interpret our modeling results, it is assumed that the scavenger provides sufficient protection if the predicted $C(\pm)P(-)$ -soman concentrations in blood are similar to the concentrations calculated after i.v. administration of $\leq 0.7 LD_{50}$ of $C(\pm)P(\pm)$ -soman in nonprotected guinea pigs, since 70% of the inhaled LD_{50} dose of $C(\pm)P(\pm)$ -soman has been reported as a maximum sign-free dose in nonprotected guinea pigs.⁹⁰ According to this criterion, sufficient protection against intoxication with $1.5-3 LD_{50}$ and $5-6 LD_{50}$ of $C(\pm)P(\pm)$ -soman is offered by a dose of human BuChE corresponding with 0.5 and 0.7 times the dose of $C(\pm)P(\pm)$ -soman, respectively. This is illustrated in Figure 2.21. The predictions correspond with results obtained in protection experiments performed at similar conditions in mice, rats, and rhesus monkeys.¹⁰⁷

The description of the model highlights the need for further refinement by discriminating between the four stereoisomers of soman. Nevertheless, the model is a promising basis for extension to other routes of administration, for scaling to other species including man, and for making predictions on the efficacy of (pre)treatment drugs.

X. THE INFLUENCE OF PROPHYLAXIS AND THERAPY UPON THE TOXICOKINETICS OF SOMAN

In principle, the influence of prophylaxis and treatment on the toxicokinetics of $C(+)P(-)$ - and $C(-)P(-)$ -soman is a major item that should be addressed. Preliminary investigations have indicated that immediate treatment with HI-6 (150

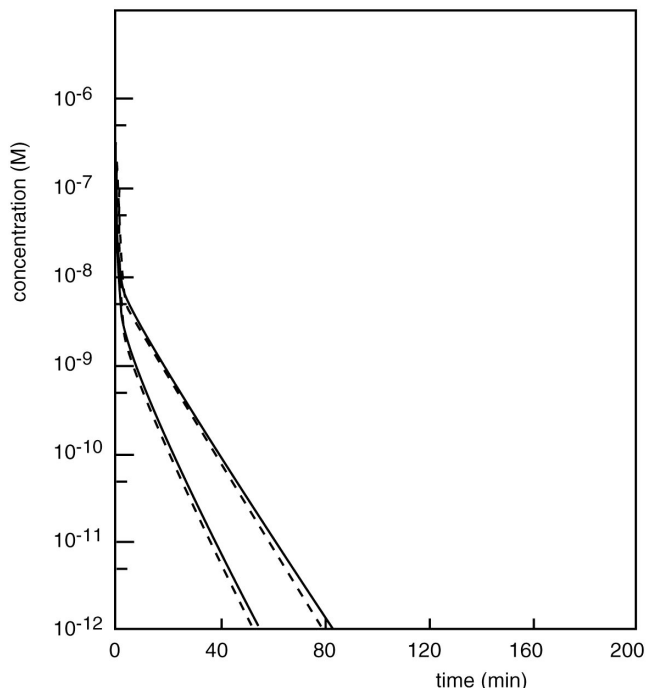


FIGURE 2.21 Physiologically-based modeling of the toxicokinetics of C(±)P(-)-soman for i.v. bolus administration of a dose corresponding with 2 LD₅₀ C(±)P(±)-soman to anesthetized, atropinized, and mechanically ventilated guinea pigs pretreated with human BuChE. The solid lines depict the predicted courses of the concentration in blood vs. time of C(±)P(-)-soman in pretreated animals in which the ratio of the initial amount of scavenger in blood over the dose of C(±)P(±)-soman is 0.3 (upper curve) or 0.5 (lower curve). For comparison, the dashed lines represent predictions for the concentrations of C(±)P(-)-soman in blood subsequent to administration of doses corresponding with 0.8 (upper curve) or 0.5 (lower curve) LD₅₀ of C(±)P(±)-soman (i.v.) to animals which were not pretreated with BuChE.

$\mu\text{mole} \cdot \text{kg}^{-1}$, i.v.) of anesthetized, atropinized, and mechanically ventilated rats had hardly any influence on the toxicokinetics of C(+)P(-)- and C(-)P(-)-soman at an i.v. dose equivalent to 3 LD₅₀ of C(±)P(±)-soman.²³ Pretreatment with pyridostigmine ($11.8 \mu\text{g} \cdot \text{kg}^{-1}$, i.p.) at 1 h before i.v. administration of a dose of 3 LD₅₀ of C(±)P(±)-soman to such rats caused a slight decrease of the blood levels of the abovementioned stereoisomers in the same time interval. However, omission of atropine in the latter experiments caused a significant increase of these blood levels in the same time interval.²⁰

Based on the abovementioned preliminary data, the influence of atropine sulfate ($50 \text{ mg} \cdot \text{kg}^{-1}$, i.p.), administered 5 min before a challenge of anesthetized and

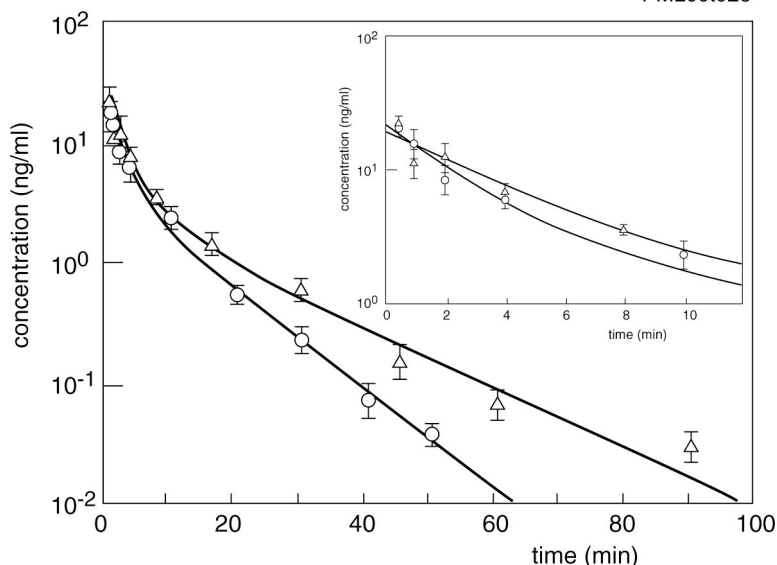


FIGURE 2.22 Semilogarithmic plot of the concentration in blood (\pm s.e.m.; $n = 6$) of C(+)-P(-)-soman vs. time after administration of 1 LD₅₀ (82.5 $\mu\text{g.kg}^{-1}$) of C(\pm)P(\pm)-soman to anesthetized and mechanically ventilated rats, pretreated (○) or not pretreated (△) by administration of atropine sulfate (50 mg.kg^{-1} , i.p.) 5 min before the challenge with C(\pm)P(\pm)-soman. The inset shows the data for the first 10 min plotted on an expanded scale.

mechanically ventilated rats with an i.v. dose of 1 LD₅₀ of C(\pm)P(\pm)-soman was investigated in more detail.²⁰ The effect of pretreatment with atropine is visualized for the C(+)-P(-)-stereoisomer in Figure 2.22, whereas the toxicokinetic data derived from the two-exponential equations fitting the concentration-time profiles are given in Table 2.12. Linear regression analysis of the two separate exponential terms of the equations showed that pretreatment with atropine had no significant influence in the first 10 min after intoxication. However, the subsequent elimination phase was significantly slower in the nonatropinized than in the atropinized animals, with terminal half-lives decreasing from 9.6–12.0 min to 6–7 min upon atropinization. Consequently, toxicologically relevant levels of C(\pm)P(-)-soman are present until 63 min after intoxication of the nonatropinized animals, but only until 37 min in the atropinized animals. In view of the antagonistic effects of atropine on the increased cardiovascular resistance caused by a dose of 1 LD₅₀ of C(\pm)P(\pm)-soman in rats, the more efficacious elimination of the toxic stereoisomers of soman in atropinized animals is probably due to a better blood circulation in the latter animals, thus leading to an increased rate of transport to sites in the animal where the stereoisomers are bound, hydrolyzed, and excreted.^{109,110}

TABLE 2.12

Toxicokinetic Parameters of Soman Stereoisomers in Anesthetized and Mechanically Ventilated Rats which Were Not Atropinized or Were Atropinized with Atropine Sulfate (i.p., 50 mg.kg⁻¹) 5 min Prior to a Challenge with an i.v. Dose of C(±)P(±)-soman Corresponding with 1 LD₅₀ (82.5 μg.kg⁻¹).

Parameter	C(+)P(-)-soman		C(-)P(-)-soman	
	Atropinized	Nonatropinized	Atropinized	Nonatropinized
Dose (μg/kg)	22.7	22.7	18.6	18.6
A (ng.ml ⁻¹) ^a	18.4	17.0	15.2	12.2
B (ng.ml ⁻¹)	3.9	2.7	5.9	4.4
a (min ⁻¹)	0.45	0.29	0.57	0.35
b (min ⁻¹)	0.096	0.058	0.12	0.072
Terminal half-life (min)	7.2	12.0	5.8	9.6
Area under curve (ng.min.ml ⁻¹)	81.5	105	76	96
Acutely toxic levels of	*—————37—————*			
C(±)P(-)soman until ^b (min)	*—————63—————*			

^aThe concentration of each stereoisomer at time t (conc.) is described by $\text{conc}^t 5 \text{Ae}^{-at} + \text{Be}^{-bt}$.

^bAfter administration of C(±)P(±)-soman. It is assumed that an AUC of 1.8 ng.min.ml⁻¹ is the minimum area with toxicological relevance (see text).

It should be concluded that the conventional treatment of C(±)P(±)-soman intoxication with atropine and oximes, such as HI-6, as well as prophylaxis with pyridostigmine have, at best, a modest beneficial effect on the elimination of the toxic stereoisomers of soman. On the other hand, the often-observed persistence of toxic levels of nerve agent for protracted periods of time after intoxication may profoundly affect the efficacy of treatment with the above-mentioned antidotes. For example, model calculations show that the efficacy of prophylaxis with pyridostigmine may be particularly limited by the persistence of C(±)P(-)-soman stereoisomers in the terminal elimination phase.¹¹¹ It follows that much can be gained in terms of efficacy of treatment if additional antidotes can be developed which aim specifically at sequestration of the toxic stereoisomers of soman. Such scavengers, e.g., phosphoryl phosphatase-type enzymes and BuChE from various sources are being developed. The investigation of the toxicokinetics of the nerve agent in the presence of scavenger will be essential to evaluate and validate the efficacy of this particular approach (see Section IX).

XI. TOXICOKINETICS OF V AGENTS

In contrast with G agents, few results of toxicokinetic investigations with V agents have been published. For various reasons it is worthwhile to compare the toxicokinetics of these two types of nerve agents. Several pathways for degradation of

G agents appear to be less effective for V agents. For example, phosphoryl phosphatases hydrolyze the P(+)-stereoisomers of G agents rapidly, but hardly degrade V agents.^{112,113} Covalent scavenging by CaE is a major pathway for the degradation of the P(-)-stereoisomers of G agents, while these enzymes are much less effective in binding V agents.¹¹⁴ Unless alternative *in vivo* pathways are available, these relative reactivities suggest a greater *in vivo* persistence of V agents than of G agents. What little is known about the toxicokinetics of V agents corroborates this assumption. Harris and co-workers investigated the toxicokinetics of the V agent O-cyclopentyl S-(2-diethylaminoethyl) methylphosphonothioate ($45 \mu\text{g} \cdot \text{kg}^{-1}$, i.v.) in anesthetized, atropinized, and mechanically ventilated rabbits (approximately 14.5 LD_{50}) and cynomolgus monkeys (approximately 10 LD_{50}).¹⁶ They estimated the concentrations of V agent on the basis of the overall inhibitory potency of blood samples towards bovine AChE. Although this approach cannot account for stereospecificity in the degradation of the two stereoisomers of the V agent, their results (Figure 2.23) indicate that $> 1 \text{ ng}$ of V agent per ml blood is still circulating at 90–120 min after intoxication, albeit that the doses were extremely high. Similar results were obtained with VX at equitoxic doses. The authors suggest that the marginal effectiveness of pretreatment with the carbamate pyridostigmine against intoxication with V agents can be explained by the remarkable persistence of these agents. Recent studies by Rocha et al.¹¹⁵ show that similar concentrations of VX can be sufficient to induce neurotoxic effects.

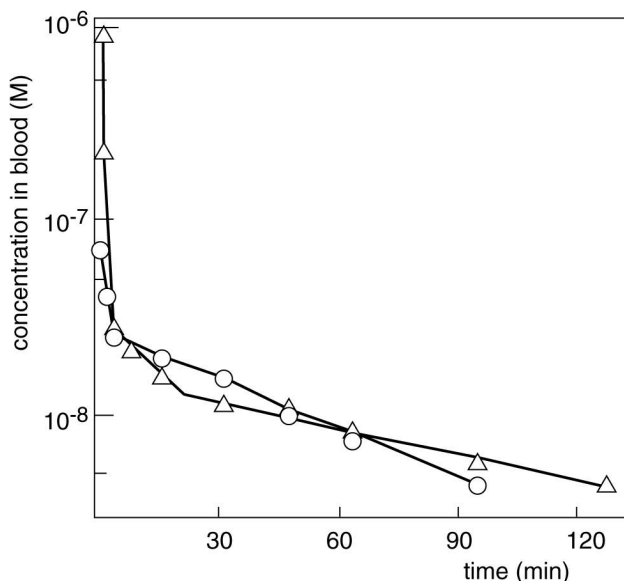


FIGURE 2.23 Semilogarithmic plot of the concentration in blood of O-cyclopentyl S-(2-diethylaminoethyl) methylphosphonothioate vs. time after administration of $45 \mu\text{g} \cdot \text{kg}^{-1}$ of this V agent to anesthetized, atropinized, and mechanically ventilated cynomolgus monkeys (Δ , approximately 10 LD_{50}) and white rabbits (\circ , approximately 14.5 LD_{50}).

Systematic investigation by Van der Schans et al.¹¹⁶ on the toxicokinetics and metabolism of VX at doses corresponding with 1–2 LD₅₀ in guinea pigs and marmosets via the i.v. and p.c. route are based on analysis of blood samples by means of chiral HPLC and gas chromatography. Preliminary results confirm the marked *in vivo* persistence of VX also at these more realistic doses and show that the overall stereospecificity for sequestration of (+)- and (–)-VX is much less pronounced than for the stereoisomers of G agents.

XII. FUTURE DIRECTIONS

With the methodology that has been developed for the analysis of nerve agents like soman, sarin, and VX, minimal detectable concentrations in the range of 1–50 pg · ml^{–1} blood have been obtained. These concentrations are at the lower limit of possibilities at the present or foreseeable state of the art in analytical chemistry. For most investigations on nerve agents, these procedures suffice to investigate the toxicokinetics at a wide range of doses and for all relevant routes of exposure. For example, many practical applications of toxicokinetic measurements can be expected in investigations dealing with the efficacy of new antidotes, e.g., in development of enzymatic scavengers for nerve agents.

The exposure studies of soman at low level (see Section VII) have shown that respiratory exposures for several hours to about 20 ppb of nerve agent are near the lower limit of what can be reached with regard to toxicokinetics based on *in vivo* measurement of intact nerve agent. If the toxicokinetics at even lower exposure levels should be investigated, e.g., at levels that are in the range of occupational exposure limits, one has to rely on measurement of nerve agent accumulated by internal scavengers such as BuChE and CaE. Methodologies for this approach have been developed for sarin and are being developed for other nerve agents, mostly based on release of the protein-bound nerve agent with fluoride ions and subsequent analysis of the generated phosphofluoridate.

It should be expected that further quantitative measurements on elimination routes of nerve agents, in combination with the wealth of available toxicokinetic data, will enable further development of physiologically-based modeling of toxicokinetics. Further model developments are needed, in particular for the respiratory and percutaneous exposure routes. Ultimately, this modeling will enable reliable interspecies extrapolation of toxicokinetic results, including extrapolation to man, which is the ultimate goal.

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