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The inhalation toxicokinetics of C(±)P(±)-soman and (±)-sarin were studied in anesthetized, atropinized guinea pigs. An apparatus was constructed for continuous generation of nerve agent vapor in air and nose-only exposure. During exposure the respiratory minute volume (RMV) and respiratory frequency (RF) were monitored. Blood samples were taken for gas chromatographic analysis of the concentrations of nerve agent stereoisomers, and to measure the progressive inhibition of acetylcholinesterase (AChE). The animals were exposed for 4-8 min to 0.4 or 0.8 LCt50 of C(±)P(±)-soman or (±)-sarin. The toxicokinetics of intravenous bolus administration of doses corresponding with 0.8 LD50 were studied as references for the inhalation experiments.

Concentrations of the P(-)-isomers increased rapidly during exposure, up to several ng/ml blood. The absorption phase of C(+)P(-)-soman lagged behind that of the C(-)P(-)-isomer. The measured progression of AChE inhibition was approximately in accordance with the observed blood levels of C(±)P(-)-soman. The results obtained of the various inhalation experiments with C(±)P(±)-soman indicated nonlinearity of the toxicokinetics, both with dose and with

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19. exposure time. Upon 8-min exposure to concentrations of (±)-sarin vapor in air yielding 0.4 and 0.8 LCt50, (-)-sarin was detectable in blood up to 2 h after exposure, whereas (+)-sarin was not detectable at all. Nonlinearity of the toxicokinetics with the (±)-sarin dose was observed.
P(-)-isomers were detected in blood upon nose-only exposure of guinea pigs for 5 h to 0.1 LCt50 of C(±)P(±)-soman, with maximum concentrations of ca. 40 pg/ml. Blood AChE was gradually inhibited up to ca. 95 % during the 5 h exposure, whereas brain AChE was not significantly inhibited.
Nerve agent-related effects on RMV and RF were absent in all of the inhalation experiments.
Intravenous infusion of C(±)P(±)-soman appeared to be a suitable substitute for the absorption phase in the technically involved respiratory exposure, unlike intramuscular bolus administration.

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SUMMARY

Previously (Grants Nos. DAMD17-85-G-5004 and DAMD17-87-G-7015) we studied the toxicokinetics of the stereoisomers of C(±)P(±)-soman in atropinized guinea pigs and marmosets at doses corresponding with 2 and 6 LD50 and with 1, 3 and 6 LD50 in rats, after administration of the nerve agent as an intravenous bolus.
These studies provided insight into the distribution and elimination of the C(±)P(±)-soman stereoisomers in these species. The guinea pig appeared to be a better model for man than the rat, as the toxicokinetics in the guinea pig closely resembles that in the marmoset, which is considered to be an adequate model for man.

In case of intoxications under conditions which prevail in chemical warfare, however, the primary route of entrance into the body of volatile nerve agents such as (±)-sarin and C(±)P(±)-soman is the respiratory route. This route will presumably lead to toxicokinetics which differ considerably from those observed after intravenous injections. As a consequence, we proposed to study the toxicokinetics of C(±)P(±)-soman and (±)-sarin after inhalation exposure. (±)-Sarin is included in these studies as it is, like C(±)P(±)-soman, a volatile nerve agent designed for intoxication via inhalation, and is stockpiled by major military powers.

In the Grant period, the following topics were studied in guinea pigs:

(a) Development of a procedure for analysis of the two stereoisomers of (±)-sarin in guinea pig blood with a minimum detectable concentration at or below the level of toxicological relevance (≤ 10 pM).
(b) The toxicokinetics in the acutely toxic Ct-range by means of dynamic exposure (i) for 8 min to a concentration yielding 0.8 and 0.4 LCt50 of C(±)P(±)-soman and (±)-sarin in order to establish the influence of dose and (ii) for 4 min to a concentration yielding also 0.8 LCt50 of C(±)P(±)-soman in order to establish the influence of the exposure time on the toxicokinetics.
(c) The inhalation toxicokinetics of C(±)P(±)-soman resulting from an exposure of 300 min to a concentration yielding 0.1 LCt50.
(d) The respiratory minute volumes of the animals during exposure as described in (b) and (c), in order to establish possible toxicant related effects on the respiration.
(e) The toxicokinetics of C(±)P(±)-soman stereoisomers resulting from (i) linear intravenous infusion in the course of 8 min yielding 0.8 LD50 (i.v., infusion) and (ii) intramuscular bolus administration of a dose of 0.8 LD50 (i.m.,bolus), as potentially simple substitutes for the experimentally difficult inhalation exposures.

(f) The progressive inhibition of acetylcholinesterase (AChE) in erythrocytes during the experiments as described in (b), (c) and (e). This will further establish the relationship between the degree of AChE inhibition in blood and measured areas under the curve of C(±)P(-)-soman and P(-)-sarin.
(g) The toxicokinetics of (+)- and (-)-sarin and of the four stereoisomers of C(±)P(±)-soman with intravenous bolus administration of 0.8 LD50 (i.v., bolus) of (±)-sarin and of C(±)P(±)-soman, respectively, as references for the toxicokinetics resulting from inhalation exposure to 0.8 LCt50 of these agents and for comparison with our previous toxicokinetic investigations in guinea pigs after intravenous administration (bolus) of C(±)P(±)-soman at doses ≥ 2 LD50.

The analyses of the blood samples were performed with the Thermal Cold Trap (TCT)/MUSIC configuration which we successfully used in the previous Grant study. In the final stage of this study, we automated the TCT injector, which eliminated the major drawback of this configuration, i.e., the limited sample throughput.

Sarin stereoisomers can be extracted from blood samples with the solid phase extraction procedure as used for C(±)P(±)-soman, with D7-(-)-sarin as the internal standard. Various stationary phases were tested for their ability to resolve the enantiomers of (±)-sarin. A commercially available ß-cyclodextrin phase was chosen for routine analysis of (±)-sarin stereoisomers in blood samples.

After intravenous administration of 0.8 LD50 C(±)P(±)-soman to anesthetized, atropinized and mechanically ventilated guinea pigs, the toxic P(-)-isomers were detectable up to 40 min after administration. The concentration of the C(+)P(-)-isomer was considerably lower than that of the C(-)P(-)-isomer. The concentration-time profile of C(-)P(-)-soman was adequately described with a two-exponential equation. The area under the curve of C(-)P(-)-soman appeared to be very small when compared with 2 and 6 LD50, which is in accordance with previous observations of nonlinear toxicokinetics of C(±)P(±)-soman in the guinea pig.

After intravenous administration of 0.8 LD50 (±)-sarin to anesthetized, atropinized and mechanically ventilated guinea pigs, (+)-sarin was not detectable, whereas the toxic (-)-sarin isomer was detectable up to at least 40 min after administration. The concentration-time profile of (-)-sarin was adequately described with a two-exponential equation. (-)-Sarin is distributed faster than C(-)P(-)-soman, whereas the elimination of (-)-sarin proceeds considerably slower.

An apparatus was constructed for the continuous generation of nerve agent vapor in a concentration range of 50 $\mu g/m^3$ to 100 mg/m^3 in air flow, with a temperature of 20 °C and a relative humidity of 70 %. The concentration of C(±)P(±)-soman in the airstream is monitored by a gas chromatographic configuration equipped with a gas-sampling

valve. Furthermore, an apparatus for simultaneous nose-only exposure of up to eight guinea pigs was constructed and tested. Software was developed for the monitoring of the respiratory frequency and the respiratory minute volume of the animals during the exposure.

During 8-min nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 0.8 LCt50 of C(±)P(±)-soman, the P(-)-isomers appeared to be rapidly absorbed. The absorption phase of the C(+)P(-)-isomer appeared to lag behind that of the C(-)P(-)-isomer. The blood acetylcholinesterase activity dropped rapidly during the 8-min exposure period, down to ca. 4 % of control activity. During the 8-min exposure period, no soman-related effects on respiratory frequency and respiratory minute volume were observed.
Upon 8 min exposure to 0.4 LCt50 of C(±)P(±)-soman, the concentrations of the P(-)-isomers were ca. fourfold lower when compared with exposure to 0.8 LCt50, indicating nonlinearity with dose at low dosages. Furthermore, inhibition of blood acetylcholinesterase proceeded somewhat slower than for exposure to 0.8 LCt50.
Upon 4-min exposure to 0.8 LCt50 of C(±)P(±)-soman, the concentrations of C(±)P(-)-soman isomers were, unexpectedly, lower than those measured for 8-min exposure to the same LCt value. This observation can be partially explained by taking into account that the respiratory minute volume for the 4-min exposure was ca. 30 % lower than that measured for the 8-min exposure. Assuming that the retention of the agent remained unchanged, this implies that only 70 % of the theoretical dose was inhaled by the animals.

During a 300-min exposure of anesthetized, atropinized and restrained guinea pigs to 0.1 LCt50 of C(±)P(±)-soman, blood concentrations of C(±)P(-)-soman were gradually built up, with maximum concentrations of ca. 40 pg/ml, leading to near complete inhibition of blood acetylcholinesterase. This was a surprising observation, since exposure to 0.1 LCt50 of C(±)P(±)-soman in a time period of 5 h was assumed to be an acceptable subchronic exposure, which obviously is not the case. With more extensive investigations, 'safe' periods of exposure to C(±)P(±)-soman can be defined.

When anesthetized, atropinized and restrained guinea pigs were nose-only exposed to 0.8 LCt50 of (±)-sarin, the (-)-isomer was rapidly absorbed. The blood acetylcholinesterase activity dropped rapidly during the 8-min exposure period, down to ca. 15 % of control activity. There were no effects on the respiratory parameters.
Upon 8-min exposure of anesthetized, atropinized and restrained guinea pigs to 0.4 LCt50 of (±)-sarin, the area under the curve was ca. 40 % of that calculated for exposure to 0.8 LCt50. Taking into account a 50% higher respiratory minute volume at the latter dose, these results indicate a significant nonlinearity with dose at low dosages. Inhibition of blood acetylcholinesterase proceeded somewhat slower than for exposure to 0.8 LCt50.

The concentration-time profiles of C(±)P(-)-soman during intravenous infusion appeared to be in close agreement with those during

respiratory exposure to an equitoxic dose. Subsequently, after the infusion had stopped, the blood levels of C(±)P(-)-soman decreased much faster than after inhalation. This observation suggests the presence of a depot in the upper respiratory tract, from which absorption continues after termination of the respiratory exposure. The time courses of blood acetylcholinesterase activity are nearly identical for both administration routes. It is concluded that intravenous infusion is a suitable substitute for the absorption phase of inhalation toxicokinetics.

After intramuscular bolus administration of a dose corresponding with 0.8 LD50, concentrations of C(±)P(-)-soman build up slowly and irregularly, leading to an irregular progression of blood acetylcholinesterase inhibition. Intramuscular bolus administration is not a suitable substitute for an equitoxic respiratory exposure.

The progression of blood AChE inhibition, as calculated from the measured concentration-time profiles of (-)-sarin and C(±)P(-)-soman using assumed bimolecular rate constants of inhibition, correlated reasonably well with the measured progression of AChE inhibition.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

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I. INTRODUCTION

Our previous and ongoing investigations (1-5) deal with the toxicokinetics of the four stereoisomers of C(±)P(±)-soman in rats, guinea pigs, and marmosets after intravenous bolus administration of C(±)P(±)-soman at doses equivalent to 1-6 LD50. These investigations show that the toxicokinetics of the relatively nontoxic C(±)P(+)-soman isomers should be strictly differentiated from those of the toxic C(±)P(-)-isomers. Whereas the C(±)P(+)-isomers are rapidly inactivated by means of enzymatic hydrolysis, the C(±)P(-)-isomers are much more persistent, being largely inactivated by irreversible binding to carboxylesterases and other binding sites. Valuable insight was obtained into interspecies differences in the toxicokinetics of C(±)P(±)-soman stereoisomers, which could explain interspecies variations in the efficacy of prophylaxis and therapy. Our studies have provided a quantitative basis for further toxicological studies of C(±)P(±)-soman as well as for the design and evaluation of new methods of prophylaxis and therapy (5-7).

In case of intoxications under conditions which prevail in chemical warfare, the primary route of entrance into the body of volatile nerve agents such as (±)-sarin and C(±)P(±)-soman is the respiratory route. The latter route is almost as effective as parenteral administration, with 70 to 90 % of an inhaled dose of (±)-sarin being retained in guinea pigs, dogs, monkeys and human volunteers (8, 9). Nevertheless, respiratory administration of nerve agents will presumably lead to toxicokinetics which differ considerably from those observed after intravenous injections as used in our earlier investigations. The inhalation and subsequent absorption of a certain dose of nerve agent vapor, largely in the upper part of the respiratory tract (10), may involve a period of seconds up to minutes or even hours, depending on the situation in the field. Therefore, it should be expected that the shapes of the toxicokinetic curves of nerve agent stereoisomers will differ considerably from those after intravenous bolus injection. Whereas the latter method of administration leads to maximum concentrations of nerve agent in the blood almost immediately after administration, the respiratory route of administration will presumably lead to a more gradual build-up of concentrations in blood and consequently to a more gradual distribution, followed by overriding elimination when the inhalation of agent ceases. Similarly Maxwell et al. (11) found that, after administration of 1 LD50 of C(±)P(±)-soman to rats, the time to maximum inhibition of AChE in various tissues is significantly longer after intramuscular than after intravenous administration.
The expected difference in the toxicokinetics of nerve agents resulting from intravenous injection and inhalation may have important consequences for the efficacy of prophylaxis and therapy of nerve agent intoxication. This hypothesis is corroborated by experiments (12) dealing with the efficacy of pyridostigmine pretreatment against C(±)P(±)-soman intoxication, which showed that this pretreatment is several times more effective when C(±)P(±)-soman is administered via inhalation or subcutaneously than after

intravenous injection. Qualitatively, this result can be understood when it is assumed that the high initial concentration of C(±)P(-)-soman upon intravenous administration will inhibit rapidly almost all critical AChE, thus shifting the dynamic equilibrium between release of free AChE from carbamylated enzyme and re-inhibition of this free enzyme by C(±)P(-)-soman towards the latter. In view of these differences, it is important to study the inhalation toxicokinetics of nerve agents. Such studies will improve insight into the toxicology of the agents and will provide a realistic support for (pre)treatment of intoxications in a field situation.

On the basis of the considerations mentioned above, we proposed to study the following aspects of inhalation toxicokinetics of nerve agents.

(a) <u>(±)-sarin and C(±)P(±)-soman as target agents</u>
Until now, our toxicokinetic studies have only involved the nerve agent C(±)P(±)-soman. (±)-Sarin is included in our studies, in addition to C(±)P(±)-soman, since these are both volatile nerve agents designed for intoxication via inhalation, and are stockpiled by major military powers. This extension of our studies demands the development of stabilization and analysis procedures for (±)-sarin in biological samples. It is expected that these procedures will be analogous to those for C(±)P(±)-soman stereoisomers (13).
As in the case of C(±)P(±)-soman, it will be essential to differentiate between the stereoisomers of (±)-sarin. Previous investigations in our laboratory have shown that the bimolecular rate constant for inhibition of electric eel AChE by (-)-sarin is ca. four orders of magnitude higher than that of (+)-sarin (13). Consequently, we expect that (+)-sarin, by analogy with the C(±)P(+)-isomers of soman, will be almost nontoxic in comparison with (-)-sarin. This assumption is strengthened by our observation that the LD50 (iv, mouse) of (-)-sarin is approximately half that of (±)-sarin (14).

(b) <u>The guinea pig as selected species for experiments</u>
In principle, primates are the species of choice to investigate the inhalation toxicokinetics of nerve agents, since data from this species should be most relevant for humans. However, the availability of primates such as marmosets for our investigations remains an uncertain factor. Since it was shown that the overall toxicokinetics of C(±)P(±)-soman stereoisomers in guinea pigs resemble those in primates more closely than the toxicokinetics in rats (1, 2), we decided to select the guinea pig for the inhalation studies. As in early studies on the respiratory toxicology of (±)-sarin (8, 9) the use of unanesthetized animals in our investigations would be preferable, to obtain as realistic toxicokinetic data as possible. Since this is no longer possible in view of the present legal regulations, guinea pigs anesthetized with ketamine will be used. In general, the latter type of anesthesia is supposed to have only a minimal influence on the respiratory minute volume (15), whereas cardiodepressant and hypotensive effects are absent. The exact influence of ketamine on vital functions of the guinea pig will be investigated prior to the main inhalation experiments. The animals

will also be atropinized in order to antagonize obstruction of airways due to bronchial secretion, whereas a cannula will be installed in the carotid artery for blood sampling. Such animals, while restrained in a tube, will be exposed in a nose-only configuration to various Ct values of C(±)P(±)-soman or (±)-sarin in a dynamic exposure set-up as developed by TNO Division of Nutrition and Food Research (16). This configuration allows continuous measurement of the respiratory minute volume of each individual animal during exposure.
The desired concentrations of C(±)P(±)-soman and (±)-sarin in air will be generated by bubbling dry air through the neat agent and further dilution with humidified air as required.
The concentration of nerve agent vapor in the humidity-controlled airstream will be monitored by means of on-line gas-sampling and gas chromatographic analysis.

(c) Exposure to various Ct values
The supralethal doses of agent as used in our previous toxicokinetic investigations will be decreased to at most 0.8 LCt50. Toxicokinetic studies involving respiratory exposure to the LCt50 and even higher Ct values are not realistic. This would require artificial respiration to keep the animals alive. On the other hand, the respiratory route is realistic and suitable for sublethal exposure. Recent experiments in our laboratory (2, 17) have shown that the blood levels of the toxic C(±)P(-)-stereoisomers of C(±)P(±)-soman can be monitored in rats for ca. 60 min after intravenous injection of a dose of C(±)P(±)-soman equivalent to 1 LD50, and for ca. 30 min after a dose corresponding to 0.5 LD50. In view of the aforementioned arguments, the toxicokinetics will be established for four scenarios (i-iv, below) of respiratory exposure, complemented by reference experiments (v, below) with intravenous bolus administration.

(i) Acute (8 min) exposure to 0.8 LCt50 of C(±)P(±)-soman and (±)-sarin

Guinea pigs will be exposed to 0.8 LCt50 of C(±)P(±)-soman or (±)-sarin with an 8-min exposure time, which is estimated to be ca. 160 $mg.min.m^{-3}$ (8, 9). This exposure time should be considered as a compromise between the often shorter exposure time to lethal concentrations of volatile nerve agents in case of chemical warfare and our desire to measure in a manageable time frame the increasing blood levels of (±)-sarin and C(±)P(±)-soman due to inhalation and absorption. Moreover, the individual variations in respiratory minute volume become extremely large when animals are exposed to almost lethal Ct's of nerve agent vapor for periods ≤ 2 min (8).
Since LCt50 values of (±)-sarin have been shown to increase with increasing exposure time and with restraint (8), the LCt50 of (±)-sarin and of C(±)P(±)-soman will be determined for an 8-min exposure time in restrained, ketamine anesthetized guinea pigs as a basis for the toxicokinetic experiments.

(ii) Acute (8 min) exposure to 0.4 LCt50 of C(±)P(±)-soman and (±)-sarin.

The incapacitating Ct (ICt50) of a nerve agent can be defined as the Ct causing beginning tremors (9). In case of chemical warfare it should be expected that large numbers of such incapacitated casualties will be amenable to effective treatment for possible return to duty. In order to obtain a quantitative basis for treatment of such casualties, the toxicokinetics of C(±)P(±)-soman and (±)-sarin will be studied at this exposure level. It is estimated (9) that the ICt50 of (±)-sarin corresponds to approximately 0.4 LCt50. For lack of data it will be assumed that the same ratio is valid for C(±)P(±)-soman. Since the exposure time for these experiments will be the same as for the exposure to 0.8 LCt50, the data from the exposures to 0.8 and 0.4 LCt50 will provide valuable information on the dose dependence for the toxicokinetics of inhaled (±)-sarin and C(±)P(±)-soman.

(iii) Acute (4 min) exposure to 0.8 LCt50 of C(±)P(±)-soman

In a field situation involving chemical warfare, the time of exposure to nerve agents is an obvious and important variable. It is known (8) that the LCt50 increases with increasing exposure time, whereas the syndromes of the intoxication will also vary with the time period needed to absorb the agent. Therefore, the influence of exposure time on the inhalation toxicokinetics of C(±)P(±)-soman will be studied by performing the experiment with C(±)P(±)-soman as mentioned under item (i) with an exposure time of 4 min instead of 8, while the concentration of C(±)P(±)-soman in the airstream is doubled.

(iv) Long-term exposure to 0.1 LCt50 of C(±)P(±)-soman

A situation involving exposure for several hours to low concentrations of nerve agent is relevant for unprotected military personnel in case of chemical warfare, when performing duty or resting in the "toxic agent-free areas" of hardened facilities and medical units where CW casualties are treated. It is assumed (18) that the personnel can be exposed to a Ct of at most 2 $mg.min.m^{-3}$ (±)-sarin or C(±)P(±)-soman. The reasoning behind the latter data is rather arbitrary, being based on the observation that it is the maximal Ct which does not cause incapacitating miosis in man, due to direct exposure of the eyes, whereas signs of systemic intoxication are supposed to appear at a tenfold higher Ct. This result has been obtained for exposure times ≤ 1 h, but has been extrapolated to exposure times up to 6 h. However, it is known that the LCt50 of (±)-sarin may increase sixfold in rats, guinea pigs and monkeys when the exposure time is increased from 0.1 min to 1 h (8, 9). By analogy it seems reasonable to assume that in a 5-h exposure to (±)-sarin or C(±)P(±)-soman a Ct appreciably in excess of 2 $mg.min.m^{-3}$ may be tolerable without an unacceptable degree of myosis. In order to contribute to a firmer toxicological basis for acceptable subchronic exposure to nerve agents, the toxicokinetics of C(±)P(±)-soman during exposure for 300 min to a concentration yielding 0.1 LCt50 will be studied, in which the LCt50 is taken as the value determined for acute (8 min) exposure. It is estimated that 0.1 LCt50 is ca. 20 $mg.min.m^{-3}$. Supposedly, this dose will cause just observable systemic

toxic effects (18) in humans after a short exposure. A dose corresponding with 0.15 LD50 (i.m. bolus; 2.3 µg/kg) is the minimal dose causing observable motoric effects in studies on behavioural toxicology with rhesus monkeys (19).

(v) Intravenous bolus administration of 0.8 LD50 of C(±)P(±)-soman and (±)-sarin

As a reference for our inhalation studies with C(±)P(±)-soman and (±)-sarin based on Ct values, toxicokinetic data are needed at "equitoxic" doses pertaining to intravenous bolus administration. Such investigations are also needed to compare the toxicokinetics of (±)-sarin, which is much more hydrophilic than C(±)P(±)-soman, with the results of our previous investigations (1, 2) of intravenously injected C(±)P(±)-soman in guinea pigs at doses ≥ 2 LD50.

(d) Intravenous infusion and intramuscular administration as possible substitutes for inhalation

From a technical point of view, toxicokinetic investigations with respiratory exposure are complicated, rather demanding on safety precautions, and costly. Such studies involve the generation, maintenance, and monitoring of desired nerve agent concentrations in air for prolonged periods, preferably in a dynamic exposure configuration. The animal experimentation involves nose-only exposure of restrained animals with pre-installed cannulas for blood sampling. The safety precautions required for handling liquid and vaporized nerve agents on a ca. 1-gram scale can only be realized in defense research-oriented laboratories by experienced personnel. Therefore, we decided to investigate whether an intravenous infusion of C(±)P(±)-soman can be used to mimic the blood curves of the C(±)P(-)-soman isomers that result from respiratory exposure to C(±)P(±)-soman. If so, intravenous infusion can be used in further toxicological studies of C(±)P(±)-soman and of other volatile nerve agents, as a convenient substitute for respiratory exposure. In order to find out whether the toxicokinetics resulting from exposure for 8 min to 0.8 LCt50 of C(±)P(±)-soman can be mimicked, the blood levels will be measured that result from intravenous infusion with an infusion pump of a dose of C(±)P(±)-soman equivalent to 0.8 LD50 at a constant rate of infusion during 8 min. The pertaining LD50 will be determined with the same method of administration.
It will also be investigated whether the gradual infusion into the general circulation resulting from intramuscular bolus administration of C(±)P(±)-soman at a dose equivalent to 0.8 LD50 (i.m., bolus) might also mimic the toxicokinetics of the above-mentioned respiratory exposure. Such experiments may also support the efforts of Maxwell et al. (11) to model the toxicodynamics of C(±)P(±)-soman exposure (in rats) after intramuscular administration of a dose equivalent to 1 LD50.

(e) Measurement of AChE levels in blood and of respiratory minute volumes

Previously (1, 2) we estimated minimum blood levels and areas under the curve of C(±)P(-)-soman isomers having toxicological relevance from rates and presumed degrees of inhibition of AChE due to these blood levels in a hypothetical situation involving free AChE which had been reactivated subsequent to complete inhibition. In contrast with our previous toxicokinetic experiments involving intravenous injection of a supralethal dose of agent, most of the experiments as described above under items (c) and (d) involve a gradual influx of a sublethal dose of nerve agent into the bloodstream. Therefore the progression of inhibition of blood AChE will be measured in conjunction with the measurement of blood levels of the C(±)P(±)-soman and (±)-sarin stereoisomers in blood samples. Since the respiratory minute volumes of the exposed animals are also measured continuously during exposure and the retained fraction of agent can be assumed to be ca. 80 % (8, 9), these measurements will allow a useful correlation between the absorbed dose of agent and degree of inhibition of blood AChE. From the point of view of a well-defined administered dose such a correlation will be even more straightforward in case of intravenous infusion and of intramuscular injection [cf. item (d)].

If possible, the data obtained in our toxicokinetic measurements should be fitted to models that have been developed by Andersen and coworkers (20, 21) specifically for inhaled compounds. Such physiologically based modelling would allow cross-species scaling, ultimately to man. These aforementioned models are based on pulmonary uptake of the investigated compounds and on the attainment during inhalation of either equilibrium, in the case of nonreactive compounds, or on the attainment of a steady-state situation, in the case of reactive compounds that are metabolized. However, nerve agents are absorbed largely in the upper part of the respiratory tract rather than in the lung (10). Moreover, in our toxicokinetic studies with C(±)P(±)-soman in case of intravenous bolus administration, we have learned that the toxic C(±)P(-)-isomers of C(±)P(±)-soman are eliminated by way of irreversible phosphonylation of binding sites in blood and in various tissues which are in large excess over the total amount of absorbed nerve agent (2). It follows that a steady-state situation during the inhalation of nerve agents will not be attained. Therefore, modelling of toxicokinetic data according to the earlier mentioned models will not be a realistic option in our case. However, efforts are made to develop models that fit the specific elimination pathways of phosphofluoridates. An example of such a model is that developed by Gearhart et al. (22) for *in vivo* inhibition of acetylcholinesterase in mice and rats after intravenous and subcutaneous administration of diisopropyl phosphorofluoridate (DFP). Furthermore, a physiologically based model for the toxicokinetics of C(±)P(±)-soman in the guinea pig is being developed as a joint effort of U.S. Army Medical Research Institute of Chemical Defense and TNO Prins Maurits Laboratory (23).

II. EXPERIMENTAL PROCEDURES

Materials

C(±)P(±)-1,2,2-Trimethylpropyl methylphosphonofluoridate [C(±)P(±)-soman], (±)-isopropyl methylphosphonofluoridate [(±)-sarin] and 2,2-dimethylpropyl methylphosphonofluoridate [(±)-neopentyl sarin, NPS] were prepared in the TNO Prins Maurits Laboratory from reaction of the appropriate alcohol with methylphosphonic difluoride and methylphosphonic dichloride according to the procedure of Bryant et al. (24). The compounds were distilled over a Spaltrohr column until a purity ≥ 99 % by gas-liquid chromatography (GLC) was obtained. The internal standard for the C(±)P(±)-soman analysis, D_{13}-C(±)P(+)-soman, was obtained as described previously (1).

Ethyl acetate (zur Rückstandanalyse) was procured from Merck (Darmstadt, Germany), and was distilled over a column packed with Dixon rings (plate number 80; NGW, Wertheim, West Germany) before use. Isopropanol (Brocacef, Rijswijk, The Netherlands) was purified by means of the same procedure (purity > 99.7 %). Aqueous solutions were prepared with high-performance liquid chromatography (HPLC)-grade water from Fisons Ltd.(Loughborough, UK).

The following products were obtained commercially and were used without further purification: saponine (BDH, Poole, UK), aluminium sulfate (BDH Analar, ≥ 98 %), sodium bicarbonate (Lamens en Indemans, 's-Hertogenbosch, The Netherlands, > 99.5 %), acetic acid (Lamens en Indemans, > 99 %), sodium acetate (Merck, zur Analyse, > 99.5 %), atropine sulfate (Brocades Stheeman, Haarlem, The Netherlands), heparin (Vitrum, Stockholm, 5000 IU/ml), ketamine hydrochloride (Vetalar^{R}, Parke-Davis, Morris Plains, NJ, USA), acetylpromazine (Vetranquil^{R} , disodium hydrogen phosphate dihydrate (analytical grade, Merck), potassium dihydrogen phosphate (analytical grade, Merck), potassium chloride (analytical grade, Merck), etopropazine hydrochloride (89.8 % purity, May & Baker Ltd.), chloroacetic acid (analytical grade, Merck), toluene (Lamens and Pleuger, 's-Hertogenbosch, The Netherlands, > 99.3 %), isoamyl alcohol (UCB, Brussels, Belgium, analytical grade), Permablend I (Packard Instruments B.V., Groningen, The Netherlands), nitromethane (analytical grade, Baker Chemicals, Phillipsburg, NJ, USA), glacial acetic acid (analytical grade, Baker Chemicals), and diethyl ether (analytical grade, Baker Chemicals).
[3H]-acetylcholine chloride (specific activity 61.4 GBq/mmol) and n-hexadecane (specific activity 340 kBq/mmol) were purchased from Amersham (Houten, The Netherlands).
Electric eel acetylcholinesterase (acetylcholine hydrolase EC 3.1.1.7) type VI-S (260 U/mg protein) and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

For work-up of blood samples for toxicokinetic measurements, C(±)P(±)-soman isomers were stabilized by addition of acetate buffer containing aluminium sulfate, NPS, and the internal standard D_{13}-C(±)P(+)-soman, before extraction over SepPak C_{18} columns (Millipore,

Waters Associates, Bedford, MA, USA) as described previously (1, 2). Analytes in ethyl acetate were concentrated at reduced pressure with a Rotavapor-M (Buchi, Switzerland). pH was measured with an Orion Ionalyzer Model 501 equipped with an Orion combination electrode type pH 91-05. The pH-meter was calibrated with a citrate/hydrochloric acid buffer, pH 4.00 at 20 °C (Merck, ready for use). Tenax TA, 60-80 mesh and the CP Cyclodex B 2,3,6,M-19 column were obtained from Chrompack (Middelburg, The Netherlands).

Synthesis of Chirasil-(D)-valine (D-Chirasil Val)

The synthesis of Chirasil-(D)-valine was analogous to that of Chirasil-(L)-valine, using D-valine tert-butylamide instead of the L-isomer (1). The reaction yield was ca. 70 %. The identity of the product was confirmed by ^{1}H-NMR, IR and elemental analysis. The nitrogen content of the product was 3.1 % (theoretically 3.2 %)

Synthesis of D_7-(±)-sarin

The synthesis of D_7-(±)-sarin was analogous to that of D_{13}-C(±)P(±)-soman (1), using D_7-isopropanol (Merck) instead of perdeuteropinacolyl alcohol, and was performed on a gram scale. The reaction yield was 86 %. Gas chromatographic analysis did not indicate any impurities. The most prominent fragment in the mass spectrum (ei) is *m/z* 101, identified as $DO(CH_3)P(O^+D)F$. Other major fragments are *m/z* 129 [$CH_3(CD_3CDO)P(O)F$] and 81 [$(CH_3)P(O)F$]. The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) is in accordance with the proposed structure: δ 1.62 [dd, 3 H, J_{PH} = 18.7 Hz, J_{FH} = 5.7 Hz, F-P-CH_3]; as is the ^{13}C-NMR spectrum ($CDCl_3$, 100.6 MHz): δ 72.2 [CD]; 22.8 [2 x CD_3]; and 10.5 [dd, 1 C, J_{PC} = 150.6 Hz, J_{CF} = 27.6 Hz, CH_3-P-F]. The internal standard, D_7-(-)-sarin, was isolated with an optical purity > 98 % by incubating the D_7-racemate in rabbit serum, by analogy with the method used to isolate the P(-)-isomers of C(±)P(±)-soman (25).

Gas chromatography

The gas chromatographic configurations used or developed for our investigations are described below. They will be referred to by number in this report.

GLC configuration 1
This configuration was used for the analysis of C(±)P(±)-soman stereoisomers.
A Carlo Erba HRGC 5360 (Mega Series) was equipped with a NP detector, a TCT injector, and a Chrompack MUSIC for two-dimensional gas chromatography. The desorption tubes for the TCT injection (length 15 cm, I.D. 2.5 mm), filled with 25 mg Tenax TA, and a loosely packed quartz wool plug (length, 7 cm) on top of the Tenax material were conditioned before use by heating under a stream of helium at 300 °C for 8 h. After the sample was spread over the quartz wool plug from a syringe, it was transferred to the Tenax material by means of a stream of nitrogen (30 ml/min for 10 min) at room temperature. Next,

the desorption tube was installed into the TCT injector. The sample was desorbed from Tenax by heating for 6 min at 150 °C (heating rate 80 °C/min) at a helium flow-rate of 17 ml/min through the desorption tube, the TCT cold trap (deactivated uncoated fused silica, length ca. 30 cm, I.D. 0.53 mm), and the precolumn of the MUSIC system in the solvent flush mode, while the TCT cold trap was held at ≤ -50 °C by means of a stream of helium cooled with liquid nitrogen. For flash injection, the TCT cold trap was heated at 180 °C (heating rate 15 °C/sec) for 3 min.

The precolumn of the MUSIC system (length 10 m, I.D. 0.53 mm) was coated with chemically bonded CPSil 8 CB (film thickness 5.25 µm), whereas the fused silica analytical column (length 50 m, I.D. 0.25 mm) was coated by Chrompack with L-Chirasil Val synthesized in the TNO Prins Maurits Laboratory (1-3) (film thickness 0.25 µm). The signal for flash heating of the TCT cold trap started the program of the gas chromatograph and of the MUSIC. The precolumn was programmed from 87 to 115 °C at 5 °C/min. The cut containing analytes was trapped between 4 and 6 min in the second (MUSIC) trap (deactivated uncoated fused silica, length ca. 30 cm, I.D. 0.25 mm) and cooled with liquid carbon dioxide at -60 to -70 °C. The oven was cooled to 80 °C during a waiting time of 1.5 min. The trapped cut was injected by heating to 200 °C in 25 sec. The analytical column was programmed from 80 to 116 °C at 4 °C/min and operated with helium gas at a constant inlet pressure of 12 kPa. The detector block temperature was kept at 250 °C. Flow-rates of air and hydrogen through the detector were 350 and 35 ml/min, respectively. Make-up gas for the detector was helium, at a flow-rate of 40 ml/min. The retention times of the C(±)P(±)-soman isomers on the custom-made Chirasil Val column were approximately 19-21 min.

GLC configuration 2 (cf. Figure 1, subsection III-1a)

This configuration was equipped for automated analysis of (±)-sarin stereoisomers. However, it has not been used in routine analysis.
A Carlo Erba HRGC 5300 gas chromatograph (Mega Series) was equipped with a NP detector and a flame ionisation detector (FID), an AS 550 autosampler for large-volume on-column injection, and a Chrompack two-dimensional column-switching system (MUSIC).
The samples, either concentrated ethyl acetate extracts from blood, or standard solution of (±)-sarin and D_7-(-)-sarin, are transferred into glass autosampler vials (Chrompack) equipped with a limited volume insert, which are sealed gastight with aluminum crimp caps with teflon-faced septa. The vials were placed in the AS 550 autosampler. Upon injection, the vials are pressurized at ca. 1.7 bar with helium. The needle of the autosampler is flushed with the sample for 3 sec, after which ca. 40 µl of the sample is injected in a time period of 15 sec into a retention gap (length 10 m, I.D. 0.53 µm, uncoated deactivated fused silica). During the injection and 10 seconds thereafter, the injector is cooled. After that, the injector and the retention gap are heated to 50 °C. The retention gap is connected to the precolumn via a press-fit connector.

The precolumn of the MUSIC system was a chemically bonded CPSil 8 CB column (length 10 m, I.D. 0.53 mm, film thickness 0.5 μm). The analytical column was a CP-Cyclodex B column (length 50 m, I.D. 0.25 mm, film thickness 0.25 μm). The precolumn is programmed at 50 °C isothermally. The cut containing the compounds of interest was trapped between 8 and 9.5 min in the second (MUSIC) trap (uncoated deactivated fused silica I.D. 0.25 mm, length ca. 30 cm) and cooled at -70 °C with liquid carbon dioxide. The oven was heated to 70 °C during a waiting time of 0.5 min. The trapped cut was injected by heating to 180 °C in 1 min. The analytical column was programmed from 70 to 100 °C at 20 °C/min and operated with helium gas at a constant inlet pressure of 1.55 kPa. The detector block was kept at 250 °C. Make-up gas for the detector was helium, at a flow-rate of 40 ml/min. Flow-rates of air and hydrogen through the detector were 350 and 35 ml/min, respectively. The (±)-sarin isomers and D_7-(-)-sarin are completely resolved on the analytical column (CP Cyclodex B). The retention times of these three components are approximately 12 min.

GLC configuration 3

This configuration was used to determine the concentration of C(±)P(±)-soman and (±)-sarin vapor in air.
A Carlo Erba HRGC 4600 gas chromatograph (Vega Series) was equipped with a NP detector, an on-column injector and a Valco gas-sampling valve. Both injectors are connected to the analytical column by means of two pieces of uncoated deactivated fused silica (length ca. 30 cm, I.D. 0.53 mm) and a glass Y-piece press-fit connector. The column was CPSil8-CB (length 10 m, I.D. 0.53 mm, film thickness 5.25 μm).
The sampling volume of the valve is 58.8 μl. Calibration of the gas-sampling valve injection was performed by comparing the results obtained for standard solutions of C(±)P(±)-soman after injection with the on-column injector.
The gas-sampling injector was flow-regulated at 10 ml/min, whereas the on-column injector was pressure-regulated at 6 kPa. The temperature of the oven was 87 °C; the detector temperature was 250 °C. Flow-rates of air and hydrogen through the detector were 350 and 35 ml/min, respectively. Make-up gas for the detector was helium, at a flow-rate of 40 ml/min. The retention time of C(±)P(±)-soman in this system was approximately 3.4 min.

GLC configuration 4

This configuration was used to analyze (±)-sarin stereoisomers in blood of guinea pigs nose-only exposed to this agent.
The configuration resembles configuration 2, but is equipped with a thermodesorption autosampler (TDAS, Carlo Erba) instead of the large-volume injector. A schematic representation of the TDAS is shown in Figure 2 (subsection III-1B).
The desorption tubes (length 10 cm, I.D. 3 mm) were partly (ca. 30 %) filled with Tenax. A glass wool plug was firmly pushed on top of the Tenax material and was fixed with a metal clamp. The tubes were

preconditioned by heating under a stream of helium at 300 °C for at least 4 h. The autosampler tray was thermostatted at 10 °C via a cooled circulator (TLC 3, Tamson, Zoetermeer, The Netherlands) filled with ethyleneglycol. The maximum capacity of the autosampler tray is 30 desorption tubes. The sample was desorbed from Tenax by heating for 3 min at 190 °C. The cold trap of the injection system was a deactivated CPSil 8 CB column (length ca. 1 m, I.D. 0.53 mm, film thickness 5.25 μm). The cold trap was kept at -60 °C with liquid nitrogen. Cooling was started before the tube to be desorbed was positioned. The analytes were reinjected from the cold trap via a temperature increase from -60 to 180 °C at a rate of 21 °C/sec. This flash heating signal started the program of the gas chromatograph and the MUSIC system. The precolumn of the MUSIC system was a chemically bonded CPSil 8 CB column (length 10 m, I.D. 0.53 mm, film thickness 5.25 μm). The precolumn was programmed at 87 °C isothermally for 2 min. The cut containing the compounds of interest was trapped in the cold trap of the MUSIC system (uncoated deactivated fused silica, length ca. 30 cm, I.D. 0.25 mm) which was cooled at -70 °C with liquid carbon dioxide. Meanwhile the GC oven was cooled from 87 to 70 °C. The trapped cut was injected onto the analytical column by heating from -60 to 180 °C in 1 min. The analytical column (CP cyclodex B, length 50 m, I.D. 0.25 mm, film thickness 0.25 μm) was programmed at 70 °C for 13 min, after which it was heated to 87 °C at 'infinite' rate, and was kept at this temperature for 4 min. Helium flow-rate was 12.4 ml/min, the inlet pressure was constantly 1.48 kPa. The retention times of the sarin stereoisomers and D_7-(-)-sarin were in the range of 13 to 15 min. The detector base was kept at 250 °C. Make-up gas for the detector was helium, at a flow-rate of 40 ml/min. Flow-rates of air and hydrogen through the detector were 350 and 35 ml/min, respectively.

Calibration curves

A solution of the C(±)P(+)-isomers of D_{13}-soman in anhydrous ethyl acetate was used as internal standard for analysis of the four stereoisomers of C(±)P(±)-soman. Calibration curves were constructed after measuring the peak heights of the C(±)P(±)-soman stereoisomers at various concentrations of C(±)P(±)-soman relative to that of the C(-)P(+)-isomer of the internal standard, as described before (1-3). An analogous procedure was used for the analysis of the (±)-sarin stereoisomers, using D_7-(-)-sarin as an internal standard.
All analyses were performed in duplicate.

Animal experiments

Male albino outbred guinea pigs of the Dunkin-Hartley type (strain Crl:(HA)BR), weighing ca. 450 g (age 6-8 weeks), were purchased from Charles River (Sulzfeld, Germany). The animals were allowed to eat and drink *ad libitum*. They were allowed to acclimatize to their new environment for at least 1 week before they were used in any

experiment. The protocols for the animal experiments were approved by the TNO Committee on Animal Care and Use.
All experiments described below were performed using animals anesthetized with racemic ketamine (Vetalar^{R}, 160 mg/kg i.m.), which, in addition, were treated with the muscle relaxant Vetranquil^{R} (0.2 ml i.m. per animal).

Determination of the LD50 of (±)-sarin in guinea pigs

The 24 h LD50 of (±)-sarin was determined after intravenous bolus administration to guinea pigs weighing 350-450 g, at five different doses. A small incision was made in the skin and some tissue was spliced in order to gain access to the jugular vein. A solution of (±)-sarin in isopropanol was diluted with distilled water just before administration of the nerve agent to the animal. This solution was injected into the jugular vein (injection volume 1 ml/kg), after which the wound was closed with a few stitches. After that, the animal was returned to its cage in the animal facilities. The animals were checked regularly to see whether they were still alive. The animals surviving the 24 h period were killed with an overdose of anesthetic (intracardial).
The mortality data were processed by probit-analysis.

Determination of the LCt50 values of C(±)P(±)-soman and (±)-sarin

Guinea pigs, weighing 450-500 g, were anesthetized and placed in the Battelle-type restrainment tubes (see III-4 c, Figure 15). The tubes were connected to the exposure apparatus. The respiratory frequency and the respiratory minute volume were recorded for 10 min, after which the animals were nose-only exposed for 8 min to air containing a controlled concentration of nerve agent vapor. At least five concentrations were used.
After exposure the animals were placed in separate cages to determine 24 h death scores. LCt50 values were calculated via probit analysis.

Determination of the LD50 of C(±)P(±)-soman in guinea pigs administered via an 8-min intravenous infusion

In groups of eight anesthetized guinea pigs, an indwelling cannula (Surflo catheter, Terumo) was inserted in the jugular vein. A standard solution of C(±)P(±)-soman in 2-propanol was diluted with distilled water just before use. The aqueous solution (1 ml/kg) was administered as an intravenous infusion using a microinjection pump (Carnegie Medicin AB, model CMA 100) in an 8-min time period. Following infusion, the indwelling cannula was removed and the skin incision was closed with a few stitches. Each animal was placed in a separate cage in the conditioned animal facility. At 24 h after the infusion, the number of dead animals per dosing group was counted. The LD50 value was calculated via probit analysis.

Determination of the LD50 of C(±)P(±)-soman in guinea pigs administered as an intramuscular bolus

To groups of 9-11 anesthetized guinea pigs C(±)P(±)-soman was administered intramuscularly in the right thigh in doses ranging from 25 to 35 μg/kg (injection volume 1 ml/kg). For this purpose, a standard solution of C(±)P(±)-soman in 2-propanol was diluted with distilled water just before use. After injection, each animal was placed in a separate cage in a conditioned room. At 24 h after administration, the number of dead animals per dose group was counted. The LD50 value was calculated via probit analysis.

Toxicokinetics of 0.8 LD50 C(±)P(±)-soman or (±)-sarin after intravenous bolus injection

Cannulas were inserted into the left carotid artery and into the trachea of anesthetized guinea pigs, weighing 450-500 g. Heparin (5000 U) was administered via the carotid cannula. The animals were mechanically ventilated via the tracheal cannula. The jugular vein was traced and made accessible. Atropine sulfate, dissolved in saline, was administered i.p. (17.4 mg/kg). A blood sample was taken from the carotid artery, after which a volume of saline corresponding with that of the blood sample was administered through the same cannula. A dose of C(±)P(±)-soman or (±)-sarin corresponding with 0.8 LD50 was injected into the jugular vein (injection volume 1 ml/kg). For this purpose, standard solutions of C(±)P(±)-soman or (±)-sarin in 2-propanol were diluted with distilled water just before use. Blood samples were taken at several time-points after administration. After taking the final blood sample of the experiment, the animals were sacrificed by an overdose of anesthetic.

Toxicokinetics of C(±)P(±)-soman and (±)-sarin via nose-only exposure

Toxicokinetic experiments were performed by nose-only exposure of anesthetized, atropinized (1 ml/kg of a solution of 17.4 mg/ml in saline) and restrained guinea pigs to concentrations of C(±)P(±)-soman or (±)-sarin vapor in air, yielding 0.4 and 0.8 LCt50. Furthermore, animals were exposed for 5 h to a C(±)P(±)-soman vapor concentration yielding 0.1 LCt50.
Blood samples were taken via a carotid cannula just before exposure, during the exposure and up to ca. 1 h after the start of the exposure. A small portion of the blood sample was used for the determination of AChE activity, the larger part was used for gas chromatographic analysis. Throughout the exposures, the respiration of the animals was monitored.

Toxicokinetics of 0.8 LD50 of C(±)P(±)-soman after an 8-min intravenous infusion

An indwelling cannula was inserted into the jugular vein of anesthetized guinea pigs. Atropine sulfate (1 ml/kg of a solution of 17.4 mg/ml in saline) was administered intraperitoneally. A dose of C(±)P(±)-soman corresponding with 0.8 LD50 was administered as an aqueous solution (injection volume 1 ml/kg), linearly in an 8-min time period via the cannula using a microinjection pump (Model CMA 100, Carnegie Medicin AB). Blood samples were drawn via a carotid cannula at several time-points just before, during and after infusion.

Toxicokinetics of 0.8 LD50 of C(±)P(±)-soman after intramuscular bolus injection

Anesthetized guinea pigs were atropinized via intraperitoneal administration of atropine sulfate (1 ml/kg of a solution of 17.4 mg/ml in saline). A dose of C(±)P(±)-soman corresponding with 0.8 LD50 was injected in the right thigh. Blood samples were drawn via a carotid cannula at various time-points before and after administration of the toxicant.

Curve-fitting of toxicokinetic data

Curve-fitting of the data in order to obtain toxicokinetic parameters was performed by nonlinear regression with the BMDP-3R program (University of California, Los Angeles, CA, USA), on a personal computer equipped with a 20-Mb hard disk and an 8087 math co-processor, as previously described (1, 2).

Radiometric determination of the acetylcholinesterase activity in blood samples

The method has been adapted from that described by Johnson and Russell (26). First of all, a quenching curve was established, using a [^{3}H]-n-hexadecane standard with nitromethane as the quenching agent. The procedure for these samples was the same as that chosen for the blood samples.
The batch of [^{3}H]-acetylcholine chloride was dissolved in 5 ml of acidified water (pH 4.0). A portion of this solution (200 μl) was mixed with acidified water (pH 4.0, 800 μl) and glacial acetic acid (20 μl). This solution was purified by extracting three times with a mixture of toluene and isoamyl alcohol (9/1, v/v; 5 ml). Next, the isoamyl alcohol was removed from the aqueous phase by extracting three times with diethyl ether (5 ml). Residual diethyl ether was removed under a gentle stream of air. The aqueous phase was mixed with a 0.03 M aqueous solution of acetylcholine perchlorate (3 ml). When not in use, this solution (activity $3.7*10^5$ Bq/ml) was stored at -20 °C.

After thawing of the tenfold diluted blood sample, 20 µl of the diluted sample was added to 0.01 M phosphate buffer (pH 7.5, 180 µl), which contained 0.1 M potassium chloride, for the determination of total cholinesterase (ChE) activity. In order to determine the AChE activity in the sample, 20 µl of the diluted blood sample was added to a solution of 25 µM etopropazine in the 0.01 M phosphate buffer (pH 7.5, 180 µl) with 0.1 M potassium chloride. The mixture was warmed to 30 °C in a thermostatted waterbath, after which the radiolabeled substrate (20 µl) was added and mixed by means of a whirlmixer. After incubation for 15 min, the reaction was stopped by addition of a buffer (pH 2.5, 100 µl), consisting of 1 M chloroacetic acid, 0.5 M sodium hydroxide and 2 M sodium chloride, and subsequent mixing. Next, the scintillation fluid (0.55 % w/v, Permablend in toluene/isoamyl alcohol 9/1, v/v; 4 ml) was added. The vial was closed with a screw cap and shaken on the whirlmixer for 10 sec. The vial was then placed in the scintillation counter.
Calibration was performed in a similar way, with solutions of electric eel acetylcholinesterase containing 10-500 mU/ml in 0.01 M phosphate buffer (pH 7.5) containing 1 mg/ml albumin, instead of the diluted blood sample. In order to compensate for the spontaneous hydrolysis of the substrate, blancs were tested consisting of the etopropazine containing phosphate buffer (200 µl).

Calculation of the time course of blood AChE inhibition from the organophosphate levels determined in blood

The time courses of the activity of AChE following nose-only exposure to C(±)P(±)-soman and to (±)-sarin as well as following intravenous infusion with C(±)P(±)-soman were simulated from the time courses of the C(±)P(-)-soman and (-)-sarin concentrations in blood by assuming that the AChE inhibition is a bimolecular reaction of the enzyme with the organophosphate. Then, at any time:

$$-(dE/dt) = k_{c+p-}*[C(+)P(-)\text{-soman}]*[E] + k_{c-p-}*[C(-)P(-)\text{-soman}]*[E] \quad (1)$$

or

$$-(dE/dt) = k_{p-}*[(-)\text{-sarin}]*[E] \quad (2)$$

in which k_{c+p-}, k_{c-p-} and k_{p-} are the rate constants for inhibition by C(+)P(-)-soman, C(-)P(-)-soman and (-)-sarin, respectively. Integration of Equation (1) for the absorption phase (0-4 or 0-8 min) yields

$$100*(E_t/E_o) = 100*\exp\{-k_{c+p-}* \int_o^t [C(+)P(-)\text{-soman}]*dt + k_{c-p-}* \int_o^t [C(-)P(-)\text{-soman}]*dt\} \quad (3)$$

in which E_o and E_t are the enzyme concentrations at time 0 and t, respectively. From the present toxicokinetic studies it was found that the functions describing the course of the blood levels for C(±)P(-)-soman in the absorption phase have the general form:

for t is 0 - τ min; [C(+)P(-)-soman] = 0 (4)

for t is τ - t min; [C(+)P(-)-soman] = A*exp(a*(t-τ)) + D (5)

and for t is 0 - t min; [C(-)P(-)-soman = A*exp(a*t) + D (6)

After substitution of the Equations (4) - (6) in Equation (3) and integration, it follows that

from 0 - τ min:

$$100*(E_t/E_o) = 100*\exp[k_{c-p-}*\{(A/a)*(1 - \exp(a*t)) - D*t\}] \quad (7)$$

from τ - t min:

$$100*(E_t/E_o) = 100*(E_\tau/E_o)*\exp[k_{c+p-}*\{(A/a)*(1 - \exp(a*(t-\tau)) - D*(t-\tau)\} + k_{c-p-}*\{(A/a)*(\exp(a*\tau) - \exp(a*t)) - D*(t-\tau)\}] \quad (8)$$

in which E_τ is the enzyme activity at time τ.
Integration of Equation (1) for the elimination phase (4-t or 8-t min) yields

$$100*(E_t/E_o) = 100*(E_{to}/E_o)*\exp\{-k_{c+p-}*\int_{t_o}^{t}[C(+)P(-)\text{-soman}]*dt + - k_{c-p-}*\int_{t_o}^{t}[C(-)P(-)\text{-soman}]*dt\} \quad (9)$$

in which t_o is the time at which exposure was stopped and E_{to} is the enzyme activity at time t_o. As follows from the present toxicokinetic studies, the functions describing the course of the blood levels of C(±)P(-)-soman in the elimination phase have the general form

$$[\text{soman isomer}] = B*\exp(bt) + C*\exp(ct) \quad (t \geq t_o) \quad (10)$$

After substitution of Equation (10) into Equation (9) and integration, it follows that

$$100*(E_t/E_o) = 100*(E_{to}/E_o)*\exp[k_{c+p-}*\{(B/b)*(\exp(b*t_o) - \exp(b*t)) + (C/c)*(\exp(c*t_o) - \exp(c*t))\} + + k_{c-p-}*\{(B/b)*(\exp(b*t_o) - \exp(b*t)) + + (C/c)*(\exp(c*t_o) - \exp(c*t))\}] \quad (11)$$

Values for the percentages of enzyme acitivity ($100*(E_t/E_o)$) were calculated for a large number of t values according to the Equations (7), (8) and (10) after substitution of initial estimates for the rate constants k_{c+p-} and k_{c-p-} and were subsequently plotted versus time. The process was iterated with various values for the inhibition rate constants until the calculated time course showed a reasonable fit to the measured values for enzyme activity.

The data obtained for the toxicokinetics of (±)-sarin were analyzed analogously.

III. RESULTS

III-1. TWO-DIMENSIONAL GAS CHROMATOGRAPHY (MUSIC) OF C(±)P(±)-SOMAN AND (±)-SARIN

a. Two-dimensional gas chromatography with large-volume injection

GLC configuration 1, equipped with a TCT injector and a MUSIC system, has been successfully used in our previous studies on the toxicokinetics of relatively low doses of C(±)P(±)-soman in various species (2). This configuration was schematically presented previously (2). The configuration has proven to be reliable and to allow for highly sensitive and selective analysis of the C(±)P(±)-soman stereoisomers. However, the hand-operated exchange of the Tenax sample tubes can be considered as a drawback of this configuration, since it limits the daily sample throughput.
In view of the expected large numbers of samples to be analyzed in our study, GLC configuration 2, equipped with an autosampler suited for large-volume injection, was constructed. Figure 1 shows this configuration schematically; technical details are described in section II.

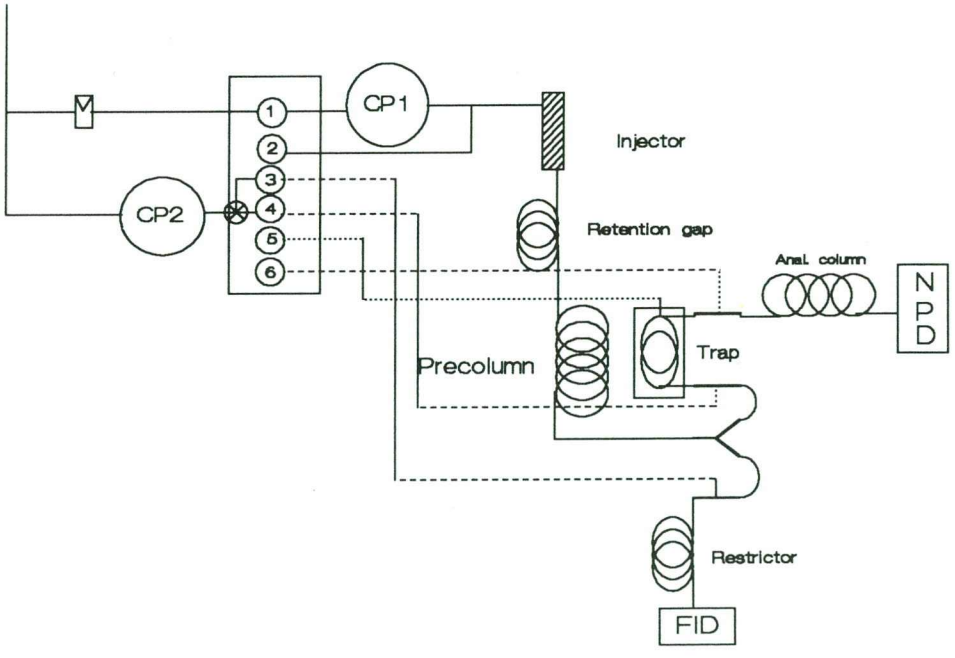

Figure 1. Schematic representation of GLC configuration 2 (see section II). CP1 and CP2 are constant pressure controllers; (1), (2), (3), (4), (5) and (6) are valves; Anal. column is the analytical column; FID and NPD are the detectors.

The autosampler increases the sample-throughput, whereas the large-volume injection should enable us to obtain the sensitivity of the analysis comparable to that of configuration 1.
For routine application, we intended to inject 40 μl of the concentrated extract (total volume 100 μl) into the system. The system is capable of injecting volumes up to 500 μl. In order to be able to inject such large volumes of liquid with the cold injector, a retention gap (10 meters of uncoated, deactivated fused silica) is installed, between the injector and the precolumn (see Figure 1). Usually, the injector/precolumn system is flow-regulated, which is satisfactory for the injection of 1-3 μl in a few seconds. The injection of 40 μl, however, will take 15 seconds, which may induce a recoil due to the back-pressure from the MUSIC system. To avoid this recoil, the injector/precolumn system has to be pressure-regulated, which has been accomplished by modification of the MUSIC configuration we used in previous studies.
During injection valves 1 and 4 (Figure 1) are opened, whereas the other valves are closed. During trapping of the components of interest, the 'Deans'-switch, which operates valves 3 and 4, closes valve 4 and opens valve 3, upon which the flow direction is reversed. Valve 5 is opened, enabling the passage of liquid carbon dioxide into the direction of the trap. Furthermore, valve number 6 is opened, after which the flow rate through the trap is increased to ca. 50 ml/min. During injection of the trapped cut onto the analytical column valves 3, 5 and 6 are closed and valve 4 is opened. Backflushing is accomplished by closing valve 1 and opening valve 2, while valve 4 remains opened.

The performance of this system was tested with C(±)P(±)-soman. First, the behaviour of the large sample volume in the retention gap and precolumn system was studied. Conditions for injection and trapping of the compounds of interest were optimized. Initially, the chromatographic conditions appeared to be satisfactory, and to the best of our knowledge, the first GLC configuration with large-volume automatic injection combined with two-dimensional chromatography had been put to work. Unfortunately, the reliability of the configuration appeared to be unsatisfactory. During continuous use the performance of the system clearly deteriorated. Results became highly irreproducible and obviously erratic.
The lack of reproducibility is probably a result of the high sensitivity of the large-volume injection to small changes in gas flow-rates and pressure in the system. In addition, the duration of the injection appears to be a critical factor in this type of sample introduction (27). However, the major problem in this particular application is the small difference between the boiling points of the solvent and the compounds of interest.
The effort needed to further increase the reliability of this configuration forced us to use configuration 1 again, in order not to endanger the continuity of the experiments.
Meanwhile, efforts were continued to optimize the conditions for large-volume injection, in this case for (±)-sarin. First, the conditions were optimized for chromatography of (±)-sarin on the pre-column. Next, the combination with MUSIC was optimized. With an

injection duration of 60 seconds, which corresponds with an injected volume of ca. 100 μl, and a pressure difference of 0.05 bar between the sample vial and the precolumn, the injection appeared to be reproducible. Unfortunately, the reliability in routine use of this chromatographic configuration continued to be problematic. As a consequence, we decided to terminate our efforts to combine large-volume injection with MUSIC.

b. Two-dimensional gas chromatography (MUSIC) with automated TCT injection

As an alternative for multidimensional gas chromatography with automated large-volume injection, GLC configuration 2 was automated by installing a thermodesorption autosampler (TDAS). A schematic representation of the injection system is shown in Figure 2.

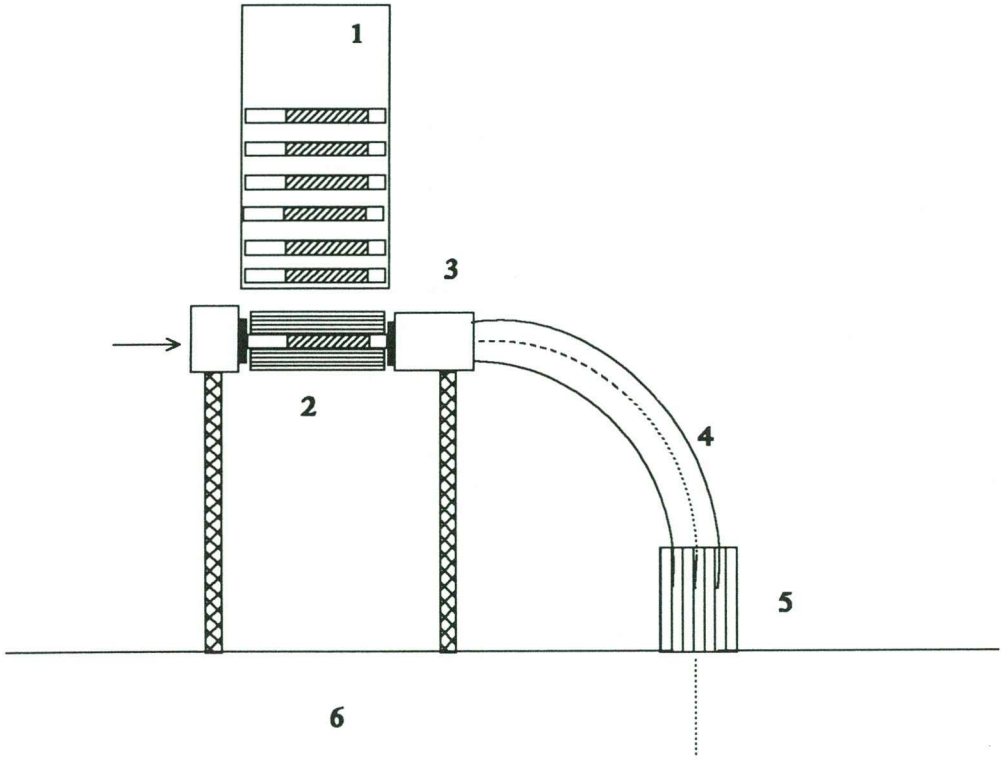

Figure 2. Schematic representation of the thermodesorption autosampler as used in GLC configuration 4 (see section II). (1) autosampler tray, (2) desorption unit, (3) valve, (4) interface, (5) cryofocussing unit, (6) gas chromatograph.

The TDAS/MUSIC combination is designated in this report as GLC configuration 4. Chromatographic details are listed in section II. The autosampler tray has a capacity of 30 samples. Since each analysis of (±)-sarin takes ca. 20 min, this means that the final sample will remain in the autosampler for about 10 h. Problems

anticipated with this procedure were (i) degradation of the analytes absorbed on Tenax within the 10-h period, and (ii) cross-contamination between the tubes. To minimize the risks of degradation of the absorbed analytes, the tray was thermostatted at 10 °C. Absence of degradation and cross-contamination was checked by analysis of known concentrations of (±)-sarin and by analysis of blanc tubes at various time-points after placing the tubes in the autosampler. The results are presented in Table 1. The within-day variation of the analysis was ca. 4% for (-)-sarin and ca. 5 % for (+)-sarin, for 11 samples which were analyzed in a 4.5-h time period after filling of the autosampler.

Table 1. Analysis of (±)-sarin in order to establish possible degradation of the analytes or cross-contamination during storage in the autosampler of GLC configuration 4.

Sample	(±)-sarin (pg)	storage time (min)	peak height (μVs)
1	250	0	184432
2	250	37	193388
3	250	81	161228
4	0	111	n.d.
5	0	152	n.d.
6	250	81	190464
7	0	177	n.d.

n.d. = not detectable

The Tenax material is introduced into the desorption tubes by using reduced pressure. In contrast with the Tenax desorption tubes used in configuration 1, the glass wool must be pushed firmly on top of the Tenax material and secured with a metal clamp. Otherwise, some Tenax material may be blown out of the tube as a result of the pressure originating from the MUSIC system. If some of these Tenax particles adhere onto the rim of the tube, a gastight fit of the desorption tube in the injector is not possible, resulting in erroneous injections.

A potential problem was also anticipated with respect to the connection with the MUSIC, which proceeds via a valve with a metallic interior, in view of previous experiences with degradation of C(±)P(±)-soman on hot metal surfaces. The temperature of the valve was varied from 100 up to 190 °C; no degradation of (±)-sarin was observed. The valve temperature was set at 190 °C, which is equal to the desorption temperature.

Although at this stage some minor problems still are encountered, which urge us still to perform the analysis during the working day to prevent any loss of samples, the introduction of the TDAS has greatly improved the performance of our gas chromatographic analysis of nerve agent stereoisomers.

III-2 DETERMINATION OF ACETYLCHOLINESTERASE ACTIVITY IN BLOOD SAMPLES

In our laboratory, blood acetylcholinesterase activities are determined radiometrically, which is sensitive, accurate and reproducible. The method is quite laborious, however. In view of the large number of samples to be analyzed in the exposure experiments, we compared the radiometric method with the colorimetric method of Ellman et al. (28), performed with microplates, which allows the determination of a large number of samples (96 per plate) in a relatively short time. A series of blood samples with an acetylcholinesterase activity ranging from 0-100 % was prepared by mixing appropriate volumes of 'active' guinea pig blood and the same blood in which acetylcholinesterase was nearly completely inhibited with C(±)P(±)-soman. The absence of free C(±)P(±)-soman in this blood sample was checked by GLC analysis, prior to mixing with 'active' blood.
A calibration curve in a comparable activity range was established with electric eel AChE. The measured substrate conversion, either in dpms or in increase of extinction, was converted into the activity in U/ml blood, via the calibration curve. The activity measured for total AChE in the 'active' blood was taken as the 100 % value. All results in the 1-50 % activity range were corrected for the residual activity measured in the 'inactive' blood samples.
The results of the validation of the radiometric and colorimetric AChE activity measurements are presented in Table 2.

Table 2. Results of the validation of the radiometric and colorimetric assay of AChE activity in guinea pig blood samples with known relative activities.

Actual AChE activity (%)	Measured AChE activity (%) ± s.d.[a] via	
	radiometry	colorimetry
100	100[b]	100[b]
50	50.0 ± 1.6	52.5 ± 1.1
20	20.8 ± 0.4	23.5 ± 4.6
10	10.3 ± 0.3	13.7 ± 3.4
5	5.1 ± 0.7	8.1 ± 1.4
2	2.5 ± 0.1	2.1 ± 0.4
1	1.2 ± 0.2	1.0 ± 0.1
0	0[c]	0[c]

[a] n=2 for radiometry, n=3 for colorimetry
[b] The measured AChE activity in the sample of the 'active' blood was defined as 100 %
[c] The measured AChE activity in the sample of the inhibited blood was defined as 0

In general, the results obtained with the microplate colorimetric method deviated more from the theoretical activity values than those obtained with the radiometric method, especially in the lower range

of AChE activity (< 20 %). Since the radiometric assay appeared to be more accurate and reproducible, this method was chosen for measurements of the time course of blood AChE in the toxicokinetic experiments.

III-3 TOXICOKINETICS OF C(±)P(±)-SOMAN IN ANESTHETIZED, ATROPINIZED GUINEA PIGS AFTER INTRAVENOUS BOLUS ADMINISTRATION OF A DOSE CORRESPONDING WITH 0.8 LD50

The toxicokinetics of the C(±)P(±)-soman stereoisomers after i.v. administration of 0.8 LD50 were studied in anesthetized, atropinized and mechanically ventilated guinea pigs. The blood samples were assayed with GLC configuration 1.
Blood samples were taken just before, and 0.5, 1, 2, 4, 7, 10, 15, 20, 30 and 40 min after administration. Since low concentrations were anticipated, fairly large blood samples (0.5-2 ml) were taken. In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used to obtain six values for each time-point. The results obtained for the individual animals are listed in Table 3.
The blood samples taken just before C(±)P(±)-soman administration were negative, confirming the absence of C(±)P(±)-soman in the materials and instruments.
The P(+)-stereoisomers were not detectable after i.v. administration of 0.8 LD50 C(±)P(±)-soman. The P(-)-stereoisomers were detectable up to 40 min after administration.
From the concentrations measured for each time-point, the mean values were calculated. These mean values with the corresponding standard error of the mean (s.e.m.) are presented in Table 4.
The time courses of the mean blood concentrations of C(+)P(-)- and C(-)P(-)-soman are shown in Figures 3 and 4, respectively.
The data in Table 4 show that the concentrations of C(+)P(-)-soman are considerably lower than those of C(-)P(-)-soman after intravenous bolus administration of 0.8 LD50 C(±)P(±)-soman, with the exception of time-point 40 min. After i.v. administration of 6 and 2 LD50, the concentration of the C(+)P(-)-stereoisomer was also lower than that of the C(-)P(-)-isomer (1, 2). Comparison of the mean blood concentrations of the stereoisomers after the various C(±)P(±)-soman doses suggests that the differences in concentrations between the two P(-)-isomers become more pronounced with decreasing dose.
An equation describing the concentration time course of C(-)P(-)-soman was determined by nonlinear regression analysis with the BMDP software. The data were fitted to two- and three-exponential equations. The F-ratio test (1, 2) indicated that an adequate fit was obtained with a two-exponential equation, whereas the curves obtained after administration of 2 and 6 LD50 C(±)P(±)-soman were described better by three-exponential equations. The toxicokinetic parameters calculated for C(-)P(-)-soman are listed in Table 5. We decided not to perform curve-fitting on the results of the C(+)P(-)-isomer, since such a fit would only lead to meaningless parameters.

Table 3. Concentrations in blood[a] (ng/ml) of C(+)P(-)- and C(-)P(-)-soman in individual anesthetized, atropinized, and mechanically ventilated guinea pigs at various time-points after i.v. administration of 0.8 LD50 (22 µg/kg) of C(±)P(±)-soman.

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 1 (548)[b]		Guinea pig 2 (559)[b]		Guinea pig 3 (448)[b]		Guinea pig 4 (451)[b]		Guinea pig 5 (621)[b]		Guinea pig 6 (638)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	0.0076	3.33	*	*	4.36	14.8	*	*	0.033	3.10	*	*
1	*	*	0.0072	0.296	*	*	0.0074	0.791	*	*	0.068	2.55
2	0.0185	0.537	*	*	*	*	0.0362	0.817	0.014	0.693	*	*
4	*	*	0.022	0.566	0.093	0.745	*	*	*	*	0.098	0.671
7	0.031	0.323	*	*	0.044	0.301	*	*	0.0068	0.080	*	*
10	*	*	0.043	0.550	*	*	0.083	0.344	*	*	0.036	0.141
15	0.056	0.137	*	*	*	*	0.052	0.186	0.017	0.012	*	*
20	*	*	0.050	0.343	0.031	0.054	*	*	*	*	0.056	0.047
30	0.0069	0.0049	*	*	0.015	0.016	*	*	0.0098	0.0054	*	*
40	*	*	0.012	0.030	*	*	0.012	0.020	*	*	0.0084	0.0032

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 7 (594)[b]		Guinea pig 8 (618)[b]		Guinea pig 9 (501)[b]		Guinea pig 10 (491)[b]		Guinea pig 11 (489)[b]		Guinea pig 12 (532)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	0.023	2.65	*	*	0.274	8.26	*	*	n.d.	1.00	*	*
1	*	*	0.140	2.61	*	*	0.232	3.93	*	*	0.23	0.38
2	*	*	0.148	1.65	0.218	3.58	*	*	0.038	0.29	*	*
4	0.078	0.482	*	*	*	*	0.110	0.823	*	*	0.14	0.24
7	0.021	0.189	*	*	0.042	0.826	*	*	0.074	0.64	*	*
10	*	*	0.257	0.105	*	*	0.072	0.166	*	*	0.054	0.047
15	*	*	0.035	0.089	0.0088	0.023	*	*	0.027	0.16	*	*
20	0.022	0.016	*	*	*	*	0.0064	0.015	*	*	0.023	0.014
30	0.031	0.0069	*	*	0.018	0.030	*	*	0.018	0.050	*	*
40	*	*	0.026	0.0074	*	*	0.021	0.0085	*	*	0.0027	0.0015

[a] Measured with GLC configuration 1 (see section II)

[b] Weight in grams

n.d.= Not detectable

* = Not measured

Table 4. Mean concentrations of C(+)P(-)- and C(-)P(-)-soman with standard error of the mean (s.e.m.) (n=6) in anesthetized, atropinized and mechanically ventilated guinea pigs after i.v. administration of 0.8 LD50 (22 μg/kg) of C(±)P(±)-soman.

Time (min)	[C(+)P(-)-soman] ± s.e.m. (ng/ml blood)	[C(-)P(-)-soman] ± s.e.m. (ng/ml blood)
0.5	0.9 ± 0.8	5.5 ± 1.9
1	0.11 ± 0.04	1.8 ± 0.6
2	0.08 ± 0.03	1.3 ± 0.5
4	0.09 ± 0.02	0.59 ± 0.08
7	0.047 ± 0.008	0.4 ± 0.1
10	0.09 ± 0.03	0.30 ± 0.07
15	0.033 ± 0.007	0.10 ± 0.03
20	0.031 ± 0.007	0.08 ± 0.05
30	0.016 ± 0.003	0.019 ± 0.007
40	0.03 ± 0.02	0.012 ± 0.004

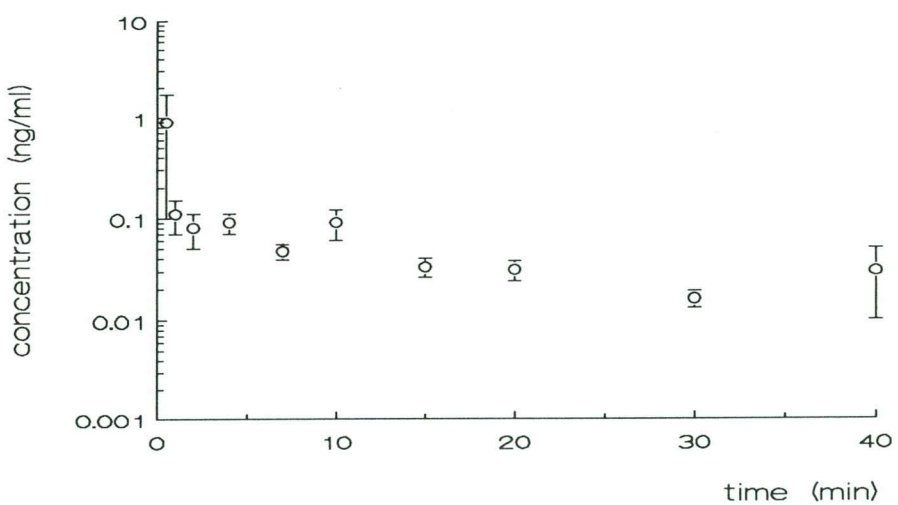

Figure 3. Semilogarithmic plot of mean concentrations in blood (± s.e.m., n=6) of C(+)P(-)-soman versus time after i.v. administration of 0.8 LD50 (22 μg/kg) of C(±)P(±)-soman to anesthetized, atropinized and mechanically ventilated guinea pigs.

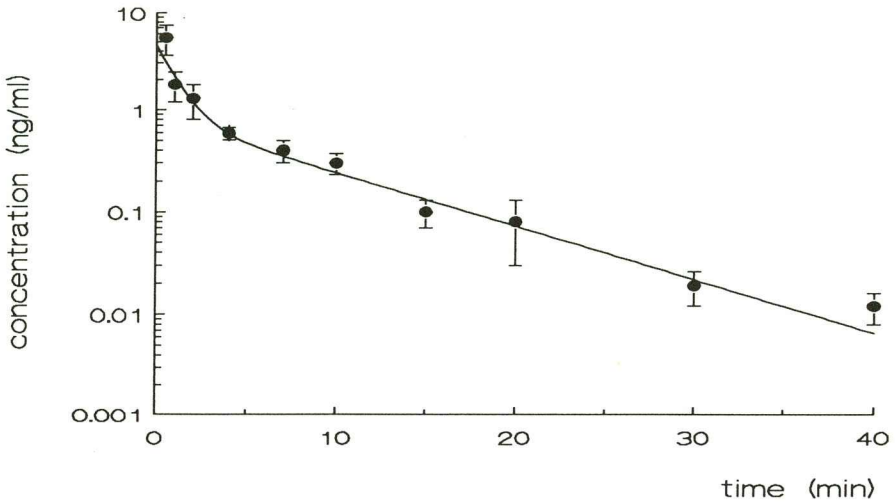

Figure 4. Semilogarithmic plot of mean concentrations in blood (± s.e m., n=6) of C(-)P(-)-soman versus time after i.v. administration of 0.8 LD50 (22 μg/kg) of C(±)P(±)-soman to anesthetized, atropinized and mechanically ventilated guinea pigs.

Table 5. Toxicokinetic parameters[a] for C(-)P(-)-soman in anesthetized, atropinized, and mechanically ventilated guinea pigs, after i.v. administration of 0.8 LD50 (22 μg/kg) of C(±)P(±)-soman.

Number of exponents	2
F-ratio	3.15[b]
A (ng/ml)	3.8
B (ng/ml)	0.80
C (ng/ml)	-
a (min^{-1})	0.95
b (min^{-1})	0.12
c (min^{-1})	-
Terminal half-life (min)	5.8
Area under the curve (ng.min/ml)	10.6

[a] The concentration of C(-)P(-)-soman at time t is described by: $[C(-)P(-)\text{-soman}] = A*e^{-at} + B*e^{-bt} + C*e^{-ct}$

[b] Critical value $F_{2,4} = 6.94$ ($p = 0.05$)

III-4 APPARATUS FOR GENERATION OF NERVE AGENT VAPOR AND EXPOSURE OF ANIMALS

a. Apparatus for generation of nerve agent vapor

An apparatus for the continuous generation of (±)-sarin and C(±)P(±)-soman vapor was constructed. A schematic representation of the apparatus is shown in Figure 5.

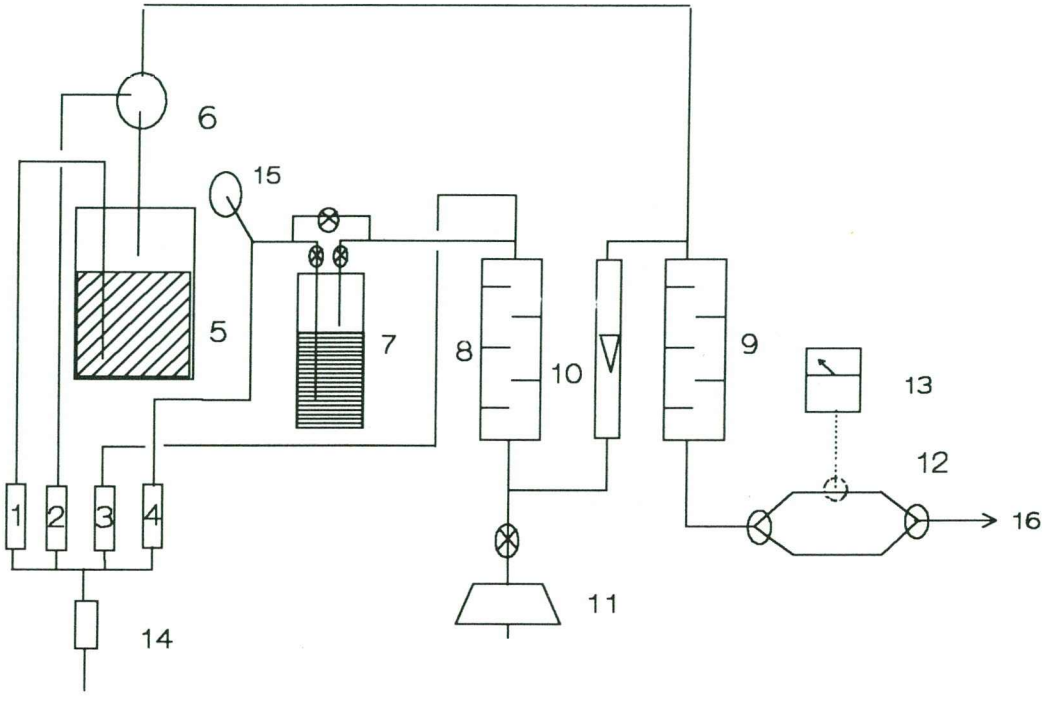

Figure 5. Apparatus constructed for generation of nerve agent vapor. (1), (2), (3) and (4) mass flow controllers; (5) thermostatted water bath ('humidifier'); (6) mixing chamber; (7) vial containing the nerve agent; (8) and (9) mixing chambers; (10) flowmeter; (11) charcoal canister; (12) bypass; (13) temperature/relative humidity meter; (14) oil filter; (15) overpressure security; (16) towards the exposure chamber.

The techniques for generation and monitoring of specified concentrations of nerve agent vapor in air are adapted from those in daily use at TNO Prins Maurits Laboratory for the testing of detection devices, canisters for gas masks, etc.(cf. also 29, 30). A supply of dry air is connected to a set of four mass flow controllers (1-4 in Figure 5) via an oil filter (14 in Figure 5). The airstream from mass flow controller 4 is bubbled through thermostatted neat (±)-sarin or C(±)P(±)-soman (7 in Figure 5) to generate sufficient

vapor for the short (4-8 min) exposures or in order to generate the low concentrations of C(±)P(±)-soman needed for the 5-h exposures. If two dilution steps of the contaminated airstream are needed, an airstream from mass flow controller 3 is mixed with the primary contaminated airstream in the first mixing chamber (8 in Figure 5). Part of this once-diluted airstream is bled via a charcoal canister (11 in Figure 5), whereas a fraction of the airstream measured with a rotameter (10 in Figure 5) is mixed in a second mixing chamber (9 in Figure 5) with airstreams from mass flow controllers 2 and 1. The relative humidity of the airstream originating from mass flow controller 1 will be ca. 100 % after passage through a thermostatted waterbath (5 in Figure 5). The desired relative humidity of the air for our study, which is 70 %, is regulated by the ratio of the flows through mass flow controllers 1 and 2, and mixing in a mixing chamber (6 in Figure 5). If only one dilution step is needed, mass flow controller 3 is closed. The air leaving the second mixing chamber passes a temperature/relative humidity meter (13 in Figure 5), connected in a bypass (12 in Figure 5), after which the airstream enters the modules for exposure of the animals (vide infra). The air leaving the modules is vented via a charcoal canister. An overpressure security (15 in Figure 5) was installed between mass flow controller 4 and vial 7, in order to minimize the risks of breaking of this vial in case of a pressure build-up in the generation unit.

Various designs for the nerve-agent-containing generation vial were tested for their convenience in everyday use. We started out with a round-bottomed, thick-walled tube in which 2 g of neat C(±)P(±)-soman was placed on the bottom. The air inlet, originating from mass flow controller 4, was led through the agent via a glass capillary with a glass frit situated at 1 cm from the end of the capillary. The presence of the frit was necessary in order to obtain a more homogeneous distribution of the air through the viscous agent. A problem with this design was the decrease of the concentration of C(±)P(±)-soman vapor in the airstream after several days of generating C(±)P(±)-soman vapor. The level of the nerve agent in the vial had dropped to such an extent that only the lower part of the frit was submerged in the nerve agent, and most of the air escaped through the part of the frit which was above the nerve agent level. As a consequence, frequent topping-up with nerve agent was necessary, with concomitant risk. This vial was modified by using a capillary with a frit situated only at the bottom; as well as by using a tube with a smaller diameter, thus producing a higher level in the tube with the same amount of C(±)P(±)-soman. Both designs proved to be more satisfactory than the original design, but still were not ideal. The design shown in Figure 6 appeared to be more suitable for our purpose.
The stream of air originating from mass flow controller 4 pushes the nerve agent into the glass frit (number 6 in Figure 6) and flows through it. Due to the large surface area of the frit, evaporization of the nerve agent is enhanced. Furthermore, the nerve agent can be used more efficiently than with the other designs. When the

generation apparatus has to function without generating nerve agent vapor, the air flow passes directly from the inlet (number 1 in Figure 6) to the outlet (number 3 in Figure 6) via the bypass (number 2 in Figure 6), by closing the valves (number 8 in Figure 6) of the vial.

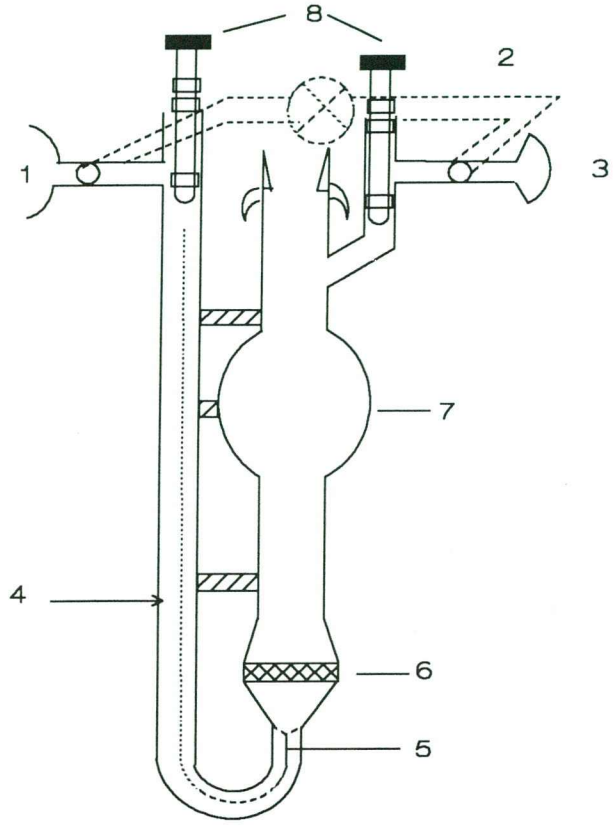

Figure 6. Outline of the vial containing the nerve agent. (1) air inlet, connected with mass flow controller 4; (2) bypass; (3) air outlet, connected with the first mixing chamber (8 in Figure 5); (4) thick-walled glass capillary; (5) neat nerve agent; (6) glass frit; (7) splasher head; (8) valves.

b. Determination of the nerve agent concentration in the airstream

The concentration of the nerve agent, i.e. C(±)P(±)-soman and (±)-sarin, in the airstream is determined by gas chromatography, i.e., with GLC configuration 3. A schematic representation of the injection device of this configuration is shown in Figure 7. This chromatograph is equipped with a cold on-column injector as well as with a heated gas-sampling device, connected to the same column using a Y-shaped press-fit connector. The on-column injector is used to calibrate the gas chromatographic performance by injecting standard solutions of C(±)P(±)-soman.

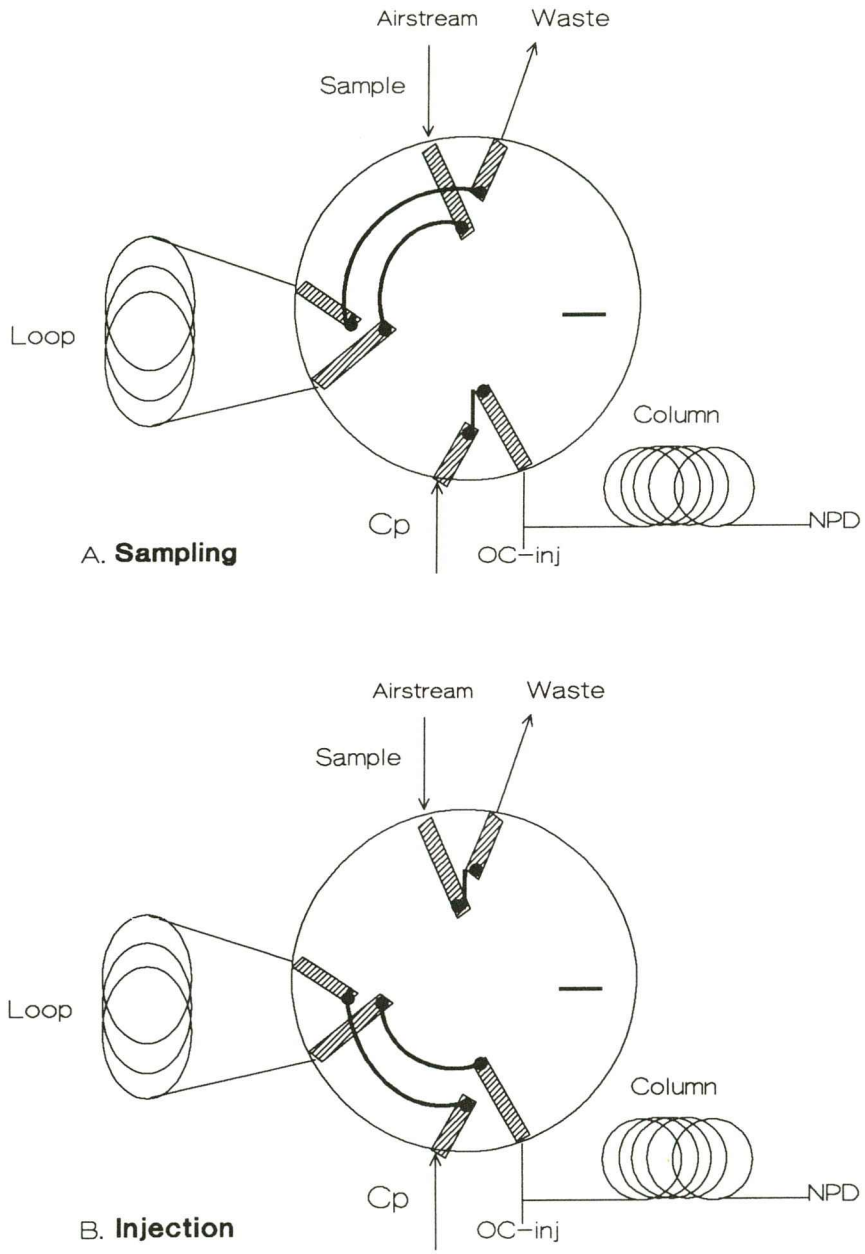

Figure 7. Schematic representation of the injection device of GLC configuration 3 (see section II). Cp is a constant pressure valve; OC-inj is the on-column injector; and NPD is the detector. Scheme A represents the sampling phase; scheme B the injection phase.

The airstream with the nerve agent vapor flows continuously through the sampling loop of the gas-sampling valve. At intervals of 4 min, the contents of the loop, 58.8 μl, are injected into the gas chromatograph. The reproducibility of the injection itself was tested with acetone vapor. The inlet capillary of the gas-sampling valve was positioned just above the fluid level in a test tube filled with acetone. With the aid of a small pump, air was drawn through the gas-sampling valve with a flow-rate of ca. 150 ml/min. A series of subsequent injections was performed. The reproducibility of the injection was assessed from the variation in the measured peak heights. The results of an experiment with four injections are presented in Table 6. The relative standard deviation (r.s.d.) of the injection is better than 0.18 %, which is a satisfactory result.

Table 6. Peak heights observed for four subsequent injections of acetone vapor in air by means of the gas-sampling valve.

Injection number	Peak height (units)
1	7017912
2	7030652
3	7046176
4	7038068

mean peak height : 7033202
standard deviation : 12004
relative standard deviation : 0.17 %

A difference in standard deviation between injections from a very simple, static system and the injection in a dynamic system, such as the generation apparatus, can be anticipated. After positioning the inlet of the gas-sampling valve into the outlet of the complete generation apparatus, the r.s.d. was increased to 2.7 % (n=10), and if the exposure unit was connected to the generation apparatus, and in use, the r.s.d. was even increased to 4 % (n=10). These latter data were obtained with injections of soman vapor in air.

Prior to testing with C(±)P(±)-soman, the stability of the generated vapor concentration was tested with dimethyl methylphosphonate (DMMP), which is frequently used in our laboratory as a relatively nontoxic nerve agent simulant. The volatility of DMMP is comparable to that of C(±)P(±)-soman.
More than 2.5 h after setting and opening the mass flow controllers, the first peak of DMMP appeared from the gas chromatograph, indicating the presence of DMMP vapor in the airstream at the sampling point. Subsequently, a stable concentration was attained within 30 min.
When the flow-rate through the generation vial was increased at that stage, the concentration stabilized at a higher level in about 15 min.

To check for possible degradation of C(±)P(±)-soman between the point of sampling and the introduction into the precolumn, the concentration determined by air sampling had to be validated. In order to perform such a validation, the airstream via the loop was led through a wash bottle filled with a suitable trapping solvent, i.e., methanol, with a known flow-rate, in order to trap all nerve agent during a fixed period of time. The contents of the wash bottle were analyzed by injecting a sample with the on-column injector on configuration 3, after addition of internal standard. Analysis of the solvent in a second wash bottle filled with methanol, connected in series with the first one, served as a check for complete trapping of nerve agent in the first wash bottle.
The total amount of trapped compound was calculated from the concentration in, and the volume of, the methanol in the wash bottles. Since both the flow-rate of the airstream and the time period during which the airstream was led through the wash bottles were known, the concentration of the compound in the airstream could be calculated. The concentration determined by direct gas chromatographic analysis of air samples was compared with the concentration, calculated by analysis of the contents of the wash bottles.
This experiment was performed first with DMMP. DMMP was efficiently trapped in the first wash bottle, since less than 0.3 % of the total DMMP amount was found in the second wash bottle. In this experiment the concentration of DMMP in the airstream, as measured via the gas-sampling valve, appeared to be 17 % higher than the actual concentration, measured via trapping in the wash bottles. This was a puzzling phenomenon. Rather than spending much time in solving this problem for DMMP, we decided to continue the experiments with C(±)P(±)-soman.
After starting the generation apparatus, it took about 45 min to reach a constant concentration of C(±)P(±)-soman in the airstream. The C(±)P(±)-soman-containing airstream was led through wash bottles filled with methanol. During the sampling period, as the airstream passed through the wash bottles, the concentration of C(±)P(±)-soman appeared to drop by ca. 2 %, probably due to the back-pressure from the methanol in the wash bottles. In later experiments with isopropanol as a trapping solvent, which is more viscous than methanol, the concentration dropped even by ca. 33 %.
The concentration determined by direct injection from the airstream appeared to be about two times higher than the calculated actual concentration. The temperature of the gas-sampling valve in that particular experiment was 75 °C. Upon increasing this temperature, the peak area for C(±)P(±)-soman decreased, as is shown in Figure 8. Apparently, the observed discrepancy between the results obtained via gas-sampling and via the wash bottle was due to condensation of C(±)P(±)-soman in the loop of the gas-sampling valve, at this relatively low temperature. At high valve temperatures, on the other hand, there is a risk of degradation of C(±)P(±)-soman as a result of contact with the hot metal sample loop of the valve. In the temperature range of 110-130 °C, the concentration as determined by gas injection was nearly the same as that determined via the wash bottle. As a result, the temperature of the gas-sampling valve was

set at 125 °C. For (±)-sarin analysis, this temperature was adequate as well.

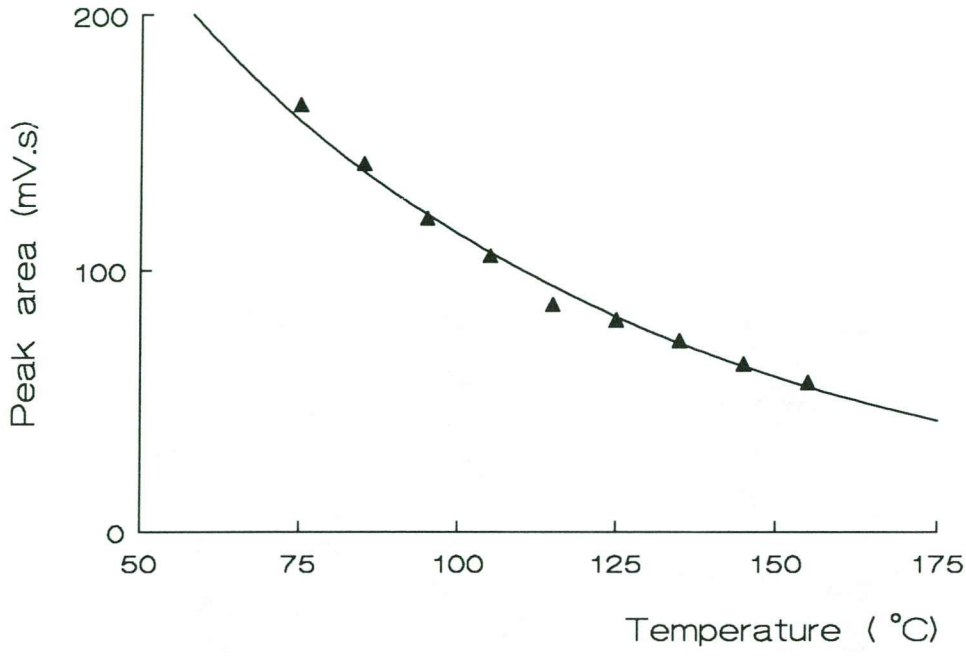

Figure 8. The relationship between the peak area measured for C(±)P(±)-soman in the airstream and the temperature of the gas-sampling valve.

c. Apparatus for exposure of guinea pigs to nerve agent vapor in air

An apparatus was constructed which enables nose-only exposure of 1 to 12 animals in individual modules. Four modules are mounted next to each other in a rack. The racks can be stacked in a frame which contains the electronics control unit. For the purpose of the study, we use only two racks, which corresponds with eight modules. See Figure 9 for a photograph of the exposure unit. A schematic representation of a module is shown in Figure 10, whereas Figure 11 shows how the guinea pig, positioned in a modified Battelle tube, is connected to the exposure unit.

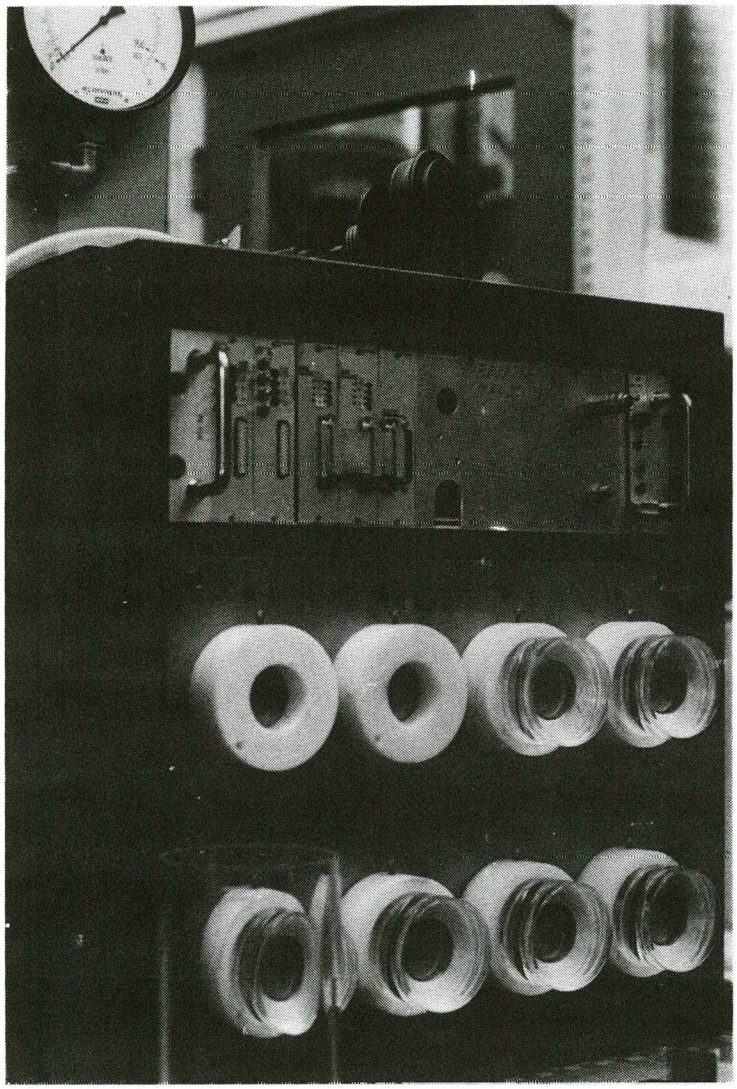

Figure 9. Photograph of the exposure unit.

When the modified Battelle tube is fitted to the exposure apparatus, it is connected gastight because of the O-rings (number 2 in Figures 10 and 11). Air is sucked through tubing number 8, while the electronic pressure monitor (number 9) checks whether a vacuum is produced, indicating gastight connection of the tube. The electronically operated three-way valve (number 5 in Figure 10) can now be switched to pass the vapor from the generation equipment via the glass tubing (number 6 in Figure 10) into the direction of the front chamber (number 1 in Figure 10), via a glass tubing (number 4, in Figure 10). Tubing number 11 (Figure 10) has a critical orifice which draws a constant bias flow of 1 l/min from the front chamber. The flow enters the front chamber through wire mesh resistance

(number 3 in Figure 10). At this point the flow as a result of the ventilation of the guinea pig is superimposed on the bias flow. The differential pressure over the resistance is measured (number 12 in Figure 10), which reflects the ventilatory flow of the animal. These measurements are transformed in a data system to calculate the respiratory minute volume during the experiment, as outlined below. In the front chamber an overpressure of about 5 cm water exists. To restrict the risk of leakage of contaminated air, associated with overpressure, air is sucked through tubing number 10 from the 'underpressure chamber' around the tube. The contaminated air coming from tubings number 10 and 11 (Figures 10 and 11) is led through a charcoal canister.

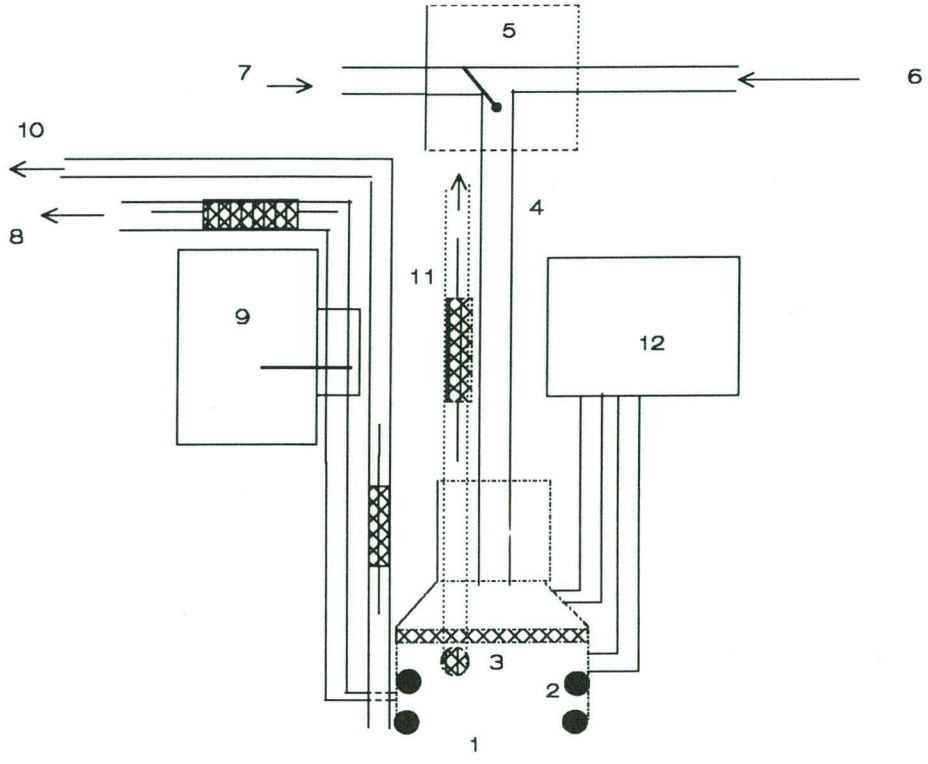

Figure 10. Schematic representation of the exposure apparatus. (1) 70-ml front chamber of the modified Battelle tube, from which the animal breathes; (2) O-rings for gastight connection of the Battelle tube; (3) wire mesh resistance; (4) tubing through which the vapor is transported to the exposure chamber; (5) 3-way valve; (6) connection with the generation apparatus; (7) inlet for ambient air; (8) tubing with a critical orifice, which is connected to (9), i.e., a vacuum check for gastight connection of the Battelle tube; (10) tubing with a critical orifice which sucks air from the 'underpressure chamber' surrounding the Battelle tube; (11) tubing with a critical orifice which is the outlet of the front chamber; (12) differential pressure measuring device.

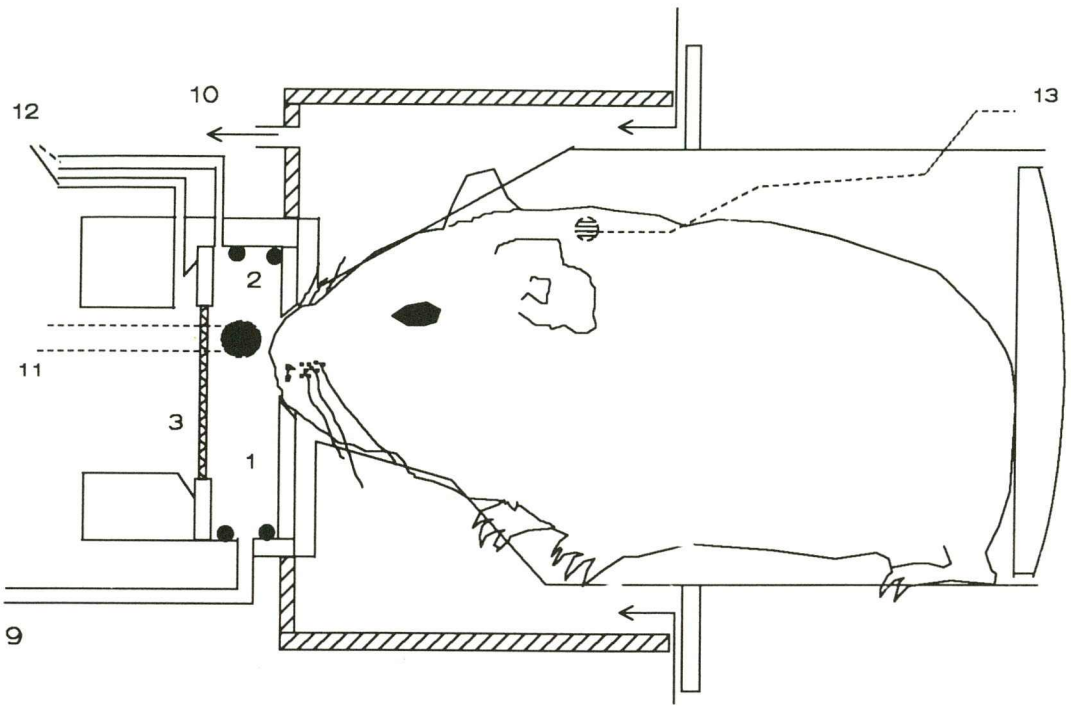

Figure 11. Position of the guinea pig in the modified Battelle tube. Numbers correspond with those of the legend of Figure 10. (13) carotid artery cannula.

As can be seen from Figure 11, only the nose of the animal is in contact with the front chamber. The guinea pig is held in position in the tube by the adjustable clamp at its rear end. In order to prevent leakage of the toxicant vapor beyond the nose area, a rubber mask is inserted into the conical end of the tube. The cannula for drawing blood samples from the animal (number 13 in Figure 11) exits from the nonsealed area of the body and protrudes from the tube.

The generation apparatus was connected to the exposure chamber. With a C(±)P(±)-soman vapor concentration of ca. 20 mg/m^3, as determined with GLC configuration 3 at the exit of the generation apparatus, the passage of the C(±)P(±)-soman vapor to each of the eight exposure chambers was checked, with the 'wash bottle' method which we also used for validation of the injection from the airstream via the gas-sampling valve. The amount of C(±)P(±)-soman, calculated by analysis of the trapping solvent, was only 16-40 % of the amount as expected from the concentration measured directly in the airstream. At first it was suspected that a stable concentration was not yet reached in the exposure unit. However, after 20 h of continuous generation, the measured concentrations were still low and irreproducible. The overpressures measured at the end of the modules varied from 34-56 mm water, which could not explain the unsatisfactory results either. A

possible explanation for the low recoveries is the fact that the airstream was sucked through the wash bottles with a pump, which should produce a constant flow-rate. The actual flow-rate, however, is influenced by the overpressure at the end of the modules and the pressure difference caused by the trapping solvent in the wash bottles. Apparently, the flow-rate of the pump was not correct under these conditions. Since calibration of the pump appeared to be rather tedious in this configuration, a different approach for determination of the C(±)P(±)-soman concentration in the exposure chambers was tried, i.e., by replacement of the Battelle tube with a plug onto each module, which was connected to GLC configuration 3 via a glass tubing. The results of this experiment are presented in Table 7.

Table 7. Peak area (μV) of C(±)P(±)-soman measured in the airstream just entering the exposure apparatus (Main) and at the 8 front chambers (1-8) of the exposure apparatus by gas chromatographic analysis via the gas-sampling valve. The time (min) passed after the start of the experiment is included for each measurement.

Position	Time (min)	Peak Area (μV)
Main	0	671220
4	20	670440
3	59	698570
2	74	722630
1	105	720720
5	161	732790
6	210	739700
7	244	732030
8	284	785940
4	321	786220
Main	340	790010

The data in Table 7 show that the concentration of C(±)P(±)-soman in the airstream has increased during the 340 min experiment. This increase in concentration was due to a rise in temperature in the laboratory, which appeared to increase from 18 °C to 23 °C during the experiment. These results emphasize the need to maintain a fairly constant environmental temperature during an exposure experiment.
As a whole, the concentrations of C(±)P(±)-soman measured in the 8 front chambers of the exposure modules do not seem to deviate much from the concentration produced in the generation apparatus.
In following experiments, the C(±)P(±)-soman concentration was measured at one of the eight exposure chambers, randomly chosen, and compared with the concentration measured in the gas stream just before entering the exposure apparatus, which did not appear to differ significantly.
These findings suggest that C(±)P(±)-soman does not degrade or disappear as a result of interaction with or diffusion through the materials in the exposure unit.

We did observe, however, an equilibration time of about 45 min in the exposure apparatus, probably due to (reversible) adsorption of C(±)P(±)-soman to the teflon tubing in the exposure unit. The equilibration time was shortened twofold by replacing all synthetic tubing, through which the soman-containing air passes from the generation apparatus to the exposure chambers, with glass tubing.

d Determination of respiratory parameters

d.1. General description

The animals restrained in the modified Battelle tubes breathe via the nose in the front chamber of the exposure module (number 1 in Figures 10 and 11). The respiration of the restrained animals is measured with specific sensors. The sensors measure the respiration from the differential pressure changes that are caused by the respiratory flow before and after the wire mesh resistance (number 3 in Figures 10 and 11). This respiratory signal is superimposed on the constant pressure difference resulting from the continuous flow of 1 l/min, which is drawn through the wire mesh. The fluctuating electrical signals of the sensors are sampled by an IBM compatible AT computer with a specifically designed homemade IO card.
Two different experimental conditions can exist:

(i) breathing of the animals without exposure to the toxicant, in which case clean ambient air from tubing number 6 (Figure 10) is drawn through the front chamber;

(ii) breathing of animals during exposure to the toxicant. A flow of the toxicant-contaminated air is maintained through the respiratory compartment. The added flow of the toxicant is slightly greater than the sucked flow, resulting in a small rise of pressure (a few mbar relative to room pressure) in the front chamber of the exposure compartment.

Since the animals can be dosed individually, both experimental conditions can occur in the exposure apparatus as a whole at the same time. The slight overpressure in case of dosing of the toxicant is adjusted via an overflow valve.

d.2. Electronics of the exposure apparatus: switches, valves and sensors

The exposure apparatus contains a unit in which the electronic parts for calibration of the sensors and for the controls of the switches of the exposure are present. The sensors, the electronic valves and the vacuum switches are part of the exposure modules. The unit with the electronic parts communicates with the computer via a data cable. The calibration of the sensors is visualized on the screen of the computer with the aid of a homemade Pascal program.
To change from clean ambient air to air containing nerve agent vapor, an electrical 3-way valve (Bürkert, Type 124, number 5 in Figure 10) has to be activated, after which a red LED lights on the module, indicating passage of nerve agent vapor containing air. This valve can only be activated by the vacuum switch (Festo pneumatic type VPE, number 9 in Figure 10) which checks the existence of a sufficient

vacuum in the connection area between the two rubber O-rings of the Battelle-type tube and the front chamber. For this purpose a tiny hole in the connecting side of the module is present, positioned between the two O-rings. Air is drawn through this hole with a constant flow of 1 l/min, via tubing number 8 (Figure 10).
The difference in pressure in front of and behind the wire mesh resistance is measured by a sensor (Honywell SenSym 142SC01D) designed to measure small pressure differences. These pressure differences (in the mbar-range) are transduced by the sensor into an electrical signal, which is amplified (output in mV-range) and sent to the computer.

d.3. Computer data acquisition and program

The signal of the sensors of the exposure unit changes continuously with time, due to the fluctuating pressure differences caused by the breathing of the animals. Every 12 sec, the computer samples 512 pressure differences per channel (= sensor) for four channels at a time, with the aid of an IO-card (PC Labcard PCL-812). After these 12 sec, the computer calculates a respiratory minute volume (RMV) and a respiratory frequency (RF) within approximately 3 sec, displays a graph of the 4*512 data points and starts again, either to sample the same four channels or, in case of eight animals, to sample the other four channels. All RMV and RF data are stored in a data file. Apart from this file with calculated data, two additional files can be generated optionally. These optional data files may contain all of the raw sampling data. In order to prevent these data files from becoming too large to handle, we chose to store only a part of the 512 data points, at adequate time intervals during the experiment. The graph of the raw data shows the respiratory profile, which is used to characterize respiratory behaviour during the exposure. The data files are constructed in such a way that they can easily be introduced into a Lotus 123 spreadsheet. Graphs and further calculations (such as Tidal Volume) are immediately at disposal.
Two Pascal programs are used for operating the exposure experiment, viz., a calibration program and the main program for all data handling. The calibration program is used to establish the offset of the sensors, in combination with the fine-tuning on the electronic module of the respiratory unit.
The major program for operation is in fact a combination of a number of unit Pascal programs which perform various tasks, such as screendriving. The program contains two algorithms to calculate the frequency of respiration and the respiratory minute volume.

(i) Frequency of respiration (RF)
The algorithm for the RF is based on a fast Fourier analysis of the sampled data points. For the performance of this routine 128 of the 512 data points are used. The actual frequency range is between 0 and 5.0 Hz.

(ii) Respiratory minute volume (RMV)
The algorithm for the RMV routine is based on the following: first, the average of the 512 data points is calculated. The respiratory signal is represented now by the data points above and below this offset. Next, the sum of all the data above this offset and the fraction of the total data points are determined.

From these data the total volume of respired air is calculated by comparison with the data points resulting from a known flow produced by an artificial ventilator.

An example of the monitoring of the respiration is shown in Figure 12.

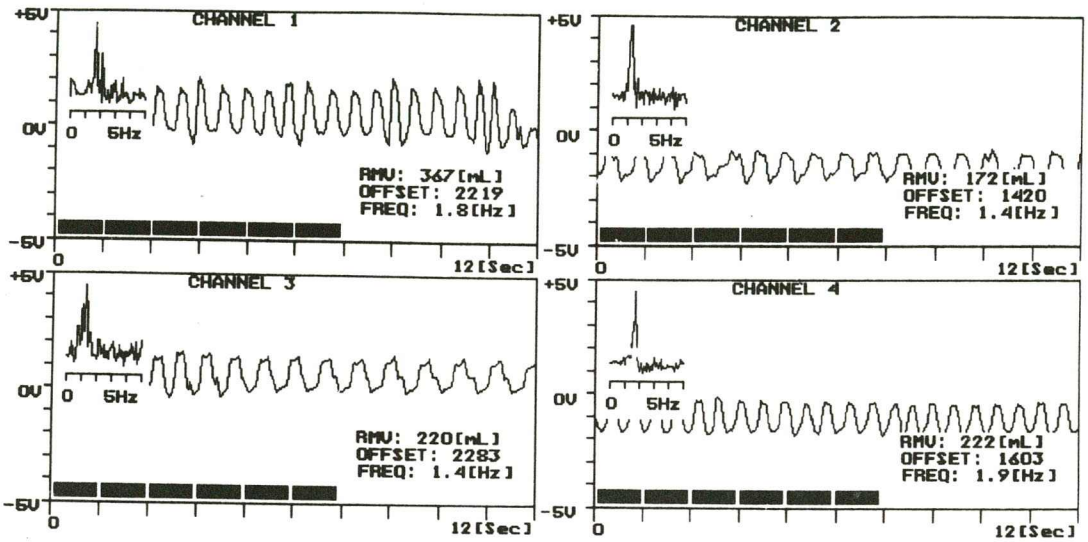

Figure 12. Typical example of a screen-dump of the respiration monitoring for 4 anesthetized guinea pigs, in channels 1, 2, 3 and 4, breathing clean air. The inserts show the 'power-spectrum' of the respiration in this 12 s measurement period and indicate the respiratory frequency. The calculated respiratory minute volume (RMV) and frequency (RF) are plotted in each graph. Note the variability in RMV and RF between the animals.

The data of eight channels are collected by a computer. The respiration frequency and respiratory minute volume are calculated during the experiment. At the end of the experiment the total inhaled volume is calculated for each channel, enabling the determination of the inhaled dose. The signals of four of the channels are shown on the monitor. Figure 12 is a screen dump made in a 12 sec period during a test run of four anesthetized guinea pigs in channels 1-4, exposed to clean air.
In anesthetized animals the respiration shows a regular pattern, with a RF of ca. 1 Hz. In unanesthetized animals the respiration was very irregular, with a RF as high as 2-3 Hz, very likely as a result of the distress that these naive animals experience in the tubes.
The determination of the RMV was unsatisfactory at first. It appeared to be difficult to obtain a gastight fit of the nose of the guinea

pig in the modified Battelle tube, leading to erratic RMV values. This problem was solved by inserting rubber masks between the nose of the animal and the cone of the tube, and increasing the pressure on the rear end of the animal, via the adjustable clamp.
The mean RMV and RF measured for 8 ketamine anesthetized guinea pigs were 91 ± 9 ml and 1.3 ± 0.3 Hz, respectively, and 114 ± 30 ml and 1.6 ± 0.4 Hz for 8 unanesthetized guinea pigs. Therefore, ketamine anesthesia has no significant effect on the respiratory parameters.

III-5 THE LCt50 OF C(±)P(±)-SOMAN IN ANESTHETIZED, RESTRAINED GUINEA PIGS AFTER NOSE-ONLY EXPOSURE FOR 8 MINUTES

Prior to the ultimate 24-h LCt50 determination, several pilot experiments were performed in order to estimate the required dosing range. First of all, the LC50 of C(±)P(±)-soman was estimated from the 24-h LD50 after intravenous bolus administration, which is 27.5 μg/kg. An intravenous dose corresponding with 1 LD50 requires administration of 11 μg of C(±)P(±)-soman to a 400-g guinea pig. Based on a respiratory frequency of 50/min and a tidal volume of 3.5 ml, which corresponds with a respiratory minute volume of 175 ml/min, and the assumption of 100 % retention of the toxicant vapor, it was calculated that a 400-g guinea pig should be exposed to a concentration of C(±)P(±)-soman vapor in air of 7.5 mg/m^3 for 8 min. In a first pilot experiment, groups of four anesthetized guinea pigs were exposed to a concentration of 7 mg C(±)P(±)-soman/m^3 for 4, 8, 12, 16 or 20 min, respectively. Most of these animals showed hardly any symptoms, just some miosis. Only two of them, exposed for 12 and 16 min, respectively, showed a slightly increased salivation two h after exposure, whereas only one of the animals died within 24 h. Obviously, the concentration used was much too low. Therefore, in succeeding experiments the concentration was increased considerably. Groups of eight anesthetized guinea pigs were exposed for 8 min to concentrations of C(±)P(±)-soman vapor in air ranging from 30 to 84 mg/m^3. After exposure, each animal was placed in a separate cage. The number of dead animals per dosing group was counted 24 h later. The results are presented in Table 8.

Table 8. Number and percentage of dead animals per dosing group, 24 h after 8-min exposure of anesthetized guinea pigs to C(±)P(±)-soman vapor in air.

[C(±)P(±)-soman] (mg/m^3)	Number of dead animals at 24 h/total number	Percentage deaths
30	0/8	0
49	1/8	12.5
55	5/8	62.5
61	6/8	75
71	3/8	38
80	6/8	75
84	8/8	100
84	7/8	88

Using probit analysis of the mortality data, the LC50 of C(±)P(±)-soman was calculated to be 60 mg/m^3 (95 % confidence limits 51-67 mg/m^3). Consequently, the LCt50 of C(±)P(±)-soman for 8-min nose-only exposure of anesthetized guinea pigs is 480 mg.min/m^3.
The results of the probit analysis are presented in Table 9 and Figure 13.

Table 9. LC10, LC30, LC50, LC70 and LC90 (24 h) with 95 % confidence limits, for 8-min nose-only exposure of anesthetized guinea pigs to C(±)P(±)-soman vapor in air, calculated via probit analysis.[a]

LC	mg/m^3	95 % confidence limits (mg/m^3)
10	40	25-48
30	51	38-58
50	60	51-67
70	70	63-82
90	88	77-124

[a] Probit equation: probit= 7.50*log[C(±)P(±)-soman] - 8.32

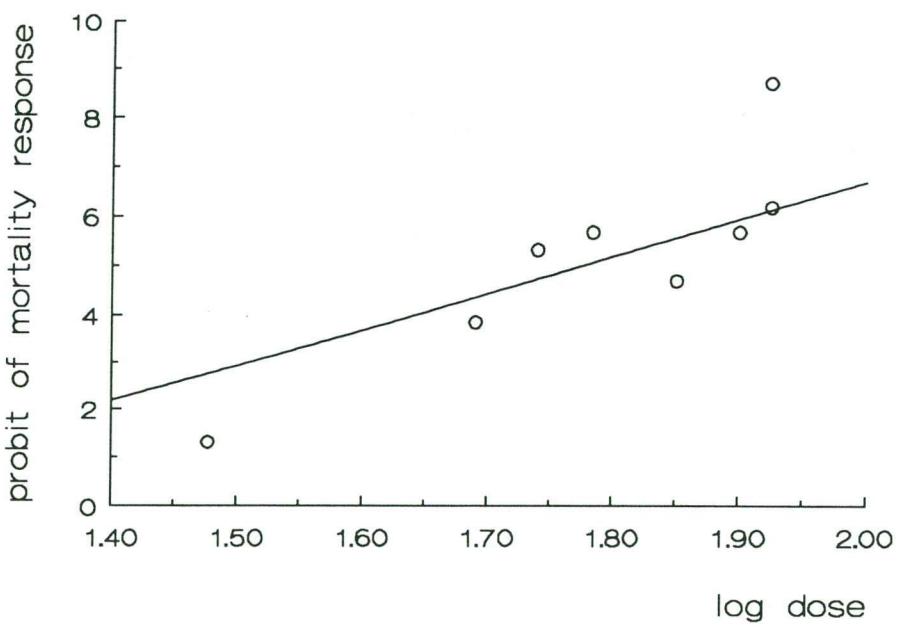

Figure 13. Probit of mortalitity of anesthetized guinea pigs nose-only exposed to C(±)P(±)-soman vapor in air for 8 min, versus the concentration of C(±)P(±)-soman vapor in air.

III-6 THE TOXICOKINETICS OF 0.8 LCt50 C(±)P(±)-SOMAN IN ANESTHETIZED, ATROPINIZED AND RESTRAINED GUINEA PIGS AFTER NOSE-ONLY EXPOSURE FOR 8 MINUTES

Anesthetized, atropinized and restrained guinea pigs were nose-only exposed to a concentration of 48 ± 5 mg/m^3 of C(±)P(±)-soman vapor in air for 8 min, yielding an exposure to 0.8 LCt50.
Blood samples were taken just before starting the exposure, at 0.5, 1, 2, 4, 8 min during exposure, and at 2, 4, 6, 8, 12, 17, 22 and 32 min after exposure. A small portion of the blood sample was used for the determination of AChE activity, the larger part was used for gas chromatographic analysis. The blood samples were analyzed with GLC configuration 1. During the exposure, the respiration of the animals was monitored.
In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used in order to obtain six values for each time-point.
Although the exposure apparatus is suitable for simultaneous exposure of up to eight animals, only two animals were exposed simultaneously in the toxicokinetic experiments, since it appeared not to be feasible to handle more than two animals at the same time.
In the blood samples taken during exposure, concentrations of the C(-)P(+)-isomer in the pg/ml range were observed, whereas the C(+)P(+)-isomer was not observed at all. The concentrations of the C(±)P(-)-isomers measured in the individual animals are listed in Table 10, whereas the mean values with standard error of the mean (s.e.m., n=6) are presented in Table 11 and Figures 14 and 15. These figures show that the absorption of the C(±)P(-)-isomers is very rapid, since the concentrations do not increase further after terminating the exposure. The data in Table 11 indicate that the absorption phase of the C(+)P(-)-isomer lags somewhat behind that of the C(-)P(-)-isomer.
Mathematical equations describing the concentration-time courses of the P(-)-isomers were obtained by nonlinear regression. The kinetics were described as a discontinuous process, with an equation for the 8-min exposure period and an equation for the post-exposure period. For the absorption phase of C(+)P(-)-soman, a lag time of 2 min was chosen. The absorption phase was described with a mono-exponential function, since the limited number of datapoints in this phase does not justify a description with more than one exponential. The post-exposure phase appears to be adequately described with a two-exponential function. As was the case for the absorption phase, the number of datapoints is insufficient for justification of a three-exponential equation. The toxicokinetic parameters are listed in Table 12. The terminal half-lives are practically the same for the two P(-)-isomers. Furthermore, the terminal half-life of C(-)P(-)-soman is in reasonable agreement with that calculated for intravenous bolus administration of 0.8 LCt50 of C(±)P(±)-soman.
The AChE activities measured during and after exposure are presented in Table 13 and Figure 16. During exposure and in the first few minutes thereafter, the blood AChE activity drops fairly rapidly down to ca. 4 % of control activity, which appears to be the residual activity under these conditions.

Table 10. Concentrations in blood[a] (ng/ml) of C(+)P(-)- and C(-)P(-)-soman in individual anesthetized, atropinized, and restrained guinea pigs at various time-points during and after nose-only exposure to 48 ± 5 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.8 LCt50.

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 1 (401)[b]		Guinea pig 2 (432)[b]		Guinea pig 3 (634)[b]		Guinea pig 4 (599)[b]		Guinea pig 5 (642)[b]		Guinea pig 6 (631)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	0.007	0.092	*	*	n.d.	0.023	*	*	n.d.	0.250	*	*
1	*	*	0.004	0.133	*	*	n.d.	0.183	*	*	n.d.	1.354
2	0.016	0.693	*	*	*	*	n.d.	0.139	0.056	0.750	*	*
4	*	*	0.028	0.873	n.d.	0.236	*	*	*	*	0.428	3.891
8	0.296	2.36	*	*	n.d.	0.873	*	*	1.328	2.354	*	*
10	*	*	0.122	0.792	*	*	0.058	0.280	*	*	2.928	3.741
12	0.301	0.762	*	*	*	*	0.114	0.127	0.719	1.245	*	*
14	*	*	0.059	0.217	0.041	0.108	*	*	*	*	1.326	1.501
16	0.323	0.755	*	*	0.047	0.061	*	*	0.280	0.768	*	*
20	*	*	0.336	0.340	*	*	0.054	0.037	*	*	0.628	0.789
25	0.150	0.373	*	*	*	*	*	*	0.070	0.260	*	*
30	*	*	0.043	0.164	*	*	0.011	0.061	*	*	0.281	0.346
40	0.039	0.089	*	*	*	*	*	*	0.052	0.094	*	*

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 7 (542)[b]		Guinea pig 8 (531)[b]		Guinea pig 9 (642)[b]		Guinea pig 10 (601)[b]		Guinea pig 11 (422)[b]		Guinea pig 12 (445)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d..	n.d..	n.d..	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	0.035	*	*	n.d.	0.323	*	*	n.d.	0.114	*	*
1	*	*	n.d.	0.683	*	*	n.d.	0.884	*	*	n.d.	0.233
2	*	*	n.d.	0.894	n.d.	0.837	*	*	*	*	n.d.	0.407
4	n.d.	0.321	*	*	*	*	0.270	3.400	n.d.	1.055	*	*
8	n.d.	1.492	*	*	4.417	5.478	*	*	1.985	3 571	*	*
10	*	*	0.940	1.966	*	*	2.762	3.255	*	*	0.616	1.175
12	*	*	1.068	1.303	0.487	1.181	*	*	*	*	0.515	0.638
14	0.145	0.296	*	*	*	*	1.062	1.250	0.438	0.694	*	*
16	0.105	0.217	*	*	0.468	0.585	*	*	0.300	0.395	*	*
20	*	*	0.239	0.436	*	*	0.502	0.656	*	*	0.114	0.159
25	*	*	0.133	0.346	0.206	0.270	*	*	*	*	0.051	0.063
30	*	*	0.294	0.107	0.185	0.363	*	*	*	*	0.031	0.042
40	0.028	0.030	*	*	*	*	0.055	0.083	0.033	0.041	*	*

[a] Measured with GLC configuration 1 (see section II)

[b] Weight in grams

n.d. = Not detectable

* = Not measured

Table 11. Mean concentrations of C(-)P(+)-, C(+)P(-)- and C(-)P(-)-soman (± s.e.m., n=6) in anesthetized, atropinized and restrained guinea pigs during and after nose-only exposure to a concentration of 48 ± 5 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.8 LCt50.

Time (min)	[C(-)P(+)-soman] ± s.e.m (n=6) (ng/ml blood)	[C(+)P(-)-soman] ± s.e.m (n=6) (ng/ml blood)	[C(-)P(-)-soman] ± s.e.m (n=6) (ng/ml blood)
0	n.d	n.d.	n.d.
0.5	0.005 ± 0.003	n.d.	0.1 ± 0.4
1	0.008 ± 0.003	n.d.	0.6 ± 0.2
2	0.004 ± 0.002	0.01 ± 0.01	0.6 ± 0.1
4	0.013 ± 0.008	0.1 ± 0.1	1.0 ± 0.4
8	0.004 ± 0.003	1.4 ± 0.4	2.7 ± 0.6
10	n.d.	1.2 ± 0.6	1.9 ± 0.5
12	n.d.	0.5 ± 0.2	0.9 ± 0.2
14	n.d.	0.5 ± 0.3	0.7 ± 0.2
16	n.d.	0.25 ± 0.07	0.5 ± 0.1
20	n.d.	0.3 ± 0.1	0.4 ± 0.1
25	n.d.	0.14 ± 0.03	0.28 ± 0.04
30	n.d.	0.11 ± 0.05	0.17 ± 0.06
40	n.d.	0.05 ± 0.01	0.07 ± 0.01

n.d. = not detectable

Table 12. Toxicokinetic parameters[a] for C(±)P(-)-soman in anesthetized, atropinized, and restrained guinea pigs, after nose-only exposure to 0.8 LCt50 C(±)P(±)-soman in 8 min.

		C(+)P(-)-soman	C(-)P(-)-soman
A (ng/ml)		-55.4	-35.8
B (ng/ml)		32.6	141
C (ng/ml)		0.83	1.9
D (ng/ml)		55.4	35.8
a (min^{-1})		0.004	0.0094
b (min^{-1})		0.42	0.54
c (min^{-1})		0.075	0.083
Terminal half-life (min)		9.2	8.4
Area under the curve (ng.min/ml)	0-8 min	7.2	8.5
	> 8 min	8.8	15.2
	total	16.0	23.7

[a] The inhalation results were fitted with a discontinuous function:
[nerve agent] = D + A*e^{-at} for the absorption phase (0-8 min), with a lag time of 2 min for C(+)P(-)-soman, and
[nerve agent]= B*e^{-bt} + C*e^{-ct} for distribution and elimination (≥ 8 min)

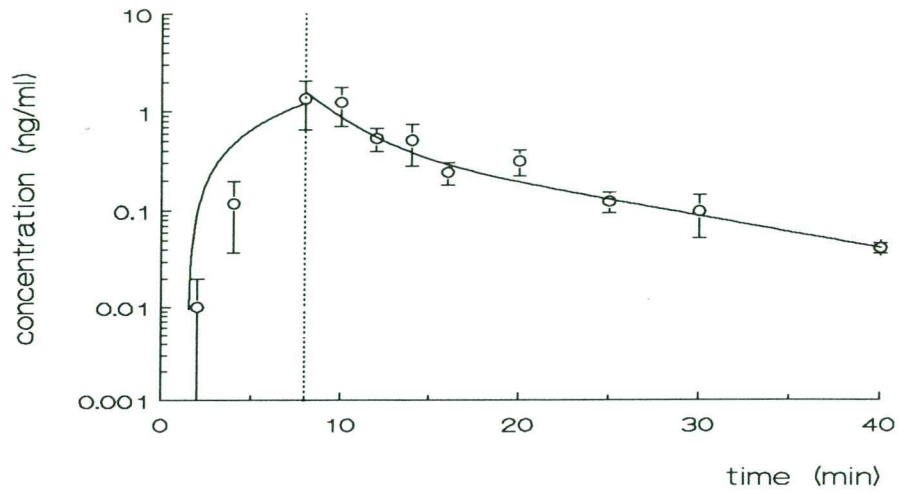

Figure 14. Semilogarithmic plot of mean concentrations in blood (± s.e.m., n=6) of C(+)P(-)-soman versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 48 ± 5 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.8 LCt50. The dotted line marks the end of the exposure period.

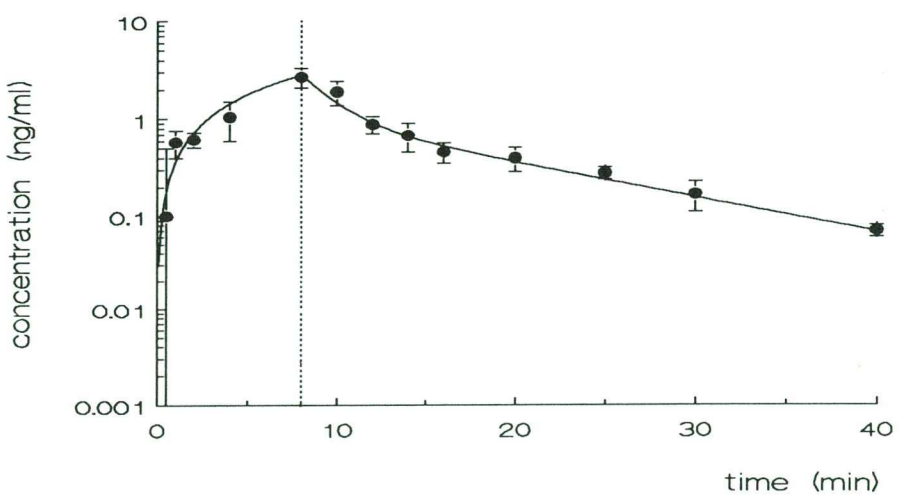

Figure 15. Semilogarithmic plot of mean concentrations in blood (± s.e.m., n=6) of C(-)P(-)-soman versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 48 ± 5 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.8 LCt50. The dotted line marks the end of the exposure period.

Table 13. Mean acetylcholinesterase (AChE) activities with s.e.m. (n=6) measured in blood samples of anesthetized, atropinized and restrained guinea pigs during and after nose-only exposure to 48 ± 5 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.8 LCt50.

Time (min)	AChE activity ± s.e.m. (%) (n=6)
0	100
0.5	92 ± 4
1	82 ± 5
2	70 ± 4
4	45 ± 12
8	12 ± 6
10	9 ± 3
12	5 ± 3
14	6 ± 4
16	6 ± 3
20	4 ± 2
25	5 ± 2
30	4 ± 2
40	4 ± 2

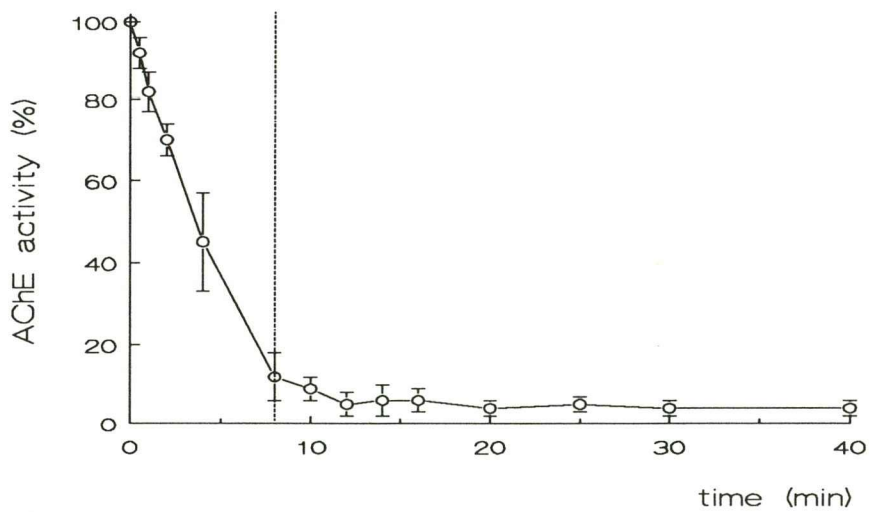

Figure 16. Time course of the acetylcholinesterase (AChE) activity (± s.e.m., n=6) in blood samples of anesthetized, atropinized, and restrained guinea pigs during and after nose-only exposure to 48 ± 5 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.8 LCt50. The dotted line marks the end of the exposure period.

An example of a respiratory profile is shown in Figure 17.

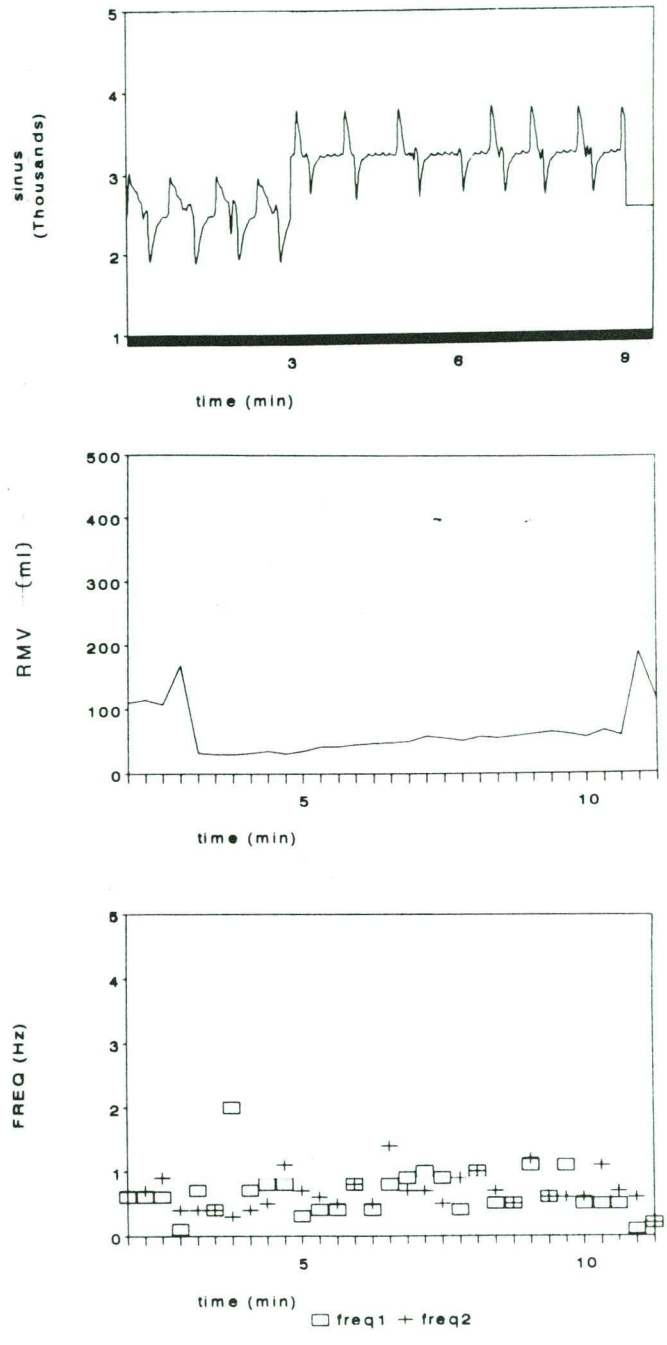

Figure 17. Respiratory sinus (top figure), respiratory minute volume (RMV, middle figure) and respiratory frequency (lower figure) of an anesthetized, atropinized and restrained guinea pig during the 8 min nose-only exposure to 48 ± 5 mg/m^3 of C(±)P(±)-soman, which corresponds with 0.8 LCt50.

During this inhalation experiment in which guinea pigs were exposed to a sublethal dose of C(±)P(±)-soman for 8 min, there were no measurable effects on the respiratory parameters. The respiratory minute volume appeared to be fairly constant during the 8-min exposure period, on average 52 ± 3 ml (s.e.m., n=12). The respiratory frequency was fairly constant as well, on average 0.65 ± 0.05 Hz (s.e.m., n=12). The respiratory profile hardly altered during the exposure.

III-7 THE TOXICOKINETICS OF 0.4 LCt50 C(±)P(±)-SOMAN IN ANESTHETIZED, ATROPINIZED AND RESTRAINED GUINEA PIGS AFTER NOSE-ONLY EXPOSURE FOR 8 MINUTES

Anesthetized, atropinized and restrained guinea pigs were nose-only exposed to a concentration of 24 ± 2 mg/m^3 of C(±)P(±)-soman vapor in air for 8 min, yielding an exposure to 0.4 LCt50.
Blood samples were taken just before and during exposure, and at various time-points after exposure. A small portion of the blood sample was used for the determination of AChE activity, the larger part was used for gas chromatographic analysis. The blood samples were analyzed with GLC configuration 1. During the exposure, the respiration of the animals was monitored.
In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used in order to obtain six values for each time-point.
P(+)-isomers were not present in detectable concentrations. The concentrations of the C(+)P(-)- and C(-)P(-)-isomers measured in the individual animals are presented in Table 14, whereas the mean concentrations with s.e.m. are shown in Table 15 and Figures 18 and 19. These figures show that the absorption of the P(-)-isomers is very rapid, since the concentrations do not increase further after terminating the exposure. The absorption phase of the C(+)P(-)-isomer lags somewhat behind that of the C(-)P(-)-isomer.
Mathematical equations describing the concentration-time courses of the P(-)-isomers were obtained by nonlinear regression. The kinetics were described as a discontinuous process, with an equation for the 8-min exposure period and an equation for the postexposure period. For the absorption phase of C(+)P(-)-soman, a lag time of 4 min was chosen. The absorption phase was described with a mono-exponential function, since the limited number of datapoints in this phase does not justify a description with more than one exponent. The post-exposure phase appears to be adequately described with a two-exponential function. By analogy with the absorption phase, the number of datapoints is insufficient for justification of a three-exponential equation. The toxicokinetic parameters are listed in Table 16.
The AChE activities measured during and after exposure are presented in Table 17 and Figure 20. During exposure and in the first few minutes thereafter, the blood AChE activity drops fairly rapidly down to ca. 10 % of control activity.
There were no C(±)P(±)-soman-related effects on the respiration, during this 8-min nose-only exposure. The mean respiratory minute volume during exposure was 44 ± 6 ml (s.e.m., n=12), whereas the respiratory frequency was 0.66 ± 0.05 Hz (s.e.m., n=12).

Table 14. Concentrations in blood[a] (ng/ml) of C(+)P(-)- and C(-)P(-)-soman in individual anesthetized, atropinized, and restrained guinea pigs at various time-points during and after nose-only exposure to 24 ± 2 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.4 LCt50.

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 1 (545)[b]		Guinea pig 2 (535)[b]		Guinea pig 3 (602)[b]		Guinea pig 4 (611)[b]		Guinea pig 5 (434)[b]		Guinea pig 6 (427)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	0.062	*	*	n.d.	0.027	*	*	n.d.	0.082	*	*
1	*	*	n.d.	0.072	*	*	n.d.	0.068	*	*	n.d.	0.127
2	n.d	0.153	*	*	n.d.	0.093	*	*	0.0005	0.134	*	*
4	*	*	n.d.	0.181	0.036	0.340	*	*	*	*	0.008	0.230
6	0.064	0.264	*	*	*	*	n.d.	0.200	0.014	0.463	*	*
8	*	*	0.016	0.356	*	*	0.010	0.298	*	*	0.118	1.316
10	0.016	0.104	*	*	0.092	0.280	*	*	0.037	0.222	*	*
12	*	*	*	*	0.069	0.152	0.017	0.122	*	*	0.018	0.080
14	*	*	*	*	*	*	0.010	0.064	0.027	0.094	*	*
16	*	*	*	*	*	*	0.016	0.054	0.019	0.063	0.016	0.044
20	0.024	0.031	*	*	0.024	0.028	*	*	0.010	0.021	*	*
25	*	*	0.011	0.012	*	*	0.006	0.013	*	*	0.006	0.006
30	*	*	0.010	0.006	*	*	0.010	0.008	*	*	0.009	0.004
40	0.019	0.006	*	*	0.012	0.006	*	*	0.005	0.016	*	*

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 7 (637)[b]		Guinea pig 8 (642)[b]		Guinea pig 9 (501)[b]		Guinea pig 10 (491)[b]		Guinea pig 11 (548)[b]		Guinea pig 12 (582)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	0.060	*	*	n.d.	0.218	*	*	n.d.	0.041	*	*
1	*	*	n.d.	0.140	*	*	n.d.	0.052	*	*	n.d.	0.053
2	n.d.	0.055	*	*	n.d.	0.428	*	*	n.d.	0.025	*	*
4	n.d.	0.232	*	*	*	*	n.d.	0.473	0.009	0.161	*	*
6	*	*	0.196	1.042	0.309	1.494	*	*	*	*	0.005	0.189
8	*	*	0.960	1.298	*	*	0.220	1.008	*	*	0.029	0.474
10	0.047	0.226	*	*	0.654	0.722	*	*	0.030	0.257	*	*
12	0.023	0.131	*	*	*	*	0.171	0.300	0.044	0.084	*	*
14	*	*	0.144	0.179	0.269	0.324	*	*	*	*	0.016	0.046
16	*	*	0.098	0.126	*	*	0.092	0.141	*	*	0.032	0.027
20	0.014	0.034	*	*	0.076	0.117	*	*	0.027	0.039	*	*
25	*	*	0.015	0.022	*	*	0.032	0.055	*	*	0.007	0.004
30	*	*	0.025	0.032	*	*	0.026	0.046	*	*	0.003	0.007
40	0.010	0.004	*	*	0.014	0.021	*	*	0.012	0.008	*	*

[a] Measured with GLC configuration 1 (see section II)

[b] Weight in grams

n.d. = Not detectable

* Not measured

Table 15. Mean concentrations ± s.e.m. (n=6) of C(+)P(-)- and C(-)P(-)-soman in anesthetized, atropinized and restrained guinea pigs after nose-only exposure to a concentration of 24 ± 2 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.4 LCt50.

Time (min)	[C(+)P(-)-soman] ± s.e.m. (n=6) (ng/ml blood)	[C(-)P(-)-soman] ± s.e.m. (n=6) (ng/ml blood)
0	n.d.	n.d.
0.5	n.d.	0.07 ± 0.03
1	n.d.	0.09 ± 0.02
2	n.d.	0.15 ± 0.06
4	0.009 ± 0.006	0.27 ± 0.05
6	0.10 ± 0.05	0.6 ± 0.2
8	0.2 ± 0.2	0.8 ± 0.2
10	0.2 ± 0.1	0.30 ± 0.09
12	0.06 ± 0.02	0.14 ± 0.03
14	0.09 ± 0.05	0.14 ± 0.05
16	0.05 ± 0.02	0.08 ± 0.02
20	0.03 ± 0.01	0.04 ± 0.02
25	0.013 ± 0.004	0.02 ± 0.01
30	0.014 ± 0.004	0.02 ± 0.01
40	0.012 ± 0.002	0.010 ± 0.003

n.d. = not detectable

Table 16. Toxicokinetic parameters[a] for C(±)P(±)-soman in anesthetized, atropinized, and restrained guinea pigs, after nose-only exposure to 0.4 LCt50 C(±)P(±)-soman for 8 min.

		C(+)P(-)-soman	C(-)P(-)-soman
A (ng/ml)		-34.7	-34.7
B (ng/ml)		1.74	24.8
C (ng/ml)		0.014	0.14
D (ng/ml)		34.7	34.7
a (min-1)		0.0014	0.0023
b (min-1)		0.28	0.45
c (min-1)		0.0054	0.0685
Terminal half-life (min)		(128)	10.1
Area under the curve (ng.min/ml)	0-8 min	1.2	2.5
	> 8 min	3.1	2.7
	total	4.3	5.2

[a] The inhalation results were fitted with a discontinuous function:
[nerve agent] = D + A*e^{-at} for the absorption phase (0-8 min), with a lag time of 4 min for C(+)P(-)- soman, and [nerve agent] = B*e^{-bt} + C*e^{-ct} for distribution and elimination (≥ 8 min)

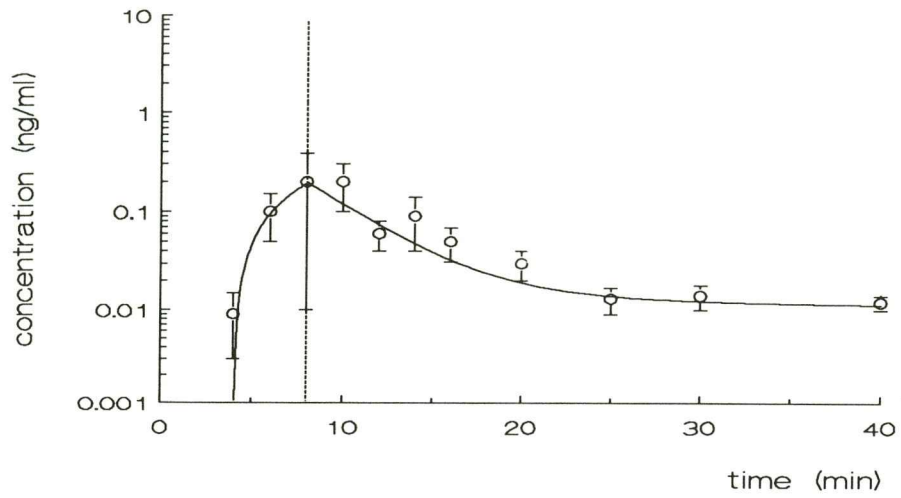

Figure 18. Semilogarithmic plot of the mean concentrations (± s.e.m., n=6) in blood of C(+)P(-)-soman versus time after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 24 ± 2 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.4 LCt50. The dotted line marks the end of the exposure period.

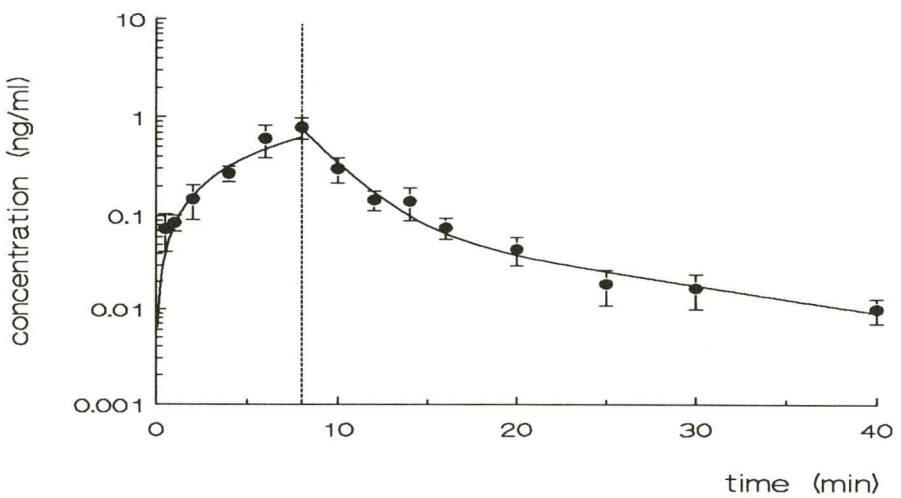

Figure 19. Semilogarithmic plot of the mean concentrations (± s.e.m., n=6) in blood of C(-)P(-)-soman versus time after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 24 ± 2 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.4 LCt50. The dotted line marks the end of the exposure period.

Table 17. Acetylcholinesterase (AChE) activities with s.e.m. (n=6), measured in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 24 ± 2 mg/m^3 C(±)P(±)-soman for 8 min, which corresponds with 0.4 LCt50.

Time (min)	AChE activity ± s.e.m. (%) (n=6)
0	100
0.5	95 ± 1
1	93 ± 4
2	78 ± 2
4	60 ± 4
6	38 ± 8
8	26 ± 5
10	17 ± 4
12	14 ± 2
14	11 ± 3
16	10 ± 2
20	9 ± 3
25	11 ± 2
30	10 ± 2
40	6 ± 3

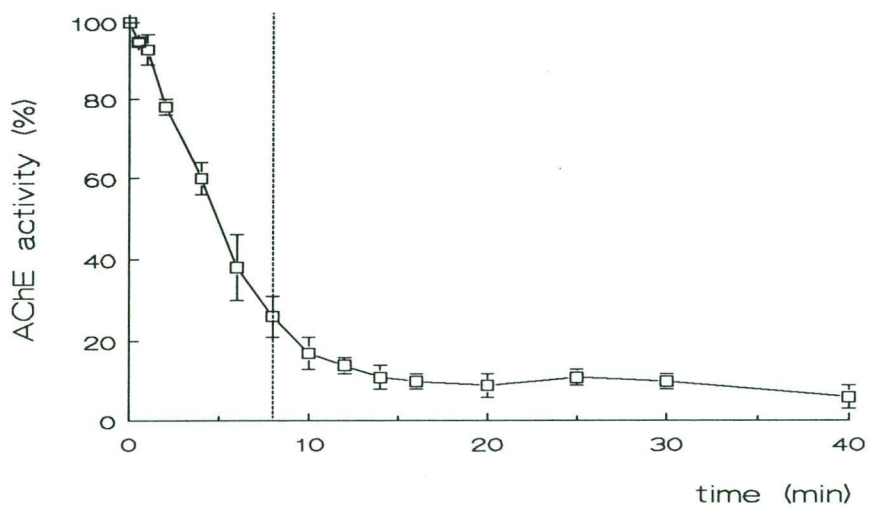

Figure 20. Time course of the acetylcholinesterase (AChE) activity ± s.e.m. (n=6) in blood samples of anesthetized, atropinized, and restrained guinea pigs nose-only exposed to 24 ± 2 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.4 LCt50. The dotted line marks the end of the exposure period.

III-8 THE TOXICOKINETICS OF 0.8 LCt50 C(±)P(±)-SOMAN IN ANESTHETIZED, ATROPINIZED AND RESTRAINED GUINEA PIGS AFTER NOSE-ONLY EXPOSURE FOR 4 MINUTES

Anesthetized, atropinized and restrained guinea pigs were nose-only exposed to a concentration of 96 ± 10 mg/m^3 of C(±)P(±)-soman vapor in air for 4 min, yielding an exposure to 0.8 LCt50. The blood samples were analyzed with GLC configuration 1.
Blood samples were taken just before and during exposure, and at various time-points after exposure. A small portion of the blood sample was used for the determination of AChE activity, the larger part was used for gas chromatographic analysis. During the exposure, the respiration of the animals was monitored.
In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used in order to obtain six values for each time-point.
Concentrations of P(+)-isomers were below detection limit in these experiments. The concentrations of C(+)P(-)-soman and C(-)P(-)-soman measured in the individual animals are presented in Table 18, whereas the mean concentrations of these isomers with s.e.m. are shown in Table 19 and Figures 21 and 22. These figures show that the absorption of the P(-)-isomers is very rapid. The concentration of the C(-)P(-)-isomer does not increase further after after terminating the exposure, whereas the concentration of the C(+)P(-)-isomer does. As a whole, the absorption phase of the C(+)P(-)-isomer lags somewhat behind that of the C(-)P(-)-isomer.
Mathematical equations describing the concentration-time courses of the P(-)-isomers were obtained by nonlinear regression. The kinetics were described as a discontinuous process, with an equation for the 4-min exposure period and an equation for the postexposure period. For the absorption phase of C(+)P(-)-soman a lag time of 2 min was arbitrarily chosen. The absorption phase of C(-)P(-)-soman was described with a mono-exponential function, since the limited number of datapoints in this phase does not justify a description with more than one exponential. For the C(+)P(-)-isomer, only two datapoints were available for the absorption phase. Consequently, mathematical description of this phase was not possible. The area-under-the-curve for 0-4 min was therefore calculated via trapezoidals. The post-exposure phase appears to be adequately described for both P(-)-isomers with a two-exponential function. By analogy with the absorption phase, the number of datapoints is insufficient for justification of a three-exponential equation. The toxicokinetic parameters are listed in Table 20.
The AChE activities measured during and after exposure are presented in Table 21 and Figure 23. During exposure and in the first few minutes thereafter, the blood AChE activity drops fairly rapidly down to ca. 5 % of control activity.
There were no C(±)P(±)-soman-related effects on the respiration during this 4-min nose-only exposure. The mean respiratory minute volume during exposure was 37 ± 5 ml (s.e.m., n=12), whereas the respiratory frequency was 0.80 ± 0.11 Hz (s.e.m., n=12).

Table 18. Concentrations in blood[a] (ng/ml) of C(+)P(-)- and C(-)P(-)-soman in individual anesthetized and atropinized guinea pigs at various time-points during and after nose-only exposure to 96 ± 10 mg/m^3 of C(±)P(±)-soman for 4 min, which corresponds with 0.8 LCt50.

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 1 (498)[b]		Guinea pig 2 (701)[b]		Guinea pig 3 (501)[b]		Guinea pig 4 (520)[b]		Guinea pig 5 (580)[b]		Guinea pig 6 (570)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d	0.048	*	*	n.d.	0.662	*	*	n.d.	0.039	*	*
1	*	*	n.d	0.095	*	*	n.d.	0.236	*	*	n.d.	0.174
2	n.d	0.398	*	*	n.d.	0.240	*	*	n.d.	0.115	*	*
3	*	*	n.d	0.590	n.d.	0.390	*	*	*	*	0.038	0.950
4	0.004	1.281	*	*	*	*	0.008	0.798	n.d.	0.265	*	*
6	*	*	0.108	0.770	*	*	0.181	0.568	*	*	0.398	0.478
8	0.086	0.360	*	*	0.026	0.223	*	*	0.010	0.050	*	*
10	*	*	0.073	0.186	0.016	0.116	*	*	*	*	0.232	0.150
12	0.048	0.160	*	*	*	*	0.086	0.264	0.172	0.022	*	*
15	*	*	0.042	0.089	*	*	0.118	0.172	*	*	0.102	0.064
20	0.026	0.092	*	*	*	*	0.043	0.086	0.282	0.009	*	*
30	*	*	0.020	0.037	0.007	0.014	*	*	*	*	0.041	0.019
40	*	*	0.011	0.017	*	*	0.008	0.017	*	*	0.012	0.012
60	0.010	0.018	*	*	0.032	0.008	*	*	0.028	0.006	*	*

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 7 (625)[b]		Guinea pig 8 (630)[b]		Guinea pig 9 (350)[b]		Guinea pig 10 (340)[b]		Guinea pig 11 (453)[b]		Guinea pig 12 (456)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	0.230	*	*	n.d.	0.206	*	*	n.d.	0.113	*	*
1	*	*	n.d.	0.162	*	*	n.d.	0.132	*	*	n.d.	0.387
2	n.d.	0.792	*	*	n.d.	0.450	*	*	n.d.	1.118	*	*
3	0.042	1.610	*	*	*	*	n.d.	0.810	0.281	2.012	*	*
4	*	*	0.012	1.205	0.083	1.288	*	*	*	*	2.170	2.930
6	*	*	0.266	0.852	*	*	1.212	1.405	*	*	0.880	1.062
8	0.245	0.382	*	*	0.216	0.320	*	*	0.312	0.380	*	*
10	0.251	0.340	*	*	*	*	0.200	0.321	0.212	0.317	*	*
12	*	*	0.042	0.144	0.136	0.283	*	*	*	*	0.075	0.207
15	*	*	0.017	0.078	*	*	0.090	0.220	*	*	0.104	0.144
20	*	*	0.106	0.094	0.034	0.049	*	*	*	*	0.048	0.081
30	0.015	0.026	*	*	*	*	0.016	0.025	0.032	0.038	*	*
40	*	*	0.008	0.006	*	*	0.009	0.056	*	*	0.014	0.027
60	0.006	0.014	*	*	0.008	0.007	*	*	0.004	0.012	*	*

[a] Measured with GLC configuration 1 (see section II)

[b] Weight in grams

n.d. = Not detectable

* = Not measured

Table 19. Mean concentrations ± s.e.m. (n=6) of C(+)P(-)- and C(-)P(-)-soman in anesthetized, atropinized and restrained guinea pigs after nose-only exposure to 96 ± 10 mg/m^3 of C(±)P(±)-soman for 4 min, which corresponds with 0.8 LCt50.

Time (min)	[C(+)P(-)-soman] ± s.e.m. (n=6) (ng/ml blood)	[C(-)P(-)-soman] ± s.e.m. (n=6) (ng/ml blood)
0	n.d.	n.d.
0.5	n.d.	0.2 ± 0.1
1	n.d.	0.20 ± 0.05
2	n.d.	0.5 ± 0.2
3	0.06 ± 0.04	1.1 ± 0.3
4	0.02 ± 0.01	1.0 ± 0.2
6	0.5 ± 0.2	0.9 ± 0.2
8	0.15 ± 0.05	0.29 ± 0.06
10	0.16 ± 0.04	0.24 ± 0.04
12	0.09 ± 0.02	0.18 ± 0.04
15	0.08 ± 0.02	0.13 ± 0.01
20	0.09 ± 0.04	0.07 ± 0.02
30	0.022 ± 0.005	0.026 ± 0.004
40	0.010 ± 0.001	0.022 ± 0.008
60	0.015 ± 0.005	0.011 ± 0.002

n.d. = not detectable

Table 20. Toxicokinetic parameters[a] for C(±)P(±)-soman in anesthetized, atropinized, and restrained guinea pigs, after nose-only exposure to 0.8 LCt50 C(±)P(±)-soman for 4 min.

		C(+)P(-)-soman	C(-)P(-)-soman
A (ng/ml)		-	-30.0
B (ng/ml)		0.72	1.70
C (ng/ml)		0.029	0.052
D (ng/ml)		-	30.0
a (min-1)		-	0.0088
b (min-1)		0.19	0.20
c (min-1)		0.011	0.026
Terminal half-life (min)		63	27
Area under the curve (ng.min/ml)	0-4 min	0.6[b]	2.1
	> 4 min	3.7	5.6
	total	4.3	7.7

[a] The inhalation results were fitted with a discontinuous function:
[nerve agent] = D + A*e^{-at} for the absorption phase (0-4 min), and [nerve agent] = B*e^{-bt} + C*e^{-ct} for distribution and elimination (≥ 4 min)

[b] Calculated via trapezoidals

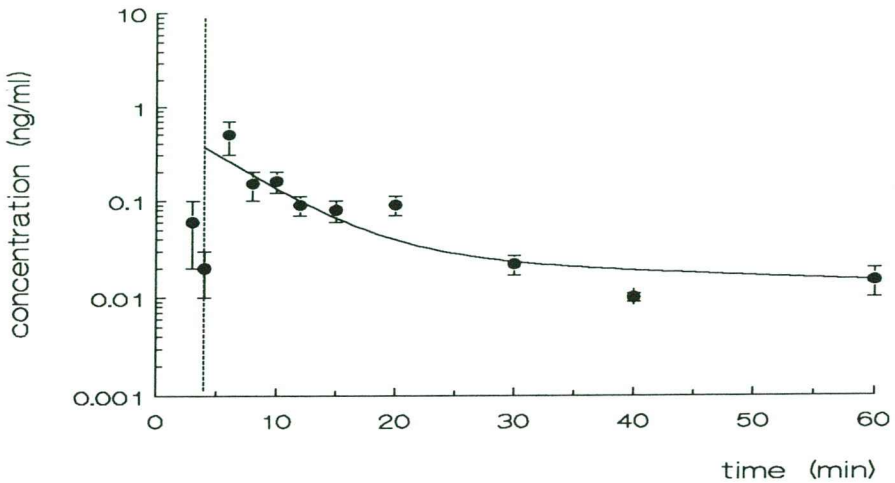

Figure 21. Semilogarithmic plot of the concentrations (± s.e.m., n=6) in blood of C(+)P(-)-soman versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 96 ± 10 mg/m^3 of C(±)P(±)-soman for 4 min, which corresponds with 0.8 LCt50. The dotted line marks the end of the exposure period.

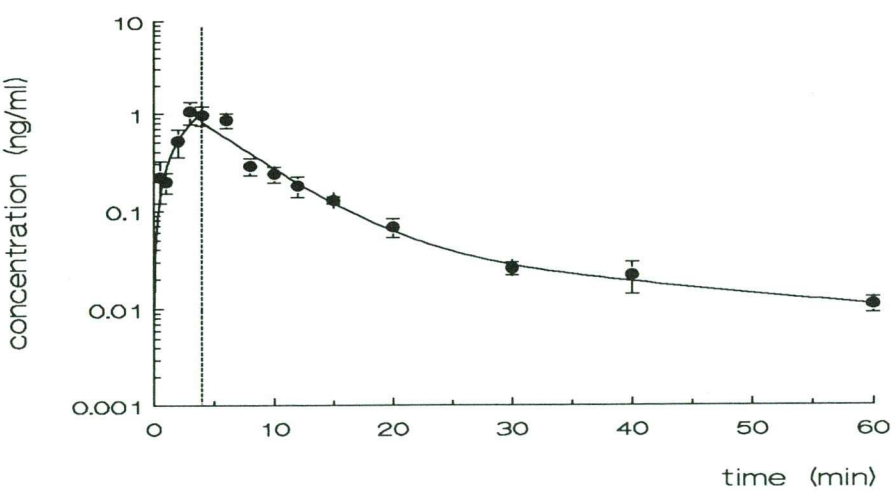

Figure 22. Semilogarithmic plot of the concentrations (± s.e.m., n=6) in blood of C(-)P(-)-soman versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 96 ± 10 mg/m^3 of C(±)P(±)-soman for 4 min, which corresponds with 0.8 LCt50. The dotted line marks the end of the exposure period.

Table 21. Acetylcholinesterase (AChE) activities with s.e.m. (n=6), measured in blood samples of anesthetized, atropinized and restrained guinea pigs exposed to 96 ± 10 mg/m^3 of C(±)P(±)-soman for 4 min, which corresponds with 0.8 LCt50.

Time (min)	AChE activity ± s.e.m. (%)
0	100
0.5	92 ± 2
1	85 ± 3
2	66 ± 6
3	35 ± 7
4	19 ± 6
6	10 ± 2
8	10 ± 4
10	7 ± 1
12	9 ± 3
15	6 ± 1
20	8 ± 3
30	5 ± 1
40	3 ± 1
60	6 ± 2

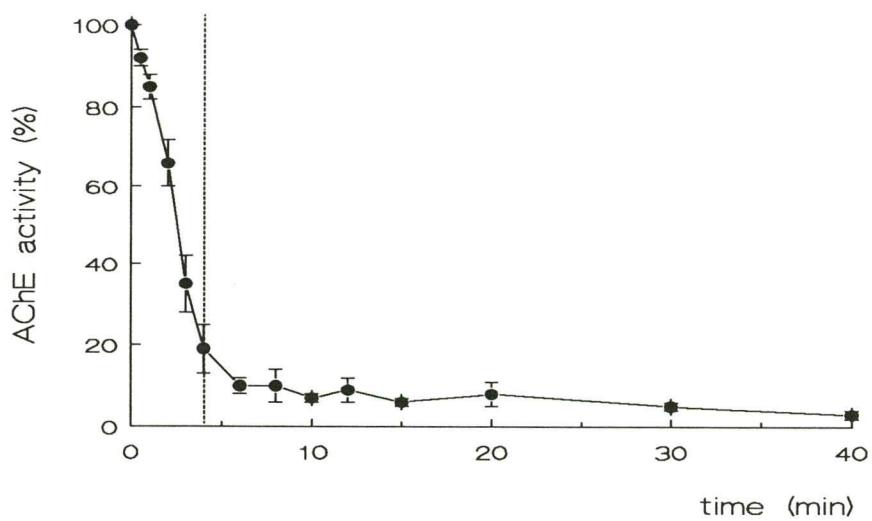

Figure 23. Time course of the acetylcholinesterase (AChE) activity ± s.e.m. (n=6) in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 96 ± 10 mg/m^3 of C(±)P(±)-soman for 4 min, which corresponds with 0.8 LCt50. The dotted line marks the end of the exposure period.

III-9 THE TOXICOKINETICS OF 0.1 LCt50 C(±)P(±)-SOMAN IN ANESTHETIZED, ATROPINIZED AND RESTRAINED GUINEA PIGS AFTER NOSE-ONLY EXPOSURE FOR 300 MINUTES

In this experiment, anesthetized, atropinized guinea pigs were exposed for 300 min to a concentration of 160 ± 16 $\mu g/m^3$ of C(±)P(±)-soman in air, yielding 0.1 LCt50. Soman stereoisomers were determined in blood samples, taken at 30-min intervals during exposure and at some time-points after terminating the exposure. Activities of acetylcholinesterase were measured in these blood samples, as well as in diaphragm and brain of the exposed animals, after sacrificing the animals at the end of the experiment. Furthermore, total cholinesterase and carboxylesterase activities were measured in the blood samples.
Some difficulties with respect to the stable generation of this low concentration of nerve agent vapor for such a long period were anticipated, presumably requiring modification of the generation vial, introduction of an additional mixing chamber for dilution and a larger sample loop for GLC configuration 3. In view of the possibility that no detectable blood levels of soman stereoisomers would be measured at any time during the exposure, a pilot experiment was performed first, with a 10-fold higher concentration of C(±)P(±)-soman, i.e., yielding 1.0 LCt50. Both P(-)-isomers were detectable from 2 h after the start of the exposure up to 5 h. The results are shown in Table 22 and Figure 24. The low concentrations of C(+)P(-)- and C(-)P(-)-soman at t=30 min in this single experiment are probably artifacts. The measured AChE activities are presented in Table 23 and Figure 25.

Table 22. Concentrations (n=1) of C(+)P(-)- and C(-)P(-)-soman in anesthetized, atropinized and restrained guinea pigs during exposure to a concentration of 1.60 ± 0.16 mg/m^3 of C(±)P(±)-soman for 300 min, which corresponds with 1.0 LCt50 (pilot experiment).

Time (min)	[C(+)P(-)-soman] n=1 (ng/ml blood)	[C(-)P(-)-soman] n=1 (ng/ml blood)
0	n.d.	n.d.
30	0.002	0.006
60	n.d.	n.d.
90	n.d.	n.d.
120	0.005	0.012
150	0.004	0.007
180	0.009	0.029
210	0.008	0.028
240	0.014	0.079
270	0.010	0.068
300	0.047	0.066

n.d. = not detectable

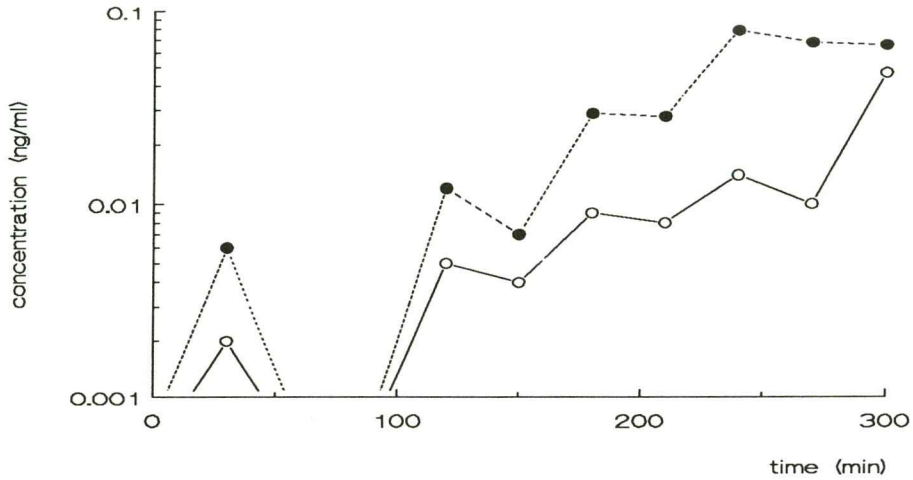

Figure 24. Semilogarithmic plot of the concentrations in blood (n=1) of C(-)P(-)-soman (●) and C(+)P(-)-soman (O) versus time during nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 1.60 ± 0.16 mg/m^3 of C(±)P(±)-soman for 300 min, which corresponds with 1.0 LCt50 (pilot experiment).

Table 23. Acetylcholinesterase (AChE) activities (n=1), measured in blood samples of anesthetized, atropinized and restrained guinea pigs during nose-only exposure to 1.60 ± 0.16 mg/m^3 C(±)P(±)-soman for 300 min, which corresponds with 1.0 LCt50 (pilot experiment).

Time (min)	AChE activity (%)
0	100
30	75.5
60	50.0
90	54.1
120	19.0
150	23.2
180	5.9
210	9.2
240	2.7
270	3.9

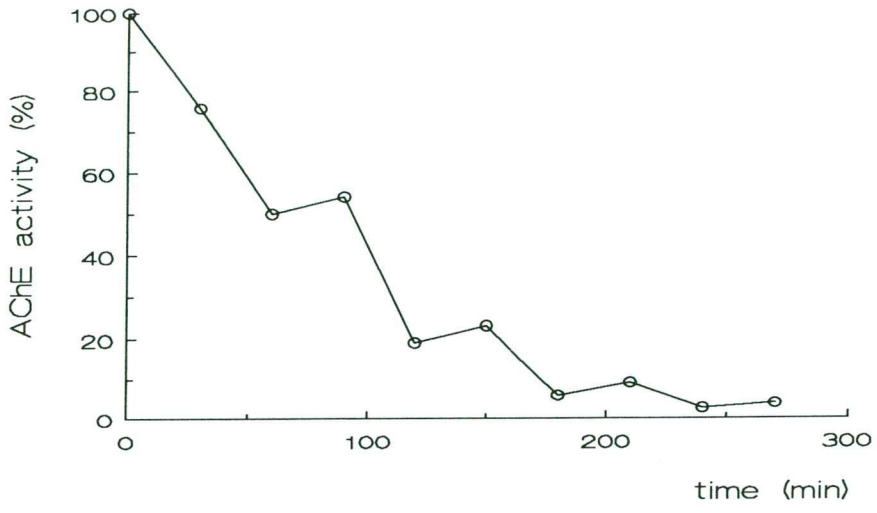

Figure 25. Time course of the acetylcholinesterase (AChE) activity (n=1) in blood samples of anesthetized, atropinized, and restrained guinea pigs during nose-only exposure to 1.60 ± 0.16 mg/m^3 of C(±)P(±)-soman for 300 min, which corresponds with 1.0 LCt50 (pilot experiment).

On the basis of these results, it seemed worthwhile to study the toxicokinetics of C(±)P(±)-soman in guinea pigs, as proposed, at 0.1 LCt50. In this study the LCt50 value was taken as the value determined for 8-min exposure.
In order to provide for a reliable measurement of the concentration of C(±)P(±)-soman vapor in air, the sample size had to be increased. A 1-ml injector loop was constructed and installed in the gas-sampling valve of the gas chromatograph of the generation apparatus. Since the internal volume of the valve is 8.8 µl, the total sampling volume is now 1008.8 µl. The generation vial was placed in melting ice, in order to decrease the evaporation of the nerve agent. With this set-up we were able to generate a stable concentration of C(±)P(±)-soman vapor in air of 160 ± 16 µg/m^3, without the need for an additional dilution chamber.
Anesthetized, atropinized and restrained guinea pigs were exposed for 300 min to a concentration of 160 ± 16 µg/m^3 of C(±)P(±)-soman in air, yielding 0.1 LCt50. In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used to obtain six values for each time point. The results obtained for the individual animals are presented in Table 24. The mean concentrations with s.e.m. of C(+)P(-)- and C(-)P(-)-soman are presented in Table 25 and Figure 26. Obviously, the absorption phase of C(+)P(-)-soman lags behind that of the C(-)P(-)-isomer, which was also observed for short-term exposures. The calculated toxicokinetic parameters are listed in Table 26, assuming a lag time of 30 min for C(+)P(-)-soman.

Table 24. Concentrations in blood[a] (ng/ml) of C(+)P(-)- and C(-)P(-)-soman in individual anesthetized, atropinized, and restrained guinea pigs at various time-points during and after nose-only exposure to 160 ± 16 µg/m^3 of C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50.

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 1 (514)[b]		Guinea pig 2 (520)[b]		Guinea pig 3 (484)[b]		Guinea pig 4 (433)[b]		Guinea pig 5 (454)[b]		Guinea pig 6 (454)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30	n.d.	n.d.	*	*	n.d.	n.d.	*	*	n.d.	n.d.	*	*
60	*	*	0.001	0.002	*	*	n.d.	n.d.	*	*	0.001	0.002
90	0.002	0.002	*	*	n.d.	n.d.	*	*	n.d.	n.d.	*	*
120	*	*	n.d.	n.d.	*	*	n.d.	n.d.	*	*	0.001	0.004
150	0.002	0.012	*	*	0.002	0.002	*	*	0.006	0.006	*	*
180	*	*	0.006	0.028	*	*	0.001	0.003	*	*	0.003	0.012
210	0.042	0.048	*	*	0.006	0.021	*	*	0.002	0.012	*	*
240	*	*	*	*	*	*	0.018	0.026	*	*	0.009	0.038
270	0.019	0.034	*	*	0.025	0.040	*	*	0.006	0.017	*	*
300	*	*	0.018	0.031	*	*	0.006	0.024	*	*	0.010	0.037
330	0.012	0.006	*	*	0.005	0.003	*	*	0.004	0.007	*	*
360	*	*	0.006	0.011	*	*	0.004	0.015	*	*	0.008	0.022
390	0.002	0.002	*	*	0.006	0.012	*	*	0.005	0.012	*	*
420	*	*	0.007	0.016	*	*	0.006	0.020	*	*	0.014	0.044

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 7 (498)[b]		Guinea pig 8 (480)[b]		Guinea pig 9 (544)[b]		Guinea pig 10 (584)[b]		Guinea pig 11 (624)[b]		Guinea pig 12 (604)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30	n.d.	0.002	*	*	n.d.	0.002	*	*	n.d.	0.004	*	*
60	*	*	n.d.	n.d.	*	*	0.001	0.004	*	*	n.d.	0.002
90	0.002	0.003	*	*	0.007	0.008	*	*	0.002	0.005	*	*
120	*	*	n.d.	n.d.	*	*	0.006	0.014	*	*	0.010	0.004
150	0.004	0.012	*	*	0.008	0.020	*	*	n.d.	0.004	*	*
180	*	*	0.004	0.008	*	*	0.006	0.022	0.002	0.006	*	*
210	0.006	0.020	*	*	0.010	0.018	*	*	0.004	0.015	*	*
240	0.005	0.019	0.004	0.025	*	*	0.004	0.010	*	*	0.005	0.014
270	0.009	0.032	*	*	0.014	0.012	*	*	0.009	0.050	*	*
300	*	*	0.009	0.006	*	*	0.008	0.052	*	*	0.021	0.034
330	*	*	*	*	0.005	0.008	0.002	0.005	0.008	0.027	*	*
360	*	*	0.004	0.018	*	*	0.012	0.012	*	*	0.003	0.003
390	0.006	0.006	*	*	0.008	0.031	*	*	0.006	0.039	*	*
420	*	*	0.004	n.d.	*	*	0.006	0.003	*	*	0.005	0.016

[a] Measured with GLC configuration 1 (see section II)

[b] Weight in grams

n.d. = Not detectable

* Not measured

Table 25. Concentrations of C(+)P(-)- and C(-)P(-)-soman (± s.e.m., n=6) in anesthetized, atropinized and restrained guinea pigs during and after nose-only exposure to a concentration of 160 ± 16 $\mu g/m^3$ of C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50.

Time (min)	[C(+)P(-)-soman] ± s.e.m., n=6 (ng/ml blood)	[C(-)P(-)-soman] ± s.e.m., n=6 (ng/ml blood)
0	n.d.	n.d.
30	n.d.	0.001 ± 0.001
60	0.001 ± 0.001	0.002 ± 0.001
90	0.002 ± 0.001	0.003 ± 0.001
120	0.003 ± 0.002	0.004 ± 0.002
150	0.004 ± 0.001	0.009 ± 0.003
180	0.004 ± 0.001	0.013 ± 0.004
210	0.012 ± 0.006	0.022 ± 0.005
240	0.006 ± 0.001	0.022 ± 0.004
270	0.014 ± 0.003	0.031 ± 0.006
300	0.012 ± 0.003	0.036 ± 0.004
330	0.006 ± 0.001	0.009 ± 0.004
360	0.006 ± 0.001	0.014 ± 0.003
390	0.006 ± 0.001	0.017 ± 0.006
420	0.007 ± 0.002	0.016 ± 0.006

n.d. = not detectable

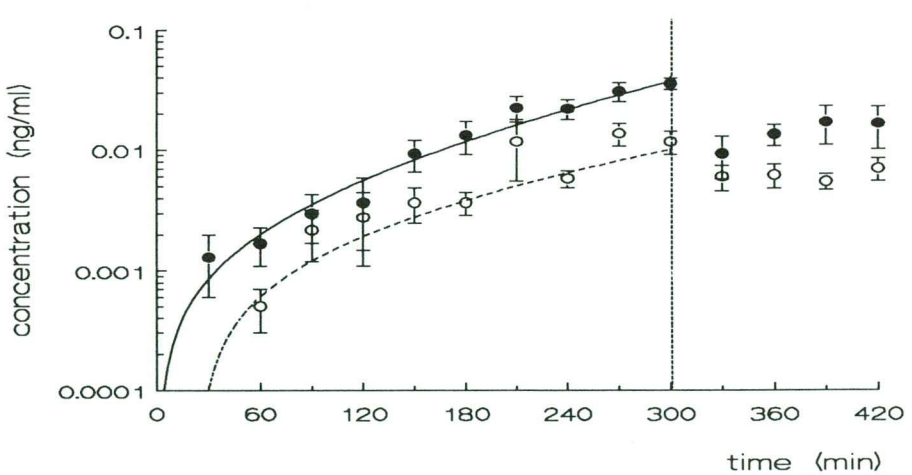

Figure 26. Semilogarithmic plot of the mean concentrations (± s.e.m., n=6) in blood of C(-)P(-)-soman (●) and C(+)P(-)-soman (○) versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 160 ± 16 $\mu g/m^3$ of C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50. The dotted line marks the end of the exposure period.

Table 26. Toxicokinetic parameters[a] for C(±)P(±)-soman in anesthetized, atropinized, and restrained guinea pigs, after nose-only exposure to 0.1 LCt50 C(±)P(±)-soman for 300 min.

	C(+)P(-)-soman	C(-)P(-)-soman
A (ng/ml)	0.0028	-1.54
B (ng/ml)	-	-
C (ng/ml)	-	-
D (ng/ml)	-0.0027	1.54
a (min-1)	-0.0058	0.000065
b (min-1)	-	-
c (min-1)	-	-
Terminal half-life (min)	-	-
Area under the 0-300 min	1.4	4.5
curve (ng.min/ml) > 300 min	-	-

[a] The inhalation results were fitted according to: [nerve agent] = D + A*e^{-at} for the absorption phase (0-300 min), with a lag time of 60 min for C(+)P(-)-soman

Curve-fitting attempts for the post-exposure toxicokinetics led to unsatisfactory fits, due to the small number of datapoints, and the slow decrease of the concentrations in this period.

The measured AChE activities are presented in Table 27 and Figure 27. The AChE activity is gradually inhibited during exposure, down to ca. 10 % of control activity. Additional AChE inhibition appears not to occur in the post-exposure period.
The carboxylesterase (CaE) activity was also measured in the blood samples taken during and after nose-only exposure. The results are presented in Table 28 and Figure 28. The residual CaE activity is ca. 75 %, which seems reasonable when compared with the observed residual AChE activity, taking into account the considerably higher concentration of CaE in guinea pig blood in comparison with AChE, and in view of the lower inhibition rate constant of soman for CaE than for AChE. The time-course of blood CaE activity looks peculiar with its oscillations. Since the samples were measured randomly over several days, each day with calibration samples, a systematic analytical error seems unlikely. It looks as if two series of samples are present. In fact, the samples taken at 1, 2, 3, 4, 5, 6 and 7 h are taken from one group of six animals, whereas the other samples are taken from another group. Variations in CaE levels between groups of animal may occur, but is unlikely in this case since the animals were used in the order as listed in Table 24, and therefore belong alternately to each of the datapoint groups. Therefore, a plausible explanation for this phenomenon is not yet at hand.

Table 27. Mean acetylcholinesterase (AChE) activity (± s.e.m., n=6), measured in blood samples of anesthetized, atropinized and restrained guinea pigs during and after nose-only exposure to 160 ± 16 $\mu g/m^3$ C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50.

Time (min)	AChE activity ± s.e.m. (n=6) (%)
0	100
30	97 ± 2
60	91 ± 2
90	80 ± 4
120	75 ± 4
150	60 ± 5
180	50 ± 7
210	30 ± 3
240	26 ± 4
270	15 ± 3
300	11 ± 2
330	10 ± 1
360	10 ± 2
390	12 ± 2
420	9 ± 2

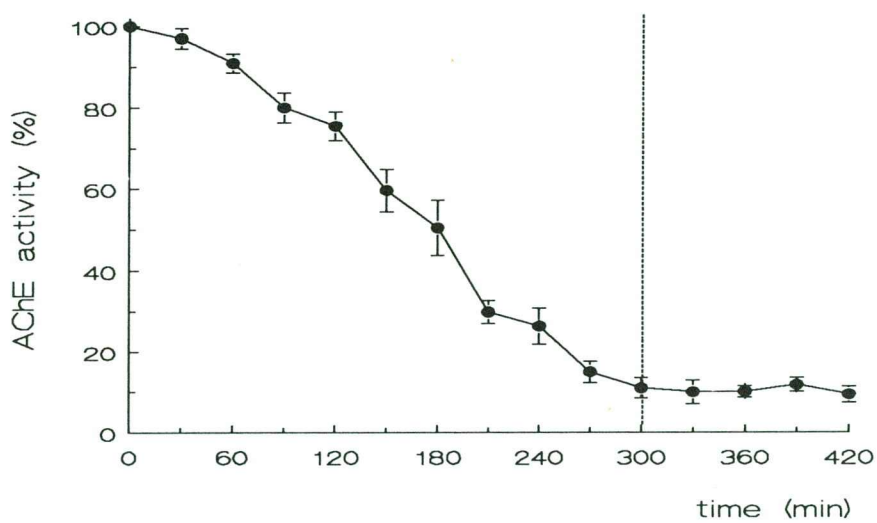

Figure 27. Mean acetylcholinesterase (AChE) activity (± s.e.m., n=6) in blood versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 160 ± 16 $\mu g/m^3$ of C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50. The dotted line marks the end of the exposure period.

Table 28. Mean carboxylesterase (CaE) activity (± s.e.m., n=6), measured in blood samples of anesthetized, atropinized and restrained guinea pigs during and after nose-only exposure to 160 ± 16 $\mu g/m^3$ C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50.

Time (min)	CaE activity ± s.e.m. (n=6) (%)
0	100
30	86 ± 6
60	98 ± 4
90	88 ± 4
120	99 ± 5
150	81 ± 4
180	94 ± 4
210	75 ± 2
240	86 ± 2
270	72 ± 3
300	85 ± 3
330	71 ± 4
360	77 ± 2
390	76 ± 7
420	76 ± 1

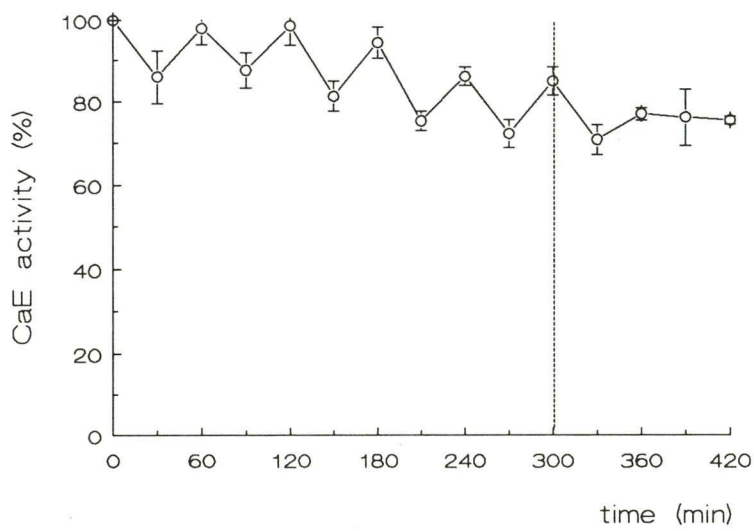

Figure 28. Mean carboxylesterase (CaE) activity (± s.e.m., n=6) in blood versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 160 ± 16 $\mu g/m^3$ of C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50. The dotted line marks the end of the exposure period.

At the end of the experiment the brain and diaphragm of the exposed animals were collected, and the AChE activity was determined. The results are presented in Table 29. Surprisingly, no significant inhibition of AChE in these tissues is observed, although blood AChE is inhibited for 90 %.

Table 29. Acetylcholinesterase (AChE) activity measured in brain and diaphragm of anesthetized, atropinized and restrained guinea pigs 120 min after nose-only exposure to 160 ± 16 $\mu g/m^3$ C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50.

Guinea pig	AChE activity (U/g tissue)	
	Brain	Diaphragm
1	15.0	1.90
2	16.1	2.15
3	12.2	2.30
4	11.7	3.05
5	10.6	1.80
6	13.8	2.70
7	10.0	1.75
8	12.8	1.90
9	18.1	1.80
10	13.6	1.70
11	16.8	2.70
12	14.4	2.40
Mean ± s.d.	13.8 ± 2.5	2.2 ± 0.4
Control values	10.7 ± 0.8 (n=10)	1.9 ± 0.3 (n=2)

In this long-term exposure experiment, there were no C(±)P(±)-soman-related effects on the respiration. A slight gradual increase in RMV and RF was observed in most animals during the experiment, which must be attributed to the slow recovery from anesthesia. Two animals had to receive an additional dose of ketamine because of restless behaviour and stress-induced irregular breathing.

III-10 THE LD50 OF C(±)P(±)-SOMAN IN ANESTHETIZED GUINEA PIGS FOR 8-MINUTE INTRAVENOUS INFUSION

Groups of eight anesthetized guinea pigs were treated with a muscle relaxant (Vetranquil^{R}, 0.2 ml) in order to facilitate the insertion of an indwelling cannula into the jugular vein. A standard solution of C(±)P(±)-soman in 2-propanol was diluted with sterile saline just before use. The aqueous solution was administered as an intravenous infusion using a microinjection pump in an 8-min time period. Following infusion, the indwelling cannula was removed and the skin incision was closed with a few stitches. Each animal was placed in a separate cage in the conditioned animal facility. At 24 h after the infusion, the number of dead animals per dosing group was counted. The results are presented in Table 30.

Table 30. Number and percentage of dead animals per dosing group, 24 h after 8-min intravenous infusion of C(±)P(±)-soman in anesthetized guinea pigs.

Dose C(±)P(±)-soman (μg/kg)	Number of dead animals at 24 h/total number	Percentage deaths
17.5	1/8	12.5
20.0	4/8	50
22.5	6/8	75
25.0	8/8	100

Using probit analysis of the mortality data, the LD50 of C(±)P(±)-soman for 8-min intravenous infusion was calculated to be 20 μg/kg (95 % confidence limits 18-22 μg/kg). The results of the probit analysis are presented in Table 31 and Figure 29.

Table 31. LD10, LD30, LD50, LD70 and LD90 (24 h) with 95 % confidence limits, for 8-min intravenous infusion of C(±)P(±)-soman in anesthetized guinea pigs, calculated via probit analysis.[a]

LD	μg/kg	95 % confidence limits (μg/kg)
10	17	14-18
30	19	16-20
50	20	18-22
70	21	20-24
90	23	22-29

[a] Probit equation:
probit= 19.4*log(dose C(±)P(±)-soman) - 20.3

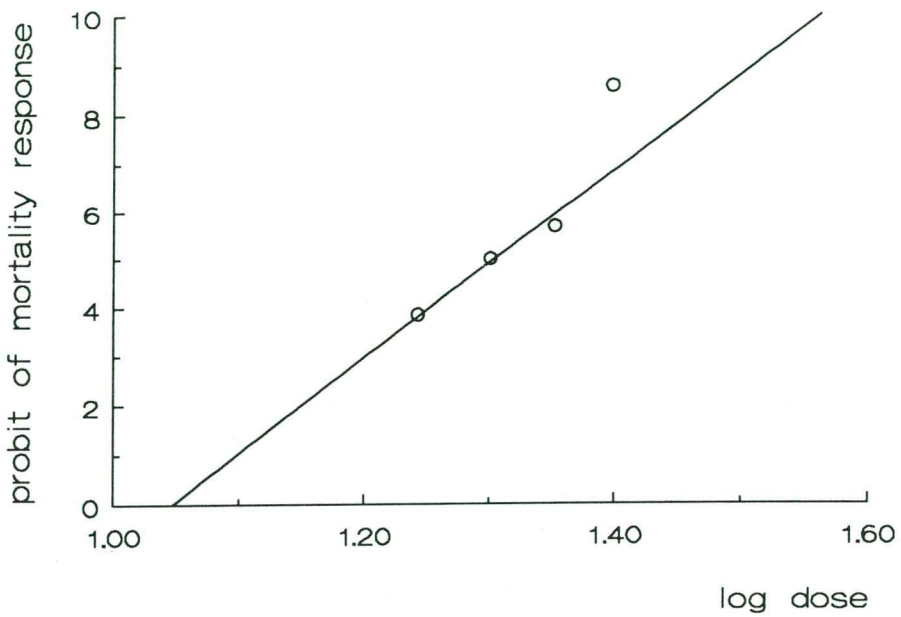

Figure 29. Relationship between the dose of C(±)P(±)-soman administered to anesthetized guinea pigs as an 8-min intravenous infusion and the probit of mortality.

III-11 TOXICOKINETICS OF 0.8 LD50 OF C(±)P(±)-SOMAN, ADMINISTERED AS AN 8-MINUTE INTRAVENOUS INFUSION TO ANESTHETIZED AND ATROPINIZED GUINEA PIGS

Ketamine anesthetized animals were treated with a muscle relaxant drug (Vetranquil^{R}; 0.2 ml, i.m.), in order to facilitate the insertion of an indwelling cannula into the jugular vein. A diluted solution of C(±)P(±)-soman in saline (16.2 μg/ml/kg) was administered at a constant infusion rate in an 8-min time period, by means of a microinjection pump. Blood samples were taken via a carotid cannula just before administration, at 0.5, 1, 2, 4, and 8 min after starting the infusion and at several time-points after infusion. A small portion of the blood sample was used for the determination of AChE activity, the larger part was used for gas chromatographic analysis. In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used in order to obtain six values for each time-point.
The concentrations of the soman stereoisomers were measured with GLC configuration 1. Traces of C(-)P(+)-soman were observed in some samples taken during the infusion. The concentrations of C(±)P(-)-soman measured in the individual animals are presented in Table 32, whereas the mean concentrations with s.e.m. are shown in Table 33 and Figures 30 and 31. The absorption phase of the C(+)P(-)-isomer clearly lags behind that of the C(-)P(-)-isomer, which was also observed after respiratory exposure (see Sections III-6 to III-9) and subcutaneous administration (34), even though the C(+)P(-)-isomer is present in a 23 % excess in synthetic C(±)P(±)-soman (1).
Mathematical equations describing the concentration-time courses of the C(±)P(-)-isomers were obtained by nonlinear regression. The kinetics were described as a discontinuous process, with an equation for the 8-min infusion period and an equation for the postinfusion period. For the absorption phase of C(+)P(-)-soman, a lag time of 2 min was chosen. The absorption phase was described with a mono-exponential function, since the limited number of datapoints in this phase does not justify a description with more than one exponent. The postinfusion phase appears to be adequately described with a two-exponential function. By analogy with the absorption phase, the number of datapoints is insufficient for justification of a three-exponential equation. The calculated toxicokinetic parameters are listed in Table 34.
The values of the acetylcholinesterase activities measured in the blood samples are presented in Table 35 and Figure 32. The residual AChE activity appears to be ca. 5 %, which is the same as for 8-min respiratory exposure to 0.8 LCt50 (see Section III-6).

Table 32. Concentrations in blood[a] (ng/ml) of C(+)P(-)- and C(-)P(-)-soman in individual anesthetized and atropinized guinea pigs at various time-points during and after 8-min i.v. infusion of 0.8 LD50 (16.2 µg/kg) of C(±)P(±)-soman.

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 1 (419)[b]		Guinea pig 2 (453)[b]		Guinea pig 3 (425)[b]		Guinea pig 4 (328)[b]		Guinea pig 5 (540)[b]		Guinea pig 6 (510)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	0.749	*	*	n.d.	0.388	*	*	n.d.	0.131	*	*
1	*	*	n.d.	0.554	*	*	n.d.	0.344	*	*	0.026	0.197
2	*	*	0.008	0.884	n.d.	0.757	*	*	*	*	0.042	0.573
4	0.019	1.747	*	*	*	*	0.146	1.239	0.355	1.266	*	*
8	2.906	3.552	*	*	0.758	1.101	*	*	0.153	1.960	*	*
10	*	*	0.08	0.279	*	*	0.155	0.208	*	*	0.297	0.632
12	*	*	0.092	0.215	0.352	0.218	*	*	*	*	0.143	0.074
14	0.106	0.222	*	*	*	*	0.334	0.158	0.071	0.166	*	*
16	0.088	0.138	*	*	0.070	0.076	*	*	0.064	0.128	*	*
20	*	*	0.148	0.053	*	*	0.037	0.068	*	*	0.042	0.045
25	*	*	0.020	0.030	0.031	0.022	*	*	*	*	0.016	n.d.
30	*	*	0.019	0.032	*	*	0.046	0.070	*	*	0.018	0.020
40	0.018	0.008	*	*	0.012	0.002	*	*	0.021	0.004	*	*

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 7 (478)[b]		Guinea pig 8 (478)[b]		Guinea pig 9 (505)[b]		Guinea pig 10 (530)[b]		Guinea pig 11 (439)[b]		Guinea pig 12 (438)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	0.879	*	*	n.d.	0.399	*	*	n.d.	0.014	*	*
1	*	*	n.d.	0.846	*	*	n.d.	0.490	*	*	n.d.	0.500
2	0.014	1.058	*	*	*	*	0.002	0.680	*	*	0.002	0.818
4	*	*	0.003	0.928	0.017	1.647	*	*	0.017	1.139	*	*
8	2.911	4.201	*	*	1.374	1.957	*	*	1.374	2.402	*	*
10	*	*	0.088	0.396	*	*	0.115	0.610	*	*	0.115	0.332
12	0.124	0.217	*	*	*	*	0.086	0.542	*	*	0.086	0.252
14	*	*	0.116	0.100	0.092	0.095	*	*	0.092	0.194	*	*
16	0.061	0.085	*	*	0.063	0.060	*	*	0.063	0.131	*	*
20	*	*	0.058	0.028	*	*	0.018	0.044	*	*	0.018	0.048
25	0.106	0.039	*	*	*	*	0.016	0.027	*	*	0.016	0.026
30	*	*	0.032	0.010	*	*	0.020	0.012	*	*	0.020	0.017
40	0.036	0.004	*	*	0.010	0.009	*	*	0.001	0.010	*	*

[a] Measured with GLC configuration 1 (see section II)

[b] Weight in grams

n.d. = Not detectable

* Not measured

Table 33. Mean concentrations ± s.e.m. (n=6) of C(+)P(-)- and C(-)P(-)-soman in anesthetized and atropinized guinea pigs during and after an intravenous infusion of 0.8 LD50 (16.2 μg/kg) of C(±)P(±)-soman in an 8-min time period.

Time (min)	[C(+)P(-)-soman] ± s.e.m. (n=6) (ng/ml blood)	[C(-)P(-)-soman] ± s.e.m. (n=6) (ng/ml blood)
0	n.d.	n.d.
0.5	n.d	0.4 ± 0.1
1	n.d	0.49 ± 0.09
2	0.019 ± 0.009	0.80 ± 0.07
4	0.10 ± 0.066	1.3 ± 0.1
8	1.4 ± 0.5	2.5 ± 0.5
10	0.16 ± 0.04	0.41 ± 0.07
12	0.17 ± 0.04	0.25 ± 0.06
14	0.13 ± 0.04	0.2 ± 0.2
16	0.070 ± 0.004	0.10 ± 0.01
20	0.06 ± 0.02	0.048 ± 0.005
25	0.06 ± 0.02	0.024 ± 0.005
30	0.029 ± 0.005	0.027 ± 0.009
40	0.023 ± 0.005	0.006 ± 0.001

n.d. = not detectable

Table 34. Toxicokinetic parameters[a] of C(+)P(-)- and C(-)P(-)-soman in anesthetized and atropinized guinea pigs, after intravenous infusion of 0.8 LD50 (16.2 μg/kg) of C(±)P(±)-soman in an 8-min time period.

		C(+)P(-)-soman	C(-)P(-)-soman
A (ng/ml)		16.1	35.8
B (ng/ml)		123694	12343
C (ng/ml)		0.18	0.72
D (ng/ml)		-16.1	-35.8
a (min-1)		-0.013	-0.0037
b (min-1)		1.44	1.08
c (min-1)		0.056	0.13
Terminal half-life (min)		12.4	5.3
Area under the curve (ng.min/ml)	0-8 min	6.7	11.8
	> 8 min	2.9	4.0
	total	9.6	15.8

[a] The infusion results were fitted with a discontinuous function:
[nerve agent] = $D + A*e^{-at}$ for the absorption phase (0-8 min), with a lag time of 2 min for C(+)P(-)-soman, and [nerve agent] = $B*e^{-bt} + C*e^{-ct}$ for distribution and elimination (≥ 8 min)

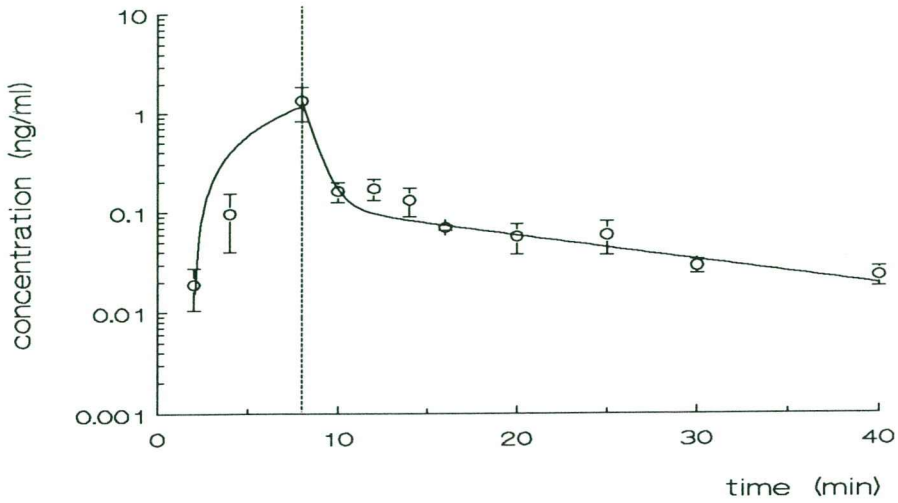

Figure 30. Semilogarithmic plot of the mean concentrations ± s.e.m. (n=6) in blood of C(+)P(-)-soman versus time during and after an 8-min intravenous infusion of 0.8 LD50 C(±)P(±)-soman (16.2 μg/kg) in anesthetized and atropinized guinea pigs. The dotted line marks the end of the infusion period.

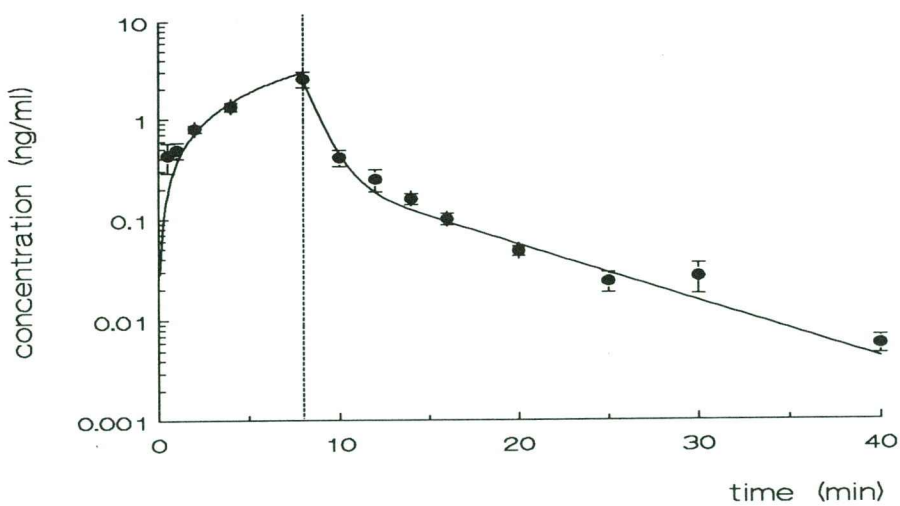

Figure 31. Semilogarithmic plot of the mean concentrations ± s.e.m. (n=6) in blood of C(-)P(-)-soman versus time during and after an 8-min intravenous infusion of 0.8 LD50 C(±)P(±)-soman (16.2 μg/kg) in anesthetized and atropinized guinea pigs. The dotted line marks the end of the infusion period.

Table 35. Mean acetylcholinesterase (AChE) activities with s.e.m. (n=6) measured in blood samples of anesthetized and atropinized guinea pigs during and after intravenous infusion of 0.8 LD50 (16.2 µg/kg) C(±)P(±)-soman in an 8-min time period.

Time (min)	AChE activity ± s.e.m. (%) (n=6)
0	100
0.5	93 ± 4
1	81 ± 2
2	66 ± 4
4	38 ± 5
8	8 ± 2
10	8 ± 2
12	8 ± 2
14	6 ± 1
16	6 ± 1
20	6 ± 1
25	6 ± 1
30	4 ± 1
40	5 ± 1

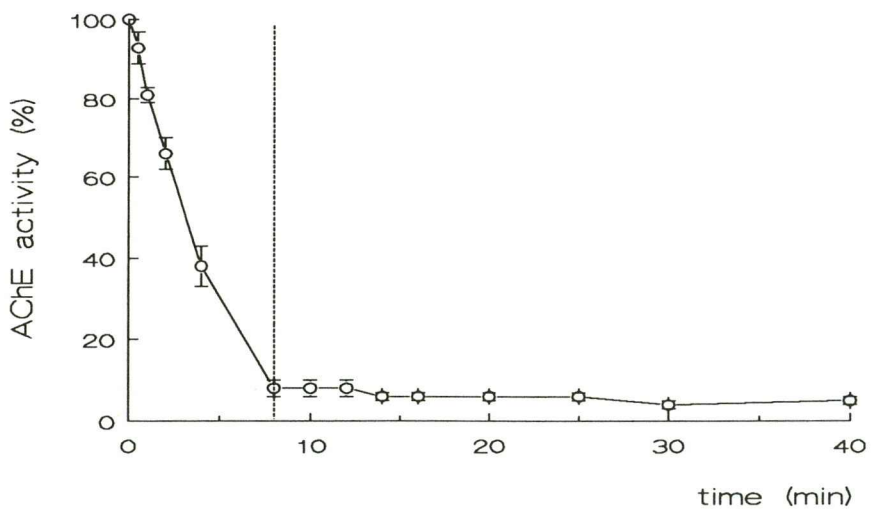

Figure 32. Time course of the mean acetylcholinesterase (AChE) activity (± s.e.m., n=6) in blood samples of anesthetized, atropinized, and restrained guinea pigs during and after an 8-min intravenous infusion of 0.8 LD50 C(±)P(±)-soman (16.2 µg/kg). The dotted line marks the end of the infusion period.

III-12 THE LD50 OF C(±)P(±)-SOMAN IN GUINEA PIGS AFTER INTRAMUSCULAR BOLUS ADMINISTRATION

To groups of 9-11 anesthetized (ketamine + Vetranquil^{R}) guinea pigs C(±)P(±)-soman was administered intramuscularly in the right thigh in doses ranging from 25 to 35 μg/kg. After injection, each animal was placed in a separate cage. At 24 h after administration, the number of dead animals per dose group was counted. The results are shown in Table 36.

Table 36. Number and percentage of dead animals per dosing group, 24 h after intramuscular administration of C(±)P(±)-soman to anesthetized guinea pigs.

Dose C(±)P(±)-soman (μg/kg)	Number of dead animals at 24 h/total number	Percentage deaths
25.0	2/9	22
27.5	4/9	44
30.0	6/10	60
32.5	5/9	55
35.0	11/11	100

Using probit analysis of the mortality data, the LD50 of C(±)P(±)-soman for intramuscular bolus administration was calculated to be 29 μg/kg (95 % confidence limits 26-31 μg/kg). The results of the probit analysis are presented in Table 37 and Figure 33.

Table 37. LD10, LD30, LD50, LD70 and LD90 (24 h) with 95 % confidence limits, for intramuscular bolus administration of C(±)P(±)-soman to anesthetized guinea pigs, calculated via probit analysis.[a]

LD	μg/kg	95 % confidence limits (μg/kg)
10	23	16-26
30	26	22-28
50	29	26-31
70	31	29-35
90	36	33-47

[a] Probit equation:
probit= 13.8*log(dose C(±)P(±)-soman) - 15.1

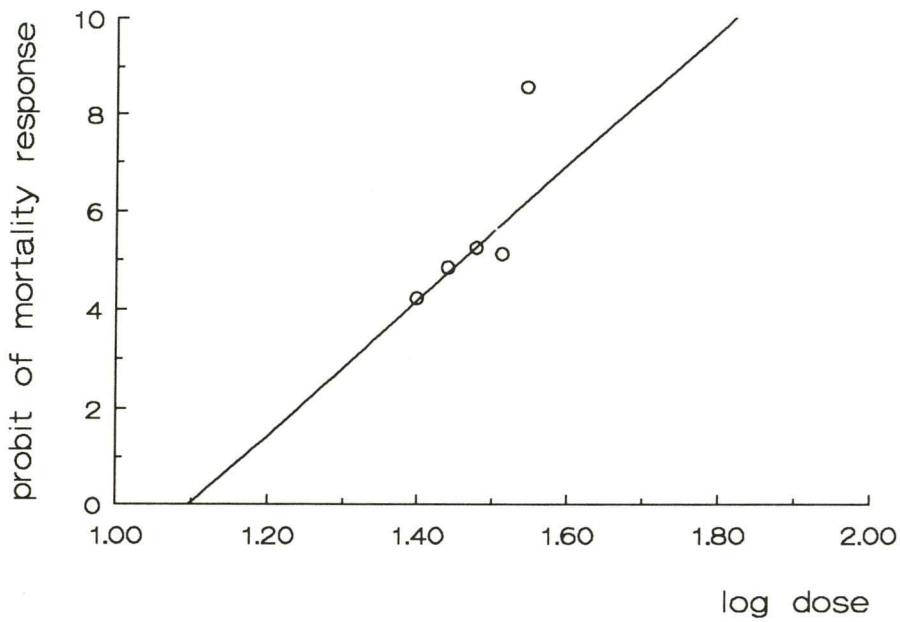

Figure 33. Relationship between the dose of C(±)P(±)-soman intramuscularly administered to anesthetized guinea pigs and the probit of mortality.

III-13 TOXICOKINETICS OF 0.8 LD50 OF C(±)P(±)-SOMAN, ADMINISTERED AS AN INTRAMUSCULAR BOLUS

C(±)P(±)-Soman was injected into the right thigh of anesthetized, atropinized guinea pigs at a dose of 23 μg/kg, which corresponds with 0.8 LD50 (i.m. bolus). Blood samples were drawn at various time-points after administration. A small portion of the blood sample was used for the determination of AChE activity, the larger part was used for gas chromatographic analysis. In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used in order to obtain six values for each time point.
The concentrations of the soman stereoisomers were measured with GLC configuration 1. The concentrations of C(±)P(-)-soman measured in the individual animals are presented in Table 38, whereas the mean concentrations with s.e.m. are shown in Table 39 and Figure 34. C(±)P(+)-isomers were not present in detectable concentrations. The absorption of the C(±)P(-)-isomers seems to be discontinuous and proceeds with some oscillation. Attempts to perform a curve-fitting on these data failed. As a result, as shown in Table 40, the only toxicokinetic parameter which could be calculated from these experiments was the area-under-the-curve, which was calculated via trapezoidals.
The measured blood acetylcholinesterase activities are presented in Table 41 and Figure 35. A relatively slow inhibition profile is observed.

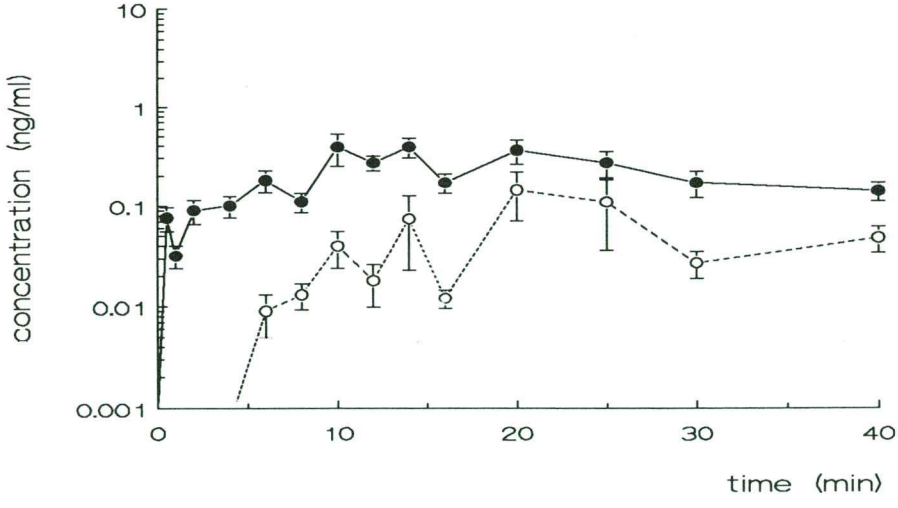

Figure 34. Semilogarithmic plot of the mean concentrations (± s.e.m., n=6) in blood of C(+)P(-)-soman (O) and C(-)P(-)-soman (●) versus time in anesthetized and atropinized guinea pigs after intramuscular bolus administration of 23 μg/kg of C(±)P(±)-soman, which corresponds with 0.8 LD50.

Table 38. Concentrations in blood[a] (ng/ml) of C(+)P(-)- and C(-)P(-)-soman in individual anesthetized and atropinized guinea pigs at various time-points after intramuscular bolus administration of 23 µg/kg C(±)P(±)-soman, which corresponds with 0.8 LD50

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 1 (353)[b]		Guinea pig 2 (349)[b]		Guinea pig 3 (462)[b]		Guinea pig 4 (455)[b]		Guinea pig 5 (432)[b]		Guinea pig 6 (433)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	0.029	*	*	n.d.	0.122	*	*	n.d.	0.025	*	*
1	*	*	n.d.	0.072	*	*	n.d.	0.024	*	*	n.d.	0.012
2	n.d.	0.041	*	*	n.d.	0.202	*	*	n.d.	0.054	*	*
4	*	*	n.d.	0.115	n.d.	0.222	*	*	*	*	n.d.	0.071
6	*	*	*	*	*	*	n.d.	0.128	n.d.	0.286	*	*
8	*	*	0.030	0.088	*	*	0.013	0.131	*	*	0.003	0.100
10	0.048	0.134	*	*	0.102	0.988	*	*	0.012	0.380	*	*
12	*	*	0.018	0.158	0.057	0.779	*	*	*	*	0.011	0.127
14	0.047	0.246	*	*	*	*	0.010	0.358	0.022	0.536	*	*
16	*	*	0.022	0.154	*	*	0.011	0.278	*	*	0.004	0.094
20	0.112	0.208	*	*	0.432	0.794	*	*	0.083	0.396	*	*
25	*	*	0.072	0.220	0.472	0.668	*	*	*	*	0.008	0.110
30	*	*	*	*	*	*	0.052	0.322	*	*	0.008	0.106
40	0.010	0.014	*	*	0.103	0.258	*	*	0.061	0.152	*	*

Time (min)	Concentration of soman isomer (ng/ml blood)											
	Guinea pig 7 (503)[b]		Guinea pig 8 (563)[b]		Guinea pig 9 (585)[b]		Guinea pig 10 (602)[b]		Guinea pig 11 (602)[b]		Guinea pig 12 (623)[b]	
	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)	C(-)P(-)
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	0.039	*	*	n.d.	0.108	*	*	n.d.	0.136	*	*
1	*	*	n.d.	0.016	*	*	n.d.	0.051	*	*	n.d.	0.016
2	n.d.	0.076	*	*	n.d.	0.129	*	*	n.d.	0.038	*	*
4	n.d.	0.100	*	*	*	*	n.d.	0.061	n.d.	0.066	*	*
6	*	*	n.d.	0.051	0.022	0.320	*	*	*	*	0.014	0.138
8	*	*	0.011	0.051	*	*	0.011	0.212	*	*	0.015	0.064
10	0.006	0.207	*	*	0.060	0.556	*	*	0.011	0.098	*	*
12	0.004	0.279	*	*	*	*	0.012	0.184	0.006	0.110	*	*
14	*	*	0.007	0.098	0.330	0.732	*	*	*	*	0.029	0.366
16	*	*	0.006	0.032	*	*	0.012	0.237	*	*	0.014	0.228
20	0.018	0.218	*	*	0.308	0.470	*	*	0.009	0.102	*	*
25	0.051	0.274	*	*	*	*	0.038	0.238	0.014	0.138	*	*
30	*	*	0.010	0.023	*	*	0.020	0.144	*	*	0.044	0.250
40	0.041	0.154	*	*	0.058	0.100	*	*	0.014	0.036	*	*

[a] Measured with GLC configuration 1 (see section II)

[b] Weight in grams

n.d. = Not detectable

* = Not measured

Table 39. Mean concentrations ± s.e.m. (n=6) of C(+)P(-)- and C(-)P(-)-soman in anesthetized and atropinized guinea pigs after intramuscular bolus administration of 0.8 LD50 (23 μg/kg) of C(±)P(±)-soman.

Time (min)	[C(+)P(-)-soman] ± s.e.m. (n=6) (ng/ml blood)	[C(-)P(-)-soman] ± s.e.m. (n=6) (ng/ml blood)
0	n.d.	n.d.
0.5	n.d.	0.08 ± 0.02
1	n.d.	0.032 ± 0.008
2	n.d.	0.09 ± 0.02
4	n.d.	0.10 ± 0.02
6	0.009 ± 0.004	0.18 ± 0.04
8	0.013 ± 0.004	0.11 ± 0.02
10	0.04 ± 0.02	0.4 ± 0.1
12	0.018 ± 0.008	0.27 ± 0.04
14	0.07 ± 0.05	0.39 ± 0.09
16	0.012 ± 0.002	0.17 ± 0.04
20	0.14 ± 0.07	0.4 ± 0.1
25	0.11 ± 0.07	0.27 ± 0.08
30	0.027 ± 0.008	0.17 ± 0.05
40	0.04 ± 0.01	0.14 ± 0.03

n.d. = not detectable

Table 40. Toxicokinetic parameters[a] for C(+)P(-)- and C(-)P(-)-soman in anesthetized and atropinized guinea pigs, after intramuscular bolus administration of 23 μg/kg of C(±)P(±)-soman, which corresponds with 0.8 LD50.

	C(+)P(-)-soman	C(-)P(-)-soman
A (ng/ml)	-	-
B (ng/ml)	-	-
C (ng/ml)	-	-
D (ng/ml)	-	-
a (min-1)	-	-
b (min-1)	-	-
c (min-1)	-	-
Terminal half-life (min)	-	-
Area under the curve[b] 0-40 min (ng.min/ml)	2	8.7

[a] The concentration of nerve agent at time t is described by: [nerve agent] = $A*e^{-at} + B*e^{-bt} + C*e^{-ct}$

[b] Calculated via trapezoidals

Table 41. Acetylcholinesterase (AChE) activities with s.e.m. (n=6), measured in blood samples of anesthetized and atropinized guinea pigs exposed to 0.8 LD50 C(±)P(±)-soman (23 µg/kg), administered as an intramuscular bolus.

Time (min)	AChE activity ± s.e.m. (n=6) (%)
0	100
0.5	94 ± 3
1	98 ± 1
2	91 ± 4
4	81 ± 5
6	74 ± 8
8	73 ± 5
10	44 ± 9
12	45 ± 6
14	33 ± 8
16	39 ± 7
20	16 ± 4
25	21 ± 6
30	1Ɛ ± 7
40	8 ± 3

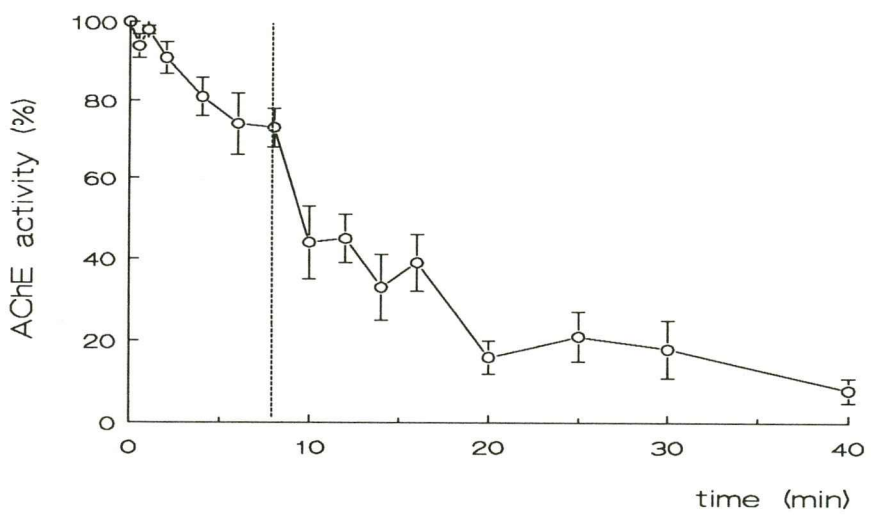

Figure 35. Time course of the acetylcholinesterase (AChE) activity ± s.e.m. (n=6) in blood samples of anesthetized and atropinized guinea pigs exposed to 0.8 LD50 C(±)P(±)-soman (23 µg/kg) administered as an intramuscular bolus.

III-14 ANALYSIS OF (±)-SARIN STEREOISOMERS

a. Chiral GLC of (±)-sarin stereoisomers

Until now, our toxicokinetic studies (1-5) have only involved the nerve agent C(±)P(±)-soman. This limitation permitted us to concentrate on the development of the analytical procedures needed for the trace analysis of the four stereoisomers of C(±)P(±)-soman in blood and tissue samples (1, 2, 13). The configurations for gas chromatographic analysis of the C(±)P(±)-soman isomers have continuously evolved in the course of our toxicokinetic investigations. The GLC-configurations using multidimensional chromatography should be very promising for toxicokinetic investigations of nerve agents more volatile than C(±)P(±)-soman, such as (±)-sarin, fulfilling the superior selectivity requirements for analysis in biological material, which contains many components with a volatility comparable to that of (±)-sarin.
First, the chromatographic resolution of the (±)-sarin stereoisomers had to be accomplished. Several years ago, we succeeded in resolving the stereoisomers of (±)-sarin by means of gas chromatography on an optically active nickel camphorate stationary phase (31). An example of such a separation is shown in Figure 36.

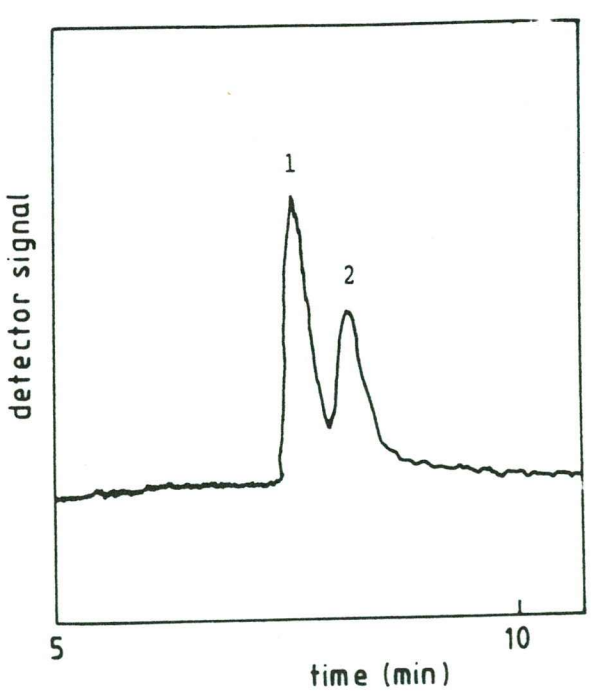

Figure 36. Gas chromatogram obtained after injection of (±)-sarin on a wide bore (length 9 m, I.D. 0.44 mm) glass column coated with bis[(1R)-3-(heptafluorobutyryl) camphorate]nickel(II) in OV-101 at 100 °C. Peak 1 is (-)-sarin; peak 2 is (+)-sarin. Chromatographic conditions are described in detail in reference 31.

The peaks obtained for the stereoisomers are quite broad, especially at lower (±)-sarin concentrations, which obviously influences the sensitivity unfavourably. Furthermore, the long-term stability of the stationary phase was problematic. This led, e.g., to variable retention times of the stereoisomers.

As an alternative, the chiral resolution of (±)-sarin was studied with the L-Chirasil Val stationary phase, which is used for the analysis of the four C(±)P(±)-soman stereoisomers (32). Figure 37 shows that the enantiomers of (±)-sarin are adequately resolved on this phase, which was also the case for the enantiomers of D_7-(±)-sarin.

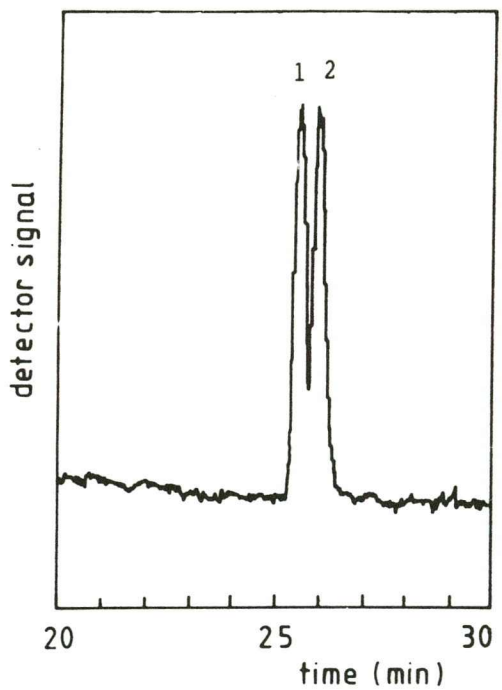

Figure 37. Gas chromatogram obtained after injection of (±)-sarin on a narrow bore (i.d. = 0.26 mm; l = 50 m) glass column coated with L-Chirasil Val at 70 °C. Peak 1 is (+)-sarin; peak 2 is (-)-sarin. Chromatographic conditions are described in detail in reference 32.

The elution order of the stereoisomers on this stationary phase is the same as for C(±)P(±)-soman, i.e., the P(+)-isomer is eluted before the P(-)-isomer. Unfortunately, the (±)-sarin enantiomers were hardly resolved from the deuterated enantiomers. The D_7-(+)-enantiomer was resolved from the (±)-sarin enantiomers, whereas

D_7-(-)-sarin co-eluted with (+)-sarin. As a result, this stationary phase can only be used for (±)-sarin analysis if optically pure D_7-(+)-sarin is available as the internal standard. Unfortunately, we did not succeed in isolating D_7-(+)-sarin from the racemic mixture. On the other hand, the (-)-enantiomer of the deuterated compound could be isolated in a relatively simple way.

We anticipated that our problem might be solved by reversing the elution order by using a D-Chirasil Val stationary phase. This phase was prepared in house (see Chapter II) and was custom-coated by Chrompack (Middelburg, The Netherlands) onto a fused silica capillary column. Indeed, on this stationary phase the (+)-enantiomer of the deuterated sarin co-eluted with the (-)-enantiomer of sarin, whereas D_7-(-)-sarin was completely resolved. This column can be used for the analysis of (±)-sarin stereoisomers, using D_7-(-)-sarin as the internal standard. Meanwhile, a glass capillary column was coated in-house with the D-Chirasil Val stationary phase, with a slightly thinner coating than on the custom-coated column, with a method described in reference 32. This column proved to be even capable of resolving the four peaks of interest, as is shown in Figure 38. For practical purposes, however, we prefer to use fused silica columns, which are much easier to handle than glass columns. Furthermore, we prefer to use commercially available columns, since the synthesis of the stationary phase and its coating onto a capillary column are tedious operations that are difficult to reproduce.

During the last several years the number of commercially available chiral stationary phases has increased. Among these, the cyclodextrin phases seem promising with respect to the resolution of chiral organophosphorus compounds, since in earlier studies in our laboratory, the inclusion of (±)-sarin and several other organophosphorus compounds in the cavity of α-cyclodextrin appeared to be stereoselective (33).

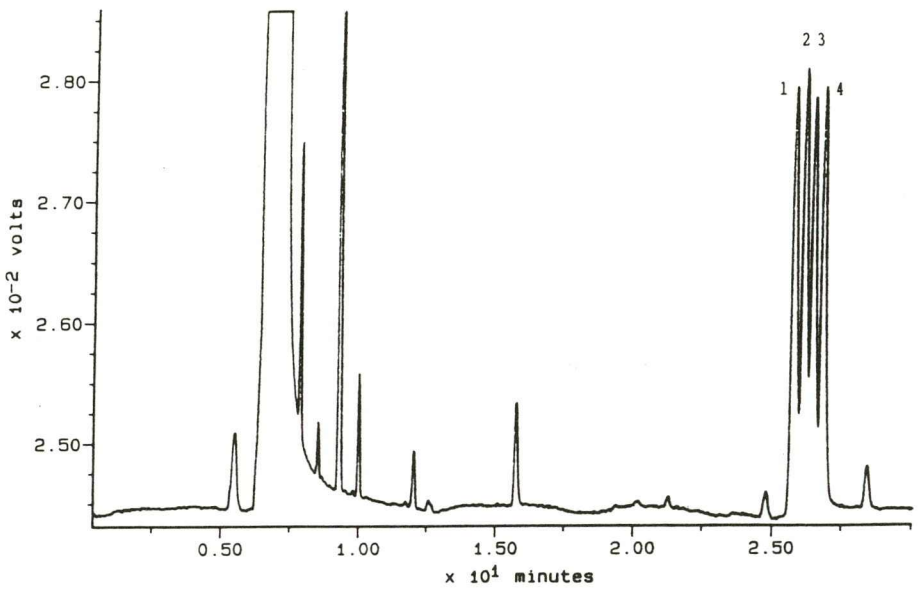

Figure 38. Chromatogram of racemic D_7-(±)-sarin and (±)-sarin on D-Chirasil Val (length 50 m, I.D. 0.24 mm, glass capillary). Carrier gas helium; oven temperature 60 °C; injector/detector (FID) temperature 180 °C. Peak 1 is D_7-(-)-sarin, peak 2 is D_7-(+)-sarin, peak 3 is (-)-sarin and peak 4 is (+)-sarin.

With the commercially available CP Cyclodex B column, a ß-cyclodextrin phase coated on fused silica, baseline resolution of D_7-(-)-sarin, (-)-sarin and (+)-sarin was accomplished, as shown in Figure 39. On this column D_7-(+)-sarin is not resolved from (-)-sarin. In view of the aforementioned considerations, we chose to use this column for the bioanalysis of (±)-sarin stereoisomers, using the optically pure D_7-(-)-sarin as the internal standard.

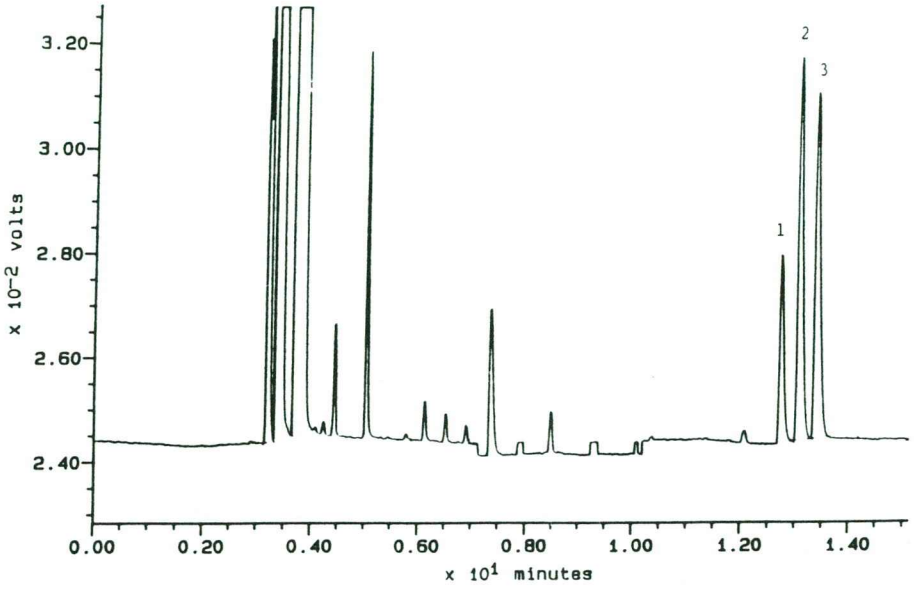

Figure 39. Chromatogram of D_7-(-)-sarin and (±)-sarin on CP Cyclodex B 2,3,6,M-19, coated onto WCOT fused silica (length 50 m, I.D. 0.25 mm). Carrier gas helium, oven temperature 70 °C; injector/detector (FID) temperature 180 °C. Peak 1 is D7-(-)-sarin, peak 2 is (-)-sarin, and peak 3 is (+)-sarin.

When used in GLC-configuration 1 with an NP detector, the limit of detection is ca. 2.5 pg per sarin enantiomer.

b. Validation of the extraction of (±)-sarin stereoisomers from blood samples

The extraction procedure with SepPak C_{18} cartridges, used for the isolation of C(±)P(±)-soman stereoisomers from biological samples, is applicable as such to the isolation of (±)-sarin. The absolute recovery of the extraction procedure appears to be ca. 60 %, which is about the same as for C(±)P(±)-soman.
The extraction procedure for (±)-sarin from guinea pig blood was validated as follows. Two hours after i.v. administration (vena

jugularis) of 2 LD50 (±)-sarin to an anesthetized, atropinized guinea pig, the animal was exsanguinated. The blood samples were stabilized with the procedure used for C(±)P(±)-soman (13). (±)-Sarin, in concentrations of 10, 1, 0.1 and 0.01 ng/ml blood, as well as the internal standard D_7-(-)-sarin, was added. Next, the blood samples, diluted with the stabilizing buffer, were subjected to solid-phase extraction on the SepPak C_{18} cartridges. Since at that time no adequate optically active stationary phase was available, the extracts were analyzed by GLC on a Carbowax 20W column in configuration 1. The results are listed in Table 42.

Table 42. Results of the verification test of the solid-phase extraction of (±)-sarin with SepPak C_{18}-cartridges for various amounts of (±)-sarin added to blood obtained from an anesthetized, atropinized guinea pig 2 h after administration of 2 LD50 of (±)-sarin.

Added (±)-sarin (ng/ml)	Measured (±)-sarin ± s.d. (n=5) (ng/ml)
10	10.0 ± 0.3
1	1.02 ± 0.01
0.1	0.098 ± 0.009
0.01	failed

The chromatogram of the extract of the 'blanc' blood showed a peak with the retention time of (±)-sarin, corresponding with a concentration of 0.04 ng (±)-sarin/ml. This could actually be (±)-sarin, still circulating 2 h after administration (cf. section III-15). Unfortunately, such a peak was also observed in extracts of the buffer solution, in spite of the use of two-dimensional chromatography, which suggests the presence of some interfering component in the chromatogram.
The validation experiment was repeated with the CP Cyclodex B column, installed in GLC configuration 1. Initially, validation of the assay of (±)-sarin at low concentrations (100 pg/ml and less) was hindered by irreproducible peak height or peak area ratios for the stereoisomers. In the past such phenomena have been caused by contaminations of the apparatus used to concentrate the ethyl acetate extracts under reduced pressure. All equipment was thoroughly rinsed and reassembled. This did not solve the problem of unreproducible peak height ratios. Initially, it seemed that the problem was caused by volume reduction of the extract under reduced pressure. The GC-configuration with TCT and MUSIC offers the possibility to transfer large sample volumes onto the Tenax material. Consequently, with this GLC configuration, there is no need for extensive volume reduction of the extract. It is possible to transfer a total volume of 400 µl of the sample onto the Tenax desorption tube, in portions of 50 µl, without sample-breakthrough. The peak height ratio of the stereoisomers was highly reproducible and accurate.

With a new set of evaporation flasks, the peak height ratio was accurate and reproducible even after our usual extraction procedure with volume reduction to 100 µl. Therefore, the problem appeared to be solved now, and there was no need for transferring large sample volumes onto Tenax.
Table 43 shows the results of the validation of the analysis of (±)-sarin at low concentrations. Known amounts of (±)-sarin were added to blood samples obtained from an anesthetized, atropinized guinea pig 2 h after intravenous administration of 2 LD50 of (±)-sarin (standard addition). Additional validation tests were performed by preincubating blank blood samples obtained from a naive guinea pig with an excess of C(±)P(±)-soman for 30 min, after which the stabilization buffer and the known amounts of (±)-sarin and D_7-(-)-sarin were added. There were no interfering peaks present in the chromatogram.
The absolute limit of detection is ca. 2.5 pg per sarin stereoisomer.

Table 43. Validation of the analysis of low concentrations of (±)-sarin in guinea pig blood (n=2).

(±)-sarin added (ng/ml)	measured (-)-sarin (ng/ml)	measured (+)-sarin (ng/ml)
0.1	0.053 ± 0.001	0.050 ± 0.001
0.05	0.027 ± 0.001	0.025 ± 0.001
0	0	0

For the solid-phase extraction of C(±)P(±)-soman stereoisomers, ethyl acetate appeared to be the most suitable solvent. For the more volatile (±)-sarin, however, this is not necessarily so. Therefore, other solvents than ethyl acetate to extract (±)-sarin from the SepPak cartridge were tested for extraction recovery and reproducibility, such as diethyl ether, dichloromethane, pentane/methanol 95/5 (v/v), butyl acetate and methyl acetate. None of these solvents proved to be advantageous over ethyl acetate.

III-15 THE LD50 (I.V. BOLUS) OF (±)-SARIN IN ANESTHETIZED GUINEA PIGS

a. Pilot experiment

Prior to the actual LD50 determination, a pilot experiment was performed, in order to get an impression of the desired dose range. This experiment was performed with eight guinea pigs. On the basis of literature data the intravenous doses to be tested were estimated to be 35, 30 and 25 μg/kg.
The pilot experiment was performed sequentially: an animal was injected with a certain (±)-sarin dose; if this animal died within 15 min, a lower dose was tested in the next animal; if not, a higher dose was tested, and so on. All animals were under ketamine anesthesia. The results of the pilot experiment are shown in Table 44

Table 44. Results of the pilot experiment for the determination of the intravenous 24-h LD50 of (±)-sarin in anesthetized guinea pigs. The '+' sign indicates whether the animal was dead or alive at 15 min after i.v. administration.

Guinea pig #	Body weight (g)	(±)-Sarin dose (μg/kg, i.v.)	Dead/alive (at 15 min)
1	390	35	+
2	358	30	+
3	338	35	+
4	379	30	+
5	394	25	+
6	378	30	+
7	394	25	+
8	420	30	+

This sequential experiment gives some indication of the actual 24-h LD50. The results in Table 44 suggest an actual 24-h LD50 dose in the range of 25-35 μg/kg.

b. The 24-h LD50 of (±)-sarin

The doses chosen for the actual LD50 experiment were 30, 27.5, 25, 22.5 and 20 μg/kg. Each dose was administered to 10 guinea pigs, except for a dose of 30 μg/kg, which was tested in six animals only. The results are presented in Table 45.
All nonsurvivors died within 30 min after (±)-sarin administration. Probit analysis was performed on the data. The results of the probit analysis are presented in Table 46 and Figure 40. From these data the intravenous bolus 24-h LD50 of (±)-sarin in guinea pigs was calculated to be 24 ± 2 μg/kg (95 % confidence interval).

Table 45. Number and percentage of dead animals per dosing group, 24 h after intravenous bolus administration of (±)-sarin to anesthetized guinea pigs.

(±)-Sarin dose (μg/kg)	Number of dead animals at 24 h/total number	Percentage deaths
30	6/6	100
27.5	7/10	70
25	6/10	60
22.5	4/10	40
20	1/10	10

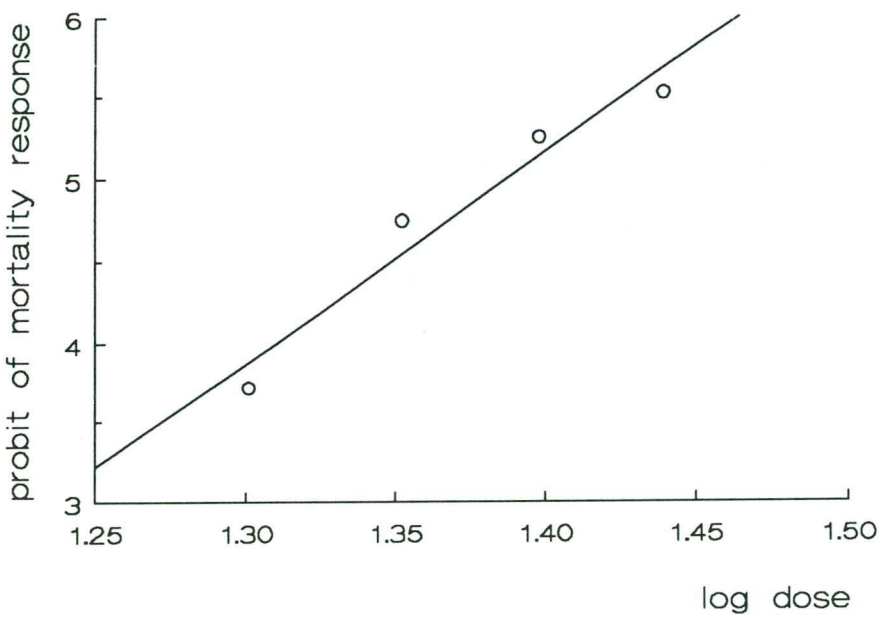

Figure 40. Probit of mortality of anesthetized guinea pigs 24 h after intravenous bolus administration of (±)-sarin versus the administered dose of (±)-sarin.

Table 46. LD10, LD30, LD50, LD70 and LD90 (24-h) with 95 % confidence limits, for intravenous bolus administration of (±)-sarin to anesthetized guinea pigs, calculated via probit analysis.[a]

LD	µg/kg	95 % confidence limits (µg/kg)
10	20	15-22
30	22	19-24
50	24	22-26
70	26	24-30
90	30	27-38

[a] Probit equation:
probit = 14.5*log dose (±)-sarin - 15.0

III-16 TOXICOKINETICS OF (±)-SARIN IN ANESTHETIZED, ATROPINIZED GUINEA PIGS AFTER INTRAVENOUS BOLUS ADMINISTRATION OF A DOSE CORRESPONDING WITH 0.8 LD50

The toxicokinetics of (±)-sarin after intravenous administration of 0.8 LD50 were studied in anesthetized, atropinized and mechanically ventilated guinea pigs. Blood samples were analyzed with GLC configuration 1 under the conditions described for configuration 2, on a CP Cyclodex-B analytical column, after preseparation on a CPSil 8 column.
(±)-Sarin was injected into a jugular vein as an i.v. bolus.
Blood samples were taken via a carotid artery cannula, from 0.5 up to 60 min after administration. In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used in order to obtain six values for each time-point.
The blood samples taken just before (±)-sarin administration were negative, confirming the absence of sarin in materials and instruments. After i.v. administration of (±)-sarin, (+)-sarin was not detectable in the blood samples. The concentrations of (-)-sarin measured in the individual animals are presented in Table 47, whereas the mean values of the concentrations of (-)-sarin with s.e.m. are shown in Table 48 and Figure 41. Mathematical equations describing the concentration-time courses of (-)-sarin were obtained by nonlinear regression. The calculated toxicokinetic parameters are presented in Table 49. The toxicokinetics of (-)-sarin is characterized by a very rapid distribution phase and a fairly slow elimination phase.

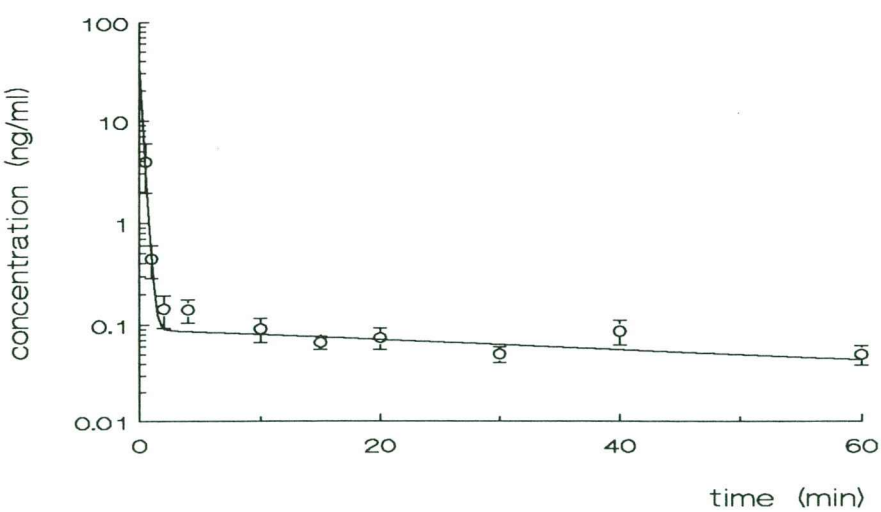

Figure 41. Semilogarithmic plot of mean concentrations in blood (± s.e.m., n=6) of (-)-sarin versus time after intravenous bolus administration of 0.8 LD50 (19.2 μg/kg) of (±)-sarin to anesthetized, atropinized and mechanically ventilated guinea pigs.

Table 47. Concentrations in blood[a] (ng/ml) of (-)-sarin in individual anesthetized, atropinized, and mechanically ventilated guinea pigs at various time-points after intravenous administration of 0.8 LD50 (19.2 µg/kg) of (±)-sarin.

Time (min)	Concentration of (-)-sarin (ng/ml blood)					
	Guinea pig 1 (532)[b]	Guinea pig 2 (556)[b]	Guinea pig 3 (444)[b]	Guinea pig 4 (454)[b]	Guinea pig 5 (483)[b]	Guinea pig 6 (498)[b]
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	12.05#	*	0.431$	*	2.900	*
1	1.077#	*	0.002$	*	*	0.400
2	*	0.319@	0.292#	*	0.100	*
4	0.182#	*	0.113$	*	*	0.276
10	0.074	*	0.013	*	0.065	*
15	*	0.062	*	0.033	*	0.077
20	0.073	*	*	0.024	0.133	*
30	*	0.029	0.042#	*	*	0.054
40	0.187	*	*	0.021	0.049	*
60	*	0.0271	0.021	*	*	0.095

Time (min)	Concentration of (-)-sarin (ng/ml blood)					
	Guinea pig 7 (565)[b]	Guinea pig 8 (550)[b]	Guinea pig 9 (553)[b]	Guinea pig 10 (558)[b]	Guinea pig 11 ( )[b]	Guinea pig 12 ( )[b]
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	0.176	*	0.630	*	0.261	*
1	*	0.012	*	0.774	*	0.169
2	*	0.045	0.020	*	*	0.003
4	0.068	*	*	0.021	0.076	*
10	0.145	*	0.064	*	0.183	*
15	*	0.095	*	0.082	*	0.046
20	*	0.078	0.107	*	*	0.029
30	0.089	*	*	0.052	0.035	*
40	0.119	*	0.075	*	0.066	*
60	*	0.048	*	0.063	*	0.044

[a] Measured with GLC configuration 1 , with chromatographic conditions of configuration 2 (see section II)

[b] Weight in grams

n.d. = Not detectable

* = Not measured

additional guinea pig (584 g)

$ additional guinea pig (602 g)

@ additional guinea pig (604 g)

Table 48. Mean concentrations of (-)-sarin with standard error of the mean (s.e.m) (n=6) in anesthetized, atropinized and artificially ventilated guinea pigs after intravenous bolus administration of 0.8 LD50 (19.2 μg/kg) of (±)-sarin.

Time (min)	[(-)-sarin] ± s.e.m. (n=6) (ng/ml blood)
0	n.d.
0.5	3.9 ± 2.0
1	0.4 ± 0.2
2	0.14 ± 0.05
4	0.14 ± 0.04
10	0.09 ± 0.02
15	0.07 ± 0.01
20	0.07 ± 0.02
30	0.050 ± 0.009
40	0.09 ± 0.02
60	0.05 ± 0.01

n.d. = not detectable

Table 49. Toxicokinetic parameters[a] for (-)-sarin in anesthetized, atropinized, and mechanically ventilated guinea pigs, after intravenous bolus administration of 0.8 LD50 (19.2 μg/kg) of (±)-sarin.

Number of exponents	2
A (ng/ml)	35.9
B (ng/ml)	0.09
C (ng/ml)	-
a (min^{-1})	4.6
b (min^{-1})	0.012
c (min^{-1})	-
Terminal half-life (min)	58
Area under the curve (ng.min/ml)	15.3

[a] The concentration of nerve agent at time t is described by: $[(-)\text{-sarin}] = A*e^{-at} + B*e^{-bt} + C*e^{-ct}$

III-17 THE LCt50 OF (±)-SARIN IN ANESTHETIZED, RESTRAINED GUINEA PIGS AFTER NOSE-ONLY EXPOSURE FOR 8 MINUTES

Groups of eight anesthetized guinea pigs were exposed for 8 min to (±)-sarin vapor concentrations in air ranging from 31-60 mg/m^3. After the nose-only exposure each animal was placed in a separate cage. The number of dead animals per dosing group was counted at 24 h after the exposure. The results are shown in Table 50.

Table 50. Number and percentage of dead animals per dosing group, 24 h after 8-min exposure of anesthetized guinea pigs to (±)-sarin vapor in air.

[(±)-sarin] (mg/m^3)	Number of dead animals at 24 h/total number	Percentage deaths
31.0	0/8	0
36.5	0/8	0
40.7	2/8	25
44.3	1/8	12.5
49.6	5/8	62.5
58.3	8/8	100
60.0	8/8	100

Using probit analysis of the mortality data, the LC50 of (±)-sarin was calculated to be 47 mg/m^3 (95 % confidence limits 44-50 mg/m^3). The results of the probit analysis are presented in Table 51 and Figure 42. As a result, the LCt50 of (±)-sarin for 8-min nose-only exposure of anesthetized guinea pigs is 376 mg.min/m^3.

Table 51. LC10, LC30, LC50, LC70 and LC90 (24-h) with 95 % confidence limits, for 8-min nose-only exposure of anesthetized guinea pigs to (±)-sarin vapor in air, calculated via probit analysis.[a]

LC	mg/m^3	95 % confidence limits (mg/m^3)
10	41	36-44
30	44	41-47
50	47	44-50
70	50	47-55
90	54	51-63

[a] Probit equation: probit= 20.6*log[(±)-sarin] - 29.4

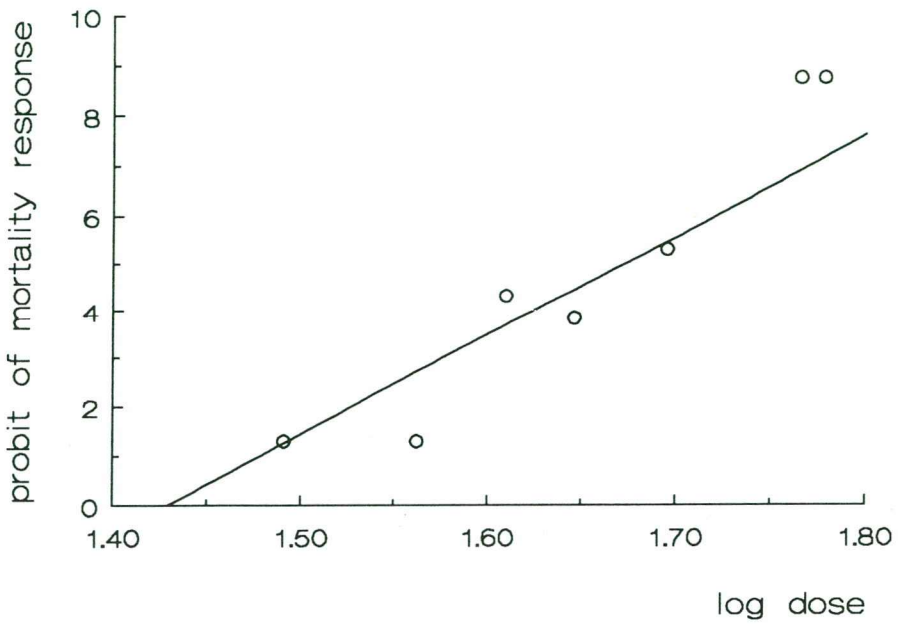

Figure 42. Probit of mortalitity of anesthetized guinea pigs nose-only exposed to (±)-sarin vapor in air for 8 min, versus the concentration of (±)-sarin vapor in air.

III-18 THE TOXICOKINETICS OF 0.8 LCt50 (±)-SARIN IN ANESTHETIZED, ATROPINIZED AND RESTRAINED GUINEA PIGS AFTER NOSE-ONLY EXPOSURE FOR 8 MINUTES

Anesthetized, atropinized and restrained guinea pigs were nose-only exposed for 8 min to a vapor concentration of 38 mg/m^3 of (±)-sarin, yielding an exposure corresponding with 0.8 LCt50.
Blood samples were taken just before and during exposure, and at various time-points after exposure. A small portion of the blood sample was used for the determination of AChE activity, the larger part was used for gas chromatographic analysis with GLC configuration 4. During the exposure, the respiration of the animals was monitored. In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used in order to obtain six values for each time-point.
The (+)-isomer was not detectable in the blood samples. The concentrations of (-)-sarin measured in the individual animals are presented in Table 52, whereas the mean concentrations with s.e.m. are shown in Table 53 and Figure 43.
A mathematical equation describing the concentration-time course of (-)-sarin was obtained by nonlinear regression. The kinetics were described as a discontinuous process, with an equation for the 8-min exposure period and an equation for the post-exposure period. The absorption phase was described with a mono-exponential function, since the limited number of datapoints in this phase does not justify a description with more than one exponent. The post-exposure phase appears to be adequately described with a two-exponential function. By analogy with the absorption phase, the number of datapoints is insufficient for justification of a three-exponential equation. The toxicokinetic parameters are listed in Table 54.
The AChE activities measured during and after exposure are presented in Table 55 and Figure 44. During exposure and in the first few minutes thereafter, the blood AChE activity drops fairly rapidly down to ca. 15 % of control activity, which is a somewhat higher activity than after 8-min exposure to 0.8 LCt50 C(±)P(±)-soman.
There were no (±)-sarin-related effects on the respiration. The mean respiratory minute volume during exposure was 31 ± 4 ml (s.e.m., n=12), whereas the respiratory frequency was 0.70 ± 0.06 Hz (s.e.m., n=12).

Table 52. Concentrations in blood[a] (ng/ml) of (-)-sarin in individual anesthetized, atropinized, and restrained guinea pigs at various time-points during and after nose-only exposure to 38 ± 4 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.8 LCt50.

Time (min)	Concentration of (-)-sarin (ng/ml blood)							
	Guinea pig 1 (577)[b]	Guinea pig 2 624)[b]	Guinea pig 3 (656)[b]	Guinea pig 4 (652)[b]	Guinea pig 5 (670)[b]	Guinea pig 6 (559)[b]	Guinea pig 7 (675)[b]	Guinae pig 8 (664)[b]
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	*	n.d.	*	n.d.	*	*	*
1	n.d.	*	*	*	*	n.d.	*	*
2	n.d.	*	*	*	0.012	*	0.042	*
4	*	n.d.	0.025	*	*	0.0445	*	*
6	*	*	*	*	0.011	0.018	*	0.302
8	*	*	*	*	1.362	1.121	*	2.782
10	1.071	*	*	*	0.934	*	0.229	*
12	*	0.514	0.322	*	*	1.064	0.198	*
14	0.314	*	*	*	0.250	*	*	0.143
16	*	*	*	*	*	0.528	*	0.110
20	*	*	0.088	*	0.114	*	0.070	0.132
30	*	0.044	*	*	*	0.086	*	*
40	*	0.027	*	*	*	*	*	0.110
60	0.017	*	0.027	*	0.050	*	*	*
120	*	*	*	*	*	*	*	*

Time (min)	Concentration of (-)-sarin (ng/ml blood)							
	Guinea pig 9 (534)[b]	Guinea pig 10 (590)[b]	Guinea pig 11 (481)[b]	Guinea pig 12 (467)[b]	Guinea pig 13 (480)[b]	Guinea pig 14 (500)[b]	Guinea pig 15 (585)[b]	Guinea pig 16 (535)[b]
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	*	*	n.d.	*	*	*	0.073	n.d.
1	*	n.d.	*	n.d.	n.d.	*	*	n.d.
2	0.049	*	*	*	*	*	0.084	0.012
4	*	0.253	n.d.	*	*	*	*	0.022
6	*	*	*	0.344	*	*	0.299	0.066
8	*	3.138	*	*	*	*	0.602	0.266
10	*	*	0.836	*	0.692	*	0.130	*
12	*	*	0.594	*	*	*	*	0.110
14	0.555	*	*	0.234	*	0.266	*	*
16	*	0.142	*	0.166	0.121	*	0.030	*
20	0.278	*	0.146	*	*	*	*	*
30	*	0.112	*	*	*	0.087	0.049	0.024
40	*	*	*	0.032	0.036	0.054	0.054	*
60	0.047	*	0.047	*	0.018	*	*	*
120	*	*	*	*	*	*	0.010	0.014

[a] Measured with GLC configuration 4 (see section II)

[b] Weight in grams

n.d. = not detectable

* = Not measured

Table 53. Mean concentrations of (-)-sarin (± s.e.m., n=6) in anesthetized, atropinized and restrained guinea pigs during and after exposure to a concentration of 38 ± 4 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.8 LCt50.

Time (min)	[(-)-sarin] ± s.e.m. (n=6) (ng/ml blood)
0	n.d.
0.5	0.01 ± 0.01
2	0.03 ± 0.01
4	0.06 ± 0.04
6	0.17 ± 0.06
8	1.5 ± 0.5
10	0.65 ± 0.2
12	0.4 ± 0.2
14	0.30 ± 0.06
16	0.18 ± 0.07
20	0.14 ± 0.03
30	0.07 ± 0.01
40	0.05 ± 0.01
60	0.03 ± 0.06
120	0.012 ± 0.002 (n=2)

n.d. = not detectable

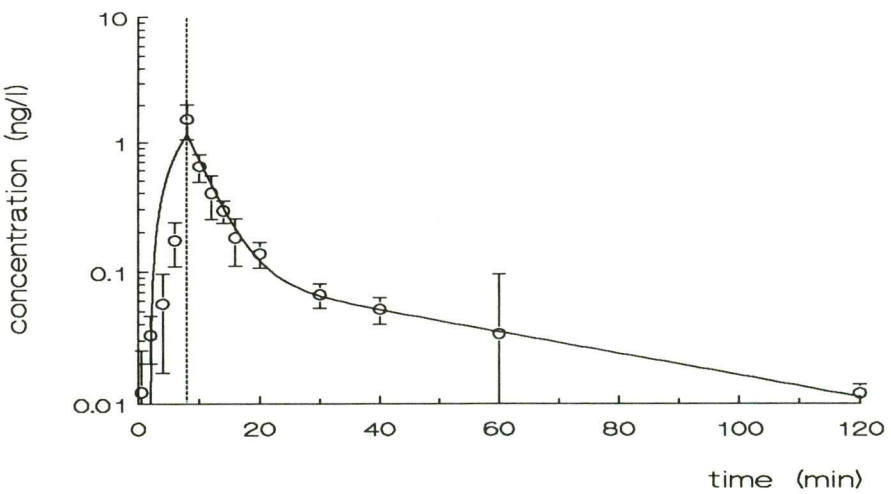

Figure 43. Mean blood concentrations (± s.e.m, n=6) of (-)-sarin versus time after nose-only exposure of anesthetized, atropinized guinea pigs to 38 ± 4 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.8 LCt50. The dotted line marks the end of the exposure period.

Table 54. Toxicokinetic parameters[a] for (-)-sarin in anesthetized, atropinized, and restrained guinea pigs, after nose-only exposure to 0.8 LCt50 (±)-sarin for 8 min.

A (ng/ml)		-164
B (ng/ml)		8.6
C (ng/ml)		0.11
D (ng/ml)		164
a (min-1)		0.0012
b (min-1)		0.26
c (min-1)		0.019
Terminal half-life (min)		36.5
Area under	0-8 min	6.3
the curve	> 8 min	9.1 (0-120 min 8.5)
(ng.min/ml)	total	15.4 (0-120 min 14.8)

[a] The inhalation results were fitted with a discontinuous function: [nerve agent] = $D + A*e^{-at}$ for the absorption phase (0-8 min), and [nerve agent] = $B*e^{-bt} + C*e^{-ct}$ for distribution and elimination (≥ 8 min)

Table 55. Mean acetylcholinesterase (AChE) activities (± s.e.m., n=6), measured in blood samples of anesthetized, atropinized and restrained guinea pigs during and after exposure to 38 ± 4 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.8 LCt50.

Time (min)	AChE activity ± s.e.m. (n=6) (%)
0	100
0.5	102 ± 2
2	95 ± 2
4	75 ± 4
6	60 ± 4
8	31 ± 6
10	31 ± 5
12	25 ± 3
14	31 ± 14
16	14 ± 4
20	19 ± 4
30	24 ± 9
40	19 ± 5
60	13 ± 3
120	27 ± 2 (n=2)

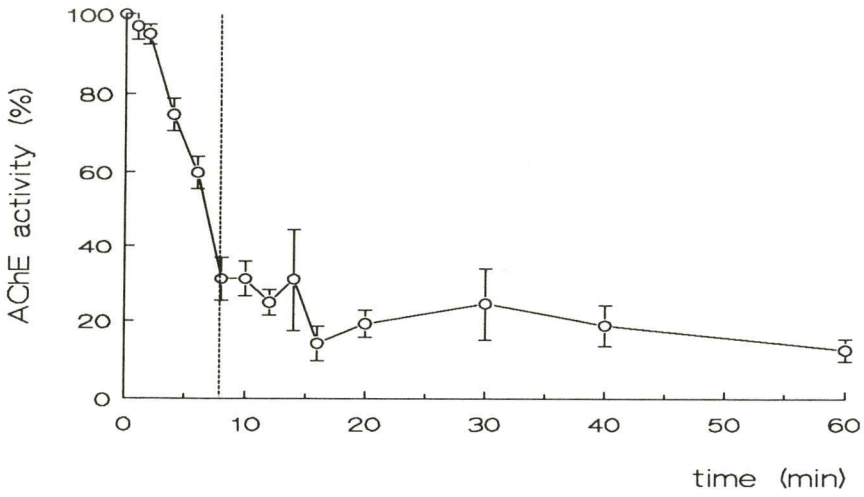

Figure 44. Mean acetylcholinesterase (AChE) activity (± s.e.m., n=6) in blood versus time during and after nose-only exposure of anesthetized, atropinized guinea pigs to 38 ± 4 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.8 LCt50. The dotted line marks the end of the exposure period.

III-19 THE TOXICOKINETICS OF 0.4 LCt50 (±)-SARIN IN ANESTHETIZED, ATROPINIZED AND RESTRAINED GUINEA PIGS AFTER NOSE-ONLY EXPOSURE FOR 8 MINUTES

Anesthetized, atropinized and restrained guinea pigs were nose-only exposed to a concentration of 19 ± 2 mg/m^3 of (±)-sarin vapor in air for 8 min, yielding an exposure to 0.4 LCt50.
Blood samples were taken just before and during the exposure, and at various post-exposure time-points. A small portion of the blood sample was used for the determination of AChE activity, the larger part was used for gas chromatographic analysis. The blood samples were analyzed with GLC configuration 4. During the exposure, the respiration of the animals was monitored.
In order to avoid too much strain on each individual animal, the sampling times were divided into two series. As a result, 12 animals were used in order to obtain six values for each time-point.
The (+)-isomer was not detectable in the blood samples. The concentrations of (-)-sarin measured in the individual animals are presented in Table 56 whereas the mean concentrations with s.e.m. are shown in Table 57 and Figure 45.
A mathematical equation describing the concentration-time courses of (-)-sarin was obtained by nonlinear regression. The kinetics were described as a discontinuous process, with an equation for the 8-min exposure period and an equation for the post-exposure period. The absorption phase was described with a mono-exponential function, since the limited number of datapoints in this phase does not justify a description with more than one exponent. The post-exposure phase appears to be adequately described with a two-exponential function. By analogy with the absorption phase, the number of datapoints is insufficient for justification of a three-exponential equation. The toxicokinetic parameters are listed in Table 58.
The AChE activities measured during and after exposure are presented in Table 59 and Figure 46. The residual AChE activity appears to be ca. 70 %, which is considerably higher than after exposure to 0.8 LCt50 (±)-sarin in 8 min (see Section III-18).
There were no (±)-sarin-related effects on the respiration. The mean respiratory minute volume during exposure was 47 ± 6 ml (s.e.m., n=12), whereas the respiratory frequency was 0.64 ± 0.04 Hz (s.e.m., n=12).

Table 56. Concentrations in blood[a] (ng/ml) of (-)-sarin in individual anesthetized, atropinized, and restrained at various time-points during and after nose-only exposure to 19 ± 2 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.4 LCt50.

Time (min)	Concentration of (-)-sarin (ng/ml blood)					
	Guinea pig 1 (568)[b]	Guinea pig 2 (527)[b]	Guinea pig 3 (575)[b]	Guinea pig 4 (600)[b]	Guinea pig 5 (586)[b]	Guinea pig 6 (626)[b]
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	n.d.	*	0.030	*	0.034	*
1	*	n.d.	*	0.024	*	0.008
2	0.056	*	0.048	*	0.035	*
4	*	0.34	0.12	*	*	0.080
6	0.51	*	*	0.19	0.062	*
8	*	*	0.36	0.046	*	0.180
10	0.36	*	0.072	*	0.180	*
12	*	*	0.065	0.028	*	0.080
14	0.11	*	*	0.068	0.058	*
16	*	*	*	0.024	*	0.078
20	0.026	*	*	*	0.099	*
30	*	*	*	*	*	0.075
40	*	0.035	*	0.055	*	0.015
60	0.049	*	*	*	0.120	*
120	0.027	0.057	*	*	0.025	0.024

Time (min)	Concentration of (-)-sarin (ng/ml blood)					
	Guinea pig 7 (557)[b]	Guinea pig 8 (547)[b]	Guinea pig 9 (557)[b]	Guinea pig 10 (547)[b]	Guinea pig 11 (537)[b]	Guinea pig 12 (544)[b]
0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5	0.044	0.033	*	0.038	*	*
1	*	0.076	*	0.036	*	0.010
2	0.031	*	0.028	*	*	*
4	0.008	*	*	0.044	0.048	*
6	*	0.39	0.71	*	*	0.016
8	*	0.69	*	0.26	*	0.072
10	0.17	*	0.16	*	0.54	*
12	0.26	*	*	0.023	*	*
14	*	0.007	*	*	0.37	0.044
16	*	0.15	0.050	0.044	*	0.10
20	0.037	*	0.17	0.068	0.052	*
30	0.045	0.016	*	0.038	0.028	0.096
40	*	0.018	*	0.026	*	*
60	0.025	*	0.080	*	0.008	0.015
120	0.012	0.055	*	*	*	*

[a] Measured with GLC configuration 4 (see section II)

[b] Weight in grams

n.d. = Not detectable

* = Not measured

Table 57. Mean concentrations of (-)-sarin (± s.e.m., n=6) in anesthetized, atropinized and restrained guinea pigs during and after nose-only exposure to a concentration of 19 ± 2 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.4 LCt50.

Time (min)	[(-)-sarin] ± s.e.m. (n=6) (ng/ml blood)	
0	n.d.	
0.5	0.03 ± 0.01	
1	0.03 ± 0.01	
2	0.04 ± 0.01	(n=5)
4	0.10 ± 0.05	
6	0.31 ± 0.11	
8	0.27 ± 0.10	
10	0.25 ± 0.07	
12	0.09 ± 0.04	(n=5)
14	0.10 ± 0.05	
16	0.07 ± 0.02	
20	0.08 ± 0.02	
30	0.05 ± 0.01	
40	0.03 ± 0.01	(n=5)
60	0.05 ± 0.02	
120	0.033 ± 0.008	

n.d. = not detectable

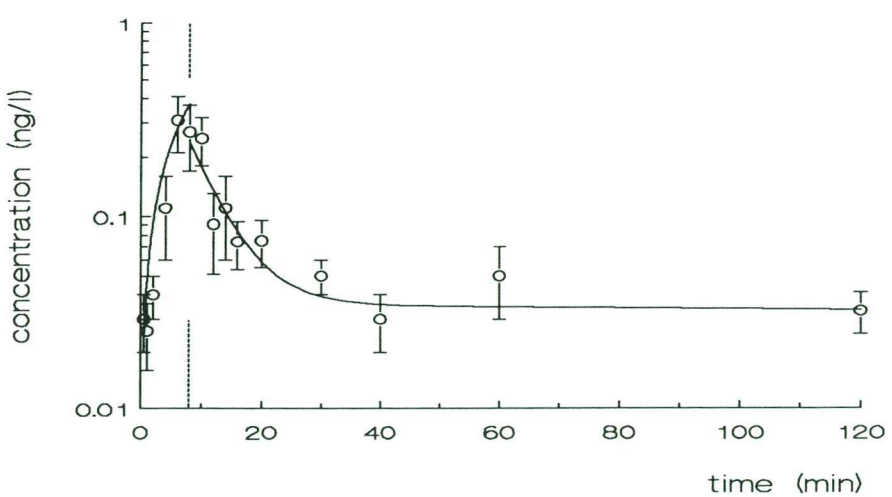

Figure 45. Mean blood concentrations (± s.e.m, n=6) of (-)-sarin versus time during and after 8-min nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 19 ± 2 mg/m^3 of (±)-sarin, which corresponds with 0.4 LCt50. The dotted line marks the end of the exposure period.

Table 58. Toxicokinetic parameters[a] for (-)-sarin in anesthetized, atropinized, and restrained guinea pigs, after nose-only exposure to 19 ± 2 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.4 LCt50.

A (ng/ml)		-55.6	
B (ng/ml)		0.86	
C (ng/ml)		0.036	
D (ng/ml)		55.6	
a (min-1)		0.00085	
b (min-1)		0.18	
c (min-1)		0.00063	
Terminal half-life (min)		(1100)	
Area under	0-8 min	0.8	
the curve	> 8 min	58.5	(0-120 min 5.5)
(ng.min/ml)	total	59.3	(0-120 min 6.3)

[a] The inhalation results were fitted with a discontinuous function: [nerve agent] = $D + A*e^{-at}$ for the absorption phase (0-8 min), and [nerve agent] = $B*e^{-bt} + C*e^{-ct}$ for distribution and elimination (≥ 8 min)

Table 59. Mean acetylcholinesterase (AChE) activities (± s.e.m., n=6), measured in blood samples of anesthetized, atropinized and restrained guinea pigs during and after nose-only exposure to 19 ± 2 mg/m^3 (±)-sarin for 8 min, which corresponds with 0.4 LCt50.

Time (min)	AChE activity ± s.e.m. (n=6) (%)
0	100
0.5	100 ± 3
1	97 ± 2
2	98 ± 2
4	89 ± 2
6	90 ± 5
8	84 ± 6
10	78 ± 6
12	75 ± 5
14	74 ± 6
16	81 ± 5
20	78 ± 6
30	80 ± 3
40	75 ± 5
60	74 ± 5
120	67 ± 5

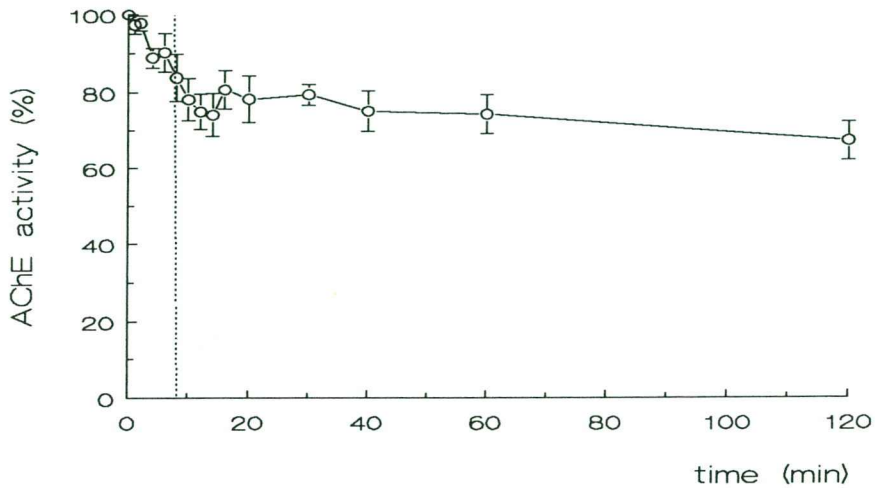

Figure 46. Mean acetylcholinesterase (AChE) activity (± s.e.m., n=6) in blood versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 19 ± 2 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.4 LCt50. The dotted line marks the end of the exposure period.

IV. DISCUSSION

The previously (1, 2) developed gas chromatographic configurations for the analysis of soman stereoisomers in biological samples have been modified with automated injectors in order to improve the daily sample throughput. Furthermore, a new configuration was developed for monitoring the generated nerve agent vapor concentrations in the airstream. In all these configurations, the highly sensitive and selective alkali flame detector was used.

The combination of two-dimensional gas chromatography (MUSIC) with on-column large-volume fluid injection (GLC configuration 2) did not, in spite of our efforts, appear to be a viable option. Nevertheless, large-volume injection in gas chromatography has been documented (27), albeit that successful applications mostly pertain to relatively nonvolatile compounds. Therefore, for the majority of the measurements we resorted to GLC configuration 1, which combines TCT injection with MUSIC. This configuration had already proven its efficacy and reliability in the previous Grant study on the toxicokinetics of C(±)P(±)-soman at low dosages in various species (2). A distinct advantage of TCT injection over on-column large-volume fluid injection is that in TCT injection, the injection volume is practically unlimited, since large volumes of extracts from biological samples can be adsorbed onto the Tenax material. However, we opted primarily for an on-column large-volume fluid injection because such an injection technique can be easily and relatively non-expensively automated, whereas automation of the TCT injection would be costly. Furthermore, the large-volume autosampler stores the samples in vials which are closed with septa, eliminating risks of cross-contamination between the samples.

Near the end of this study, we had the opportunity to invest in a TCT autosampler. Since the results with the on-column large-volume injection were far from promising, we decided to purchase the TCT autosampler, in order to benefit from such an automated system for the determination of the toxicokinetics of (±)-sarin during and after nose-only exposure of guinea pigs. Our experiences with the TCT injector had already proven that this injection principle is most suited for combination with MUSIC. In the TCT autosampler which we purchased, the Carlo Erba TDAS, a valve with a metallic interior serves as the connection with the MUSIC system. Previously, C(±)P(±)-soman stereoisomers were found to degrade upon contact with hot metal surfaces, which occurred occasionally upon manipulations with the metal connectors of the MUSIC system. However, with valve temperatures up to 190 °C, we did not observe degradation of the sarin stereoisomers in the TCT autosampler.

Other potential anticipated problems were degradation of the sarin isomers adsorbed on Tenax and cross-contamination between the samples during storage in the autosampler. As a preventive measure, the tray of the autosampler was thermostatted at 10 °C. By analyzing samples stored up to 4.5 h in the autosampler, we did not find indications that the analytes degraded during this period. Furthermore, no (±)-sarin was detected in the analysis of control tubes which were stored amidst the Tenax tubes containing the analytes, confirming absence of cross-contamination under these conditions.

At this moment some minor technical problems prevent us from performing analyses unattended overnight, such as an unsatisfactory communication between the MUSIC system and the TDAS. In the present situation, the TDAS continues the injection of samples in the event of malfunctioning of the MUSIC system. This may occur, e.g., when the system runs out of solid carbon dioxide, resulting in the loss of these samples. Obviously, this particular problem can be solved easily if the MUSIC sends a signal to the TDAS that an error in the MUSIC system occurs. On the other hand, it would be convenient if the TDAS is restarted by the MUSIC after errors have been rectified by the MUSIC itself.

Upon the start of this project, gas chromatographic resolution of (±)-sarin stereoisomers was already accomplished on an optically active nickel camphorate stationary phase (31,32), as well as on the L-Chirasil Val phase used for analysis of C(±)P(±)-soman stereoisomers. On the former phase the peaks of the two (±)-sarin stereoisomers were very broad, which increased the limit of detection. On the L-Chirasil Val stationary phase the peaks were narrow and fairly symmetrical. Unfortunately the internal standard we intended to use, D_7-(±)-sarin was not completely resolved from the (±)-sarin. The (+)-enantiomer of the internal standard was completely resolved from (±)-sarin, but unfortunately we did not succeed in isolating this enantiomer. As was anticipated, the problem was solved by using the in-house prepared D-Chirasil Val stationary phase, which was custom-coated on a glass capillary column. Although this stationary phase is capable of fully resolving (±)-sarin and D_7-(±)-sarin, we decided not to use this phase in routine analysis of (±)-sarin in biological samples, since we were not absolutely sure that we would be able to produce a new equally well-performing column if our column would break down during the experiments. Obviously, we could make this decision only when we found out that a commercially available ß-cyclodextrin phase resolved D7-(-)-sarin from (±)-sarin, as well as (±)-sarin itself. The peaks of the analytes of interest were narrow and fairly symmetrical, allowing a high sensitivity in combination with NP detection. Since the (-)-isomer of the deuterated internal is easily isolated, this stationary phase was suitable for our purposes.

In recent years the number of commercially available stationary phases for liquid and gas chromatography has increased considerably. Unfortunately, it is very difficult to predict which stationary phase is likely to resolve the analytes of interest. In the case of (±)-sarin, however, earlier studies in our laboratory (33) showed a highly stereoselective interaction of (±)-sarin with α-cyclodextrin in aqueous solution. Based on this observation we anticipated that an optically active stationary phase based on interaction with a cyclodextrin would be suitable. Various cyclodextrin columns were tested, of which only the Cyclodex B column appeared to be capable of resolving (±)-sarin. So far, the performance of this column has not deteriorated, despite the injection of large numbers of extracts from blood samples. Obviously, this has to be mainly accredited to the two-dimensional chromatography, which protects the analytical column from contamination.

We found that the extraction procedure for C(±)P(±)-soman from blood samples is applicable as such to (±)-sarin, albeit with a different internal standard. This is convenient and unexpected. Although (±)-sarin is more volatile than C(±)P(±)-soman, loss of sarin isomers due to co-evaporation when removing ethyl acetate under reduced pressure was not observed. Validation of the extraction procedure demonstrated that the stabilizing procedure with an acidic buffer containing neopentyl sarin and aluminium ions is adequate.
The limit of detection for the sarin isomers, i.e., routinely ca. 5 pg of injected isomer should be sufficient for toxicokinetic studies, since in a 1-ml blood sample this limit of detection corresponds with ca. 10 pg (-)-sarin/ml (ca. 7 pM), based on an absolute extraction recovery of 50 %.

In earlier investigations (1, 2), the toxicokinetics of soman stereoisomers in the guinea pig were determined after intravenous bolus administration of doses corresponding with 2 and 6 LD50. We have now extended this series of studies with a sublethal dose corresponding with 0.8 LD50. The concentrations measured for the two P(-)-soman stereoisomers after administration of this dose, as well as the area under the curve values are very low in comparison with those measured after administration of 2 and 6 LD50, as shown in Figures 47 and 48. An overview of the toxicokinetic parameters calculated from all toxicokinetic experiments is presented in Table 60.
Figure 47 shows that the mean concentration time course of C(+)P(-)-soman seems to be erratic. We have no indications that an interfering peak is present in the chromatograms. We decided not to perform curve fitting on these results, since such a fit would only lead to meaningless parameters.
The terminal half-life after administration of 0.8 LD50 is about three times shorter than at doses corresponding with 2 and 6 LD50 C(±)P(±)-soman (Table 60). The area under the curve values for C(-)P(-)-soman after 2 and 6 LD50 are 228 and 520 ng.min/ml, respectively, i.e., considerably higher values than that obtained after 0.8 LD50 (Table 60). This extreme nonlinearity with dose of the toxicokinetics might be explained by assuming that a relatively large fraction of the C(±)P(±)-soman dose will be rapidly bound to carboxylesterases and cholinesterases in blood.

Table 60. Survey of toxicokinetic parameters[a] of C(+)P(-)-soman, C(-)P(-)-soman and (-)-sarin in anesthetized and atropinized guinea pigs after nose-only exposure for 4-8 min to 0.4 - 0.8 LCt50 of C(±)P(±)-soman or (±)-sarin. Comparative parameters for intravenous bolus, intravenous infusion and intramuscular administration are also given.

	0.8 LCt50 (4 min)		0.8 LCt50 (8 min)		0.4 LCt50 (8 min)		0.1 LCt50 (300 min)		0.8 LD50 (i.v.bolus)		0.8 LD50 (8 min i.v.inf.)		0.8 LD50 (i.m., bolus)		0.8 LCt50 (8 min)	0.4 LCt50 (8 min)	0.8 LD50 (i.v. bolus)
Parameter	C(+)P(-)	C(-)P(-)	C(+)P(-)[b]	C(-)P(-)	C(+)P(-)[c]	C(-)P(-)	C(+)P(-)[d]	C(-)P(-)	C(+)P(-)	C(-)P(-)	C(+)P(-)[b]	C(-)P(-)	C(+)P(-)	C(-)P(-)	(-)-sarin	(-)-sarin	(-)sarin
C_{max}[e] ($ng.ml^{-1}$, ± s.e.m., n=6)	0.5±0.2[f]	1.1±0.3	1.4±0.4	2.7±0.6	0.2±0.2	0.8±0.2	0.0012 ±0.003	0.036 ±0.004	-	-	1.4±0.5	2.5±0.5	0.14 ±0.07	0.4±0.1	1.5±0.5	0.3±0.1	-
A ($ng.ml^{-1}$)	-	-30	-55.4	-35.8	-34.7	-34.7	0.0028	-1.54	-	3.8	16.1	35.8	-	-	-164	-55.6	35.9
B ($ng.ml^{-1}$)	0.72	1.70	32.6	141	1.74	24.8	-	-	-	0.80	123694	12343	-	-	8.6	0.86	0.09
C ($ng.ml^{-1}$)	0.029	0.052	0.83	1.9	0.014	0.14	-	-	-	-	0.18	0.72	-	-	0.11	0.036	-
D ($ng.ml^{-1}$)	-	30	55.4	35.8	34.7	34.7	-0.0027	1.54	-	-	-16.1	-35.8	-	-	164	55.6	-
a (min^{-1})	-	0.0088	0.004	0.0094	0.0014	0.0023	-0.0058	0.000065	-	0.95	- 0.013	-0.0037	-	-	0.0012	0.00085	4.6
b (min^{-1})	0.19	0.20	0.42	0.54	0.28	0.45	-	-	-	0.12	1.44	1.08	-	-	0.26	0.18	0.012
c (min^{-1})	0.011	0.026	0.075	0.083	0.0054	0.0685	-	-	-	-	0.056	0.13	-	-	0.019	0.00063	-
Terminal half-life (min)	63	27	9.2	8.4	-	10.1	-	-	-	5.8	12.4	5.3	-	-	36.5	-	58
Area under curve ($ng \cdot min.ml^{-1}$)																	
0-end exp.	0.6[g]	2.1	7.2	8.5	1.2	2.5					6.7	11.8			6.3	0.8	
end exp.-∞	3.7	5.6	8.8	15.2	3.1	2.7					2.9	4.0			9.1[h]	5.5[i]	
Total	4.3	7.7	16.0	23.7	4.3	5.2	1.4	4.5	-	10.6	9.6	15.8	2.0[g]	8.7[g]	15.4	6.3	15.3
RMV[j] ($ml.min^{-1}$, ± s.e.m.)	37 ± 5		52 ± 3		43 ± 6										31 ± 4	47 ± 6	

[a] The inhalation and infusion results were fitted with discontinuous functions: [nerve agent] = $D + A*e^{-at}$ for the absorption or infusion phase, and nerve agent = $B*e^{-bt} + C*e^{-ct}$ for distribution and elimination.
[b] Assuming a lag time of 2 min.
[c] Assuming a lag time of 4 min.
[d] Assuming a lag time of 60 min.
[e] At end of exposure or infusion period, unless noted otherwise.
[f] At t = 6 min (2 min after end exposure).
[g] Area under the curve measured with the trapezoidal method.
[h] Area under the curve 8 - 120 min: 8.5 $ng.ml.min^{-1}$.
[i] Area under the curve 8 - 120 min.
[j] RMV = respiratory minute volume.

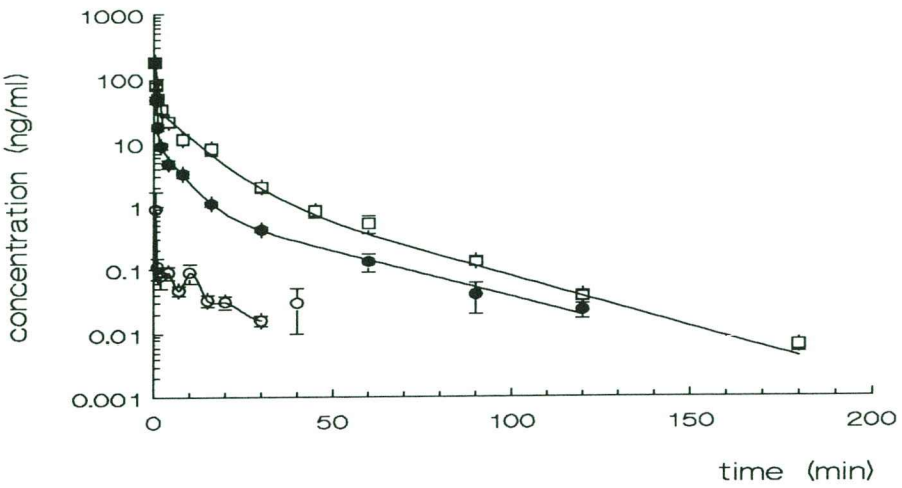

Figure 47. Semilogarithmic plots of mean concentrations in blood (± s.e.m., n=6) of C(+)P(-)-soman versus time after i.v. administration of 6 LD50 (□, 165 μg/kg), 2 LD50 (●, 55 μg/kg), and 0.8 LD50 (O, 22 μg/kg) of C(±)P(±)-soman to anesthetized, atropinized and mechanically ventilated guinea pigs.

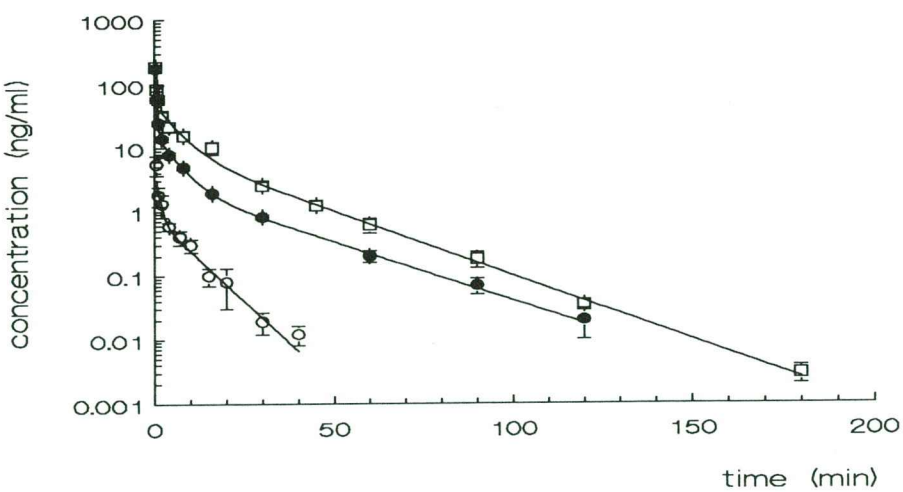

Figure 48. Semilogarithmic plots of mean concentrations in blood (± s.e.m., n=6) of C(-)P(-)-soman versus time after i.v. administration of 6 LD50 (□, 165 μg/kg), 2 LD50 (●, 55 μg/kg), and 0.8 LD50 (O, 22 μg/kg) of C(±)P(±)-soman to anesthetized, atropinized and mechanically ventilated guinea pigs.

An apparatus for generation of nerve agent vapor in air and subsequent nose-only exposure of anesthetized, restrained guinea pigs was developed. The generation of nerve agent vapor via mass flow controllers appeared to be very suitable for our purposes. Finding the optimum design for the generation vial containing the neat nerve agent required some experimentation. The design shown in Figure 6 appeared to provide the most economic use of the agent present in the vial, and was most suitable for generation of stable nerve agent vapor concentrations during long time periods. GLC configuration 3 offered sufficient sensitivity and reproducibility for monitoring of the concentration of nerve agent vapor in air. Due to the injection of air samples with a relative humidity of ca. 70 %, the performance of the detector deteriorated more rapidly than we generally encounter in the toxicokinetic studies. A remedy for this decrease in sensitivity was renewal of the alkali tablet.

A drawback of the original design of the exposure apparatus was the use of synthetic tubing. The adsorption of C(±)P(±)-soman to this synthetic tubing gave rise to long equilibration times after starting nerve agent generation. This problem was solved by replacing the synthetic tubing with glass tubing.

Obviously, much attention was paid to the safety aspects of working with this equipment. An important safety measure is the electronic pressure monitor, which checks whether the Battelle tube is connected gastight to the exposure unit. In addition, in order to minimize risks of accidental fracture of the glass tubing, the generation apparatus, the exposure apparatus and the gas chromatograph were fixed with screws on a mobile working bench. The generation and exposure apparatus were placed inside a cabinet made of perspex, connected to a ventilator which created a slight underpressure in this cabinet. An additional ventilated cabinet was connected to the front side of the exposure unit, surrounding the Battelle tubes, in order to ensure suction of any nerve agent vapor passing the 'underpressure chamber' (see Figure 10) surrounding the Battelle tube. With these additional measures we were confident that risks for the researchers operating the apparatus were minimized.

Upon starting the inhalation studies, we observed only a marginal respiratory signal. This indicated that the nose of the guinea pig did not fit adequately into the rubber mask of the modified Battelle tube. After increasing the pressure on the rear end of the animal, an acceptable respiratory signal was obtained. This solution was suitable for the experiments in which the LCt50 values of C(±)P(±)-soman and (±)-sarin were determined. Unfortunately, in the toxicokinetic studies the carotid cannula became blocked as a result of compressing the animal. After some experimenting we were able to obtain an adequate fit of the animal in the tube by mounting a fork in the tube, which is positioned around the neck of the animal. With this modification, the nose of the animal can be pushed into the rubber mask tightly, without compressing the thorax of the animal or interfering with the cannula.

The inhalation toxicokinetic studies with C(±)P(±)-soman involved nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 0.8 and 0.4 LCt50 of C(±)P(±)-soman in an 8-min period, and to 0.8 LCt50 of C(±)P(±)-soman in a 4-min period. The mean concentration time courses for C(+)P(-)- and C(-)P(-)-soman for these exposures are compared in Figures 49 and 50, respectively. These figures show that the absorption of C(+)P(-)- and C(-)P(-)-soman is very rapid, since the concentrations do not increase further after terminating the exposure. The measured concentrations are reasonably high in the period after exposure, when compared with those measured after intravenous bolus administration of 0.8 LD50 of C(±)P(±)-soman (see Figures 3 and 4). This may suggest the presence of a depot in the upper respiratory tract, from which absorption continues after termination of the exposure.

In all of these respiratory exposures, the absorption phase of the C(+)P(-)-isomer lags somewhat behind that of the C(-)P(-)-isomer, in spite of the ca. 20 % excess of the former isomer over the latter isomer in C(±)P(±)-soman. We observed this phenomenon previously after subcutaneous administration (34). A straightforward explanation for this difference between the two P(-)-isomers seems not available. Possibly, this phenomenon is partly caused by differences in binding of the two isomers at the absorption site in the respiratory tract. The value for the terminal half-life observed for respiratory exposure to 0.8 LCt50 of C(±)P(±)-soman is in reasonable agreement with the value calculated for an equitoxic intravenous bolus (Table 60).

During and after exposure to 0.4 LCt50 of C(±)P(±)-soman in 8 min the maximum concentrations of C(±)P(-)-soman in this experiment are ca. fourfold lower than the mean maximum concentrations after an 8-min respiratory exposure to a dose corresponding with 0.8 LCt50 of C(±)P(±)-soman. Furthermore, the area under the curve for the former experiment is ca. fourfold lower, indicating nonlinear toxicokinetics at low dosages.

In comparison with exposure to a dose corresponding with 0.8 LCt50 for 8 min, the concentrations of the soman stereoisomers are lower after exposure for 4 min. It was anticipated that the concentrations would be higher, since the animals are exposed to a twofold higher concentration of C(±)P(±)-soman. Furthermore, the time course of AChE inhibition is quite similar to that for 8-min exposure. These observations suggest that the animals inhaled a lower fraction of the dose than in the 8-min exposure, which is supported by the data obtained for the respiratory parameters. Comparison of the respiratory minute volumes for both experiments suggests that in the 4-min exposure, the animals have only inhaled 71 % of the dose, when compared with the 8-min exposure, assuming that the retention of the agent is not altered. The AUC value of the 4-min exposure is ca. fourfold lower than that of the 8-min exposure, which suggest nonlinear toxicokinetics due to shorter exposure time periods.

The data in Table 39 suggest that the maximum blood level of C(+)P(-)-soman is reached a few minutes after cessation of the exposure, which is not observed for the 8-min exposures. Further investigations should reveal whether this phenomenon is real and

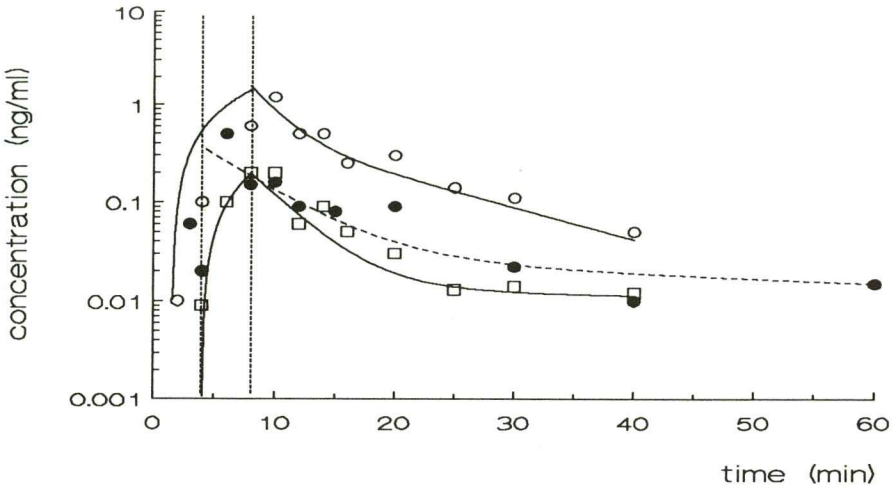

Figure 49. Semilogarithmic plots of mean concentrations in blood (± s.e.m., n=6) of C(+)P(-)-soman versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to C(±)P(±)-soman vapor in air yielding 0.4 (□) 0.8 (○) LCt50 in 8 min, or 0.8 LCt50 in 4 min (●)

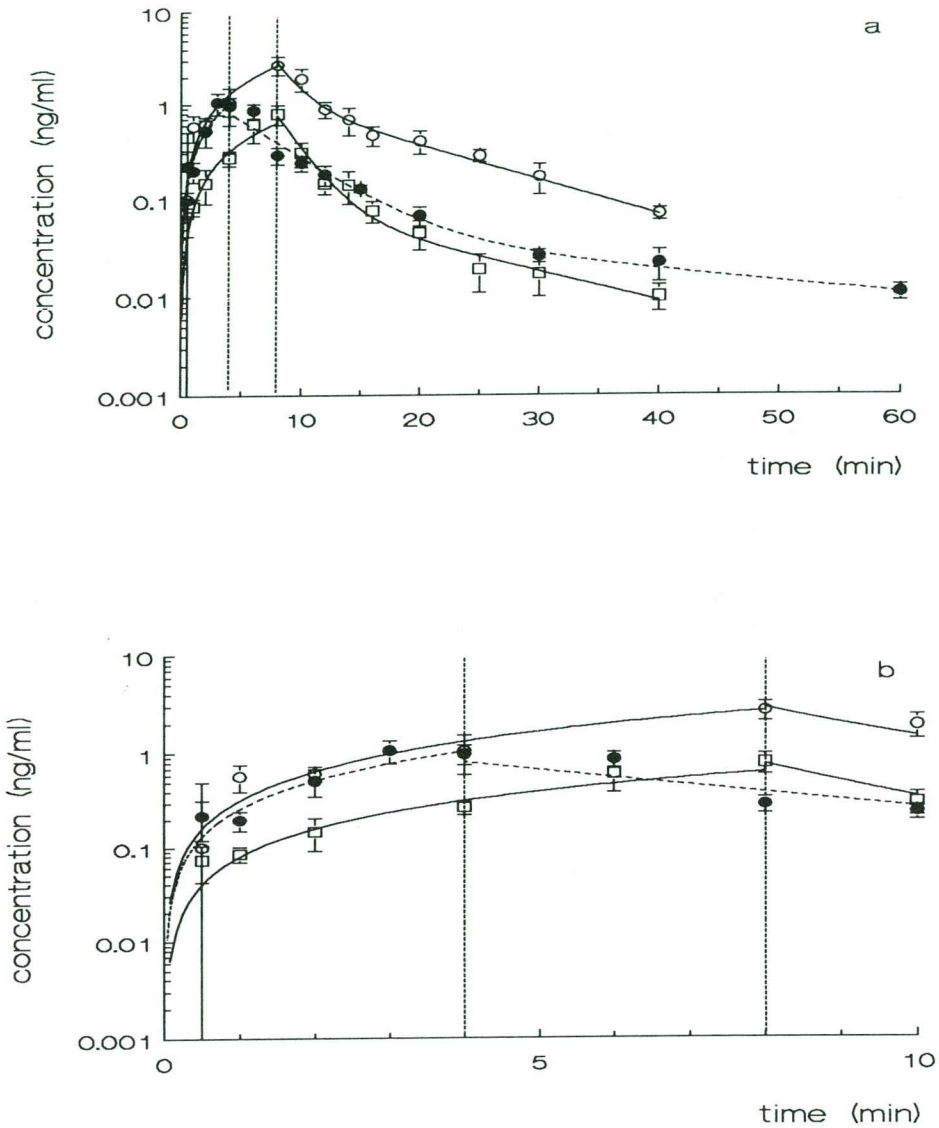

Figure 50. Semilogarithmic plots of mean concentrations in blood (± s.e.m., n=6) of C(-)P(-)-soman versus time during and after nose-only exposure of anesthetized, atropinized and restrained guinea pigs to C(±)P(±)-soman vapor in air yielding 0.4 (□) 0.8 (○) LCt50 in 8 min, or 0.8 LCt50 in 4 min (●). a. all data; b. data for the first 10 min plotted on expanded scale.

might occur more explicitly at exposure times < 4 min, which are likely to be encountered under battlefield conditions.

The retention of C(±)P(±)-soman after nose-only exposure of guinea pigs is unknown. Upon inspiration, the strongly hydrophilic nerve agent is likely to be deposited on the mucosa in the respiratory tract. Upon expiration, redistribution between the mucosa and the air to be exhaled will occur. Only a limited number of data are available on the retention of (toxic) vapors and gases in the respiratory tract (8, 9, 35). In human studies, for hydrogen cyanide gas inhaled through the mouth, the retention in the lung appeared to be ca. 60 %, whereas nasal retention was as low as ca. 20 % (35). For (±)-sarin, retention in resting and exercising men was ca. 85 and 79 %, respectively, whereas retention in the monkey and dog was 77 and 73 %, respectively (8). Obviously, these data cannot be differentiated for the two sarin isomers. An indication of the retention of C(±)P(±)-soman in the guinea pig can be obtained as follows: from the area under the curve for intravenous infusion the AUC per ng of administered C(±)P(±)-soman was calculated. Next, the amount of systemically available C(±)P(±)-soman was calculated from the AUC of the respiratory exposure. This value was related to the intake, which was calculated as the product of RMV, C(±)P(±)-soman concentration and exposure time. For the 8-min nose-only exposure to 0.8 LCt50, a systemic availability of ca. 70 % was calculated for C(±)P(-)-soman in comparison with the 8-min intravenous infusion of 0.8 LD50 (cf. AUC values in Table 60).
During exposure, and in the first few minutes thereafter, the blood AChE activity drops fairly rapidly down to ca. 4-10 % of control activity, depending on the concentration of C(±)P(±)-soman to which the animals were exposed. Figure 51 shows that in comparison with exposure to C(±)P(±)-soman yielding 0.8 LCt50, the AChE activity decreases more slowly after exposure to 0.4 LCt50, whereas during the 4-min exposure to 0.8 LCt50, the AChE activity appears to decrease more rapidly than after 8-min equitoxic exposure.

With respect to the reliability of the respiratory monitoring in the present study, it must be reported that the flow-rate of 1 l/min per animal, which is necessary to maintain the desired C(±)P(±)-soman or (±)-sarin concentration, causes a slight (≤ 5 mbar) increase in pressure which influences the respiration of the animals. Figure 52 represents an example of a respiratory sinus of an anesthetized control guinea pig in the nose-only exposure apparatus before and during exposure to an air flow of 1 l/min with an overpressure of ca. 5 mbar. The first part of the curve (panel A) represents the respiratory pattern when the animal is breathing ambient air at atmospheric pressure, whereas the second part (panel B) represents breathing during overpressure.
The slight overpressure displaces the offset of the differential pressure reading, decreases the respiratory frequency (panel C, RF), increases the durations of inspiration (TI) and expiration (TE), and induces a time pause (TP) between expiration and inspiration, resulting in a depression of the respiratory minute volume (panel D, RMV). Not all animals are equally sensitive to this overpressure. In

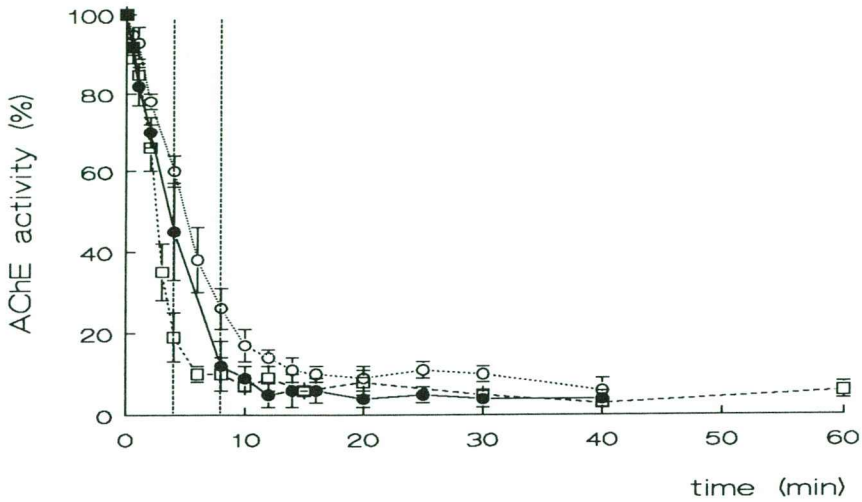

Figure 51. Time course of mean acetylcholinesterase (AChE) activity (± s.e.m., n=6) in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to concentrations of C(±)P(±)-soman yielding 0.4 (O) or 0.8 (●) LCt50 in 8 min, or 0.8 LCt50 in 4 min ( ).

most animals respiration was less affected than shown in Figure 52 (RMV decrease ≤ 30 %).

Exposure to C(±)P(±)-soman or (±)-sarin under the conditions described in this report did not influence the respiratory profile as observed when the animal was breathing clean air with overpressure (panel B). Although blood AChE activity was inhibited to a large extent at the end of the short-term (4 or 8 min) and long-term (300 min) exposures, the enzyme activity in the diaphragm and brain was inhibited to a much smaller extent. This may explain why the RMV is not influenced in the exposure experiments. Evidence for this assumption is obtained from the results of the 5-h exposure to 0.1 LCt50 of C(±)P(±)-soman, in which residual AChE activities were measured in the diaphragm and brain at the end of the experiments. In these target organs, the AChE activity was not significantly inhibited. These suggestions appear to be in discrepancy with the findings of Wolthuis et al. (36), who reported that nearly lethal doses of C(±)P(±)-soman, administered as an intravenous bolus to rats, appeared to act predominantly centrally. It may well be that the route and rate of administration strongly influence the distribution of the toxicant, with major consequences for the level of inhibition of AChE in the target organs and thus for (pre)treatment. This aspect needs to be further explored. Experiments beyond the scope of this study showed a clear depression of RMV during exposure of animals for longer time periods or to higher concentrations of the nerve agent vapor than performed in this study. A similar depression was observed after intravenous injection of

doses corresponding with 1-2 LD50 of C(±)P(±)-soman. Ultimately, this resulted in the death of these animals.

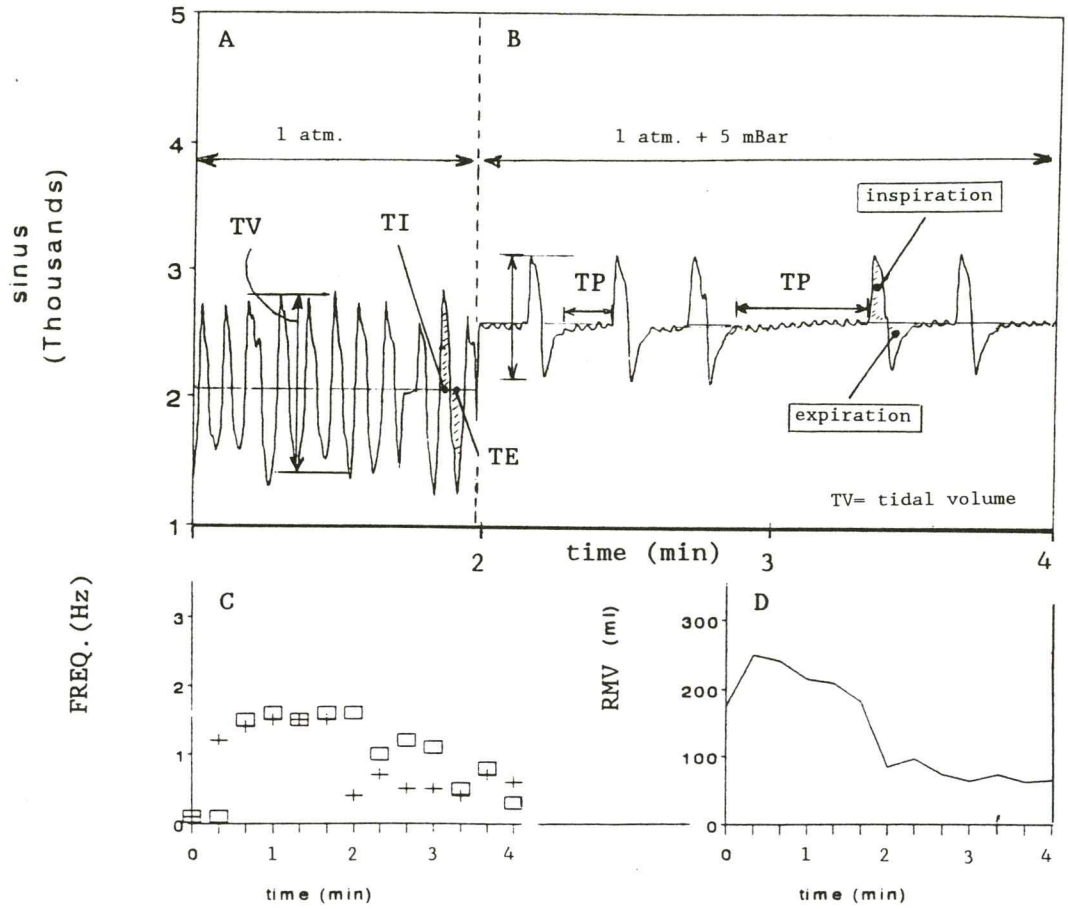

Figure 52. Recording of the respiration of an anesthetized control guinea pig in the nose-only exposure apparatus, before and during breathing clean air with a slight overpressure.

In the 5-h nose-only exposure of anesthetized, atropinized and restrained guinea pigs to a concentration of C(±)P(±)-soman vapor in air, yielding 0.1 LCt50, problems were anticipated with respect to the generation of a constant nerve agent vapor concentration for such a long period. Fortunately, it appeared that the generated concentrations were very stable throughout the 5-h period and variation was well within the 10 % margin. The use of a larger sample loop in GLC configuration 4 enabled a reliable monitoring of the generated concentrations. The animals endured their 5-h restraint in the Battelle tube very well. Most of them gradually became conscious during the experiment. Our initial plan to reinject anesthesia during the experiment was abandoned, since generally the animals remained calm showing regular respiratory signals. During the experiment, the respiratory minute volume gradually increased ca. 20 % in most

animals, due to the diminishing anesthesia. Only two of the animals received additional ketamine, since they became restless, which was apparent from irregular respiratory patterns.
Figure 26 indicates that there is a lag time of ca. 30 min for the appearance of the C(+)P(-)-soman in blood. The concentrations of C(+)P(-)- and C(-)P(-)-soman are gradually built up during the exposure to values which are surprisingly high for such a low dose, especially when compared with the concentrations measured after exposure to 0.4 LCt50 in 8 min. This is reflected in the area under the curve for C(-)P(-)-soman in these experiments (Table 60). Prolonged exposure to low concentrations of nerve agent is relevant for unprotected military personnel performing duty in 'toxic-agent-free areas', e.g., in medical units where CW casualties are treated. Supposedly, exposure to 0.1 LCt50 in a 5-h time period is an acceptable subchronic exposure to nerve agents, since acute exposure to a dose corresponding with 0.1 LD50 will cause just observable systemic toxic effects in humans (19). However, this experiment indicates that a 5-h exposure yielding 0.1 LCt50 of C(±)P(±)-soman leads to blood levels of C(±)P(-)-soman which can and are indeed observed to cause significant blood AChE inhibition (> 30 %) after ca. 2.5 h. Nevertheless, brain AChE is only inhibited to a small extent, minimizing the risk of a centrally induced incapacitation. These results suggest that, with more extensive investigations, 'safe' periods of exposure to such concentrations of nerve agent can be defined.

Figures 53 and 54 show that the concentration-time profiles of C(+)P(-)- and C(-)P(-)-soman during 8-min intravenous infusion are in close agreement with those measured during 8-min respiratory exposure for an equitoxic dose, which suggests that indeed the absorption of C(±)P(±)-soman after respiratory exposure is very rapid. Furthermore, after intravenous infusion, the concentration build-up of C(+)P(-)-soman lags behind that of the C(-)P(-)-isomer to more or less the same extent as observed for 8-min nose-only exposure to 0.8 LCt50 of C(±)P(±)-soman. Since no absorption process takes place after intravenous infusion, this phenomenon is likely to be due to a more rapid binding of C(+)P(-)-soman to covalent binding sites in comparison with C(-)P(-)-soman. After nose-only exposure stereoselective binding at the absorption site may contribute to the intensity of this phenomenon.
Subsequently, after the infusion has stopped, the blood levels of C(±)P(-)-soman decrease much faster than after respiratory exposure. The area-under-the-curve values for the absorption phases of both administration routes are nearly identical, whereas the postexposure value for respiratory exposure is ca. fourfold higher than that for intravenous infusion (cf. Table 60). This may have important consequences for (pre)treatment.

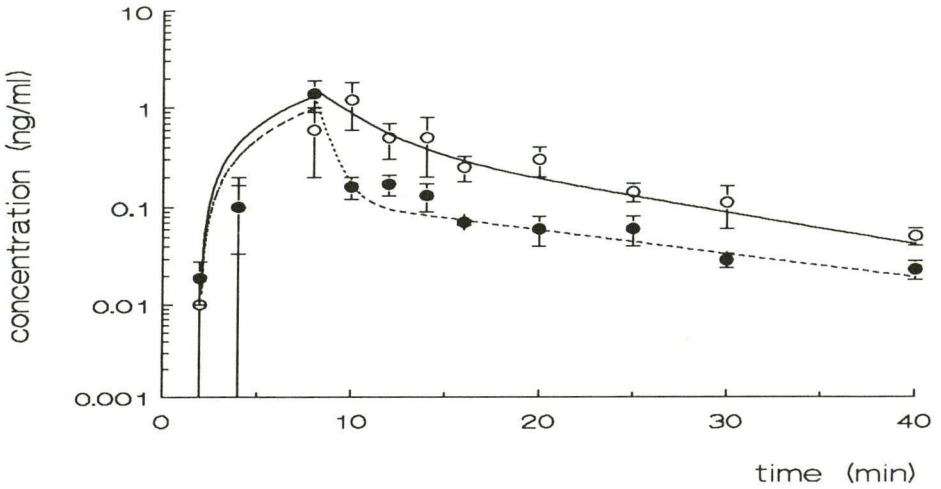

Figure 53. Semilogarithmic plot of the mean concentrations of C(+)P(-)-soman in blood of anesthetized and atropinized guinea pigs during and after an 8-min nose-only exposure to 0.8 LCt50 (O) and an 8-min intravenous infusion of 0.8 LD50 (●) of C(±)P(±)-soman.

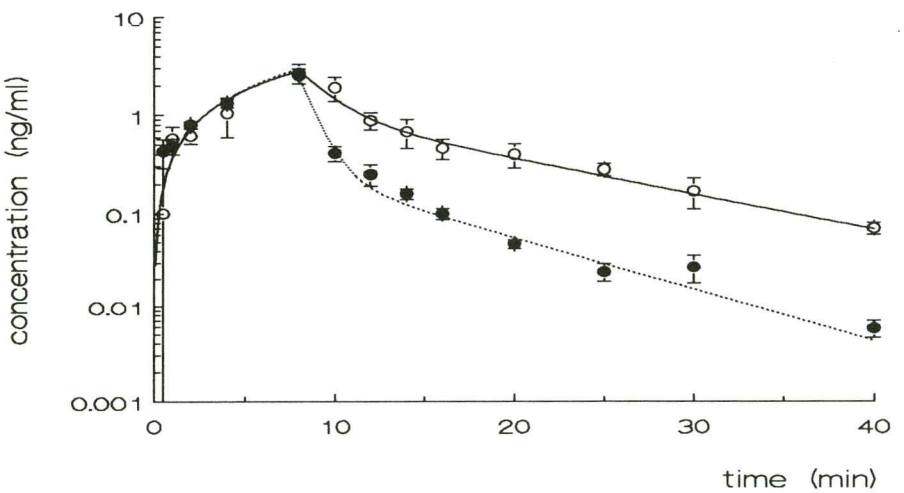

Figure 54. Semilogarithmic plot of the mean concentrations ± s.e.m. (n=6) of C(-)P(-)-soman in blood of anesthetized and atropinized guinea pigs during and after an 8-min nose-only exposure to 0.8 LCt50 (O) and an 8-min intravenous infusion of 0.8 LD50 (●) of C(±)P(±)-soman.

Since the relationship between exposure to 0.8 LCt50 and intravenous infusion of a dose equivalent to 0.8 LD50 is not straightforward, we suggest that with some additional infusion subsequent to the time span of the respiratory exposure, such an infusion may become a suitable substitute for respiratory exposure.
Figure 55 shows that the time courses of AChE activity after intravenous infusion and respiratory exposure to an equitoxic dose are nearly identical.

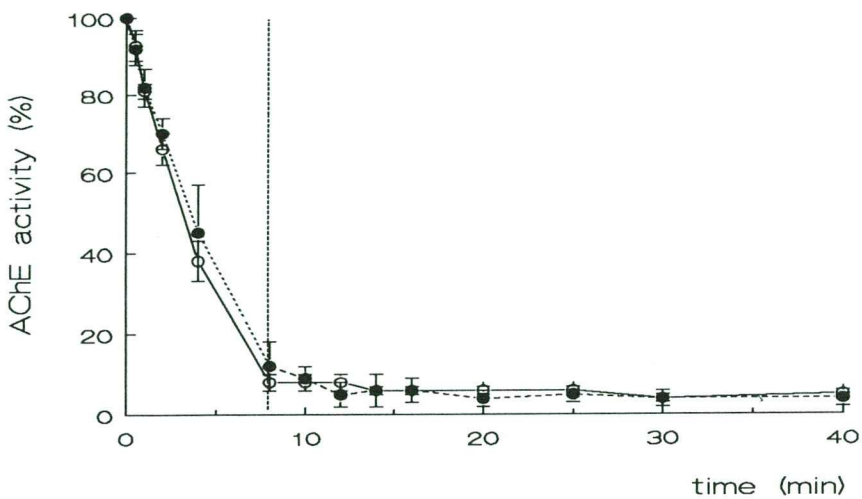

Figure 55. Mean acetylcholinesterase (AChE) activities ± s.e.m. (n=6) in blood of anesthetized and atropinized guinea pigs during and after an 8-min nose-only exposure to 0.8 LCt50 (●) and an 8-min intravenous infusion of 0.8 LD50 (O) of C(±)P(±)-soman.

After intramuscular bolus administration it seems that the absorption of soman stereoisomers is discontinuous and proceeds with some oscillation, as if the compound is influencing its own absorption from the injection site. Figures 56 and 57 show that up to 20 min after intramuscular bolus administration of C(±)P(±)-soman the concentration-time profiles of C(+)P(-)- and C(-)P(-)-soman are quite different from those measured after nose-only exposure to an equitoxic dose. After that time-point, the concentrations for the two enantiomers are in reasonable agreement for both administration routes. The low area under the curve value calculated for intramuscular bolus administration (0-40 min), i.e., 10.7 ng.min/ml for the two P(-)-isomers, is 46% less than the corresponding value for intravenous infusion (cf. Table 60), indicating a low systemic availability for the intramuscular route.
A relatively slow inhibition profile of blood AChE is observed, which is in accordance with the slow build-up of C(±)P(-)-isomers. The discontinuities in the progression of AChE inhibition after

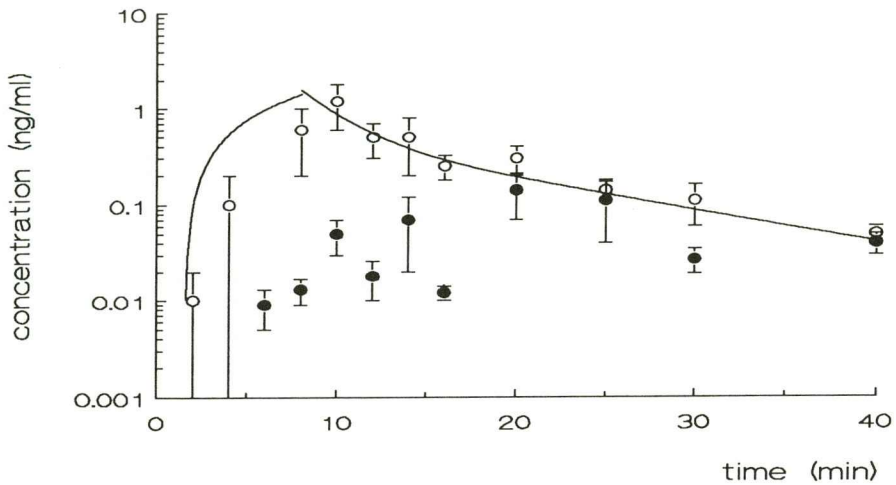

Figure 56. Semilogarithmic plot of the mean concentrations ± s.e.m. (n=6) of C(+)P(-)-soman in blood of anesthetized and atropinized guinea pigs during and after an 8-min nose-only exposure to 0.8 LCt50 (O) and after intramuscular bolus administration of 0.8 LD50 (●) of C(±)P(±)-soman.

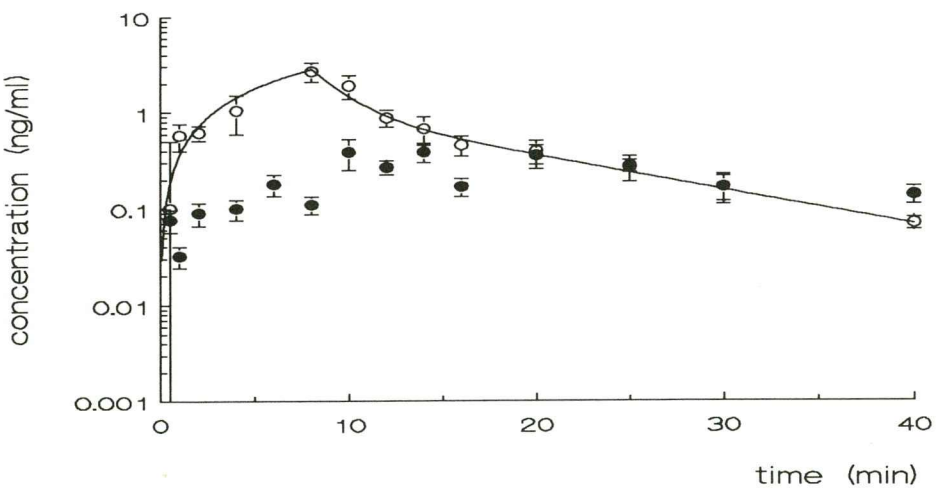

Figure 57. Semilogarithmic plot of the mean concentrations ± s.e.m. (n=6) of C(-)P(-)-soman in blood of anesthetized and atropinized guinea pigs during and after an 8-min nose-only exposure to 0.8 LCt50 (O) and after intramuscular bolus administration of 0.8 LD50 (●) of C(±)P(±)-soman.

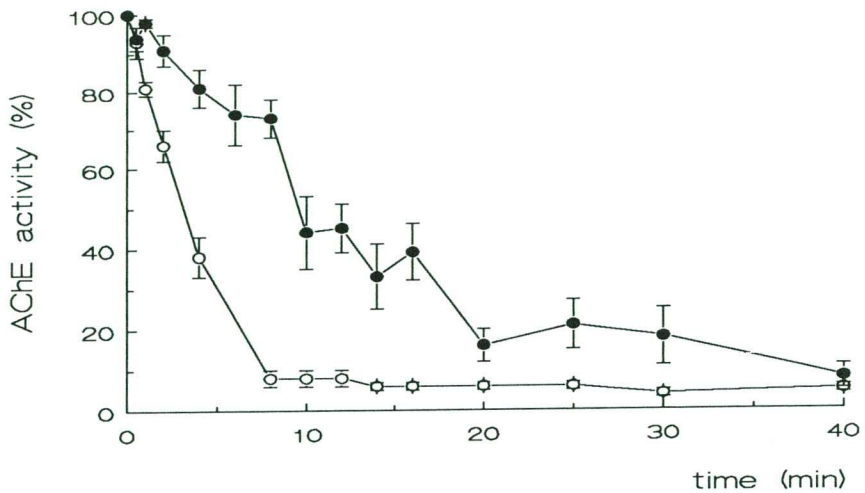

Figure 58. Mean acetylcholinesterase (AChE) activities ± s.e.m. (n=6) in blood of anesthetized and atropinized guinea pigs during and after an 8-min nose-only exposure to 0.8 LCt50 (O) and after intramuscular bolus administration of 0.8 LD50 (●) of C(±)P(±)-soman.

intramuscular administration reflect roughly the above-mentioned oscillations in the blood levels of the C(±)P(-)-soman isomers. Figure 58 shows that the progressive inhibition of blood AChE after intramuscular bolus injection is not comparable to that measured after 8-min nose-only exposure to an equitoxic dose.
These results suggest that intramuscular bolus administration is not a suitable substitute for respiratory exposure to C(±)P(±)-soman.

In this study we have now performed the first toxicokinetic studies with (±)-sarin. The 24-h LD50 of (±)-sarin in guinea pigs after intravenous bolus administration appeared to be slightly lower than that of C(±)P(±)-soman, i.e., 24 μg/kg versus 27.5 μg/kg.
When comparing (Table 60) the toxicokinetic data for (-)-sarin with those of C(-)P(-)-soman after intravenous bolus administration of doses of (±)-sarin and C(±)P(±)-soman corresponding with 0.8 LD50, some striking differences can be noted. The distribution phase of (-)-sarin is nearly 10 times more rapid than that of C(-)P(-)-soman, whereas its elimination phase is ca. 10 times slower. The latter finding is rather surprising, since it was not anticipated that (-)-sarin would be more persistent than C(-)P(-)-soman. We are certain that this is not an artefact, resulting from a malfunctioning stabilizing buffer. Our validation experiments (cf. III-14b) indicate that (-)-sarin is not regenerated from binding sites during the sample pretreatment. Further studies should reveal whether slower rates of (enzymatic) hydrolysis and of binding might explain the persistence of (-)-sarin relative to that of C(±)P(-)-soman.

The 24-h LCt50 for 8-min exposure of anesthetized, restrained guinea pigs, in this study determined to be 376 mg.min/m^3, is in close agreement with a reported value (8) of 340 mg.min/m^3 for 0.86 min exposure of unanesthetized(?) guinea pigs. As was the case with the acute toxicity after intravenous bolus administration, the acute respiratory toxicity is higher for (±)-sarin than for C(±)P(±)-soman when expressed as LD50 and LCt50 values, respectively. However, on a molar basis, the toxicities of these agents are not significantly different.

The maximum concentration of (-)-sarin after respiratory exposure is comparable to that of C(-)P(-)-soman for an equitoxic dose.

The terminal half-life of (-)-sarin appears to be nearly an order of magnitude longer than that of C(-)P(-)-soman.

Comparison of the concentration-time courses for (-)-sarin after 8-min nose-only exposure yielding 0.4 and 0.8 LCt50 (Figure 59) seems to indicate nonlinearity of the toxicokinetics with dose. The areas under the curve (Table 60) cannot be compared for these experiments, since the $AUC_{0-\infty}$ for exposure to 0.4 LCt50 is unrealistically high due to the extremely slow elimination phase as determined via curve fitting. The AUC values for 0-120 min (Table 60) for both exposures do not indicate a significant nonlinearity. However, taking into account that the RMV during exposure to the lower dose is approximately 1.5-fold higher than for exposure to 0.8 LCt50 (Table 60), it should be concluded that the toxicokinetics are nonlinear with dose, as is the case for similar exposures to C(±)P(±)-soman.

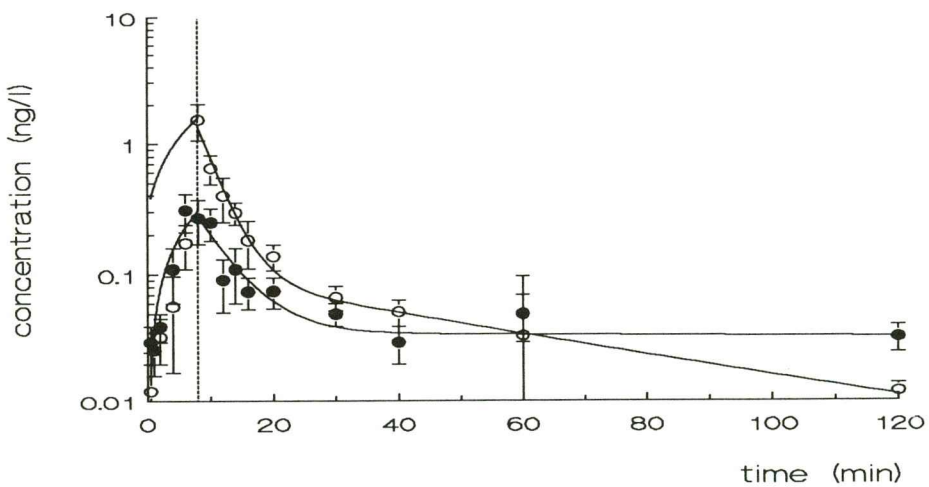

Figure 59. Semilogarithmic plots of mean concentrations of (-)-sarin in blood versus time during and after nose-only exposure to (±)-sarin vapor in air yielding 0.4 (●) or 0.8 (○) LCt50 in 8 min. The dotted line marks the end of the exposure period.

Figure 60 shows the blood AChE inhibition during and after nose-only exposure to 0.4 and 0.8 LCt50 of (±)-sarin. Upon exposure to 0.4 LCt50 of (±)-sarin, AChE is only inhibited to a small extent, when compared with exposure to 0.8 LCt50. Furthermore, the extent of blood AChE inhibition is less than upon exposure to C(±)P(±)-soman, in particular for exposure to 0.4 LCt50.

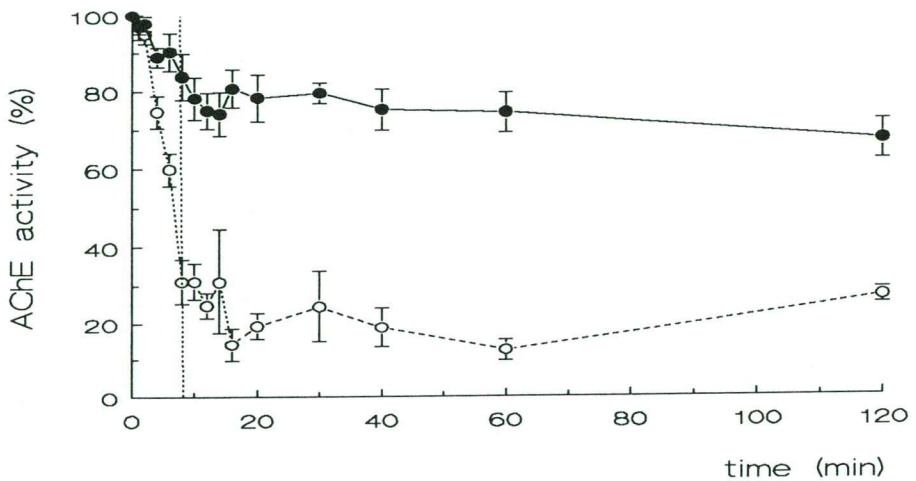

Figure 60. Mean acetylcholinesterase (AChE) activity ± s.e.m. (n=6) in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to concentrations of (±)-sarin for 8 min, yielding 0.4 (●) or 0.8 (O) LCt50. The dotted line marks the end of the exposure period.

The course of blood AChE inhibition was calculated from the measured concentration-time profiles of (-)sarin and of the C(+)P(-)- and C(-)P(-)-isomers of soman in order to try to correlate these profiles with the measured progressive inhibition of blood AChE. The calculations were simply based on a bimolecular reaction of the organophosphate present in blood with blood AChE. Rate constants of inhibition were assumed in these calculations and were varied until the optimum fit was achieved. The results of these calculations for the two (±)-sarin studies and for one C(±)P(±)-soman study (4-min exposure to 0.8 LCt50) are shown in Figures 61-63. Reasonable correlations between the predicted and measured AChE activity profiles were obtained for the two (±)-sarin studies, since only slightly different rate constants of inhibition had to be assumed for the two studies, i.e., $1.1*10^7$ and $1.6*10^7$ $M^{-1}min^{-1}$.
For the studies performed with C(±)P(±)-soman, it was not possible to obtain an adequate fit, except for the 5-h exposure study in which only the absorption phase of the toxicokinetic experiments was processed. In general, a much higher rate constant had to be used for inhibition during the absorption phase than during the elimination phase in order to obtain reasonable fits in both phases (see Figure 63 for an example). Since the blood levels of C(+)P(-)-soman lag

behind those of C(-)P(-)-soman, the relative concentrations of the two P(-)-isomers, and consequently the contributions of the isomers to AChE inhibition, are different in the absorption and elimination phases. However, no differentiation was made in these calculations between the inhibition rate constants for C(+)P(-)- and C(-)P(-)-soman, which probably explains the discrepancy between the calculated and measured AChE activities. This explanation is supported by the observation that the calculated AChE activities for the 5-h exposure study, in which the concentrations of the two isomers differ by an approximately constant factor, correlate very well with the measured values. Furthermore, the predictions of AChE inhibition by (-)-sarin following exposure to (±)-sarin are in close agreement with the measured values.

As a further support, calculations were carried out assuming a 10-fold higher inhibition rate constant for C(-)P(-)-soman than for C(+)P(-)-soman. Results are presented in Figures 64-68. Reasonable fits were obtained for four out of five toxicokinetic studies, using almost identical inhibition rate constants in all cases for C(-)P(-)-soman, i.e., $(0.8\text{-}0.9)*10^8$ $M^{-1}min^{-1}$, and at a 10-fold lower level for C(-)P(-)-soman, i.e., $(0.08\text{-}0.09)*10^8$ $M^{-1}min^{-1}$. Only the AChE activities measured in the 8-min exposure to 0.8 LCt50 C(±)P(±)-soman could not be predicted in this way from the measured C(±)P(-)-soman blood levels (Figure 64). Although these results indicate a simple correlation between the organophosphate levels present in blood and the extent of blood AChE inhibition following exposure of the guinea pig to the agent, *in vitro* data on the rate constant for inhibition of blood AChE from the guinea pig by C(±)P(-)-soman and (-)-sarin will be needed for final proof.

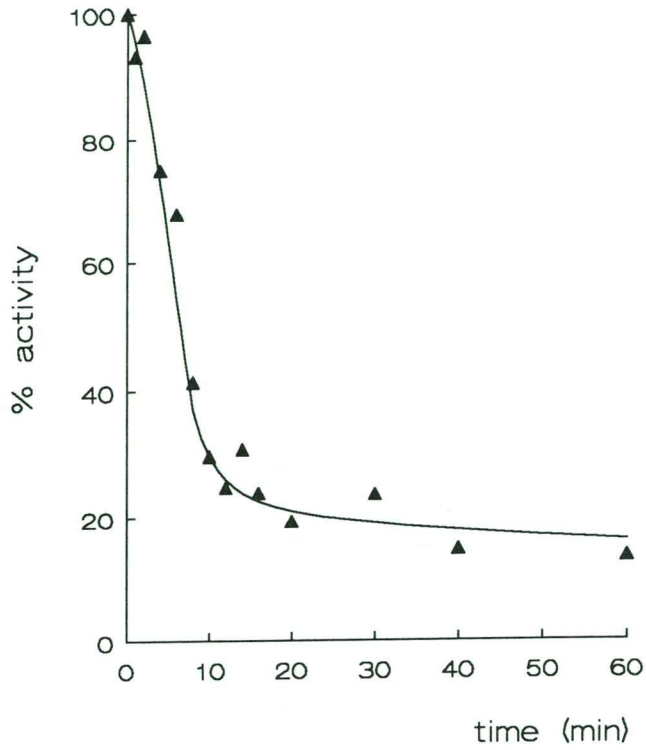

Figure 61. Acetylcholinesterase (AChE) activities in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 38 ± 4 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.8 LCt50. The solid line depicts the profile for AChE inhibition calculated from the measured blood concentrations of (-)-sarin, assuming an inhibition rate constant of $1.6*10^7$ $M^{-1}min^{-1}$.

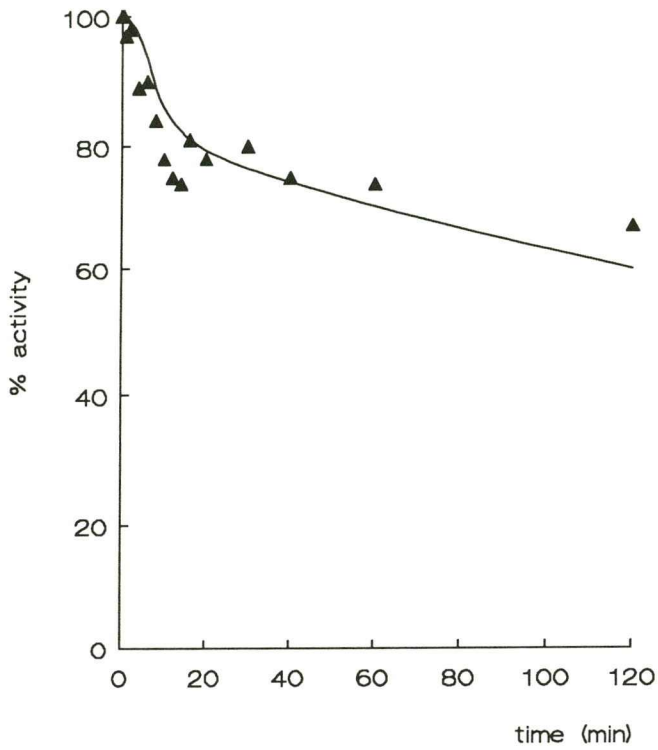

Figure 62. Acetylcholinesterase (AChE) activities in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 19 ± 2 mg/m^3 of (±)-sarin for 8 min, which corresponds with 0.4 LCt50. The solid line depicts the profile for AChE inhibition calculated from the measured blood concentrations of (-)-sarin, assuming an inhibition rate constant of $1.1*10^7$ $M^{-1}min^{-1}$.

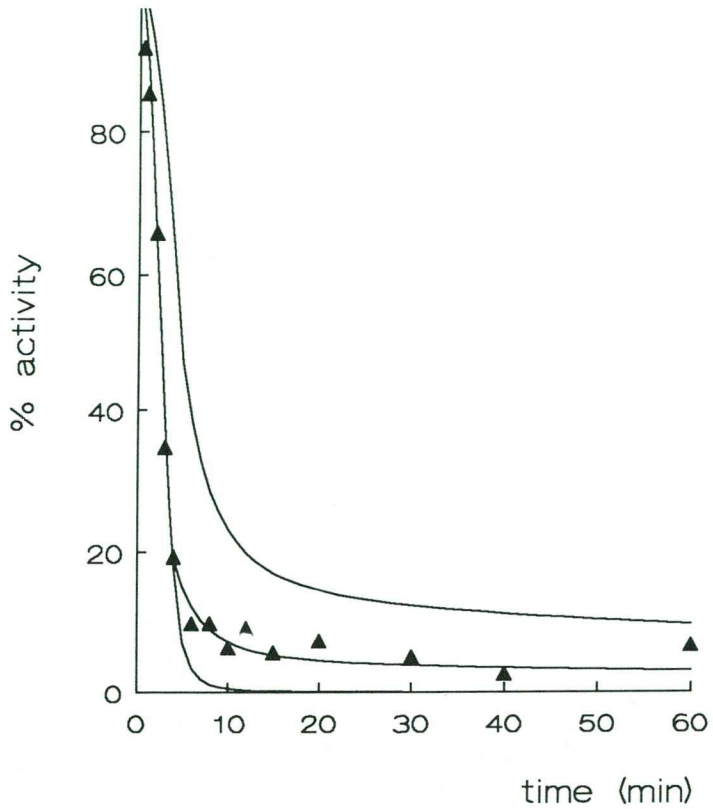

Figure 63. Acetylcholinesterase (AChE) activities in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 96 ± 10 mg/m^3 of C(±)P(±)-soman for 4 min, which corresponds with 0.8 LCt50. The lines depict the profile for AChE inhibition calculated from the measured blood concentrations of C(±)P(-)-soman, assuming inhibition rate constants for C(±)P(-)-soman of $1.5*10^8$ $M^{-1}min^{-1}$ (——) and $0.4*10^8$ $M^{-1}min^{-1}$ (--- and ...). One of the profiles (...) was only calculated for the elimination phase of the toxicokinetics, i.e., the time period 4 - 60 min.

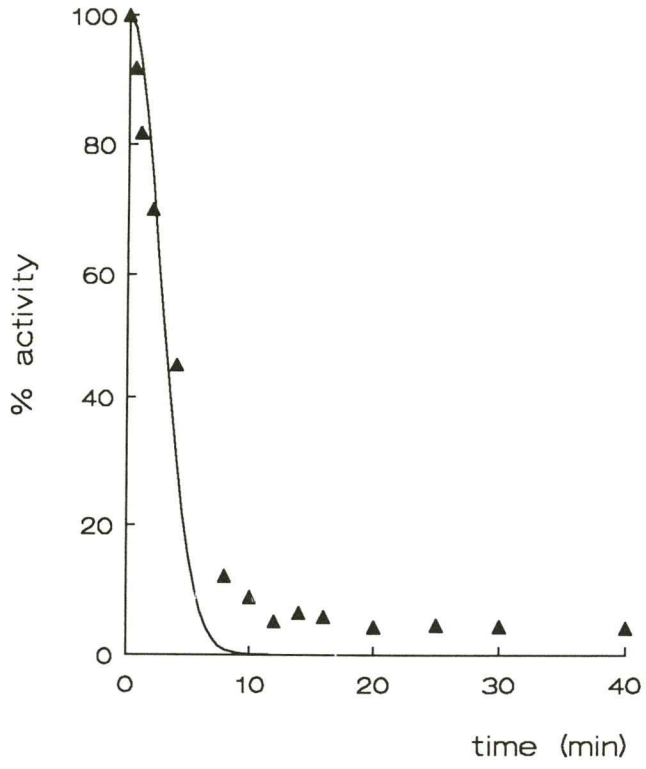

Figure 64. Acetylcholinesterase (AChE) activities in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 48 ± 5 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.8 LCt50. The solid line depicts the profile for AChE inhibition calculated from the measured blood concentrations of C(±)P(-)-soman, assuming inhibition rate constants for C(-)P(-)- and C(+)P(-)-soman of $0.8*10^8$ and $0.08*10^8$ $M^{-1}min^{-1}$, respectively.

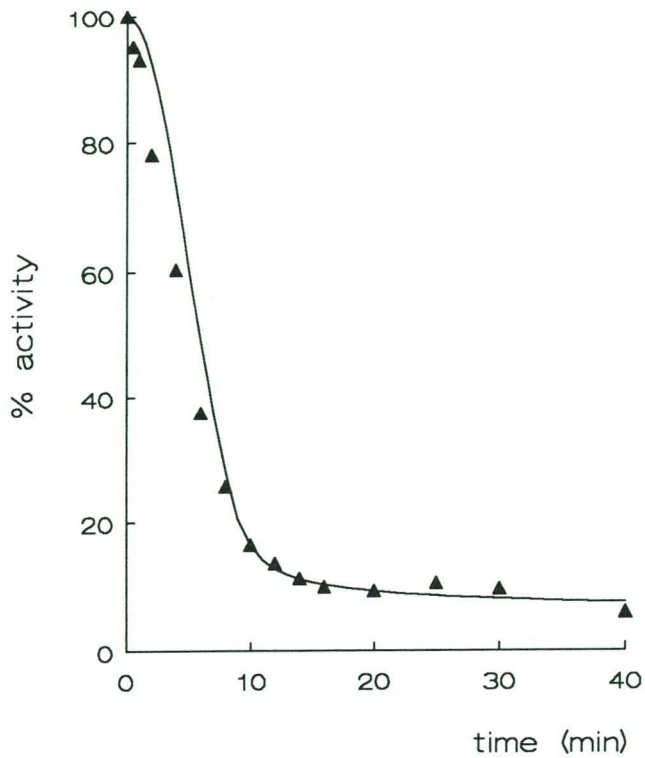

Figure 65. Acetylcholinesterase (AChE) activities in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 24 ± 2 mg/m^3 of C(±)P(±)-soman for 8 min, which corresponds with 0.4 LCt50. The solid line depicts the profile for AChE inhibition calculated from the measured blood concentrations of C(±)P(-)-soman, assuming inhibition rate constants for C(-)P(-)- and C(+)P(-)-soman of $0.9*10^8$ and $0.09*10^8$ $M^{-1}min^{-1}$, respectively.

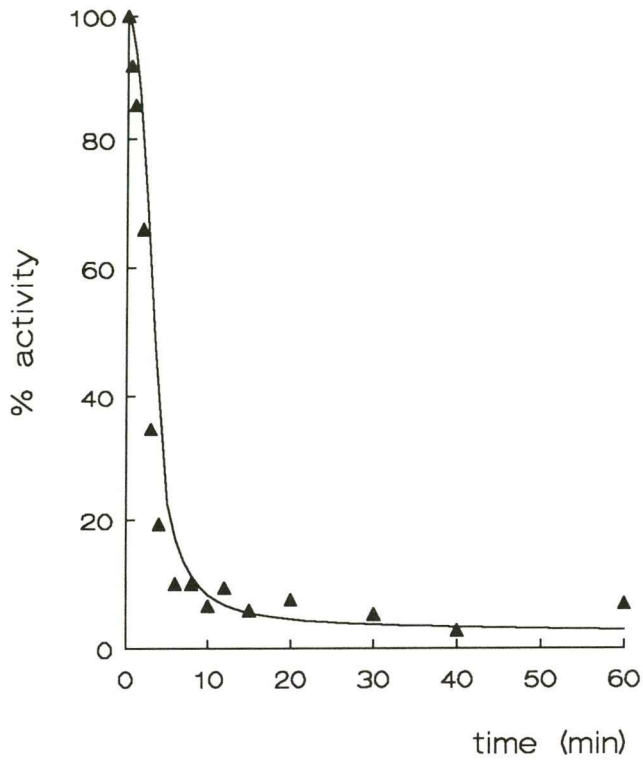

Figure 66. Acetylcholinesterase (AChE) activities in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 96 ± 10 $\mu g/m^3$ of C(±)P(±)-soman for 4 min, which corresponds with 0.8 LCt50. The solid line depicts the profile for AChE inhibition calculated from the measured blood concentrations of C(±)P(-)-soman, assuming inhibition rate constants for C(-)P(-)- and C(+)P(-)-soman of $0.8*10^8$ and $0.08*10^8$ $M^{-1}min^{-1}$, respectively.

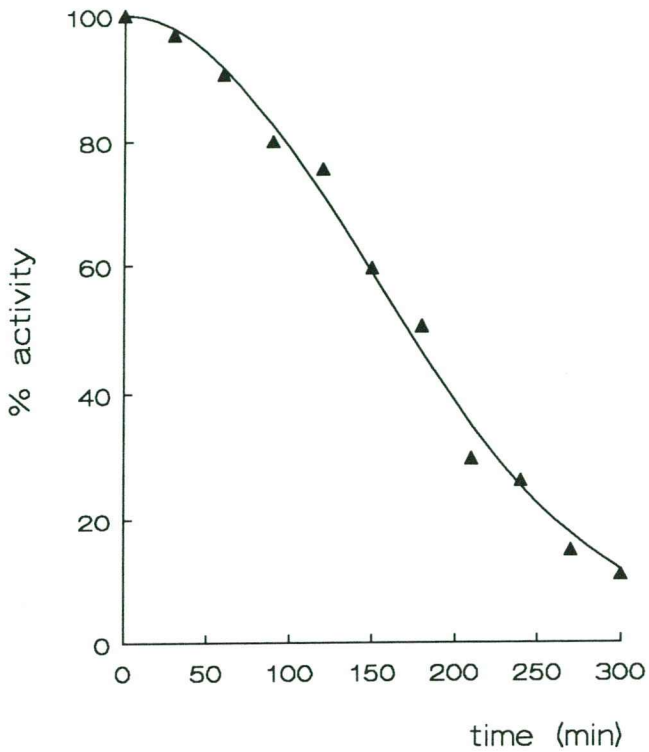

Figure 67. Acetylcholinesterase (AChE) activities in blood samples of anesthetized, atropinized and restrained guinea pigs nose-only exposed to 160 ± 16 $\mu g/m^3$ of C(±)P(±)-soman for 300 min, which corresponds with 0.1 LCt50. The solid line depicts the profile for AChE inhibition calculated from the measured blood concentrations of C(±)P(-)-soman, assuming inhibition rate constants for C(-)P(-)- and C(+)P(-)-soman of $0.85*10^8$ and $0.085*10^8$ $M^{-1}min^{-1}$, respectively.

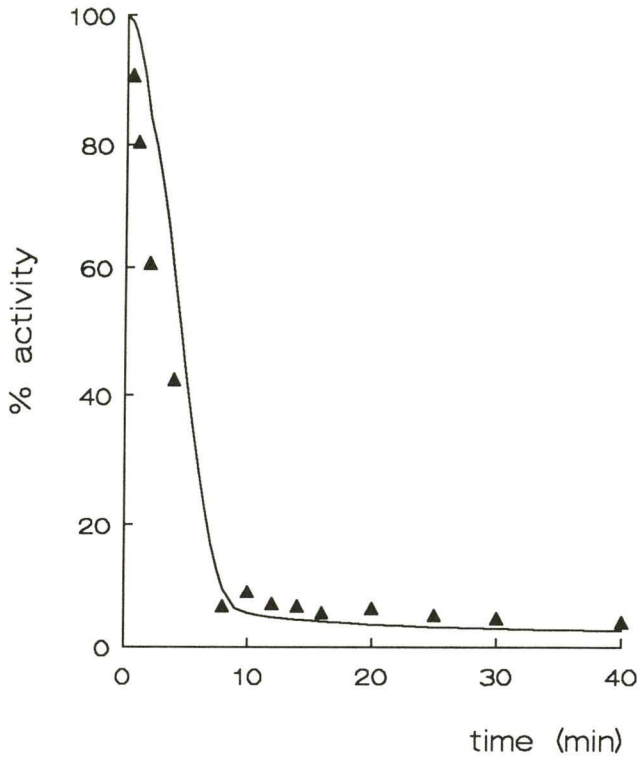

Figure 68. Acetylcholinesterase (AChE) activities in blood samples of anesthetized and atropinized guinea pigs during and after an 8 min intravenous infusion of 0.8 LD50 of C(±)P(±)-soman. The solid line depicts the profile for AChE inhibition calculated from the measured blood concentrations of C(±)P(-)-soman, assuming inhibition rate constants for C(-)P(-)- and C(+)P(-)-soman of $0.8*10^8$ and $0.08*10^8$ $M^{-1}min^{-1}$, respectively.

The overall results of the present study indicate that the techniques for nose-only exposure of guinea pigs, with respiratory monitoring, are sufficiently developed for toxicokinetic investigations. The technique can be applied to toxicokinetic studies of other relatively volatile nerve agents as well, e.g., GF, tabun and VX. Further studies are needed to obtain insight into the retention of nerve agents upon respiratory exposure, as well as into the sites of absorption in various species. Such studies will contribute to physiologically based modeling of the toxicokinetics of these agents. Furthermore, efficacies of pretreatment and treatment regimens can be tested via nose-only exposure.
For extrapolation to battlefield conditions insight into the absorption phase after short (0.5-2 min) respiratory exposure is required.

V. CONCLUSIONS

1. Our newly developed gas chromatographic configuration for analysis of soman and sarin stereoisomers, based on automated large-volume injection, two-dimensional chromatography, and alkali flame detection increases the daily sample throughput, whereas the large-volume injection allows for minimum detectable concentrations of nerve agent stereoisomers in biological samples below 10 pM. However, this complex and delicate configuration is not very suitable for routine application. In contrast herewith, the gas chromatographic configuration equipped with an automated thermodesorption injector and multidimensional chromatography proved to fulfill our requirements with respect to sensitivity, sample throughput and reliability in routine use.

2. The stereoisomers of (±)-sarin can be resolved on various chiral stationary phases. On a homemade D-Chirasil Val column, the stereoisomers of (±)-sarin and of its deuterated analogue D_7-(±)-sarin, which can be used as an internal standard in bioanalysis, are completely resolved. For routine application, however, we prefer to use a CP Cyclodex B column. This column does not resolve D_7-(+)-sarin from (-)-sarin, but has the advantage of being commercially available, whereas optically pure D_7-(-)-sarin can be easily isolated for use as an internal standard.

3. (±)-Sarin stereoisomers, with D_7-(-)-sarin as the internal standard, can be extracted from blood samples by a procedure similar to that used for C(±)P(±)-soman, as verified for blood concentrations of (±)-sarin of ≥ 50 pg/ml.

4. After intravenous administration of 0.8 LD50 (±)-sarin to anesthetized, atropinized and mechanically ventilated guinea pigs, (+)-sarin was not detectable, whereas (-)-sarin was detectable up to at least 40 min after administration. The concentration-time profile of (-)-sarin was adequately described with a two-exponential equation. In comparison with C(-)P(-)-soman, (-)-sarin is distributed faster, whereas the elimination of (-)-sarin proceeds considerably slower than that of C(-)P(-)-soman.

5. After intravenous administration of 0.8 LD50 C(±)P(±)-soman to anesthetized, atropinized and mechanically ventilated guinea pigs, the P(+)-isomers were not detectable, whereas the P(-)-isomers were detectable up to 40 min after administration. The concentration of the C(+)P(-)-isomer was considerably lower than that of the C(-)P(-)-isomer. The concentration-time profile of C(-)P(-)-soman was adequately described with a two-exponential equation, whereas the profile of the C(+)P(-)-isomer could not be fitted. At a dose corresponding with 0.8 LD50, the area under the curve of C(-)P(-)-soman is very small when compared with the areas corresponding with 2 and 6 LD50.

6. An apparatus was constructed for the continuous generation of nerve agent vapor in a concentration range of 50 $\mu g/m^3$ to 100 mg/m^3 in air flow, with a temperature of 20 °C and a relative humidity of 70 %. The concentration of C(±)P(±)-soman in the airstream is monitored by a gas chromatographic configuration equipped with a gas-sampling valve. Furthermore, an apparatus for simultaneous nose-only exposure of up to eight guinea pigs was constructed and tested. Software was developed for the monitoring of the respiratory frequency and the respiratory minute volume of the animals during the exposure.

7. Upon 8-min nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 0.8 LCt50 of C(±)P(±)-soman, the C(-)P(-)-isomer is rapidly absorbed, with blood levels starting to decrease immediately upon termination of exposure. The absorption phase of the C(+)P(-)-isomer appeared to lag behind that of the C(-)P(-)-isomer, a phenomenon which was observed in all respiratory exposure experiments. There were no soman-related effects on the respiratory parameters.

8. Upon 8-min exposure of anesthetized, atropinized and restrained guinea pigs to 0.4 LCt50 of C(±)P(±)-soman, the area under the curve of the C(±)P(-)-isomers was ca. fourfold lower when compared with exposure to 0.8 LCt50, indicating nonlinearity with dose at low dosages.

9. Upon 4-min exposure of anesthetized, atropinized and restrained guinea pigs to 0.8 LCt50 of C(±)P(±)-soman, the areas under the curve of the C(±)P(-)-isomers were fourfold lower than those measured for 8-min exposure to the same dose. The results indicate nonlinearity of the toxicokinetics with exposure time. The blood level of C(+)P(-)-soman increased for an additional 2 min after termination of the exposure.

10. During a 300-min exposure of anesthetized, atropinized and restrained guinea pigs to 0.1 LCt50 of C(±)P(±)-soman, blood concentrations of C(±)P(-)-soman are gradually built up, with maximum concentrations of ca. 40 pg/ml. This exposure leads to nearly complete inhibition of blood acetylcholinesterase and very little central inhibition. Therefore, exposure to 0.1 LCt50 of C(±)P(±)-soman in 5 h is <u>not</u>, as was assumed, an acceptable subchronic exposure. With more extensive investigations, 'safe' periods of exposure to C(±)P(±)-soman can be defined. There were no significant soman-related effects on respiratory parameters.

11. Upon 8-min nose-only exposure of anesthetized, atropinized and restrained guinea pigs to 0.8 LCt50 of (±)-sarin, the (-)-isomer was rapidly absorbed. (+)-Sarin was not detectable in the blood samples. The blood acetylcholinesterase activity dropped rapidly during the 8-min exposure, down to ca. 15 % of control activity. There were no sarin-related effects on the respiratory parameters.

12. Upon 8-min exposure of anesthetized, atropinized and restrained guinea pigs to 0.4 LCt50 of (±)-sarin, the area under the curve of the (-)-isomer was ca. twofold lower when compared with exposure to 0.8 LCt50. In combination with a 1.5-fold higher respiratory minute volume at the lower dose, this indicating nonlinearity with dose at low dosages. Inhibition of blood acetylcholinesterase proceeded somewhat slower than for exposure to 0.8 LCt50. There were no sarin-related effects on the respiratory parameters.

13. The concentration-time profiles of C(±)P(-)-soman during intravenous infusion appeared to be in close agreement with those during respiratory exposure to an equitoxic dose. Subsequently, after the infusion has stopped, the blood levels of C(±)P(-)-soman decrease much faster than after inhalation. The time courses of blood acetylcholinesterase activity are nearly identical for both administration routes. It is concluded that intravenous infusion is a suitable substitute for the absorption phase of inhalation toxicokinetics. The observation that the blood levels of C(+)P(-)-soman lags behind that of the C(-)P(-)-isomer may suggest a more rapid binding or hydrolysis of the former epimer.

14. After intramuscular bolus administration of a dose corresponding with 0.8 LD50, concentrations of C(±)P(-)-soman are built up slowly and irregularly, leading to a slow inhibition of blood acetylcholinesterase. Intramuscular bolus administration is not a suitable substitute for an equitoxic respiratory exposure.

15. The progression of blood AChE inhibition, as calculated from the measured concentration-time profiles of (-)-sarin and C(±)P(-)-soman using assumed bimolecular rate constants of inhibition, correlates reasonably well with the measured progression of AChE inhibition.

16. Since the blood concentrations of C(±)P(-)-soman decrease more slowly after respiratory exposure than after an equitoxic intravenous infusion, we assume the presence of a depot in the upper respiratory tract from which absorption continues after termination of the exposure. This phenomenon may become more important with shorter exposure times, and suggests the possibility for decontamination of the airways after respiratory exposure.

17. Toxicokinetic evaluation of the respiratory exposure would greatly benefit from knowledge of the fraction of the inhaled dose which is retained in the animal, and from insight into the absorption sites of C(±)P(±)-soman and (±)-sarin in the respiratory tract.

18. The technique to perform nose-only exposure of guinea pigs to controlled nerve agent concentrations has been sufficiently developed to study the absorption phase of C(±)P(-)-soman and of other nerve agents during a short exposure period (0.5-2 min). Such a short exposure may be encountered on the battlefield.

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