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# BIODISPOSITION OF ORGANOPHOSPHATES IN GUINEA PIGS AND MICE AFTER INTRAMUSCULAR, INTRAVENOUS AND INHALATION EXPOSURE

FINAL REPORT

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Inhalation exposure of $^{3}$H-soman in mice resulted in rapid distribution from the lungs to all tissues, with concentrations remaining elevated as long as 24 hr after exposure. The intramuscular administration of $^{3}$H-soman also resulted in a rapid distribution to all tissues, particularly the lungs and kidneys, with high concentrations still remaining in most tissues after 24 hr. Soman rapidly phosphorylated protein, and was quickly hydrolyzed to free $^{3}$H-pinacolylmethylphosphonic acid ($^{3}$H-PMPA). The biodisposition of $^{3}$H-soman and its metabolites was quite similar after both routes of administration, but contrasted with previous studies showing rapid clearance of organophosphates following intravenous administration.


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The biodisposition of $^{3}$H-soman and its metabolites in guinea pigs was highly dependent upon the route of administration. Following inhalation, the highest concentrations were found in lung, trachea, fat, and kidney 5 min after exposure. Concentrations of $^{3}$H-soman fell dramatically by the 30 min time point but then remained relatively constant for the duration of the time period studied. In contrast, intramuscular administration resulted in a sustained release of $^{3}$H-soman, with maximal concentrations achieved in most tissues (highest in lung, fat, heart, and kidney) at the 60 min time point and extremely elevated levels still remaining at 24 hr. Intravenous administration resulted in a rapid transfer to all tissues. Organs which contained the greatest quantity of $^{3}$H-soman were again lung, kidney, and fat at the 5 min time point. However, these concentrations dissipated much more quickly than with intramuscular injection or inhalation. With all routes of administration, $^{3}$H-soman was rapidly hydrolyzed to free $^{3}$H-PMPA which was present in all tissues. This reaction appeared to occur most rapidly after intravenous administration.

The most striking difference between the mice and guinea pigs occurred with $^{3}$H-soman concentration following the inhalation exposure. All tissues of the mice contained higher concentrations of $^{3}$H-soman despite exposure to a dose that was less than that administered to guinea pigs. However, it appeared that there were no major differences between mice and guinea pigs in the biodisposition and metabolism of $^{3}$H-soman which would directly account for the differences in sensitivities of these two species to organophosphates. Although the time-course varied across routes of administration, relatively high levels of organophosphate were invariably found in lung, fat, and kidney, with initial distribution and clearance occurring most rapidly with intravenous exposure in both species. Regardless of the route of administration, the kidney plays an important role in the elimination of $^{3}$H-soman and its metabolites.

## SUMMARY

The time course of $^{3}$H-soman and its metabolites was determined in tissues obtained from guinea pigs exposed to a sublethal dose of $^{3}$H-soman by either inhalation, intramuscular, or intravenous administration and mice exposed to a sublethal dose of $^{3}$H-soman by inhalation or intramuscular injection.

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Billy R. Martin 2/11/92

PI Signature Date

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# INTRODUCTION

The organophosphate esters, such as diisopropylfluorophosphate (DFP), soman and sarin, are highly toxic compounds that affect a variety of physiological processes. Their best characterized pharmacological effect is cholinesterase inhibition, although it has been demonstrated in the past few years that numerous other biological processes are affected by these agents. These organophosphate esters are truly unique compounds in that they are highly toxic, reactive, volatile and labile, which imposes severe constraints on the study of their pharmacological effects. However, these properties exert important influences on the activity of the organophosphates. For example, they rapidly phosphorylate tissue proteins (and thereby exert their pharmacological effect) or they are quickly hydrolyzed to inactive metabolites. It would appear that alterations in the biodisposition of the organophosphates could have profound effects on their toxicity.

One aspect that is crucial to organophosphate toxicity is the route of administration. It is important to point out that humans are most likely to be exposed to organophosphates via inhalation or percutaneous absorption. The respiratory tract is the most rapid and most complete of these routes of absorption (1). Intoxication by inhalation manifests itself rapidly through severe bronchial constriction and excess accumulation of secretions from bronchial and salivary glands (2). Most pharmacological studies in laboratory animals have been carried out using oral (p.o.), intravenous (i.v.) or subcutaneous (s.c.) administration due to the ease and accuracy of these routes. Fonnum *et al.* (3) examined the ability of tri-o-cresyl phosphate to alter the toxicity of inhaled soman. They concluded that the respiratory system did not act as a barrier to soman because cholinesterase in plasma and brain was inhibited after the inhalation. However, they did not report the time of exposure nor the time at which cholinesterase activity was measured. In addition, Mosberg *et al.* (4) conducted studies in order to determine the significance of species variation and route of administration with regard to the toxicity of soman. They found clear differences between the physiological consequences of soman administration by the intravenous and inhalation routes. Death occurred by different means after these two routes of administration which prompted them to suggest that different modalities of therapy may be required depending upon the route of exposure.

There have been numerous implications of the importance of biodisposition with regard to delayed toxicity of organophosphates. Studies in which animals were injected with high doses of soman suggested that it is stored in tissue depots and therefore is unavailable for inhibition of acetylcholinesterase or hydrolysis (5, 6). Two sites suggested to be depots are plasma aliesterases (7) and muscle (8). While the existence of storage depots for intact soman remains controversial, these studies underscored the importance of biodisposition. Of course, it has also been shown that aliesterases serve as an important mechanism for the detoxification of soman (9).

Another important dependent variable related to toxicity of organophosphates is species differences. It has been reported that mice and guinea pigs respond differently with regard to magnitude of response to both agent challenge and oxime/cholinolytic therapy (10-12). Futhermore, Maxwell *et al.* (13) speculated that these interspecies differences resulted from interspecies variations in soman metabolism and biodisposition. Indeed, they were able to show that pretreatment with cresylbenzodioxaphosphorin oxide, which blocks nonspecific binding of soman without inhibiting acetylcholinesterase, eliminated the differences in species susceptibility to soman. These results suggest that the pharmacokinetics differ dramatically among different species.

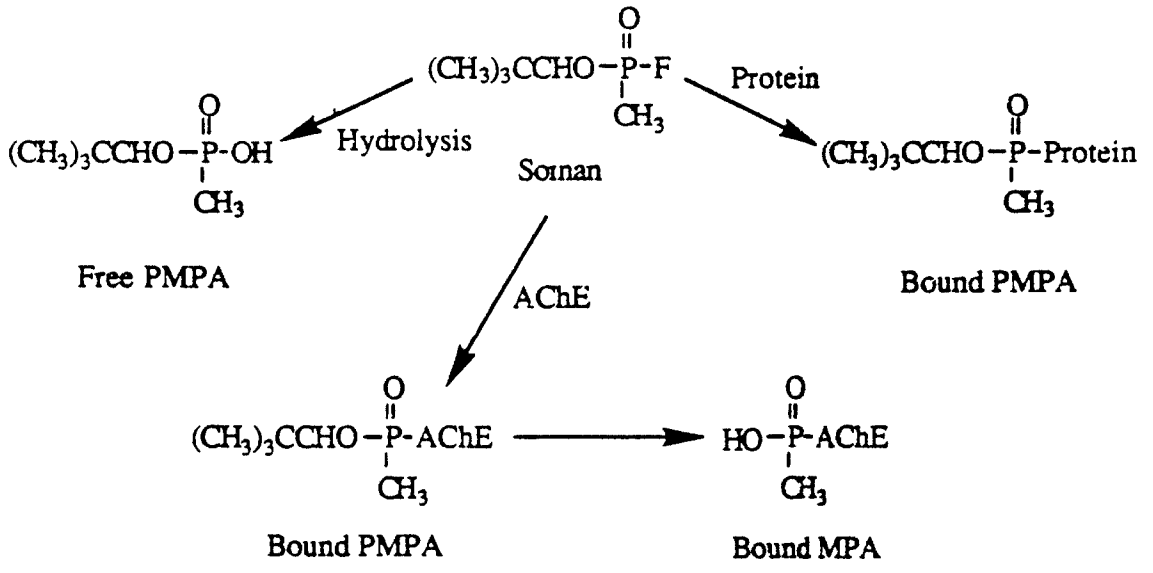
The first biodisposition studies were carried out with $DF^{32}P$ in mice (14-16), rabbits (17), humans (18) and cats (19). However, none of these studies was carried out in a comprehensive

fashion, such as delineation between parent compound and metabolites, complete time courses, etc. Shuh (20) examined the distribution of $^{3}$H-DFP between atrial tissue and blood from guinea pigs but no *in vivo* biodisposition was studied. There was relatively little information in the literature regarding the pharmacokinetics of soman. Benschop *et al.* (21) determined the time course of soman in the blood of rats, and Beck *et al.* (22) measured soman in brain and blood of mice 30 sec after an i.v. injection of soman. While tissue biodisposition of radioactivity had been studied after administration of $^{32}$P-sarin, no attempts had been made to qualify or quantify sarin and metabolite concentrations (23). Studies were then undertaken in our laboratory to determine the time course of the parent compound as well as that of its individual metabolites in all of the major organs of mice that had received an i.v. injection of radiolabeled organophosphate. Futhermore, we correlated the time course of the organophosphates to that of pharmacological activity. In the first study, $^{3}$H-DFP was administered i.v. to mice so that the time course of non-bound $^{3}$H-DFP, non-bound $^{3}$H-DIP (hydrolyzed $^{3}$H-DFP), bound $^{3}$H-DIP ($^{3}$H-DFP phosphorylation of tissue) and $^{3}$H-MIP could be established (24). It was clear from these experiments that DFP readily penetrated all tissues, whereupon either it was rapidly hydrolyzed to free $^{3}$H-DIP or it phosphorylated protein to form bound $^{3}$H-DIP. There were high concentrations of $^{3}$H-DFP in brain at the early time points but these levels fell rapidly. Within 2 hr all tissue concentrations of $^{3}$H-DFP were below 50 pg/mg tissue. One of the most important findings from this experiment was the extent and rapidity with which $^{3}$H-DFP was hydrolyzed. Approximately one third of the $^{3}$H-DFP was inactivated to free $^{3}$H-DIP, either by enzymatic or spontaneous hydrolysis, within 1 min after administration. Appropriate control experiments were carried out to eliminate the possibility of spontaneous hydrolysis before the injection or during tissue preparation and extraction. There was a rather quick decline in tissue concentrations of $^{3}$H-DIP which was consistent with the earlier findings of Ramachandran (16) who measured the clearance of DI$^{32}$P administered to mice. The major portion of the radioactivity was bound to tissue as a result of DFP phosphorylation. The time course of bound $^{3}$H-DIP differed from that of either free $^{3}$H-DFP or free $^{3}$H-DIP in that it persisted for long periods of time. The concentrations of bound $^{3}$H-DIP were low for several days in all tissues except liver.

The DFP-induced cholinesterase inhibition did not correlate with the time course of bound $^{3}$H-DIP in brain, diaphragm and plasma, which underscores the fact that DFP readily phosphorylates protein other than cholinesterase. In addition, the motor hypoactivity induced by DFP recovered much earlier than brain cholinesterase inhibition which suggested that cholinesterase inhibition may not be responsible for this effect. It is possible that cholinesterase inhibition may be the major determinant for lethality, while the effects produced by lower doses of DFP could be due to other mechanisms of action.

The biological fate of $^{3}$H-soman was also studied in mice in a fashion similar to that described for $^{3}$H-DFP (25). Only trace quantities of $^{3}$H-soman were found in all tissues as early as 1 min following the i.v. administration of 25 µg/kg of $^{3}$H-soman. Within 1 min almost one-half of the radioactivity was present in all tissues as free $^{3}$H-pinacolylmethylphosphonic acid (PMPA), the pharmacologically inactive hydrolysis product of $^{3}$H-soman (see scheme 1 for the metabolic profile of soman). The concentrations of free $^{3}$H-PMPA fell by almost 50% by 1 hr in most tissues. High concentrations of bound $^{3}$H-PMPA ($^{3}$H-soman phosphorylation of tissue) were found in all tissues immediately after $^{3}$H-soman treatment, particularly in lung, heart and kidney. These concentrations declined to < 50% by 8 hr. The quantities of radioactivity that were not extractable from tissue homogenates after basic hydrolysis were assumed to be $^{3}$H-methylphosphonic acid (MPA) which is the "aged" form of $^{3}$H-soman. These quantities of nonextractable radioactivity were relatively small in all tissues except brain, which is rich in cholinesterase.

Scheme 1. Metabolism of soman




PMPA = Pinacolymethylphosphonic acid
MPA = Methylphosphonic acid

The data from this experiment showed that i.v. treatment does not result in appreciable storage of $^{3}$H-soman in any tissue in mice. The highest quantities were found in lung but they were only a fraction of the concentrations of bound and unbound $^{3}$H-PMPA. Any storage depot would be composed of bound $^{3}$H-PMPA or $^{3}$H-MPA rather than intact $^{3}$H-soman.

A major finding from the time course studies was the lack of correlation between cholinesterase inhibition in brain and decreased motor activity and rectal temperature. These pharmacological effects had subsided by 24 hr but cholinesterase activity remained depressed for at least 3 days in brain as well as diaphragm. As with the DFP studies, the time course of pharmacological effects did not correspond to the time course of $^{3}$H-soman and its metabolites in brain.

A detailed biodispositional study of $^{3}$H-sarin was carried out at an i.v. dose of 80 µg/kg which, as was the case with $^{3}$H-soman, represented approximately 65% of the $LD_{50}$ (26). A comparison of the biodisposition of $^{3}$H-sarin and $^{3}$H-soman revealed that the amount of tissue phosphorylation was similar for both despite the lower dose of $^{3}$H-soman. The major difference between the two was the higher degree of spontaneous and/or enzymatic hydrolysis of $^{3}$H-sarin (to $^{3}$H-isopropylmethylphosphonic acid, IMPA). All tissues contained much higher concentrations of $^{3}$H-IMPA than $^{3}$H-PMPA, the most notable of which was lung, which contained concentrations of $^{3}$H-IMPA that were 15 times greater than those for $^{3}$H-PMPA. There was also extensive phosphorylation (bound $^{3}$H-IMPA) in lung. High concentrations of free and protein-bound $^{3}$H-IMPA, in addition to non-extractable radioactivity, remained in lung even at 24 hr. These data suggest that the lung may play an important role in the tissue disposition and detoxification of $^{3}$H-sarin, even after i.v. administration.

In our inhalation studies with $^{3}$H-DFP, mice were exposed to the vapor generated from 4 mg of $^{3}$H-DFP, resulting in a dose of 2.1 mg/kg body weight, which was sublethal [the $LC_{50}$

(C.L.) was found to be 5.4 (4.6-6.2) mg/kg]. The tissue concentrations of free $^3$H-DFP and $^3$H-DIP were higher after this inhalation exposure than after i.v. injection which was probably a reflection of the lower i.v. dose (1 mg/kg). The concentrations of bound $^3$H-DIP were actually lower in lung following inhalation exposure as compared to i.v. treatment. These studies clearly show that DFP is readily absorbed from the lungs.

The lack of a correlation between the time course of whole brain concentrations of organophosphates and behavioral effects prompted us to examine the biodisposition within the brain. Surprisingly, there were much higher concentrations of organophosphates and metabolites in hypothalamus than in other brain areas (27). It would appear that the neurochemistry in the hypothalamus is affected to a greater degree than that in other brain areas of the mouse.

There are numerous factors that may be important with regard to the expression of organophosphate toxicity. The rate at which the organophosphates penetrate a particular tissue is undoubtedly an important factor regulating their toxicity. These agents are highly reactive with most proteins so that the tissues exposed initially to the organophosphates may suffer the greatest degree of phosphorylation or these tissues may represent major sites of hydrolysis or inactivation. Therefore, we hypothesized that the biodisposition of the organophosphates, or the rate at which they penetrate tissues, differs with regard to species as well as route of administration. Our specific hypothesis was that the biodisposition and/or hydrolysis of soman in guinea pig differs depending upon the route of administration and is responsible for the differences in toxicity observed following administration of the organophosphate by various routes. Additionally, the difference in sensitivity of mice and guinea pigs to the organophosphates may be due to differences in the biodisposition and metabolism between the two species.

The first specific objective in the current contract was to study the biodisposition of soman and its metabolites in guinea pigs at sublethal doses with the emphasis on determining the importance of route of administration with regard to metabolism and biodisposition of $^3$H-soman. The time course of $^3$H-soman and its metabolites [including non-protein bound $^3$H-PMPA, protein-bound $^3$H-PMPA and $^3$H-MPA] was determined in tissues obtained from animals exposed to $^3$H-soman by inhalation, intramuscular or intravenous administration. The second objective was to evaluate species differences. The biodisposition and metabolism of $^3$H-soman were evaluated in mice following inhalation and intramuscular injections of sublethal doses of $^3$H-soman.

## MATERIALS AND METHODS

**Analytical procedures.** Thin-layer chromatography (TLC) and gas chromatography (GC) were used to determine the purity of our organophosphates as well as the selectivity of the extraction scheme. TLC analysis was carried out by applying radiolabeled standards or solvent extracts to 5 x 10 cm silica gel plates (Analtech) which are developed in either 5 % methanol:chloroform or acetone:methanol:chloroform:conc. ammonia (25:25:45:5). The silica gel (1 cm sections) was scraped into scintillation vials containing 1 ml of methanol:distilled water (1:1, v/v). The samples were sonicated for 30 min and aqueous counting scintillant (RPI) was added for counting. Soman had an $R_F$ value of 0.64 while PMPA remained at the origin in the 5 % methanol:chloroform system. In the acetone:methanol:chloroform:conc. ammonia solvent system the $R_F$ values of soman and PMPA were 0.90 and 0.39, respectively, while MPA did not move from the origin. The Hewlett Packard GC was equipped with a nitrogen/phosphorus detector and a 2 mm x 6 ft column packed with 6 %/4 % OV 210/OV 101 on WHP (80/100 mesh). The carrier gas (He) flow rate was 20 ml/min and the injector and detector temperatures were 250 and 300 °C, respectively. The column was maintained at isothermal conditions. At a column temperature of 160 °C, soman and PMPA had retention times of 2.7 and 5.3 min, respectively.

**Synthesis of reference metabolites.** $^3$H-PMPA was synthesized following the methodology of Harris *et al.* (28). $^3$H-Soman (687 µCi) was added to soman (100 µg) which was then placed in 2 ml of 0.5 N NaOH and mixed by vortex at frequent intervals for 1 hr. TLC analysis of the reaction mixture indicated a single spot with an $R_F$ of 0.39 when the plates were developed in acetone:methanol:chloroform:conc. ammonia (25:25:45:5) which coincided with PMPA. $^3$H-MPA was also synthesized as described by Harris *et al.* (28).

**Measurement of tissue concentrations of $^3$H-soman, $^3$H-PMPA and nonextractable radioactivity.** Previous studies have shown that solvent extraction can be used to separate DFP and its two metabolites in a quantitative fashion (15, 24). Therefore, an extraction scheme was developed for the separation of $^3$H-soman, free $^3$H-PMPA, protein-bound $^3$H-PMPA and $^3$H-MPA so that tissue concentrations of each could be measured. In order to establish the assay, 0.1 µCi of either $^3$H-soman, $^3$H-PMPA or $^3$H-MPA was added to 2-ml aliquots of heat-denatured liver homogenates (500 mg tissue/2 ml). Toluene (10 ml) was added and the samples were shaken for 15 min. The toluene removed 91 ± 0.2, 0.5 ± 0.0 and 0.2 ± 0.0 % (means ± S.E.M., n =3) of the $^3$H-soman, $^3$H-PMPA and $^3$H-MPA, respectively. The samples were then made acidic with $H_2SO_4$ and reextracted with 4 ml of isobutanol:toluene (1:1, v/v) which removed 2.3 ± 0.1, 95 ± 3, 5.5 ± 2.6 % of the $^3$H-soman, $^3$H-PMPA and $^3$H-MPA, respectively. Approximately 7, 5 and 95 % of the $^3$H-soman, $^3$H-PMPA and $^3$H-MPA, respectively, remained in the aqueous phase. Therefore, the following procedure was adopted. Blood samples from the cervical wound were collected in heparinized tubes and centrifuged at 1000 x *g* to obtain plasma and erythrocytes. Tissues were removed, weighed and homogenized with a polytron (Brinkman Instruments Co., Westbury, NY) for 1 min at high speed in a combination of 2 ml of phosphate buffer:sucrose (9:1, v/v) and 10 ml of toluene. The remaining carcasses were skinned and homogenized with two volumes of buffer in a blender. Two-ml aliquots of the carcass homogenates were removed, added to 10 ml of toluene and mixed with a polytron as described above. All homogenates were centrifuged at 1000 x *g* for 10 min and 5 ml of the toluene extracts removed and added directly to spectrofluor PPO-POPOP (Amersham-Searle) in toluene for determination of $^3$H-soman concentrations by liquid scintillation

spectrometry. Counting efficiency was determined by external standardization. One-ml aliquots of the toluene extract of the five replicates of each tissue were pooled and concentrated to approximately 0.3 ml under a slow stream of nitrogen for subsequent TLC analysis.

**Table 1. Percent recovery of $^3$H-soman from various tissues[a].**

| | Percent Recovery | Mean | | Standard Deviation | | Standard Error |
|---|---|---|---|---|---|---|
| **LIVER** | | | | | | |
| Free soman | 75.00% | 563,755 | ± | 10,093 | ± | 5,826.9 |
| Free PMPA | 7.50% | 56,820 | ± | 1,596.6 | ± | 921.8 |
| Bound PMPA | 14.00% | 105,690 | ± | 3,145.4 | ± | 1,816.0 |
| Residual Metabolite | 3.80% | 12,575 | ± | 324.3 | ± | 187.2 |
| **LUNG** | | | | | | |
| Free soman | 88.00% | 573,833 | ± | 12,617.8 | ± | 7,284.9 |
| Free PMPA | 6.40% | 41,739 | ± | 605.17 | ± | 349.3 |
| Bound PMPA | 2.00% | 12,725 | ± | 424.7 | ± | 245.2 |
| Residual Metabolite | 4.00% | 10,969 | ± | 704.4 | ± | 406.7 |
| **BRAIN** | | | | | | |
| Free soman | 87.00% | 721,529 | ± | 7,270.8 | ± | 4,197.8 |
| Free PMPA | 6.70% | 55,638 | ± | 3,382.5 | ± | 1,952.8 |
| Bound PMPA | 2.00% | 14,504 | ± | 486.9 | ± | 281.1 |
| Residual Metabolite | 4.00% | 15,468 | ± | 454.1 | ± | 262.1 |

**[a]All tissues were prepared using 10 µl (6.7ng/µCi) of $^3$H-Soman per 0.5g of tissue. Data are expressed as percent recovery as well as DPM's (means, N=6).**

An aliquot (0.3) of the aqueous layer of each sample that remained after extraction was solubilized (TS-2 Reagent, RPI) overnight prior to liquid scintillation spectrometry. Quench was corrected by external standardization. The remaining aqueous portion was acidified with 180 µl of 12 N $H_2SO_4$ prior to extraction with 4 ml of isobutanol:toluene (1:1, v/v). These samples were homogenized with a polytron for 20 sec and then centrifuged at 1000 x *g* for 10 min. Aliquots (2 ml) of the isobutanol:toluene were counted for radioactivity as described above for the determination of concentrations of free $^3$H-PMPA. One-ml aliquots of the solvent extracts for the five replicates of each tissue were pooled and evaporated under nitrogen to approximately 0.3 ml for TLC analysis. The aqueous samples were then made basic by the addition of 0.5 ml of 10 N NaOH and heated in an autoclave (22 psi at 270 °C) for 1 hr. The hydrolyzed samples were adjusted to 1N $H_2SO_4$ and homogenized again with 4 ml of isobutanol:toluene. After

**Table 2. Percent recovery of $^3$H-PMPA from various tissues.**

| | Percent Recovery | Mean | | Standard Deviation | | Standard Error |
|---|---|---|---|---|---|---|
| **LIVER** | | | | | | |
| Free soman | 0.15% | 117.1 | ± | 2.0 | ± | 1.15 |
| Free PMPA | 85.00% | 33,746 | ± | 4,680.6 | ± | 2,702 |
| Bound PMPA | 11.20% | 4,453 | ± | 120.9 | ± | 69.80 |
| Residual Metabolite | 4.00% | 1410.7 | ± | 221.2 | ± | 127.7 |
| **LUNG** | | | | | | |
| Free soman | 0.15% | 138.2 | ± | 11.5 | ± | 6.6 |
| Free PMPA | 83.40% | 36,580.4 | ± | 963.3 | ± | 556.18 |
| Bound PMPA | 13.30% | 5,830.4 | ± | 54.76 | ± | 31.6 |
| Residual Metabolite | 3.20% | 1,212.8 | ± | 97.7 | ± | 56.4 |
| **BRAIN** | | | | | | |
| Free soman | 0.24% | 227.6 | ± | 88.3 | ± | 51.0 |
| Free PMPA | 85.40% | 39,899.1 | ± | 661.7 | ± | 382.0 |
| Bound PMPA | 11.60% | 5,398.9 | ± | 212.3 | ± | 122.5 |
| Residual Metabolite | 2.80% | 1,140.6 | ± | 50.29 | ± | 29.0 |

**[a]All tissues were prepared using 10 µl (0.67ng/µCi) of $^3$H-PMPA per 0.5g of tissue. Data are expressed as percent recovery as well as DPM's (means, N=6).**

homogenizing the samples with the polytron for 20 sec, the samples were centrifuged at 1000 x *g* for 10 min. A 1-ml aliquot of each isobutanol:toluene extract was removed and counted for radioactivity and the remaining solvent extracts were prepared for TLC analysis as described above. The radioactivity found in this extraction step represented bound $^3$H-PMPA. An aliquot of the remaining aqueous layer was solubilized as described above and counted for determination of unextractable radioactivity.

Controls were carried out with each experiment by homogenizing tissues from untreated mice in 2 ml of buffer with a polytron. Either $^3$H-soman, $^3$H-PMPA or $^3$H-MPA (1 µCi each) was added to these homogenates, as well as to 2 ml of buffer followed immediately by the addition of 10 ml of toluene. The samples were homogenized again with the polytron and then processed at the same time and in the same manner as the tissue samples from treated mice. In this way, the stability of the agent as well as the extraction efficiencies could be determined.

**Animals.** Male ICR mice weighing 25-30 g and male Hartley guinea pigs weighing 450-500 g were used for these experiments.

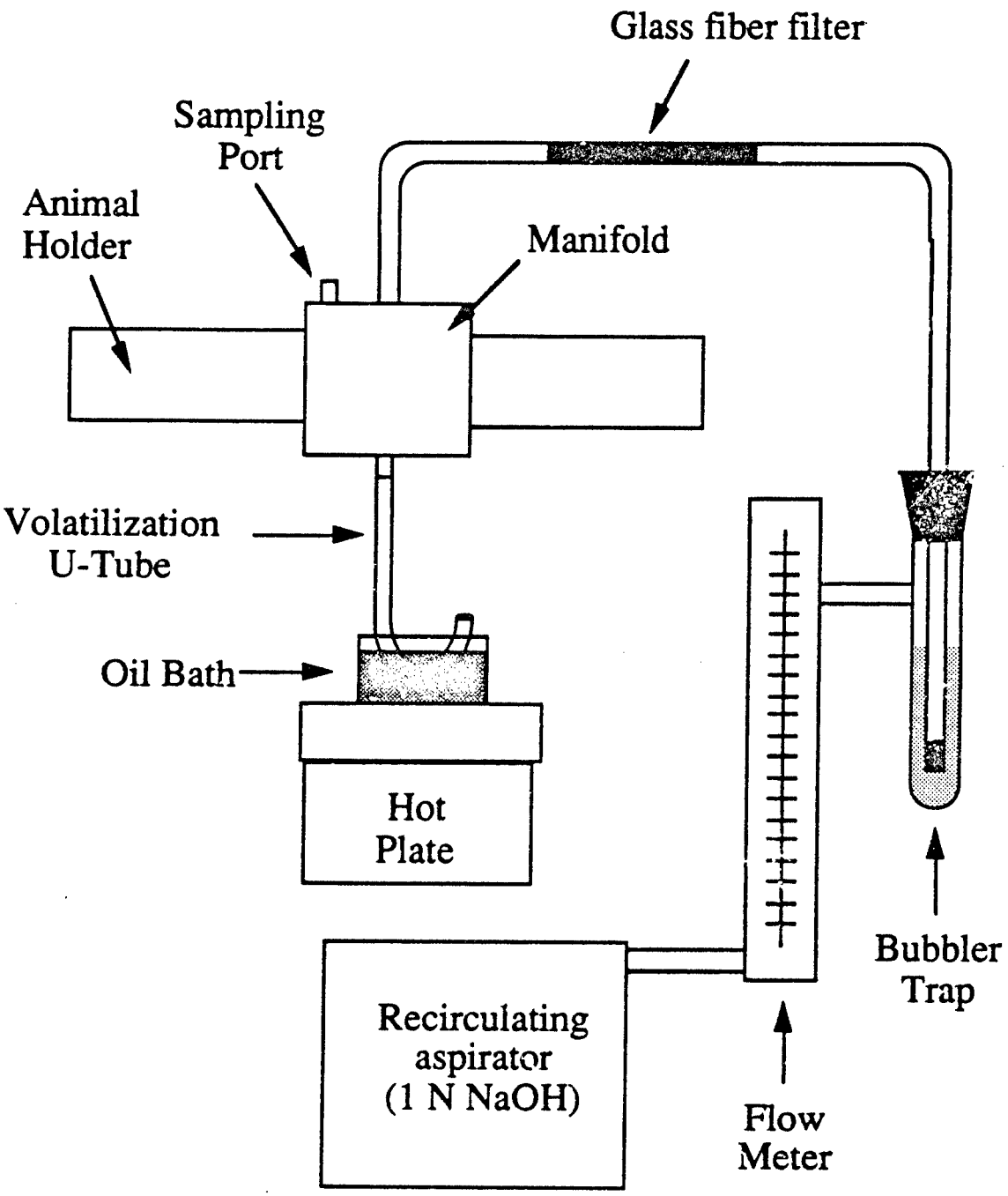
**Measurement of total radioactivity in urine and feces.** Urine was counted directly in aqueous counting scintillant. Feces was homogenized with a polytron at high speed in five volumes of phosphate buffer. Aliquots of these homogenates were combusted in a Hewlett Packard Sample Oxidizer for liquid scintillation spectrometry.

**Measurement of acetylcholinesterase activity.** The exposed animals were decapitated, 6 ml of blood was collected from guinea pigs and 500 µl of blood was collected from mice in heparinized tubes. The samples were centrifuged 30 min for guinea pigs and 10 min for mice in order to separate the plasma from the red blood cells. Total, pseudo-, and true cholinesterase activity were measured in all samples. The substrate was prepared by adding [$^3$H]-acetylcholine iodide and acetylcholine iodide to a buffer solution (10 mM TES, pH 7.4) to produce a 2.5 mM solution containing 2 µCi/ml. Tissue was diluted 10-fold with buffer. The samples were incubated for 30 min at 37°C. The incubation mixture contained 60 µl of red blood cells, 100 µl of [$^3$H]- acetylcholine, and either 40 µl of TES buffer or 40 µl of a $2.5 \times 10^{-5}$M solution of 1,5-bis(4-allyldimethylammoniumphenyl) pentan-3-one dibromide (BW284C51). This compound is an inhibitor of true cholinesterase at this concentration. All samples were run in triplicate. After incubation, the reaction was terminated by the addition of 40 µl of 1 N HCl and the incubated tubes were placed on ice. 100 µl of the reaction mixture was removed and added to 5 ml of the special scintillation cocktail, which contained 2,5-diphenyloxazole and p-bis-[-2-(5-phenyloxazolyl)] benzene in a solution of toluene:isoamyl alcohol (9:1). The addition of HCl reduced the polarity of the acetate (which contains the tritium) thus allowing it to be extracted into the scintillation cocktail. Samples were counted by using the liquid scintillation counter (LS1801 from Beckman). Background values were determined and samples contained 60 µl of TES buffer without RBC's. Values from sample tubes that were not incubated with BW284C51 represented the total cholinesterase activity, whereas those that contained the inhibitor produced values that represented the pseudocholinesterase activity. The latter was subtracted from the former to provide the true cholinesterase activity. The amount of substrate hydrolyzed was determined by dividing the true activity by the specific activity of the [$^3$H]-acetylcholine solution. Enzymatic activity was then determined by dividing the amount of hydrolyzed [$^3$H]-acetylcholine by the incubation time and the protein content of the sample. The protein concentration was determined by the method described by Bradford (29).

**Inhalation exposure.** There are numerous methods for exposing animals to vapors. However, there are added restrictions when dealing with highly toxic agents that are also radioactive. Therefore, a relatively simple apparatus was designed in our laboratory that allows for the dynamic exposure of a small quantity of material to mice, rats or guinea pigs. It is possible to expose animals to pharmacologically active doses while at the same time safely collecting the remaining material. In addition, only the noses of the animals come into direct contact with the volatilized material which minimizes percutaneous absorption.

The inhalation apparatus for guinea pigs is an enlargement of the apparatus used to expose mice to volatilized organophosphates as described in detail by Scimeca *et al.* (30) and as depicted in Scheme 2. The system consisted of three major components: volatilization tube and exposure manifold, collecting traps, and a vacuum system. Four guinea pigs were placed in their individual animal holders such that their heads remained snugly in place. The animal holders were then attached to the manifold, which was designed to allow air flow past the animals' noses. The agent was pipetted into the U-shaped volatilization tube and then the oil bath (maintained at 80 °C) raised so that the U-shaped tube was immersed in the oil, as shown in the diagram. This latter step initiated the exposure period which has a duration of 10 min. The second component of the system was the collecting traps, which were included for the purpose of capturing the

Scheme 2. Inhalation exposure apparatus.



organophosphate that was not inhaled. The first trap was a 15-cm section of tygon tubing containing approximately 2.5 g of glass wool (which mimics a Cambridge filter). Air flow passed from this filter through a bubbler trap (with a fritted end) that was immersed in equal parts of propylene glycol and ethyl acetate. A flow meter was placed after the collecting system and regulated the air flow at 1120 ml per min. The last component was the vacuum system which was comprised of a water pump that recirculated four liters of 1 N NaOH to create the negative pressure that drove the air flow through the entire apparatus. This particular design placed the entire system under negative pressure thereby preventing loss of the organophosphate. In addition to the glass wool and propylene glycol/ethyl acetate traps, the recirculating 1 N NaOH also served as an efficient means for detoxifying any organophosphate which has not been trapped. The entire inhalation apparatus was contained within the hood in the Dilute Organophosphate Laboratory.

The mouse exposure apparatus was similar to the guinea pig system with the exception that it was capable of administering vapor to six mice rather than four guinea pigs. In addition, the sample compartment in the mouse apparatus was designed somewhat differently, although both systems work on the sample principle of pulling air over a heated sample of organophosphate.

**Determination of vapor concentration during inhalation.** The manifold of the inhalation apparatus contains a port which allows sampling of the vapor with a 100 µl gas-tight syringe. In order to establish the concentration of vapor during the 10-min inhalation period, samples were taken at 1-min intervals with syringes that had been heated to 50 °C. The volatilization was done with $^{3}$H-soman so that the vapor concentration could be determined by liquid scintillation spectrometry. The sample in the syringe was injected into 10 ml of scintillation fluid. The syringe was washed by drawing scintillation fluid into the syringe several times and then injecting it back into the scintillation vial. The samples were then subjected to liquid scintillation spectrometry.

**Intravenous injections.** The i.v. injections of soman (15µg/kg) in guinea pigs were performed through the ear vein of lightly restrained guinea pigs using a 31 or 32 gauge needle.

**Experimental protocol for the biodisposition and metabolism of $^{3}$H-soman in guinea pigs following administration by different routes.** The first requirement for the inhalation studies was to establish a general protocol for exposing guinea pigs to $^{3}$H-soman. Exposure conditions were chosen so that the guinea pigs received a pharmacologically active dose that was sublethal. $^{3}$H-Soman (54 µCi/238 µg) suspended in 244 µl of propylene glycol was volatilized at 65-70 °C for 10 min. at a flow of 1035 ml/min. Following exposure, the guinea pigs were decapitated and skinned. The carcasses were homogenized in 2 volumes of water and an aliquot was solubilized for determination of radioactivity by liquid scintillation spectrometry. The results from these experiments demonstrated that the animal carcasses contained 2.31 ± 0.2 µg (mean ± SEM) of soman. This quantity represented 0.97 ± 0.08 % of the starting material. The remainder of the radioactivity was collected in the traps. The average dose ± S.E.M. for six animals was 5.03 ± 0.38 µg/kg.

Once the experimental protocol had been established, guinea pigs were exposed to the vapor of $^{3}$H-soman for 10 min and then six animals per group were decapitated at the following times: 5, 15, 30, 60, 120, 240, 480 and 1440 min. Six animals were needed per group due to greater variability with inhalation exposure than with i.v or i.m. injections. Due to the fact that only four guinea pigs could be exposed at a time, it was necessary to pool four animals from one inhalation exposure with two animals from a subsequent exposure. The animals which were

decapitated at 240, 480 and 1440 min were placed in metabolic chambers for the collection of urine and feces. Intact $^3$H-soman, free $^3$H-PMPA, bound $^3$H-PMPA (phosphorylated protein) and $^3$H-MPA were measured in plasma, erythrocytes, brain areas, diaphragm, liver, lung, trachea, kidney, fat, urine and feces. Total radioactivity was measured in the carcasses and added to that in the other tissues in order to establish dosimetry. The concentration of $^3$H-soman vapor in the manifold was measured at the beginning, middle and end of each exposure.

The biodisposition of $^3$H-soman and its metabolites was studied in guinea pigs after intramuscular (hind leg) and intravenous (ear vein) injections of a sublethal injection of $^3$H-soman. Again, it was necessary to establish a dose of soman which was sublethal. Doses of 20, 30 and 65 μg/kg produced 100% deaths in 3 animals, 1 animal and 1 animal injected intravenously with the respective doses. An intravenous dose of 15 μg/kg produced no deaths in three animals. Therefore, a dose of 15 μg/kg was chosen for both intramuscular and intravenous studies. Guinea pigs (5 per group) were treated with $^3$H-soman and decapitated at the times indicated above for the measurement of $^3$H-soman and individual metabolites in all of the specified tissue.

Erythrocyte cholinesterase activity was the biological marker which was measured in all animals.

**Experimental protocol for the biodisposition and metabolism of $^3$H-soman in mice following administration by different routes.** The inhalation studies were similar to that outlined for guinea pigs. The inhalation apparatus was the same as that described previously (28). $^3$H-Soman (780 μCi/150 μg) suspended in 244 μl of propylene glycol was volatilized at 65-70 °C for 10 min at a flow of 1035 ml/min. Following exposure, the mice were decapitated and skinned. In order to determine dosimetry, the whole body carcasses were homogenized in 2 volumes of sucrose/phosphate buffer, and an aliquot was solubilized for determination of radioactivity by liquid scintillation spectrometry. The results from these initial experiments demonstrated that the animal carcasses contained 2.06 ± 0.16 μg/kg of $^3$H-soman. Mice were exposed to this sublethal concentration of $^3$H-soman and six mice were decapitated at each of the following times: 5, 15, 30, 60, 120, 240, 480 and 1440 min. The animals decapitated at 240, 480 and 1440 min were placed in metabolic chambers for the collection of urine and feces. Intact $^3$H-soman, free $^3$H-PMPA, bound $^3$H-PMPA and $^3$H-MPA were measured in plasma, erythrocytes, brain areas, diaphragm, liver, lung, trachea, kidney, fat, urine and feces. Total radioactivity was measured in the carcasses which remained after dissection. The total radioactivity levels in the remaining carcasses are presented in the tables describing residual metabolites. The concentration of $^3$H-soman vapor in the manifold was measured at the beginning, middle and end of each exposure. Cholinesterase activity was measured in erythrocytes in all mice.

The biodisposition of $^3$H-soman and its metabolites was studied in mice after intramuscular (hind leg) injection of a sublethal dose of $^3$H-soman. A dose of soman which was sublethal yet produced cholinesterase inhibition was found to be 25 μg/kg. Therefore, mice (6 per group) were treated with this dose of $^3$H-soman and decapitated at the times indicated above for the measurement of $^3$H-soman and individual metabolites in all of the specified tissues. Cholinesterase activity was measured in erythrocytes in all animals as mentioned above.

The protocol for determining acetylcholinesterase inhibition following i.v. administration was the same as that described above for the guinea pig experiments. The only exception was that the mice received a 10-sec infusion of $^3$H-soman (25 μg/kg) via the tail vein rather than the ear vein.

## RESULTS

**Biodisposition and metabolism of $^3$H-soman in mice after inhalation.** Mice were allowed to breathe vapor generated from 150 µg of $^3$H-soman as described above and then decapitated at various times thereafter in order to measure the time course of tissue concentrations of $^3$H-soman and its metabolites. Five min after the termination of the inhalation exposure, $^3$H-soman levels were highest in fat, diaphragm, kidney and lung (Table 3). The time course of $^3$H-soman was somewhat erratic in that the levels fell dramatically by 15 min but then appeared to rebound by 30 min in several tissues. It is not clear why the $^3$H-soman concentrations are elevated as long as 24 hr after inhalation exposure. However, these results suggest that soman is slowly eliminated after inhalation exposure in contrast to our previous results (25) which clearly showed a rapid clearance after intravenous administration. $^3$H-Soman was readily hydrolyzed to form unbound (free) $^3$H-PMPA which was present in all tissues (Table 4). Concentrations in blood, brain, lung, liver, and fat remained relatively constant during the 24 hr period. Maximal concentrations in trachea occurred during the 30-240 min time period. As expected, lung contained high concentrations of bound $^3$H-PMPA which remained quite high throughout the 24 hr observation period (Table 5). It is also not surprising that relatively high concentrations of bound $^3$H-PMPA were located in trachea. However, it is not clear why the maximal concentrations were not obtained until 240 min. The concentrations in brain were notably low but constant throughout. The pattern of distribution of $^3$H-MPA (Table 6) closely resembled that of $^3$H-PMPA. Due to the high content of acetylcholinesterase in brain, it was not unexpected to find the concentrations of $^3$H-MPA to be almost double those of $^3$H-PMPA. Highest $^3$H-MPA concentrations were found in the diaphragm at 5 and 120 min, the lungs at 5, 60 and 480 min and the trachea at 240 min. $^3$H-MPA levels in the kidneys varied moderately with time as expected with excretory organs, while $^3$H-MPA levels in the liver remained relatively constant.

**Biodisposition and metabolism of $^3$H-soman in mice after intramuscular administration.** Free $^3$H-soman concentrations after intramuscular injection in mice are depicted in Table 7. The highest quantities of free $^3$H-soman, found in the trachea, lung, heart, diaphragm and testicular fat at 5 min, declined over the next 30 min. It is interesting to note that the concentrations of $^3$H-soman then rose at 120, 240, and 1440 min in most tissues. Actually, the levels at 1440 min were similar to those at the 5-min time point for trachea, fat, liver, kidney, heart, diaphragm, and brain. It appears that this route of administration results in a sustained release of $^3$H-soman. Hydrolysis of $^3$H-soman occurred rapidly to form free $^3$H-PMPA. In most tissues the highest amounts were found between 5 and 30 min after administration, except for the trachea which had the highest levels at 1440 min (Table 8). Free $^3$H-PMPA concentrations were highest in blood at 30 min and in the lung and brain at 5 min. Testicular fat levels were highest at 15 min in the early time points and then declined until rising again to reach a maximum concentration at 1440 min. The concentrations in brain also declined and then rebounded at 1440 min. Bound $^3$H-PMPA tissue concentrations were highest in the lung, trachea and blood at the 5-min time point (Table 9). The highest levels in lung, trachea and heart were found at 240 min. An increase in bound $^3$H-PMPA was observed in blood at the 15 and 30 min time points, followed by dramatic fluctuations for the remaining time. Bound $^3$H-PMPA levels in the kidney were highest and most consistent during the 15-240 min time period. The highest $^3$H-MPA concentrations in the diaphragm were found at 60 and 120 min, in the lungs at 5, 60 and 120 min and in the trachea at 5 and 240 min. $^3$H-MPA levels in the kidneys varied moderately with time

**Table 3.** Tissue concentrations of $^{3}H$-soman in mice after inhalation exposure[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 4.28 ± 0.22 | 1.76 ± 0.23 | 1.78 ± 0.10 | 3.38 ± 0.33 | 2.15 ± 0.34 | 3.30 ± 0.40 | 3.16 ± 0.21 | 3.70 ± 0.32 |
| Brain | 2.71 ± 0.21 | 1.17 ± 0.25 | 1.21 ± 0.13 | 1.90 ± 0.32 | 1.30 ± 0.19 | 2.03 ± 0.38 | 2.55 ± 0.33 | 2.24 ± 0.43 |
| Diaphragm | 15.85 ± 0.22 | 1.47 ± 0.25 | 2.01 ± 0.22 | 12.50 ± 1.37 | 7.43 ± 1.59 | 6.77 ± 1.21 | 3.76 ± 0.52 | 10.65 ± 1.80 |
| Heart | 7.46 ± 1.02 | 2.72 ± 0.55 | 7.57 ± 1.16 | 8.50 ± 0.86 | 3.98 ± 0.57 | 4.12 ± 0.73 | 7.10 ± 1.21 | 11.31 ± 1.46 |
| Kidney | 14.23 ± 1.20 | 2.01 ± 0.35 | 5.88 ± 1.44 | 8.27 ± 0.88 | 3.60 ± 0.50 | 6.02 ± 0.84 | 8.79 ± 0.98 | 10.06 ± 0.83 |
| Liver | 5.90 ± 0.62 | 2.09 ± 0.27 | 3.65 ± 0.20 | 4.19 ± 0.29 | 2.06 ± 0.19 | 4.28 ± 0.84 | 4.10 ± 0.38 | 7.44 ± 0.79 |
| Lung | 11.94 ± 1.53 | 3.37 ± 0.70 | 12.90 ± 2.13 | 14.70 ± 2.02 | 6.42 ± 1.50 | 8.68 ± 1.72 | 11.67 ± 1.08 | 21.37 ± 2.22 |
| Trachea | 8.86 ± 0.85 | 2.93 ± 0.21 | 15.93 ± 0.90 | 15.45 ± 1.29 | 12.75 ± 1.06 | 27.98 ± 3.92 | 7.55 ± 0.99 | 21.67 ± 3.94 |
| Fat | 19.31 ± 2.16 | 3.41 ± 0.77 | 6.01 ± 0.70 | 21.32 ± 2.59 | 7.11 ± 1.20 | 8.07 ± 0.86 | 11.47 ± 2.22 | 15.62 ± 1.73 |

[a] Mice were exposed to the vapor of 150 µg of $^{3}H$-soman which resulted in a dose of 2 µg/kg. Each time point represents the mean of six individual animals.

**Table 4.** Tissue concentrations of free $^{3}$H-PMPA in mice after inhalation exposure[a].

| Tissue | Time (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 2.19 ± 0.21 | 3.33 ± 0.58 | 1.99 ± 0.38 | 1.87 ± 0.19 | 1.71 ± 0.15 | 1.82 ± 0.15 | 1.50 ± 0.19 | 1.30 ± 0.11 |
| Brain | 1.29 ± 0.20 | 1.17 ± 0.18 | 0.79 ± 0.15 | 0.95 ± 0.14 | 1.20 ± 0.26 | 1.19 ± 0.19 | 1.07 ± 0.16 | 0.80 ± 0.11 |
| Diaphragm | 7.04 ± 1.21 | 1.47 ± 0.24 | 1.25 ± 0.08 | 7.55 ± 1.55 | 6.12 ± 1.23 | 3.46 ± 0.47 | 1.58 ± 0.26 | 2.98 ± 0.57 |
| Heart | 3.17 ± 0.65 | 1.96 ± 0.34 | 2.58 ± 0.60 | 3.85 ± 0.66 | 2.35 ± 0.38 | 2.06 ± 0.33 | 2.81 ± 0.80 | 1.53 ± 0.30 |
| Kidney | 9.45 ± 1.05 | 4.87 ± 0.72 | 5.75 ± 1.21 | 6.52 ± 0.72 | 3.43 ± 0.38 | 2.85 ± 0.31 | 3.51 ± 0.42 | 3.17 ± 0.44 |
| Liver | 2.95 ± 0.32 | 3.34 ± 0.34 | 2.68 ± 0.35 | 2.29 ± 0.15 | 1.96 ± 0.22 | 1.62 ± 0.18 | 1.73 ± 0.25 | 2.00 ± 0.25 |
| Lung | 5.67 ± 0.76 | 2.81 ± 0.60 | 6.21 ± 1.05 | 8.03 ± 1.14 | 5.09 ± 1.09 | 5.05 ± 0.97 | 4.20 ± 0.77 | 4.48 ± 0.83 |
| Trachea | 3.20 ± 0.44 | 3.32 ± 0.39 | 10.78 ± 0.88 | 8.12 ± 0.83 | 9.96 ± 1.81 | 11.68 ± 2.87 | 3.26 ± 0.34 | 7.27 ± 0.74 |
| Fat | 5.88 ± 0.62 | 2.51 ± 0.67 | 2.82 ± 0.68 | 7.73 ± 1.10 | 4.13 ± 0.96 | 3.30 ± 0.27 | 4.89 ± 1.05 | 4.07 ± 0.53 |

[a] Mice were exposed to the vapor of 150 µg of $^{3}$H-soman which resulted in a dose of 2 µg/kg. Each time point represents the mean of six individual animals.

**Table 5.** Tissue concentrations of bound $^{3}$H-PMPA in mice after inhalation exposure[a].

| Tissue | Time (Min) 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
|---|---|---|---|---|---|---|---|---|
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 17.45 ± 1.78 | 29.05 ± 3.32 | 12.22 ± 0.83 | 16.49 ± 0.91 | 32.78 ± 4.18 | 14.14 ± 0.55 | 40.12 ± 2.39 | 8.82 ± 0.91 |
| Brain | 0.80 ± 0.11 | 1.10 ± 0.25 | 0.91 ± 0.14 | 1.14 ± 0.07 | 0.80 ± 0.11 | 0.78 ± 0.12 | 1.02 ± 0.21 | 0.71 ± 0.06 |
| Diaphragm | 5.78 ± 1.00 | 1.47 ± 0.25 | 1.57 ± 0.24 | 7.00 ± 0.79 | 6.70 ± 0.91 | 3.34 ± 0.42 | 1.63 ± 0.22 | 2.86 ± 0.49 |
| Heart | 4.12 ± 1.47 | 1.86 ± 0.31 | 2.84 ± 0.39 | 7.12 ± 1.01 | 20.04 ± 4.67 | 2.24 ± 0.47 | 3.31 ± 1.12 | 3.44 ± 0.91 |
| Kidney | 7.14 ± 1.29 | 5.12 ± 0.89 | 5.36 ± 1.14 | 6.40 ± 0.79 | 4.72 ± 0.54 | 3.38 ± 0.83 | 5.17 ± 0.55 | 4.91 ± 0.34 |
| Liver | 2.87 ± 0.37 | 3.32 ± 0.29 | 4.18 ± 0.74 | 3.32 ± 0.29 | 2.50 ± 0.34 | 2.20 ± 0.10 | 1.77 ± 0.21 | 4.03 ± 1.40 |
| Lung | 22.79 ± 4.70 | 9.40 ± 1.04 | 22.14 ± 4.94 | 28.26 ± 2.16 | 38.53 ± 7.05 | 17.31 ± 1.53 | 8.69 ± 0.73 | 15.67 ± 2.05 |
| Trachea | 4.99 ± 0.28 | 7.17 ± 0.99 | 22.15 ± 2.35 | 16.73 ± 1.62 | 16.81 ± 2.28 | 33.14 ± 5.96 | 5.00 ± 0.44 | 10.43 ± 2.17 |
| Fat | 6.00 ± 1.06 | 4.19 ± 1.27 | 4.78 ± 0.75 | 8.41 ± 1.98 | 5.96 ± 1.68 | 3.11 ± 0.57 | 3.81 ± 0.55 | 5.52 ± 0.96 |

[a] Mice were exposed to the vapor of 150 µg of $^{3}$H-soman which resulted in a dose of 2 µg/kg. Each time point represents the mean of six individual animals.

**Table 6.** Tissue concentrations of residual $^{3}H$-MPA in mice after inhalation exposure[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 5.13 ± 0.48 | 9.12 ± 2.95 | 3.22 ± 0.79 | 3.80 ± 0.48 | 2.81 ± 0.31 | 6.10 ± 0.66 | 4.28 ± 0.47 | 3.77 ± 0.29 |
| Brain | 1.68 ± 0.16 | 1.53 ± 0.20 | 1.11 ± 0.32 | 2.04 ± 0.47 | 2.35 ± 0.51 | 1.93 ± 0.58 | 1.70 ± 0.47 | 1.29 ± 0.27 |
| Diaphragm | 11.09 ± 2.48 | 1.96 ± 0.29 | 1.33 ± 0.23 | 15.32 ± 3.29 | 9.16 ± 1.30 | 8.18 ± 2.90 | 2.75 ± 0.56 | 3.91 ± 1.30 |
| Heart | 4.27 ± 0.86 | 2.39 ± 0.55 | 4.22 ± 0.74 | 7.14 ± 1.92 | 3.24 ± 0.52 | 4.27 ± 1.04 | 3.43 ± 0.69 | 2.08 ± 0.25 |
| Kidney | 11.45 ± 1.65 | 6.46 ± 1.26 | 9.22 ± 2.08 | 9.89 ± 1.92 | 7.62 ± 1.29 | 6.52 ± 1.40 | 5.67 ± 1.26 | 7.38 ± 0.77 |
| Liver | 3.15 ± 0.43 | 3.15 ± 0.34 | 3.74 ± 0.37 | 3.67 ± 0.51 | 3.80 ± 0.72 | 4.07 ± 0.67 | 3.74 ± 0.44 | 3.57 ± 0.23 |
| Lung | 11.81 ± 3.18 | 3.96 ± 0.67 | 8.87 ± 1.57 | 12.03 ± 1.42 | 12.96 ± 2.42 | 9.70 ± 2.80 | 9.88 ± 2.62 | 5.93 ± 1.04 |
| Trachea | 5.78 ± 0.64 | 4.85 ± 1.26 | 21.84 ± 4.53 | 19.08 ± 3.09 | 14.58 ± 2.19 | 30.77 ± 5.69 | 7.39 ± 1.25 | 18.67 ± 3.79 |
| Fat | 11.22 ± 1.74 | 4.88 ± 1.16 | 7.42 ± 1.54 | 16.89 ± 1.94 | 6.63 ± 1.73 | 7.46 ± 1.32 | 8.00 ± 2.06 | 10.98 ± 2.12 |
| Carcass | | 1082 ± 142 | 1493 ± 101 | 1884 ± 360 | 1026 ± 81 | | | 1048 ± 173 |

[a] Mice were exposed to the vapor of 150 µg of $^{3}H$-soman which resulted in a dose of 2 µg/kg. Each time point represents the mean of six individual animals.

**Table 7.** Tissue concentrations of $^{3}H$-soman in mice after intramuscular administration of $^{3}H$-soman[a].

| Tissue | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 4.09 ± 0.74 | 3.20 ± 0.74 | 1.55 ± 0.30 | 1.66 ± 0.28 | 1.51 ± 0.10 | 2.32 ± 0.52 | 2.56 ± 0.36 | 2.17 ± 0.30 |
| Brain | 1.98 ± 0.20 | 1.40 ± 0.22 | 1.22 ± 0.05 | 0.78 ± 0.09 | 1.60 ± 0.35 | 2.75 ± 0.41 | 0.91 ± 0.19 | 1.88 ± 0.55 |
| Diaphragm | 5.74 ± 0.52 | 6.64 ± 1.67 | 3.39 ± 0.53 | 2.51 ± 0.49 | 6.32 ± 1.32 | 7.33 ± 0.71 | 3.67 ± 0.95 | 7.15 ± 1.17 |
| Heart | 6.28 ± 0.72 | 2.73 ± 0.64 | 5.35 ± 0.50 | 3.73 ± 0.61 | 2.97 ± 0.41 | 7.13 ± 1.83 | 7.06 ± 0.86 | 5.51 ± 1.00 |
| Kidney | 3.72 ± 0.31 | 4.86 ± 0.60 | 4.65 ± 0.52 | 3.85 ± 0.45 | 6.98 ± 1.39 | 7.22 ± 1.29 | 3.14 ± 0.30 | 5.34 ± 0.84 |
| Liver | 2.46 ± 0.12 | 2.23 ± 0.41 | 4.20 ± 0.38 | 1.94 ± 0.24 | 3.36 ± 0.96 | 3.78 ± 0.60 | 3.53 ± 0.35 | 2.71 ± 0.39 |
| Lung | 10.84 ± 0.87 | 6.89 ± 0.79 | 7.83 ± 0.88 | 4.93 ± 0.37 | 8.34 ± 1.72 | 11.95 ± 1.01 | 10.02 ± 0.96 | 6.79 ± 1.08 |
| Trachea | 16.34 ± 3.29 | 18.38 ± 5.11 | 4.61 ± 0.35 | 3.03 ± 0.52 | 4.74 ± 0.96 | 15.85 ± 4.07 | 3.61 ± 0.42 | 15.35 ± 3.20 |
| Fat | 5.92 ± 0.28 | 9.67 ± 1.46 | 4.32 ± 0.75 | 4.04 ± 0.45 | 10.15 ± 2.00 | 8.01 ± 1.23 | 4.16 ± 0.38 | 7.14 ± 1.29 |

[a] $^{3}H$-Soman dose was 25 µg/kg. Each time point represents the mean of six individual animals.

**Table 8.** Tissue concentrations of free $^{3}$H-PMPA in mice after intramuscular injection of $^{3}$H-soman[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 6.81 ± 2.02 | 3.94 ± 0.55 | 13.80 ± 6.14 | 3.55 ± 0.74 | 1.65 ± 0.14 | 1.18 ± 0.22 | 3.12 ± 0.70 | 2.17 ± 0 30 |
| Brain | 1.92 ± 0.57 | 0.98 ± 0.11 | 0.60 ± 0.04 | 0.51 ± 0.06 | 0.59 ± 0.13 | 0.98 ± 0.14 | 0.63 ± 0.10 | 1.88 ± 0.55 |
| Diaphragm | 4.05 ± 0.68 | 4.89 ± 0.81 | 1.83 ± 0.08 | 1.43 ± 0.21 | 2.49 ± 0.46 | 2.57 ± 0.30 | 2.33 ± 0.63 | 7.15 ± 1.17 |
| Heart | 2.73 ± 0.57 | 2.35 ± 0.24 | 1.20 ± 0.18 | 1.38 ± 0.19 | 1.49 ± 0.08 | 1.38 ± 0.28 | 2.19 ± 0.21 | 5.51 ± 1.00 |
| Kidney | 15.79 ± 3.66 | 16.87 ± 2.88 | 13.54 ± 3.54 | 8.06 ± 2.09 | 6.03 ± 0.86 | 4.30 ± 1.15 | 3.18 ± 0.43 | 5.34 ± 0.84 |
| Liver | 7.91 ± 0.31 | 5.65 ± 0.95 | 6.29 ± 0.64 | 3.83 ± 0.60 | 2.38 ± 0.57 | 2.08 ± 0.60 | 3.26 ± 0.55 | 2.71 ± 0.39 |
| Lung | 10.86 ± 1.32 | 7.21 ± 0.63 | 3.17 ± 0.23 | 2.66 ± 0.44 | 4.77 ± 0.73 | 4.14 ± 0.81 | 4.43 ± 0.39 | 6.79 ± 1.08 |
| Trachea | 9.56 ± 1.07 | 9.53 ± 1.36 | 2.62 ± 0.56 | 1.49 ± 0.42 | 2.24 ± 0.31 | 4.50 ± 0.97 | 2.12 ± 0.28 | 15.35 ± 3.20 |
| Fat | 2.95 ± 0.36 | 6.95 ± 1.82 | 3.52 ± 1.06 | 2.43 ± 0.44 | 2.05 ± 0.23 | 1.90 ± 0.35 | 1.89 ± 0.28 | 7.14 ± 1.29 |

[a] $^{3}$H-Soman dose was 25 µg/kg. Each time point represents the mean of six individual animals.

**Table 9.** Tissue concentrations of bound $^{3}$H-PMPA in mice after intramuscular injection of $^{3}$H-soman[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 18.98 ± 3.77 | 58.58 ± 9.05 | 108.72 ± 23.1 | 41.36 ± 3.53 | 41.22 ± 2.75 | 85.08 ± 22.6 | 10.09 ± 1.58 | 47.95 ± 9.11 |
| Brain | 0.81 ± 0.14 | 1.18 ± 0.11 | 0.87 ± 0.19 | 0.69 ± 0.04 | 0.98 ± 0.19 | 2.19 ± 0.29 | 0.66 ± 0.09 | 0.96 ± 0.11 |
| Diaphragm | 4.04 ± 0.57 | 6.24 ± 0.66 | 3.03 ± 0.46 | 3.55 ± 0.65 | 5.02 ± 0.90 | 7.71 ± 2.29 | 2.12 ± 0.59 | 5.07 ± 0.39 |
| Heart | 4.51 ± 0.71 | 10.92 ± 5.03 | 3.59 ± 0.66 | 3.92 ± 0.59 | 5.16 ± 0.76 | 24.65 ± 11.4 | 3.04 ± 0.33 | 10.47 ± 3.18 |
| Kidney | 6.59 ± 0.96 | 11.91 ± 2.84 | 18.93 ± 7.20 | 13.71 ± 4.01 | 11.46 ± 4.02 | 12.50 ± 3.81 | 5.19 ± 0.67 | 7.48 ± 0.93 |
| Liver | 4.12 ± 0.69 | 5.06 ± 1.00 | 10.44 ± 2.78 | 8.34 ± 1.73 | 5.30 ± 1.59 | 6.14 ± 2.57 | 6.51 ± 1.76 | 5.01 ± 1.60 |
| Lung | 25.99 ± 3.77 | 35.67 ± 12.3 | 21.88 ± 1.73 | 16.64 ± 1.78 | 22.34 ± 3.21 | 64.86 ± 17.4 | 14.66 ± 2.30 | 31.55 ± 5.58 |
| Trachea | 10.51 ± 0.89 | 13.84 ± 2.18 | 4.78 ± 0.88 | 4.01 ± 0.86 | 3.59 ± 0.07 | 18.11 ± 4.81 | 2.20 ± 0.30 | 7.57 ± 0.73 |
| Fat | 2.39 ± 0.35 | 10.23 ± 3.84 | 8.69 ± 3.23 | 9.27 ± 2.25 | 5.22 ± 0.92 | 5.50 ± 0.94 | 6.49 ± 2.98 | 5.99 ± 1.11 |

[a] $^{3}$H-Soman dose was 25 µg/kg. Each time point represents the mean of six individual animals.

as expected with excretory organs, while $^3$H-MPA levels in the liver remained relatively constant (Table 10).

**Biodisposition and metabolism of $^3$H-soman in guinea pigs after inhalation exposure.** The biodisposition of $^3$H-soman in guinea pigs following inhalation of a dose of 2 µg/kg is presented in Table 11. As expected the highest concentrations were found in lung 5 min after exposure. Trachea, fat and kidney contained relatively high concentrations at this first time point whereas the remaining tissues contained lower concentrations which were approximately equivalent to each other. The concentrations of $^3$H-soman fell dramatically by the 30 min time point but then remained relatively constant for the duration of the time period studied. In addition, the $^3$H-soman was distributed relatively evenly throughout all tissues, with the possible exception of the trachea, during the 30-1440 time period. Within 5 min, the major portion of the $^3$H-soman had either been hydrolyzed to free $^3$H-PMPA (Table 12) or had phosphorylated tissue in the form of bound $^3$H-PMPA (Table 13). At the earliest time point, the greatest quantity of free $^3$H-PMPA was found in the kidneys followed by lung and trachea. Interestingly, the lowest quantities were found in brain. The concentrations in kidneys and liver increased until they reached maximal quantities at 30 min and then declined during the remainder of the time course. A similar time course was observed in the trachea which is not readily explainable. In most tissues, there was a steady decline in free $^3$H-PMPA for the entire time course. As for bound $^3$H-PMPA (Table 13), the highest concentrations were found in trachea which remained high for at least 6 hr. The next highest concentrations were measured in lung and kidney. Maximal concentrations in brain occurred at 15 min but were relatively low thereafter. The distribution pattern of $^3$H-MPA (Table 14) was somewhat similar to that of $^3$H-PMPA. The highest concentrations were found in trachea and there was a relatively even distribution in the other tissues at the 5 min time point. One of the major differences between their biodisposition was the higher concentrations of $^3$H-MPA in brain which would be expected due to the high concentration of acetylcholinesterase in brain.

**Biodisposition and metabolism of $^3$H-soman in guinea pigs after intramuscular administration.** This route of administration resulted in a sustained release of $^3$H-soman following treatment with a dose of 15 µg/kg (Table 15). In most tissues maximal concentrations were attained at the 60 min time point. These tissues included diaphragm, heart, kidney, kiver, lung and fat. Maximal concentrations were achieved immediately in blood and only declined by 50% after 6 hr. Levels in all tissues remained basically constant through the 60 min time point and then declined slowly through the 480 min time point, except for the lung and testicular fat which had increasing and maximum concentrations over the 480 and 1440 min time points. As with the other routes of administration, $^3$H-soman was rapidly hydrolyzed to free $^3$H-PMPA which was present in all tissues (Table 16). Free $^3$H-PMPA tissue concentrations were highest in the kidney and lung at 15 and 30 min following treatment. while free $^3$H-PMPA levels in the blood were greatest at 5 min and declined over time. It is interesting to note that the lowest concentrations of free $^3$H-PMPA were found in brain. Bound $^3$H-PMPA tissue concentrations were highest for the blood, kidney, and lung (Table 17) after 5 min. In general, the highest tissue levels, found after either 5 or 30 min, declined slowly thereafter. An increase in bound $^3$H-PMPA was observed in blood from 15 to 30 min, and then varied only slightly with time until increases occurred at the 240 and 480 min marks. One of the most striking features of the bound $^3$H-PMPA was the exceedingly high concentrations which were present in lung. Very low concentrations of bound $^3$H-PMPA were present in brain which were similar to those of free $^3$H-PMPA. High concentrations of $^3$H-MPA, consistent with phosphorylation of cholinesterase in areas with high acetylcholinesterase activity, were found in the brain and in excretory organs such as the kidneys (Table 18).

**Table 10.** Tissue concentrations of residual $^{3}$H-MPA in mice after intramuscular injection of $^{3}$H-soman[a].

| Tissue | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 6.38 ± 0.25 | 4.13 ± 0.43 | 12.43 ± 1.17 | 19.62 ± 5.65 | 5.00 ± 1.55 | 5.83 ± 0.89 | 4.51 ± 0.34 | 2.30 ± 0.28 |
| Brain | 1.17 ± 0.17 | 1.87 ± 0.29 | 2.22 ± 0.31 | 3.28 ± 0.37 | 1.47 ± 0.65 | 3.11 ± 0.54 | 1.31 ± 0.25 | 1.07 ± 0.26 |
| Diaphragm | 3.76 ± 1.05 | 6.96 ± 0.61 | 4.81 ± 0.97 | 8.14 ± 0.77 | 7.39 ± 1.45 | 6.96 ± 1.74 | 2.93 ± 0.49 | 5.84 ± 0.94 |
| Heart | 3.09 ± 0.31 | 2.78 ± 0.17 | 3.94 ± 0.74 | 4.97 ± 0.93 | 3.72 ± 0.52 | 5.27 ± 1.26 | 3.45 ± 0.69 | 4.06 ± 0.75 |
| Kidney | 9.92 ± 1.08 | 7.43 ± 1.42 | 12.50 ± 1.72 | 13.03 ± 1.14 | 7.16 ± 1.24 | 8.64 ± 1.28 | 4.35 ± 0.53 | 5.38 ± 1.09 |
| Liver | 2.57 ± 0.31 | 2.97 ± 0.29 | 4.57 ± 0.55 | 7.16 ± 1.50 | 3.82 ± 1.00 | 5.11 ± 0.62 | 3.24 ± 0.63 | 1.99 ± 0.07 |
| Lung | 16.75 ± 1.18 | 7.32 ± 1.80 | 10.25 ± 0.90 | 15.27 ± 1.82 | 15.18 ± 3.48 | 10.41 ± 2.83 | 6.97 ± 2.08 | 4.46 ± 1.03 |
| Trachea | 20.71 ± 4.64 | 16.41 ± 1.67 | 10.20 ± 1.50 | 10.68 ± 0.78 | 5.15 ± 0.55 | 17.96 ± 5.95 | 3.84 ± 0.69 | 11.66 ± 1.90 |
| Fat | 6 38 ± 0.75 | 6.33 ± 0.46 | 9.15 ± 1.96 | 12.78 ± 2.58 | 5.90 ± 0.90 | 3.28 ± 0.87 | 4.26 ± 0.97 | 3.67 ± 0.67 |
| Carcass | 1697 ± 146 | 2312 ± 0.467 | 2472 ± 122 | 2111 ± 254 | 1131 ± 153 | 1595 ± 205 | 1138 ± 134 | |

[a] $^{3}$H-Soman dose was 25 µg/kg. Each time point represents the mean of six individual animals.

**Table 11.** Tissue concentrations of $^{3}H$-soman in guinea pigs after inhalation exposure[a].

| Tissue | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 1.78 ± 0.08 | 1.38 ± 0.08 | 0.59 ± 0.08 | 0.54 ± 0.04 | 0.41 ± 0.02 | 0.48 ± 0.03 | 0.27 ± 0.01 | 0.39 ± 0.03 |
| Brain | 1.86 ± 0.15 | 1.43 ± 0.07 | 0.49 ± 0.03 | 0.50 ± 0.03 | 0.43 ± 0.02 | 0.42 ± 0.03 | 0.31 ± 0.02 | 0.44 ± 0.05 |
| Diaphragm | 1.97 ± 0.24 | 1.29 ± 0.10 | 0.49 ± 0.03 | 0.55 ± 0.05 | 0.47 ± 0.04 | 0.55 ± 0.04 | 0.27 ± 0.02 | 0.40 ± 0.03 |
| Heart | 2.05 ± 0.16 | 1.32 ± 0.07 | 0.54 ± 0.03 | 0.53 ± 0.04 | 0.46 ± 0.02 | 0.54 ± 0.05 | 0.29 ± 0.01 | 0.42 ± 0.01 |
| Kidney | 3.81 ± 0.22 | 1.57 ± 0.11 | 0.54 ± 0.03 | 0.65 ± 0.08 | 0.51 ± 0.04 | 0.66 ± 0.13 | 0.27 ± 0.02 | 0.43 ± 0.02 |
| Liver | 2.07 ± 0.25 | 1.35 ± 0.11 | 0.55 ± 0.03 | 0.54 ± 0.45 | 0.45 ± 0.01 | 0.57 ± 0.08 | 0.26 ± 0.02 | 0.42 ± 0.02 |
| Lung | 4.58 ± 0.21 | 1.60 ± 0.11 | 0.58 ± 0.04 | 0.59 ± 0.03 | 0.50 ± 0.03 | 0.72 ± 0.05 | 0.32 ± 0.01 | 0.45 ± 0.03 |
| Trachea | 3.88 ± 0.93 | 2.28 ± 0.30 | 1.16 ± 0.05 | 1.14 ± 0.13 | 1.27 ± 0.09 | 1.64 ± 0.42 | 0.32 ± 0.04 | 0.79 ± 0.06 |
| Fat | 4.17 ± 0.47 | 1.28 ± 0.22 | 0.56 ± 0.07 | 0.49 ± 0.02 | 0.45 ± 0.04 | 0.87 ± 0.23 | 0.30 ± 0.02 | 0.46 ± 0.04 |

[a] The $^{3}H$-soman dose was 5 µg/kg. Each time point represents the mean of six individual animals.

**Table 12.** Tissue concentrations of free $^{3}$H-PMPA in guinea pigs after inhalation exposure[a].

| Tissue | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 4.79 ± 0.62 | 7.32 ± 0.73 | 5.03 ± 0.70 | 4.76 ± 0.59 | 1.82 ± 0.18 | 1.03 ± 0.08 | 2.87 ± 0.25 | 1.16 ± 0.05 |
| Brain | 2.37 ± 0.22 | 2.81 ± 0.51 | 1.29 ± 0.15 | 1.12 ± 0.06 | 0.93 ± 0.07 | 0.71 ± 0.03 | 2.86 ± 0.39 | 0.69 ± 0.09 |
| Diaphragm | 3.28 ± 0.49 | 4.85 ± 0.77 | 3.14 ± 0.69 | 3.62 ± 0.39 | 1.47 ± 0.27 | 1.25 ± 0.15 | 2.10 ± 0.07 | 0.88 ± 0.07 |
| Heart | 3.76 ± 0.59 | 3.87 ± 0.59 | 2.67 ± 0.19 | 2.71 ± 0.17 | 1.57 ± 0.15 | 1.09 ± 0.11 | 2.57 ± 0.34 | 0.94 ± 0.06 |
| Kidney | 12.58 ± 1.49 | 19.91 ± 1.84 | 22.94 ± 3.33 | 16.64 ± 0.56 | 5.65 ± 1.09 | 1.71 ± 0.10 | 2.73 ± 0.13 | 0.82 ± 0.07 |
| Liver | 4.22 ± 0.52 | 5.63 ± 0.77 | 7.69 ± 1.16 | 7.19 ± 0.55 | 2.84 ± 0.29 | 1.35 ± 0.13 | 2.96 ± 0.25 | 0.80 ± 0.05 |
| Lung | 8.09 ± 0.40 | 8.55 ± 2.10 | 7.45 ± 1.08 | 7.12 ± 0.62 | 3.83 ± 0.63 | 1.92 ± 0.22 | 3.21 ± 0.21 | 1.02 ± 0.08 |
| Trachea | 6.15 ± 0.64 | 15.38 ± 3.58 | 17.83 ± 2.94 | 14.74 ± 1.89 | 11.02 ± 1.33 | 4.70 ± 0.45 | 2.61 ± 0.36 | 1.73 ± 0.17 |
| Fat | 5.31 ± 0.99 | 5.46 ± 1.08 | 3.23 ± 0.52 | 2.58 ± 0.28 | 1.12 ± 0.14 | 1.79 ± 0.45 | 3.35 ± 0.22 | 0.68 ± 0.09 |

[a] The $^{3}$H-soman dose was 5 µg/kg. Each time point represents the mean of six individual animals.

**Table 13.** Tissue concentrations of bound $^{3}$H-PMPA in guinea pigs after inhalation exposure[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 5.59 ± 0.81 | 14.60 ± 0.65 | 14.85 ± 1.22 | 11.73 ± 1.75 | 8.72 ± 0.92 | 11.09 ± 0.68 | 7.30 ± 0.17 | 9.33 ± 0.20 |
| Brain | 3.13 ± 0.28 | 4.68 ± 0.20 | 1.53 ± 0.17 | 1.48 ± 0.07 | 1.16 ± 0.12 | 1.39 ± 0.18 | 0.78 ± 0.06 | 0.79 ± 0.05 |
| Diaphragm | 3.43 ± 0.44 | 9.11 ± 1.64 | 2.44 ± 0.18 | 2.73 ± 0.25 | 1.79 ± 0.24 | 2.21 ± 0.36 | 0.92 ± 0.16 | 1.07 ± 0.09 |
| Heart | 4.26 ± 0.75 | 6.70 ± 0.84 | 3.06 ± 0.15 | 2.94 ± 0.21 | 2.00 ± 0.25 | 2.09 ± 0.17 | 1.98 ± 0 33 | 1.40 ± 0.18 |
| Kidney | 11.82 ± 2.34 | 24.03 ± 6.58 | 12.67 ± 1.30 | 10.98 ± 1.68 | 6.90 ± 1.26 | 3.12 ± 0.25 | 1.42 ± 0.16 | 1.28 ± 0.11 |
| Liver | 4.97 ± 1.25 | 13.63 ± 1.35 | 19.66 ± 0.89 | 3.30 ± 0.30 | 2.19 ± 0.18 | 2.24 ± 0.39 | 1.11 ± 0.04 | 1.47 ± 0.07 |
| Lung | 12.13 ± 3.88 | 12.20 ± 2.26 | 7.21 ± 0.96 | 7.47 ± 0.87 | 4.94 ± 0.75 | 4.58 ± 0.32 | 6.26 ± 1.80 | 2.58 ± 0.33 |
| Trachea | 20.18 ± 5.37 | 26.67 ± 3.07 | 17.06 ± 1.97 | 16.26 ± 1.27 | 14.42 ± 1.84 | 13.53 ± 2.67 | 4.14 ± 0.58 | 4.45 ± 0.77 |
| Fat | 3.69 ± 0.38 | 8.12 ± 2.37 | 2.36 ± 0.31 | 2.21 ± 0.14 | 1.46 ± 0.11 | 2.26 ± 0.43 | 0.98 ± 0.10 | 1.01 ± 0.13 |

[a] The $^{3}$H-soman dose was 5 µg/kg. Each time point represents the mean of six individual animals.

**Table 14.** Tissue concentrations of residual $^{3}H$-MPA in guinea pigs after inhalation exposure[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 12.06 ± 1.69 | 9.34 ± 0.90 | 11.27 ± 1.52 | 7.64 ± 1.44 | 7.96 ± 1.16 | 11.09 ± 2.67 | 2.33 ± 0.38 | 3.32 ± 1.30 |
| Brain | 9.83 ± 1.37 | 8.49 ± 1.94 | 5.21 ± 0.44 | 3.98 ± 0.28 | 4.64 ± 0.42 | 2.71 ± 0.53 | 1.33 ± 0.17 | 2.18 ± 0.28 |
| Diaphragm | 9.71 ± 1.47 | 5.56 ± 0.33 | 4.43 ± 0.34 | 4.40 ± 0.36 | 4.03 ± 0.16 | 3.21 ± 0.40 | 1.36 ± 0.52 | 2.18 ± 0.31 |
| Heart | 10.40 ± 1.58 | 6.55 ± 1.26 | 5.18 ± 0.43 | 4.81 ± 0.64 | 4.71 ± 0.40 | 3.68 ± 0.36 | 1.46 ± 0.02 | 3.64 ± 1.22 |
| Kidney | 12.91 ± 1.43 | 10.70 ± 1.40 | 7.63 ± 0.42 | 6.16 ± 0.36 | 5.85 ± 0.42 | 3.47 ± 0.44 | 1.55 ± 0.25 | 2.55 ± 0.17 |
| Liver | 10.58 ± 1.21 | 8.47 ± 1.10 | 5.91 ± 0.69 | 4.61 ± 0.52 | 4.32 ± 0.34 | 3.64 ± 0.35 | 1.35 ± 0.09 | 2 59 ± 0.12 |
| Lung | 12.44 ± 1.12 | 9.39 ± 1.51 | 6.87 ± 0.57 | 6.41 ± 0.85 | 7.05 ± 1.27 | 4.16 ± 0.43 | 1.61 ± 0.11 | 3.40 ± 0.25 |
| Trachea | 20.42 ± 2.25 | 12.92 ± 1.94 | 14.10 ± 1.52 | 11.07 ± 0.92 | 14.56 ± 1.83 | 7.54 ± 1.41 | 1.90 ± 0.25 | 5.84 ± 0.98 |
| Fat | 11.67 ± 1.71 | 7.89 ± 1.73 | 6.22 ± 0.67 | 4.57 ± 0.35 | 5.02 ± 0.31 | 4.51 ± 1.38 | 1.25 ± 0.16 | 1.90 ± 0.20 |
| Carcass | 2089 ± 159 | 2704 ± 292 | 3279 ± 315 | 2599 ± 472 | 1742 ± 145 | 709 ± 36 | 540 ± 68 | 484 ± 75 |

[a] The $^{3}H$-soman dose was 5 µg/kg. Each time point represents the mean of six individual animals.

**Table 15.** Tissue concentrations of $^{3}H$-soman in guinea pigs after intramuscular injection[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 11.49 ± 1.20 | 11.18 ± 0.44 | 11.35 ± 0.83 | 8.63 ± 1.43 | 9.04 ± 0.84 | 6.16 ± 0.88 | 6.90 ± 1.09 | 8.57 ± 0.69 |
| Brain | 6.73 ± 0.53 | 5.57 ± 0.36 | 6.19 ± 0.94 | 6.32 ± 0.49 | 4.63 ± 0.41 | 5.10 ± 0.66 | 7.03 ± 1.51 | 9.20 ± 0.84 |
| Diaphragm | 5.23 ± 0.55 | 6.42 ± 0.34 | 3.94 ± 0.85 | 8.38 ± 0.96 | 4.86 ± 0.71 | 4.74 ± 0.63 | 5.76 ± 0.76 | 12.16 ± 1.35 |
| Heart | 5.93 ± 0.92 | 6.01 ± 0.22 | 5.65 ± 0.70 | 15.97 ± 1.23 | 7.38 ± 1.14 | 5.40 ± 0.32 | 9.30 ± 0.73 | 13.14 ± 0.94 |
| Kidney | 11.33 ± 1.50 | 10.62 ± 1.42 | 10.69 ± 1.85 | 13.31 ± 1.04 | 9.48 ± 0.52 | 10.90 ± 1.33 | 11.27 ± 1.61 | 22.88 ± 1.04 |
| Liver | 6.20 ± 0.68 | 6.14 ± 0.61 | 4.19 ± 0.84 | 9.70 ± 0.51 | 5.68 ± 0.36 | 4.76 ± 0.44 | 5.96 ± 0.90 | 9.73 ± 1.04 |
| Lung | 11.42 ± 0.66 | 10.76 ± 1.97 | 14.09 ± 1.20 | 26.84 ± 1.78 | 11.57 ± 0.93 | 10.79 ± 1.17 | 16.35 ± 0.85 | 22.45 ± 2.09 |
| Trachea | 7.03 ± 1.05 | 4.04 ± 0.34 | 4.70 ± 1.30 | 7.68 ± 0.83 | 6.51 ± 0.64 | 8.61 ± 0.94 | 6.19 ± 0.80 | 14.04 ± 2.10 |
| Fat | 14.67 ± 1.42 | 13.88 ± 0.23 | 10.57 ± 1.58 | 16.06 ± 0.88 | 11.24 ± 0.76 | 10.87 ± 0.90 | 17.90 ± 0.77 | 29.79 ± 2.09 |

[a] The $^{3}H$-soman dose was 15 μg/kg. Each time point represents the mean of six individual animals.

**Table 16.** Tissue concentrations of free $^{3}$H-PMPA in guinea pigs after intramuscular injection of $^{3}$H-soman[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 10.03 ± 3.30 | 3.21 ± 0.90 | 4.80 ± 0.83 | 2.07 ± 0.60 | 2.28 ± 0.13 | 1.76 ± 0.12 | 1.48 ± 0.22 | 2.55 ± 0.15 |
| Brain | 1.58 ± 0.24 | 1.36 ± 0.11 | 1.21 ± 0.13 | 1.49 ± 0.21 | 1.81 ± 0.13 | 1.15 ± 0.10 | 1.61 ± 0.23 | 1.96 ± 0.07 |
| Diaphragm | 2.62 ± 0.67 | 1.84 ± 0.30 | 1.85 ± 0.29 | 1.50 ± 0.23 | 2.37 ± 0.40 | 1.50 ± 0.11 | 1.63 ± 0.15 | 2.54 ± 0.14 |
| Heart | 3.40 ± 0.66 | 2.24 ± 0.64 | 2.55 ± 0.40 | 2.16 ± 0.19 | 3.41 ± 0.25 | 2.36 ± 0.22 | 1.82 ± 0.36 | 3.27 ± 0.15 |
| Kidney | 30.38 ± 8.17 | 23.23 ± 9.52 | 21.77 ± 6.49 | 5.36 ± 1.43 | 6.88 ± 0.52 | 6.62 ± 0.11 | 5.10 ± 1.00 | 8.32 ± 0.93 |
| Liver | 3.71 ± 0.74 | 4.43 ± 1.22 | 4.26 ± 1.18 | 2.53 ± 0.70 | 2.85 ± 0.34 | 2.11 ± 0.21 | 1.66 ± 0.24 | 2.93 ± 0.32 |
| Lung | 15.27 ± 5.37 | 16.04 ± 6.26 | 13.62 ± 4.47 | 6.42 ± 1.70 | 10.30 ± 0.31 | 8.52 ± 0.58 | 5.90 ± 0.83 | 11.05 ± 1.34 |
| Trachea | 3.04 ± 0.83 | 2.56 ± 0.79 | 1.84 ± 0.20 | 1.62 ± 0.19 | 1.95 ± 0.32 | 2.24 ± 0.17 | 1.53 ± 0.15 | 2.39 ± 0.32 |
| Fat | 4.19 ± 1.30 | 3.20 ± 0.69 | 2.86 ± 0.26 | 2.28 ± 0.33 | 3.59 ± 0.21 | 2.92 ± 0.40 | 2.65 ± 0.36 | 5.70 ± 0.44 |

[a] The $^{3}$H-soman dose was 15 µg/kg. Each time point represents the mean of six individual animals.

**Table 17.** Tissue concentrations of bound $^{3}$H-PMPA in guinea pigs after intramuscular injection of $^{3}$H-soman[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 25.26 ± 5.58 | 18.20 ± 0.84 | 36.08 ± 7.38 | 11.76 ± 1.57 | 14.38 ± 1.78 | 14.45 ± 1.89 | 25.52 ± 3.90 | 3.97 ± 0.19 |
| Brain | 1.15 ± 0.16 | 1.37 ± 0.27 | 1.50 ± 0.16 | 1.11 ± 0.10 | 1.12 ± 0.15 | 1.41 ± 0.04 | 1.37 ± 0.22 | 0.67 ± 0.12 |
| Diaphragm | 2.14 ± 0.40 | 1.69 ± 0.29 | 1.50 ± 0.26 | 1.81 ± 0.35 | 1.43 ± 0.27 | 2.03 ± 0.30 | 1.26 ± 0.19 | 1.09 ± 0.24 |
| Heart | 4.80 ± 1.12 | 1.76 ± 0.30 | 1.89 ± 0.26 | 3.91 ± 0.98 | 1.70 ± 0.31 | 3.07 ± 0.21 | 2.40 ± 0.47 | 1.52 ± 0.39 |
| Kidney | 12.46 ± 4.96 | 16.25 ± 7.28 | 17.03 ± 5.95 | 5.81 ± 1.29 | 14.14 ± 2.05 | 20.33 ± 2.86 | 10.79 ± 3.30 | 8.52 ± 1.69 |
| Liver | 2.42 ± 0.62 | 2.70 ± 0.43 | 2.28 ± 0.39 | 2.00 ± 0.30 | 2.28 ± 0.37 | 2.91 ± 0.47 | 1.93 ± 0.39 | 1.60 ± 0.13 |
| Lung | 39.13 ± 10.3 | 19.55 ± 6.61 | 27.05 ± 7.94 | 21.75 ± 6.61 | 27.56 ± 6.41 | 28.75 ± 2.77 | 27.91 ± 5.58 | 11.15 ± 0.93 |
| Trachea | 3.76 ± 1.37 | 1.95 ± 0.35 | 1.98 ± 0.28 | 4.61 ± 1.02 | 1.65 ± 0.13 | 3.58 ± 0.46 | 1.36 ± 0.20 | 0.81 ± 0.15 |
| Fat | 2.57 ± 0.57 | 2.79 ± 0.43 | 3.21 ± 0.35 | 3.27 ± 0.38 | 2.82 ± 0.37 | 3.51 ± 0.55 | 3.43 ± 0.29 | 1.55 ± 0.34 |

[a] The $^{3}$H-soman dose was 15 µg/kg. Each time point represents the mean of six individual animals.

**Table 18.** Tissue concentrations of residual $^{3}$H-MPA in guinea pigs after intramuscular injection of $^{3}$H-soman[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 4.55 ± 1.24 | 14.20 ± 2.38 | 9.21 ± 1.09 | 6.95 ± 0.62 | 4.84 ± 0.29 | 6.47 ± 0.76 | 9.65 ± 1.66 | 2.57 ± 0.28 |
| Brain | 1.61 ± 0.24 | 6.83 ± 0.49 | 2.32 ± 0.23 | 4.32 ± 0.50 | 4.11 ± 0.34 | 1.45 ± 0.24 | 12.58 ± 1.72 | 1.25 ± 0.27 |
| Diaphragm | 1.72 ± 0.24 | 8.00 ± 0.87 | 2.58 ± 0.25 | 4.04 ± 0.39 | 4.68 ± 0.54 | 1.62 ± 0.11 | 8.91 ± 1.60 | 1.96 ± 0.19 |
| Heart | 2.20 ± 0.31 | 8.04 ± 0.26 | 3.10 ± 0.48 | 4.31 ± 0.44 | 3.72 ± 0.58 | 2.58 ± 0.36 | 8.72 ± 1.53 | 1.60 ± 0.15 |
| Kidney | 7.44 ± 1.48 | 20.82 ± 2.03 | 11.18 ± 2.49 | 11.36 ± 2.11 | 10.79 ± 1.29 | 5.44 ± 0.59 | 12.13 ± 1.03 | 3.09 ± 0.76 |
| Liver | 2.01 ± 0.16 | 7.40 ± 0.67 | 3.05 ± 0.48 | 3.59 ± 0.35 | 3.73 ± 0.49 | 2.20 ± 0.41 | 5.81 ± 0.81 | 1.86 ± 0.17 |
| Lung | 5.80 ± 0.39 | 16.18 ± 1.25 | 7.81 ± 0.53 | 9.21 ± 0.98 | 9.11 ± 1.03 | 5.94 ± 0.27 | 31.05 ± 2.74 | 4.53 ± 0.63 |
| Trachea | 1.50 ± 0.29 | 7.14 ± 0.69 | 3.20 ± 0.38 | 5.02 ± 0.43 | 4.93 ± 0.69 | 3.03 ± 0.57 | 3.29 ± 0.78 | 1.37 ± 0.22 |
| Fat | 2.49 ± 0.82 | 14.68 ± 1.84 | 2.45 ± 0.20 | 7.45 ± 0.64 | 6.43 ± 0.71 | 4.51 ± 0.70 | 9.21 ± 0.60 | 2.67 ± 0.29 |
| Carcass | 964 ± 204 | 2873 ± 362 | 1408 ± 205 | 1992 ± 262 | 1235 ± 196 | 1951 ± 86 | 1267 ± 372 | 930 ± 147 |

[a] The $^{3}$H-soman dose was 15 µg/kg. Each time point represents the mean of six individual animals.

**Table 19.** Tissue concentrations of $^{3}H$-soman in guinea pigs after intravenous injection[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 9.80 ± 0.51 | 4.75 ± 0.47 | 2.32 ± 0.15 | 2.96 ± 0.23 | 1.51 ± 0.06 | 0.87 ± 0.06 | 0.96 ± 0.08 | 0.70 ± 0.06 |
| Brain | 8.86 ± 0.79 | 1.32 ± 0.15 | 2.91 ± 0.34 | 4.14 ± 0.82 | 0.97 ± 0.18 | 1.35 ± 0.23 | 1.25 ± 0.11 | 0.81 ± 0.08 |
| Diaphragm | 8.69 ± 0.41 | 1.64 ± 0.14 | 3.64 ± 0.17 | 3.63 ± 0.39 | 2.23 ± 0.54 | 1.56 ± 0.12 | 1.50 ± 0.16 | 0.81 ± 0.08 |
| Heart | 12.32 ± 0.68 | 3.76 ± 0.41 | 4.69 ± 0.34 | 3.69 ± 0.31 | 2.00 ± 0.11 | 1.95 ± 0.10 | 1.64 ± 0.20 | 0.83 ± 0.04 |
| Kidney | 20.53 ± 2.52 | 4.07 ± 0.69 | 9.37 ± 1.17 | 7.48 ± 0.50 | 4.99 ± 0.61 | 3.09 ± 0.65 | 1.52 ± 0.19 | 1.33 ± 0.13 |
| Liver | 7.37 ± 0.55 | 2.76 ± 0.18 | 2.59 ± 0.07 | 3.66 ± 0.45 | 2.04 ± 0.16 | 1.32 ± 0.15 | 1.33 ± 0.11 | 0.70 ± 0.04 |
| Lung | 22.06 ± 1.63 | 11.26 ± 1.13 | 14.40 ± 0.98 | 10.38 ± 0.99 | 5.55 ± 0.51 | 3.78 ± 0.47 | 1.55 ± 0.18 | 1.69 ± 0.14 |
| Trachea | 9.99 ± 0.42 | 2.04 ± 0.12 | 3.17 ± 0.31 | 4.76 ± 0.20 | 1.72 ± 0.25 | 1.53 ± 0.13 | 1.52 ± 0.11 | 0.56 ± 0.13 |
| Fat | 15.54 ± 0.63 | 2.41 ± 0.53 | 4.42 ± 0.25 | 5.50 ± 0.25 | 4.61 ± 0.26 | 2.81 ± 0.26 | 1.70 ± 0.14 | 1.84 ± 0.12 |

[a] The $^{3}H$-soman dose was 15 µg/kg. Each time point represents the mean of six individual animals.

**Biodisposition and metabolism of $^3$H-soman in guinea pigs after intravenous administration.** The intravenous administration of 15 µg/kg of $^3$H-soman resulted in a rapid transfer to all tissues. Organs which contained the greatest quantity of $^3$H-soman at the 5 min time point were lung, kidney and, surprisingly, fat (Table 19). Concentrations were also quite high in brain at this initial observation time. However, the concentrations

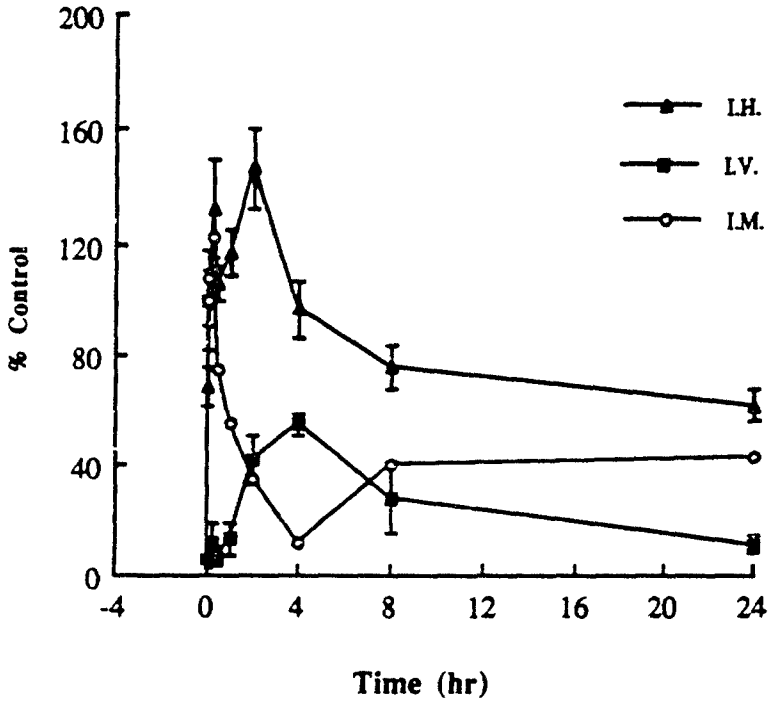



**Figure 1. True cholinesterase activity in blood in guinea pigs after exposure to soman by different routes of administration.** The results are expressed as a percent of control animals which were treated with saline either i.v or i.m. or exposed to air in a manner similar to the soman inhalation.

True cholinesterase activities (means ± SEM) for control guinea pigs were 1.35 ± 0.14, 2.96 ± 0.51, 5.96 ± 1.32 nmol of ACh hydrolyzed per mg protein per min following vehicle administration by inhalation (I.H.), intravenous (I.V.) and intramuscular (I.M.) administration, respectively. Each data point represents a mean of six animals. Despite the fact that intra-assay variability (represented by the triplicate values of any given time point) was very low, the inter-assay variability was often large.

- For I.H. group, the variability in the data was 6%-13% of the mean values.
- For I.V. group, the variability in the data was 7%-55% of the mean values.
- For I.M. group, the variability in the data was 10%-46% of the mean values.

dissipated rather quickly in most tissues. $^{3}$H-Soman appeared to be hydrolyzed faster to free $^{3}$H-PMPA after intravenous administration than after intramuscular administration. Free $^{3}$H-PMPA concentrations were extremely high in kidney and lung at 5 min and remained so up to the 1440 min mark (Table 20). Free $^{3}$H-PMPA levels in the blood peaked at 5 min and declined steadily over time. Free $^{3}$H-PMPA concentrations declined rapidly through the 60 min time point and then declined to low but variable levels for the remainder of the time course. Bound $^{3}$H-PMPA tissue concentrations were highest for the blood, followed by kidney and lung (Table 21). Very low quantities were found in brain. The highest levels, found in tissues between 5 and 60 min, declined slowly thereafter. An unexplained increase in bound $^{3}$H-PMPA was observed in most tissues at the 60 min time point. The appearance of $^{3}$H-MPA was somewhat erratic although the high concentrations of $^{3}$H-MPA were typically found within 30 min of administration (Table 22).

**Inhibition of acetylcholinesterase activity by soman.** Total, pseudo-, and true cholinesterase activities were measured in tissue samples as described in Methods section (p. 12). The effects of soman on cholinesterase activity in guinea pigs after either inhalation (5 µg/kg), intravenous (15 µg/kg) or intramuscular (15 µg/kg) administration is presented in Figure 1.

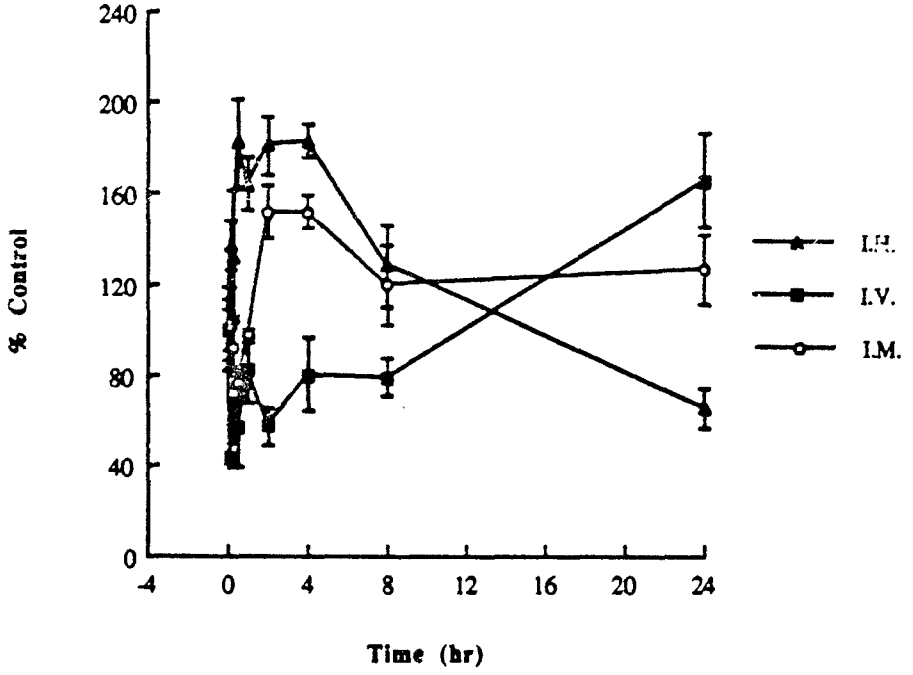



**Figure 2. True cholinesterase activity in blood in mice after exposure to soman by different routes of administration.** The results are expressed as a percent of control animals which were treated with saline either i.v or i.m. or exposed to air in a manner similar to the soman inhalation.

**Table 20.** Tissue concentrations of free $^{3}$H-PMPA in guinea pigs after intravenous injection of $^{3}$H-soman[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 15.73 ± 3.79 | 7.46 ± 1.05 | 5.89 ± 0.74 | 5.23 ± 1.73 | 1.98 ± 0.30 | 2.34 ± 0.47 | 1.23 ± 0.19 | 0.50 ± 0.03 |
| Brain | 3.36 ± 0.49 | 1.68 ± 0.33 | 1.48 ± 0.28 | 1.14 ± 0.23 | 1.24 ± 0.17 | 0.86 ± 0.13 | 1.16 ± 0.17 | 0.36 ± 0.05 |
| Diaphragm | 8.13 ± 0.85 | 3.12 ± 0.47 | 4.66 ± 0.41 | 2.43 ± 0.61 | 1.61 ± 0.22 | 1.51 ± 0.27 | 1.29 ± 0.19 | 0.37 ± 0.03 |
| Heart | 12.83 ± 1.43 | 4.12 ± 0.71 | 8.02 ± 1.54 | 5.76 ± 1.14 | 2.64 ± 0.05 | 2.53 ± 0.60 | 2.15 ± 0.26 | 0.59 ± 0.08 |
| Kidney | 133.40 ± 6.05 | 91.70 ± 19.2 | 57.90 ± 9.97 | 44.61 ± 8.69 | 10.71 ± 2.06 | 12.26 ± 4.05 | 5.63 ± 0.42 | 1.22 ± 0.06 |
| Liver | 12.76 ± 1.71 | 7.49 ± 1.04 | 7.18 ± 1.22 | 6.87 ± 1.13 | 2.35 ± 0.66 | 2.80 ± 0.48 | 1.72 ± 0.11 | 0.37 ± 0.04 |
| Lung | 72.38 ± 15.5 | 48.82 ± 8.48 | 48.26 ± 12.7 | 32.32 ± 7.94 | 17.03 ± 4.93 | 13.98 ± 2.65 | 8.03 ± 1.05 | 2.12 ± 0.21 |
| Trachea | 6.68 ± 0.56 | 2.94 ± 0.33 | 4.40 ± 0.69 | 2.23 ± 0.55 | 1.50 ± 0.17 | 1.19 ± 0.15 | 1.22 ± 0.08 | 0.30 ± 0.05 |
| Fat | 6.64 ± 0.53 | 4.77 ± 0.91 | 5.88 ± 0.56 | 4.55 ± 1.25 | 2.23 ± 0.34 | 1.93 ± 0.26 | 2.22 ± 0.21 | 0.54 ± 0.07 |

[a] The $^{3}$H-soman dose was 15 µg/kg. Each time point represents the mean of six individual animals.

**Table 21.** Tissue concentrations of bound $^{3}$H-PMPA in guinea pigs after intravenous injection of $^{3}$H-soman[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 182.05 ± 11.3 | 159.20 ± 9.80 | 173.70 ± 8.18 | 62.93 ± 15.1 | 21.17 ± 0.54 | 17.44 ± 2.55 | 7.92 ± 1.21 | 5.53 ± 0.34 |
| Brain | 2.85 ± 0.54 | 1.77 ± 0.20 | 1.61 ± 0.28 | 4.91 ± 0.72 | 0.75 ± 0.09 | 1.77 ± 0.26 | 1.41 ± 0.15 | 0.55 ± 0.12 |
| Diaphragm | 6.88 ± 0.76 | 3.21 ± 0.36 | 4.69 ± 0.58 | 6.97 ± 1.20 | 1.45 ± 0.27 | 2.42 ± 0.22 | 1.97 ± 0.45 | 0.64 ± 0.10 |
| Heart | 5.86 ± 0.43 | 4.81 ± 0.69 | 5.10 ± 0.74 | 14.89 ± 3.96 | 1.51 ± 0.28 | 3.02 ± 0.59 | 4.12 ± 0.78 | 0.92 ± 0.14 |
| Kidney | 80.42 ± 6.82 | 41.33 ± 4.05 | 48.96 ± 8.90 | 91.02 ± 23.8 | 12.82 ± 1.40 | 16.34 ± 2.14 | 19.81 ± 2.23 | 5.17 ± 0.77 |
| Liver | 8.05 ± 1.41 | 3.91 ± 0.45 | 4.49 ± 0.76 | 15.98 ± 1.65 | 1.81 ± 0.31 | 3.54 ± 0.46 | 3.16 ± 0.39 | 0.92 ± 0.14 |
| Lung | 91.20 ± 8.71 | 62.41 ± 5.89 | 66.86 ± 11.9 | 161.60 ± 27.4 | 18.79 ± 4.60 | 44.52 ± 6.32 | 40.28 ± 5.15 | 12.09 ± 0.47 |
| Trachea | 4.48 ± 0.21 | 2.81 ± 0.63 | 3.75 ± 0.51 | 7.22 ± 0.89 | 1.45 ± 0.23 | 2.67 ± 0.32 | 1.52 ± 0.18 | 0.73 ± 0.16 |
| Fat | 2.64 ± 0.14 | 3.48 ± 0.49 | 4.12 ± 0.46 | 10.80 ± 2.06 | 1.52 ± 0.11 | 4.08 ± 0.29 | 2.56 ± 0.28 | 0.59 ± 0.07 |

[a] The $^{3}$H-soman dose was 15 µg/kg. Each time point represents the mean of six individual animals.

**Table 22.** Tissue concentrations of residual $^{3}$H-MPA in guinea pigs after intravenous injection of $^{3}$H-soman[a].

| | Time (Min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | 5 | 15 | 30 | 60 | 120 | 240 | 480 | 1440 |
| | ng/g tissue (means ± S.E.M.) | | | | | | | |
| Blood | 3.21 ± 0.48 | 11.53 ± 1.05 | 26.83 ± 8.13 | 3.76 ± 0.41 | 2.35 ± 0.59 | 4.30 ± 0.66 | 12.96 ± 3.48 | 2.68 ± 0.77 |
| Brain | 2.82 ± 0.30 | 2.40 ± 0.36 | 1.98 ± 0.49 | 1.86 ± 0.46 | 1.18 ± 0.25 | 1.59 ± 0.34 | 1.87 ± 0.25 | 0.62 ± 0.10 |
| Diaphragm | 4.23 ± 1.22 | 2.55 ± 0.17 | 3.14 ± 0.20 | 2.11 ± 0.47 | 0.99 ± 0.27 | 1.20 ± 0.25 | 2.04 ± 0.54 | 0.52 ± 0.07 |
| Heart | 5.34 ± 1.63 | 2.38 ± 0.35 | 3.71 ± 0.64 | 2.88 ± 0.56 | 1.35 ± 0.38 | 2.30 ± 0.40 | 2.57 ± 0.80 | 0.67 ± 0.16 |
| Kidney | 20.21 ± 2.16 | 6.28 ± 0.47 | 28.85 ± 3.79 | 17.54 ± 4.21 | 3.44 ± 0.27 | 4.00 ± 0.95 | 5.94 ± 0.86 | 0.58 ± 0.10 |
| Liver | 2.62 ± 0.43 | 2.01 ± 0.17 | 3.61 ± 0.38 | 2.91 ± 0.47 | 1.32 ± 0.20 | 1.73 ± 0.45 | 2.78 ± 0.48 | 0.44 ± 0.05 |
| Lung | 8.24 ± 1.45 | 5.37 ± 0.33 | 23.18 ± 3.09 | 6.55 ± 1.47 | 2.96 ± 0.81 | 4.94 ± 1.17 | 8.47 ± 2.40 | 1.13 ± 0.30 |
| Trachea | 4.32 ± 0.99 | 1.96 ± 0.30 | 3.71 ± 0.63 | 1.68 ± 0.14 | 1.70 ± 0.17 | 1.72 ± 0.51 | 2.04 ± 0.37 | 0.67 ± 0.11 |
| Fat | 4.64 ± 0.52 | 1.82 ± 0.09 | 3.29 ± 0.83 | 3.87 ± 0.86 | 2.50 ± 0.78 | 3.01 ± 0.82 | 2.91 ± 0.46 | 0.44 ± 0.10 |
| Carcass | 1571 ± 0.218 | 5433 ± 280 | 4915 ± 198 | 2976 ± 279 | 1314 ± 235 | 1693 ± 440 | 1132 ± 94 | 375 ± 33 |

[a] The $^{3}$H-soman dose was 15 µg/kg. Each time point represents the mean of six individual animals.

True cholinesterase activities (means ± SEM) for control mice were 1.52 ± 0.47, 1.41 ± 0.24, 2.25 ± 0.24 nmol of ACh hydrolyzed per mg protein per min following vehicle administration by inhalation (I.H.), intravenous (I.V.) and intramuscular (I.M.) administration, respectively. Each data point represents a mean of six animals. Despite the fact that intra-assay variability (represented by the triplicate values of any given time point) was very low, the inter-assay variability was often large.

* For I.H. group, the variability in the data was 4%-22% of the mean values.
* For I.V. group, the variability in the data was 9%-32% of the mean values.
* For I.M. group, the variability in the data was 3%-18% of the mean values.

Soman administered either intravenously or intramuscularly produced profound and long-lasting acetylcholinesterase inhibition. As would be expected, the onset was much faster with the intravenous administration, whereas the maximal inhibition with the intramuscular administration did not occur until 4 hr later. It can be seen that inhalation of a relatively low dose of 5 µg/kg had very little effect on acetylcholinesterase activity. In fact, there appeared to be a parodoxical increase in acetylcholinesterase activity 2 hr after the inhalation exposure. It is certainly clear that the pharmacological effects following soman inhalation are dramatically different from those observed following the other routes of administration which could influence both biodisposition and metabolism of soman. The results in Figure 2 depict the acetylcholinesterase activity in mice following either intramuscular injection of 25 µg/kg or inhalation of 2 µg/kg of soman. Neither of these low doses produced suppression of acetylcholinesterase activity with the exception of slight depression at 24 hr after inhalation exposure. Again, it would appear that these low doses actually stimulated acetylcholinesterase activity up to 4 hr after treatment. In order to determine whether procedural problems could be responsible for this anomaly, acetylcholinesterase activity was quantitated in mice which were injected intravenously. As can been seen from the results in Figure 2, intravenous administration resulted in the expected decrease in acetylcholinesterase activity which occurred immediately after injection and returned to control quantities by 24 hr.

## DISCUSSION

To reiterate the goals of the research, the first specific objective was to characterize the biodisposition of soman and its metabolites in guinea pigs at sublethal doses in order to establish the importance of route of administration with regard to metabolism and biodisposition of $^{3}$H-soman. The second objective was to determine the biodisposition and metabolism of $^{3}$H-soman in mice following inhalation and intramuscular injections of sublethal doses of $^{3}$H-soman in order to assess species differences.

In summary, inhalation exposure of $^{3}$H-soman in mice resulted in rapid distribution from the lungs to all tissues. It was rather surprising that the $^{3}$H-soman levels were sustained throughout the 24 hr time course. There were considerable fluctations in the tissue concentration of both $^{3}$H-soman and its metabolites. However, there were several similarities between the results from this study and previous ones from our laboratory. The high concentrations of $^{3}$H-soman and its metabolites in kidney and lung are consistent with previous results. The intramuscular administration of $^{3}$H-soman also resulted in a rapid distribution to all tissues, particularly the lungs and kidneys. It is particularly important to note that the trachea did not serve as a storage depot for $^{3}$H-soman and its metabolites following inhalation exposure. In addition, the concentrations of $^{3}$H-soman and $^{3}$H-MPA in trachea following intramuscular administration were higher than would be expected. It rapidly phosphorylated protein and was quickly hydrolyzed to free $^{3}$H-PMPA. The biodisposition of $^{3}$H-soman and its metabolites was quite similar after both routes of administration.

Most of the differences in the biodisposition and metabolism of $^{3}$H-soman between inhalation exposure and intramuscular administration could be logically explained based upon the route of treatment. For example, it was to be expected that relatively higher concentrations of $^{3}$H-soman would be present in blood following intravenous administration than after inhalation. On the other hand, high concentrations of $^{3}$H-soman were present in lung and trachea after inhalation. The higher concentrations in trachea are consistent with what would be expected following inhalation exposure. On the whole, the biodisposition and metabolism of $^{3}$H-soman after inhalation exposure exhibited a pattern which was similar to that after intravenous administration. Regardless of the route of administration, the kidney obviously plays an important role in the elimination of $^{3}$H-soman and its metabolites. Additionally, high concentrations of the organophosphates were found in lung after all routes of administration. Of course, the dose of $^{3}$H-soman administered by the different routes is an important consideration.

The most striking difference between the mice and guinea pigs occurred with $^{3}$H-soman concentration following the inhalation exposure. All tissues of the mice contained higher concentrations of $^{3}$H-soman despite exposure to a dose that was less than that administered to guinea pigs. It is interesting to note that tracheal concentrations of soman and its metabolites were comparable following inhalation exposure and intramuscular injection in mice, whereas their tracheal levels in guinea pigs were considerably higher after inhalation exposure. In addition, the concentrations were highly variable in mice which complicated the interpretation. However, it appeared that there were no major differences between the biodisposition and metabolism of $^{3}$H-soman in guinea pigs and mice after these routes of administration, which would directly account for the difference in sensitivities of these two animal species to soman.

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