Central effects of pc VX exposure

1 Introduction

In contrast to volatile nerve agents of the so-called G-type such as sarin and soman, which act primarily via the respiratory route, nerve agents of the V-type are several orders of magnitude less volatile due to their physicochemical properties and act primarily as a liquid via the percutaneous route (Maynard and Beswick 1992). The most important representative of the V-agents is VX ([O-ethyl-S-(2-diisopropylaminoethyl) methylphosphonothioate], which is several orders of magnitude more lethal via the skin than for example sarin, but also highly toxic as a vapor via the respiratory and ocular route (National Research Council 1997; Reuter et al. 2000). Several nations have VX-stockpiles awaiting destruction. The threat of using nerve agents in military conflicts (Macilwain 1993) and in terrorist attacks (Nagao et al. 1997) urges the necessity for development of effective treatment regimens.

Following exposure, nerve agents induce several cholinergic signs, such as salivation, miosis, bradycardia, bronchoconstriction, seizures and respiratory depression due to irreversible inhibition of acetylcholinesterase (AChE). VX also showed direct pharmacological effects on several receptor types, for example muscarinic (Bakry et al. 1988; Pittel et al. 2006; Silveira et al. 1990) and nicotinic acetylcholine receptors (Bakry et al. 1988), inhibited evoked GABA and glutamate release and enhanced spontaneous release of the latter transmitters (Rocha et al. 1998).

Since the percutaneous route of exposure is less predictive in terms of onset times and severity of clinical signs, Van der Schans et al. (2003) have determined the toxicokinetics of VX after percutaneous exposure in anaesthetized hairless guinea pigs. Maximum plasma levels of VX were not reached until several hours after exposure, followed by a slow elimination phase. In spite of the use of anaesthetized animals, the variability in toxicokinetics was considerable. The latter variable and delayed absorption of VX from the skin may have important implications for military or civilian first responders with respect to triage, decontamination and therapeutic drug regimens (Dalton et al. 2006; Hamilton et al. 2004). The present study was designed to investigate the central and peripheral effects of exposure to percutaneous VX in hairless guinea pigs, in order to broaden the scope for possible treatment. The hairless guinea pig is considered to be a more appropriate model in this respect than the common guinea pig since its skin structure is more comparable to that of man (Panchagnula et al. 1997; Sueki et al. 2000).

Most previous experiments evaluating physiological effects of VX after percutaneous exposure were conducted in anaesthetized animals. In the present study, the effects of percutaneous exposure to VX (500 μg/kg, ~4LD50) were studied in conscious freely moving animals. Acetylcholine (ACh) and choline (Ch) levels were measured in the striate body of the brain using microdialysis. Simultaneously, cortical EEG and cholinergic signs of poisoning were recorded. At the end of the experiment, AChE activities in different brain regions, liver and diaphragm were determined.
2 Methods

2.1 Animals
Male hairless guinea pigs [Crl:IAF(HA)BR] (375 ± 10 g), obtained from Charles River (Maastricht, The Netherlands) were used in the present study. Prior to the experiments they were housed with 2 animals per cage, and allowed to get accustomed to standard conditions for two weeks. Room temperature was kept at 19-22 °C and relative humidity was maintained at 55-65% and lights were on from 7 am to 7 pm. Acidified water and standard guinea pig chow (Teklad global diet 2040, Harlan, Horst, The Netherlands) were available ad libitum. The experiments described received prior approval from the TNO Animal Ethical Committee.

2.2 Chemicals
Acetylcholinesterase, choline oxidase, acetylcholine, choline and Triton-X100 were obtained from Sigma Chemical Co. (Zwijndrecht, The Netherlands). Kathon CG (1.5% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) was provided by Rohm and Haas (Croyden, UK). LiChrosorb® NH₂ was purchased from E. Merck (Amsterdam, The Netherlands). Hypnorm® (fentanyl/fluanisone) was purchased from Janssen Pharmaceutica (Beerse, Belgium) and Dormicum® (midazolam) was delivered by Roche Nederland BV (Mijdrecht, The Netherlands). (±) VX [O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate] was obtained from the stocks of TNO Defence, Security and Safety and of purity ≥98% (GC). The other chemicals used were of standard purity and purchased from renowned companies. For HPLC analysis the highest purity grade was used. All solutions were prepared with water tapped from a Milli-Q system (Millipore SA, Molsheim, France).

2.3 Surgical procedures
Guinea pigs were anaesthetized with 6 ml/kg FFM-mix (1.25 mg/ml midazolam, 2.5 mg/ml fluanisone, 0.079 mg/ml fentanyl citrate) via a single i.p. injection. Premedication with 0.05 mg/kg atropine sulphate (s.c.) was administered to prevent respiratory arrest. A concentric microdialysis probe was stereotactically inserted into the ventral striatum (P1.8, L 2.8, V 8.3 mm relative to bregma and the dura mater). Two stainless steel screws (A8.0 and P1.2 mm relative to bregma and 1 mm from the sagittal suture) placed at the dura mater were fixed to the skull with dental cement and fixed to a plug to serve as EEG electrodes. A heating pad was used to maintain body temperature. The microdialysis probes used were constructed in-house and made of a polycrylonitril/sodiummethyl sulfonate copolymer dialysis membrane (Filtral 12, Hospal BV, Breda, The Netherlands), of which 4 mm was exposed.

2.4 HPLC Instrumentation and acetylcholine analysis
Microdialysate samples were assayed for acetylcholine and choline using a HPLC system previously described by Damsma et al. (1987) with some modifications. The system consisted of a LC-10AD VP pump (Shimadzu, Den Bosch, The Netherlands), a pulse damper (SSI, Alltech, Breda, The Netherlands), a refillable guard column (silica pellicular packing material, Alltech, Breda, The Netherlands), an EC 125/2
Nucleosil 100-5 C18 AB analytical column (Aurora Borealis Control, Schoonebeek, The Netherlands), preloaded with 0.5% sodium lauryl sulfate, an enzyme reactor, an electrically actuated injector (VALCO VICI AG, Schenkon, Switzerland) with a 20 ul sample-loop, an INTRO potentiostate equipped with a VT03 flow cell with Pt work electrode (Antec Leyden BV, Hazerswoude, The Netherlands) and a chromatography data acquisition system (Chromeleon®, Gynkotek, Germering, Germany). The post-column enzyme reactor contained immobilized acetylcholinesterase (80 U), which converted acetylcholine to choline, and choline oxidase (40 U), which converted choline to hydrogen peroxide that was electrochemically detected at +450 mV. The mobile phase consisted of a 166 mM potassium phosphate buffer (pH 8.5) containing 1 mM tetramethylammonium chloride, 0.79 mM EDTA and 250 µl/l Kathon CG. The system was run at 0.35 ml/min and temperature was maintained at 30 °C. Limit of quantification was 1 pmol/ml. Linear calibration curves were obtained in the range from 5 – 1000 pmol/ml (r > 0.990). The intra-assay coefficients of variation for 10 and 100 pmol/ml were 2.9 % and 4.1 %, respectively. Inter-assay variability could not be determined due to variable enzyme activities in the post-column reactor between days.

2.5 Microdialysis procedure for acetylcholine determination

Following surgery, the guinea pigs were individually placed in a perspex cage (25 x 25 x 40 cm) with free access to food and water. Experiments started 14-20 hours after recovery from the surgical procedure, between 9 and 10 AM. At the beginning and end of each experiment a 10 nM acetylcholine/choline calibration standard was injected in duplicate, and if necessary corrections for reduced sensitivity of the enzyme reactor during the experiment were made. The microdialysis probe was perfused with a Ringer solution at 2.0 µl/min delivered by a microinjection pump (CMA 100, CMA microdialysis AB, Solna, Sweden). To obtain detectable quantities of acetylcholine in the dialysate, 100 nM neostigmine was added to the perfusate. The guinea pig was connected directly to the sample loop, allowing on-line analysis of the microdialysates. The injection valve was automatically activated every 10 min, resulting in an injection volume of 20 µl.

2.6 Clinical signs

After obtaining one hour of baseline data of EEG and microdialysis samples, the guinea pig was gently held by hand, and a solution of VX in Isopropylalcohol (32 mg/ml), or IPA alone (control animals), was applied at the level of the lumbar 3 plexus on the belly, on an area of about 2 cm², at a dose of 500 µg/kg (16 µl/kg), which corresponds to 4LD50 (van der Schans et al. 2003). After absorbance of the fluid, the spot was covered with a stainless-steel cup of 2 cm², which was strapped to the skin with surgical tape and removed after one hour. The area was not decontaminated.

Following exposure, the guinea pigs were monitored and the onset of the following cholinergic signs were registered: Chewing: A clear chewing-like movement of the guinea pig in which the entire head is involved as a consequence of increasing saliva production; Shivering: Continuous shivering of the entire body. The animal is able to move around; Salivation: Extensive drooling from the mouth, which is often accompanied by tears fluid; Tremor: Extensive shivering combined
with involuntary movements in which the entire body is involved. The guinea pig looks mentally dissociated from the environment and has lost control over its movements; Respiratory Distress: Low respiratory rate and heavy breathing.

Immediately after obtaining the last microdialysis sample after registration of a flat EEG, organs were quickly removed and homogenized for determination of enzyme activity, as described below.

2.7 EEG acquisition and analysis

EEG was obtained using PhysioTel telemetry system from Data Sciences Inc. (DSI) using the TA11ETA-F40 transmitter body, which was attached to the plug fixed on the guinea pig’s skull. A receiver board measured the signal from the transmitter, which was consolidated and stored on an IBM-compatible personal computer via a Data Exchange matrix at 100 Hz sampling frequency. After storage, the data were converted into EDF format. Every 10 seconds power spectra were calculated using Fast Fourier Transformation (FFT). A low cut off filter at 1.6 Hz was used to filter eye movement artifacts and high digital filter at 50 Hz was used. Animals were awake during the measurement.

Relative powers of EEG bands (Delta 1.6-3.5 Hz, Slow Theta 4.0-6.5 Hz, Fast Theta 7.0-8.5 Hz, Alpha 9.0-14.0, Beta1 14.5-24 Hz, Beta2 24.5-30 Hz, Gamma1 30.5-35 Hz and Gamma2 35.5-50 Hz) were calculated by dividing the sum of the absolute power in the frequency range by the total power of the spectrum according to Timofeeva and Gordon (2001). EEG data were synchronized with acetylcholine determinations using 10 minute averages.

2.8 Enzyme activity

Diaphragm, liver and different brain parts (Fig. 2) were homogenized (900 rpm, 10 % w/v homogenate) in ice-cold TENT buffer, which consisted of 50 mM Tris, 5 mM EDTA, 1 M NaCl and 1% v/v Triton X-100, pH 7.4. The homogenates were centrifuged at 12000 g in an Eppendorf centrifuge at 4 °C and supernatants were immediately frozen in liquid nitrogen and stored at -20 °C until analysis of enzyme activity, within one month.

Samples were analyzed for AChE activity using a modification of the method by Ellman et al. (1961). Shortly, after appropriate dilution, 10 µl samples were incubated with 0.8 mM 5,5'-dithio-bis-(2-nitrobenzoic acid) (Sigma Aldrich B.V.) and 0.8 mM acetylthiocholine iodide. The delta OD per µl homogenate per min at 415 nm at ambient temperature served as measurement for AChE activity. Activities were normalized versus enzyme activities of 6 control animals.

2.9 Study design, data presentation and statistical analysis

Two groups of 6 animals each were used. This group size is based on expected ACh accumulation of approximately 750 ± 300% (mean ± SD) at time of seizure development/death (Bueters et al. 2002) compared to control animals, resulting in a statistical power of 90% for detecting a scientifically significant ACh accumulation. Whenever appropriate, microdialysis and EEG data were analyzed using Repeated measures one-way ANOVA followed by Dunnett’s post-hoc test. Enzyme activities were analyzed using Student’s t-test. Results were considered significant if p<0.05.
3 Results

3.1 Clinical signs

Cholinergic signs were registered upon percutaneous application of 500 µg/kg VX. Although all animals received the same dose of VX, in particular the time course in which the cholinergic signs appeared showed great variability (Table 1).

Table 1. First occurrence of a cholinergic sign following percutaneous exposure to VX (500 µg/kg). The time points at which a sign was observed are indicated in minutes.

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Chewing</th>
<th>Shivering</th>
<th>Salivation</th>
<th>Tremor</th>
<th>R. Distress</th>
<th>EEG seizure</th>
<th>Death</th>
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<tr>
<td>1</td>
<td>124</td>
<td>374</td>
<td>320</td>
<td>414</td>
<td>467</td>
<td>518</td>
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<td>180</td>
<td>180</td>
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<tr>
<td>3</td>
<td>104</td>
<td>70</td>
<td>70</td>
<td>190</td>
<td>262</td>
<td>107</td>
<td></td>
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<td>4</td>
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<td>75</td>
<td>287</td>
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<td>120</td>
<td>101</td>
<td>190</td>
<td>262</td>
<td>287</td>
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</tr>
<tr>
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<td>55</td>
<td>55</td>
<td>80</td>
<td>80</td>
<td>92</td>
<td></td>
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</tr>
</tbody>
</table>

Mean: 97  232  193  261  273  128  318
SEM: 13  78  69  76  85  53  95

Figure 1 shows the first appearance of the cholinergic signs versus the % of time elapsed between VX application and death (100%). It is clear that, on average, the onset time to the first occurrence of each sign is predictive for the time to the occurrence of more severe signs. The symptom chewing occurs at 25±4 % of the total survival time, followed by shivering and salivation at 58±5% and 63±7% of survival time, respectively. On average, animals start showing tremor at 75±4% and the most severe symptom, respiratory distress, is only seen shortly before death at 85±4% of total time. Remarkably, only 2 out of 6 animals showed seizure activity on their EEG.

Figure 1. First appearance of cholinergic signs in individual animals (vertical axis) at percentages of total experimental time (horizontal axis) between VX application (0%) and death (100%). The small triangles show individual first appearances of signs, vertical lines show average first appearance (n=6). For absolute times see Table 1.
3.2 Enzyme activities

In figure 2 the relative inhibition of AChE in several brain regions as well as in liver and diaphragm is shown. In animals showing no seizures, AChE activity was significantly inhibited by approximately 85-90% in all organs, except for the striate body, in which inhibition was only 75%. Two animals with seizures showed higher levels of AChE inhibition, to approximately 90-95% of that of control animals, in particular in rest of brain, striate body, liver and diaphragm. However, because of the small number of animals displaying seizures, no statistics were calculated.

![Figure 2](image_url)

**Figure 2.** Relative AChE activity in brain parts, liver and diaphragm. The AChE activities of 4 animals after pc intoxication with VX (500 ug/kg) not displaying seizures (grey bars) and 2 animals displaying seizures (open bars) after VX were calculated as percentage from AChE activities from control animals (n=6, black bars). Enzyme activities from Medulla Oblongata (MO), striate body (STR), hippocampus (HIPP), rest of brain (REST), liver and diaphragm (DIAPHR) are displayed. AChE activity is significantly different in all organs of intoxicated animals. Only the 4 non-seizing animals were compared statistically to controls (Student’s t-test, *, p<0.05)

3.3 Acetylcholine, choline levels and total EEG power

Figure 3 shows microdialysis results and EEG total power changes at several experimental time points. Out of 6 animals in the VX-exposed group, only two animals exhibited seizure activity on their EEG. The ACh level in one of these 2 animals had increased early in the experiment (at 50% of experimental time), ending at a very high level at the end of the experiment (Panel A). The seizures started at approximately 65% of experimental time, indicating that the primary moderate increase in ACh (in this animal) did not induce generalized seizures. The ACh measurement in the other animal showing seizures, failed due to a malfunctioning probe. The 4 animals without seizures showed very slight and not significant increases in ACh levels. In contrast to the early increase in EEG power in the 2 animals with seizures, the EEG power in the non-seizing animals gradually decreased, which became significant at 90% of experimental time (Panel C). Both seizing as well as non-seizing animals showed a gradual increase in Choline levels (Panel B).
3.4 EEG bands

The changes in relative powers in different EEG bands following percutaneous intoxication with VX are shown in figures 4 and 5. For each animal, the 30 minutes of recording before intoxication served as individual baseline value for EEG, and were set at 100%. At 4 experimental time points, the average relative power per band was normalized to the baseline value. Figure 4 shows the EEG parameters for the 4 non-seizing animals. At 25% of experimental time no overt changes were observed in any of the EEG bands. At 50% of experimental time, however, the relative power in some bands had started to change. The relative power in the Delta and Gamma2 band transiently increased and had returned to baseline value.
by the end of the experiment. In contrast to the increase in the latter bands, most other bands (Theta1, Theta2, Alpha, Beta2 and Gamma1) showed a significant and in some bands persistent decrease compared to baseline. The Beta1 band showed a less consistent effect, but towards the end of the experiment its relative power had increased significantly.

The EEG changes in the 2 animals with seizures are shown in figure 5. The time courses in relative EEG-band powers are to some extent similar to those of animals that did not seize. Because of the low number of animals showing seizures, statistics regarding the deflections from baseline could not be calculated. However, the relative power returned to baseline values approximately at 75% of the experimental time.
4 Discussion

The toxicokinetics of VX following skin application in the guinea pig have shown a delayed penetration through the skin (van der Schans et al. 2003), resulting in a delayed appearance of cholinergic signs (Chilcott et al. 2003; Hamilton et al. 2004). It is obvious that this delayed penetration of VX through the skin contributes to the variation with regard to onset times of clinical signs in the present study. The high VX dose used in the present study (4LD50) was chosen in order to minimize inter-individual variability. Regarding signs of poisoning, the latter goal was not completely achieved. Not all animals showed all signs. In animals showing a rapid onset of signs and a relatively short survival time for example, the chewing sign did not appear. However, looking closer at the onset time of the cholinergic signs, a less variable picture emerged. At an average of 25% of survival time, the animals showed chewing movements, shivering at 58%, salivation at 63%, tremor at 78% and respiratory distress at 85% of survival time. It is of particular interest that the deviations of these relative times belonging to the different signs are very small, indicating that this parameter is a reliable tool to predict the survival time.

The AChE activity was highly inhibited in the investigated organs of all animals. This is in line with observations from Maxwell et al. (2006), who ascribed 90% of toxicity of organophosphorous compounds to AChE inhibition. In spite of a relatively high AChE inhibition in the brain, no overt or seizure-inducing ACh levels were reached in all animals. Only 2 out of 6 animals developed seizures. The latter animals

Figure 5. Normalized changes in relative powers of EEG bands after 500 μg/kg VX p.c. of the 2 individual animals which demonstrated seizures on their EEG at several time points during the experiment (% of experimental time, horizontal axis). Due to the small number, statistical analysis was not carried out. For clarity reasons, values of control animals were left out, see fig. 4.
tended to have higher levels of AChE inhibition in striate body and rest of brain. The inhibition levels in these 2 animals were approximately 90 and 95%, in striate body and brain respectively, compared to 75 and 90%, in animals that did not have seizures on their EEG. These high levels of central AChE inhibition, expected to be a requirement for seizure development, are in line with previous studies (Bueters et al. 2003; Joosen and van Helden 2007; Testylier et al. 1999). Other workers have shown in guinea pigs, using a different route of administration, that of all nerve agents tested, VX was least likely to produce seizure activity in all animals (Shih et al. 2003; Shih and McDonough 2000). Because of the failure of the microdialysis probe in one seizing animal, extracellular ACh levels could only be obtained from one of 2 seizing animals. In the latter animal, the seizures were accompanied by a high increase of extracellular ACh levels, i.e. over 10 times of basal level. From other studies it appeared that seizures did not occur until ACh levels rise over 200 times compared to basal levels and that brain AChE inhibition must have been inhibited over 65% (Tonduli et al. 1999). In a previous study, we have shown that over 75% of the AChE must be inhibited in the striate body to induce significant elevations over baseline levels of ACh, likely to induce seizures (Joosen and van Helden 2007). The other animals, 4 out of 6, showed elevations of ACh levels to approximately 5 times that of basal levels, which apparently was much too low to induce seizures. Although initiation and early expression of seizure activity are cholinergic in nature, other neurotransmitters, including glutamate and GABA can influence the maintenance of the seizures (McDonough, Jr. and Shih 1997).

All animals showed increases in extracellular levels of choline. Although choline itself has no affinity for receptors, choline dynamics are affected in several conditions of brain injury (Scremin et al. 2006). An increase of brain choline levels is found in case of cerebral trauma (Nortje and Menon 2004) and in focal and global cerebral ischemia (Scremin and Jenden 1989a; Scremin and Jenden 1989b). In these models, activation of phospholipases led to an enhanced rate of phospholipid degradation, resulting in an increasing production of free choline (Phillis and O’Regan 2003). Also in our study the net production of free choline from phospholipids may contribute to the observed increase of choline levels and may point to cerebral ischemia following VX intoxication.

Apart from increased choline levels, also some of the observed EEG changes might be the result of ischemia. Although in animals without seizures the total EEG power was not affected until the end of the experiments, significant changes of relative power in different EEG bands were observed at earlier stages in the experiment, similar to those found in animals not having seizures. Significant increases in Delta, Beta1 and Gamma2 were observed, whereas the relative power in other bands was significantly decreased. A similar significant shift to lower frequency EEG activity in the Delta band was seen within one hour following permanent occlusion of a cerebral artery in a rat model of ischemia (Cohen et al. 1994). In man, diffuse generalized slowing of the EEG is also associated with a malignant outcome of ischemia due to middle cerebral artery infarction (Burghaus et al. 2007). Additionally, short periods of circulatory arrest in man also induced increases in Delta power and more consistent decreases in Beta and Alpha bands.
Central effects of pc VX exposure

(Visser et al. 2001). A similar increase in relative power in the Delta band, not cholinergically mediated, was observed by Timofeeva and Gordon (2001) after oral administration of chlorpyrifos.

In case of organophosphate poisoning, it is expected that EEG changes are cholinergically mediated due to increases in ACh levels. In this respect, increases in Theta1 and Gamma2 and decreases in Theta2, Alpha, Beta1, Beta2 and Gamma1 were observed after subcutaneous administration of the muscarinic agonist oxotremorine in rats (Timofeeva and Gordon 2001). Although only slight increases in ACh were observed in animals without seizures, and not all EEG changes observed were similar to that reported by Timofeeva and Gordon, some changes in EEG bands observed in this study might be related to cholinergic effects. Particularly the increase in Gamma2 and decrease in Beta2 might be related to increased ACh levels (Joosen and van Helden 2007; Timofeeva and Gordon 2001).

Until approximately 65% of experimental time had elapsed, the EEG changes in animals that developed seizures were similar to that in animals that did not. It is not clear why some animals developed seizures and some did not. This might be related to the influence of neurotransmitters other than ACh (McDonough, Jr. and Shih 1997). One could speculate that small amounts of anaesthesia were still present while the EEG recording started, that is 14-20 hrs after surgery. However, this is highly unlikely since the half life of elimination of midazolam is about 3 hrs, that of fluanisone and fentanyl even shorter. Moreover, the latter 2 drugs are not reported to have any anti-convulsive property in case of OP poisoning. Finally, the low dose of atropine (50 µg/kg), used as premedication for surgery, is not even anti-convulsive when given immediately after OP intoxication. In conclusion, the observed EEG changes do not clearly indicate development of seizure activity, the general course of EEG changes, however, points to progressive effects of intoxication.

A major part of toxicity in case of percutaneously applied VX may be due to impact on the major organs such as the diaphragm muscle and heart. Direct effects on cardiac M2 receptors cannot be ruled out (Silveira et al. 1990). Additionally, in AChE knockout mice, which are highly sensitive to VX toxicity in spite of lacking the primary toxicity target, atropine treatment was ineffective. The latter observation implies that VX initiated the neurotoxic cascade following OP intoxication via other pathways than through cholinergic innervation (Duysen et al. 2001). The EEG effects in non-seizing animals as discussed above may be related to a decreased cerebral blood flow and ischemia due to cardiorespiratory failure. Cardiorespiratory failure was also observed in case of intoxication with VR, a structural isomer of VX. VR seems to perturb the peripheral cardiorespiratory system in a progressive way, which ultimately led to collapse of central mechanisms and death (Chang et al. 1998).

In the present study, direct central cholinergic effects, such as ACh accumulation and seizure development were not observed in most animals after percutaneous exposure to VX. Although we have only measured ACh in the striate body, it is likely to be representative for other brain regions, such as the medulla oblongata, in which the respiratory centre is located. Finding no ACh accumulation is congruent with the EEG measurements, which did not show any convulsive activity or overt cholinergic
activation in particular EEG bands. Nevertheless, we cannot completely rule out that there might have been cholinergic overactivation in the respiratory centre since the AChE activity was more inhibited in the brain stem than in the striate body in non-seizing animals. These results are similar to that of another study, in which the AChE activity in the striate body was only 40% inhibited after VX, whereas that in hippocampus and brain stem was 60% inhibited (Chang et al. 1998). At the latter levels of inhibition, no cholinergic signs were reported in that particular study, in contrast to the animals in the present study.

Taken together, our results point to a more prominent action of VX in the peripheral compartment, attributing to clinical signs and death, which is in line with results found by others (Shih and McDonough 2000). Additionally, the agent has shown much more rapid inhibition of AChE in peripheral tissues compared to that in brain tissue following VX intoxication, although the different administration route used in the latter study may have influenced the inhibition rate in different tissues (Shih et al. 2005). In contrast to red blood cell AChE, which was completely inhibited within 10 min, the whole blood ChE showed only 70% inhibition at 1 hour after injection with VX. The differential inhibition between red cells and whole blood is likely to be due to the lesser inhibition of the plasma ChE content by VX, which contributes to the inhibition of whole blood. It is apparent that most blood ChE must be inhibited before substantial inhibition of organ AChE can occur, (Sidell 1997), although it is known that most ChE-inhibitors, including nerve agents, produce differential inhibition of different enzymes and tissues. Inhibition of red blood cell AChE over 90% has shown to be associated with a severe impairment of neuromuscular transmission, whereas the latter was only slightly affected at 70-90% inhibition (Thiermann et al. 2005). At physiological pH, the VX molecule is a protonated amine, which complicates its penetration into the brain (Epstein et al. 1974; Maxwell et al. 1997). Therefore, the supposed primary toxicity in the peripheral organs may be explained by the structure of VX.

The present study has shown that percutaneous exposure to VX leads to a progressive, but predictable course of clinical signs and survival time, with overt changes in EEG. It also demonstrates that peripheral toxic effects mainly contribute to the primary cause of death, which will have implications for treatment. In conclusion, clinical signs will mainly serve as indicators for the onset and maintenance of treatment in subsequent studies.
Reference List


Maynard RL and Beswick FE (1992) Organophosphorous compounds as chemical warfare agents. 373-385.


Chapter 3