Chapter 6

General Discussion
Although the probability of nerve agents being used for military purposes has diminished considerably, use by non-state actors on military or civilian targets is a realistic scenario, which has a high impact on society. Consequently, exposures to OPs are most likely to be unanticipated. Given this, the present medical countermeasures should be efficacious and not reliant on pretreatment. The risk of human exposure to OP insecticides mainly arises from agricultural use in developing countries leading regularly incidents, or are even used in suicide attempts; estimated at approximately 3 million exposures and 200 thousand fatalities each year (Eddleston et al. 2005; Eyer 2003). Relevant animal models allowing a more integrated analysis of sequelae of the toxicological impact of different nerve agents are required to enable advances in medical countermeasures, which is one of the main goals of this thesis. Furthermore, the efficacy of current treatment protocols and possible improvements were investigated in the models developed. In addition, long term-effects of OP-exposure and possible interventions were investigated. Due to the similarity in chemical structure and in mechanism of action, insights acquired by studying nerve agents can also be used to improve treatment of intoxications with OP insecticides, the latter of which represents a worldwide problem.
Improved animal models to study nerve agent poisoning

The differences in chemical structure of nerve agents result in different risk profiles for exposure, distribution, and toxic effects. Volatile agents such as soman, sarin and tabun, will most likely enter the body via inhalation leading to a rapid development of signs. Although inhalation exposure would be the most relevant route for these agents, experimental approaches are difficult and were therefore replaced by the subcutaneous exposure route in the present studies. In contrast, for low volatile agents, such as VX and most OP insecticides, exposure via the skin is more likely and the persistence of these agents in the circulation results in a delayed and continuous development of clinical signs. An important requirement of animal models is that they allow assessment of rapid diagnosis, differential toxicological profiles and cognitive impairments to enable adaptations of the emergency protocols presently in use.

1.1 Rapid diagnosis

A crucial point in treatment of OP poisoning is the timing of treatment, which is highly dependent on the ability to diagnose exposure as early as possible. To this end, animal models were developed in which toxicological effects of nerve agents could be measured at physiological and biochemical level upon different risk and exposure scenarios. Animals were either subcutaneously exposed to soman or percutaneously to VX. Biochemical verification of exposure is best accomplished by determination of AChE activity. Direct measurement of tissue AChE activity is technically not possible; the inhibition levels of both cholinesterases in blood can be regarded as measures of exposure. Blood AChE activity closely reflects the AChE activity at the neuromuscular junction (Thiermann et al. 2009). In the experiments described in chapter 2 and 4, the type of nerve agent and exposure route showed to affect the predictive validity of AChE inhibition for the appearance of a clinical sign. The rapid evolvement of clinical signs after soman exposure was associated with a very rapid full inhibition of blood and probably also brain AChE, thereby lowering the use of AChE screening as a predictor for the toxicological status of the animal. In contrast, the slower profile of blood AChE inhibition exhibited after pc exposure to VX, showed to be more applicable for screening. Both after sc soman and pc VX, full inhibition of blood AChE was not always associated with death. This implies that in addition to clinical signs, monitoring AChE activity is reliable to establish the toxicological status in scenarios of slowly progressing poisoning or to follow up recovery, whereas development of clinical signs is the most reliable in case of a rapid inhalation exposure to a nerve agent.

1.2 Differential toxicological profiles

Although both soman and VX act via a similar mechanism, that is inhibition of acetylcholinesterase, physiology was affected quite differently. As expected, toxic signs upon sc exposure to soman developed very rapidly and seizures were always present, as discussed in Chapter 2. Progression of poisoning was accompanied by a dramatic increase in respiratory minute volume, while heart rate remained practically unaffected in animals surviving the experimental period of 90 minutes. In contrast, after pc exposure to VX, animals showed a delay in development of clinical
Cholinergic signs compared to subcutaneous soman exposure. In addition, only a few animals showed seizures on their EEG (Joosen et al. 2008; van der Schans et al. 2003). Most animals suffered from life threatening respiration problems such as bronchoconstriction and decreased minute volume, and a severely lowered heart rate. The differential toxicological profiles have implications for timing and type of treatment.

An overview of the generic and differential patterns upon exposure to both nerve agents is shown in figure 1. In particular the difference in seizure development in animals exposed to either sc soman or pc VX is striking. A likely explanation is the difference in distribution of both agents due to their chemical structures on the one hand, and the exposure route on the other hand. Both VX and soman consist of mixtures of stereo-isomers. In contrast to the stereo-isomers of VX, the isomers of soman show very different profiles of persistence in the circulation and toxicities, resulting from their selective binding to AChE (van der Schans et al. 2008). Since the type and number of binding sites for soman and VX are assumed to be the same, the higher persistence of VX, charged at physiological pH, in the circulation increases the chance for VX to encounter binding sites before entering the brain compared to soman. The results presented in this thesis indicate that soman acts predominantly in the CNS, whereas VX is more confined to the peripheral compartment, indicating that different treatment approaches are required.

Figure 1. Overview of differential nerve agent effects and intervention strategies. The subcutaneous route is an approximation of the inhalation route regarding toxicokinetics and effects. Grey arrows indicate current countermeasures in use. Dotted grey arrows indicate novel countermeasures.

Apart from different toxicokinetics, different effects independent from AChE inhibition and determined mostly in vitro, might contribute to differential seizure development of both agents. Very low levels of VX have shown to lower the firing rate and amplitude of action potentials in hippocampal neurons in vitro (Rocha et al. 1999). Translated to the in vivo situation, this may cause a more
generalized reduction of action potentials in the brain and a shift of EEG power to lower frequency ranges by VX opposed to the expected increase induced by ACh accumulation. In contrast to VX, soman has shown to induce internalization of GABAA receptors in cultured hippocampal neurons (Wang et al. DRDC Canada, personal communication), which probably contributes to the resistance of soman-induced seizures to benzodiazepines.

The differential effects on heart rate and respiratory control are more difficult to explain. Cholinergic overstimulation due to AChE inhibition is expected to result in a dramatic decrease in heart rate, which was present in animals pc exposed to VX. In addition, these animals suffered from suppression of respiration, probably resulting from muscle paralysis and stimulation of the vagal nerve. In animals exposed to sc soman, heart rate remained normal, but the development of seizures was accompanied by an increase in respiratory minute volume. This might be explained by stimulation of the respiratory centers in the brain. In addition, bronchorrhea and bronchoconstriction mediate lower pO2 levels in blood, thereby stimulating chemoreceptors in the respiratory centers (Wiener and Hoffman 2004). These results indicate that in case of pc exposure to VX treatment should be aimed at restoring these vital parameters, whereas soman exposure requires a higher protection against seizure development.

1.3 Cognitive impairment

Long term effects on cognition after nerve agent poisoning are connected with brain injury resulting from excitotoxicity following seizure initiation by excessive ACh accumulation. A rat model was used to evaluate such effects in Chapter 5. However, the results obtained in the rat model imply that cognition deficits can be present, even when soman-induced seizures were only short-lasting (Joosen et al. 2009). We have not investigated long-term effects after pc exposure to VX. It might be anticipated that the decrease in RMV and heart rate result in ischemia (Joosen et al. 2008). Ischemia induces brain injury, which is associated with cognitive decline (Bendel et al. 2005). The results imply that apart from diminishing seizure activity, stabilization of the cholinergic pathway, that regulates neurogenesis in the hippocampus, and prevention of ischemic injury following pc exposure to VX might be targets for treatment of long-term cognition deficits (Fig. 1).

2 Advances in treatment protocols

2.1 Physostigmine efficacy: importance of timing

To obtain a high efficacy, it is recommended to initiate treatment after exposure to a nerve agent as early as possible, within one minute after exposure. However, a more practical time point for starting treatment is the appearance of first clinical signs. To date, a combination of pretreatment and therapy is required to provide the most optimal protection against nerve agent poisoning (Aas 2003). The risk of asymmetric and unpredictable terrorist use, targeting not only at armed forces but also at civilian population, makes a therapy necessary which is less reliant on pretreatment. A proof of principle study in guinea pigs showed that a combination
of scopolamine, HI-6 and physostigmine administered immediately after poisoning was effective in protecting animals from the lethal effects of tabun, sarin, soman and VX (Wetherell et al. 2006). An alternative to physostigmine could be galantamine, which has additional neuroprotective properties (Pereira et al. 2009).

In Chapter 2, the added therapeutic value of physostigmine was investigated in a soman-exposed guinea pig model, either in the presence of obidoxime and/or atropine or in the presence of a single drug. Single physostigmine treatment showed an U-shape efficacy, with low efficacy at the lowest dose, high efficacy at the middle dose and no efficacy or aggravating toxic signs of soman at the highest dose of physostigmine. In combination with obidoxime and atropine, results were similar, however, the middle physostigmine dose even showed a higher ability in preventing spreading and continuation of seizures compared to single treatment with physostigmine. Delay of treatment with physostigmine to appearance of first clinical signs at 10 minutes after poisoning, without addition of atropine, showed to be dangerous and aggravated signs at a dose that was highly effective when administered at one minute after exposure. This demonstrated that physostigmine is effective by competing with soman for binding sites.

Although there is room for inclusion of a carbamate in the immediate treatment regimen against nerve agent toxicity, it should be employed with care. An antimuscarinergic agent is required to prevent unwanted effects if physostigmine is applied at time points when AChE is fully inhibited. In addition, the type of nerve agent and the oxime to be used is of great importance. The combination HI6, scopolamine and physostigmine showed to be ineffective against tabun poisoning (Wetherell et al. 2006). HI6 is unable to reactivate tabun-inhibited AChE, implying that the efficacy also depends on the efficacy of the oxime. In case of soman poisoning, obidoxime is a bad reactivator, but probably sufficient to provide a pool of AChE subsequently protected by physostigmine (Chapter 2). In general, soman-inhibited AChE ‘ages’ very rapidly, making the enzyme refractory to treatment with the currently available oximes (Maxwell et al. 2006). This implies that in particular after soman exposure carbamate treatment should be applied as soon as possible.

2.2 Percutaneous VX exposure: need for continuous treatment

It is anticipated that the current treatment regimen in case of percutaneous exposure to VX will provide insufficient protection. The slow penetration of VX after pc exposure and its high persistence in the circulation, are not aligned with pharmacokinetics of drugs administered in case of exposure to a nerve agent (Joosen et al. 2008; van der Schans et al. 2003). The present regimen, consisting of administration of 3 autoinjector doses at once used at first signs showed to postpone severe toxicity. The efficacy of the treatment was improved when the timing of the antidotes was adjusted to the appearance of clinical signs (Chapter 4). This is in line with results obtained from case studies from patients exposed to OP insecticides, who required intravenous treatment with oximes up to one week to reanimate AChE, which was correlated to restoration of neuromuscular function (Thiermann et al. 2009).

The high incidence of the absence of seizures initially indicated that anticonvulsive treatment by diazepam would not be necessary in a pc VX exposure scenario
(Chapter 3). However, microdialysis results showed high levels of ACh accumulation in the brain, indicating that VX entered the brain in sufficient amounts to cause dangerously high levels of ACh (Chapter 4). While in animals left untreated after pc exposure to VX, the decline in heart rate most likely prevents full distribution of VX into the brain, the preservation of heart rate by atropine and obidoxime probably enabled a more continuous distribution of VX into the brain (Chapter 4). Because obidoxime only enters the brain in very limited amounts, thereby insufficiently reactivating inhibited AChE, addition of atropine and diazepam are necessary to prevent the development of seizures in spite of high amounts of ACh.

Treatment of pc VX exposure with a carbamate, such as physostigmine, combined with an effective reactivator might be an advisable approach to investigate further. The slow entrance of VX into the circulation provides a suitable time window for protection of AChE by physostigmine.

2.3 Impairment of cognition: treatment options

Regarding long-term effects, animals showed a decline in neurogenesis and cognition at 8 weeks after exposure to soman, in spite of effective and life saving treatment with the oxime HI-6 and atropine. As seizures were only present for a short time, not inducing observable brain injury, the cognitive deficits may not have resulted from excitotoxicity, but probably were due to an imbalance in cholinergic input into the dentate gyrus. In the present thesis, it was aimed to enhance neurogenesis by means of subchronic antipsychotic treatment with olanzapine (Chapter 5). This drug, however, was not able to induce neurogenesis at 8 weeks after poisoning and was, probably therefore, not effective in restoring cognitive deficits. However, other pharmacological agents might be able to increase neurogenesis in such a way that they could improve cognition. To this end, it is recommended to test growth factors (Collombet et al. 2005) and anticholinesterases such as physostigmine or galantamine when cognition deficits show up (Kotani et al. 2008). In addition, physical exercise might help to restore the balance in neurogenesis in the DG of the hippocampus (Van Praag et al. 1999).

3 Conclusion

The animal models developed allow an integrative assessment of toxicology and treatment efficacy. The toxicological profiles of sc soman exposure and pc VX exposure showed to be very different and accordingly demand a very different treatment approach. In particular, timing of treatment is an important issue in both cases. Soman exposure requires rapid treatment, directly aimed at restoring AChE activity and terminating seizure activity, whereas pc VX exposure requires repeated treatment on appearance of clinical signs until all nerve agent has entered the circulation and has been hydrolyzed. The results from the VX experiments are useful to the clinical practice of OP insecticide poisoning, because of their similar persistence in the body. Another important conclusion of the present study is that an effective life-saving treatment at short term, may not be sufficient to prevent long term cognition deficits. This implies that a more thorough understanding of the cause of such deficits is needed to design improved treatment strategies. In this
thesis, impaired neurogenesis is proposed as a cause of cognition deficits, which could be a starting point for treatment.

Extrapolation of the results to humans remains difficult. Sensitivity for OPs and antimuscarinergics, oxime-induced reactivation and ‘aging’ of OP-inhibited AChE markedly differs between species. Whereas the guinea pig is a suitable model to investigate the impact of AChE protection, it is less suitable for the assessment of subtle cognition impairments, due the lack of proper tests; the rat model is more suitable to assess impact on cognition. To provide the best translational results, it is recommended to include both models in screening of treatment efficacy.

Reference List