Summary

Towards improved models for treatment of organophosphate poisoning
Organophosphates (OPs) are highly toxic compounds. They represent a large class of pesticides used worldwide, and (un)intentional poisoning is recognized as a global problem. The research towards more effective insecticides led to the discovery of nerve agents. Although the Chemical Weapons Convention, an agreement to ban the use, proliferation and stockpile of OP nerve agents, is ratified by most nations this cannot prevent the threat of such compounds being used by terrorists. Therefore research into mechanism of OP action and into clinical countermeasures when poisoning occurs is necessary.

Exposure to an OP leads to inhibition of Acetylcholinesterase activity and to an excessive buildup of Acetylcholine (ACh) in cholinergic nerve terminals. The overstimulation of muscles and nerve cells by ACh leads to muscular paralysis, excessive mucus secretion and to epileptiform brain activity. In case of exposure to high levels of OP, these symptoms can lead to death. The current medical countermeasures for OP poisoning are effective to a reasonable extent; yet, there is room for improvement. In order to be able to improve medical countermeasures a thorough understanding of the routes of entry, fate within the body and the mechanism of action is needed.

This thesis focussed on nerve agents, which can be divided into different classes based on their chemical structure. The first type of these chemical warfare agents, such as soman, sarin and tabun, are highly volatile and upon dispersion these agents will most likely enter the body via inhalation. In contrast, other types of nerve agents, such as VX, have a much lower volatility, making exposure via the skin most likely. In addition, their chemical structures encompass a nitrogen atom, charging the molecule in a physiological environment, which hampers their entrance into brain. These differential properties of chemical agents result in different risk profiles with regard to exposure, distribution, and toxic effects. The differential toxicology might urge for adaptations in the emergency protocols presently in use. Due to the similarity in chemical structure and mechanism of action, the insight acquired by studying nerve agents can also be used to improve treatment of intoxications with OP-pesticides.

In order to enable improvement of medical management, research in this thesis focussed on optimizing animal models to further investigate the mechanism of OP toxicity and treatment efficacy. Two types of OPs were used: soman, representing a highly toxic volatile OP, entering the body via inhalation and producing toxic signs very quickly, and VX, representing a much less volatile OP entering the body via the skin and demonstrating a clinical delay of toxic signs and long persistence. In particular treatment efficacy was tested.

In Chapter 2, an integrative guinea pig model was established to evaluate biochemical and vital physiological parameters following soman poisoning. Then, the efficacy of a current treatment regimen consisting of obidoxime and atropine was tested at 1 minute after exposure, as well as after appearance of first clinical signs after exposure to different doses of soman. This regimen appeared to be hardly effective; it provided low efficacy following challenge with a high dose of soman, and also when the treatment was postponed. Addition of the carbamate physostigmine to this treatment appeared to improve the outcome dramatically when administered at 1 minute after poisoning. However, when physostigmine
treatment was delayed to 10 minutes after poisoning, its efficacy showed to be redundant or even harmful.

The VX-exposed hairless guinea pig model is presented in Chapter 3. Exposure of VX via the skin was shown to be unpredictable and variable in terms of toxicokinetics. After a lag period following skin application, VX levels in blood continue to rise for several hours. The physiological consequences of accumulating central ACh levels, clinical signs and AChE activity were investigated. It was shown that the highly variable development of clinical signs upon exposure was predictive for the clinical outcome. Only few animals exposed to VX developed seizures, while most of them suffered from EEG signs that pointed to ischemia.

In Chapter 4 the VX model was extended to investigate toxicokinetics in freely moving animals combined with physiology, e.g. respiration, EEG and heart rate effects. Exposure to VX led to an increase in bronchoconstriction and a dramatic decline in heart rate, which was in line with the signs of ischemia on the EEG pointed out in Chapter 3. After full establishment of all parameters, a treatment protocol consisting of a one-shot injection consisting of 3 auto-injector equivalents of atropine, obidoxime and diazepam was tested. Initially, this treatment effectively postponed clinical signs of skin VX poisoning, but clinical signs reappeared after some time, progressing in a similar way as in untreated animals. Repetitive treatment on reappearance of signs, however, showed to be effective as long as treatment was continued. These findings imply that in case of skin exposure with VX, treatment should be continued until all VX has been eliminated from the body.

Long-term behavioural deficits of OP-poisoning and a possible intervention by drug-induced neurogenesis were investigated in a soman-poisoned rat model in Chapter 5. The rats were injected with a seizure-inducing dose of soman, combined with life-saving treatment consisting of atropine and HI-6. All animals suffered from seizures for a short period of time, which was accompanied by a huge increase of ACh levels in the brain. Although brain damage was very limited at 24 hours after poisoning, rats showed an impaired learning curve in a spatial memory task at 8 weeks after exposure. This was accompanied by a decrease in neurogenesis in the hippocampus, which could not be restored by the antipsychotic Olanzapine, which was reported to enhance neurogenesis. Although Olanzapine was ineffective in enhancing neurogenesis and normalizing behaviour, influencing neurogenesis by drug or other means might be an attractive approach in the treatment of long-term cognitive deficits after OP poisoning.

Overall, the results described in this thesis led to improved insights in the treatment of exposure to the agents soman and VX. The animal models developed allow an integrative assessment of toxicology and treatment efficacy. The sensitivity for these OPs and anti-muscarinic receptor compounds, the oxime-induced reactivation and ‘aging’ of OP-inhibited acetylcholinesterase markedly differs between species, complicating the extrapolation of these results to man. Whereas the guinea pig model is most suitable to investigate drugs for acetylcholinesterase protection, it is less suitable for the assessment of subtle cognition impairments, due to the lack of proper tests; the rat model is more suitable to assess impact of drugs on cognition. Thus, to provide the best translational results, it is recommended to include both the guinea pig and rat model in screening treatment efficacy. In the
rat model it was shown that an effective life-saving treatment at short term might not be sufficient to prevent long-term cognition deficits. This implies that a better understanding of the cause of such deficits is needed in order to design improved treatment strategies. It is assumed that impaired neurogenesis attributes to cognition deficits, which might be a starting point for research into future treatment of OP poisoning.

The toxicological profiles of soman and VX exposure in the guinea pig model showed to be very different and accordingly demand a completely different treatment approach. In particular the timing of treatment is an important factor to enhance efficacy in case of exposure to both VX and soman. Whereas soman exposure requires fast treatment, aimed at immediate recovery of acetylcholinesterase activity and terminating seizure activity, VX exposure requires repeated treatment on (re)appearance of clinical signs until all nerve agent has entered the circulation and has been hydrolysed. The results from the VX experiments are useful to the clinical treatment of OP insecticide poisoning, because of similarity in persistence in the body.