Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	50100
1.2	Provide the name of the licenced establishment.	Netherlands Organization for Applied Scientific Research TNO
1.3	Provide the title of the project.	Toxicology of chemical threat agents; efficacy and adverse effects of medical countermeasures against these threats

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☐ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or animal
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.1.

In order to protect people against the adverse effects of exposure to highly toxic compounds, research is needed to improve our understanding of the toxicology of these threats and develop efficacious medical countermeasures. These toxic exposures range from chemical threats against military personnel,

occupational incidents with exposure to toxic industrial chemicals, but also potential terrorist attacks on (large) groups of civilians.

Actors

In spite of the success of the Chemical Weapons Convention (CWC, established in 1997) the chemical threat is still perceived as realistic. To date 4 'state parties' have not ratified the CWC, and these are nations located in areas of high concern. Some of these nations are suspected of having an interest in chemical weapons. And even though Syria has ratified the CWC, the repeated use of chemical weapons within this nation has been reported and proven in several instances. In addition, 'non-state actors' such as terrorist groups may be tempted to using toxic compounds to achieve their means. Toxic compounds have also been used for (politically driven) assassination attempts, such as the killing of Kim Jong-nam at Kuala Lumpur airport (Malaysia) in 2017 with the nerve agent VX, the intoxication in 2018 of Sergei and Yulia Skripal in Salisbury (UK) with a nerve agent of the so-called Novichok type, and the recent (2020) poisoning of the Russian politician Aleksej Navalny in Tomsk with a similar compound. In the case of Sergei and Yulia Skripal, also a police officer and two British civilians were severely injured, of which one civilian did not survive the accidental exposure. Another example of chemical exposure which affects civilians is that in some Asian countries, such as India, there is a high rate of accidents but also attempted suicides with organophosphate pesticides that resemble organophosphate nerve agent intoxications. These examples highlight that use of these chemical threat agents still has the potential to impact a broad range of people.

Scenarios

Recently, concerns have increased with respect to the low volatility nerve agent VX, which was one of the agents present in Syria during the civil war. Due to its low volatility the presence of VX is very hard to detect and the agent is highly persistent in the environment as well as in the body. Rumors that low volatility nerve agents had been developed by Russia, the Novichok agents, were confirmed by proof of their use in the recent poisonings in the UK and Tomsk. Novichok agents appear to be highly persistent in the body, which complicates treatment of intoxication by these compounds. Apart from the mechanism of action, not much is known about this type of nerve agent, and more research is needed to develop effective countermeasures. Moreover, low- and non-volatile compounds, such as VX, would have to be dispersed by aggressors as liquid or solid aerosols, for which the technology has evolved considerably over the past decades, increasing the risk of abuse and expanding the scenarios of potential use.

Spectrum of compounds

In recent years, not only the actors and scenarios of potential use of chemical agents have broadened, but also the spectrum of compounds that may be used as a weapon. In the Cold War era, it was mostly relatively volatile liquids, such as the 'classical' nerve agents tabun, sarin and soman as well as blistering agents such as sulfur mustard and lewisite, that were considered a threat. After the Cold War ended, the awareness grew that toxic industrial chemicals, which are less toxic than traditional chemical warfare agents but are present in much higher quantities around the world, may also be used as chemical weapons by potential adversaries, for instance by blowing up storage facilities. Toxicological research into these types of compounds also aids in the management of patients exposed during (occupational) incidents, such as accidents involving chlorine and phosgene. Both toxic chemicals are widely used in various industries which leads to incidents on a regular basis [1].

Besides toxic industrial chemicals, concerns are growing that pharmaceutically-based agents, such as synthetic opioids and other potent pharmaceuticals with an effect on critical physiological processes, may be used by malicious parties. Meanwhile, the number of illegal labs where drugs are being synthesized is increasing, which feeds the aforementioned concern.

The available treatments for intoxications with certain chemical warfare agents have not essentially changed in the past decades, while for some still no (causal) therapy is available. Besides discovery and development of new antidotes, a new approach is to try whether a therapeutic that is registered for other indications is effective in counteracting the adverse physiological effects of chemical warfare agents. This makes sense, as expanding the list of indications for which a therapeutic is registered is much easier and quicker, and less risky, than designing and licensing a brand new antidote.

This proposal is a follow-up of the project granted under number AVD50102016583 (August 1,2016-January 31, 2022) entitled 'Exposure to chemical threats: Medical interventions, Toxicology, and Safety'. Within that project studies were performed on, among others, the development of a delayed treatment against VX nerve agent intoxication tailored specifically to civilian incident scenarios [2], pharmacokinetics and efficacy of atropine and obidoxime combination therapy against VX and sarin [3,4], optimization of anticholinergic treatment against sarin by comparing scopolamine against the standard of care atropine [5], comparing the efficacy of various combinations of pharmaceuticals to counteract seizures induced by nerve agents and other toxicants [6,7]. Also, a start was made with establishing a translational guinea pig model for acute fentanyl intoxication in which the efficacy of the opioid antidote naloxone in combination with respiratory stimulant doxapram can be evaluated. Moreover, polytherapy with clinically available anticonvulsants was found to be efficacious against intoxications with different classes of chemical threat agents, indicating potential use as a generic therapy.

Thus far, in the previous project around 450 rats and 950 guinea pigs were used, the foreseen studies with mice and pigs were not performed. The results of the various animal studies have enabled us to better indicate the threat posed by various compounds and have given direction to which measures should be taken to mitigate the effects of these compounds, should these be used by malicious parties. The estimated number of animals in this project proposal is higher as compared to the used numbers in the previous project to account for the diversification of the research scope in case current events or developments prompt the intensification of specific types of studies.

The above mentioned changes in actors, scenarios and chemicals are reflected in the scope of this project which main objective is to determine the efficacy of medical interventions in qualified models of toxic chemical exposures.

3.2 Purpose

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The main objective of the proposed project is to assess the toxicology of chemical threat agents and design and/or evaluate interventions to counteract and/or minimize the adverse health effects of intentional or accidental exposures. These interventions may include preventive treatment, treatment with a single or a combination of therapeutics, decontamination procedures with or without use of active pharmaceutical ingredients (for example to aid destruction or scavenging of the toxicant). This is for the benefit of military personnel, first responders, as well as the general public.

As it is currently not possible to study the complex toxicological processes in full using exclusively *in silico* and/or *in vitro* models, animal studies are still necessary to reach this objective. Physiological animal studies do provide input for *in silico* model development which ultimately leads to an increased knowledge gain per study and a reduction in the total amount of necessary animal studies. Moreover, in contrast to drug development trajectories for diseases such as cancer or atherosclerosis, for intoxications with prohibited chemical substances there is no patient population and thus no possibility to perform clinical trials. This means that the development and approval of treatments relies on animal studies with translational animal models, by the US Food and Drug Administration (FDA) known as the Animal Rule [8]. The studies performed within this project aim to contribute to this development and can be divided into the following three components.

Component 1: Development of translational animal models to study the toxicology of chemical threat agents

For (classes of) chemical substances for which no adequate treatments and no established animal models are available, new animal models will be developed, based on *in vitro* and *in silico* studies and evaluation of scientific literature. Animal models will be developed that adequately reflect the aspect to be studied for the situation in humans. This will include characterization of the toxic effects (toxidrome) induced by the

compound under study, *in vivo* verification of the underlying mechanism of toxicity as established via *in vitro* experiments, as well as the toxicokinetics and metabolic pathways.

Component 2: Pharmacokinetics and pharmacodynamics of therapeutics and adverse effects
Though some of the therapeutics to be studied are clinically available and approved for other indications (e.g. the anticholinergic scopolamine is approved for treatment of nausea and motion sickness) with readily available data on pharmacokinetics and adverse effects, oftentimes different information (i.e. dose range, administration routes, interactions with other substances, etc.) is needed to establish their suitability as medical countermeasure for intoxications with highly toxic agents.
Possible adverse effects of therapeutics in the dose-range required for efficacy against chemical threat agents will be observed in the animal models, as these dose-ranges are oftentimes higher than those tested for licensed indications. Also, different types of adverse effects might be relevant for the evaluation of efficacy of therapeutics in intoxication scenarios. An example of such an adverse effect is incapacitation (i.e. the inability to perform a specified act or function) of personnel by therapeutics, and could for instance occur in a false alarm situation, where no exposure to a compound has occurred. Incapacitation of personnel by therapeutics is an unwanted phenomenon in military context, as this may endanger completion of the mission.

Component 3: Efficacy of medical countermeasures against intoxication by chemical threat agents
The efficacy of interventions to counter the adverse effects of chemical threat agents will be studied in already available or newly developed animal models. This includes the evaluation of the efficacy of therapeutics, but also other types of interventions may be studied, such as procedures to decontaminate the skin. The studies will be aimed at establishing both short term efficacy, such as survival for up to 96 h, reduction of life-threatening effects and/or incapacitation induced by the compound, as well as intermediate term efficacy, such as recovery from the effects of the intoxication after treatment, which will be studied for a period of up to 14 days post-intoxication.

3.2.2 Provide a justification for the project's feasibility.

TNO Defence, Safety and Security, has a long standing tradition in research on chemical defence, with assignments mainly from the Netherlands Ministry of Defence (MoD) and Armed Forces, but also [redacted]
International cooperation is well-established within the chemical defence domain, and TNO cooperates among others within [redacted] Within these cooperations, research programs are lined up and in some cases actual joint projects are performed.

Within this cooperative efforts there is a reasonably high degree of task specialisation. TNO has a worldwide dominant knowledge position on toxicokinetics and elimination pathways of extremely toxic compounds as well as on *in vitro* diagnosis of exposure to such compounds. TNO is a designated laboratory for analysis of environmental and biomedical samples for the Organisation for the Prohibition of Chemical Weapons (OPCW), which underscores its status in the worldwide chemical defence domain. Moreover, TNO is the only organisation in the Netherlands where compounds that are forbidden under the CWC may be present and used, obviously only for research and testing for protective purposes.

For each research question the most suitable animal model is chosen. In the chemical defence domain most research questions can be addressed using rats or guinea pigs as a model. TNO has decennia of experience with these species (i.e., since the 1950s) and works with qualified personnel to safely perform animal studies within the high tox laboratory facilities. Over recent years TNO has built-up the capability to generate aerosols of highly toxic chemicals in a controlled and safe way, also with the possibility to expose animals to these aerosols via the respiratory or dermal route. Besides other routes of exposure used in the previous projects, this technology will be added in the current proposal for further optimisation.

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

☒ No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

3.3 Relevance

3.3.1 What is the scientific and/or social relevance of the objectives described above?

Although the Chemical Weapons Convention (CWC) is highly successful, the chemical threat is considered to be realistic, both in a military and civilian context. The CWC only applies to the 'scheduled' compounds, which are defined by their Chemical Abstract Services (CAS) number. Other compounds could be developed and used without violating the CWC to the letter. In 2018 in the UK, several individuals were poisoned with a nerve agent that was not listed as a CWC-scheduled compounds. In 2019 several variants of these so-called Novichok agents were added to the list of scheduled compounds. This illustrates that the list of scheduled compounds will always lag behind the development of chemical threats, warranting the necessity to continuously anticipate new threats.

Characteristics to be considered in threat evaluation include the availability of the compound, ease of dispersion, stability, detectability, the toxicology of a compound, etc. For emerging threats not all critical aspects of the toxicology may be known, such as the mechanism of toxicity, toxicity in qualitative and quantitative sense, toxicokinetics and elimination pathways. Use of animals models may be necessary to obtain some of this information. If a compound is considered to be a realistic threat, the armed forces will have to establish whether their capabilities in the field of detection, (physical) protection and decontamination are adequate to mitigate the threat and be able to fall back on medical countermeasures if protective measures fail. Currently, medical countermeasures are not available for all (classes of) chemical threat agents. Existing treatments can reduce lethality, but mostly do not adequately prevent or terminate detrimental endpoints, or are difficult to administer on the site of the incident.

In a military setting, the risk of exposure may vary and depends on the quality of intelligence concerning chemical threats prior to a mission. Chemical attacks in military context will most likely affect a relatively small number of personnel, who are well trained to cope with chemical threats, and are in possession of protective measures, such as a gasmask, protective clothing, skin decontaminant and autoinjectors containing antidotes. The military population consists mainly of healthy, relatively young people. In a civilian setting however, the situation may be quite different. The general public, including children and elderly, is not prepared at all for chemical attacks and protective measures are not available. First responders are trained to deal with such events, but precious time ticks away before they arrive on the scene and establish which chemical agent has been used. A mass casualty situation may develop. In such a situation, the doses that individuals may be exposed to will range from supralethal, leading to a very rapid death, to individuals exposed to lower doses, who will develop (severe) signs or lethality over a broader time window. It is particularly the group exposed to lower doses that could be saved in such a situation, albeit that the pharmacological countermeasures should be safe, effective, and easy to administer in a mass casualty situation. However, in contrast to the military situation, only approved drugs can be used as a medical intervention for the general population.

As establishing the efficacy of medical countermeasures against chemical threat agents is not feasible in humans, translational animal models are needed, that can convince agencies like the FDA and the European Medicines Agency (EMA). In such cases, the FDA applies The Animal Rule to grant approval based on well-controlled animal studies that proof that a treatment is reasonably likely to produce clinical benefit in humans. Because these pre-clinical studies are not followed by extensive clinical testing, animal research for the development of medical countermeasures is crucial.

3.3.2 Who are the project's stakeholders? Describe their specific interests.

The main stakeholder for the project is the Netherlands Ministry of Defence (MoD). Protection against chemical, biological, radiological and nuclear (CBRN-)threats is high on the agenda of the MoD, with an emphasis on chemical threats. For decades the MoD has been investing millions of EUROs on an annual basis in knowledge build-up on the various aspects of CBRN-defence, i.e. threat analysis (in which the intrinsic toxicity of a compound is one of the determining factors), detection, identification, physical protection, decontamination, diagnosis and medical countermeasures. Animal studies are performed within

the context of threat analysis, diagnosis and medical countermeasures. The MoD supports the animal work done by TNO, as well the development of alternatives for using animals. Besides the Netherlands MoD as stakeholder, TNO performs research for and in collaboration with [redacted]

A second stakeholder is the scientific community that will benefit from both the fundamental and applied research that is performed by TNO. The fundamental research performed at TNO within this project on prohibited substances cannot be performed by other parties and is key in advancing the scientific understanding and approach to the treatment of exposure to highly toxic compounds.

A third stakeholder is the medical community which benefits from the research performed in the military domain for example with treating intoxications of patients. In addition, due to the diverse nature of TNO as a whole, TNO oftentimes has the ability to bring together innovations from different scientific fields to create new applications that serve as a stepping-stone for advancement in other scientific fields.

A fourth category stakeholder is the pharmaceutical industry that develops medical countermeasures which are also applicable to the military domain, such as skin decontaminants, autoinjectors containing antidotes against chemical warfare agents, and new antidotes that are more efficacious than the existing ones or are effective against a broader spectrum of chemicals. TNO acquires assignments from these companies to, for instance, evaluate the efficacy of the countermeasures against the actual chemical warfare agents and establish the pharmacokinetics of antidotes, in animal models.

The experimental animals used for the studies are also stakeholders. The animals are specifically bred for the purpose of the studies by licensed animal suppliers and will live within the restraints of the animal facility settings. Care is given to provide housing that fits the animals needs and resembles their preferred circumstances as much as possible within the animal facility settings. Animals will be housed in groups where possible, provided with appropriate bedding, shelter and cage enrichment. Environmental circumstances such as a day/night light cycle, temperature and humidity will be kept within preferred limits. During experiments, measures will be taken to prevent unnecessary discomfort and in the context of life-long-learning all personnel involved will keep up with new scientific insights and developments regarding animal care, handling and experimenting. Humane endpoints and surrogate indicators of severe intoxication effects will be established and incorporated where possible to minimize any unnecessary suffering, with euthanasia in case these endpoints are reached.

Finally, victims intoxicated by highly toxic compounds are also stakeholders. The shared ultimate goal of the MoD, scientific and pharmaceutical stakeholders is to provide the highest quality protection and medical care to anyone at risk at risk of exposure or exposed to highly toxic compounds. Gaining more insight in the toxicology of highly toxic chemicals will also benefit first responders and medical personnel in providing the best possible on-site and hospital care post-incident. In addition, protection against terrorist attacks by malicious parties will aid in the protection of society as a whole and the democratic values that bind it.

3.4 Strategy

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

1. Development of translational animal models

In order to study threats posed by a chemical substance and test new or improved treatments or interventions it is necessary to have appropriate animal models available. The animal models represent victims in a civilian mass casualty situation, or military personnel in a mission setting. As the time frame to possible first responder intervention will be variable, an important requirement for an animal exposure model is that typical time to effect and treatment profiles can be mimicked. In a military situation, this ranges from self-aid and buddy aid within minutes after exposure, to field medical care by medics or in a field hospital, followed by medical evacuation. In a civilian setting, medical care providers can be overwhelmed by the number of victims and treatment can be delayed for hours.

A number of prerequisites for performing *in vivo* research towards medical countermeasures in animals exist (See figure 1). First, there should be a need for effective treatments or interventions; if they are available, no animal research is warranted. Available treatments are considered not effective when it is not life-saving, when long lasting effects are observed (such as brain injury due to the inability of innate repair of affected organs) or when a treatment has substantial side effects that do not fully outweigh the potential

therapeutic effects. Additionally, treatment concepts from other disease models could be employed to CBRN relevant intoxications when similar organs or organ effects are targeted, like anti-epileptic drugs that could mitigate organophosphate-induced convulsions. In case of a known treatment, this treatment may not be effective at the extremely high doses of chemical threat agent which can occur during intoxication, allowing room for improvement.

After the decision that the research question ratifies the use of animals, the most suitable animal model for the agent under study will be selected based on literature and possibly prior efforts towards approval of certain drug use for FDA/EMA regulation. For the challenge agent of interest, the mechanism of toxicity should be known, either from literature, or *in vivo* (toxicokinetics and toxicodynamics) and/or *in vitro* collected data. *In vitro* data consists of target characterization, and identification of the mechanism of toxicity, which can be either direct, or resulting from the initiation of a toxic cascade. Similar prerequisites are required for the potential treatments to be tested.

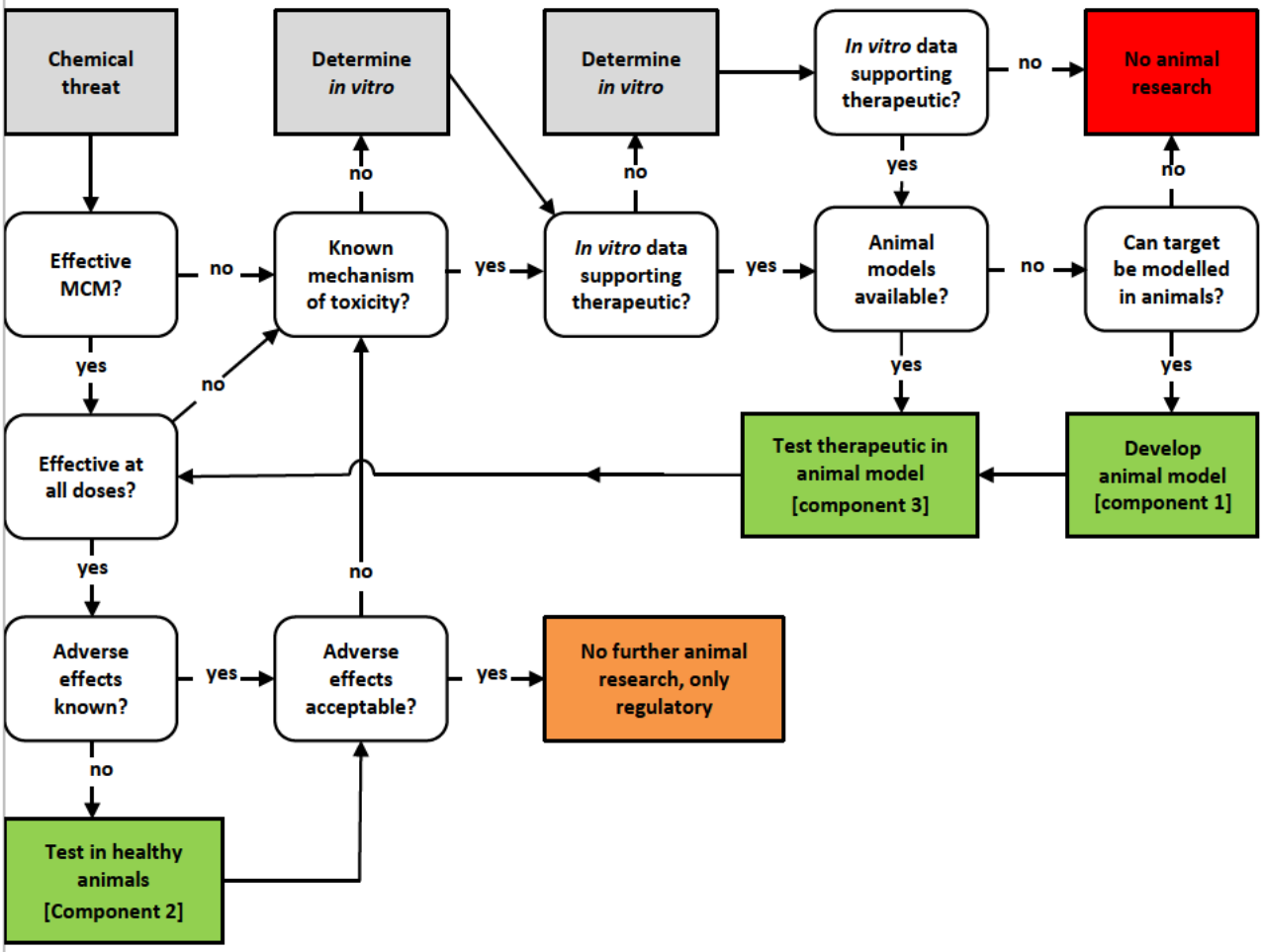

Figure 1: Strategy for determining the necessity of animal research and the prerequisites for performing studies belonging to the three different components of this project. MCM = medical countermeasure

The selection of an animal species depends on the availability of the identified (human) targets and mechanisms of interest. In addition, the parameters to be assessed should be reliably measurable in the species of choice. If those have already been modelled in animals and the model mimics the chemical threat specific context, potential therapeutics or interventions can be tested using available models. If there are no models readily available, but the mechanisms of interest are known and can be modelled in the species of choice, the required animal model can be developed inhouse. In some cases, the mechanisms of interest have been modelled in animals in a non-intoxication specific context and need to be optimized to fit the chemical threat context. For example, a lot of research has been conducted on opioid toxicity in

animals, but model either addiction (with different mechanisms involved, such as tolerance and withdrawal) or small overdoses relevant to post-operative analgesia. Both are not suited for efficacy testing of potential treatments against acute, very high, opioid overdoses in otherwise healthy individuals. The choice of animal species based on these considerations will follow a case-by-case approach, ensuring selection of the most appropriate species for each research question.

Readout parameters will be established for an animal model depending on the type of compound, and be representative for the compound under study. The range of chemical threat agents in this context covers among others irritants (e.g. chlorine, phosgene, PFIB), vesicants such as sulfur mustard and Lewisite, blood toxicants such as cyanides, and nerve blocking agents such as nerve agents e.g. sarin, VX, Novichoks), carbamate and organophosphorus pesticides, and toxins such as botulinum toxin, and highly potent pharmaceuticals such as the synthetic opioids. In the models developed, several triggers to treat (for example clinical signs), will be identified from the observed toxidrome that will correspond to time frames encountered by first responders or physicians. Relevant time frames include up to 96 hours for studies focusing on acute intoxications and up to 14 days for recovery trajectories. The objective for studies up to 14 days is identification of recovery mechanisms and potential early indicators of recovery as to select appropriate humane endpoints and keep future studies as short as possible. A prerequisite for these studies is that the animals are subjected to challenge doses below the range that would be labeled with severe discomfort in acute intoxication settings; the focus is on the mechanism, not the extend of intoxication for high doses for a longer period of time.

2. Pharmacokinetics and pharmacodynamics of therapeutics and adverse effects

In case of a search for improved medical countermeasures, consisting of new therapeutics or off-label use of a therapeutic already available for other diseases, information on the required dose range and possible side effects in healthy animals is needed. If this information is not available from the literature, the dose range and possible side effects will be characterized in healthy animals in phase 2. This can also include the study of alternative routes of administration of a countermeasure that is already approved. Critical physiological parameters and/or pharmacokinetics will be determined to estimate best practices for intervention in a field situation and will complement the data collected from intoxicated animals (phase 3) Data collected in this phase can be used for the development of PK-PD models: These models can provide useful links between animal models and clinical data, with the ultimate aim of predicting therapeutic doses in humans.

3. Efficacy of medical countermeasures against intoxication by chemical threat agents

Finally, the new treatment or intervention will be tested in the animal model selected or developed above. For this purpose, the triggers to treat as defined in the animal model development/ selection phase will be used, representative for the field situation to be studied (such as challenge dose and route of exposure), and the read out parameters for toxicity should show improvements over control animals. One of the parameters will be survival, for chemical threat agents with a delayed effect such as sulfur mustard, up to 96 h. Another parameter is recovery of the animal from the effects of a chemical challenge, up to 14 days after intoxication.

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

As shown in the research strategy above, certain circumstances will ratify animal research.

1) In case no qualified animal model is available, the primary phase consists of selection and/ or development of an animal model for a specific highly toxic compound. The animal species will be selected based on the mechanism of toxicity of the compound, and will be either rat or guinea pig (see appendix for selection rationale). After selection, a dose – time to effect range will be determined of a specified chemical. To that end, the animals can be equipped with for example telemetric devices for physiological monitoring, or cannulas for blood sampling. Relevant exposure routes, including respiratory exposure to gases, vapors and aerosols, percutaneous exposures to liquids, vapors and aerosols, as well as invasive routes, either as bolus administration or prolonged infusion, may be studied. A variety of physiological read-out parameters will be established, such as indicator effects on the nervous system (e.g. electroencephalography (EEG)), respiration (a.o.

respiratory frequency, tidal volume, minute volume) and cardiovascular system (a.o. heart rate, blood pressure), to characterize the toxidrome. Also, biomarkers of exposure and/or effects may be developed, as well as physiological triggers to treat. This will lead to a characterized response at (sub)lethal doses, with specified triggers for intervention. These procedures will also be appropriate to develop forensic tools and identification of biomarkers of exposure, or determine the biological fate of the compound to which the animal has been exposed.

2) In parallel, the antidote response in animals not exposed to the toxicant will be characterized. These types of experiments are necessary for dose range finding, determination of pharmacokinetics following different exposure routes and characterization of possible side effects specifically relevant to acute toxicity scenarios. The pharmacokinetics of prospective therapeutics will be studied for various relevant routes of administration, such as intramuscular, intranasal, subcutaneous, intraosseous, topical/percutaneous, etc. Telemetric readouts can be applied, in addition to pharmacokinetic determinations in tissues or fluids.
3) After establishing the animal model, treatment efficacy of drugs or interventions will be evaluated in the intoxicated animal model at specified triggers to treat. Treatments will be administered to the animals via the routes selected for the new treatment in a prior phase.

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

As stated in 3.4.2, the proposal consists of three components. Model development and determination of pharmacokinetics, pharmacodynamics and adverse effects (components 1 and 2, respectively) can be performed in parallel, and are independent of each other. Experiments from component 3, efficacy testing, can only be performed if information from components 1 and 2 from own studies or from other labs are available.

3.4.2 Provide a justification for the strategy described above.

Performing animal studies is not the default choice to answer questions related to the toxicology of chemical threats, the efficacy of medical countermeasures and potential adverse side-effects of these countermeasures. By following the strategy describe above, useful results that contribute to reaching the goal of the study will be obtained, using a minimum number of animals.
With funding from the Netherlands Ministry of Defence. TNO Rijswijk is actively exploring and developing alternatives to animal testing, via *in silico* and *in vitro* approaches. Mostly, the alternative approaches cannot fully replace animal studies but will provide guidance on which in vivo research is needed, in many cases reducing the number of animals needed to obtain an adequate answer to the research questions.

3.4.3 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Rat and Guinea Pig models to study the toxicology of chemical threat agents and countermeasure evaluation

References

[1] R. voor V. en Milieu, Vijftien jaar incidentanalyse, (2019). https://doi.org/10.21945/RIVM-2019-0042.

[2] [redacted]

[3] [redacted]

[4] [redacted]

[5] [redacted]

[redacted]

[6] [redacted]

[7] [redacted]

[8] Animal Rule Information | FDA, (n.d.). https://www.fda.gov/emergency-preparedness-and-response/mcm-regulatory-science/animal-rule-information.