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 our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

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.	TNO
1.3	Provide the title of the project.	Exposure to chemical threats: Medical interventions, toxicology and safety

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
- ☐ 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.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

In 1997 the Chemical Weapons Convention (CWC) entered into force. To date 192 state parties have ratified the CWC, which has greatly reduced the threat of chemicals being used in acts of war between states. However, 3 state parties have not ratified the CWC. Since these states are located in sensitive

regions of the globe, there is still a concern for chemical warfare between states. The use of chemicals by non-state actors, such as terrorist groups, is nowadays perceived as a realistic threat. As terrorist have proven to be opportunists, they will not only revert to 'classical' chemical warfare agents such as nerve agents and sulfur mustard, but will also have an interest in chemicals that are relatively easy to obtain in large quantities, such as 'toxic industrial chemicals' (TICs). TICs are generally less toxic than the classical chemical warfare agents, but sufficiently hazardous to cause mass casualties and to disrupt societies. For example in the case of intentional exposure as described above, as well as in the case of unintentional exposure, eg after catastrophic incidents in chemical plants. Obviously, in such scenarios both military personnel, first responders (police, firemen, paramedics) and civilians are at risk. The impact on civilians may be even higher, as they are untrained for and unprotected against such threats.

Several forms of toxic agents exist, ranging from solid salts to volatile liquids and gasses. Irrespective of the form of dissemination and consequent route of exposure, the chemical moiety determines the mechanism of toxicity. The toxicodynamics depend on the route and duration of exposure and accordingly the dose and the chemical form. In case of human exposure, the possibility to provide adequate and safe medical countermeasures, including decontamination, for casualties and first responders is of importance. This requires insight into the mechanism of toxicity, the toxicokinetics and the toxic effects of relevant chemicals. Such insights provide a basis for development of antidotes and supportive treatment to mitigate the effects of exposure to toxic chemicals on the short and longer term.

Ideally, newly developed (experimental) antidotes may be approved as drugs, or existing drugs may be approved for use as antidotes against chemical threats. In Europe, EMA regulates approval of new drugs. For Drug approval for use in the general population in the USA, FDA approval is required. Both regulatory agencies differ in their approaches for drug approval in case the phase III (Clinical efficacy) trials, a crucial part of the drug approval process, cannot be pursued. Whereas the FDA in such cases requires testing of medical countermeasures following the FDA animal rule* in a qualified animal model, the EMA would benefit from the results achieved in such models to allow fast track approval. The animal rule states that efficacy of a medical countermeasure in case of an exposure should be shown in an animal model that meets at least 4 criteria: construct validity, predictive validity, face validity and defined outcome of the medical countermeasure. Currently, such animal models in which medical countermeasures against chemical exposure can be tested, are available. However, adjustments to operational settings are needed, making such models qualified for a certain context of use, by a regulatory agency. The supporting evidence for drug approval in that context can be achieved by testing drug efficacy in a qualified animal model under agency-approved quality standards.
*:http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM399217.pdf

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

Main objective

The project proposal is aimed at supporting research towards medical countermeasures, forensics and safety evaluations for military personnel, first responders and civilians in case of a catastrophic incident in which highly toxic chemicals are intentionally or accidentally released, leading to life-threatening human exposures.
For the military domain it is anticipated that the focus will be on the development of new strategies for medical countermeasures, supported by research on toxicological pathways and toxicokinetics, in appropriate animal models.
For the civilian domain it is anticipated that the focus will be on evaluating the efficacy of existing antidotes, other pharmaceuticals and supportive treatments in qualified animal models. The difference between the domains originates from the differences between military and civilian settings, with different regulations, and average population. For example, the military population consists of relatively young and healthy people, of which the majority are men, whereas the civilian population consists of a variety

of age groups and 50% men/women.
In more detail the following issues will be addressed:

1. Development of animal models

One of the main objectives of the project proposal is to develop qualified animal models for intentional, or accidental, life-threatening exposure to highly toxic chemicals, including chemical warfare agents, via various routes to allow regulatory testing of newly developed pharmaceuticals or interventions as medical countermeasures, or testing of new applications of existing drugs for regulatory approval. The qualified animal models should represent a civilian mass casualty or military casualty situation, in which typical time to effect and treatment profiles can be mimicked. The mechanism of toxicity in the model used should be similar to the human situation, as well as the intended mechanism of action of a prospective medical countermeasure.
The models used should allow variability in doses and accordingly time to effect. A certain degree of effect, such as a measurable change in heart rate, labored breathing, or other objectively measurable changes, will be defined as a trigger to initiate treatment. Representative available readout parameters will be selected to monitor treatment effects on acute toxicity, and in later stages long term quality of life. In this context, the animal models can also be employed to validate biomarkers of exposure, biochemically or physiologically, for example to develop forensic or diagnostic tools. Furthermore, the behavior of highly toxic chemicals in deceased bodies could pose a hazard for exposure of responders handling the remains. For that purpose, the fate of the chemicals in a body after death could be an aim of a study.

2. Safety and pharmacokinetics of different treatments

Additionally, safety and pharmacokinetic studies will be performed with regard to prospective treatments in the respective animal species. Treatments can be aimed at newly identified targets, or could be aimed at operationalizing more optimal administration routes for certain settings and timing of administration.

3. Short and long term efficacy

Therapeutic efficacy in case of exposure to highly toxic chemicals will be tested in available or developed animal models in a follow up stage. Initially, such studies will be aimed at short term efficacy, i.e. enhancing survival or minimizing life threatening signs of toxicity in the first 48 hours, whereas in final stages, the effects at the long term of an effective, or partially effective countermeasure can be the aim of evaluation, to investigate the benefits for quality of life.

Achievability

Research towards medical chemical countermeasures is only performed in a very limited number of laboratories in the world. Extensive international collaborations exist. Studies are generally demanded by national or international governments responsible for safety of civilians, first responders and military personnel. The establishment has an excellent experience with a range of animal models for exposure to highly toxic chemicals, including chemical warfare agents, with applications in forensics, toxicology and medical interventions. Furthermore, the establishment has close relationships with another facility which is AAALAC and GLP accredited, and which could perform certain study parts in collaboration. The project will be carried out at the establishment or under supervision of toxicologists from the establishment, experienced in the field of medical chemical countermeasures who will advise on for example triggers to treat and read out parameters.
For selection of animal models, where applicable, requirements of the FDA animal rule will be followed, as covered in: "Guidance for Industry: Product Development under the Animal Rule". An extract of the considerations in animal model selection and development is presented in the table below (from p. 41 of the guidance). In other cases, this guideline provides a transparent list of elements that have to be considered for selecting appropriate animal species.
Technology developed in the project could become part of technology transfers to support for example FDA regulation. Appropriate training of personnel handling animals will be ensured by adequate training. Training of personnel to master new techniques is covered in a separate project at the establishment.

3.3 Relevance

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

In spite of the Chemical Weapons Convention entering into force in 1997, the possible use of chemicals as warfare agents remains a threat, in particular for military forces, but also in a civilian setting.

Although current treatments can reduce lethality, they do not adequately prevent or terminate detrimental endpoints, or are difficult to administer on the site of the incident. Additionally, the availability and use of TICs in industrialized areas poses a risk of exposure of the civil population due to accidental or intentional release. In all cases, forensic measures to prove exposure to such chemicals, and ensuring safety of first responders are of importance.
In a military setting, the risk of exposure can vary and depends on the quality of intelligence concerning chemical threats prior to a mission
In a mass casualty 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, only approved drugs can be used as medical intervention. This requires testing for approval in line with FDA or EMA regulations as indicated above. Currently, a range of animal models is in use for research towards medical chemical countermeasures. However, there is a need for availability of standardized qualified models (at sufficiently high quality standards) that will more routinely allow testing of new drugs or drug applications, for approval of human use, including children and elderly.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

In order to test new or improved therapeutics or interventions it is necessary to have appropriate animal models available. The animal models will 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.
A number of prerequisites for performing *in vivo* research towards medical chemical countermeasures in animals exist (See figure 1). When no effective treatment exists, and *in vitro* mechanistic data argue for favourable outcomes *in vivo*, for example in case of a new concept intervening in the toxic cascade. Additionally, treatment concepts from other disease models could be employed 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 all realistic challenge doses, allowing room for improvement. A treatment is not effective when it is not life-saving, or when long lasting effects are observed, such as brain injury due to the inability of innate repair of affected organs. An alternate reason to conduct such research, is when a medical countermeasure has substantial side effects, that do not fully outweigh the potential therapeutic effects.
The species determination depends on a variety of factors. For a challenge agent, the mechanism of toxicity should be known, either from literature, or *in vitro* and/ or *in vivo* collected data (toxicokinetics and toxicodynamics). *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 drugs. The selection of an animal species depends on the availability of these identified (human) targets in the animals to be tested. In addition, the parameters to be assessed should be reliably measurable in the suspect species.

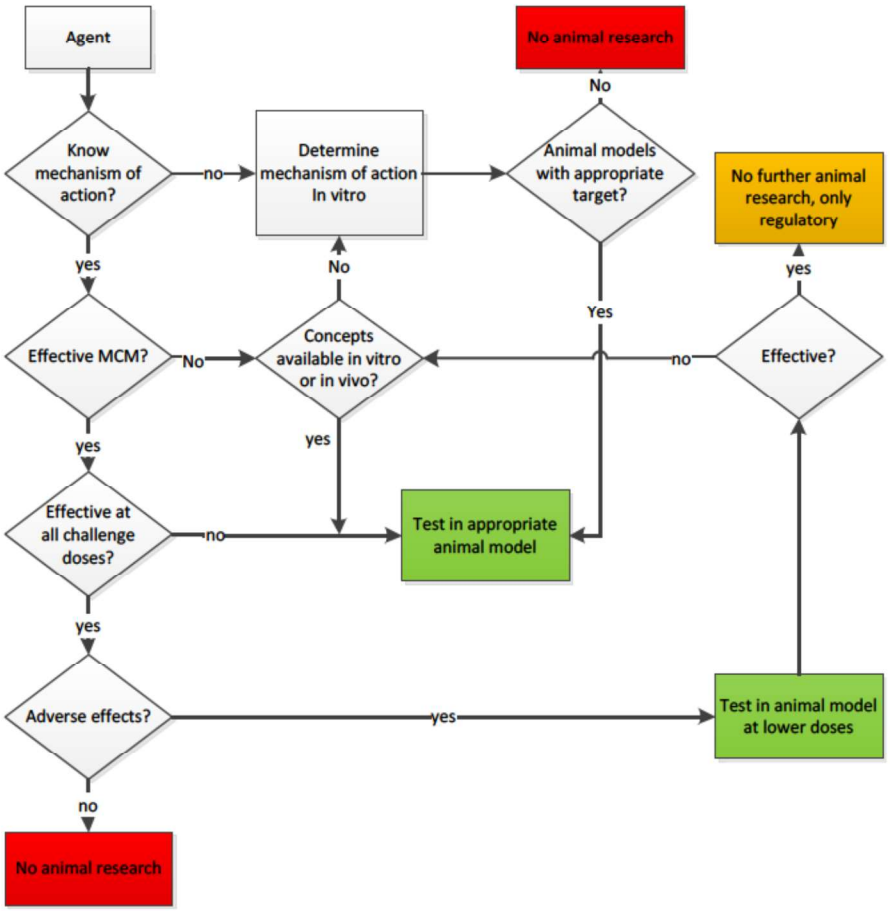

Figure 1. Strategy for determining the necessity of animal research.

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. In the prospective animal model, the mechanism of toxicity should be similar to the human situation, as well as the intended mechanism of action of a prospective medical countermeasure (See for elements Table 1).

Table 1. Elements related to the choice of animal species with regard to agents and drugs

Elements related to the etiologic or challenge-induced disease or condition (Context of Use)
CHARACTERISTICS OF THE ETIOLOGIC OR CHALLENGE AGENT
• The Challenge Agent
• Pathophysiological Mechanisms of Toxicity
• Route of Exposure
• Dose and Quantification of Exposure
HOST SUSCEPTIBILITY AND RESPONSE
NATURAL HISTORY OF THE DISEASE OR CONDITION -PATHOPHYSIOLOGICAL COMPARABILITY
• Time to Onset
• Progression
• Manifestations
TRIGGER FOR INTERVENTION
ELEMENTS RELATED TO THE INVESTIGATIONAL DRUG AND THE SELECTION OF AN EFFECTIVE DOSE IN HUMANS
THE INVESTIGATIONAL DRUG

• Mechanism of Action
• Drug Class
• Dosage Form and Route of Administration
SELECTION OF AN EFFECTIVE DOSE IN HUMANS
• PK and PD Information to Be Obtained in Animals and Humans
• PK/PD Considerations for Human Dose Selection

Readout parameters will be established for an animal model depending on the type of chemical, and be representative for the chemical under study. The range of chemical threat compounds in this context covers among others lung damaging agents (eg phosgene, PFIB, chlorine), blister agents such as sulfur mustard and Lewisite, blood toxicants such as cyanides, and organophosphate pesticides and nerve agents. In the models developed, several triggers to treat (for example clinical signs), will be identified that will correspond to time frames encountered by first responders or physicians.
In case of a search for improved medical countermeasures, consisting of new drugs or off-label use of a drug already available for other diseases, dose range finding and possible side effects of medical pharmacological interventions will be characterized in healthy animals in a second or parallel phase. This can also indicate alternative routes of administration of a countermeasure than already approved. Critical physiological parameters and/ or pharmacokinetics will be determined to estimate best practices for intervention in a field situation.
Finally, the new pharmaceutical or medical 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. This final stage can be expanded to cover longer time frames, to evaluate the efficacy at the long term after a successful or partially successful countermeasure. Depending on the chemical and animal species, this period may cover up to 1 year.

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. A schematic overview of the approaches and components in the framework of the project are shown below in Figure 2. The numbers point to the respective appendices.

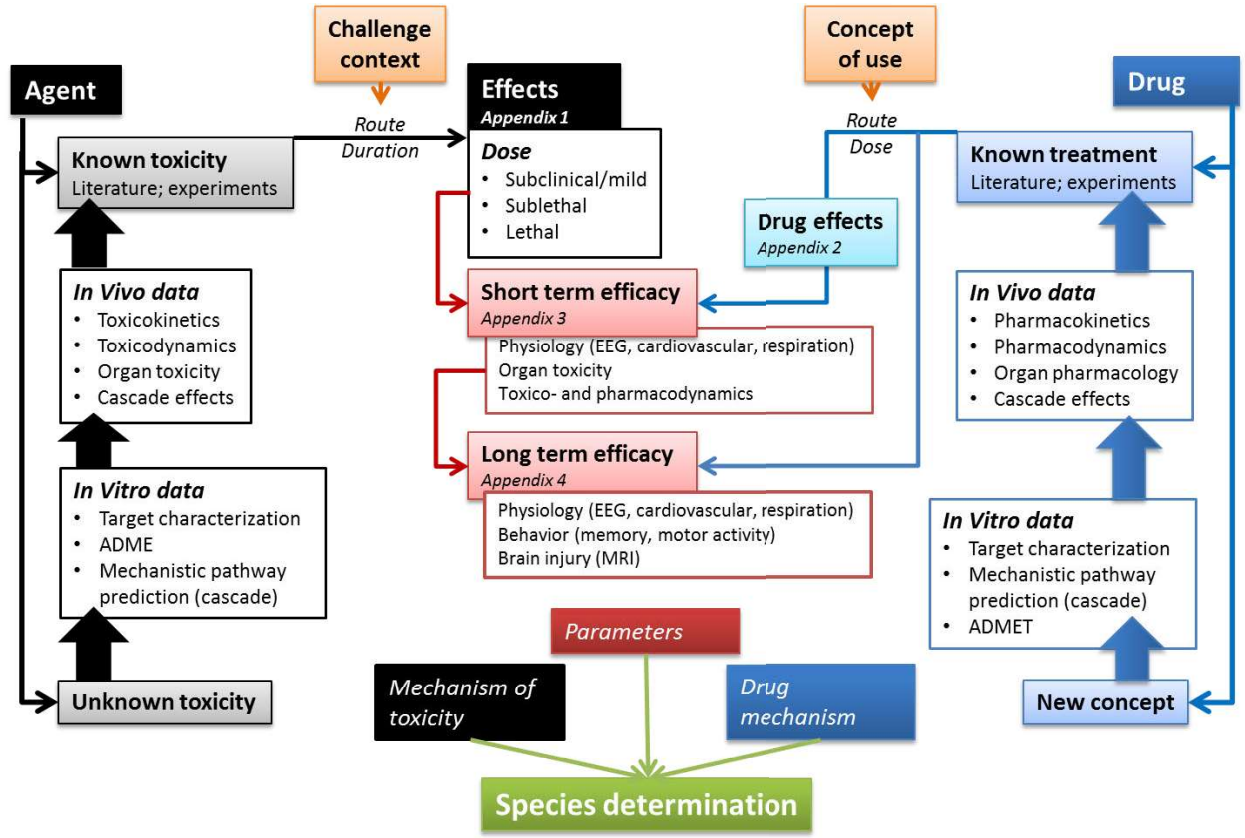

Figure 2. Schematic overview of the approaches and components in the framework of the project

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 chemical. The animal species will be selected based on the mechanism of toxicity of the chemical, and will, most likely, be mouse, rat, guinea pig or minipig (see appendix 1 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. 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 chemical 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 (safety evaluation). Telemetric readouts can be applied, in addition to pharmacokinetic determination. (see appendix 2)
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 (see appendix 3).
4) Effective or partially effective regimens selected from phase 3 experiments will be evaluated for long term efficacy. This will provide insight into possible improvements of quality of life (see appendix 4)

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.

Research as described in appendices (1) and (2) can be performed in parallel, and are independent of each other. Experiments from appendix (3), efficacy testing, can only be performed if information from appendices (1) and (2) from own studies or other labs are available. Consequently, research as described in (4) will only be pursued if long term effects are expected from either chemical, treatment or a

combination thereof.

3.4.4 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	Models for exposure to highly toxic chemicals
2	Characterization of drug responses, including pharmacokinetics
3	Determination of treatment efficacy of drugs against the effects of exposure to highly toxic chemicals
4	Long term effects of exposure to highly toxic chemicals and treatment
5	
6	
7	
8	
9	
10	