National Toxicology Program
U.S. Department of Health and Human Services

DRAFT NTP MONOGRAPH ON

SYSTEMATIC REVIEW OF LONG-TERM NEUROLOGICAL EFFECTS FOLLOWING ACUTE EXPOSURE TO THE ORGANOPHOSPHORUS NERVE AGENT SARIN

Revised December 19, 2018

Office of Health Assessment and Translation
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TABLE OF CONTENTS

* Erratum: see page 44.

LIST OF TABLES AND FIGURES

TABLES:

FIGURES:

DATA TABLES:

DATA FIGURES:

ABSTRACT

Background: Sarin (CAS #: 107-44-8) is a highly toxic organophosphorus nerve agent that was developed for chemical warfare during World War II and continues to be used in conflicts. Immediate effects of sarin exposure are well known, and although there are suggestions in the literature of neurological effects persisting after the initial signs have subsided, long-term neurological effects of acute exposure to sarin are not well characterized in humans.

Objective: The National Toxicology Program (NTP), on behalf of the National Institutes of Health (NIH) Countermeasures Against Chemical Threats (CounterACT) program, conducted a systematic review to evaluate the evidence for long-term neurological effects in humans and animals following acute exposure to sarin.

Method: A systematic review protocol was developed and utilized for this evaluation that followed the Office of Health Assessment and Translation (OHAT) approach for conducting literature-based health assessments. Any effect observed 24 hours after exposure (including days to years after exposure) was considered *long-term* for this assessment. Since effects might vary based on time after exposure, the development of hazard conclusions was considered for three different time periods: initial (>24 hours–7 days after exposure), intermediate (8 days–1 year after exposure), and extended (more than 1 year after exposure) periods.

Results: The literature search and screening process identified 32 datasets within the 34 human studies and 47 datasets within the 51 animal studies (from 6,837 potentially relevant references) that met the objective and the inclusion criteria. Four main health-effect categories of neurological response were identified as having sufficient data to reach hazard conclusions: (1) cholinesterase levels; (2) visual and ocular effects; (3) learning, memory, and intelligence; and (4) morphology and histopathology in nervous system tissues.

Cholinesterase: Taken together, the human and animal bodies of evidence provide a consistent pattern of findings in the initial period after exposure that acute sarin exposure is associated with decreased cholinesterase levels. This is supported by similar findings in the intermediate period. There is a high level of evidence from the human studies that sarin decreased cholinesterase levels in the initial time period (primarily supported by two controlled exposure studies) and a moderate level of evidence for decreased cholinesterase from experimental animal studies. The evidence for cholinesterase effects in the intermediate period is more limited with an inadequate level of evidence from human case report studies, and a moderate level of evidence from experimental animal studies. The evidence for potential effects on cholinesterase in the extended period is inadequate with no experimental data and only a single study in humans.

Visual and ocular effects: The human body of evidence in the initial period provides a moderate level of evidence that acute sarin exposure constricts pupil diameter in humans and decreases the pupil: iris ratio from 24 hours through the first week following exposure. There is a consistent pattern of findings that this decrease gradually normalizes in the following week to several months. There is a moderate level of evidence from human studies that sarin has negative effects on vision in the intermediate time period including decreases in visual evoked potentials. There is inadequate evidence of decreased pupil size in animals in the initial and intermediate periods. In addition to changes in pupil diameter and response, case reports or case series have reported that subjects exposed to sarin occupationally or via terrorist attacks complained of vision problems for weeks to years after exposure. The evidence for

visual and ocular effects in the extended period is inadequate with one experimental animal study with very serious risk-of-bias concerns that did not observe an effect, one prospective study in humans, and four case reports with serious risk-of-bias concerns.

Learning, memory and intelligence: Taken together, the human and animal bodies of evidence provide a consistent pattern of findings across all three time periods that acute exposure to sarin is associated with effects on learning and memory. Conclusions that sarin affects learning and memory in the initial and intermediate periods are based on a moderate level of evidence from experimental animal studies during these periods. Experimental studies in rats found consistent sarin-related effects on learning and memory that were apparent for days, weeks, and months after sarin exposure. The evidence from human studies for effects on learning and memory during the initial period is inadequate with no studies identified, and there is a low level of evidence that sarin affects memory during the intermediate period. In the extended period, there is a moderate level of evidence that sarin exposure is associated with impaired learning and memory based on epidemiological studies and a low level of evidence from experimental animal studies.

Morphology and histopathology in nervous system tissue: Collectively, the human and animal bodies of evidence provide a consistent pattern of findings that acute exposure to high doses of sarin is associated with morphological and histological changes in nervous tissue across all three time periods. Conclusions for the initial and intermediate periods are based on a moderate level of evidence from experimental animal studies that sarin exposure affects nervous tissue within the first 7 days and through 90 days thereafter. The evidence from human studies for the initial and intermediate time periods is inadequate with only a single case report identified. Although there were no experimental animal studies available to evaluate morphological and histological changes at the extended time period after exposure, one cross-sectional study and one case report, which evaluated adults from the Tokyo subway attack, provide evidence that acute exposure to high levels of sarin is associated with morphological and histological changes in human nervous system tissues in the years following sarin exposure.

Other neurological effects are included in this review; however, the evidence was not considered for reaching conclusions due to having few studies on a given outcome, inconsistency in findings, heterogeneity of the data, and study limitations.

Conclusions: Hazard conclusions were considered for the four main health-effect categories at all three time-periods after exposure. The conclusions with the highest level of evidence for each time period were used to reach the overall conclusions. NTP concludes that acute sarin exposure is *known to be a neurological hazard to humans* in the initial time period of >24 hours–7 days after exposure based on suppression of cholinesterase. NTP concludes that acute sarin exposure is *suspected to be a neurological hazard to humans* in the intermediate time period of 8 days–1 year after exposure based on multiple effects, including suppression of cholinesterase, visual and ocular effects, effects on learning and memory, and morphological and histological changes in nervous system tissues. NTP concludes that acute sarin exposure is *suspected to be a neurological hazard to humans* in the extended time period of greater than 1 year after exposure based on multiple effects, including effects on learning and memory and morphological and histopathological changes in nervous system tissues.

This evaluation identified data gaps that contribute to lower confidence in the bodies of evidence for some endpoints and time periods after exposure. Future targeted research to assess the long-term neurological effects of sarin exposure should address areas with low confidence in the findings. Future research would benefit from the use of well-characterized human exposure data, use of exposed and appropriately matched control populations for neurological tests, and animal models that address the

inconsistencies identified in this review using study design, conduct, and reporting practices to minimize bias. Based on the hazard conclusions from this review, additional research on the 4 main health effect categories above may impact the confidence in the conclusions. Research may also be informative on a diverse range of neurological endpoints identified in the report appendices where there is inadequate evidence to determine whether there is an association with acute sarin exposure.

INTRODUCTION

Sarin is a nerve agent developed for chemical warfare during World War II. This highly toxic nerve agent is liquid at ambient temperatures. It is also known as GB, which is a two-character identifier assigned by NATO. Sarin belongs to a chemically diverse group of OP chemicals that have at least one carbon atom bound to a phosphorous atom. The group includes other chemical weapons and many agricultural and residential pesticides. It is likely that sarin continues to be used in conflict, as reported by the United Nations in Syria in 2013 (Sellström *et al.* 2013).

Due to the nature of exposure to sarin (i.e., rare events that often occur as a result of occupational accidents or terror attacks), the available studies are primarily case reports, case series, or cross-sectional studies and also include two controlled trials. The majority of human data come from individuals studied following two terrorist attacks in Japan. One occurred in the Tokyo subway system in 1995 (Okumura *et al.* 1996), and the other attack occurred in Matsumoto city in 1994 in a residential area near the center of the city (Morita *et al.* 1995).

The persistence, or time period of effects following acute exposure to sarin, is a key factor in this review. Acute effects of sarin immediately after exposure are well characterized and are not the focus of the review. The median lethal dose (LD_{50}) of dermal exposure to sarin for a 70 kg person is only 1 - 10 mL (ATSDR 2011). Acute effects include a progression from miosis (constriction of the pupils), excessive secretions and muscle fasciculation to seizures that may progress to status epilepticus, muscle paralysis, cardiorespiratory depression, and death due to respiratory failure. The signs and symptoms of acute exposure are generally referred to as cholinergic signs. From experimental studies of humans and animals where dose is known, cholinergic signs are generally characterized as “mild, moderate, or severe.” When dose is unknown, a reasonable approximation of dose may be estimated by observing clinical signs and symptoms. This is an established approach in which a threshold detection level may be translated into estimates of high-, intermediate- and low-level exposures with an accepted degree of consistency (Brown and Brix 1998). The assessment of quantitative exposure levels at which long-term neurological effects occur is beyond the scope of this review.

The focus of this review is on neurological effects that are considered “long term” or observed at any time after cholinergic signs have subsided. Such long-term neurological health effects[1] may be observed several hours, days, weeks or years after the cholinergic crisis subsides. Long-term effects may be pathophysiological and/or behavioral. Therefore, in this evaluation, “long term” is considered any effect

[1] Throughout this document, “Long-term” neurological effect is defined as any neuropathological, pathophysiological, or behavioral effect observed that occurs at least 24 hours after the acute sarin exposure. Therefore, long-term neurological effects, as defined in this document, may occur immediately after the cholinergic signs and symptoms caused by an initial sublethal acute exposure have subsided, or they may overlap with the initial cholinergic crisis. Long-term neurological effects may also be observed over a range of time periods including days, weeks, months or years after the cholinergic symptoms subside. Long-term neurological effects may resolve or may persist.

occurring more than 24 hours after exposure. The 24-hour time point was selected to reflect the possible variation in time for cholinergic signs to subside due to differing exposure levels and individual responses. For the purpose of characterizing outcomes, the time after exposure was broken down into three time periods to better capture effects related to sarin exposure in the days (initial time period, potentially including cholinergic effects), weeks (intermediate time period, not anticipated to include cholinergic effects), and years (extended time period) after exposure. This will help determine if the long-term effects resolve or persist.

Several literature reviews of the long-term neurological effects following exposure to sarin have been published (SIPRI 1975, Defense Science Board 1994, Brown and Brix 1998, Augerson 2000, Binns *et al.* 2004, IOM 2004, Brown 2009, White *et al.* 2016). However, many of these reviews have been an assessment of health effects in military personnel during conflicts such as the Gulf War, and are confounded by concurrent mixed exposures to other chemicals including other chemical warfare agents. This systematic review was developed to focus on a specific data set where sarin nerve agent is the only suspected exposure. Additionally, a systematic review of the evidence has not been performed in which selection criteria were clearly stated and consistently applied; where a broad hierarchy of evidence is considered including all evidence streams (human, animal and mechanistic); where a broad range of human study designs are considered including uncontrolled studies and case-reports or case series; and in which individual studies were assessed for internal validity or risk of bias. Given that the majority of the evidence for potential long-term health effects of sarin addresses neurological endpoints, this review focused on neurological outcomes.

In partnership with NTP, the NIH Countermeasures Against Chemical Threats (CounterACT) program conducted a systematic review to evaluate the evidence for long-term neurological effects in humans and animals following acute exposure[2] to the organophosphorus (OP) nerve agent sarin (CAS #: 107-44-8). This review was initiated because of suggestions in the literature of long-term neurological effects of sarin poisoning in humans [e.g., case reports of victims in the Matsumoto and Tokyo subway attacks suffering long-term behavioral abnormalities and alteration of brain morphology (Murata *et al.* 1997, Yamasue *et al.* 2007)], as well as animal studies reporting long-term neurological effects of sarin [e.g., experimental animal studies of neurotoxicity (Burchfiel and Duffy 1982) and behavioral and neurophysiological functions Kassa *et al.* (2001c)].

OBJECTIVE AND SPECIFIC AIMS

Objective

The overall objective of this evaluation is to undertake a systematic review to understand the long-term neurological effects following acute, sublethal exposure to sarin based on integrating levels of evidence from human and animal studies and consideration of the degree of support from mechanistic data.

Specific Aims

- Identify literature that assessed long-term neurological health effects following acute exposure to sarin in human, animal, and *in vitro*/mechanistic studies.

[2] Throughout this document, an “acute exposure” is defined as exposure to sarin occurring in a period <24 hours that causes cholinergic signs and symptoms.

- Extract data on potential long-term (as described above) neurological health effects from relevant studies.
- Assess the internal validity (risk of bias) of individual studies using pre-defined criteria.
- Summarize the extent and types of health effects evidence available.
- Describe limitations of the systematic review, limitations of the evidence base, identify areas of uncertainty, as well as data gaps and research needs on long-term neurological health effects of sarin.

Dependent on the extent and nature of the available evidence:

- Synthesize the evidence using a narrative approach or meta-analysis (if appropriate) considering limitations on data integrating such as study design heterogeneity.
- Rate confidence in the body of evidence for human and animal studies separately according to one of four statements: High, Moderate, Low, or Very Low/No Evidence Available.
- Translate confidence ratings into level of evidence of health effects for human and animal studies separately according to one of four statements: High, Moderate, Low, or Inadequate.
- Combine the level of evidence ratings for human and animal data to reach one of five possible hazard identification conclusions: Known, Presumed, Suspected, Not classifiable, or Not identified to be a hazard to humans.

METHODS

Problem Formulation and Protocol Development

The research question and specific aims stated above were developed and refined through a series of problem formulation steps including: (1) consideration of reports in the literature as to whether long-term neurological effects of OP agents in humans are a common occurrence; (2) discussion of these reports and related questions on long-term neurological health effects of OP agents at the NIH CounterACT Workshop in February 2014; and (3) development of the Systematic Review Subcommittee (chaired by David A. Jett, Ph.D.; Pamela J. Lein Ph.D., and Mark Kirk M.D.). The Systematic Review Subcommittee was convened in June 2015 and began to refine the research question, specific aims, and develop a draft protocol for conducting the systematic review. The focus of the current project on sarin, rather than all OP agents, was selected to aid in reaching conclusions (i.e., equivalent exposure to the same agent could be more directly compared than exposure across multiple agents). Similarly, the focus on neurological health effects was selected as an aid to reaching conclusions and because the majority of data on potential health effects of sarin are for neurological effects. The protocol was peer reviewed and finalized in April of 2017 and used to conduct this review (https://ntp.niehs.nih.gov/go/sarin). A brief summary of the methods is presented below.

PECO Statements

PECO (**P**opulation, **E**xposure, **C**omparators and **O**utcomes) statements were developed as an aid to identify search terms and inclusion/exclusion criteria as appropriate for addressing the overall research question (long-term neurological effects of acute sarin exposure) for the systematic review (Higgins and Green 2011).The PECO statements are listed below for human (**Table 1**), animal (**Table 2**), and *in vitro*/mechanistic studies (**Table 3**).

Using the PECO statements, the evaluation searched for evidence of long-term neurological effects associated with acute sarin exposure from human studies across a broad range of study design types along with controlled exposure animal studies and mechanistic/*in vitro* studies. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes *in vitro* and *in vivo* laboratory studies directed at cellular, biochemical, and molecular mechanisms that explain how a chemical produces particular adverse health effects.

Table 1. Human PECO (Population, Exposure, Comparator and Outcome) Statement	
PECO Element	**Evidence**
Population	Humans without restriction as to age or sex, geographic location, or life stage at exposure or outcome assessment; and no restriction as to whether military or civilian/non-combatant
Exposure	Single acute exposure to sarin based on: • known dose or concentration in an experimental protocol • diagnostic biomonitoring data (e.g., sarin or biomarkers in plasma or urine) • environmental detection (e.g., air, soil) • corroboration by assessment of direct (in hospital, in clinic) or indirect observation of symptoms of acute cholinergic signs (video or reported by patient's family) • dose may be extrapolated from clinical signs and symptoms per Brown and Brix (1998) and as adapted from Namba *et al.* (1971) No restriction on whether exposure is accidental or intentional
Comparators	For controlled and uncontrolled studies, comparable populations not exposed to sarin; and for case series-reports, no comparable populations
Outcomes	Neurological outcomes including changes in nervous system function (e.g., cognitive, sensory, motor), and neuropathology (e.g., imaging and post-mortem)

Table 2. Animal PECO (Population, Exposure, Comparator and Outcome) Statement	
PECO Element	**Evidence**
Population	Without restriction as to species, age, or sex, or life stage at exposure or outcome assessment
Exposure	Single acute exposure to sarin based on administered dose or concentration or biomonitoring data (e.g., urine, blood, or other specimens)
Comparators	Comparable untreated animal subjects or animals exposed to vehicle-only treatment
Outcomes	Neurological outcomes including changes in nervous system function (e.g., cognitive, sensory, motor), neuropathology (e.g., imaging and post-mortem), and neurophysiology (e.g., ion channel and receptor function)

Table 3. *In Vitro*/Mechanistic PECO (Population, Exposure, Comparator and Outcome) Statement	
PECO Element	**Evidence**
Population	Human or animal cells, tissues or model systems with *in vitro* exposure regimens
Exposure	Exposure to sarin based on administered dose or concentration
Comparators	Comparable cells or tissues exposed to vehicle-only treatment or untreated controls
Outcomes	Measurements of the survival and the morphology of neurons or glia, including histochemical and immunohistochemical techniques such as H&E, Nissl, Rapid Golgi, Fluoro-Jade, Silver Stain, HRP, GFAP, neurotransmitter stains, axon/dendrite-specific markers, and others

Literature Search

Search terms were developed to identify all relevant published evidence that addresses the research question on long-term neurological health effects potentially associated with acute, sublethal exposure to sarin in humans and animals by (1) using the search term "sarin" and related synonyms "GB" or sarin's IUPAC ID "(RS)-propan-2-yl methylphosphonofluoridate", (2) without restriction by health outcome or key words to identify long-term neurological effects. A test set of relevant studies was used to ensure that the search terms retrieved 100% of the test set. The following eight electronic databases were searched using a search strategy tailored for each database by an informationist on the evaluation team (specific search terms used for the PubMed search are presented in **Appendix 1**); the search strategy for other databases are available in the protocol (https://ntp.niehs.nih.gov/go/sarin). No language restrictions or publication year limits were imposed and the databases were searched in April 2016, January 2017, and April 2018 with a final updated search on October 25, 2018.

Databases Searched

- Cochrane Library
- DTIC
- EMBASE
- NIOSHTIC
- PubMed
- Scopus
- Toxline
- Web of Science

Searching Other Resources

The reference lists of all included studies, relevant reviews or reports, commentaries or letters on specific studies, and other non-research articles were manually searched for additional relevant publications.

Given that incidents of human exposure to sarin includes terrorist attacks and military personnel, the search was conducted to identify the anticipated range of evidence for human studies; original papers may include non-peer-reviewed studies, for example, reports from US military observational studies, as well as uncontrolled studies, case series, or case reports. In all instances, the paper must: (1) document

exposure to sarin; (2) confirm both acute symptoms, *i.e.*, cholinergic crisis; and (3) assess and report some long-term neurological health effects from the exposure.

Unpublished Data

Unpublished data were eligible for inclusion provided the owner of the data was willing to have the data made public and peer reviewed (see protocol for more details https://ntp.niehs.nih.gov/go/sarin).

Study Selection

Evidence Selection Criteria

In order to be eligible for inclusion, studies had to comply with the type of evidence specified by the PECO statements (**Table 1**, **Table 2**, and **Table 3**). The following additional exclusion criteria were applied:

(1) Human or animal studies with an exposure duration greater than or equal to 24 hours, except repeat dose studies where the outcome is first measured at least 24 hours after the first dose but before any subsequent exposure after 24 hours;

(2) Human controlled studies where the purpose was only to apply treatment for acute sarin effects;

(3) Human or animal studies with acute exposures to several different chemicals;

(4) Animal treatment/recovery studies that administer sarin and a treatment, unless there is a sarin-only control group;

(5) Human studies with no assessment of health effect outcomes after cholinergic crisis has subsided;

(6) Animal studies with neurological effects only measured within 24 hours after exposure

(7) Articles without original data (e.g., editorials or reviews); and

(8) Studies published in abstract form only (grant awards and conference abstracts).

Screening Process

References retrieved from the literature search were screened for relevance and eligibility using DistillerSR® by Evidence Partners, a web-based, systematic-review software program with structured forms and procedures to ensure standardization of the process. Search results were first consolidated in Endnote reference management software and duplicate articles were removed prior to uploading the references into DistillerSR®. Screeners from the evaluation team were trained with an initial pilot phase to improve clarity of the evidence selection criteria and to improve accuracy and consistency among screeners. All references were independently screened by two trained screeners at the title and abstract level to determine whether a reference met the evidence selection criteria. Studies that were not excluded by reviewing the title and abstract were screened with a full-text review. Screening conflicts were resolved through discussion. Following full-text review, the remaining studies were “included” and used for the evaluation.

Data Extraction

Extraction Process and Data Warehousing

Data were collected (i.e., extracted) from included studies by one member of the evaluation team and checked by a second member for completeness and accuracy. Any discrepancies in data extraction were resolved by discussion or consultation with a third member of the evaluation team. Information that was inferred, converted, or estimated during data extraction is annotated, e.g., using brackets [n = 10]. Data were extracted as presented in the publications, including reported levels of statistical significance. NTP did not conduct independent statistical analyses to confirm levels of statistical significance reported in the publications nor did they determine statistical significance when study authors did not conduct statistical analyses.

Data extraction was completed using the Health Assessment Workspace Collaborative (HAWC), an open source and freely available web-based interface application.[3] Data extraction elements are listed separately for human, animal, and *in vitro* studies in the protocol (https://ntp.niehs.nih.gov/go/sarin). The data extraction results for included studies will be publicly available and will be able to be downloaded in Excel format through HAWC.

Quality Assessment of Individual Studies

Risk of bias was assessed for individual studies using a tool developed by OHAT that outlines a parallel approach to evaluating risk of bias from human, animal, and mechanistic studies to facilitate consideration of risk of bias across evidence streams with common terms and categories. The risk-of-bias tool is comprised of a common set of 11 questions that are answered based on the specific details of individual studies to develop risk-of-bias ratings for each question. Study design determines the subset of questions used to assess risk of bias for an individual study (**Table 4**).

Assessors were trained with an initial pilot phase undertaken to improve clarity of rating criteria and to improve consistency among assessors. Studies were independently evaluated by two trained assessors who answered all applicable risk-of-bias questions with one of four options in **Table 5** following pre-specified criteria detailed in the protocol (https://ntp.niehs.nih.gov/go/sarin). The criteria describe aspects of study design, conduct, and reporting required to reach risk-of-bias ratings for each question and specify factors that can distinguish among ratings (e.g., what separates “definitely low” from “probably low” risk of bias).

Key Risk-of-Bias Questions

In the OHAT approach, some risk-of-bias questions or elements are considered potentially more important when assessing studies because there is more empirical evidence that these areas of bias have a greater impact on estimates of the effect size or because these issues are generally considered to have a greater effect on the credibility of study results in environmental health studies (Rooney *et al.* 2016). There were three Key Questions for observational human studies: confounding, exposure characterization, and outcome assessment. There were also three Key Questions for experimental animal studies: randomization, exposure characterization, and outcome assessment. When there was insufficient information to assess the potential bias for a risk-of-bias question and authors did not

[3] HAWC (Health Assessment Workspace Collaborative): A Modular Web-based Interface to Facilitate Development of Human Health Assessments of Chemicals (https://hawcproject.org/portal/).

respond to an inquiry for further information, a conservative approach was followed, and the studies were rated as probably high risk of bias for that question.

Table 4. OHAT Risk-of-bias Questions and Applicability by Study Design

	Experimental Animal*	Human Controlled Trials**	Cohort	Case-Control	Cross-Sectional***	Case Series
1. Was administered dose or exposure level adequately randomized?	X	X				
2. Was allocation to study groups adequately concealed?	X	X				
3. Did selection of study participants result in the appropriate comparison groups?			X	X	X	
4. Did study design or analysis account for important confounding and modifying variables?			X	X	X	X
5. Were experimental conditions identical across study groups?	X					
6. Were research personnel blinded to the study group during the study?	X	X				
7. Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	X
8. Can we be confident in the exposure characterization?	X	X	X	X	X	X
9. Can we be confident in the outcome assessment (including blinding of outcome assessors)?	X	X	X	X	X	X
10. Were all measured outcomes reported?	X	X	X	X	X	X
11. Were there no other potential threats to internal validity?	X	X	X	X	X	X

*Experimental animal studies are controlled exposure studies. Non-human animal observational studies can be evaluated using the design features of observational human studies such as cross-sectional study design.

**Human Controlled Trials are studies in humans with controlled exposure (e.g., Randomized Controlled Trials, non-randomized experimental studies)

***Cross-sectional studies include population surveys with individual data (e.g., NHANES) and surveys with aggregate data (i.e., ecological studies).

Any discrepancies in ratings between assessors were resolved through discussion to reach the final recorded risk-of-bias rating for each question along with a statement of the basis for that rating. Members of the evaluation team were consulted for assistance if additional expertise was necessary to reach final risk-of-bias ratings based on specific aspects of study design or performance reported for individual studies. Information or study procedures that were not reported is assumed not to have been conducted, resulting in an assessment of "probably high" risk of bias. Although the protocol defines a purity of 95% with independent confirmation to be necessary for a rating of probably low risk of bias, Munroe *et al.* (1999) indicates that sarin must be at least 93% pure; therefore, 93% without independent confirmation was considered probably low risk of bias unless there were other reasons (e.g., inhalation study without chamber concentrations) for the exposure characterization to be considered probably high risk of bias.

Table 5. The Four Risk-of-bias Rating Options	
Answers to the risk-of-bias questions result in one of the following four risk-of-bias ratings	
++	***Definitely Low*** *risk of bias*: There is direct evidence of low risk-of-bias practices
+	***Probably Low*** *risk of bias*: There is indirect evidence of low risk-of-bias practices **OR** it is deemed that deviations from low risk-of-bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
– NR	***Probably High*** *risk of bias*: There is indirect evidence of high risk-of-bias practices (indicated with "-") **OR** there is insufficient information provided about relevant risk-of-bias practices (indicated with "NR" for not reported). Both symbols indicate probably high risk of bias.
––	***Definitely High*** *risk of bias*: There is direct evidence of high risk-of-bias practices

Organizing and Rating Confidence in Bodies of Evidence

Health Outcome and Endpoint Grouping by Four Main Categories of Neurological Effects

The main category for long-term neurological health outcomes includes all neurological effects. After data were extracted for all studies, the health effects results were grouped across studies to develop bodies of evidence or collections of studies with data on the same or related outcomes for the 4 main categories of neurological effects: 1) cholinesterase, 2) visual and ocular, 3) learning, memory, and intelligence, and 4) morphology and histopathology. Technical advisors and subject matter experts were consulted as needed to determine: 1) endpoints that can be grouped as similar or related endpoints, and 2) if downgrades are warranted based on the reliability or quality of specific endpoints or groups of endpoints for determining neurological effects. The remaining neurological endpoints will be discussed briefly.

Considerations for Pursuing a Narrative or Quantitative Evidence Synthesis

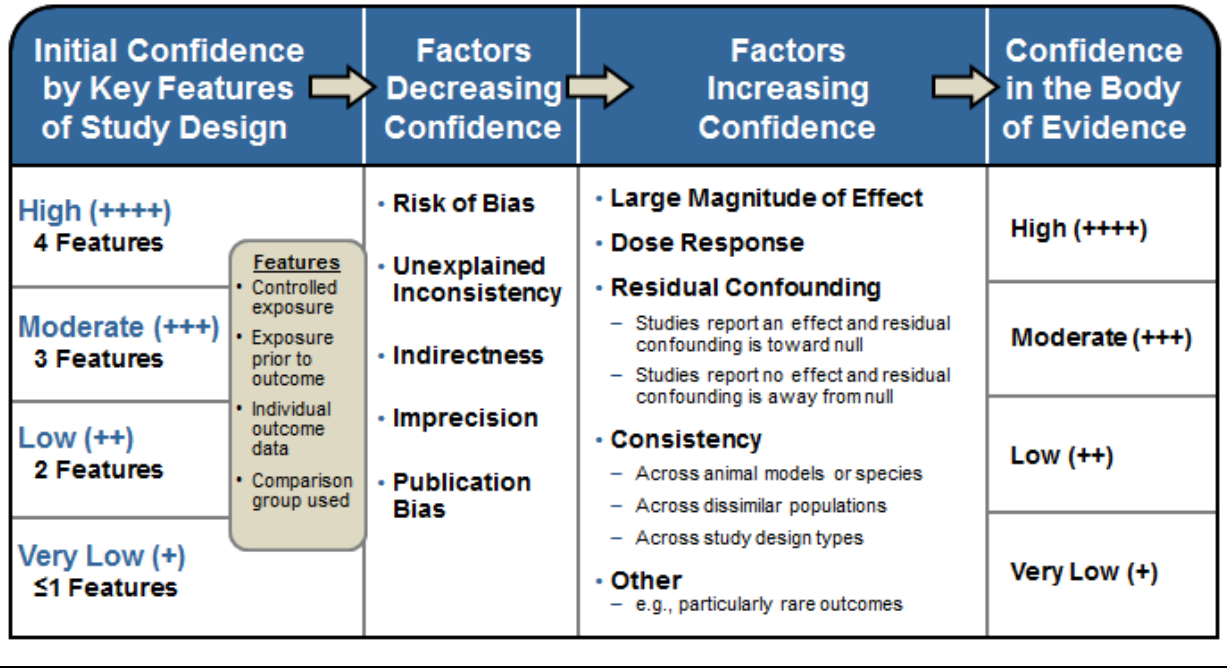
Heterogeneity within the available evidence was used to determine the type of evidence integration that was appropriate: either a quantitative synthesis (meta-analysis) or narrative approach for evidence integration. Heterogeneity within the available human and animal evidence was so high that only a narrative, not a quantitative or meta-analysis, was appropriate for evidence integration. Meta-analysis approaches are considered most suitable if there are at least six to ten studies for a continuous variable

and at least four studies for a categorical variable (Fu *et al.* 2011). ChE effects had the most data available with two controlled trials, one cross-sectional study, and six case reports/series, as well as fourteen non-human animal studies; however, the data are not amenable for a meta-analysis because they were not collected during the same time frame, or in the case of the animals from the same biological metric (i.e., blood and different areas of the brain). While studies on memory and visual and ocular effects were also available, the specific tests were diverse. Therefore, the data do not lend itself to conducting a meta-analysis as there were generally only three to five studies available for any specific endpoint for these continuous variables.

Confidence Rating: Assessment of Body of Evidence

The quality of evidence within groups of neurological effects was evaluated using the GRADE system for rating the confidence in the body of evidence (Guyatt *et al.* 2011, Rooney *et al.* 2014). More detailed guidance on reaching confidence ratings in the body of evidence as "high," "moderate," "low," or "very low" is provided in the OHAT Handbook for Conducting a Literature-Based Health Assessment (http://ntp.niehs.nih.gov/go/38673, see STEP 5). In brief, available human and animal studies on a particular health outcome were initially grouped by key study design features, and each grouping of studies was given an initial confidence rating by those features. Starting at this initial rating (column 1 of **Figure 1**), potential downgrading of the confidence rating was considered for factors that decrease confidence in the results (column 2 of **Figure 1** [risk of bias, unexplained inconsistency, indirectness or lack of applicability, imprecision, and publication bias]); and potential upgrading of the confidence rating was considered for factors that increase confidence in the results (column 3 of **Figure 1** [large magnitude of effect, dose response, consistency across study designs/populations/animal models or species, consideration of residual confounding, and other factors that increase our confidence in the association or effect]). Consideration of consistency across study designs, human populations, or animal species is not included in the GRADE guidance (Guyatt *et al.* 2011); however, it is considered in the modified version of GRADE used by OHAT (Rooney *et al.* 2014, NTP 2015).

Figure 1. Assessing Confidence in the Body of Evidence

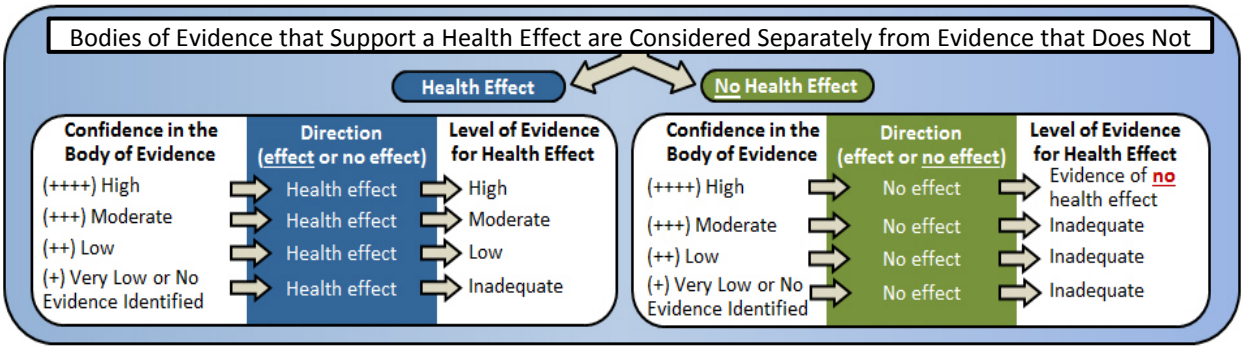


Confidence ratings were independently assessed by the evaluation team, CounterACT personnel, and the analyst-contractors for accuracy and consistency, and discrepancies were resolved by consensus and consultation with technical advisors as needed. Confidence ratings for the primary outcomes are summarized in evidence profile tables for each outcome.

Preparation of Level of Evidence Conclusions

The confidence ratings were translated into level of evidence of health effects for each type of health outcome separately according to one of four statements: (1) High, (2) Moderate, (3) Low, or (4) Inadequate (**Figure 2**). The descriptor "evidence of no health effect" is used to indicate confidence that the substance is not associated with a health effect. Because of the inherent difficulty in proving a negative, the conclusion "evidence of no health effect" is only reached when there is high confidence in the body of evidence.

Figure 2. Translate Confidence Ratings into Evidence of Health Effect Conclusions



Evidence Descriptors	Definition
High Level of Evidence	There is high confidence in the body of evidence for an association between exposure to sarin and the health outcome(s).
Moderate Level of Evidence	There is moderate confidence in the body of evidence for an association between exposure to sarin and the health outcome(s).
Low Level of Evidence	There is low confidence in the body of evidence for an association between exposure to sarin and the health outcome(s), or no data are available.
Inadequate Evidence	There is insufficient evidence available to assess if exposure to sarin is associated with the health outcome(s).
Evidence of No Health Effect	There is high confidence in the body of evidence that exposure to sarin is not associated with the health outcome(s).

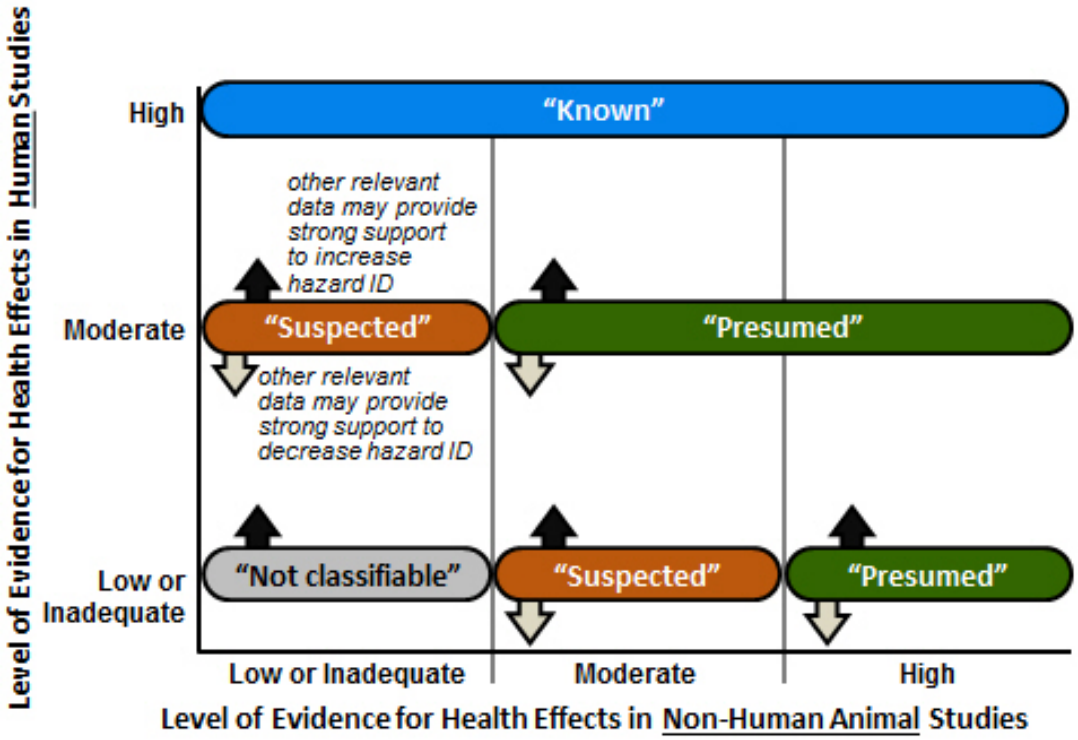
Integrate Evidence to Develop Hazard Identification Conclusions

Finally, the levels of evidence ratings for human and animal data were integrated with consideration of *in vitro*/mechanistic data to reach one of five possible categories of evidence of long-term neurological health effect: (1) Known, (2) Presumed, (3) Suspected, (4) Not classifiable, or (5) Not identified to be a long-term neurological effect in humans (**Figure 3**).

Consideration of Human and Animal Data

Initial hazard identification conclusions were attempted by integrating the highest level-of-evidence conclusion for long-term neurological health effect(s) on an outcome basis for the human and the animal evidence streams. The level of evidence conclusion for human data from Step 6 of the OHAT Handbook for that health outcome was considered together with the level of evidence for animal data to reach one of four initial hazard identification conclusions as to the evidence of long-term neurological effects in humans: Known, Presumed, Suspected, or Not classifiable. When either the human or animal evidence stream was characterized as "Inadequate Evidence" for a particular health effect, then conclusions were based on the remaining evidence stream alone (which is equivalent to treating the missing evidence stream as "Low" in **Figure 3**).

Figure 3. Hazard Identification Scheme for Long-term Neurological Effects



Consideration of Mechanistic Data

There is no requirement to consider mechanistic or mode-of-action data to reach a hazard identification conclusion regarding long-term neurological health effects. However, when available, this and other relevant supporting types of evidence may be used to raise (or lower) the category of the hazard identification conclusion. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes *in vitro* and *in vivo* laboratory studies directed at cellular, biochemical, genetic, and molecular mechanisms that explain how a chemical produces particular adverse effects.

For the evaluation of long-term neurological health effects associated with acute exposure to sarin, NTP was interested in mechanistic or *in vitro* measures that may support the biological plausibility of corresponding neurological outcomes reported from *in vivo* studies in animals or humans. The PECO

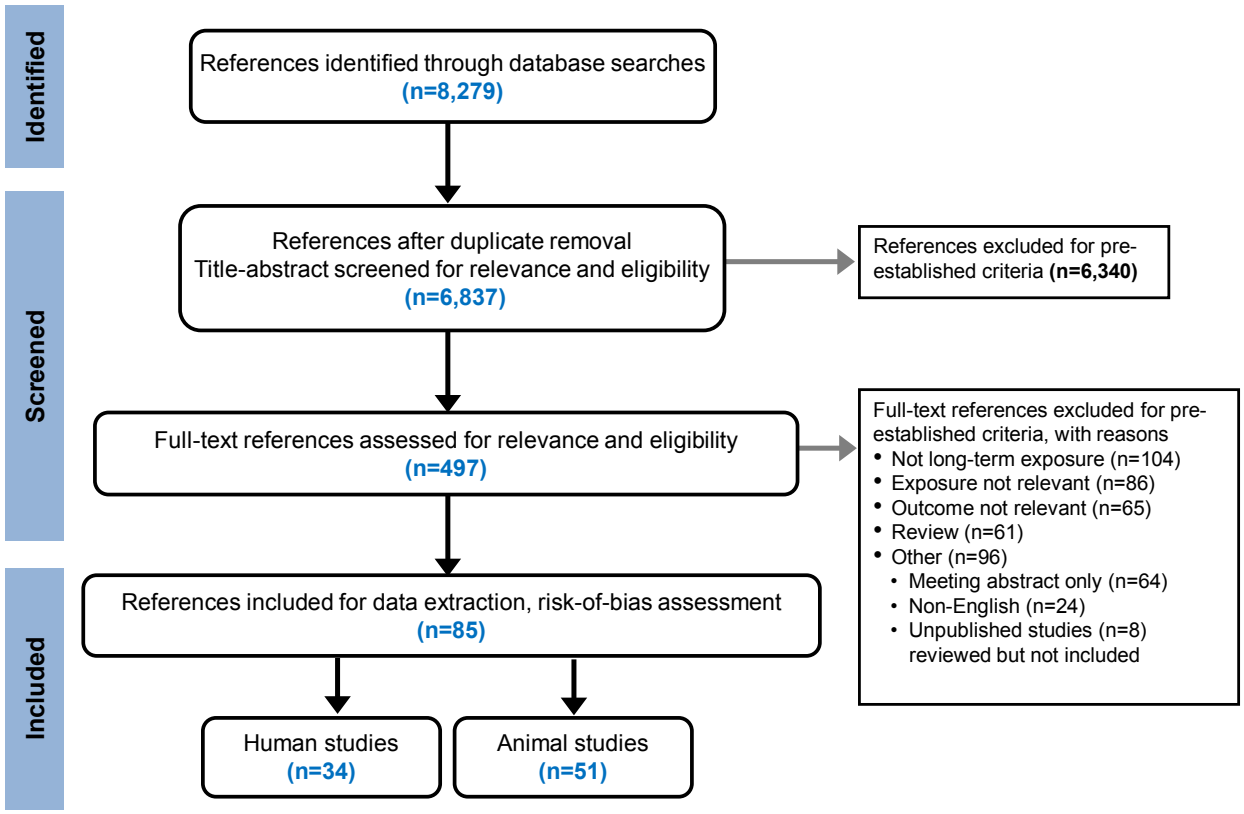
statement in **Table 3** provides the specific endpoints considered, mainly including survival and morphology of neurons or glia. For this assessment, no *in vitro* studies following these criteria were identified for any time period following exposure (including less than 24 hours).

RESULTS AND EVIDENCE SYNTHESIS

Literature Search Results

The electronic database searches retrieved 6,837 references. Ninety-three percent of the total references retrieved (6,340) were excluded during the title and abstract screening and 412 references were excluded during the full text review for not satisfying the PECO criteria. The screening results are outlined in the study selection diagram with reasons for exclusion documented at the full text review stage (**Figure 4**) [using reporting practices outlined in Moher *et al.* (2009)]. After full text review, 85 studies were considered relevant, which included 34 human studies and 51 animal studies. However, 2 of the human publications and 4 of the animal publications included data published in another study, so there were 32 human datasets within the 34 human studies and 47 animal datasets within the 51 animal studies. When multiple publications presented the same data, a single study was selected for extraction (e.g., the first or most complete reporting) and all of the studies were included in the HAWC project database for this sarin evaluation (https://hawcproject.org/assessment/302/) and were reviewed to answer risk-of-bias questions regarding the datasets that were extracted. Eight studies were identified that included unpublished data. The unpublished data were reviewed, but determined not to have any data that would change the hazard conclusions because the data were either subsequently published, were not published by authors who had published several other studies on the topics, the data did not add any useful evidence to the sections, or the data only added to the heterogeneity of the data; therefore, it was not extracted or included in the assessment. The list of included references are provided in **Appendix 2**.

Figure 4. Study Selection Diagram



Health Effects Results

The human and animal neurological data across all studies were sorted into four main health effect categories of neurological response: (1) cholinesterase (ChE) levels; (2) visual and ocular effects; (3) learning, memory, and intelligence; and (4) morphology and histopathology in nervous system tissues. As the objective of this assessment was to focus on long-term neurological effects following acute sarin exposure, data pertaining to all non-neurological health outcomes associated with sarin exposure were not categorized and synthesized in this report. Results were grouped across studies within each category to develop bodies of evidence or collections of studies with data on the same or related outcomes. Human and animal studies were identified on potential effects of sarin in all four neurological categories. Specific endpoints were comparable for some effects (e.g., memory), but not all cases (e.g., P100 latency to measure visual evoked potential, and self-reported difficulty seeing). The human evidence described a range of neurological symptoms. To the extent possible, neurological symptoms in humans were related to neurological observations in animals, although for many human symptoms there are no data on a similar endpoint in experimental animals. These specific symptom data are provided in **Table A5-1**. In some cases, the neurological effects observed in humans did not fit in any larger category and preclude any synthesis of the data. For the animal data, there was a set of data with no human equivalent. Brain chemical changes in animals are presented in **Table A5-8**, but these data will not be discussed in detail because the heterogeneity of the data precludes an informative synthesis of the data.

There were many additional neurological health outcomes reported where there is inadequate evidence to determine whether there is an association with acute sarin exposure. These outcomes included sensory effects other than visual, sleep disruption, anxiety and fear, avoidance and depression, activity

and strength, other neurological symptoms, and EEG data. The body of evidence was inadequate to evaluate potential effects for these health outcomes because there was heterogeneity in the endpoints examined, too few human or animal studies to make any conclusions due to inconsistencies, small sample sizes (e.g., a single case report), and/or there were serious risk-of-bias concerns that contributed to very low confidence in the data. The results, evidence synthesis, and risk-of-bias assessment for the health outcomes with inadequate evidence are in **Appendix 4**.

It should be noted that the majority of the human data come from individuals studied following two terrorist attacks in Japan. One occurred in the Tokyo subway system in 1995 where sarin was released in five subway cars on three separate subway lines during the morning rush hour. Eleven of the commuters died and there were more than 5,000 subjects that required emergency medical evaluation (Okumura *et al.* 1996). Many of the publications on this attack were based on the initial 640 patients admitted to St. Luke's International Hospital. This was the closest hospital to one of the subway stations hit and treated the largest patient population exposed to sarin (Ohbu *et al.* 1997). Of these 640 subjects, 111 were admitted to the hospital and 528 were discharged and considered to be mild cases. The other attack occurred in Matsumoto city in 1994 in a residential area near the center of the city (Morita *et al.* 1995). Sarin was later detected in a pond in the city center. It was estimated that about 600 residents were exposed based on a survey of the residents in the area 3 weeks after exposure with an 84.9% response rate (Nakajima *et al.* 1998, Nakajima *et al.* 1999). Fifty-eight people were reportedly admitted to hospitals, and seven deaths occurred (Morita *et al.* 1995).

Due to the nature of exposure to sarin (i.e., rare events that often occur as a result of occupational accidents or terror attacks), the majority of available studies are case reports, case series, or cross-sectional studies. These types of study designs are generally considered to be of lesser quality than cohort studies due to a lack of control group or baseline data and an inability to demonstrate that exposure occurred prior to the development of the health outcome; however, data from these study types are still considered highly useful for assessing long-term neurological effects following acute sarin exposure. With regard to the uncertainty related to temporality of exposure and outcome, sarin exposures that occurred during terror attacks occurred during a known time period prior to the assessment of the outcome, and it is unlikely that sarin exposure would occur in control populations in cross-sectional studies. The case reports and case series may not have included controls for comparison, but are representative of larger exposed populations, and the results can generally be compared to some standard normal value. While this may not be ideal when the standard normal encompasses a range, which would make it difficult to determine individual-level effects, some determination can be made based on these standard values as would typically be done by doctors in a clinical setting. Uniquely, a major advantage of the human data for sarin is the availability of two controlled trials in addition to the cross-sectional studies and case reports/series, considering the high toxicity of sarin. The inclusion of these two controlled trials in this assessment provides strong evidence of an effect in the initial time period following exposure. These control trials provide valuable insight that is often not available in most assessments.

Results Organization

Each section is organized to present and explain the rating of confidence in the body of evidence that sarin exposure is associated with the effect described in that section for human and animal data separately. The confidence in the data was determined as described in **Figure 1**. Human data are discussed prior to animal data. Sections with sufficient data were organized so that the first paragraph discusses the confidence in the data, the second paragraph provides a brief overview of the available

studies, followed by an overview of overall risk-of-bias concerns and summarizes of the data organized by time frames when appropriate.

Although this systematic review collected and considered mechanistic data, limited information was identified to support the biological plausibility of corresponding neurological outcomes. The mechanisms that explain how chemicals produce many neurological effects are unknown. Although cholinesterase inhibition is evaluated in this review as a neurological effect from acute sarin exposure, cholinesterase can potentially be a mechanism for the other neurological effects evaluated. However, there are insufficient data to determine if the cholinesterase effects are responsible for the other neurological effects (e.g., secondary neuronal damage occurring in cholinergic regions of the brain due to prolonged over activation of the cholinergic receptors) or if other non-cholinergic mechanisms are involved (Pope 2006).

Organization by Time after Exposure

While any effect observed 24 hours after exposure is considered long-term for this assessment (see **Introduction** for description), review of the data determined that this covered several different time periods after exposure over which results may vary; therefore, the evaluation of hazard conclusions was evaluated for three different time periods (see **Table 6**). Effects observed after 24 hours through 7 days are considered effects over the initial time period after exposure. Due to variation in the duration of cholinergic effects, this time range was selected as the initial time period. The initial time period likely includes cholinergic effects as well as side effects related to cholinergic hyperstimulation. The intermediate period after exposure is considered 8 days to 1 year after exposure in humans and non-human primates. It is expected that cholinergic effects would have entirely subsided either prior to or during the initial time period after exposure and would not be included in the intermediate time period. Because rodents have a different life span, the intermediate period was considered to be 8 days to 90 days. The 90-day time point was selected based on standard subchronic study dosing guidelines due to the lack of any common comparison for time after exposure guidelines. The extended period after exposure is considered any time greater than 1 year in humans and non-human primates and greater than 90 days in rodents.

Table 6. Definition of Time Periods After Exposure

Time period after exposure	Humans and non-human primates	Animals except non-human primates
Initial time period	>24 hours–7 days	>24 hours–7 days
Intermediate time period	8 days–1 year	8 days–90 days
Extended time period	>1 year	>90 days

Risk-of-Bias Considerations

Risk-of-bias ratings for all of the individual studies for all questions are available in **Appendix 3**. The risk of bias of individual studies in the body of evidence was considered in developing the confidence ratings for each health effect. The key risk-of-bias questions (e.g., confounding, exposure characterization, and outcome assessment for human studies and randomization, exposure characterization, and outcome assessment for experimental animal studies) are discussed in the consideration of the body of evidence for each health effect. These are the questions considered for this assessment to potentially have the greatest impact on the results if not addressed appropriately. No study was excluded based on concerns for risk of bias, but if present, confidence conclusions were considered with and without high risk-of-bias studies (e.g., studies rated probably high or definitively high risk of bias for two key risk-of-bias

questions) to assess the impact of the high risk-of-bias studies. However, it was determined that none of the time period-specific bodies of evidence evaluated in this assessment contained a sufficient number of higher quality studies to be able to stratify and evaluate studies based on quality (i.e., lower quality studies vs. higher quality studies). Confidence in the bodies of evidence was downgraded twice if studies were consistently rated probably high or definitely high risk of bias for all three key risk-of-bias questions. Confidence in the bodies of evidence was downgraded once if studies were consistently rated probably high or definitely high risk of bias for two of the three key risk-of-bias questions. Although risk-of-bias ratings for the non-key risk-of-bias questions were considered in the evaluation, confidence in the bodies of evidence were only downgraded based on the ratings for the key risk-of-bias questions unless specific studies had serious issues in other areas.

When assessing study quality, it is very important to consider how the design and conduct of the study may have increased or decreased the validity of the study results. Bias is a systematic error that occurs in results or inferences. Biases can operate in either direction, leading to an underestimation or overestimation of the true effect. Biases can vary in magnitude. Some biases are small (and trivial compared with the observed effect) and others are substantial (so that an apparent finding may be entirely due to bias). Even a particular source of bias may vary in direction. Bias due to a particular design flaw (e.g., lack of allocation concealment) may lead to underestimation of an effect in one study but overestimation in another study. It is usually impossible to know to what extent biases have affected the results of a particular study although there is good empirical evidence that particular flaws in the design, conduct, and analysis of randomized studies lead to bias. Based on these factors, it is important that all studies are evaluated for risk for bias (NTP 2015).

Cholinesterase (ChE)

Sarin is an organophosphate that causes inhibition of acetylcholinesterase (AChE) (Lee 2003). This inhibition leads to an increase in acetylcholine leading to cholinergic hyperstimulation (i.e., overstimulation of the muscarinic and nicotinic acetylcholine receptors in the central and peripheral nervous system due to excess acetylcholine). The signs and symptoms of acute exposure are generally referred to as cholinergic crisis (e.g., miosis, salivation, lacrimation, rhinorrhea, difficulty breathing, convulsions, seizures, diarrhea), which generally subside in a few days. Although sarin is known to inhibit AChE related to the cholinergic signs, a summary of the cholinesterase effects occurring more than 24 hours after exposure are discussed in this section. The human studies generally measured total blood ChE with only a few studies specifically reporting AChE measurements. The animal studies primarily measured AChE in the blood or brain. Many animal studies also evaluated butyrylcholinesterase (BChE), but these results are not discussed because the physiological function of BChE related to neurological effects is unclear and, in general, these studies also measured AChE. For consistency throughout the section, “ChE” will be the term used for all cholinesterase measurements discussed. Although tests for ChE are considered standard tests, there are different kits and methodologies used to determine ChE (or AChE more specifically) that may vary depending on the medium in which ChE was tested.

Ideally, ChE measurements after exposure would be compared to a baseline measurement (i.e., before exposure) for an individual, as ChE levels can vary by individual. These baseline data are unlikely to be available for many subjects unless they were specifically working with anticholinesterases; therefore, the ChE levels of subjects were likely compared to a “normal” value. Although many of the studies did not state the value that was used to calculate percent of control, percent of normal is considered to be valid in determining inhibition; however, it may affect the results depending on where the subjects’ initial baseline values fell in the range of “normal” and which value was used as the control. Subjects with baseline values on the lower end of normal may indicate a greater ChE inhibition than what

actually occurred. On the contrary, ChE inhibition in subjects with baseline values on the higher end of normal may have been understated or not detected.

Human Cholinesterase Data

***Summary*:** There is high confidence in the body of evidence that acute sarin exposure suppresses ChE blood levels in humans over the initial period of 1–7 days following initial exposure, with very low confidence that suppression continues over a period of months after exposure. The studies providing data on ChE response in blood for a period of days, including two non-randomized controlled trials (Grob and Harvey 1958, Baker and Sedgwick 1996) and two case reports (Grob 1956, Sidell 1974), reported consistent evidence of ChE inhibition following acute sarin exposure (see **Figure D1**). Similarly, the studies that provide data on ChE response for a period of weeks to months, including six case reports (Grob 1956, Sidell 1974, Rengstorff 1985, Sekijima *et al.* 1995, Ohtomi *et al.* 1996, Okumura *et al.* 1996), showed consistent lowering of blood levels of ChE following acute sarin exposure (see **Figure D2** and **Figure D3**). One cross-sectional study that evaluated ChE blood levels 5 years after exposure did not observe a difference in ChE compared to controls (Tochigi *et al.* 2002). Although results show consistent lowering of blood levels of ChE for a period of days to months following acute sarin exposure, there are limitations in the body of evidence including small sample sizes (n = 8–10 for the controlled exposure studies), risk-of-bias concerns, and uncertainties related to study design for the case reports. High confidence in the body of evidence for the initial period following acute sarin exposure is primarily based on the two controlled trials with support from two case report studies. The high initial confidence from the controlled trials was downgraded once for risk-of-bias concerns (i.e., lack of reporting for the key risk-of-bias questions regarding outcome assessment and, in one study, randomization and exposure characterization) and upgraded for evidence of large magnitude of effect (i.e., mean RBC ChE levels were 60.5% of control values at 3 days) to support a final rating of high confidence in the body of evidence for the initial period following acute sarin exposure. This high confidence rating for suppressed ChE in the days following acute exposure is also supported by the well-established response for immediate ChE inhibition in the first 24 hours following acute sarin exposure (NRC 1997, Abu-Qare and Abou-Donia 2002, Lee 2003, Tokuda *et al.* 2006, Yanagisawa *et al.* 2006, Gupta 2015). There is biological support for consistency of sarin-related suppression of ChE over the period of days to months, but the body of evidence is limited to six case report studies. These case reports result in a low initial confidence rating for the body of evidence for the intermediate time period. This initial confidence for the intermediate time period is downgraded further for risk-of-bias concerns, and results in a final rating of very low confidence in the body of evidence. The body of evidence for the extended time period following acute sarin exposure consists of one cross-sectional study and is considered inadequate to evaluate whether sarin exposure is associated with changes in ChE a year or more after exposure due to the limited number of studies, small sample size, and risk-of-bias concerns (see **Table 8**).

The available epidemiological studies that evaluated the association between acute exposure to sarin and ChE effects varied greatly in the timing of the outcome measurement (ranging from 26 hours to 5 years after exposure). Two non-randomized controlled trials as well as two case reports of accidental occupational exposure report ChE effects 1–7 days after exposure, six case reports of accidental occupational exposure or from the Matsumoto or Tokyo subway sarin attacks report ChE effects weeks to months after exposure, and one cross-sectional analysis reports ChE levels 5 years after the terrorist attack in the Tokyo subway system (see **Table 7** for study details). Studies were all conducted in adults with a range in sample size from a single case report to 68 subjects (34 exposed and 34 control subjects). Exposure scenarios varied greatly and included accidental occupational exposures; exposures resulting from two separate terrorist attacks that varied in location, population, and exposure conditions; and

two controlled trials – one that used an inhalation chamber and the other that used oral administration. Sarin exposure levels were known only for the two controlled trials. For effects, some studies measured ChE in red blood cells (RBCs) or plasma or serum, while others specifically measured AChE in RBCs.

Table 7. Studies on Activity of Circulating Cholinesterase in Humans

Study	Study design (Location/ Subjects) [n]	Exposure measure timing	ChE assessment timing	Analysis	ChE activity summary
Initial time period after exposure (>24 hours–7 days)					
Baker and Sedgwick (1996)	Non-randomized controlled trial (United Kingdom/male servicemen) [8]	Inhalation (experimental chamber) for 30 min, 0.5 mg/m^3	3 days	RBC ChE activity % of control values	Depression of RBC ChE activity (57.9–66.1% of control values; mean 60.5% of control values) at 3 days
Grob and Harvey (1958)[a]	Non-randomized controlled trial (United States/adult volunteer subjects) [10]	Oral, initial dose range 0.0005–0.022 mg/kg (dose within 24 hours)	26 hours	Plasma and RBC ChE activity % of control values	Depression of ChE activity of RBCs (17.5% of control values at 26 hours) greater than that of the plasma (41.2% of control values at 26 hours)
Grob (1956)	Case-reports (Unspecified location/males) [3]	Accidental occupational exposures (each a separate occasion; not measured)	1.5, 2, 2.5, 3, 4, 5, 6, 7 days	Plasma and RBC ChE activity % of control values	Depression of ChE activity of RBCs (as low as 3% and 8% of control values at 2 days and 6 days, respectively) greater than that of the plasma (as low as 7.5% and 28% of control values at 2 days and 7 days, respectively)
Sidell (1974)	Case reports (United States/ males) [3]	Accidental occupational exposures (not measured)	3, 6 days	Plasma and RBC ChE activity % of control values	Depression of ChE activity of RBCs (as low as 38.1% of control values at 3 days) and plasma (as low as 64.8% of control values at 3 days)
Intermediate time period after exposure (8 days–1 year)					
Grob (1956)	Case-reports (Unspecified location/males) [3]	Accidental occupational exposures (each a separate occasion; not measured)	8–14 (daily), 16–24 (daily), 26–34 (daily), 42, 43, 52, 53, 55, 56, 60, 74, 88 days	Plasma and RBC ChE activity % of control values	Depression of ChE activity of RBCs ranged from 10–21% of control values at 8 days and gradually returned to normal activity over a period of 3 months; depression of ChE activity of plasma ranged from 30–65% of control values at 8 days and gradually returned to normal activity over a period of 3–8 weeks.
Rengstorff (1985)	Case-reports (United States/ males) [2]	Single accidental occupational exposure (not measured)	13, 34, 62, 90 days	Mean plasma and RBC ChE activity uM/ml/min	Depression of ChE activity of RBCs (4.5 uM/ml/min at 13 days v. 13 uM/ml/min at 90 days for higher exposed individual) and plasma (4.2 uM/ml/min at 13 days v. 5.4 uM/ml/min at 90 days for higher exposed individual); gradual increase to baseline over period of up to 90 days

Table 7. Studies on Activity of Circulating Cholinesterase in Humans					
Study	**Study design (Location/ Subjects) [n]**	**Exposure measure timing**	**ChE assessment timing**	**Analysis**	**ChE activity summary**
Sekijima *et al.* (1995)	Case report (Japan/ Matsumoto sarin attack victim) [1]	Terrorist attack, single exposure (not measured)	10–90 days, every 10 days	RBC ChE and serum ChE activity % of normal values	Depression of ChE activity of RBCs (13.2% of normal values at 10 days) and serum(52.7% of normal values at 10 days); returned to normal at approximately 3 months
Sidell (1974)	Case reports (United States/ males) [3]	Accidental occupational exposures (not measured)	13, 20, 27, 41, 62, 69, 90 days	Plasma and RBC ChE activity % of control values	gradual return to normal activity over period of up to 90 days (see initial effect above under 1–7 days)
Okumura *et al.* (1996)	Case reports (Japan/Tokyo subway attack, St. Luke's Hospital) [4]	Terrorist attack, single exposure (not measured)	2 months	RBC ChE, plasma ChE	RBC ChE increased over time in 4 severe cases with resolution taking about 2 months; in 1 patient that died it returned to normal in 13 days with treatment
Ohtomi *et al.* (1996)	Case series (Japan/Tokyo subway attack, SDF Central Hospital) [62]	Terrorist attack, single exposure (not measured)	3–4 months	RBC ChE, plasma ChE	RBC ChE: all patients back to normal range by 3 months; plasma ChE: 7 patients still below normal range by 3–4 months
Extended time period after exposure (>1 year)					
Tochigi *et al.* (2002)	Cross-sectional (Japan/Tokyo subway sarin attack, adult victims and controls) [68]	Terrorist attack, single exposure (not measured)	5 years	Mean serum ChE activity IU/L	No significant difference in mean serum ChE (313 IU/L at 5 years v. 347 IU/L for control); ChE was significantly lower among victims with PTSD compared with controls

[a] Grob and Harvey (1958) administered multiple exposures and assessed ChE levels at multiple time points, however the ChE assessment at 26 hours after the first sarin exposure but before the subsequent sarin exposures is the only data that satisfies the PECO statement.

Overall risk-of-bias discussion of the body of evidence

Confidence in the body of evidence for the human studies was downgraded because of serious concern for risk of bias. Risk-of-bias ratings for individual studies for all questions are available in **Figure D6** through **Figure D9**. There are a number of risk-of-bias issues in the evidence relating to design and conduct of individual studies in addition to general limitations (i.e., not risk-of-bias issues) based on the case-report study design. Most of the human studies were case reports and were rated as probably high risk of bias across multiple key risk-of-bias questions including confounding and lack of blinding of outcome assessors. The controlled trials have fewer issues regarding risk of bias, but lack of reporting for the key risk-of-bias questions (i.e., questions regarding randomization and exposure assessment for the oral study, and blinding during outcome assessment) occurred. Attempts to contact the study authors to obtain additional information when information pertinent for determining risk of bias was not reported was generally not successful, likely because the publications are more than 20 years old. When information was lacking to assess the potential bias for a risk-of-bias question, a conservative approach was followed, and the studies were rated probably high risk of bias for that question. Because the time period-specific bodies of evidence contain few higher quality studies, the studies were not stratified based on quality (i.e., lower quality studies vs. higher quality studies). For the same reason,

NTP was unable to compare the results of lower risk-of-bias studies to higher risk-of-bias studies as a way to assess the impact of the unreported information.

Lack of information on specific exposure measures was a principal issue in many of the studies. The two controlled trials had known administered levels of exposure; however, Grob and Harvey (1958) administered different dose levels of sarin to each of the 10 subjects without discussing if they were randomly assigned. In addition, the solution containing sarin was 90% pure with the remaining 10% consisting of other ChE inhibitors. The authors did not report which ChE inhibitors made up the remaining 10%. Although it is likely that sarin caused the initial ChE inhibition reported in the study, it is unknown if the remaining 10% of ChE inhibitors was associated with long-term effects; however, this study only measures ChE through 26 hours after exposure. The majority of the studies were based on accidental occupational exposure or one of the two terror attacks. For these studies, it is acknowledged that exposure to sarin occurred despite lack of information on level or exposure, and many of the studies provide some information on initial symptoms indicative of exposure. These are considered to not pose a risk-of-bias concern and were assigned a rating of probably low risk of bias because there is little concern for exposure misclassification. Sarin is not found in the general population and can be evaluated as ever/never exposed in any of the scenarios (occupational exposure or terror attack). Controls would not have been exposed to sarin, but there is potential concern about exposure to other organophosphates, which was considered a confounder of concern.

Failure to address potential confounders was a main issue in the case reports and cross-sectional study. None of the studies address potential exposure to other anticholinesterases (such as drugs used for Alzheimer's disease or dementia), which could occur in an occupational setting, or other potential confounders (e.g., age, sex, race/ethnicity, smoking, body mass index, alcohol consumption, and variables that represent socioeconomic status). Although it is recognized that sarin may be a more potent anticholinesterase than other anticholinesterases, assessing sarin's long-term effects could be biased away from or towards the null depending on the long-term effects in controls exposed to other anticholinesterases. Although Tochigi *et al.* (2002), the one cross-sectional study, generally matched the controls by age and sex, the authors did not indicate that controls potentially exposed to anticholinesterases were excluded, and no other potential confounders were considered (e.g., smoking, alcohol consumption, body mass index [BMI]). The majority of the case reports compared ChE levels to a normal or control level, but no information was provided on whether the normal/control level was standardized for the sex and age of the subjects. Although the studies did not address potential confounders, there is not enough information available to indicate how this would impact the results. No studies were excluded based in concerns for risk of bias, but the lack of information available on the potential confounders adds to the serious concerns for risk of bias.

The main limitation in the outcome assessment was the lack of reporting if the outcome was assessed without knowledge of exposure. Neither of the controlled trials provided information on whether the outcome assessors were adequately blinded to the study group or exposure level, or if samples were sent to an independent laboratory or were coded and stored for analysis all at the same time. Regarding biases in the outcome assessment of ChE levels in case reports, testing conducted as part of a hospital examination is considered probably low risk of bias because it is unlikely that the lab technician would have knowledge of exposure or knowledge of specific results that may be associated with the exposure; however, many of the studies did not state that the ChE testing was part of routine hospital examinations and may be biased due to lack of blinding if the assessors were aware of the possible exposure. The cross-sectional study by Tochigi *et al.* (2002) also did not report if the outcome assessors were blinded, and it is unlikely that the test was conducted as part of a routine hospital exam, as both

the exposed and control groups were evaluated 5 years after the exposure. This lack of data on blinding during outcome assessment adds to the serious concern for risk of bias, and combined with the lack of information on potential confounders, caused the confidence rating in the body of evidence to be downgraded once.

Effects in the initial period after exposure
The results from two controlled trials (8–10 participants) and two case reports (3 subjects in each publication all on accidental occupational exposure) provided consistent results for depressed ChE through 7 days after exposure. Two controlled trials reported evidence of ChE inhibition following acute sarin exposure from 1–3 days after exposure. In both controlled trials, subjects were used as their own controls (Grob and Harvey 1958, Baker and Sedgwick 1996), and this body of evidence supports a high initial confidence based on study design considerations. One controlled trial administered sarin vapor with an ambient concentration of 0.5 mg/m^3 for 30 minutes and measured ChE in RBCs 3 hours and 3 days after exposure in 8 subjects (Baker and Sedgwick 1996). Three hours after exposure, the mean RBC ChE levels were 57.5% of the baseline values. By 3 days post-exposure, the RBC ChE levels were largely unchanged with a mean value of 60.5% of the baseline. Grob and Harvey (1958) administered a solution containing sarin (90% purity with 10% other compounds with anticholinesterase activity) at varying concentrations (initial dose ranged from 0.0005–0.022 mg/kg with an average value of 0.012 mg/kg). ChE levels at 26 hours were reduced by at least 40% after oral exposure (Grob and Harvey 1958). Although the study provided known levels of sarin to the subjects, each subject appeared to have received a different dose of sarin. The authors noted that doses of 0.002 to 0.022 mg/kg resulted in 15–75% reduction in plasma and RBC ChE activity decreasing in a dose-related manner. Maximum depression occurred within 1–2 hours, but RBC and plasma ChE levels were not restored within 26 hours and were still depressed by approximately 17.5% and 40% by 26 hours, respectively.

The limitations of the two controlled trials include the risk-of-bias concerns discussed above and small sample size. Confounding is less likely to cause bias in the controlled trials because the subjects were used as their own controls; however, because blinding of the outcome assessors was not reported, this could increase the likelihood of bias since baseline levels of the subjects were used as control levels, and the outcome assessors could know which ChE assessment took place before and after the sarin exposure. In addition, there are risk-of-bias concerns about exposure in one of the controlled trials (Grob and Harvey 1958) because different doses were administered to the subjects without reporting if the subjects were randomly selected for the different exposures, and the study used sarin that was 90% pure with other anticholinesterases in the remaining 10%. These risk-of-bias issues support a single downgrade from the initial confidence; however the large magnitude of effect supports an upgrade in confidence. Therefore, the final high confidence rating for the body of evidence considers risk-of-bias concerns but also the large magnitude of effect, robust study design, and substantial support from evidence of acute ChE effects within the first 24 hours after sarin exposure (NRC 1997, Abu-Qare and Abou-Donia 2002, Lee 2003, Tokuda *et al.* 2006, Yanagisawa *et al.* 2006, Gupta 2015), and reflects the consistency in the results regardless of exposure route.

Case reports of accidental occupational exposures also demonstrated decreases in ChE 1–7 days after acute sarin exposure, but the levels and lengths of depressions varied by subject and by exposure scenario (Grob 1956, Sidell 1974). In addition, recovery rates for RBC ChE levels were consistently longer than those for plasma ChE levels. Grob (1956) details three different case reports of accidental exposure to sarin (presumably occupational). In each case, there was established exposure to an unknown amount of sarin, but each case had suppression of ChE ranging from 8–30% of normal levels through 7 days after exposure. Sidell (1974) reports on four cases of accidental exposure to sarin, but ChE

measurements were only taken in 3 of the cases. The 3 cases all worked in an area where sarin was stored, and exposure occurred after one of the sarin containers leaked. Initial ChE levels ranged from approximately 55–80% of control for plasma ChE and 30–55% of control for RBC ChE. All 3 cases had reduced RBC and plasma ChE at 3 and 6 days following exposure with a reduction of at least 40% depending on subject, time, and type of ChE. The variation in ChE levels could be related to differences in exposure, but the lack of exposure information precludes a determination. RBC ChE levels for all three cases in Sidell (1974) decreased further from normal levels from day 3 to day 6, while plasma ChE levels recovered closer to normal levels during the same time period. A similar trend in RBC and plasma ChE levels was reported for two of the three cases reported on in Grob (1956).

These case reports provide supporting evidence to the controlled trials that ChE levels are depressed 1–7 days after exposure to sarin. In both case reports, it is unclear what the control levels were and whether they represented baseline levels for the subjects or if they were based on an assumed "normal" range, as results were only presented as percent control. In addition, as exposure occurred in both case reports via occupational accidents, it is possible that the subjects experienced lower, asymptomatic chronic exposure to sarin.

Effects in the intermediate period after exposure

The results from six case reports/series (1–62 cases per publication) demonstrated that decreases in ChE can last weeks to months after the exposure, but the levels and lengths of depressions varied by subject and by exposure scenario (Grob 1956, Sidell 1974, Rengstorff 1985, Sekijima *et al.* 1995, Ohtomi *et al.* 1996, Okumura *et al.* 1996). The body of evidence for this time period is limited to case reports and therefore supports a low initial confidence based on study design considerations. In the cases discussed by Grob (1956) mentioned above, each case had suppression of ChE ranging from 8–30% of normal levels with a gradual return to normal at 3–8 weeks for plasma ChE and at 3 months for RBC ChE. In cases from the Sidell (1974) study discussed above, it took between 10 and 90 days after exposure for plasma ChE to return to normal and at least 90 days for RBC ChE to return to normal in all subjects. Rengstorff (1985) reported on two of the Sidell (1974) cases. The subject closest to the sarin exposure experienced an initial depression of RBC ChE level at 19% of baseline levels and plasma ChE levels at 35% of baseline. The plasma ChE levels returned to normal within 30 days, while the RBC ChE levels took 90 days to return to normal. The other exposed co-worker experienced little change in plasma ChE and an initial depression of RBC ChE at 84% of baseline. The RBC ChE level for this worker gradually increased and reached baseline around 60 days. A case report of a victim of the Matsumoto sarin attack was found to have depressed serum and RBC ChE by at least 50% 10 days after sarin exposure (Sekijima *et al.* 1995). Serum ChE levels returned to normal approximately 30 days following exposure, but the RBC ChE took 80 days to return to normal. Okumura *et al.* (1996) described 4 of the 640 subjects that were admitted into St. Luke's Hospital after the Tokyo subway attack. These 4 subjects all exhibited signs of severe sarin poisoning. In the patient that died, RBC ChE did not return to normal for 13 days even though plasma ChE was noted to return to normal within 7 hours of treatment. The other 3 cases were all unresponsive when admitted to the hospital, and it was noted that it took between 51 and 72 days for RBC ChE values to return to normal. All of these subjects were stated to have no evidence of sequelae when they were discharged. Cases brought to the SDF Central Hospital were described by Ohtomi *et al.* (1996). Twenty-seven of the 62 patients (44%) admitted had plasma ChE levels below normal range. Recovery was slow with or without treatment with 7 patients still below normal levels 3–4 months after exposure. Twenty-eight of 53 patients (53%) had RBC ChE levels below normal range at admission. All patients had levels return to normal range by 3 months after exposure.

These case reports provide consistent evidence that acute sarin exposure causes ChE suppression that can last up to 90 days before returning to normal levels. Each case report was established to have been exposed to sarin, but the levels of exposure are unknown and therefore cannot be related to the severity in depression of ChE or the length of time for ChE levels to return to normal. The initial low confidence was downgraded to very low confidence in the body of evidence due to the risk-of-bias concerns discussed above and the problematic study design.

Effects in the extended period after exposure
Only a single study was available that evaluated ChE levels several years after sarin exposure, but ChE levels were not reported to be significantly depressed. Tochigi *et al.* (2002), a cross-sectional study with a control group for comparison, evaluated ChE levels 5 years after exposure during the Tokyo subway attack. Controls were selected from the staff at the Tokyo University Hospital, and although they were age-matched and mostly sex-matched, there was no information provided to indicate that they had not been exposed to substances that could have altered their ChE levels. Tochigi *et al.* (2002) found that victims of the Tokyo subway attack did not have decreased serum ChE levels 5 years after the attack when compared with control subjects; however, a significant decrease in serum ChE level was observed in subjects that developed post-traumatic stress disorder (PTSD) (n = 8) compared with controls 5 years after exposure.

The Tochigi *et al.* (2002) study had several limitations. The sample size was small (34 exposed and 34 control subjects), which may have limited the power of the study to detect an effect. No information was provided as to the selection of exposed participants from the cohort of Tokyo terror attack victims, except that they had been treated in the emergency room after the attack and were followed up at a general hospital in Tokyo. It was noted that serum ChE levels were available in 25 of the exposed subjects within 1–3 days of exposure. The mean ChE levels in these 25 subjects were lower 1–3 days after the attack than they were at 5 years, which provides evidence of exposure in at least 25 of the 34 subjects, but the authors did not report any symptoms. Although the controls were generally matched to the victims by age and sex, the authors did not indicate that controls potentially exposed to anticholinesterases were excluded, and no other potential confounders (e.g., smoking, alcohol consumption, BMI) were considered.

Taken together, epidemiological studies provide evidence that acute sarin exposure is associated with decreased ChE from 1 day through 12 months. The data present a consistent pattern of findings from 26 hours to 90 days after exposure with different levels of confidence depending on the time period after exposure. There is high confidence in the body of evidence in the initial period and very low confidence in the body of evidence in the intermediate period. There is inadequate evidence to evaluate whether there are effects in the extended period, based on a single study that showed no significant effects in 68 subjects (34 controls and 34 exposed) evaluated at 5 years.

Animal Cholinesterase Data
***Summary*:** There is moderate confidence in the body of evidence that acute sarin exposure suppressed ChE blood and brain levels in animals over a period of days to months after the initial exposure. The results show a consistent lowering of ChE blood levels following acute sarin exposure across multiple studies and at different time periods following exposure (see **Figure D4** and **Figure D5**). However, there are limitations in the body of evidence, including small sample sizes (n = 2–6 for most studies) and risk-of-bias concerns. The consistent evidence support suppression of ChE within days following acute sarin exposure, but the length of the suppression varied by study, and there was less evidence for suppression 1 week to 90 days (which is considered relevant for humans 1 week to 12 months after exposure).

Confidence in the body of evidence for the animal studies was downgraded for both the initial period and intermediate period (up to 90 days) from an initial high confidence because of serious concern for risk of bias to support the final confidence rating of moderate (see **Table 8**).

There are 15 experimental studies in the animal body of evidence that evaluated the association between acute exposure to sarin and ChE effects. Experimental animal studies used various species, strains, methods of exposure, and locations from which ChE levels were measured (e.g., blood, plasma, different areas of the brain). The studies primarily measured AChE in the blood, plasma, or in sections of the brain (see **Figure D4** and **Figure D5**). Activity in the blood was measured from 26 hours to 90 days after exposure in rats or monkeys. Activity in the brain was measured in the brainstem, cerebellum, corpus striatum, cortex, and hippocampus from 3–90 days after exposure in rats (Sprague-Dawley or Wistar). Of the studies measuring ChE, varying injection methods for administering the sarin were used (i.e., subcutaneous, intramuscular, intraperitoneal, or intravenous).

Overall risk-of-bias discussion of body of evidence
Confidence in the body of evidence for the animal studies was downgraded because of serious concern for risk of bias (see **Figure D10** and **Figure D11**). The main risk-of-bias concern with the animal studies was lack of reporting of important details for key risk-of-bias questions (i.e., questions regarding randomization, exposure assessment, and outcome assessment). Only 3 of the 15 studies indicated that the animals were randomized to treatment. None of the other studies provided details on randomization or how animals were assigned to treatment. In one study in monkeys (Genovese *et al.* 2007), animals had been treated with other compounds more than 6 months prior to treatment with sarin, but the compounds were not reported nor was it reported if they were ChE inhibitors. The lack of information regarding previous exposures coupled with the lack of information on randomization results in the possibility of serious risk of bias. On the other hand, the strength of the exposure in the animal studies is that they have a known administered sarin dose. Because the sarin was administered via injection, the animals are known to receive that specific dose; however, the majority of the animal studies (9 of 15) lacked information on the purity of the sarin administered or other possible contaminants (such as other ChE inhibitors) that may have biased the results. Four studies administered sarin with >98% purity, of which three studies stated that the purity was verified. ChE levels were measured using acceptable methods in all studies; however, none of the studies reported that the outcome assessors were adequately blinded to the study group. Because the time period-specific bodies of evidence contain few higher quality studies, the studies were not stratified based on quality (i.e., lower quality studies vs. higher quality studies). For the same reason, NTP was unable to compare the results of lower risk-of-bias studies to higher risk-of-bias studies as a way to assess the impact of the unreported information.

Effects in the initial period after exposure
The results from 11 experimental studies in animals demonstrated depressed ChE from 1–7 days after exposure. Experimental studies in male and female rats demonstrated relatively consistent decreases in blood and plasma ChE levels 1–7 days after exposure. In female rats, RamaRao *et al.* (2011) found that acute subcutaneous sarin exposure at 120 µg/kg decreased plasma ChE up to 3 days after exposure, but levels normalized by day 7. Decreased blood ChE was also observed in male rats up to 3 days after intraperitoneal sarin exposure to 12.5 or 50 µg/kg, but the authors did not evaluate blood ChE levels after 3 days (Nieminen *et al.* 1990). Chaubey *et al.* (2016) and Chaubey *et al.* (2017) observed a statistically significant decrease in blood ChE activity through day 7 in male rats exposed subcutaneously to sarin at 80 µg/kg. Although Chaubey *et al.* (2016) did not measure the levels after day 7 to determine if or when levels returned to control levels, Chaubey *et al.* (2017) measured blood ChE activity at 11 and

90 days and reported that levels returned to normal by day 11. In contrast to studies that reported depressed ChE from 1–7 days after sarin exposure, one study (Bansal *et al.* 2009) did not observe a decrease in plasma ChE in female rats 3 days after subcutaneous sarin exposure to 80 µg/kg. Experimental studies in monkeys also demonstrated consistent decreases in blood ChE levels 1–7 days after exposure. In monkeys (both African Green and Rhesus), Genovese *et al.* (2007) observed a decrease in blood ChE activity from 26 hours to 7 days after exposure. Results for this study were not statistically significant, however, this is not unexpected, as there were only 2–3 monkeys per treatment group.

There is also evidence that tissue ChE activity is decreased in the initial time period of 1–7 days after exposure, although several studies did not find decreased ChE. Gupta *et al.* (1991) observed a statistically significant decrease in brain ChE levels in the brainstem, cortex, hippocampus, and striatum of Sprague-Dawley rats 7 days after exposure to 110 µg/kg sarin. Similarly, Chaubey *et al.* (2017) observed a statistically significant decrease in brain ChE levels in the cortex, corpus striatum, and hippocampus of male Wistar rats 1 and 7 days after exposure to 80 µg/kg sarin. Brain ChE levels returned to normal in the hippocampus by day 11 but remained depressed in the cortex and corpus striatum. RamaRao *et al.* (2011) also observed a statistically significant decrease in brain ChE levels in the cerebellum and cortex of Wistar rats 3 days after exposure to sarin at 120 µg/kg, but levels were back to control levels by day 7. Whalley and Shih (1989) measured ChE activity in the brain of male albino rats after exposure to sarin at 120 µg/kg. ChE activity was depressed in the hippocampus and cortex of the brain by approximately 35% at 168 hours (i.e., 7 days). In the striatum, ChE levels were near control levels by day 7. Lower sarin doses of 12.5 and 50 µg/kg caused a significant decrease in brain ChE only in the striatum of Wistar rats 3 days after exposure (Nieminen *et al.* 1990). This was the only time period evaluated. Decreases were observed in other sections of the brain, but were not statistically significant. Tripathi and Dewey (1989) reported decreases in brain ChE levels in mice 1–7 days after exposure to sarin at 80 µg/kg. In contrast, several studies did not find decreased ChE in brain tissue at similar doses. Bansal *et al.* (2009) did not observe a change in brain ChE in Wistar rats 3 days after exposure to 80 µg/kg, but they did observe a decrease in brain ChE mRNA. Scaife and Shuster (1960) also did not observe a change in brain ChE in rats (90 µg/kg) or guinea pigs (30 or 35 µg/kg) through 7 days. Koelle *et al.* (1977) observed a decrease in ChE in various nerves and muscle tissue in cats from 2–6 days after exposure to 2 µmol/kg.

Effects in the intermediate period after exposure

The eight experimental studies that described effects on ChE during this time period also provide evidence of effects on blood ChE in the weeks and months after exposure, but the evidence is less consistent. In rats, as noted above, RamaRao *et al.* (2011) observed that blood ChE levels were back to control levels by day 7 and were unchanged at 14 and 30 days. Similarly, Chaubey *et al.* (2017) observed that blood ChE activity in rats returned to normal by day 11 and were unchanged at day 90 after being depressed through day 7. In monkeys, Genovese *et al.* (2007) observed a decrease in blood ChE activity that was maintained at week 2 following acute exposure to sarin; however, ChE levels began normalizing by week 6. In the Rhesus monkey, blood ChE levels were not back to control levels in the 5.87-µg/kg group at 10 weeks following exposure; however, they were back to control levels for the African Green monkey at 10 weeks. Results for this study were not statistical significance because there were only 2–3 monkeys per treatment group. Pearce *et al.* (1999) also studied monkeys, but only provided qualitative results indicating that mean erythrocyte ChE inhibition was 51.3% at 3 hours but returned to baseline 4–45 weeks later and was not statistically significant at 3 months post-exposure.

As noted above, there is evidence that acute sarin exposure alters ChE activity in different regions of the brain in rats during the first week following exposure, but there may be compensatory upregulation of activity since there were statistically significant increases in ChE observed in the brainstem, cerebellum, and midbrain of rats 15 days after exposure (Abou Donia *et al.* 2002). Conversely, levels in the cortex were significantly decreased. Jones *et al.* (2000) only evaluated ChE activity 90 days after exposure in the cortex and brainstem of rats administered sarin via intramuscular injection. Cortex ChE was inhibited by about 30% in the animals receiving 100 µg/kg sarin, although the effect was not statistically significant due to high variability. The brainstem ChE, however, was significantly increased 90 days after exposure. Chaubey *et al.* (2017) observed statistically significant decreases in brain ChE levels in the cortex and corpus striatum of rats at 11 and 90 days following exposure to 80 µg/kg sarin via subcutaneous injection. ChE levels in the hippocampus were depressed through day 7 but returned to normal by day 11 (Chaubey *et al.* 2017). For mice, decreases in ChE levels in the brain were maintained 2 weeks after exposure to 80 µg/kg (Tripathi and Dewey 1989). Koelle *et al.* (1977) observed a decrease in ChE levels in various nerves and muscle tissue in cats through 18 days. Although significance was not measured in terms of control levels, ChE levels were <10% of the control within 30 minutes and increased gradually through the 18 days.

Effects in the extended period after exposure
There is no animal evidence to evaluate the potential association between sarin exposure and effects greater than 90 days after exposure in rodents or 1 year in non-human primates.

Integration of Evidence for Cholinesterase
There is consistent evidence that ChE levels are reduced in humans and animals after acute exposure to sarin, however, the evidence varies based on the length of time after exposure. There is high confidence in the human data in the initial period after exposure, but lower confidence in the intermediate period after exposure based on limitations in the body of evidence largely due to the relative paucity of clinical studies other than case reports. The data in the extended period after exposure are inadequate to evaluate potential effects based on the limited number of studies and the limitations in the one study that was available. There is moderate confidence in the animal data for both the initial period and intermediate period with no data for the extended period after exposure. The uncertainty in the animal evidence is mainly due to lack of reporting information necessary to evaluate risk-of-bias concerns and the heterogeneity of the data with regards to the outcomes measured, when the outcomes were measured, the species or strain used, and the method for administering sarin. These confidence ratings translate directly into level-of-evidence conclusions and support an initial hazard identification conclusion based on the different times as detailed below.

Effects initial period after exposure

- **Human body of evidence:** High Confidence = High Level of Evidence
- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Initial hazard conclusion (High Human x Moderate Animal)** = Known to be a Neurological Hazard to Humans
- **Final hazard conclusion for the initial period (after consideration of biological plausibility)** = Known to be a Neurological Hazard to Humans

Effects intermediate period after exposure

- **Human body of evidence:** Very Low Confidence = Inadequate Level of Evidence

- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Initial hazard conclusion (Inadequate Human x Moderate Animal)** = Suspected to be a Neurological Hazard to Humans
- **Final hazard conclusion for the intermediate period (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to Humans

Effects extended period after exposure

- **Human body of evidence:** Low Confidence with no evidence= Inadequate Level of Evidence
- **Animal body of evidence:** No studies = Inadequate Level of Evidence
- **Initial hazard conclusion (Inadequate Human x Inadequate Animal)** = Not Classifiable
- **Final hazard conclusion for extended period (after consideration of biological plausibility)** = Not Classifiable

Taken together, the human and animal bodies of evidence provide a consistent pattern of findings in the initial period after exposure that acute sarin exposure is associated with decreased ChE levels. The body of evidence for the intermediate period is more limited as it is based primarily on case reports in humans, and the data are less consistent in the animal literature during this time period. The body of evidence is inadequate in the extended period due to only a single study in humans, which did not observe an effect at 5 years after exposure. It is known that sarin binds to and inactivates AChE (Gunderson *et al.* 1992, Spradling and Dillman 2011, Hargreaves 2012). The build-up of the acetylcholine is associated with the cholinergic effects observed with higher exposures to organophosphates including sarin. Humans are typically treated with oximes to break the bond between sarin and AChE before the dealkylation process referred to as aging has occurred, which results in irreversible enzyme inhibition. The half-life for sarin aging is 5 hours (Brown and Brix 1998). It can take up to 3 months for the ChE to regenerate (Brown and Brix 1998), which correlates to the timing for much of the human data evaluated for this assessment. Although this may explain the findings through 3 months after exposure, there are no mechanistic data available to help determine if there are potential effects longer than 3 months. Although ChE was evaluated, as it is a known acute effect of sarin, potential health effects due to prolonged ChE inhibition are unknown.

Table 8. Cholinesterase Evidence Profile for Sarin

INITIAL CONFIDENCE for each body of evidence (# of studies)	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
Human										
Initial period - Initial High (2 nonrandomized controlled trials*)[a]	↓	---	---	---	---	↑	---	---	---	**High**
Intermediate period - Initial Low (6 case reports/case series)[b]	↓	---	---	---	---	---	---	---	---	**Very Low**
Extended period - Initial moderate (1 cross-sectional study)[c]	↓	---	---	---	---	---	---	---	---	**Low**
Animal										
Initial period - Initial High (11 mammal study)[d]	↓	---	---	---	---	---	---	---	---	**Moderate**
Intermediate period - Initial High (8 mammal study)[e]	↓	---	---	---	---	---	---	---	---	**Moderate**
Extended period No studies	--	---	---	---	---	---	---	---	---	**No rating**

References:

Human: Baker and Sedgwick (1996)[a], Grob and Harvey (1958)[a], Grob (1956)[b], Ohtomi *et al.* (1996)[b], Okumura *et al.* (1996)[b], Rengstorff (1985)[b], Sekijima *et al.* (1995)[b], Sidell (1974)[b], Tochigi *et al.* (2002)[c]

Animal: Abou Donia *et al.* (2002)[e], Bansal *et al.* (2009)[d], Chaubey *et al.* (2016)[d], Chaubey *et al.* (2017)[de], Damodaran *et al.* (2003)**, Genovese *et al.* (2007)[de], Gupta *et al.* (1991)[d], Jones *et al.* (2000)[e], Koelle *et al.* (1977)[de], Nieminen *et al.* (1990)[d], Pearce *et al.* (1999)[e], RamaRao *et al.* (2011)[de], Scaife and Shuster (1960)[d], Tripathi and Dewey (1989)[de], Whalley and Shih (1989)[d]

*There are also data from 2 case reports (Grob 1956, Sidell 1974), but confidence is based on the 2 non-randomized control trials.

** Evaluated AChE mRNA in the brain

Visual and Ocular

Initial signs of acute intoxication with OP nerve agents include narrowing of the pupil of the eye (miosis). This is considered a sensitive and early presentation of acute exposure (Brown and Brix 1998). Miosis is also used as the basis for establishing threshold exposure limits for military occupational exposure and is considered as a sign of possible high-level exposure (Brown and Brix 1998). This section describes long-term visual or ocular effects (e.g., miosis or narrowing of the pupil) that are reported to occur after acute sarin exposure. The data include outcomes from medical evaluations [e.g., pupillary response, miosis, pupil diameter, visual evoked potential (VEP)] and self-reported symptoms (e.g., blurred vision, dimmed vision, double vision, ocular pain, etc.).

Human Visual and Ocular Data

***Summary*:** Based on the available studies, there is moderate confidence in the body of evidence that acute sarin exposure is associated with visual or ocular effects in humans over the initial period of 1–7 days following initial exposure, moderate confidence that visual or ocular effects persist over the intermediate period of 8 days to 1 year, and very low confidence over the extended period of more than 1 year. The studies that provide visual or ocular data from 1–7 days after acute exposure to sarin, including five case reports/series (Sidell 1974, Morita *et al.* 1995, Sekijima *et al.* 1995, Nohara and Segawa 1996, Ohtomi *et al.* 1996), showed consistent effects of miosis and other visual or ocular parameters (e.g., visual-field abnormalities, conjunctival hyperaemia, etc.). The studies that provide data on visual or ocular effects for a period of weeks to months, including eight case series (Sidell 1974, Rengstorff 1985, Morita *et al.* 1995, Nohara and Segawa 1996, Ohtomi *et al.* 1996, Nakajima *et al.* 1998, Ogawa *et al.* 1999, Okudera 2002) and two cross-sectional studies (Murata *et al.* 1997, Yokoyama *et al.* 1998a), showed consistent evidence that miosis occurred but recovered within the first 1–2 months after exposure while other visual or ocular effects persisted from weeks to months in some of the study subjects, and VEPs were found to be significantly slower 6 to 8 months following exposure. The studies that provide data on visual or ocular effects for a period of years, including four case reports/series (Sekijima *et al.* 1997, Kawana *et al.* 2001, Ohtani *et al.* 2004, Okumura *et al.* 2005) and one prospective cohort study (Nakajima *et al.* 1999), showed evidence that other visual or ocular effects (e.g., ocular pain, blurred vision) persisted from 1–5 years following exposure. Although results provide evidence of visual or ocular effects from days to years following acute sarin exposure, there are limitations in the human body of evidence due to risk-of-bias concerns and uncertainties related to the availability of only case reports/series. Moderate confidence in the body of evidence for the initial period following exposure is primarily based on the consistent pattern of findings of miosis from the five case series/reports. Although the prevalence of miosis and the magnitude of pupil constriction in the initial time period following acute sarin exposure is considered fairly consistent and large, respectively [e.g., Ohtomi *et al.* (1996) reported that 95% of 62 subjects had miosis 1 day following exposure with 39% of subjects having miotic pupils of ≤1mm, and Morita *et al.* (1995) observed pupil diameters ≤1.5mm during the first 2 days following exposure in approximately 50% of 219 subjects], these factors alone might not warrant an upgrade in confidence due to the limited number of studies and limitations due to study design (i.e., case reports/series only); however, when considered collectively and supported by the well-established response of immediate constriction of the pupils in the first 24 hours following acute sarin exposure, the initial low confidence was upgraded once to support a final rating of moderate confidence in the body of evidence for the initial period. Moderate confidence in the body of evidence of visual or ocular effects for the intermediate period following exposure is based on two cross-sectional studies with an initial and final confidence of moderate. This is supported by data from eight case series, which also reported visual or ocular effects. The case series that reported effects at the intermediate period had an initial confidence of low and were downgraded once for serious risk-of-bias concerns. Very low confidence in the body of evidence for visual or ocular effects for the extended period

following exposure is based on one cohort study with support from four case reports/series. The initial confidence of moderate for the cohort study was downgraded twice for very serious risk-of-bias concerns (i.e., failure to control for confounding, potential biases in outcome assessment from self-reporting of symptoms via questionnaires, and loss of subjects over time) to support a final rating of very low confidence in the body of evidence for extended period following acute sarin exposure. This is supported by data from four case reports/series, which also reported visual or ocular effects. These case reports/series had an initial confidence rating of low that was downgraded once for serious risk-of-bias concerns.

The available epidemiologic studies in the human body of evidence that evaluated the association between acute exposure to sarin and visual or ocular effects varied in the timing of the outcome measurement (>24hrs to up to 5 years), the parameters that were measured, and study design (see **Table 9**). The majority of the data are from case reports/series, and therefore there is lower confidence in much of the data due to limitations in that study design (e.g., no controlled exposure and generally no concurrent control). Exposure scenarios varied greatly and included accidental occupational exposures and exposures resulting from two separate terrorist attacks. However, specific exposure levels were not known in any of the studies and in many cases were based on initial symptoms (including the ocular effects) in some subjects, which were obtained via questionnaire in some cases. The data set included one prospective cohort study (Nakajima *et al.* 1999) conducted on all inhabitants in the area around the Matsumoto sarin release site, two cross-sectional studies that report on the same subjects from the Tokyo subway attack (Murata *et al.* 1997, Yokoyama *et al.* 1998a), and 13 case series/reports on subjects involved in the Matsumoto and Tokyo attacks (Morita *et al.* 1995, Sekijima *et al.* 1995, Nohara and Segawa 1996, Ohtomi *et al.* 1996, Sekijima *et al.* 1997, Nakajima *et al.* 1998, Ogawa *et al.* 1999, Kawana *et al.* 2001, Okudera 2002, Ohtani *et al.* 2004, Okumura *et al.* 2005) or exposed accidentally in the workplace (Sidell 1974, Rengstorff 1985). Studies were all conducted in adults with a range in sample sizes from 2–1,743 subjects. The only studies with control groups were the cohort study, Nakajima *et al.* (1999) (318 exposed, 919 control – grouped based on self-reported diagnosis of sarin-related symptoms immediately following sarin release), and the two cross-sectional studies (18 of the approximately 5,000 passengers exposed to sarin during the Tokyo subway attack and 15 to 18 sex- and age-matched control subjects) (Murata *et al.* 1997, Yokoyama *et al.* 1998a). The visual effects reported included outcomes diagnosed by a clinician (e.g., miosis), measurements during a medical exam (e.g., pupil diameter), and self-reported symptoms via questionnaires.

Table 9. Studies on Visual or Ocular Effects in Humans

Study	Study design (Location/Study) [n]	Exposure measure timing	Assessment timing	Analysis	Visual/ocular outcome summary
Initial time period after exposure (>24 hours–7 days)					
Morita *et al.* (1995)	Case series (Japan/Matsumoto city) [219]	Terrorist attack, single exposure (not measured)	2 days	Miosis (pupil size measured in 219 people with 49 examined by an ophthalmologist)	Pupil diameter was less than 1 mm for 21 individuals, 1.5 mm for 87, 2 mm for 6, and 2.5 mm for 32 during the first 2 days after exposure. 124 patients had decreased visual acuity with miosis. Examination revealed a decreased amplitude of accommodation which recovered within several days. 39 people complained of visual-field abnormalities.

Table 9. Studies on Visual or Ocular Effects in Humans

Study	Study design (Location/Study) [n]	Exposure measure timing	Assessment timing	Analysis	Visual/ocular outcome summary
Nohara and Segawa (1996)	Case series (Japan/ Matsumoto sarin attack victims) [51]	Terrorist attack, single exposure (not measured)	1, 2, 3, 4, and 7, days	Pupil diameter as measured during medical examination [n = 4–15]; self-reported symptoms and ocular signs	Pupillary diameter was small (mean 1.55 mm day 1) and appeared to increase over time (mean 3.9 mm day 7). There were several ocular symptoms, but the timing is unclear. Conjunctival hyperaemia and concentric contraction of the visual fields were common within the first 4 days.
Ohtomi *et al.* (1996)	Case series (Japan/ Tokyo sarin attack victims) [62]	Terrorist attack, single exposure (not measured)	1 day, 1 week	Miotic pupils ≤1mm (right eye) as measured during medical examination	Day 1: 95% of victims had miosis, while 39% of victims had miotic pupils ≤1mm.; other ocular manifestations at 1 week: ciliary and conjunctival congestion (16 subjects), ocular and periorbital pain (28 subjects), dim vision (6 subjects), blurred vision (33 subjects), ocular irritation (8 subjects), and visual field abnormality (8 subjects)
Sekijima *et al.* (1995)	Case report (Japan/Matsumoto city) [1 19-year old man]	Terrorist attack, single exposure (not measured)	1 week	Pupil diameter	Pupil effects diminished after approximately 1 week.
Sidell (1974)	Case series (United States/accidental occupational exposure) [3 men; 27, 50, and 52 years old]	Accidental exposure to vapors while working, single exposure (not measured); symptoms included respiratory distress and marked miosis with slight eye pain	3 and 6 days following sarin exposure	Ratio of diameter of the pupil to diameter of the iris calculated from a greatly enlarged photograph; calculated as % of the ratio obtained 3–4 months after exposure	All men had marked miosis during the first 24 hours; recovery of miosis reported to be prolonged; pupil/iris diameter was 50.5–53.9% (mean 52.1%) of control values at 3 days and 64.1–75.5% (mean 69.8%) of control values at 6 days;. about 60–70% of the lost ability to dark adapt returned in two weeks
Intermediate time period after exposure (8 days–1 year)					
Morita *et al.* (1995)	Case series (Japan/Matsumoto city) [219]	Terrorist attack, single exposure (not measured)	2 days, 1 month	Miosis (pupil size measured in 219 people with 49 examined by an ophthalmologist)	Only qualitative statement indicating that Miosis disappeared within a month in all people examined
Murata *et al.* (1997)	Cross-sectional (Japan/ Tokyo subway attack victims) [36]	Terrorist attack, single exposure (not measured)	6 months	VEP	P100 latency in sarin cases were significantly prolonged when compared to matched controls.
Nakajima *et al.* (1998)[a]	Case series (Japan/Matsumoto city) [1,743 at 3 weeks and 105 at 4 months]	Terrorist attack, single exposure (not measured)	3 weeks and 4 months	Self-reported symptoms	Diplopia (i.e., double vision): 3 subjects at 3 weeks and 4 months (presumably the same subjects)

Table 9. Studies on Visual or Ocular Effects in Humans

Study	Study design (Location/Study) [n]	Exposure measure timing	Assessment timing	Analysis	Visual/ocular outcome summary
Nohara and Segawa (1996)	Case series (Japan/ Matsumoto sarin attack victims) [51]	Terrorist attack, single exposure (not measured)	9 days following sarin exposure	Pupil diameter as measured during medical examination	Pupillary diameter was small during the first week (mean 1.55 mm day 1) and appeared to increase over time (mean 4 mm day 9).
Ogawa *et al.* (1999)	Case series (Japan/Tokyo subway attack) [681]	Terrorist attack, single exposure (not measured)	2 months	Self-reported symptoms	At 2 months subjects still reported: dimness of vision (2.6%), constricted visual field (2.2%), eye irritation (4.4%), blurred vision (6.5%), eye pain (4.6%), increased lacrimation (0.4%), and double vision (1%)
Ohtomi *et al.* (1996)	Case series (Japan/ Tokyo subway attack victims) [62]	Terrorist attack, single exposure (not measured)	4, 8 weeks and 3 months	Miotic pupils ≤1mm (right eye) as measured during medical examination	Recovery of miosis was complete within 2 months. Other ocular manifestations still reported to occur at 3 months: ciliary and conjunctival congestion (5 subjects), ocular and periorbital pain (10 subjects), dim vision (1 subject), blurred vision (5 subjects), ocular irritation (3 subjects), and visual field abnormality (2 subjects)
Okudera (2002)	Case series (Japan/Matsumoto city) [155]	Terrorist attack, single exposure (not measured)	3 weeks	Examination most results self-reported symptoms	Ocular symptoms reported after 3 weeks include: ocular pain (14 subjects), darkness of visual field (13 subjects), and eye weakness (10 subjects). The pupil size was smaller in subjects complaining of eye weakness, but no measurements were provided. Although blurred vision was noted at 1 year, no details including number of subjects with effect were reported.
Rengstorff (1985)	Case series (United States/accidental occupational exposure) [2 men; 46 and 53 years old]	Accidental exposure to vapors while working, single exposure (not measured); exposure confirmed by depressed ChE activity and miotic pupils	Over 90-day period (starting at 11 days after exposure)	Pupil diameter and reaction assessed in darkened room using "black light" (ultraviolet) stimulation and mm ruler, a procedure sensitive to ± 0.5 mm	Both men had fixed, slightly irregular pupils (< 1 mm in diameter) which remained unchanged for 24 hours; 11 days after exposure, pupils widened to about 2 mm; pupils continued to increase in size and stabilized between 30 and 45 days.
Sidell (1974)	Case series (United States/accidental occupational exposure) [3 men; 27, 50, and 52 years old]	Accidental exposure to vapors while working, single exposure (not measured); symptoms included respiratory distress and marked miosis with slight eye pain	13, 20, 27, 41, and 62 days following sarin exposure	Ratio of diameter of the pupil to diameter of the iris calculated from a greatly enlarged photograph; calculated as % of the ratio obtained 3–4 months after exposure	About 60–70% of the lost ability to dark adapt returned in two weeks, but complete recovery took 2 months; pupil/iris diameter was 78.4–89.7% (mean 83.9%) of control values at 13 days and gradually returned to approximately 100% of control values by 62 days.

Table 9. Studies on Visual or Ocular Effects in Humans

Study	Study design (Location/Study) [n]	Exposure measure timing	Assessment timing	Analysis	Visual/ocular outcome summary
Yokoyama *et al.* (1998a)[a]	Cross-sectional (Japan/ Tokyo subway attack victims) [36]	Terrorist attack, single exposure (not measured)	6–8 months	VEP	P100 latency in sarin cases were significantly prolonged when compared to matched controls.
Extended time period after exposure (>1 year)					
Kawana *et al.* (2001)	Case series (Japan/Tokyo subway attack victims) [191-283 depending on survey year]	Terrorist attack, single exposure (not measured)	2, 3, 5 years after exposure	Self-reported symptoms	Subjects reported tiredness of eye (33.5-39.3%), dim vision (23.3-25.7%), difficulty seeing distance (18.0-21.5%), and difficulty seeing close (13.1-17.7%), eye discharge (8.9-11.0%), strange feeling in eyes (5.3-9.4%), and other eye symptoms (3.9-5.2%)
Nakajima *et al.* (1999)	Prospective cohort (Japan/Matsumoto city) [1,237 at 1 year and 836 at 3 years]	Terrorist attack, single exposure (not measured, and self-reported exposure based on hospital patient status)	1 and 3 years after exposure	Self-reported symptoms	Blurred vision, narrowing of visual field, and asthenopia (not significantly different between patients and non-patients at 1 year, but significantly increases in victims at 3 years)
Ohtani *et al.* (2004)	Case series (Japan/Tokyo subway attack victims) [34]	Terrorist attack, single exposure (not measured)	5 years	Self-reported symptoms	Eyes tend to become easily tired (19 subjects), blurred vision (20 subjects), difficulty seeing far (17 subjects), difficulty seeing nearby objects (13 subjects), difficulty in focusing (23 subjects), eye mucus (11 subjects), feeling of a foreign object in the eye (9 subjects), other eye symptoms (2 subjects)
Okumura *et al.* (2005)	Case series (Japan/Matsumoto city) [303]	Terrorist attack, single exposure (not measured)	1 year	Self-reported symptoms	18.5% reported eye symptoms after 1 year
Sekijima *et al.* (1997)	Case report (Japan/Matsumoto city) [1 46-year old man]	Terrorist attack, single exposure (not measured)	12-17 months	Self-reported symptoms	One subject reported visual field defects at the 1 year follow-up exam, but was noted to be completely recovered by 17 months.

[a] The data from these two studies for this effect appear to be from the same subjects.

Overall risk-of-bias discussion of the body of evidence

Confidence in the body of evidence for the human studies was downgraded because of serious concern for risk of bias. Risk-of-bias ratings for individual studies for all questions are available in **Figure D15** through **Figure D18**. There are a number of risk-of-bias issues in the evidence relating to design and conduct of individual studies as well as general limitations (i.e., not risk-of-bias issues) based on the case-report/series study design. All but three of the human studies are case reports/series, and the majority of the human studies were rated as probably high risk of bias across multiple key risk-of-bias questions including confounding and lack of blinding of outcome assessors. The prospective cohort study (Nakajima *et al.* 1999) was also rated probably high or definitely high risk of bias over two key questions due to failure to control for confounding and potential biases in outcome assessment from self-reporting of symptoms via questionnaires, as well as loss of subjects over time (the first survey had a response rate of 60.3% [1,237/2,052], and the second survey excluded 52 rescuers and had a response rate of 41.8% [836/2,000], and no comparison was made between those lost to follow-up and those

remaining in the study). The two cross-sectional studies had little potential for bias, but they apparently reported on the same subjects.

As discussed previously for the studies on ChE, confounding and lack of blinding of outcome assessors were the principal risk-of-bias issues in the majority of studies, which were based on one of the two terror attacks or accidental exposures. The majority of studies do not address potential exposure to other organophosphates or potential confounders to address prolonged visual and ocular effects. Blinding of outcome assessors was also a general limitation. For self-reported symptoms, blinding is not possible. Doctors would also not have been blind to exposure. The potential bias of doctor evaluation based on knowledge of exposure cannot be determined. However, in the two cross-sectional studies, blinding of outcome was not an issue because it was reported that assessors were blind or the test was computerized. The lack of addressing potential confounders and the lack of data on blinding at outcome assessment lead to the serious risk-of-bias concern and the single downgrade in the confidence in the body of evidence. Lack of information or specific exposure measures was an issue in many of the studies, but this is not considered to pose a risk-of-bias concern. The majority of the studies were based in accidental occupational exposure or one of the two terror attacks. Some of the studies based exposure status on self-reported diagnosis and/or hospital admission, and only in some of the severe cases was the exposure based on the initial decrease in ChE, miosis, or on other initial symptoms at examination. However, it is acknowledged that exposure to sarin occurred in these studies, and therefore, they are considered to not pose a risk-of-bias concern and were assigned a rating of probably low risk of bias for exposure characterization because there is little concern for exposure misclassification, as sarin is not found in the environment.

Effects in the initial period after exposure

Case reports/series (336 total subjects) provided consistent results for visual or ocular effects 1–7 days after acute exposure to sarin (Sidell 1974, Morita *et al.* 1995, Sekijima *et al.* 1995, Nohara and Segawa 1996, Ohtomi *et al.* 1996). Miosis was consistently diagnosed in almost all subjects by examination within hours of exposure and generally persisted during the first week (later resolved by 1–2 months; for some of the studies it is not clear when the miosis resolved as it was only noted to be resolved by a specific time). Ohtomi *et al.* (1996) reported that 95% of 62 subjects had miosis 1 day following exposure with 39% of subjects having miotic pupils of ≤1mm. Nohara and Segawa (1996) reported miosis in subjects (<4 mm: 80% of 51 subjects; <2 mm: 41% of 51 subjects) following acute sarin exposure (presumably within the first 24 hours following exposure). In subjects who received pupil examinations overtime, pupillary diameter gradually increased through day seven (mean 1.55 mm at 1 day [n = 13]; mean 3.9 mm at day 7 [n = 5]) (see **Figure D12**). A case report on one severe case from the Matsumoto attack did not report quantitative results on pupil effects, but reported that pupil effects were resolved within a week of exposure (Sekijima *et al.* 1995). Morita *et al.* (1995) reported that miosis was the most common sign in 219 subjects evaluated, with a diameter less than 1 mm for 21 subjects, 1.5 mm for 87 subjects, 2 mm for 6 subjects, and 2.5 mm for 32 subjects during the first 2 days after exposure. A case series on 3 subjects with occupational exposures to sarin reported marked miosis during the initial examinations following exposure (within the first 24 hours) with “prolonged recovery” (recovery time not reported). Three days after exposure, the mean ratio of diameter of the pupil to diameter of the iris was 52.1% of control values, which increased to 69.8% of control values by 6 days following exposure (Sidell 1974) (see **Figure D13**).

In addition to miosis and pupil-related ocular effects, the available studies consistently reported other visual or ocular effects (e.g., visual-field abnormalities, conjunctival hyperaemia, etc.) in subjects 1–7 days after acute exposure to sarin; however, the data were more heterogeneous and in many cases

based on self-reported symptoms. Morita *et al.* (1995) reported that 124 of 219 patients (57%) had decreased visual acuity with miosis. Some subjects showed concentric defects that recovered within a few days despite continued miosis, and examinations revealed decreased amplitude of accommodation which recovered within several days. Thirty-nine of 219 patients (18%) also complained of visual-field abnormalities. Nohara and Segawa (1996) reported qualitatively that conjunctival hyperaemia and concentric contraction of the visual fields were common within the first 4 days following the acute exposure; but these conditions generally improved. Contrary to Morita *et al.* (1995), visual acuity did not appear to be diminished in most subjects (Nohara and Segawa 1996). At 1 week following the Tokyo subway sarin attack, Ohtomi *et al.* (1996) reported that 33 of 62 hospital patients (53%) reported blurred vision (compared to 87% of the 62 patients initially after exposure), 28 of 62 patients (45%) reported ocular and periorbital pain (76% initially), and 16 of 62 patients (26%) reported ciliary and conjunctival congestion (87% initially). In addition, some patients still reported decreased intraocular pressure (15% at 1 week; 48% initially), ocular irritation (13% at 1 week; 58% initially), visual field abnormality (13% at 1 week; 31% initially), and dim vision (10% at 1 week; 87% initially)(Ohtomi *et al.* 1996).

These case reports/series provide a consistent pattern of findings that miosis and other visual or ocular parameters persist 1–7 days after acute exposure to sarin. Other ocular and visual effects were not examined or reported in all studies, but additional ocular and visual effects beyond miosis are consistently reported to persist over the first week following exposure. The limitations of the case reports/series include the risk-of-bias concerns discussed above and small sample sizes in two of the studies. However, risk of bias was not considered serious since two of the five studies (Sidell 1974, Sekijima *et al.* 1995) were assigned a rating of probably low risk of bias for outcome assessment since hospital tests would not likely be biased based on knowledge of exposure.

Effects in the intermediate period after exposure

The results from eight case series (2,916 total subjects) and two cross-sectional studies (n = 36, assuming both studies report on the same participants) provided consistent results for recovery of miosis within the first 2 months after exposure, the persistence of other ocular and visual effects (e.g., ocular pain, blurred vision) in some of the study subjects, for weeks to at least months after exposure, and for slower VEPs 6–8 months following exposure. The studies that followed sarin-exposed subjects diagnosed with miosis beyond the first 1–2 weeks reported complete recovery or stabilization within 1–2 months (Sidell 1974, Rengstorff 1985, Morita *et al.* 1995, Ohtomi *et al.* 1996). The findings on miosis from the case series include 5 occupationally-exposed subjects, 62 individuals involved in the Tokyo sarin attack, and 4–15 subjects involved in the Matsumoto attack.

The majority of visual and ocular symptoms reported were generally reduced from 3 weeks to at least four months following acute sarin exposure; however, small percentages of individuals involved in the Tokyo and Matsumoto sarin attacks reported one or more remaining ocular and visual symptoms during this time period. Rengstorff (1985) reported that visual acuity and amplitude of accommodation improved within 3–5 weeks in two subjects accidently exposed to sarin. Nakajima *et al.* (1998) reported that 3 weeks after exposure, blurred vision persisted in 25% of the 87 subjects who reported blurred vision immediately after the terror attack. In addition, blurred vision was reported by Ohtomi *et al.* (1996) in 17 of 62 subjects (27%) at four weeks, by Ohtomi *et al.* (1996) and Ogawa *et al.* (1999) in 10 of 62 subjects (16%) at eight weeks and 44 of 681 subjects (6.5%) at 2 months, respectively, by Ohtomi *et al.* (1996) in 5 of 62 subjects (8.1%) at 3 months, and by Nakajima *et al.* (1998) in 5 of 105 subjects (4.8%) at 4 months after exposure. Nakajima *et al.* (1998) reported that 3 weeks after exposure, ocular pain persisted in 21% of the 114 subjects who reported ocular pain immediately after the terror attack.

Ocular pain was also reported by Okudera (2002) in 14 of 155 subjects (9.0%) at 3 weeks, by Ohtomi *et al.* (1996) in 9 of 62 subjects (15%) at four weeks, by Ohtomi *et al.* (1996) and Ogawa et al. (1999) in 6 of 62 subjects (9.7%) at eight weeks and 31 of 681 subjects (4.6%) at 2 months, respectively, by Ohtomi *et al.* (1996) in 10 of 62 subjects (16%) at 3 months, and by Nakajima et al. 1998 in 4 of 105 subjects (3.8%) at 4 months after exposure. Other visual or ocular effects that persisted in small percentages of subjects from 3 weeks to at least four months after exposure include dimness of vision, eye irritation or weakness, increased lacrimation, ciliary and conjunctival congestion, and visual field abnormalities including constricted or narrowing of visual field, darkness of visual field, flickering of vision, and double vision (Ohtomi *et al.* 1996, Nakajima *et al.* 1998, Ogawa *et al.* 1999, Okudera 2002). The gradual decline in these other reported visual and ocular effects in subjects involved in the Tokyo and Matsumoto sarin attacks also supports recovery over several months following exposure, although some of subjects continued to report symptoms beyond four months (**Table 10.**). In the only two studies with controls that reported effects less than 1 year after acute sarin exposure, VEPs were found to be significantly ($p<0.05$) slower in sarin cases compared to unexposed controls 6 to 8 months following exposure (Murata *et al.* 1997, Yokoyama *et al.* 1998a).

These case series and cross-sectional studies provide a consistent pattern of findings that miosis persists past 1 week and recovers within 1–2 months after exposure, other visual or ocular parameters (e.g., ocular pain, blurred vision) generally recover but persist in small percentages of subjects from 3 weeks to at least 4 months after exposure, and that slower VEPs persist 6–8 months following acute exposure to sarin. The limitations of the case series include the risk-of-bias concerns discussed above. The two cross-sectional studies had little potential for bias, but they apparently reported on the same subjects.

Effects in the extended period after exposure

The results from four case reports/series (621 total subjects) and one prospective cohort (n = 1,237) provide evidence of the persistence of other ocular and visual effects (e.g., ocular pain, blurred vision) in participants for 1–5 years after exposure. Nakajima *et al.* (1999) found that blurred vision, asthenopia, and narrowing of visual field were significantly higher among sarin victims (those diagnosed with, or reporting, sarin symptoms immediately after the attack) than non-victims (those not reporting symptoms immediately after exposure) at 3 years after the Matsumoto sarin attack. Small percentages of participants reported blurred vision, asthenopia, and narrowing of visual field 1 year after the sarin attack as well, but the prevalence was not statistically different compared to controls. Four case reports/series reported visual or ocular symptoms in exposed subjects 1, 2, 3, and 5 years after sarin exposure in the Tokyo and Matsumoto attacks (Sekijima *et al.* 1997, Kawana *et al.* 2001, Ohtani *et al.* 2004, Okumura *et al.* 2005). In all of the studies, symptoms were self-reported via survey. Okumura *et al.* (2005) reported that 56 of 303 subjects (18.5%) reported eye symptoms 1 year after the Matsumoto attack. Sekijima *et al.* (1997) reported on 1 subject following the Matsumoto attack who reported visual field defects 1 year following the sarin exposure with complete recovery by 17 months. Kawana *et al.* (2001) reported higher rates of eye symptoms (tiredness of eyes, dim vision, difficulty focusing, difficulty seeing distance) compared to other physical symptoms at 2, 3, and 5 years after exposure. However, the authors suggested the physical symptoms may be related to post-traumatic stress disorder. Ohtani *et al.* (2004) reported eye effects in 34 victims of the Tokyo subway attack by questionnaire 5 years after the incident, which included difficulty focusing (n = 23 of 34; 68%), blurred vision (n = 20 of 34; 59%), eyes tend to become easily tired (n = 19 of 34; 56%), difficulty seeing far (n = 17 of 34; 50%), difficulty seeing nearby objects (n = 13 of 34; 38%), eye mucus (n = 11 of 34; 32%), feeling of a foreign object in the eye (n = 9 of 34; 26%), and other eye symptoms (n = 2 of 34; 5.9%).

These case reports/series and prospective cohort study provide a consistent pattern of findings that visual and ocular parameters (e.g., blurred vision, difficulty focusing) persist in subjects from 1–5 years after exposure. The limitations of the case reports/series and prospective cohort include the risk-of-bias concerns discussed above, which include failure to control for confounding and potential biases in outcome assessment from self-reporting of symptoms via questionnaires, as well as loss of subjects over time.

Collectively, epidemiological studies provide evidence that acute sarin exposure is associated with miosis from 1–7 days after exposure with complete recovery in most cases from 1–2 months after exposure, slower VEPs 6–8 months following exposure, and the persistence of other ocular and visual effects (e.g., ocular pain, blurred vision, difficulty focusing) in small percentages of subjects from days to several years after exposure. The data present a consistent pattern of findings with different levels of confidence depending on the time period after exposure. The majority of studies are from case reports/series, which results in less confidence in the association between sarin and visual effects. There is moderate confidence in the body of evidence in the initial period based on the consistency of the evidence of miosis from case reports/series, moderate confidence in the body of evidence in the intermediate period based on two cross-sectional studies, and very low confidence in the body of evidence in the extended period based on one prospective cohort study and four case reports/series.

Animal Visual and Ocular Data

Summary: There is very low confidence in the body of evidence that acute sarin exposure is associated with visual or ocular effects in animals over the intermediate and extended periods. The animal body of evidence consists of three studies (Mioduszewski *et al.* 2002, Gore *et al.* 2012, Egoz *et al.* 2017) that evaluated pupil diameter over the initial period of 1–7 days (Mioduszewski *et al.* 2002, Gore *et al.* 2012, Egoz *et al.* 2017) and one study (Kassa *et al.* 2001a) that evaluated visual functional observational battery (FOB) scores 3–12 months after acute sarin exposure. In the initial period of 1–7 days following exposure, two studies found no sarin-related ocular effects (Gore *et al.* 2012, Egoz *et al.* 2017). One study did observe a sarin-related effect on pupil diameter in the initial period following exposure (Mioduszewski *et al.* 2002); however, the pattern of effect (i.e., a reduction in pupil diameter during the first hour followed by an increase above normal after 24 hours which mostly diminished by 7 days after exposure) was not consistent with other animal data or the human data (see **Figure D14**). In the intermediate and extended periods following exposure, one study explored visual parameters 3–12 months after an acute exposure and reported no effect in visual FOB scores (Kassa *et al.* 2001a). There are limitations in the body of evidence, including a small number of available studies and risk-of-bias concerns. For the initial time period, the initial rating of high confidence for the animal body of evidence was downgraded once for unexplained inconsistencies. Although it is recognized that study results differed by strain and method of exposure, there are too few studies to definitively explain the inconsistency in results. Overall, because two of three studies in the initial period did not observe sarin-related ocular effects after 24 hours following exposure, and the pattern of effect observed in the one study that reported an ocular effect after 24 hours did not correspond with other animal data or the human data, the body of evidence for the initial time period following acute sarin exposure is considered inadequate to evaluate whether sarin exposure is associated with ocular effects. For the intermediate and extended periods, the initial high confidence ratings for the animal body of evidence were downgraded twice for very serious risk of bias including lack of randomization, lack of blinding of outcome assessors, lack of information regarding methods for inhalation exposure, and the use of sarin with 90% purity without providing information on the remaining 10% to indicate that there were no impurities that could affect the results. Confidence in the body of evidence for intermediate and extended periods were also downgraded once for the inability to evaluate consistency based on a single

study available to support a final confidence rating of very low for the intermediate and extended periods (see **Table 10**).

There are four animal studies in the animal body of evidence that evaluated the association between acute sarin exposure and long-term ocular effects (Kassa *et al.* 2001a, Mioduszewski *et al.* 2002, Gore *et al.* 2012, Egoz *et al.* 2017). All studies were conducted in rats but in different strains. Three studies evaluated ocular effects over the initial period of 1–7 days following sarin exposure (Mioduszewski *et al.* 2002, Gore *et al.* 2012, Egoz *et al.* 2017). All three studies evaluated changes in pupil diameter but differed by the strain of rats used and methods of exposure (two male Long-Evans rat topical exposure studies and one male and female Sprague-Dawley rat inhalation study). One study evaluated ocular effects in male albino SPF rats 3, 6, and 12 months following a single inhalation exposure to sarin (Kassa *et al.* 2001a). Effects included pupil size, pupillary response to light, endo-exophthalmos, palpebral closure, or lacrimation as part of a FOB designed to evaluate behavioral and neurophysiological function in exposed animals (Kassa *et al.* 2001a).

Overall risk-of-bias discussion of body of evidence
Confidence in the body of evidence for the animal study that evaluated ocular effects over the intermediate and extended periods (Kassa *et al.* 2001a) was downgraded because of very serious risk-of-bias concerns (see **Figure D19** and **Figure D20**). Risk-of-bias concerns included a lack of (or lack of reporting of) randomization of animals, lack of information regarding methods for inhalation exposure, lack of blinding of outcome assessors, and the use of sarin with 90% purity without providing information on the remaining 10% to indicate that there were no impurities that could affect the results. In addition, details on how the pupil diameter measurements were taken as part of the FOB were not reported. Although there were some risk-of-bias concerns in the animal studies that evaluated ocular effects over the initial period following exposure, including a lack of (or lack of reporting of) randomization of animals and lack of blinding of outcome assessors in the inhalation study, the concerns were not considered serious enough to downgrade the confidence in the body of evidence for the initial period following exposure.

Effects in the initial period after exposure
Three experimental studies in rats examined sarin-related effects on pupil diameter within the first 7 days after sarin exposure and reported inconsistent results. Mioduszewski *et al.* (2002) evaluated pupil diameter in male and female Sprague-Dawley rats (n = 10/sex/treatment group) prior to and up to 7 days after a single inhalation exposure to sarin of varying durations. Pupil constriction was evident within the first hour of exposure. This was followed by increased pupil size to almost pre-exposure size by 24 hours and continued dilation above pre-exposure levels by day 2 (see **Figure D14**). The expansion in pupil diameter was reduced but was still larger than pre-exposure size by day 7, and the effect was observed at all time points and was dose dependent (Mioduszewski *et al.* 2002). The exposure levels used in the study were selected for the purpose of deriving an LC50, and high incidences of sub-lethal signs of toxicity were often observed (Mioduszewski *et al.* 2002). Exposures and associated effects on pupil size may, therefore, not be directly comparable to those in humans. Gore *et al.* (2012) and Egoz *et al.* (2017) evaluated pupil diameter in male Long-Evans rats (n = 12/treatment group) prior to and up to 7 days after a single topical sarin exposure of 1 µg (Egoz *et al.* 2017) or varying topical sarin exposures ranging from 0.002 to 10 µg (Gore *et al.* 2012). Gore *et al.* (2012) and Egoz *et al.* (2017) also observed a pupil constriction during the first few hours after exposure; however, both studies observed a return to baseline or close to baseline by 24 hours without further changes in pupil diameter beyond 24 hours.

Effects in the intermediate period after exposure
There was only one study that evaluated ocular effects in rats (male albino SPF rats; n = 10/treatment group) 3 months after a single exposure (Kassa *et al.* 2001a). The study found no effects on pupil size, pupillary response to light, endo-exophthalmos, palpebral closure or lacrimation based on FOB scores after a single inhalation exposure up to 2.5 µg/L. Endpoints were scored as part of an FOB designed to evaluate behavioral and neurophysiological function in exposed animals.

Effects in the extended period after exposure
There was only one study that evaluated ocular effects in rats (male albino SPF rats; n = 10/treatment group) at 6 and 12 months after a single exposure (Kassa *et al.* 2001a). The study found no effects on pupil size, pupillary response to light, endo-exophthalmos, palpebral closure or lacrimation based on FOB scores after a single inhalation exposure up to 2.5 µg/L. Endpoints were scored as part of an FOB designed to evaluate behavioral and neurophysiological function in exposed animals.

Integration of Evidence for Visual and Ocular Effects
There is evidence that pupil size is reduced (i.e., miosis) in humans 1–7 days after acute exposure to sarin, VEPs are reduced 6–8 months after acute exposure, and other visual and ocular effects (e.g., blurred vision, ocular pain, difficulty focusing) persist in humans during the first week and remain for several months to years after exposure. Although there are limitations in the body of evidence in the initial period largely due to study design (i.e., case reports/series only), there is moderate confidence in the human data in the initial period following acute sarin exposure based on the consistent pattern of findings that miosis occurs 1–7 days after exposure with data supporting miosis persisting for the first several weeks. Across all time points, the evidence for other visual or ocular effects is less consistent due to the limited data and considerable heterogeneity between studies on the visual or ocular parameters that were measured. There is moderate confidence for sarin-associated reductions in VEPs 6–8 months after acute exposure in humans based on two cross-sectional studies with little potential for bias; however, the two studies are presumed to have reported on the same subjects. There is very low confidence in the persistence of other visual or ocular effects in the extended period after exposure based on one perspective cohort and four case reports/series due to risk-of-bias concerns.

There is very low confidence in the animal data for the intermediate and extended periods following exposure based on one animal study that evaluated ocular effects (e.g., pupil size, pupillary response to light) and did not find evidence of an effect. Although a decrease in pupil diameter from 1–7 days was consistently observed in the human data, two experimental animal studies found no effect on pupil diameter beyond 24 hours after exposure, and one experimental animal study observed an increase in pupil diameter from 1–7 days following exposure. Therefore, the animal body of evidence in the initial period following exposure is inadequate to evaluate potential sarin-related effects based on the limited number of studies and no evidence of an effect that corresponds with the human data. These confidence ratings for human and animal bodies of evidence translate directly into level-of-evidence conclusions and support an initial hazard identification conclusion based on the different times as detailed below.

Effects initial period after exposure

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Animal body of evidence:** Moderate Confidence with no discernible effect = Inadequate Level of Evidence

- **Initial hazard conclusion (Low Human x Inadequate Animal)** = Suspected to be a Neurological Hazard to Humans

- **Final hazard conclusion[4] for the initial period (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to Humans

Effects intermediate period after exposure

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence

- **Animal body of evidence:** Very Low Confidence = Inadequate Level of Evidence

- **Initial hazard conclusion (Moderate Human x Inadequate Animal)** = Suspected to be a Neurological Hazard to Humans

- **Final hazard conclusion for the intermediate period (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to Humans

Effects extended period after exposure

- **Human body of evidence:** Very Low Confidence = Inadequate Level of Evidence

- **Animal body of evidence:** Very Low Confidence = Inadequate Level of Evidence

- **Initial hazard conclusion (Inadequate Human x Inadequate Animal)** = Not Classifiable

- **Final hazard conclusion for extended period (after consideration of biological plausibility)** = Not Classifiable

[4] Erratum: The final hazard conclusion for visual and ocular effects in the initial period (after consideration of biological plausibility) was changed from “Not Classifiable” to “Suspected to be a Neurological Hazard to Humans” on December 19, 2018.

Table 10. Visual and Ocular Evidence Profile for Sarin

INITIAL CONFIDENCE for each body of evidence (# of studies)	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
Human										
Initial period – Initial Low (5 case reports/case series)[a]	---	---	---	---	---	---	---	---	↑	**Moderate**
Intermediate period – Initial Moderate (2 cross-sectional studies)[c]	--	--	--	--	--	--	--	--	--	**Moderate**
Intermediate period – Initial Low (8 case reports/case series)[b]	↓	---	---	---	---	---	---	---	---	**Very Low**
Extended period - Initial Moderate (1 prospective cohort)[d]	↓↓	---	---	---	---	---	---	---	---	**Very Low**
Extended period - Initial Low (4 case reports/case series)[e]	↓	---	---	---	---	---	---	---	---	**Very Low**
Animal										
Initial period - Initial High (3 mammal studies)[f]	---	↓	---	---	---	---	---	---	---	**Moderate**
Intermediate period- Initial High (1 mammal study)[g]	↓↓	↓	---	---	---	---	---	---	---	**Very Low**
Extended period- Initial High (1 mammal study)[g]	↓↓	↓	---	---	---	---	---	---	---	**Very Low**

References:

Human: Kawana *et al.* (2001)[e], Morita *et al.* (1995)[a,b], Murata *et al.* (1997)[c], Nakajima *et al.* (1998)[b], Nakajima *et al.* (1999)[d], Nohara and Segawa (1996)[a,b], Ogawa *et al.* (1999)[b], Ohtani *et al.* (2004)[e], Ohtomi *et al.* (1996)[a,b], Okudera (2002)[b], Okumura *et al.* (2005)[e], Rengstorff (1985)[b], Sekijima *et al.* (1995)[a], Sekijima *et al.* (1997)[e], Sidell (1974)[a,b], Yokoyama *et al.* (1998a)[c]

Animal: Egoz *et al.* (2017)[f], Gore *et al.* (2012)[f], Kassa *et al.* (2001a)[g], Mioduszewski *et al.* (2002)[f]

Learning, Memory, and Intelligence

Acetylcholine is a major neurotransmitter involved in learning, memory, and intelligence (Chen 2012, Gais and Schonauer 2017). AChE is responsible for the breakdown of acetylcholine in the synapses of nerve cells. As described earlier in this report, organophosphates including sarin inhibit AChE, which disrupts cholinergic neurotransmission (Lee 2003, Chen 2012). This inhibition leads to increased levels of synaptic acetylcholine and subsequent cholinergic hyperstimulation. Because sarin interacts with the cholinergic pathway, based on mechanism alone, there is an expectation that sarin may impact a variety of behavioral measures. Evidence presented by Chen (2012) suggests that exposure to sarin results in secondary neuronal damage in the cholinergic regions of the brain, and this secondary damage is thought to be a major contributor to neurological impairments related to memory and other cognitive functions.

Learning, memory, and intelligence are considered related cognitive functions; therefore, endpoints related to these cognitive functions in humans and animals are discussed together in the section. Tests in humans that are specific for learning (e.g., California Verbal Learning Test [CVLT]), memory (e.g., digit span, self-reported memory loss, memory function tests), and intelligence (e.g., Wechsler Adult Intelligence Scale [WAIS-III]) are considered relevant. Other tests that include a learning or memory component (e.g., digit symbol test, Thurstone word fluency test, Boston naming test) are also considered. For animals, studies that assess maze performance and discrimination learning activities are included. The differential-reinforcement-of-low-rate (DRL) test measures various components of cognition of which short-term memory is only a small portion. It also involves vigilance, patience, time estimation, excitability of the animal, etc. This test is not discussed here, although it is recognized that it might provide some supporting data. It is also recognized that lack of attention or concentration is a symptom in humans or animals that could affect learning and memory, but these endpoints are not considered in this section, as they were not specifically evaluated in relation to learning and memory issues.

Human Learning, Memory, and Intelligence Data

***Summary*:** There is low confidence in the body of evidence that acute sarin exposure is associated with impairments to learning, memory, and intelligence in humans over the intermediate period of 8 days to 1 year after exposure and moderate confidence in the body of evidence for the extended period of greater than 1 year after exposure. The studies that provide memory data for the intermediate period, including one cross-sectional study (Yokoyama *et al.* 1998c) and two case reports (Sekijima *et al.* 1995, Loh *et al.* 2010), demonstrated some effect on memory or cognitive function, but there is no consistency in the endpoints measured across studies. The studies that provide memory data for the extended period, including two case series studies (Kawana *et al.* 2001, Ohtani *et al.* 2004) and two cross-sectional studies (Nishiwaki *et al.* 2001, Miyaki *et al.* 2005), report evidence of lingering effects on memory and cognitive function years after sarin exposure using different tests for evaluating memory and cognitive function. Although results show a pattern of findings of impaired learning, memory and intelligence for a period of weeks to years following acute sarin exposure, there are limitations in the body of evidence including risk-of-bias concerns and uncertainties related to study design of case reports. There is low confidence in the body of evidence for the intermediate period following acute sarin exposure based on one cross-sectional study with a small sample size (n = 18) and two case report studies. None of the studies were downgraded for risk-of-bias concerns. The final rating of low confidence in the intermediate period was supported by heterogeneity of the endpoints evaluated, small sample sizes, and the small number of available studies. Moderate confidence in the body of evidence for the extended period following acute sarin exposure is primarily based on the two cross-sectional studies, which had an initial and final confidence of moderate with support from two case report studies. The

two case reports had an initial low confidence rating which was downgraded to very low confidence for serious risk-of-bias concerns (i.e., failure to control for confounding, potential biases in outcome assessment from self-reporting of symptoms, and few of the initial subjects responded or were included in the study). For the initial period covering 1–7 days following acute sarin exposure, no studies were available; therefore, the body of evidence for this time period is considered inadequate to evaluate whether acute sarin exposure is associated with impairments to learning, memory, and/or intelligence (see **Table 12**).

The available epidemiological studies in the human body of evidence that evaluated the association between acute exposure to sarin and effects on learning, memory, and intelligence generally evaluated the outcomes months to years after the initial exposure (see **Table 11**). There are no studies that specifically evaluated these outcomes in the initial period of 1–7 days after exposure. The majority of studies were conducted in adults who were exposed during the terrorist attack on the Tokyo subway. One case report study reports on an individual exposed during the Matsumoto city attack, and one case report study reports on a military man exposed while disarming an improvised explosive device (IED) containing sarin. Studies used different methods for determining effects on learning, memory, and intelligence (e.g., self-reported memory effects as well as memory and other cognitive function tests). Although it cannot be known if the same subjects participated in more than one study evaluating the Tokyo subway attack, there are a couple of studies that focused on subway workers and rescue personnel. This would indicate that these subjects are different from the studies evaluating the hospital victims.

Table 11. Studies on Learning and Memory Functions in Humans

Study	Study design (Location/Study) [n]	Exposure measure timing	Assessment timing	Analysis	Learning/memory outcome summary
Initial time period after exposure (>24 hours–7 days)					
No studies available.					
Intermediate time period after exposure (8 days–1 year)					
Sekijima *et al.* (1995)	Case report (Japan/Matsumoto city) [1 19-year old man]	Terrorist attack, single exposure (not measured)	10 days	Not reported	Forgetfulness persisted until the 10th day
Yokoyama *et al.* (1998c)	Cross-sectional (Japan/Tokyo subway system attack victims) [33]	Terrorist attack, single exposure (not measured)	6 to 8 months	Forward, backward digit span test, paired-associate learning, digit symbol, picture completion	Digit symbol test score significantly lower in sarin cases than in controls; no significant differences in digit span test, paired-associate learning, and picture completion scores

Table 11. (continued)

Study	Study design (Location/Study) [n]	Exposure measure timing	Assessment timing	Analysis	Learning/memory outcome summary
Loh *et al.* (2010)	Case report (U.S. Military) [1]	Disarming an IED containing colorless liquid determined to be sarin, subject had decreased RBC ChE and symptoms	8 months	Wechsler memory scale-III, Rey complex figure recall T-scores, self-reported symptoms of memory loss WRAT-III reading test; Boston naming test; Thurstone verbal fluency test; WAIS-III IQ test, PSI, and subtest scaled-arithmetic; California Verbal Learning Test	Self-reported short-term memory loss; although was not noted to have an impairment in any of the memory scores, subject was noted to have impaired recall of words and numbers Decreased verbal fluency T-score; reduced WAIS-III PSI; impaired WAIS-III subtest scaled-arithmetic score; impaired CVLT performance; no IQ impairments/ inefficiencies
Extended time period after exposure (>1 year)					
Kawana *et al.* (2001)	Case series (Japan/ Tokyo subway system attack victims) [582]	Terrorist attack, single exposure (not measured)	2, 3, and 5 years	Self-reported difficulty with memory Of 582 (St. Luke's Hospital), 283 questionnaires received in 1997, 206 in 1998, and 191 in 2000. % incidence	1997–11.7%; 1998–11.2%; 2000–12.6% Data from other cohorts provided for comparison: 24.3% (Tokyo NGO), 19.5% (Matsumoto victims), 12.6% (Matsumoto controls)
Ohtani *et al.* (2004)	Case series (Japan/ Tokyo subway system attack victims) [34]	Terrorist attack, single exposure (not measured)	5 years	Self-reported forgetfulness count of subjects reporting symptom	Severity of self-reported forgetfulness: 18 none; 14 mild; 2 severe

Table 11. (continued)

Study	Study design (Location/Study) [n]	Exposure measure timing	Assessment timing	Analysis	Learning/memory outcome summary
Miyaki *et al.* (2005)	Cross-sectional (Japan/Tokyo subway system attack victims–subway workers) [36] Cross-sectional (Japan/Tokyo subway system attack victims–subway workers, rescue staff, and police) [145]	Terrorist attack, single exposure (not measured)	7 years 3 years (rescue staff, police); 7 years (subway workers)	Memory function tests (forward, backward digit span test; Benton visual memory retention test)	Exposed group performed less well on memory function tests; differences not significant likely due to small number of subjects; results suggest causal relationship between sarin exposure and memory function; ORs were generally increased, but had large 95% CI
Nishiwaki *et al.* (2001)	Cross-sectional (Japan/Tokyo subway system attack victims–rescue staff and police) [106]	Terrorist attack, single exposure (not measured)	2 years, 10 months to 3 years, 9 months	Memory function tests (forward, backward digit span tests; Benton visual memory retention test)	Effects related to exposure suggested (although not significant) for digit span tests; dose-response increase in adjusted OR

Overall risk-of-bias discussion of the body of evidence

Confidence in the body of evidence for the human case series studies was downgraded because of serious concern for risk of bias. Risk-of-bias ratings for individual studies for all questions are available in **Figure D25** through **Figure D28**. There are a number of risk-of-bias issues in the evidence relating to design and conduct of individual studies as well as general limitations (i.e., not risk-of-bias issues) based on the case-report/series study design. The majority of the human studies were rated as probably high risk of bias for lack of blinding of outcome assessors, and two of the four studies had confounding issues.

As discussed previously for the studies on ChE, confounding and lack of blinding of outcome assessors were the principal risk-of-bias issues in the majority of studies, which were based on one of the two terror attacks or accidental exposures. The majority of studies do not address potential confounders for learning, memory, or intelligence effects. Blinding of outcome assessors was also a general limitation. For self-reported symptoms, blinding is not possible. The lack of addressing potential confounders and the lack of data on blinding at outcome assessment lead to the serious risk-of-bias concern for some time points and a single downgrade in the confidence in the body of evidence; however, for some time points these are not considered to be serious concerns, and the studies were not downgraded.

Lack of information or specific exposure measures was an issue in many of the studies, but this is not considered to pose a risk-of-bias concern. The victims of the terrorist attack are recognized as being exposed to sarin, although the levels of exposure or the proximity of the victims to the release of sarin were not reported. One study (Yokoyama *et al.* 1998c) was rated as definitely low risk of bias for exposure characterization, because all subjects were admitted to the hospital after the Tokyo subway attack and had signs and symptoms indicative of sarin exposure. The authors provided data indicating that subjects had decreased pupil diameter and serum ChE activity when they arrived at the hospital after the attack to provide supporting data on exposure and also stated that controls were not exposed

to sarin. Loh *et al.* (2010) provided data on ChE levels in the subject based on the subjects own baseline levels. Although the study did not report the purity of the sarin (i.e., if there were other anticholinesterases in IED), the decreased ChE and sarin identified in the IED are sufficient evidence of exposure. The other reports may have indicated that the subjects were hospitalized after the attack, but in many cases this was based on questioning of the victims and not from hospital records.

Effects in the initial period after exposure
There is no human evidence to evaluate effects on learning and memory at 1–7 days after exposure.

Effects in the intermediate period after exposure
Results from one cross-sectional study (33 subjects) and two case reports (one terrorist attack victim; one Army sergeant with accidental exposure) provided some evidence of impaired learning and memory during the intermediate period after sarin exposure, but the data are limited to a single significant test and a general memory issue in a case report (Loh *et al.* 2010) that was not supported by test results. Yokoyama *et al.* (1998c), a cross-sectional study, evaluated learning and memory function 6–8 months after exposure from the Tokyo subway attack. Eighteen patients who had been admitted to St. Luke's International Hospital compared with 15 unexposed individuals had a significantly lower digit symbol test score; however, the victims of the Tokyo subway attack did not have a significant decrease in memory function as measured by the digit span score. There were also no significant differences in paired-associate learning or picture completion scores for the victims versus controls. The confidence in the study is limited by a small sample size and only one of the three tests showing an effect. Two case reports (Sekijima *et al.* 1995, Loh *et al.* 2010) indicated that the two subjects had some memory loss during this time period. A 19-year old male with severe initial symptoms after the Matsumoto city terrorist attack had forgetfulness (not reported how determined) through 10 days after exposure (Sekijima *et al.* 1995). A 34-year-old male senior Army sergeant who was exposed to sarin when disarming an IED containing sarin complained of short-term memory loss 8 months after exposure (Loh *et al.* 2010). Although many of the memory tests did not observe levels that were considered impaired, the man was noted to have issues recalling words and numbers within minutes and had issues recalling words that began with the letter F. No IQ impairments or inefficiencies were noted for this individual; however, he demonstrated reduced speed of information processing and impaired performance on the CVLT, Thurstone verbal fluency test, and one of the WAIS-III verbal subtests. The results were generally considered within normal range, and there are no previous results in this subject for comparison. Although the human body of evidence suggests that acute sarin exposure may result in neurological impairments related to learning and memory in the intermediate period, there is generally low confidence in the body of evidence because of limitations such as small sample size, lack of overlap in endpoints evaluated, and lack of strong or consistent evidence of effect. Based on a single cross-sectional study with a single significant effect, and the case reports with either a presumably self-reported symptom of forgetfulness or results within standard normal ranges without a comparison, the human data are determined to have inadequate evidence for evaluating the effects of acute sarin exposure on learning, memory, and intelligence in the intermediate period.

Effects in the extended period after exposure
The results in the two case series studies (Kawana *et al.* 2001, Ohtani *et al.* 2004) and two cross-sectional studies (Nishiwaki *et al.* 2001, Miyaki *et al.* 2005), that evaluated learning and memory function in victims of the 1995 Tokyo subway sarin attack in the extended period of years after sarin exposure, provided more consistent evidence. However, again many different tests were used and results did not always achieve significance. Kawana *et al.* (2001) found 11–12% of subjects reported difficulty with memory 2–5 years after exposure. However, when the study authors compared results

from the 191 respondents in 2000 (at 5 years) to 87 controls identified from the Matsumoto sarin attack, they found a similar incidence of difficulty with memory 12.6% for both groups. However, there was no information provided to indicate that controls identified after the Matsumoto sarin attack would be appropriate to compare to the Tokyo subway victims. Ohtani *et al.* (2004) investigated the mental and somatic symptoms of 34 Tokyo subway system sarin attack victims 5 years after the attack and found that severity of forgetfulness was none for 18 cases, mild for 14 cases, and severe for 2 cases. In the two cross-sectional studies (Nishiwaki *et al.* 2001, Miyaki *et al.* 2005), the authors examined memory function in Tokyo subway sarin attack victims who were subway workers and rescue personnel (including police officers) at 3 or 7 years after exposure. Miyaki *et al.* (2005) reported that exposed subway workers (in 1998) and rescue personnel (in 2002) performed less well on memory function tests, although differences were not statistically significant (80 total exposed, 65 total referents). Similarly, Nishiwaki *et al.* (2001) evaluated memory function for 56 exposed rescue personnel who had worked at the disaster site compared with 52 referent subjects matched for age and occupation approximately 3 years after the attack. The investigators used the same memory function tests as Miyaki *et al.* (2005) and also found a suggested (but not statistically significant) relationship between sarin exposure and memory disturbance.

The case series studies had several limitations. Both studies were rated as probably high risk of bias for the key question regarding outcome assessment due to lack of blinding because all outcomes were self-reported and participants would have been aware of their exposure. One of the two studies (Kawana *et al.* (2001) was rated as probably high risk of bias for the key question regarding confounding (authors reported demographic information for survey respondents [gender ratio, age, employment status, and marital status]; however, this information was not accounted for when evaluating the symptoms) (see **Figure D25** and **Figure D26**). In addition, there were risk-of-bias concerns due to attrition in both case series, as no information was provided on the subjects that participated compared to those who did not, and few participated in either study (Kawana *et al.* (2001) received a 33% to 49% response rate depending on the assessment year, and Ohtani *et al.* (2004) had 34 out of 565 victims of the Tokyo subway attack that visited St. Luke's International Hospital participate). The cross-sectional studies were rated as probably high risk of bias for one key question—outcome assessment, due to lack of blinding for outcome assessors (see **Figure D27** and **Figure D28**).

Taken together, the epidemiological evidence suggests that acute sarin exposure is associated with impaired learning and memory in the intermediate period and extended period after sarin exposure. There are no human studies available to evaluate the potential association between sarin exposure and effects on learning and memory in the initial period after exposure.

Animal Learning and Memory Data

***Summary*:** There is moderate confidence in the animal body of evidence that acute sarin exposure affects learning and memory over the initial and intermediate time periods and low confidence in the extended time period. In rats, the results show consistent evidence of impaired learning and memory following acute sarin exposure across multiple studies and at different time periods following exposure. In monkeys, results were inconsistent and were of limited utility due to small sample sizes. There are limitations in the body of evidence, including small sample sizes and risk-of-bias concerns for the key risk-of-bias questions regarding randomization, exposure assessment, and outcome assessment. The initial high confidence ratings for the animal body of evidence were downgraded once for all time periods for risk-of-bias concerns. For the initial and intermediate time periods, confidence ratings for the animal body of evidence were also downgraded once for imprecision (due to wide ranges in confidence intervals and large standard deviations in the data) and upgraded once for evidence of dose response to

support a final rating of moderate confidence. The body of evidence for the animal studies in the extended time period was downgraded for inconsistency to support a final rating of low confidence (see **Table 12**).

There are nine experimental studies in the animal body of evidence that evaluated the association between acute exposure to sarin and effects related to learning and memory (see **Table A5-3** through **Table A5-5**). The heterogeneity in the behavioral tests and study design presented some challenges to evaluating the body of evidence. Health endpoints related to learning and memory in experimental animal studies included maze performance (using water maze, T maze, Y maze, and radial arm maze) and discrimination learning activities. The studies focused on rats (Sprague-Dawley or Wistar) or marmoset monkey. While the monkey studies used both sexes, the studies in rats all used male rats. While the rat studies administered sarin via inhalation, the monkey studies administered sarin via intramuscular injection. Doses and timing of outcome measure varied by study.

Overall risk-of-bias discussion of body of evidence
Confidence in the body of evidence for the animal studies was downgraded because of serious concern for risk of bias (see **Figure D29** and **Figure D30**). The main risk-of-bias concern with the animal studies was lack of reporting of important details for key risk-of-bias questions (i.e., questions regarding randomization, exposure assessment, and outcome assessment). Only one of the nine studies indicated that the animals were randomized to treatment (Grauer *et al.* 2008). None of the remaining eight studies provided details on randomization or how animals were assigned to treatment. While the majority of authors did not respond to inquiries on whether animals were randomized, Kassa *et al*. responded that the animals were not randomized to treatment. In one study in monkeys (Wolthuis *et al.* 1995), animals served as their own controls, but 5 of the 154 animals had already been trained on hand-eye coordination and had been injected once at least 2 months previously with other ChE inhibitors (stated to be highly reversible). The main limitation of the exposure characterization in the majority of the animal studies (five of nine) was lack of data on the purity of the sarin administered. Five studies administered sarin with ≥95% purity, but only two of the studies stated that the purity was verified and provided methods. Of the inhalation studies, Genovese *et al.* (2009) was the only study to provide data on chamber measurements. The Kassa *et al*. studies indicated target doses and provided information on symptoms and AChE levels to indicate the differences in the doses. Although correspondence with the study authors indicated that they measured the concentrations in the chamber, they were not able to provide us with the levels in the chambers. The information provided in the study indicated that the animals likely received different levels of sarin, but this cannot be confirmed. In addition, the study authors indicated that purity of the sarin was 90%. Memory-related endpoints were measured using acceptable methods in all studies; however, most of the endpoints can be subjective and none of the studies reported that the outcome assessors were adequately blinded to the study group. For five of the ten studies, however, blinding was not expected to appreciably bias the results because the tests were automated.

Effects in the initial period after exposure
Experimental studies in rats (Kassa *et al.* 2001b, Kassa *et al.* 2002, Kassa *et al.* 2004, Genovese *et al.* 2009) and common marmoset (Wolthuis *et al.* 1995, Pearce *et al.* 1999, Muggleton *et al.* 2003) found consistent sarin-related effects related to memory within the first week after sarin exposure. Results from three studies in rats for this time period suggest that there are learning and memory issues associated with sarin that can occur within the first week after the acute exposure (Kassa *et al.* 2002, Kassa *et al.* 2004, Genovese *et al.* 2009). In monkeys, results of discrimination learning tasks within 1 week following acute sarin exposure were inconsistent. In monkeys, the majority of the results did not

achieve statistical significance even if the results demonstrated a change from control. This is likely due to the small number of animals used (n = 2–5) and may also be related to potential differences by sex that were not controlled for with such small sample sizes. Genovese *et al.* (2009) exposed male rats to sarin vapor and evaluated performance on an operant conditioning task and radial-arm maze spatial memory task after 48 hours. Single sarin exposures did not affect performance on the VI56 and had little effect on acquisition of the radial-arm maze task. The only statistically significant results for the radial-arm maze task occurred during the first 5-block session (out of 11 total 5-block sessions), in which reference memory errors and working memory errors significantly increased in a dose-dependent manner. Kassa *et al.* studies (2001b, 2002, 2004) exposed male rats to sarin vapor and evaluated learning and spatial memory using a T-maze or Y-maze (see **Figure D21** and **Figure D22**). Kassa *et al.* (2001b) tested cognitive function using the T-maze and observed no significant effect on T-maze completion time at 1 week. Kassa (2002, 2004) evaluated spatial discrimination (time of reaction) using the Y-maze and observed a dose-dependent increase in reaction time at week 1, with a significant increase in reaction time at the highest dose.

In monkeys, Pearce *et al.* (1999) and Wolthuis *et al.* (1995) conducted discrimination performance tests and found no statistically significant learning deficits in the week following exposure. Conversely, using a discrimination serial reversal task, Muggleton *et al.* (2003) found improved reversal learning (i.e., statistically significant fewer mean errors) in monkeys following sarin administration at 0–12 days after exposure.

The body of evidence in rats suggests that acute sarin exposure results in neurological impairments related to learning and memory within the initial period after exposure; however, there was heterogeneity in the behavioral tests used across studies. Results in monkeys were inconsistent and were of limited utility due to small sample sizes.

Effects in the intermediate period after exposure

Experimental studies in the rat (Kassa *et al.* 2001b, Kassa *et al.* 2002, Kassa *et al.* 2004, Grauer *et al.* 2008, Allon *et al.* 2011) observed sarin-related effects related to learning and memory 2 weeks to 6 weeks after sarin exposure. Results from three studies in rats for this time period suggest that there are learning and memory issues associated with sarin that can last for weeks after the acute exposure (Kassa *et al.* 2002, Kassa *et al.* 2004, Grauer *et al.* 2008) (see **Figure D21** through **Figure D24**. In monkeys (Pearce *et al.* 1999, Muggleton *et al.* 2003), results of discrimination learning tasks after 1 week following acute sarin exposure were inconsistent.

Allon *et al.* (2011) exposed male rats to sarin vapor and evaluated latency to reach the platform in a water maze working/reference memory task. At 1 month following exposure, no significant differences between the groups were detected. Water maze acquisition of both control and exposed rats showed a decrease in latency to reach the platform, indicating no effect of sarin on working and reference memory. In another water maze study, Grauer *et al.* (2008) exposed male rats to sarin vapor and evaluated latency to reach the platform at 5 weeks. Sarin-exposed rats showed an increased latency to reach the platform, indicating that both working memory and reference memory were impaired; however, the statistical significance of these results is unclear. Kassa *et al.* studies (2001b, 2002, 2004) exposed male rats to sarin vapor and evaluated learning and spatial memory using a T-maze or Y-maze. Kassa *et al.* (2001b) tested cognitive function using the T-maze and observed no significant effect on T-maze completion time at week 2 through week 5. Kassa *et al.* (2002, 2004) evaluated spatial discrimination (time of reaction) using the Y-maze and observed a dose-dependent increase in reaction time at weeks 2 and 3, with significant increases in time of reaction at the highest dose. At 4–6 weeks,

times of reaction were more consistent among doses, and no significant results were observed at any dose.

In monkeys, Pearce *et al.* (1999) observed no deleterious effects on discrimination performance tasks (number of errors to reach criterion) at 2–6 weeks after exposure. As noted above, using a discrimination serial reversal task, Muggleton *et al.* (2003) found improved reversal learning (i.e., fewer mean errors) in monkeys following sarin administration (11.15 µg/kg) at 0–12 days after exposure.

Consistent with the initial period after exposure, the body of evidence in rats for the intermediate period suggests that there are learning and memory issues associated with sarin that can last for weeks after exposure; however, the evidence in monkeys was inconsistent and of limited utility due to small sample sizes.

Effects in the extended period after exposure

Results from the two experimental studies in the rat (Grauer *et al.* 2008, Allon *et al.* 2011) evaluating sarin-related effects related to working memory and reference memory 4 or 6 months after sarin exposure were inconsistent. Results from one of the two studies suggest that there are memory issues associated with sarin that can last for months after the acute exposure (Grauer *et al.* 2008) (see **Figure D23** and **Figure D24**). Allon *et al.* (2011) exposed male rats to sarin vapor and evaluated latency to reach the platform in a water maze working/reference memory task. At 6 months following exposure, the authors found no effect of sarin on working and reference memory. The lack of an effect of sarin by Allon *et al.* (2011) continues what the study found for the intermediate period. In another water maze study, Grauer *et al.* (2008) exposed male rats to sarin vapor and evaluated latency to reach the platform at 4 months and 6 months. At both long-period time points (as well as during the intermediate period), sarin-exposed rats showed an increased latency to reach the platform, indicating that both working memory and reference memory were impaired (statistical significance not indicated). The report of impaired memory by Grauer *et al.* (2008) continues what the study found at the intermediate time period.

Integration of Evidence for Learning, Memory, and Intelligence

There is evidence to suggest learning and memory impairments in humans and animals following acute exposure to sarin. In humans, evidence suggests lingering effects on learning and memory (with no clear evidence to suggest deficits in intelligence as measured by IQ) in the intermediate and extended periods after sarin exposure, with low to moderate confidence in the data. There is a low level of human evidence in the intermediate period that acute sarin exposure affects learning, memory, and intelligence due to the low confidence in the memory data, small sample size, and heterogeneity of the available data. In animals, there is moderate confidence that acute sarin exposure is associated with learning and memory effects in the initial and intermediate periods after exposure based on the consistency of the findings in rats. For the extended period, there were only two animal studies available, and the results were inconsistent between studies. Considering the entire body of evidence for animal studies (across initial, intermediate, and extended time periods), there is low confidence that acute sarin exposure affects learning and memory in rats in the extended time period as well. These confidence ratings translate directly into level-of-evidence conclusions and support an initial hazard identification conclusion of *suspected to be a neurological hazard to humans* at all time points.

Effects for the initial period after exposure

- **Human body of evidence:** No studies = Inadequate Level of Evidence
- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence

- **Initial hazard conclusion (Inadequate Human x Moderate Animal)** = Suspected to be a Neurological Hazard to Humans

- **Final hazard conclusion for the initial period (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to Humans

Effects for the intermediate period after exposure

- **Human body of evidence:** Low Confidence = Low Level of Evidence

- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence

- **Initial hazard conclusion (Low Human x Moderate Animal)** = Suspected to be a Neurological Hazard to Humans

- **Final hazard conclusion for the intermediate period (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to Humans

Effects for the extended period after exposure

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence

- **Animal body of evidence:** Low Confidence = Low Level of Evidence

- **Initial hazard conclusion (Moderate Human x Low Animal)** = Suspected to be a Neurological Hazard to Humans

- **Final hazard conclusion for extended period (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to humans

Collectively, the human and animal bodies of evidence provide consistent patterns of findings that acute exposure to sarin is associated with long-term issues with learning and memory. The human data are mainly based on cross-sectional studies evaluating subjects from the Tokyo subway attack. The animal data in rats support that an acute sarin exposure can affect memory in the initial, intermediate, and extended periods following exposure.

A mechanism by which organophosphates—and sarin in particular—could cause learning and memory effects has been suggested (Lee 2003, Chen 2012, Gais and Schonauer 2017) and could be related to secondary neuronal damage occurring in the cholinergic regions of the brain. While there are data to suggest that ChE levels are affected (increased and decreased) in different regions of the brain, there are not sufficient data to indicate that this was associated with neuronal damage to the cholinergic regions of the brain. One study (Yamasue *et al.* 2007) noted a decrease in regional white matter volume in victims from the Tokyo subway attack. The study, however, did not indicate that this was associated with damage in the cholinergic region of the brain or that the subjects were tested for learning, memory, or intelligence. Given this information, it is unlikely that there is sufficient mechanistic information to increase or change the hazard determination.

Table 12. Learning and Memory Evidence Profile for Sarin										
	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					**Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence**				
INITIAL CONFIDENCE for each body of evidence (# of studies)	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	FINAL CONFIDENCE RATING
Human										
Initial period (1–7 days)	No studies available.									**No rating**
Intermediate period - Initial Moderate (1 cross-sectional study)[a]	---	↓*	---	---	---	---	---	---	---	**Low**
Intermediate period - Initial Low (2 case reports)[b]	---	---	---	---	---	---	---	---	---	**Low**
Extended period - Initial Moderate (2 cross-sectional studies)[c]	---	---	---	---	---	---	---	---	---	**Moderate**
Extended period - Initial Low (2 case series)[d]	↓	---	---	---	---	---	---	---	---	**Very Low**
Animal										
Initial period - Initial High (7 mammal studies)[e]	↓	---	---	↓	---	---	↑	---	---	**Moderate**
Intermediate period - Initial High (7 mammal studies)[f]	↓	---	---	↓	---	---	↑	---	---	**Moderate**
Extended period - Initial High (2 mammal study)[g]	↓	↓	---	---	---	---	---	---	---	**Low**

References:
Human: Yokoyama *et al.* (1998c)[a], Loh *et al.* (2010)[b], Sekijima *et al.* (1995)[b], Nishiwaki *et al.* (2001)[c], Miyaki *et al.* (2005)[c], Ohtani *et al.* (2004)[d], Kawana *et al.* (2001)[d]
Animal: Allon *et al.* (2011)[f,g], Genovese *et al.* (2009)[e,], Grauer *et al.* (2008)[f,g], Kassa *et al.* (2001b)[e,f], Kassa *et al.* (2002)[e,f], Kassa *et al.* (2004)[e,f], Muggleton *et al.* (2003)[e,f], Pearce *et al.* (1999)[e,f], Wolthuis *et al.* (1995)[e]

* The body of evidence was downgraded for inability to evaluate consistency based on the single cross-sectional study (Yokoyama et al. 1998c) with small sample size (n = 18), positive results in a single test, and no other study characteristics that would provide confidence in the effect such as large magnitude or dose response, etc.

Nervous System Morphological and Histological Changes

Morphological or histological changes in neural tissue are direct measures of neurological damage. It is important to note that the types of pathology observed in the human nervous system are readily modeled in experimental mammalian models. There is a general paucity of relevant human data, in part because the resources required, such as magnetic resonance imaging (MRI) or positron emission tomography (PET), are not routinely available for assessing neurological damage in living individuals, and histopathological analyses, which can only be performed after death, are difficult to conduct in such a way as to obtain high quality data. However, there are some human studies available that examined morphological and histological changes in nervous system tissues (including brain, spine, and sural nerve) of subjects who were accidently exposed to sarin during a military operation (low-level sarin exposure) or during the Tokyo subway attack. Despite the small number of studies, any human data on this endpoint are insightful and therefore have been assessed in this report. Changes to muscle tissue are not considered in this section because the focus of the section is neurological effects; however, it should be noted that any muscle effects could also be related to some of the neuromuscular effects observed.

Human Morphological and Histological Data

***Summary*:** There is moderate confidence in the body of evidence that acute sarin exposure is associated with morphological and histological changes in human neurological tissues in the extended period. The human body of evidence consists of two case reports (Himuro *et al.* 1998, Loh *et al.* 2010) and one cross-sectional study (Yamasue *et al.* 2007) (see **Table 13** and **Table 14**) that evaluate effects months to years after sarin exposure. None of the studies provide data on morphological and histological changes over a period of days or weeks after exposure. A single case report was available that evaluated morphological or histological changes in neurological tissue at 8 months following sarin exposure but found no abnormalities during an MRI examination of the brain and spine (Loh *et al.* 2010). The studies that provide data on morphological and histological changes in nervous tissue more than 1 year after exposure, including one cross-sectional (Yamasue *et al.* 2007) and one case report (Himuro *et al.* 1998), report evidence of morphological and histological changes to human nervous system components following acute sarin exposure. The moderate confidence in the body of evidence is based mainly on the cross-sectional study with an initial and final confidence of moderate and support from one case report. While the case report had an initial and final rating of low confidence due to general limitations based on the case report study design (e.g., mainly not having a control for comparison), the study assessed damage to the brain against a "normal" standard, which could potentially increase the confidence in the case reports. There are inadequate data to assess the relationship between sarin and morphological changes in the initial and intermediate time periods after exposure due to the lack of data available.

The available epidemiological studies in the human body of evidence that evaluated the association between acute exposure to sarin and morphological and histological changes in the human nervous system tissues evaluated the outcomes months to years after the initial exposure (see **Table 13**). There are no studies that specifically evaluated morphological and histological changes in the human nervous system within days or weeks after exposure. Two studies (one cross-sectional and one case report) were conducted in adults who were exposed during the Tokyo subway sarin terrorist attack. The third study was a case report of a military man deployed in Iraq who was exposed while disarming an IED containing sarin.

Table 13. Studies on Morphological and Histological Changes to Nervous System Tissues in Humans					
Study	**Study design (Location/Study) [n]**	**Exposure measure timing**	**Assessment timing**	**Analysis**	**Morphological/ histological outcome summary**
Initial time period after exposure (>24 hours–7 days)					
No studies available.					
Intermediate time period after exposure (8 days–1 year)					
Loh *et al.* (2010)	Case report (U.S. Military) [1]	Disarming an IED containing colorless liquid determined to be sarin, subject had decreased RBC ChE and symptoms	8 months	Magnetic Resonance Imaging (MRI) of the brain and spine	MRI examination of brain and spine was normal
Extended time period after exposure (>1 year)					
Himuro *et al.* (1998)	Case report (Japan/Tokyo subway system attack victim) [1]	Terrorist attack, single exposure (not measured)	15 months (man died 15 months after attack without regaining consciousness)	Autopsy (pathologic examination)	In sural nerve, severe reduction in both large and small myelinated fibers with preferential loss of large myelinated fibers; in spinal cord, total loss of myelinated fibers in the white matter and severe neuronal loss in the central gray matter; no changes observed in dorsal root ganglia, dorsal roots, posterior column of the spinal cord; in brain, severe hypoxic-ischemic encephalopathy
Yamasue *et al.* (2007)	Cross-sectional (Japan/ Tokyo subway system attack victims) [38 victims +76 controls]	Terrorist attack, single exposure (not measured)	5–6 years	Diffusion tensor MRI; voxel-based morphometry	Reduced regional gray matter volume in the right insular and temporal cortices; significant regional gray matter volume reduction in left hippocampus; significant regional white matter volume reduction in left temporal stem close to the insular cortex; negative correlation between reduced regional white matter volume in the left temporal stem and severity of symptoms; reduced regional white matter volume correlated with decreased serum ChE and severity of chronic somatic complaints

Overall risk-of-bias discussion of the body of evidence
Risk-of-bias ratings for individual studies for all questions are available in **Figure D31** and **Figure D32**. Although there were a few risk-of-bias concerns in two of the three key risk-of bias questions (i.e., questions regarding confounding and outcome assessment), it is unlikely that the risk-of-bias concerns in the body of evidence seriously altered the results. The cross-sectional study (Yamasue *et al.* 2007) had a single risk-of-bias concern related to confounding, but the study adjusted for the majority of potential confounders. The authors treated age, sex, SES, and intracranial volume as confounding factors. However, body mass index (BMI), alcohol consumption, and smoking were not reported or addressed, which has potential to bias the results. One case report (Himuro *et al.* 1998) was also rated as probably high risk of bias for confounding. It was noted that before the attack the patient was a healthy 51-year-old man with no neuropathy; however, there were little details provided on the subject to indicate that there were no potential confounders for the outcomes of interest. The Loh *et al.* (2010) case report was rated as probably high risk of bias for outcome assessment due to lack of blinding of outcome assessors, although with regards to the MRI results reported in the case report, the lack of blinding would likely bias towards an effect and no effect was observed; therefore, it is not considered a serious risk of bias.

Effects in the initial period after exposure
There is no human evidence to evaluate the potential association between acute sarin exposure and morphological and histological changes in human nervous system tissues at 1–7 days after exposure.

Effects in the intermediate period after exposure
A single case report was available that evaluated morphological or histological changes in neurological tissue at 8 months following sarin exposure (Loh *et al.* 2010). A 34-year-old male senior Army sergeant who was exposed to sarin while disarming an IED was examined for brain and spine abnormalities. The MRI examination of the brain and spine was determined to be normal. Loh *et al.* (2010) was rated as probably high risk of bias for one key question—outcome assessment—due to lack of blinding of outcome assessors (see **Figure D31** and **Figure D32**), although this may be less of a concern for the MRI assessment. However, the study used a standard MRI, which is not comparable to the diffusion tensor MRI and voxel-based morphometry used by Yamasue *et al.* (2007).

Effects in the extended period after exposure
One cross-sectional and one case report were available that evaluated morphological and histological changes in nervous tissue of adults who were exposed during the terrorist attack on the Tokyo subway. Yamasue *et al.* (2007) evaluated nervous tissue changes of 38 victims of the Tokyo subway attack who had been treated in the emergency department compared to 76 healthy controls. The study was conducted 5–6 years after the exposure. Recruitment methods for the 38 subjects from the 149 who participated in the 2000 survey done by Kawana *et al.* (2001) was not specified, but the controls were matched by age and sex. There were no significant differences in total gray matter, total white matter, total cerebrospinal fluid volume, or intracranial volume measured by diffusion tensor MRI; however, the voxel-based morphometry demonstrated that exposed subjects had a significantly reduced regional gray matter volume in the right insular and temporal cortices. A significant regional gray matter volume reduction in the left hippocampus and a significant regional white matter volume reduction in the left temporal stem close to the insular cortex were also observed. The study also found a negative correlation between the reduced regional white matter volume in the left temporal stem and the severity of symptoms. The reduced regional white matter volume was noted to be correlated with decreased serum ChE and the severity of chronic somatic complaints.

Himuro *et al.* (1998) reported a case of a 51-year-old man who was exposed to sarin during the Tokyo subway attack and died 15 months later without regaining consciousness. During autopsy, a neuropathological examination showed a severe reduction in large and small myelinated fibers with preferential loss of large myelinated fibers of the sural nerve. The spinal cord examination revealed total loss of myelinated fibers in the white matter and severe neuronal loss in the central gray matter. No changes were observed in the dorsal root ganglia, dorsal roots, and posterior column of the spinal cord. Examination of the brain revealed severe hypoxic-ischemic encephalopathy. The authors concluded that the revealed pathology is consistent with dying-back neuropathy and could represent a late sequela of sarin intoxication.

Both studies were rated as probably high risk of bias for one key risk-of-bias question (confounding) (see **Figure D31** and **Figure D32**). However, it is unlikely that this plausible bias seriously altered the results. In Himuro *et al.* (1998), the authors noted that before the attack the patient was a healthy man with no neuropathy, but the authors did not provide any details on the subject to indicate that there were no potential confounders for the outcomes of interest. However, given the damage observed and the subject's symptoms after the exposure, it is likely that the effects are related to the sarin exposure. In Yamasue *et al.* (2007), statistical analyses treated intracranial volume, age, and sex as confounding covariates, but did not address BMI, alcohol consumption, and smoking status, leading to a probably high risk of bias. Although the confounders not evaluated could potentially bias results, it is highly unlikely that they would occurred in one group at a rate that would significantly impact the brain morphology, which is assumed to likely be a result of the sarin exposure. In addition, Yamasue *et al.* (2007) selected subjects that had sufficient evidence of sarin exposure after the attack, and outcomes were assessed using reliable methods.

Taken together, the epidemiological evidence suggests that acute exposure to high levels of sarin is associated with morphological and histological changes in human nervous system tissues in the years following sarin exposure. There is inadequate human evidence available to evaluate the potential association between sarin exposure and nervous tissue effects in the days to months following exposure.

Animal Morphological and Histological Data

***Summary*:** There is moderate confidence in the body of evidence that acute sarin exposure is associated with morphological and histological changes in neurological tissues in animals over the initial period and intermediate period after exposure (see **Table 14**). The results consistently provide evidence of sarin-related effects related to nervous tissue changes within the first 7 days and through 90 days following acute sarin exposure. However, there are limitations in the body of evidence, including serious risk-of-bias concerns. In addition, while the staining methods used [e.g., hematoxylin and eosin (H&E) staining or Nissl] allowed the authors to detect morphologic changes in nervous tissue, modern techniques that provide a more comprehensive assessment of underlying neuropathology not revealed by classical Nissl/H&E staining were not employed, and therefore the full extent of the morphological changes may not have been detected and reported. The initial high confidence in the animal body of evidence was downgraded for risk-of-bias concerns related to randomization, exposure assessment, and outcome assessment to support a final rating of moderate confidence for a period of days to months following acute sarin exposure.

There are six experimental studies in the animal body of evidence that evaluated the association between acute exposure to sarin and morphological and histological changes in neurological tissues (Singer *et al.* 1987, Kawabuchi *et al.* 1991, Kadar *et al.* 1995, Grauer *et al.* 2008, Lazar *et al.* 2016,

Chaubey *et al.* 2017). All studies used rats (five male Sprague-Dawley rat studies; one Wistar female rat study). Sarin administration methods varied (i.e., subcutaneous injection, intramuscular injection, or inhalation); doses and timing of outcome measure also varied by study.

Overall risk-of-bias discussion of body of evidence
Confidence in the body of evidence for the animal studies was downgraded because of serious concerns for risk of bias (see **Figure D33** and **Figure D34**). The main risk-of-bias concern with the animal studies was lack of reporting of important details for key risk-of-bias questions (i.e., questions regarding randomization, exposure assessment, and outcome assessment). Only two of the five studies indicated that the animals were randomized to treatment (Singer *et al.* 1987, Grauer *et al.* 2008). None of the others provided sufficient details on randomization or how animals were assigned to treatment. The main limitation of the exposure characterization in three of the five animal studies was lack of data on the source and/or purity of the sarin administered. Three studies administered sarin with ≥95% purity, but only one study (Kadar *et al.* 1995) indicated that the purity was verified and provided methods. Morphology- and histology-related endpoints were measured using acceptable methods in all studies; however, none of the studies reported that the outcome assessors were adequately blinded to the study group or reported methods to reduce potential bias.

Effects in the initial period after exposure
Four experimental studies in rats examined sarin-related effects related to nervous tissue changes within the first 7 days after sarin exposure. Results from the four studies in rats for this time period suggest that there are nervous tissue effects associated with sarin that can occur within the first week after acute exposure. In Grauer *et al.* (2008), male rats were exposed to 34.2 µg/L of sarin via inhalation for 10 minutes, and brain morphology was examined at 1 week. Brain damage was found in 6 of the 10 exposed animals and included enlargement of ventricles and cell death in the piriform cortex, the hippocampus (including the CA1, CA3, and dentate gyrus), and the thalamus. No brain damage was observed in controls. Authors noted that the severity of brain damage was correlated with initial signs of toxicity (convulsions). Kawabuchi *et al.* (1991) exposed female Wistar rats to a single subcutaneous injection of sarin at 80 µg/kg and evaluated motor nerve fiber degeneration on days 1, 3, and 6. Neural degeneration was observed over that time period, but recovery was apparent by day 6, evidenced by restored neural sites, nerve sprouting, and endplate regeneration. In Lazar *et al.* (2016), male rats received an intramuscular injection of sarin at 80 µg/kg, and brains were removed and examined at 1, 2, 6, 24 and 48 hours after exposure. The authors observed a time-dependent increase in the severity of brain damage, most notably in the hippocampus and piriform cortex. Picnotic and necrotic cells seen in the CA1 and CA3 subregions of the hippocampus increased over time. In the piriform cortex, neuronal cell death was almost complete at 48 hours following exposure to sarin and was due mostly to necrosis associated with severe astrocytosis. Singer *et al.* (1987) administered a single subcutaneous injection of sarin (111–197 µg/kg) to rats and evaluated brain damage on days 2, 6, and 7. Moderate or severe neuronal necrosis was observed in 2 of the 3 animals sacrificed at 2 days (moderate at 157 and 170 µg/kg), 3 of the 6 rats sacrificed at 6 days (moderate at 170 µg/kg and severe at 125 and 197 µg/kg), and 1 rat sacrificed at 7 days (severe at 170 µg/kg).

Effects in the intermediate period after exposure
Three experimental studies in rats (Singer *et al.* 1987, Kadar *et al.* 1995, Chaubey *et al.* 2017) examined sarin-related effects related to nervous tissue changes in the intermediate period after sarin exposure. Results from all studies suggest that nervous tissue effects associated with sarin that can last for weeks or months after the acute exposure. Kadar *et al.* (1995) exposed rats to a single LD_{50} dose of sarin (95 µg/kg intramuscular) and examined the brains of surviving animals for histological and morphometric

changes. Seventy percent of surviving rats developed brain lesions with varying degrees of severity primarily in the hippocampus, piriform cortex, and thalamus. The severity of the lesions was related to the presence or absence of convulsions, and the damage was exacerbated over time. At 3 months, damage had extended to areas of the brain that had not been affected initially. In addition, the authors observed almost complete degeneration of the CA1 cell layer and severe necrosis in the CA2 and CA3 regions. There was an initial decrease in single-cell surface area in the hippocampal CA1 and CA3 subfields of exposed animals with gradual increases back towards control; however, results were still significantly decreased 90 days after the exposure. Singer *et al.* (1987) administered a single subcutaneous injection of sarin (111–197 µg/kg) to rats and evaluated brain damage on days 9, 21, 28, and 35. Mild, moderate, or severe neuronal necrosis was observed in 2 of 5 rats sacrificed at 9 days (moderate at 125 and severe at 170 µg/kg), 0 of 5 rats sacrificed at 21 days, 1 of 5 rats sacrificed at 28 days (moderate at 140 µg/kg), and 1 of 6 rats sacrificed at 35 days (mild at 125 µg/kg). Chaubey *et al.* (2017) administered a single subcutaneous 0.5-LD_{50} dose (i.e., 80 µg/kg) to rats and evaluated brain histopathology at 3 months. The authors observed necrotic regions with degenerative neurons and neuroglia in the cortex, similar effects in the corpus striatum characterized by formation of perineuronal and perivascular spaces, and necrotic regions in the hippocampus with hypocellularity of neurons and neuroglia prominent.

Effects in the extended period after exposure
There is no animal evidence to evaluate the potential association between sarin exposure and morphological and histological changes in nervous tissue in the extended period after exposure.

Integration of Evidence for Morphological and Histological Changes
There is evidence to suggest morphological and histological changes in human and animal nervous tissue following acute exposure to higher doses of sarin. There is moderate confidence in the human data for sarin-associated nervous tissue effects in the extended period based on one cross-sectional study with support from one case report with little potential for bias. The body of evidence prior to a year (i.e., in the initial and intermediate periods) is inadequate in humans. There is moderate confidence that acute sarin exposure is associated with nervous tissue effects in animals based on the consistency of the findings in rats through 90 days after exposure. These confidence ratings translate directly into level-of-evidence conclusions and support an initial hazard identification conclusion of *suspected to be a neurological hazard to humans*.

Effects in the initial period after exposure

- **Human body of evidence:** No studies = Inadequate Level of Evidence
- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Initial hazard conclusion (Inadequate Human x Moderate Animal)** = Suspected to be a Neurological Hazard to Humans
- **Final hazard conclusion (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to Humans

Effects in the intermediate period after exposure

- **Human body of evidence:** Low Confidence with no effect = Inadequate Level of Evidence
- **Animal body of evidence:** Moderate Confidence = Moderate Level of Evidence

- **Initial hazard conclusion (Inadequate Human x Moderate Animal)** = Suspected to be a Neurological Hazard to Humans

- **Final hazard conclusion (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to Humans

Effects in the extended period after exposure

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence

- **Animal body of evidence:** No studies = Inadequate Level of Evidence

- **Initial hazard conclusion (Moderate Human x Inadequate Animal)** = Suspected to be a Neurological Hazard to Humans

- **Final hazard conclusion (after consideration of biological plausibility)** = Suspected to be a Neurological Hazard to Humans

Collectively the human and animal bodies of evidence provide consistent patterns of findings that acute exposure to higher doses of sarin is associated with morphological and histological changes in nervous tissue. The human data are based on one cross-sectional study and one case report evaluating adults from the Tokyo subway attack. The animal data support that an acute sarin exposure can cause nervous tissue effects.

Mechanistic data support the evidence of morphological and histological changes in humans and animals associated with acute sarin exposure. OP nerve agents including sarin cause hyperactivity in the nervous system triggered by hyperstimulation of cholinergic receptors which leads to respiratory failure via peripheral and central mechanisms and seizures via central mechanisms. The increased cholinergic drive in the central nervous system (CNS) results in an overactivation of glutamate receptors and a subsequent rise in intracellular calcium levels, which culminates in an excitotoxic response in the CNS (Chen 2012). Significant elevations in hippocampal calcium levels after OP-induced status epilepticus persist for weeks, and drugs inhibiting intracellular calcium-induced calcium release reduce neuronal cell damage and death (Deshpande *et al.* 2016). Secondary effects, such as extensive intracellular edema, increased blood-brain barrier permeability, cerebral hemorrhages, and increased neuroinflammatory and stress responses, likely also contribute to morphological changes caused by OP exposure (Chen 2012). Studies in animals and humans have linked oxidative stress with acute and chronic exposures to OP nerve agents and pesticides (Pearson and Patel 2016), and the highly potent OP nerve agent soman causes changes in brain region oxygenation after sublethal doses that cause seizures (Lee *et al.* 2018). Hypoxia and oxidative stress are important considerations since they may be effects unrelated to OP-induced seizures, and both hypoxia and oxidative stress have been linked to morphological and histological changes in central and peripheral nervous systems in other disease contexts (Tonni *et al.* 2014, Pomara *et al.* 2015, Raz *et al.* 2016). The few mechanistic animal studies conducted specifically with sarin are consistent with the above studies of other OP nerve agents and pesticides. For example, rats exposed to sublethal doses of sarin exhibited significant cell death and neurodegeneration in the CNS associated with changes in apoptotic proteins and an early bi-phasic activation of astrocytes (Lazar *et al.* 2016), and proteomic studies of sarin-exposed rats are beginning to unmask details of the excitotoxicity and other mechanisms described above (Chaubey *et al.* 2017).

Table 14. Morphological and Histological Changes to Nervous System Tissues Evidence Profile for Sarin

INITIAL CONFIDENCE for each body of evidence (# of studies)	Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence					Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
Human										
Initial period	No studies available									**No rating**
Intermediate period - Initial Low (1 case report)[a]	---	---	---	---	---	---	---	---	---	**Low**
Extended period - Initial Moderate (1 cross-sectional study)[b]	---	---	---	---	---	---	---	---	---	**Moderate**
Extended period - Initial Low (1 case report)[c]	---	---	---	---	---	---	---	---	---	**Low**
Animal										
Initial period - Initial High (4 mammal studies)[d]	↓	---	---	---	---	---	---	---	---	**Moderate**
Intermediate period - Initial High (3 mammal studies)[e]	↓	---	---	---	---	---	---	---	---	**Moderate**
Extended period	No studies available									**No rating**

References:

Human: Himuro *et al.* (1998)[c], Loh *et al.* (2010)[a], Yamasue *et al.* (2007)[b]

Animal: Chaubey *et al.* (2017)[e], Grauer *et al.* (2008)[d], Kadar *et al.* (1995)[e], Kawabuchi *et al.* (1991)[d], Lazar *et al.* (2016)[d], Singer *et al.* (1987)[d,e]

DISCUSSION

Based on the systematic review of the evidence, the NTP reached conclusions on long-term neurotoxicity following acute sarin exposure that are specific for the length of time following sarin exposure. The NTP concludes that sarin is *known to be a neurological hazard to humans in the initial time period (>24 hours–7 days) after exposure* based on a high level of evidence that sarin inhibits ChE in the days after exposure in humans and a moderate level of evidence in the animal studies in the same time period. The NTP concludes that sarin is *suspected to be a neurological hazard to humans in the intermediate time period (8 days–1 year) after exposure* based on ChE (inadequate level of evidence from studies in humans and moderate level of evidence from studies in animals); visual and ocular (moderate level of evidence from studies in humans and inadequate level of evidence from studies in animals); learning, memory, and intelligence (inadequate level of evidence from studies in humans and moderate level of evidence from studies in animals); and morphology and histological changes (inadequate level of evidence from studies in humans and moderate level of evidence from studies in animals). The NTP concludes that sarin is *suspected to be a neurological hazard to humans in the extended time period (>1 year) after exposure* based on learning, memory, and intelligence (moderate level of evidence from studies in humans and inadequate level of evidence from studies in animals); and morphology and histological changes (moderate level of evidence from studies in humans and inadequate level of evidence from studies in animals). These conclusions represent the bodies of evidence with the greatest confidence and therefore the strongest conclusions for each time period after exposure. There is additional weaker or limited evidence of other sensory effects, self-reported symptoms (including but not limited to sleep disruption, depression), and activity and strength, supported by some evidence of disruption in EEGs (see **Appendix 4**). Although biological plausibility of effects was considered, mechanistic data did not significantly impact the conclusions. The mechanism(s) of long-term neurological effects of sarin are not clearly understood.

The high level of evidence in the human data was primarily based on controlled trials with support from case report studies. Although there were risk-of-bias concerns in the controlled trials (i.e., lack of reporting for the key risk-of-bias questions regarding outcome assessment and, in one study, randomization and exposure characterization), these studies provided evidence of a large magnitude of effect, which increased the confidence in the body of evidence and resulted in a high level of evidence. The moderate level of evidence in the human data was primarily based on cross-sectional studies that did not have serious risk-of-bias concerns. Although the human body of evidence mainly consisted of case reports and case series, there were sufficient cross-sectional studies with supporting evidence from the case reports/series to reach a moderate level of evidence. The moderate level of evidence from animal studies is supported by moderate confidence in the body of evidence from animal studies of sarin exposure on ChE; learning, memory, and intelligence; and morphology and histopathology. These confidence ratings are based on results that consistently showed an effect during a specific time period after exposure. Animal data were limited mainly due to the heterogeneity of the outcomes measured. In addition, the animal data generally had serious risk-of-bias issues related to lack of information provided on randomization, blinding of outcome assessor, and exposure assessment.

The systematic review format in this evaluation adds transparency (e.g., clear statement of the objective, PECO criteria and literature search terms) and rigor (e.g., risk-of-bias assessment of individual studies) to the process for reviewing evidence of long-term neurological effects of sarin. This review focuses on the 4 main health-effect categories of neurological response with sufficient data to reach hazard conclusions and clearly outline the evidence forming the basis of those conclusions:
(1) cholinesterase levels; (2) visual and ocular effects; (3) learning, memory, and intelligence; and

(4) morphology and histopathology in nervous system tissues. The conclusions of this systematic review align with conclusions from a published narrative review of long-term neurological effects following exposure to sarin by the National Academies of Sciences (NAS) in concluding that there is strong evidence for effects of sarin on cholinergic effects (e.g., ChE) in the period covering hours to days after exposure and less conclusive evidence for visual effects and other symptoms over longer time periods. In 2010, the Institute of Medicine (IOM) of the National Academies of Sciences, Engineering, and Medicine published a review of long-term health effects of Gulf War veterans associated with sarin as the only suspected exposure and concluded that there was sufficient evidence of a causal relationship between exposure to sarin and a dose-dependent acute cholinergic syndrome that is evident seconds to hours following sarin exposure and resolves in days to months. The 2004 IOM report also concluded that there was limited/suggestive evidence of an association between exposure to sarin at doses sufficient to cause acute cholinergic signs and symptoms and a variety of subsequent long-term (i.e., longer than several months following exposure) neurological effects including visual disturbances, visual evoked potential, symptoms of PTSD, psychomotor performance, EEG records of sleep, headache, and other endpoints (IOM 2004). The data on PTSD, headaches, sleep, anxiety and other neurological effects were evaluated and considered inadequate evidence to reach hazard conclusion in this systematic review (see Appendix 4).

Limitations of the Evidence Base

There are a number of serious limitations in the body of evidence from human studies that apply across the different neurological outcomes. The major limitation in the epidemiological studies is study design. The majority of studies followed subjects from two terrorist attacks (Matsumoto city and Tokyo subway attacks) without including any control groups. The case series following the Tokyo subway attack victims were also limited because many of the studies followed only the subjects who were brought to one hospital (St. Luke's hospital) after the attack, which only accounted for 640 off the approximate 5,000 exposed subjects. In addition, subjects were lost over time with no information provided on subjects lost, including the reasons for the loss of subjects (e.g., because of death, non-participation in follow-up surveys, etc.) and whether the subjects lost to follow-up were more likely to be milder cases. Of the 640 subjects brought to St. Luke's, it has been reported that 111 were admitted to the hospital and 528 were discharged and considered mild cases (Morita *et al.* 1995); however, in the majority of studies they did not report any details on the exposure of the subjects. Most studies only indicated that they were victims of the attacks. In some cases, subjects self-reported if they had been admitted to the hospital or not, which was used as a proxy for level of exposure. This can lead to exposure misclassification even based on a never/ever exposure scenario. Few studies provided details on cholinergic symptoms and ChE levels immediately after exposure for all subjects in the study to indicate sarin exposure or levels of sarin exposure, which could be used to qualitatively demonstrate exposure gradient. It is recognized that the subjects of the sarin attacks were likely exposed to some level of sarin, and controls would not have been exposed, as sarin is not found in the environment. Although exposure details are a limitation, it is the lack of controls for comparison that are the major limitation in the body of evidence. Even when a study included a control, exposure was mainly assessed as a never/ever scenario. This is a limitation because it may be the level of exposure that is associated with prolonged neurological effects, and there are few if any studies that address this limitation.

Another limitation to the epidemiology evidence base is that many of the studies only included self-reported symptoms. Because the subjects knew they were exposed, there is potential bias in the reporting of symptoms. In some of the studies that followed subjects over time, symptoms were added to questionnaires after they had been noted to occur by some of the subjects as a "write-in" symptom.

This presents a limitation in the information available for each time period and decreases the potential to follow the symptom for resolution over time. Even in studies where results were not self-reported, there is no indication that the outcome assessors were blind to the exposure group. The majority of the studies also did not account for any potential confounders such as age. While these limitations were true across the majority of the endpoints, the cross-sectional studies on memory did account for potential confounders, which made these limitations less of an issue for the memory effects. Regarding morphology and histopathology data in humans, this information would only be evaluated during an autopsy or if subjects had lingering effects after the exposure, which decrease the likelihood that information would be available to evaluate these effects in the initial period.

Similarly, there are limitations in the body of evidence from experimental animal studies. The principal limitation is the lack of reporting details for determining risk of bias and failure of author response to address the lack of reporting, for which a conservative approach was followed (i.e., when there was not enough information to assess the potential bias for a risk-of-bias question and authors did not respond to an inquiry for further information, studies were rated probably high risk of bias for that question). The majority of animal studies did not report if the animals were randomized to treatment. One author for several of the animal studies (i.e., all Kassa *et al.* studies) reported that they did not randomize their animals to treatment. More than half of the animal studies did not report the purity of the sarin, or they used sarin of 90% purity without providing information on the remaining 10% to indicate that there were no impurities that could affect the results. However, 21% (9 of 43) of the animal studies used sarin of sufficient purity and reported verification of the purity. The majority of the studies also did not report if the outcome assessors were blinded to the experimental condition or did not report enough details to indicate that the lack of blinding would likely not bias the results. Kassa *et al*. (2002, 2004) responded to our inquiry and indicated that the outcome assessors were not blinded to experimental conditions. However, in two of their memory studies, a Y-maze test noted to be fully automated was used, which reduced the potential risk of bias.

Another limitation in the animal data is the heterogeneity of the data. Few studies used similar endpoints and several of the endpoints were subjective FOB scores. These limitations occurred across all of the endpoints making the data inadequate for reaching hazard conclusions for many of the long-term neurological effects. In addition, due to the limited number of studies and heterogeneity of the data in both the human and animal bodies of evidence, NTP was unable to thoroughly evaluate the data by additional variables that are known to affect organophosphate toxicity and neurotoxicity, including strain/species differences, genetics and epigenetic effects, body temperature, and presence of physiological stressors.

The staining methods employed during histopathological examinations is also considered a limitation in the animal data. While the staining methods used (e.g., H&E staining or Nissl) allowed the authors to detect morphologic changes in nervous tissue, modern techniques that provide a more comprehensive assessment of underlying neuropathology not revealed by classical Nissl/H&E staining were not employed (even in the most recent study in 2017), and therefore the full extent of the morphological changes may not have been detected and reported.

Targeted research addressing improving human characterization of exposure with neurological tests compared to a control population, in addition to targeted research in animal models addressing the inconsistencies identified in this review regarding study design and conduct practices to minimize bias would help improve the body of evidence to critically assess the long-term neurological effects from an acute exposure to sarin.

Key Data Gaps

Considering the context in which humans are typically exposed to sarin (i.e., during wartime situations and terrorist attacks), researchers are somewhat limited in their opportunities to study human populations acutely exposed to sarin in comparison to appropriate control groups, which can make the data gaps identified in this review difficult to address. Although there were two terrorist attacks in Japan, they both occurred more than 20 years ago, so additional studies on any remaining subjects are not likely to provide the additional data needed, as age is an important confounder for many of the outcomes detailed in this report. Because these are rare and unpredictable events, there could be value to developing a rapid research response capability so that emergency response would include the latest treatment knowledge for the victims. The response could also collect vital human clinical data soon after chemical exposures. Well designed, pre-planned, epidemiological studies would add valuable data to the body of evidence that would be likely to impact conclusions or the confidence in the conclusions reached in this systematic review, given the lack of human data on many endpoints and time periods.

More rigorous human data would add greater confidence to conclusions reached in this evaluation across all outcomes described above except in the body of evidence for suppression of cholinesterase activity in the initial time period, which already is rated as high confidence and a high level of evidence. Human evidence for sarin-related health effects that would benefit from additional data include visual and ocular effects, learning and memory effects, and morphology and histological changes in nervous system tissues following acute sarin exposure in the three time periods (i.e., initial, intermediate, extended) and alterations of cholinesterase activity in the intermediate and extended periods. Data on other persistent symptoms and neurological effects would also be valuable, as a range of effects have been reported, but the evidence was inadequate to evaluate these health outcomes due to serious limitations in the bodies of evidence (e.g., heterogeneity in the endpoints examined, too few human or animal studies, small sample sizes, serious risk-of-bias concerns).

Prospective longitudinal cohort studies would be the most informative to better assess neurological effects associated with sarin exposure. Studies that assess visual and ocular effects 1 year or more after exposure in humans would address a data gap in reaching conclusions. However, studies that evaluate measurable results beyond pupil size and self-reported symptoms would add value to the assessment of potential visual and ocular effects resulting from acute sarin exposure. Human cohort studies would be invaluable in characterizing the relationship between ChE activity and neurological effects over time, as well as the potential relationship between acute sarin exposure and the development of PTSD as it relates to other neurological effects. Only one available study (Tochigi *et al.* 2002) addressed subjects with PTSD symptoms as a subgroup and found evidence of a long-term depression in serum ChE levels in the PTSD subset of patients (n = 8) compared with controls, whereas the same association was not seen in the entire study population compared to controls.

Because of the ability to conduct controlled-exposure studies, experimental animal studies are particularly important for addressing research gaps identified by this systematic review, especially for identifying specific effects that could be targeted for medical mitigation. Animal studies indicate differences in inhibition and recovery of ChE activity in different areas of the brain; however, the data are insufficient to identify if there are particularly vulnerable areas of the brain. Future research could focus on these effects to help identify potentially vulnerable areas that could be targeted. Studies in appropriate animal models are also needed for rigorous, well-controlled experimental assessments of the dose-response relationship between sarin exposure and long-term neurological effects. Research is needed to further characterize the morphological and histological effects of sarin observed in humans and animals to determine their clinical significance and the potential therapeutic approaches that may

preclude these effects (e.g., neuroprotectants). Research is also needed to address the heterogeneity in the behavioral tests and study design among studies evaluating the learning and memory effects of sarin observed in animals. Another gap in both the human and animal data are the effects of sarin on the developing and aging brain. It currently cannot be assessed if children, the very young, or the very old are more susceptible populations.

Most human studies provide self-reported symptoms. While it was attempted to match animal tests to the human symptoms, animal studies that specifically attempt to examine endpoints in animals that directly correspond to commonly self-reported symptoms in humans would strengthen the evaluation of human and animal data together.

Limitations of the Systematic Review

The hazard identification conclusions in this evaluation were developed for long-term neurological effects associated with acute sarin intoxication based on integrating levels of evidence from human and animal studies. However, the available mechanistic data were not sufficient to impact the confidence ratings. Although there were a few *in vivo* studies that evaluated mechanistic data, they were very limited in number and there was no overlap in endpoints evaluated (none of the studies evaluating the same endpoints or potential mechanisms). The NTP literature search was focused on mechanistic data that were clearly relevant for evaluating the biological plausibility of neurological outcomes reported from *in vivo* studies in animals or humans. The literature search only included *in vitro* data if the endpoint was directly relevant to survival or morphology of neuronal or glial cells. This focused approach may have missed mechanistic studies of earlier events such as inhibition of neuropathy target esterase (Brown and Brix 1998) or broader mechanistic categories such as oxidative stress, neuroinflammation, or other mechanisms separate from the cholinergic pathway that may inform the overall evaluation of potential neurotoxicity associated with exposure to sarin.

The NTP systematic review did not consider unpublished data for this review. There were publicly-available, unpublished data identified from the literature search. Data from the identified unpublished studies were either subsequently published (and therefore included in this review), or were from authors who had published several other studies on the topic. Based on a review of the unpublished data identified, it was determined that the inclusion of the unpublished data to the body of evidence would not change any of the hazard conclusions; therefore, unpublished data were not included in the review. However, because sarin is a nerve agent used in chemical warfare, there are likely to be unpublished studies that are not publicly available that might provide additional support for the effects observed and discussed in this review. Therefore, not including unpublished data from non-publicly available sources may be a limitation of the systematic review.

This systematic review also was limited to acute sarin exposure. Although the intent of the review was to evaluate the effects of acute sarin exposure on long-term neurological effects, there may have been data available from short-term or chronic exposures that may have relevance to the findings described in this report.

Exposure characterization and dose-response assessment were beyond the scope of this review. The evaluation did not attempt to quantitatively characterize exposure or identify exposure levels of sarin at which long-term neurological effects occur. In general, there was a lack of quantitative exposure data in the human studies identified, and there may not be sufficient data from these studies to identify a threshold or exposure level for long-term neurological effects of sarin.

CONCLUSION

Hazard conclusions were considered for the four main health effect categories at all three time periods after exposure. The conclusions with the highest level of evidence for each time period were used to reach the overall conclusions. NTP concludes that acute sarin exposure is *known to be a neurological hazard to humans* for effects in the initial period of 1–7 days after exposure based on ChE data. NTP concludes that acute sarin exposure is *suspected to be a neurological hazard to humans* for multiple effects in the intermediate period of 1 week to 12 months after exposure based on the ChE, visual and ocular, learning and memory, and morphology and histological data. NTP concludes that acute sarin exposure is *suspected to be a neurological hazard to humans* for multiple effects in the extended period greater than 1 year after exposure based learning and memory and morphology and histological data.

This evaluation identified data gaps that contribute to lower confidence in the bodies of evidence for some endpoints and time periods. Multiple other symptoms and neurological effects have been reported in the days, months, and years after acute sarin exposure, but the evidence was inadequate to reach a hazard conclusion. Future targeted research to assess the long-term neurological effects of sarin exposure could help to address areas with lower confidence, including the use of well-characterized human exposure data with neurological tests compared to a control population and research in animal models addressing the inconsistencies identified in this review using study design and conduct practices to minimize bias. Based on the hazard conclusions, potential endpoints identified by this evaluation for further research include ChE, visual and ocular effects, effects on learning and memory, and morphological and histological changes in nervous system tissues.

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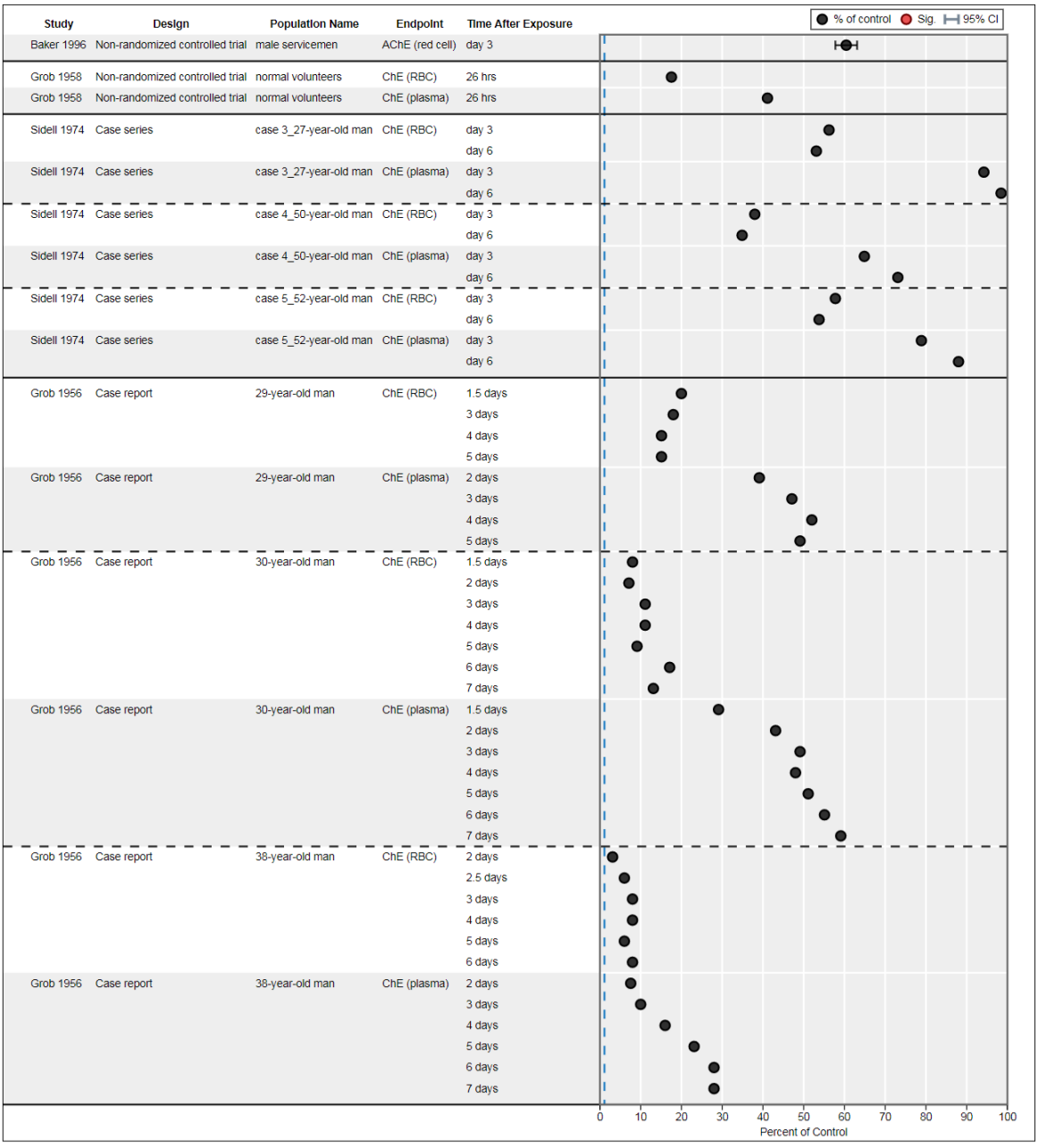
DATA FIGURES

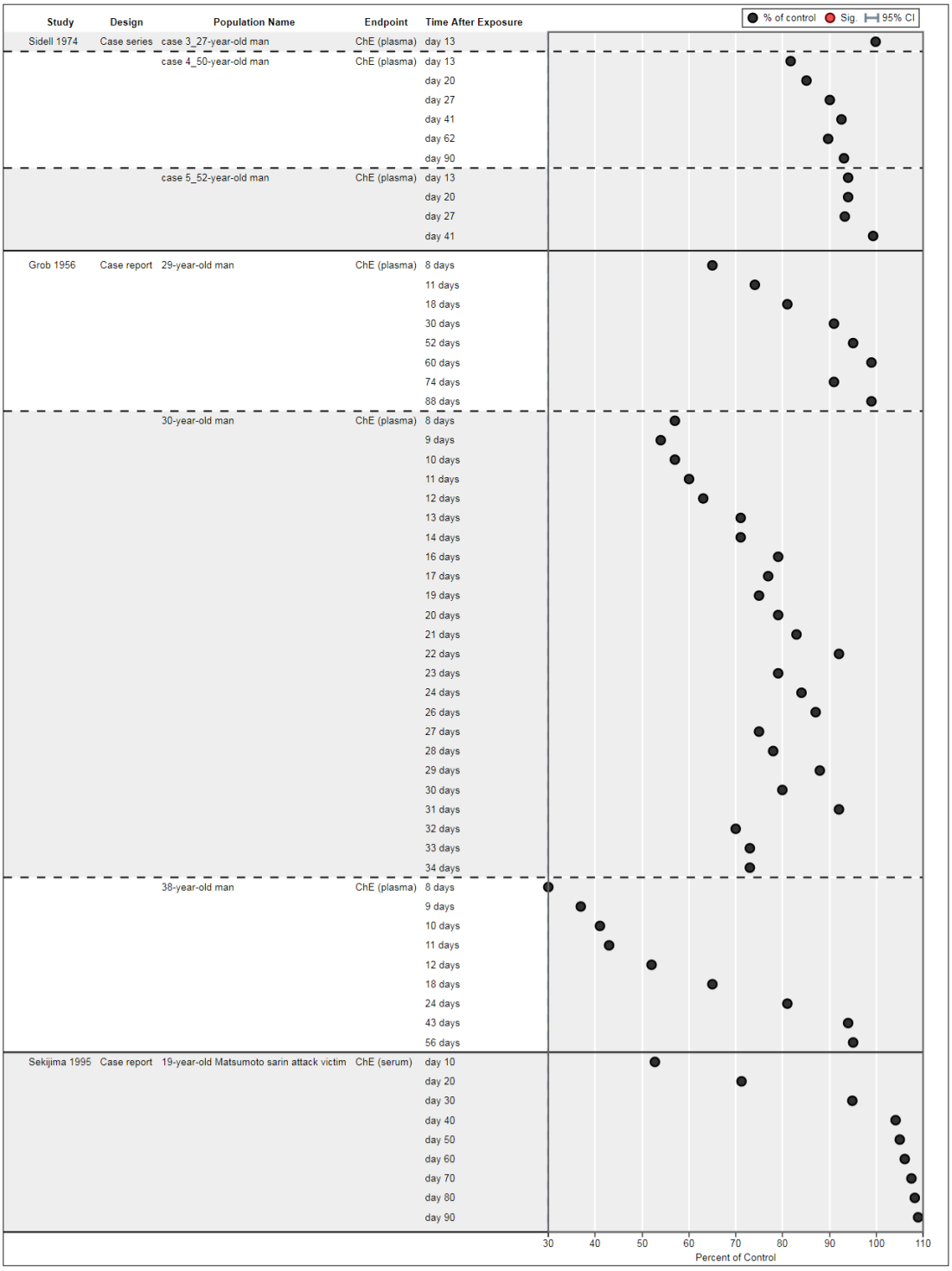
Cholinesterase-related Effects and Outcomes

Figure D1. ChE Levels in Blood in Humans Following Acute Sarin Exposure (Initial Period)



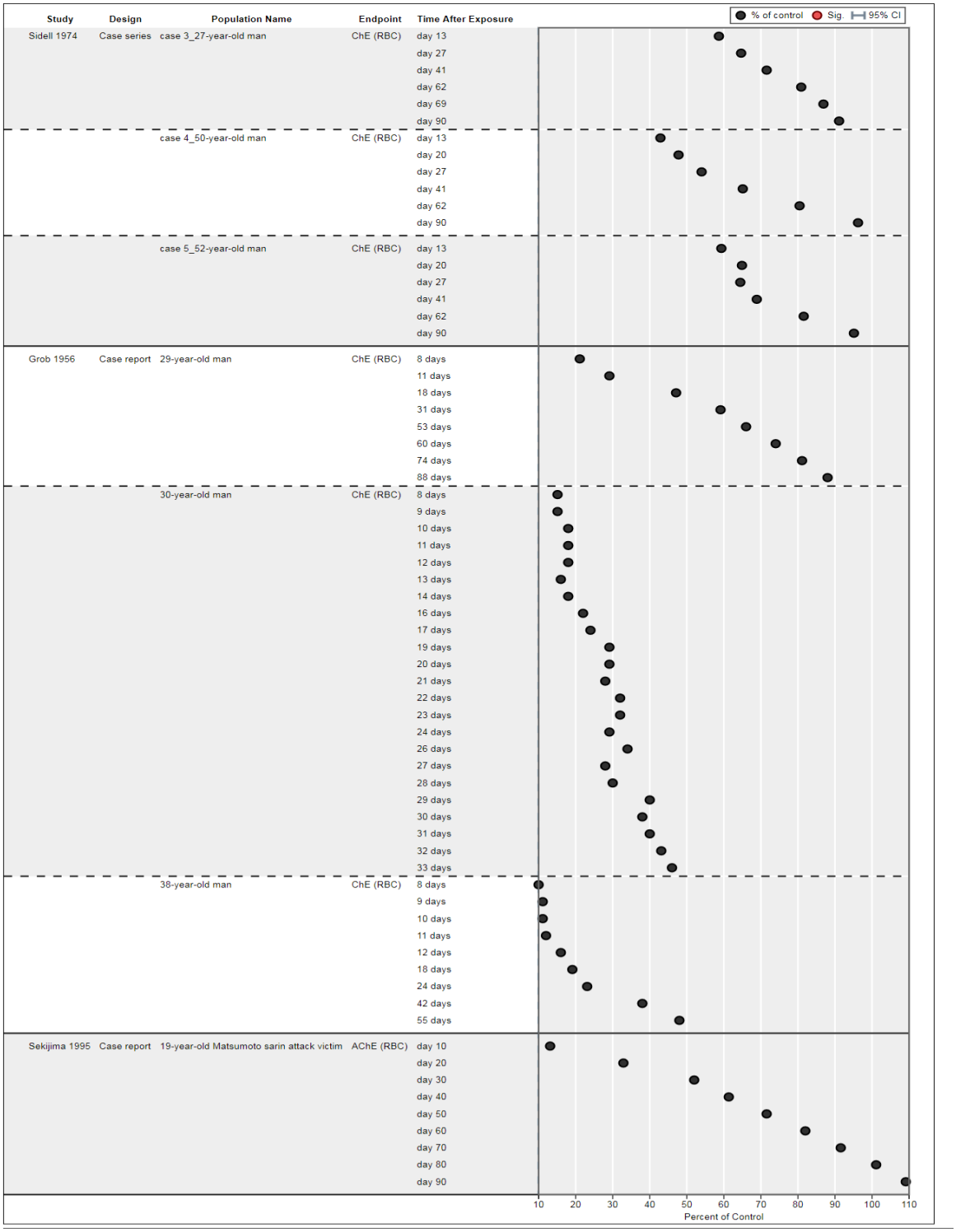
Interactive figure and additional study details in HAWC here. For the studies presented, statistical analyses were not conducted. Results are based on a change from normal or subjects' baseline. Subjects in the case reports/series were exposed to an unknown amount of sarin and were evaluated in terms of time after exposure. Baker and Sedgwick (1996) administered sarin vapor with an ambient concentration of 0.5 mg/m^3 for 30 minutes. Grob and Harvey (1958) administered a solution containing sarin at varying concentrations (initial dose ranged from 0.0005–0.022 mg/kg with an average value of 0.012 mg/kg). Figures for the intermediate period (one for serum/plasma and one for red blood cells) are provided below.

Figure D2. ChE Levels in Blood in Humans Following Acute Sarin Exposure (Intermediate Period—Serum or Plasma)



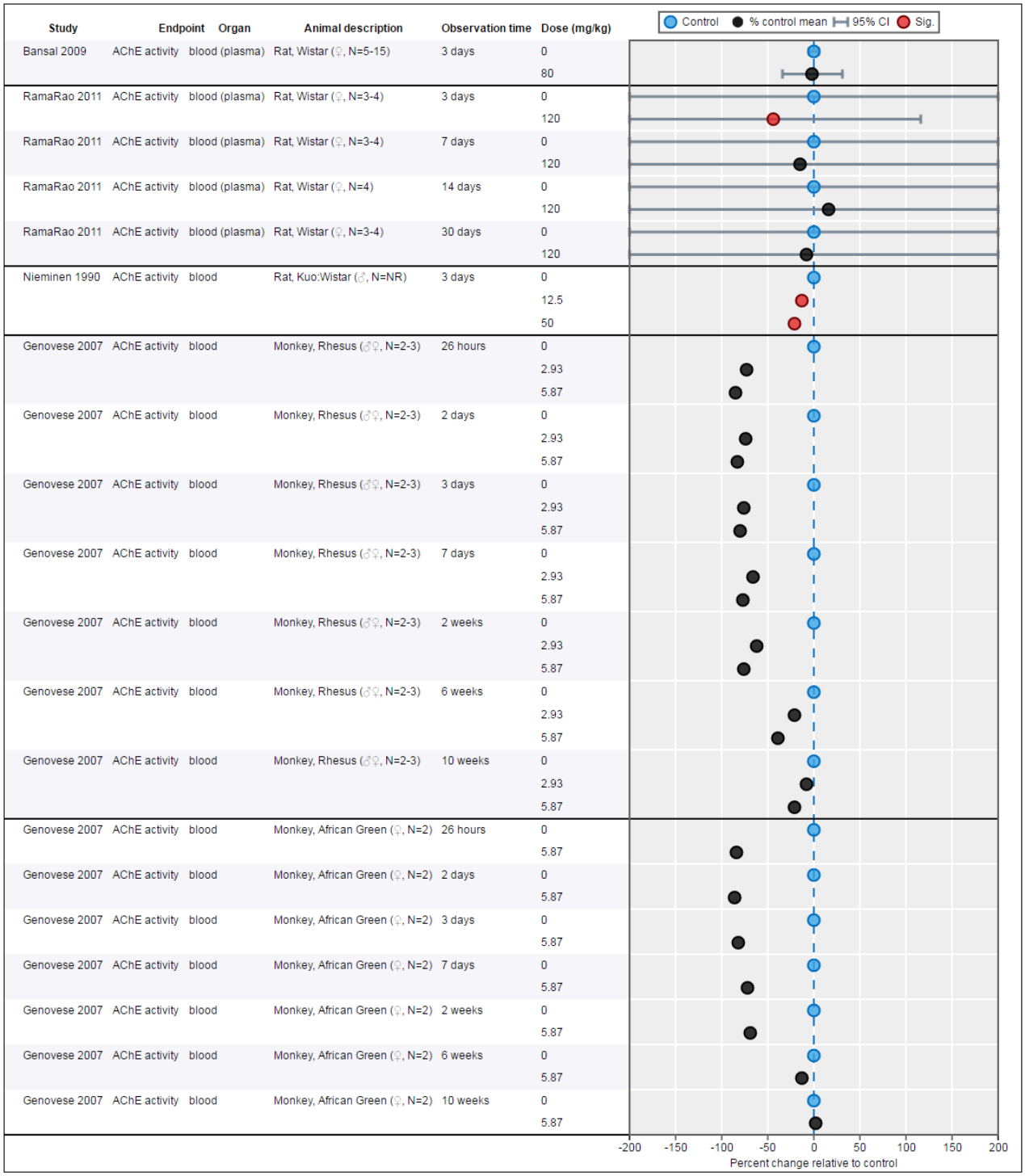
Interactive figure and additional study details in HAWC here. For the studies presented, statistical analyses were not conducted. Results are based on a change from normal or subjects' baseline. Subjects in the case reports/series were exposed to an unknown amount of sarin and were evaluated in terms of time after exposure. Rengstorff (1985) results are not shown in the visualization because of differing units.

Figure D3. ChE Levels in Blood in Humans Following Acute Sarin Exposure (Intermediate Period—Red Blood Cells)



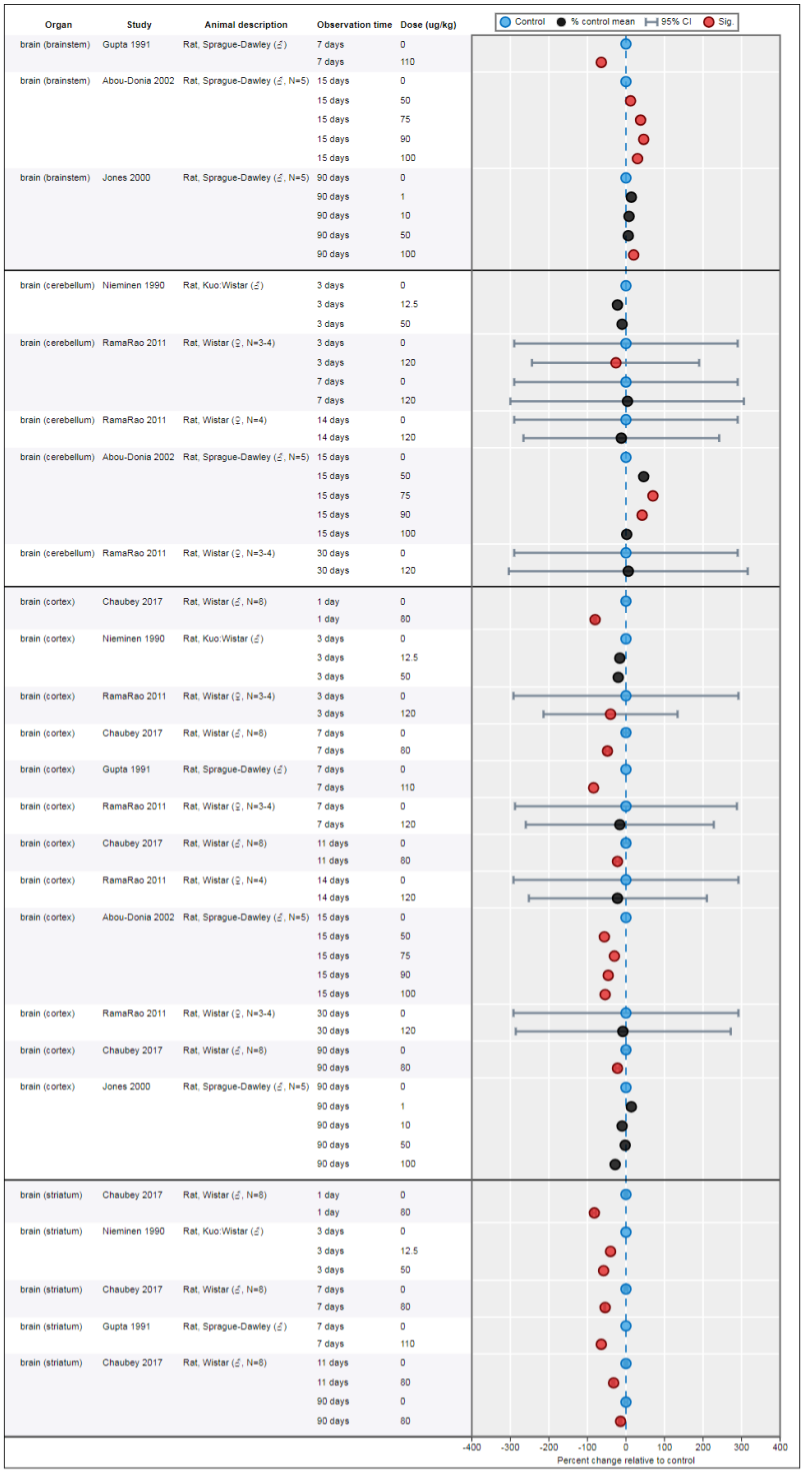
Interactive figure and additional study details in HAWC here. For the studies presented, statistical analyses were not conducted. Results are based on a change from normal or subjects' baseline. Subjects in the case reports/series were exposed to an unknown amount of sarin and were evaluated in terms of time after exposure. Rengstorff (1985) results are not shown in the visualization because of differing units.

Figure D4. AChE Levels in Blood in Animals Following Acute Sarin Exposure



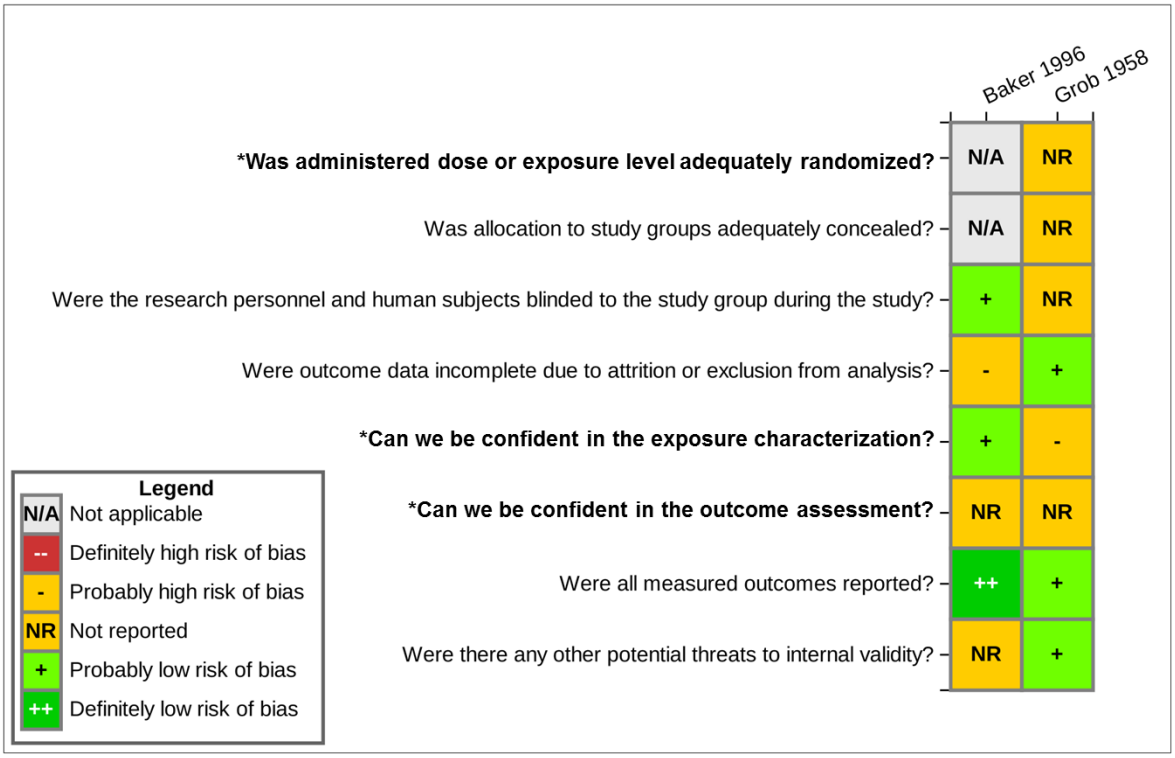
Interactive figure and additional study details in HAWC here. Some animal studies also specifically evaluated BChE, but these results are not included in the figure because its physiological function related to neurological effects is unclear. Studies with ChE activity or AChE mRNA results only (Whalley and Shih 1989, Damodaran *et al.* 2003, Chaubey *et al.* 2016, Chaubey *et al.* 2017) are not included in the figure. See the text for relevant information about these studies.

Figure D5. AChE Levels in the Brain of Animals Following Acute Sarin Exposure



Interactive figure and additional study details in HAWC here. This figure includes brain regions with results from more than 2 studies. Some animal studies also specifically evaluated BChE, but these results are not included in the figure because its physiological function related to neurological effects is unclear. Studies with ChE activity or AChE mRNA results only (Whalley and Shih 1989, Damodaran *et al.* 2003, Chaubey *et al.* 2016) are not included in the figure. See the text for relevant information about these studies.

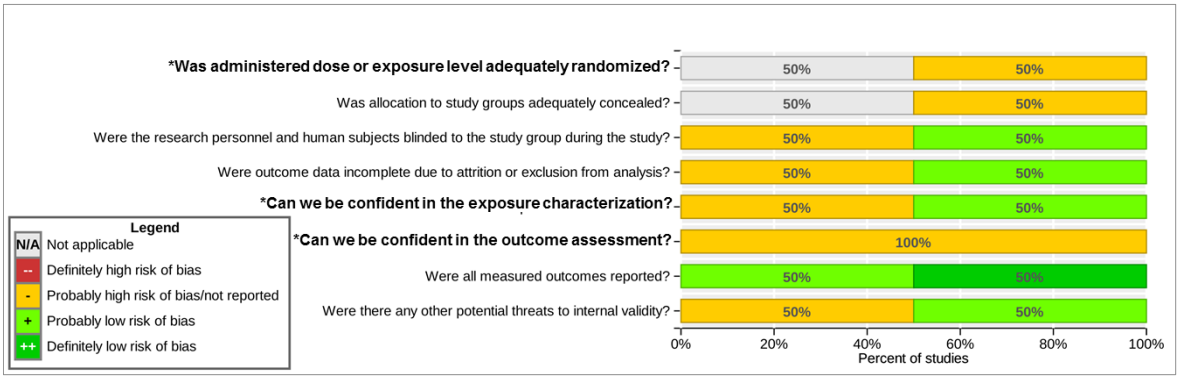
Figure D6. Risk-of-bias Heat Map for Controlled Trials Assessing ChE Levels in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human controlled exposure studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

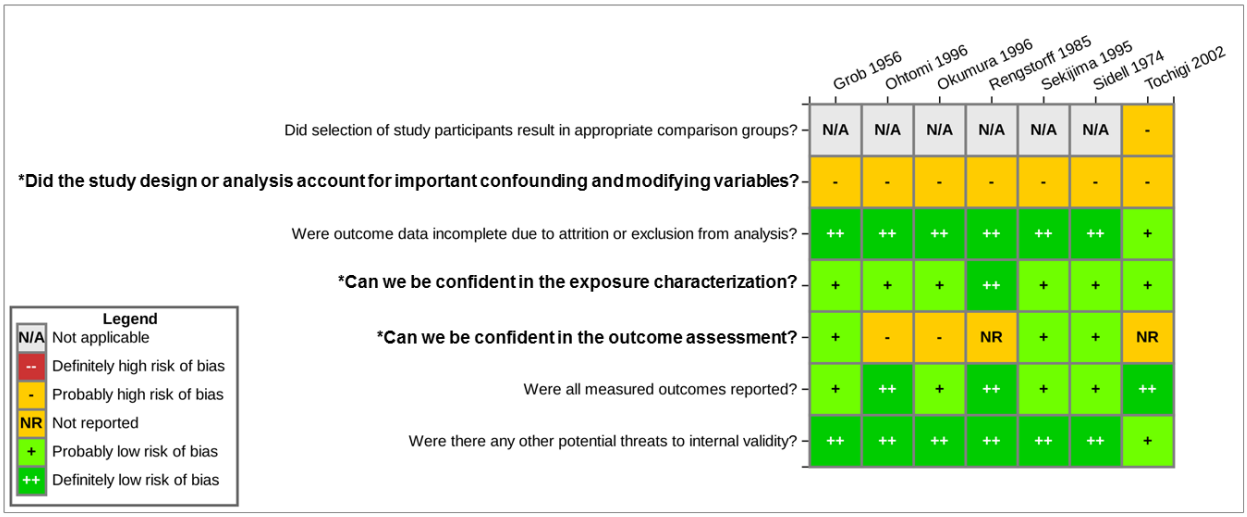
Figure D7. Risk-of-bias Bar Chart for Controlled Trials Assessing ChE Levels in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human controlled exposure studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

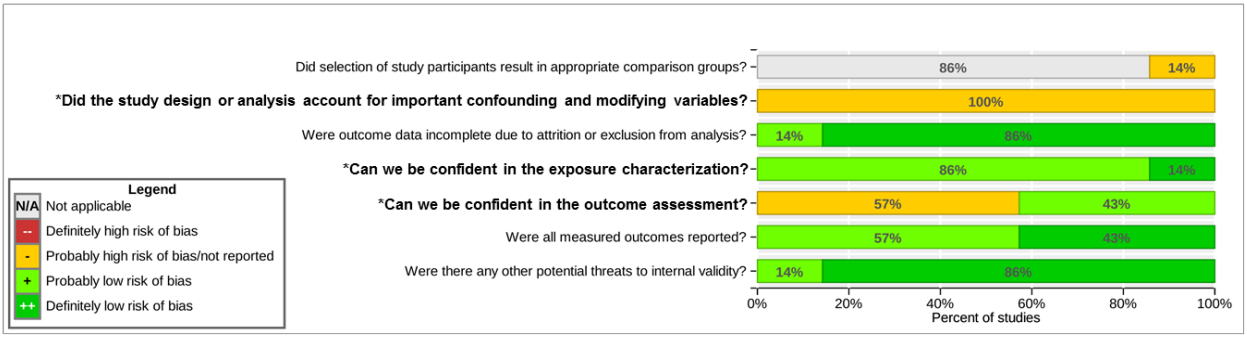
Figure D8. Risk-of-bias Heat Map for Case Reports/Series and Cross-Sectional Studies Assessing ChE Levels in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and cross-sectional studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

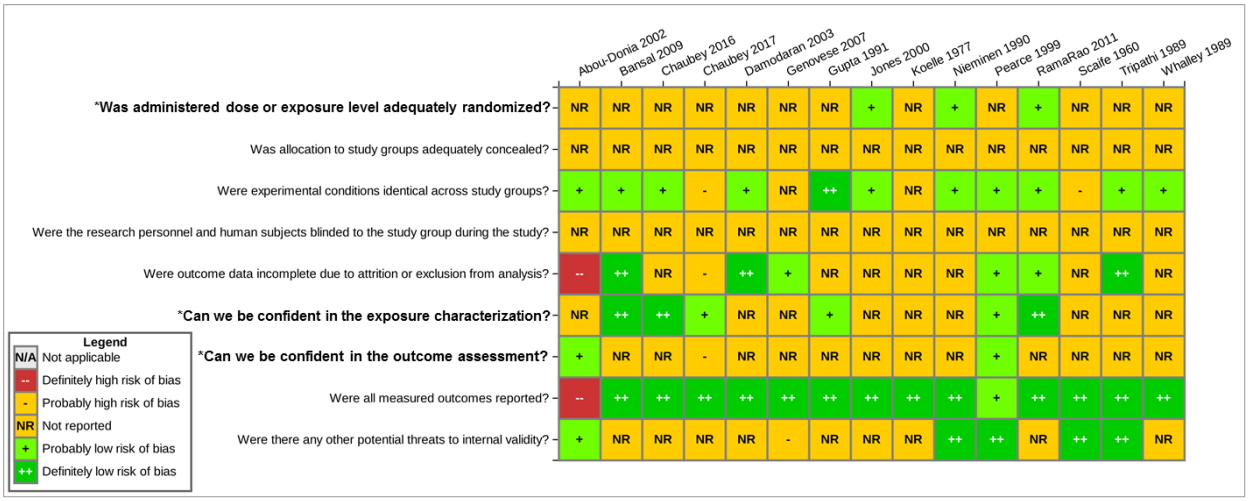
Figure D9. Risk-of-bias Bar Chart for Case Reports/Series and Cross-Sectional Studies Assessing ChE Levels in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and cross-sectional studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

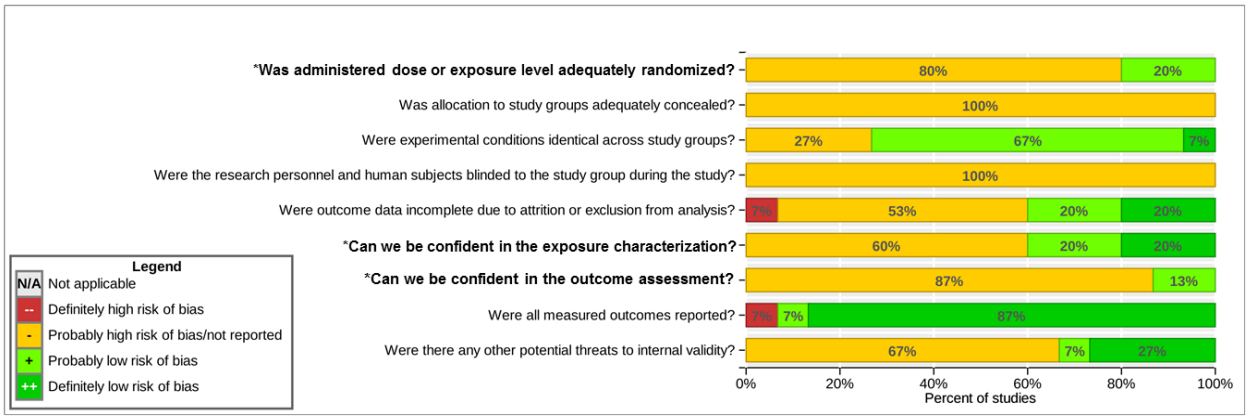
Figure D10. Risk-of-bias Heat Map for Individual Studies Assessing AChE Levels in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure D11. Risk-of-bias Bar Chart for Individual Studies Assessing AChE Levels in Animals Following Acute Sarin Exposure

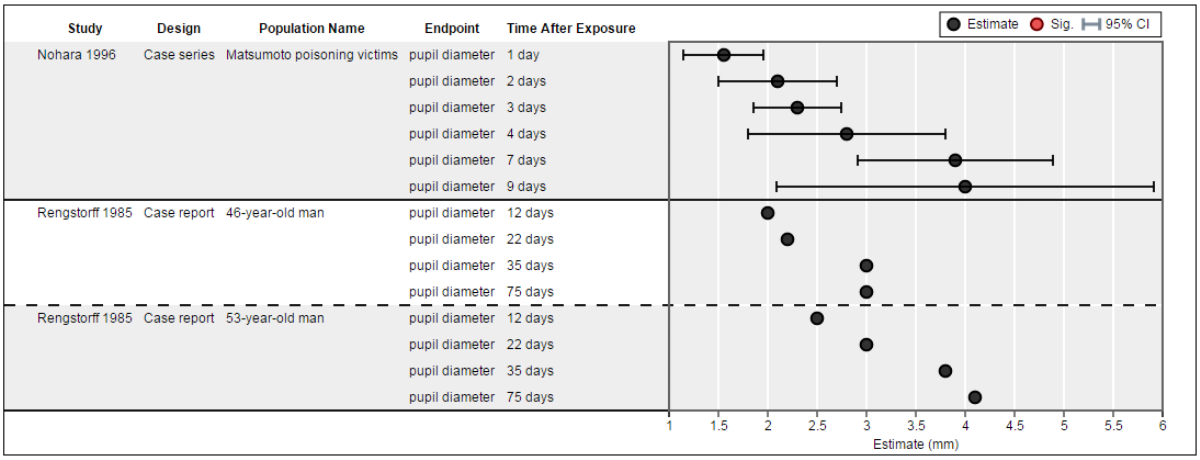


*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

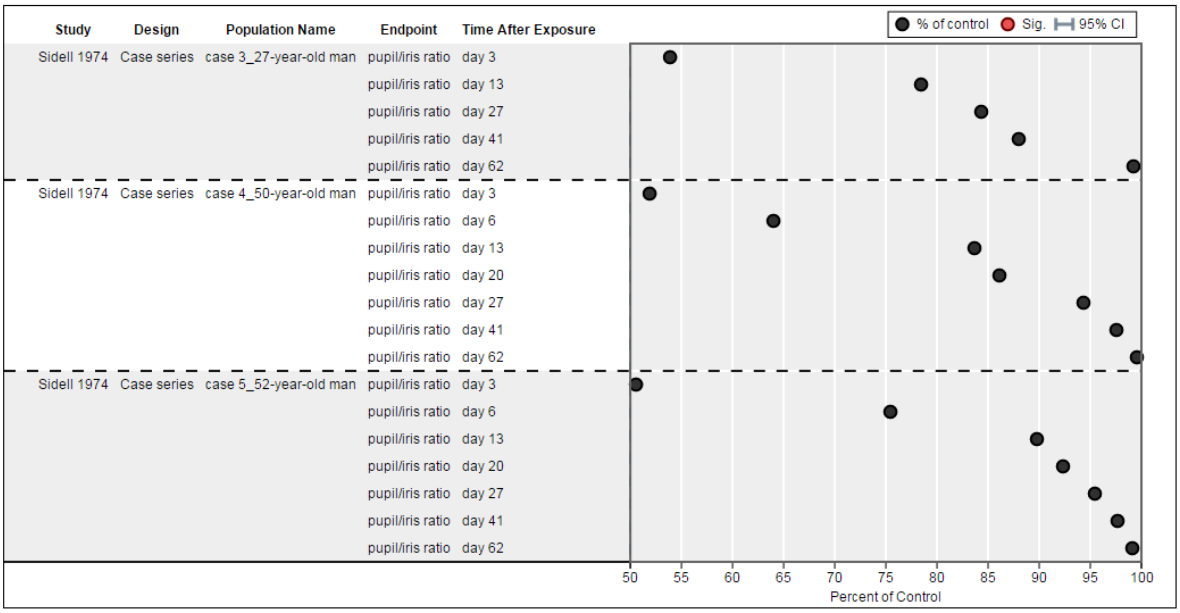
Visual and Ocular Effects

Figure D12. Pupil Diameter in Humans Following Acute Sarin Exposure



Interactive figure and additional study details in HAWC here. For the studies presented, statistical analyses were not conducted. Subjects in the case reports/series were exposed to an unknown amount of sarin and were evaluated in terms of time after exposure.
Note: Normal pupil size varies from 2 to 4 mm (bright light) to 4 to 8 mm (dark) (see https://www.ncbi.nlm.nih.gov/books/NBK381/).

Figure D13. Pupil/Iris Ratio in Humans Following Acute Sarin Exposure



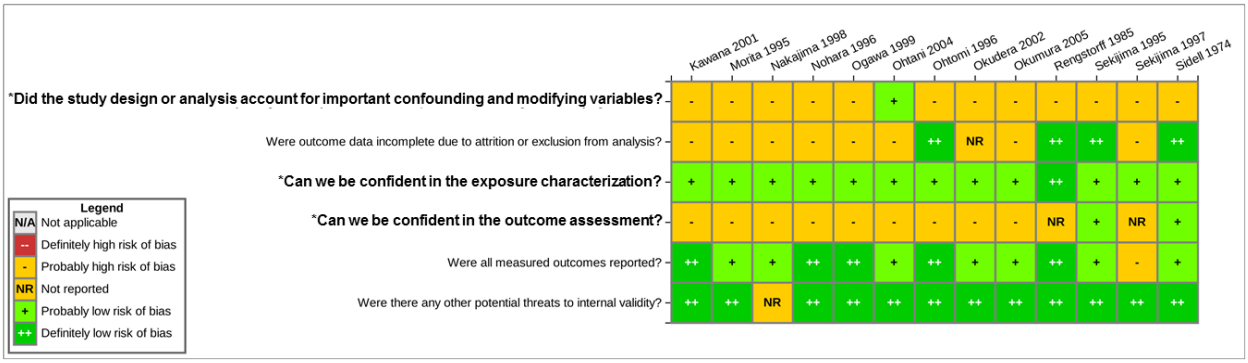
Interactive figure and additional study details in HAWC here. For the study presented, statistical analyses were not conducted. Results are based on a change from normal or subjects' baseline. Subjects in the case series were exposed to an unknown amount of sarin and were evaluated in terms of time after exposure.

Figure D14. Pupil Diameter in Animals Following Acute Sarin Exposure

Study	Animal description	Treatment period	Endpoint	Units	Observation time	Dose	Units
Egoz 2017	Rat, Long-Evans (♂, N=12)	acute (single topical exposure)	pupil diameter	% baseline	1.5 days	0	ug
						1	ug
					2 days	0	ug
						1	ug
					3 days	0	ug
						1	ug
					4 days	0	ug
						1	ug
					7 days	0	ug
						1	ug
Gore 2012	Rat, Long-Evans (♂, N=12)	acute (single topical exposure)	pupil diameter	% baseline	2 days	0	ug
						0.002	ug
						0.02	ug
						0.035	ug
						0.05	ug
						0.1	ug
						0.2	ug
						1	ug
						2	ug
						10	ug
					3 days	0	ug
						0.002	ug
						0.02	ug
						0.035	ug
						0.05	ug
						0.1	ug
						0.2	ug
						1	ug
						2	ug
						10	ug
Mioduszewski 2002	Rat, Sprague-Dawley (♀, N=10)	acute (5 minutes)	pupil diameter	number of reticule lines	2 days	0	mg.min/m3
						181.5	mg.min/m3
	Rat, Sprague-Dawley (♂, N=10)	acute (5 minutes)	pupil diameter	number of reticule lines	2 days	0	mg.min/m3
						181.5	mg.min/m3
	Rat, Sprague-Dawley (♀, N=10)	acute (60 minutes)	pupil diameter	number of reticule lines	2 days	0	mg.min/m3
						360	mg.min/m3
	Rat, Sprague-Dawley (♂, N=10)	acute (60 minutes)	pupil diameter	number of reticule lines	2 days	0	mg.min/m3
						360	mg.min/m3
	Rat, Sprague-Dawley (♀, N=10)	acute (240 minutes)	pupil diameter	number of reticule lines	2 days	0	mg.min/m3
						504	mg.min/m3
	Rat, Sprague-Dawley (♂, N=10)	acute (240 minutes)	pupil diameter	number of reticule lines	2 days	0	mg.min/m3
						504	mg.min/m3
	Rat, Sprague-Dawley (♀, N=10)	acute (360 minutes)	pupil diameter	number of reticule lines	2 days	0	mg.min/m3
						828	mg.min/m3
	Rat, Sprague-Dawley (♂, N=10)	acute (360 minutes)	pupil diameter	number of reticule lines	2 days	0	mg.min/m3
						828	mg.min/m3
	Rat, Sprague-Dawley (♀, N=10)	acute (5 minutes)	pupil diameter	number of reticule lines	7 days	0	mg.min/m3
						181.5	mg.min/m3
	Rat, Sprague-Dawley (♂, N=10)	acute (5 minutes)	pupil diameter	number of reticule lines	7 days	0	mg.min/m3
						181.5	mg.min/m3
	Rat, Sprague-Dawley (♀, N=10)	acute (60 minutes)	pupil diameter	number of reticule lines	7 days	0	mg.min/m3
						360	mg.min/m3
	Rat, Sprague-Dawley (♂, N=10)	acute (60 minutes)	pupil diameter	number of reticule lines	7 days	0	mg.min/m3
						360	mg.min/m3
	Rat, Sprague-Dawley (♀, N=10)	acute (240 minutes)	pupil diameter	number of reticule lines	7 days	0	mg.min/m3
						504	mg.min/m3
	Rat, Sprague-Dawley (♂, N=10)	acute (240 minutes)	pupil diameter	number of reticule lines	7 days	0	mg.min/m3
						504	mg.min/m3
	Rat, Sprague-Dawley (♀, N=10)	acute (360 minutes)	pupil diameter	number of reticule lines	7 days	0	mg.min/m3
						828	mg.min/m3
	Rat, Sprague-Dawley (♂, N=10)	acute (360 minutes)	pupil diameter	number of reticule lines	7 days	0	mg.min/m3
						828	mg.min/m3

Interactive figure and additional study details in HAWC here.

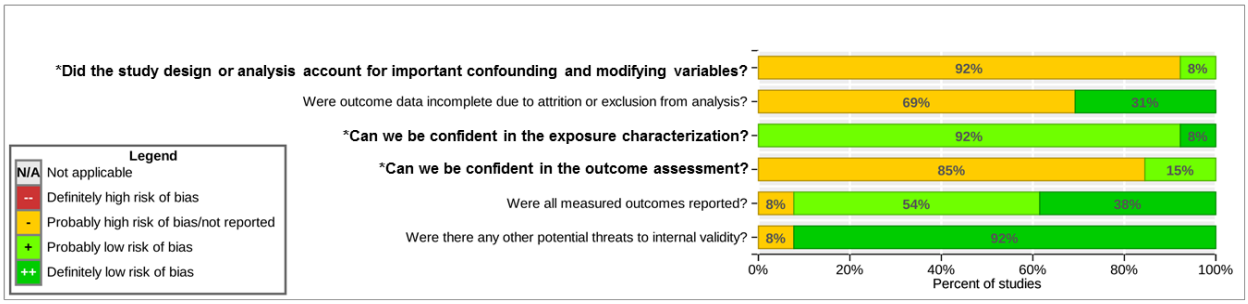
Figure D15. Risk-of-bias Heat Map for Case Reports/Series Assessing Visual and Ocular Effects in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

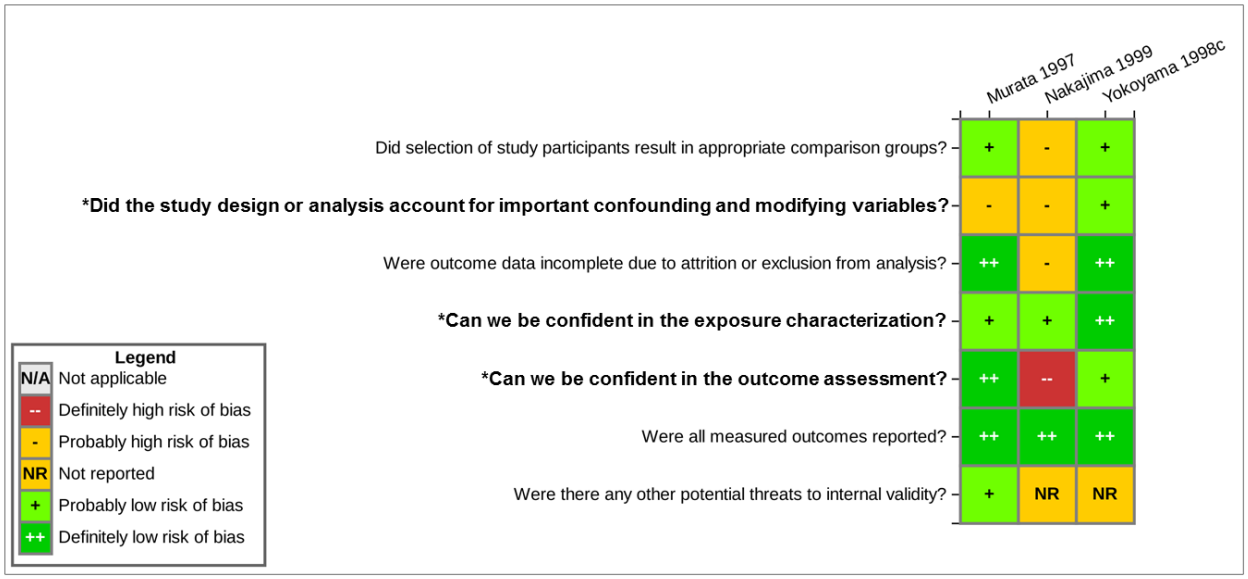
Figure D16. Risk-of-bias Bar Chart for Case Reports/Series Assessing Visual and Ocular Effects in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

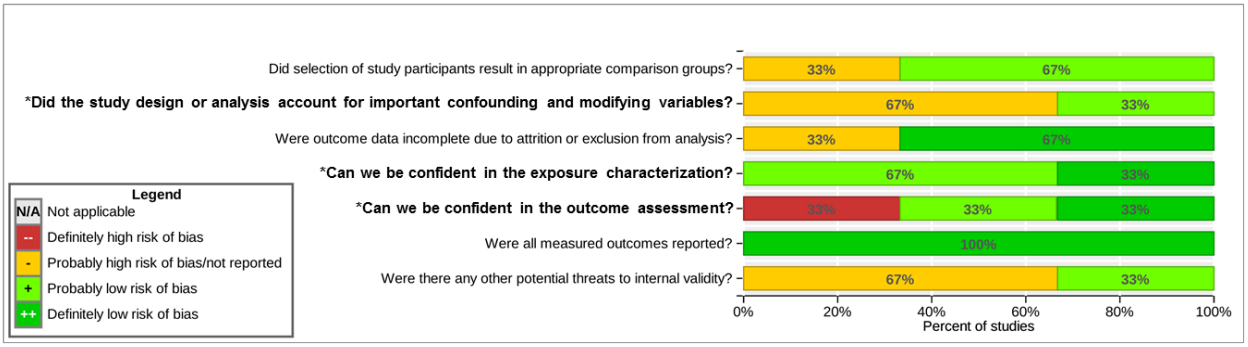
Figure D17. Risk-of-bias Heat Map for Standard Observational Studies Assessing Visual and Ocular Effects in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

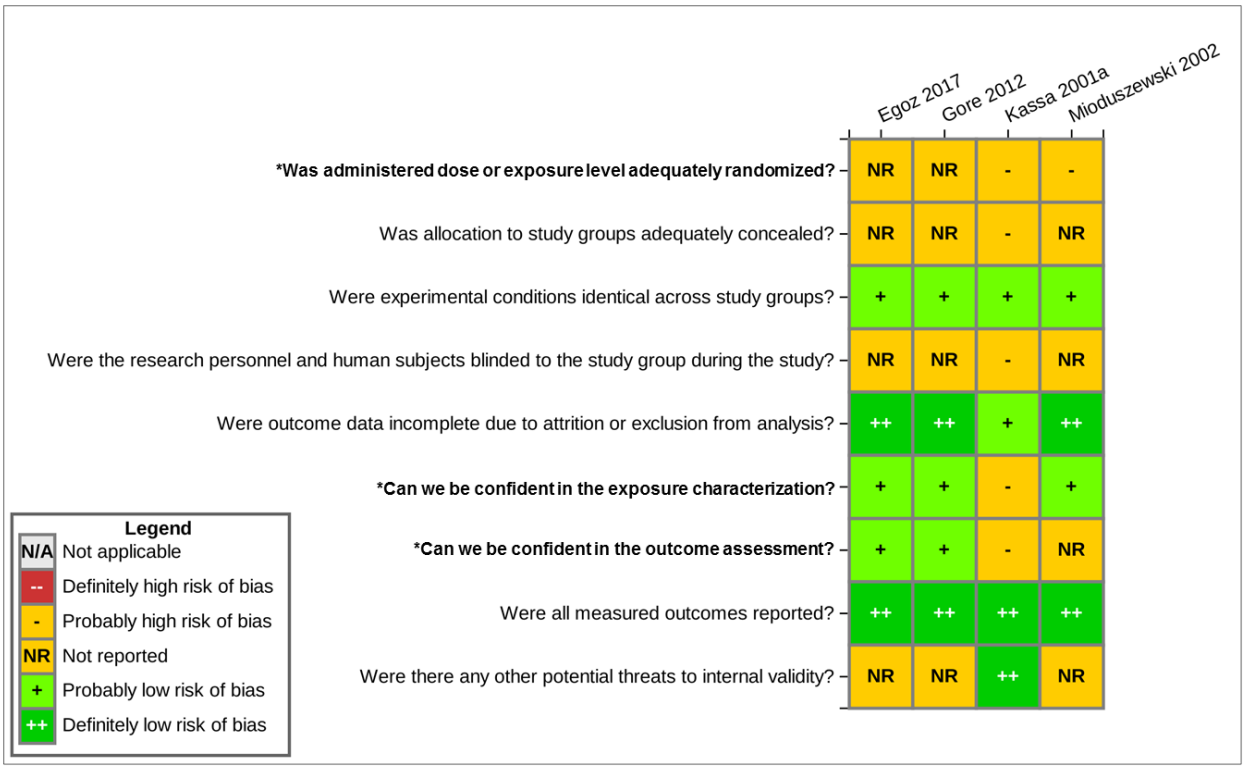
Figure D18. Risk-of-bias Bar Chart for Standard Observational Studies Assessing Visual and Ocular Effects in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

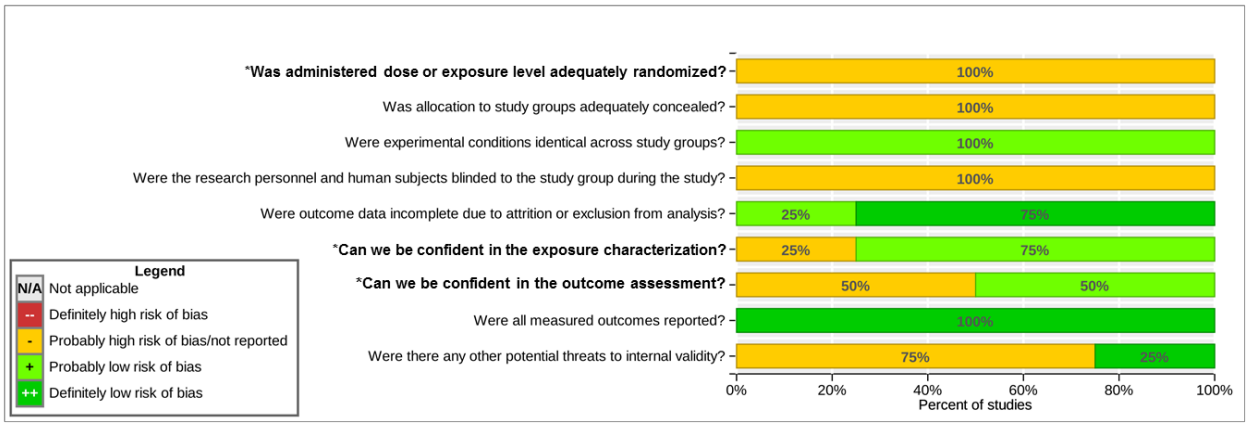
Figure D19. Risk-of-bias Heat Map for Individual Studies Assessing Visual and Ocular Effects in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure D20. Risk-of-bias Bar Chart for Individual Studies Assessing Visual and Ocular Effects in Animals Following Acute Sarin Exposure

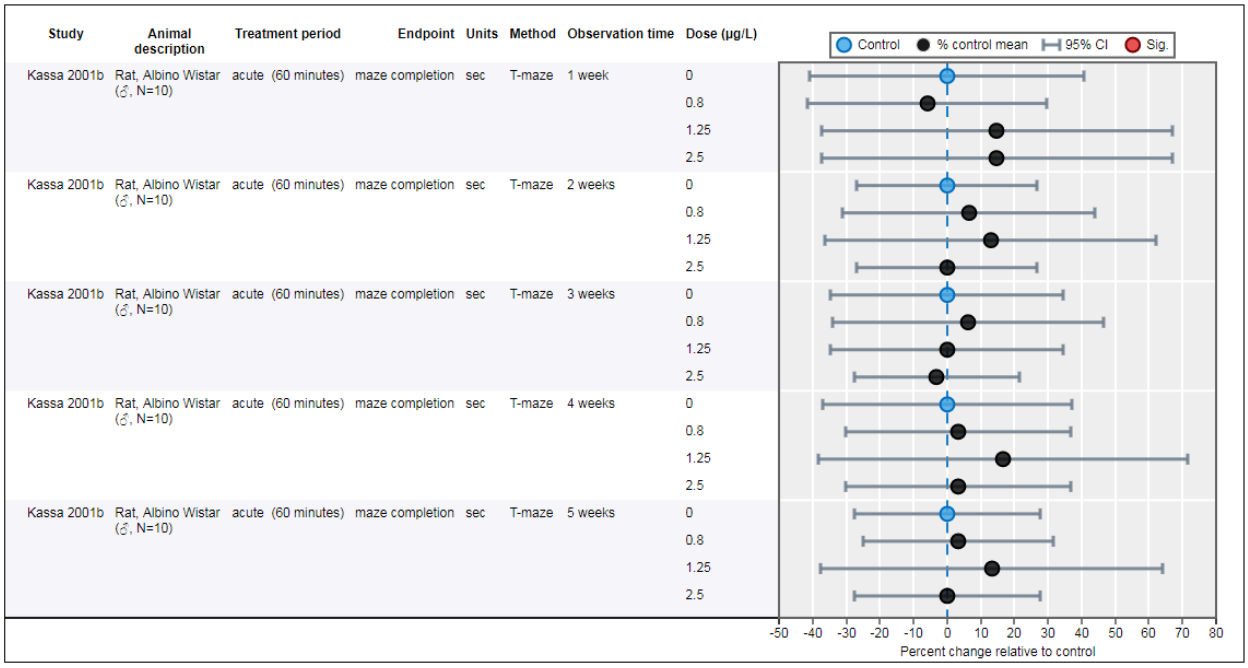


*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

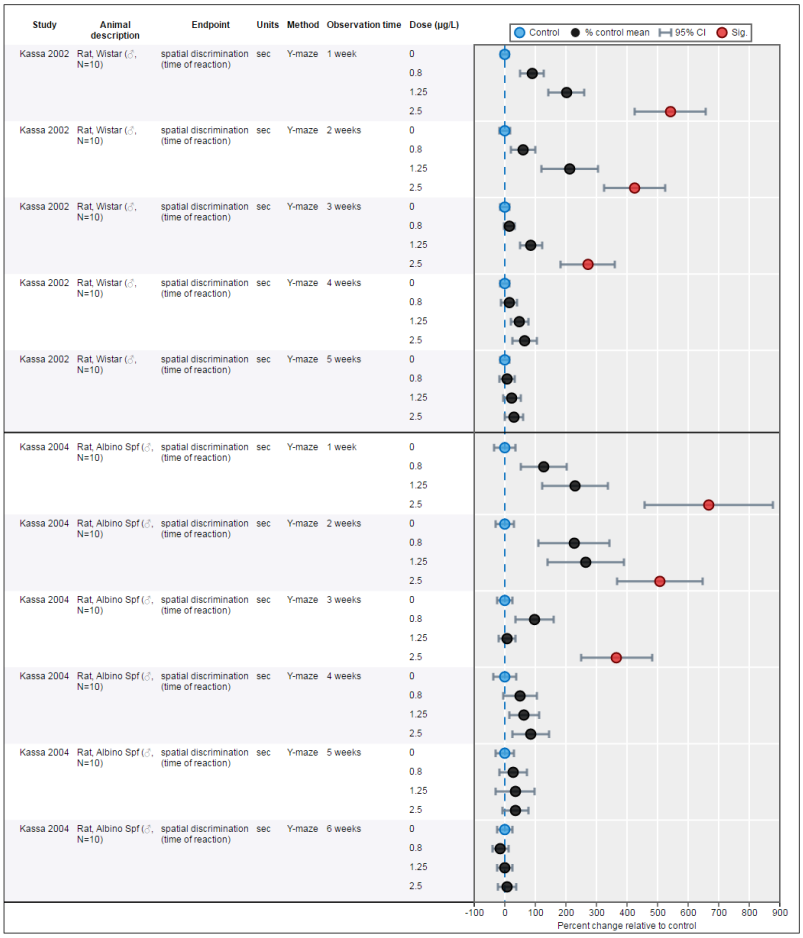
Effects on Learning, Memory, and Intelligence

Figure D21. T-Maze Results in Animals Following Acute Sarin Exposure



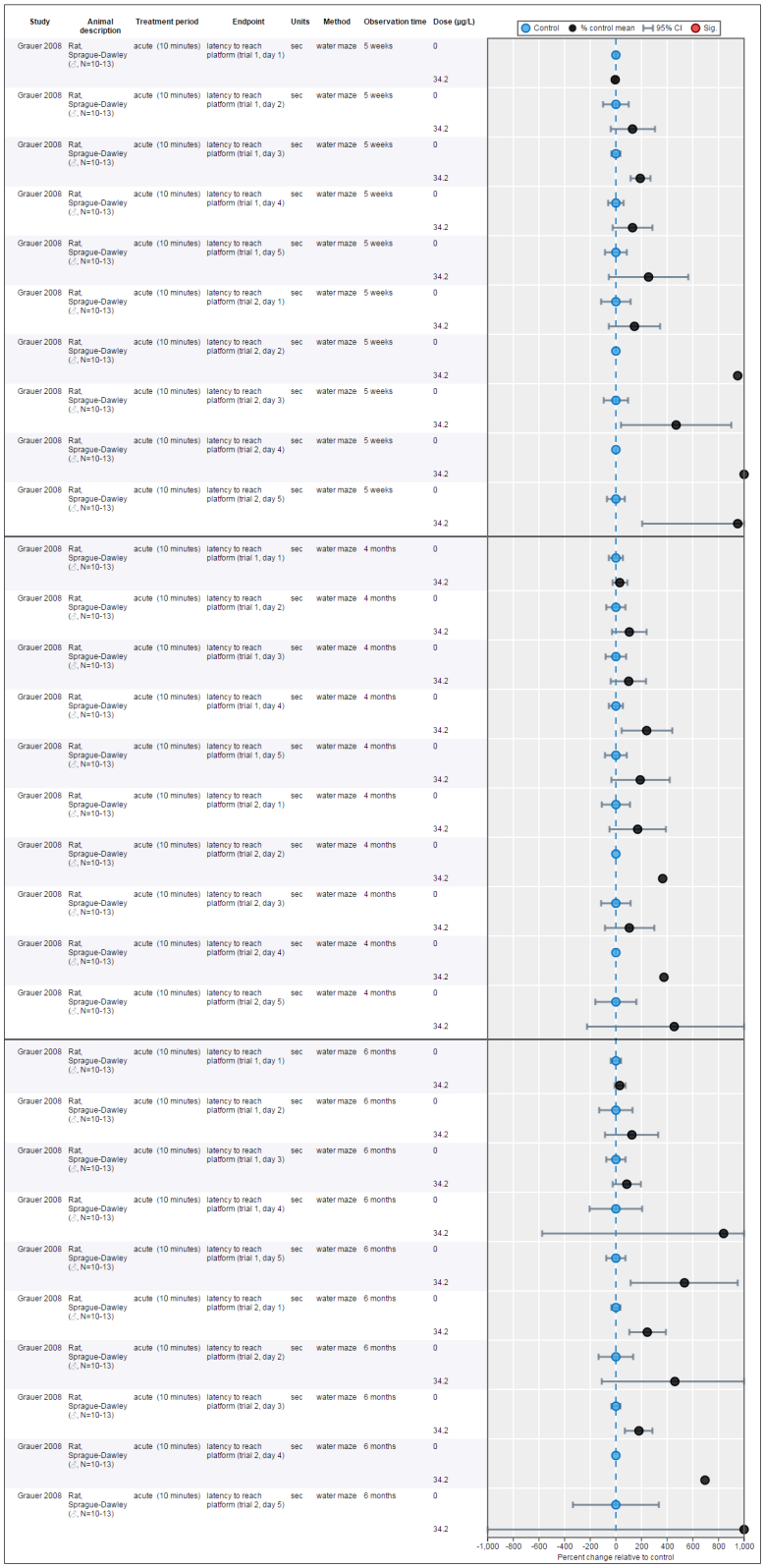
Interactive figure and additional study details in HAWC here.

Figure D22. Y-Maze Results in Animals Following Acute Sarin Exposure



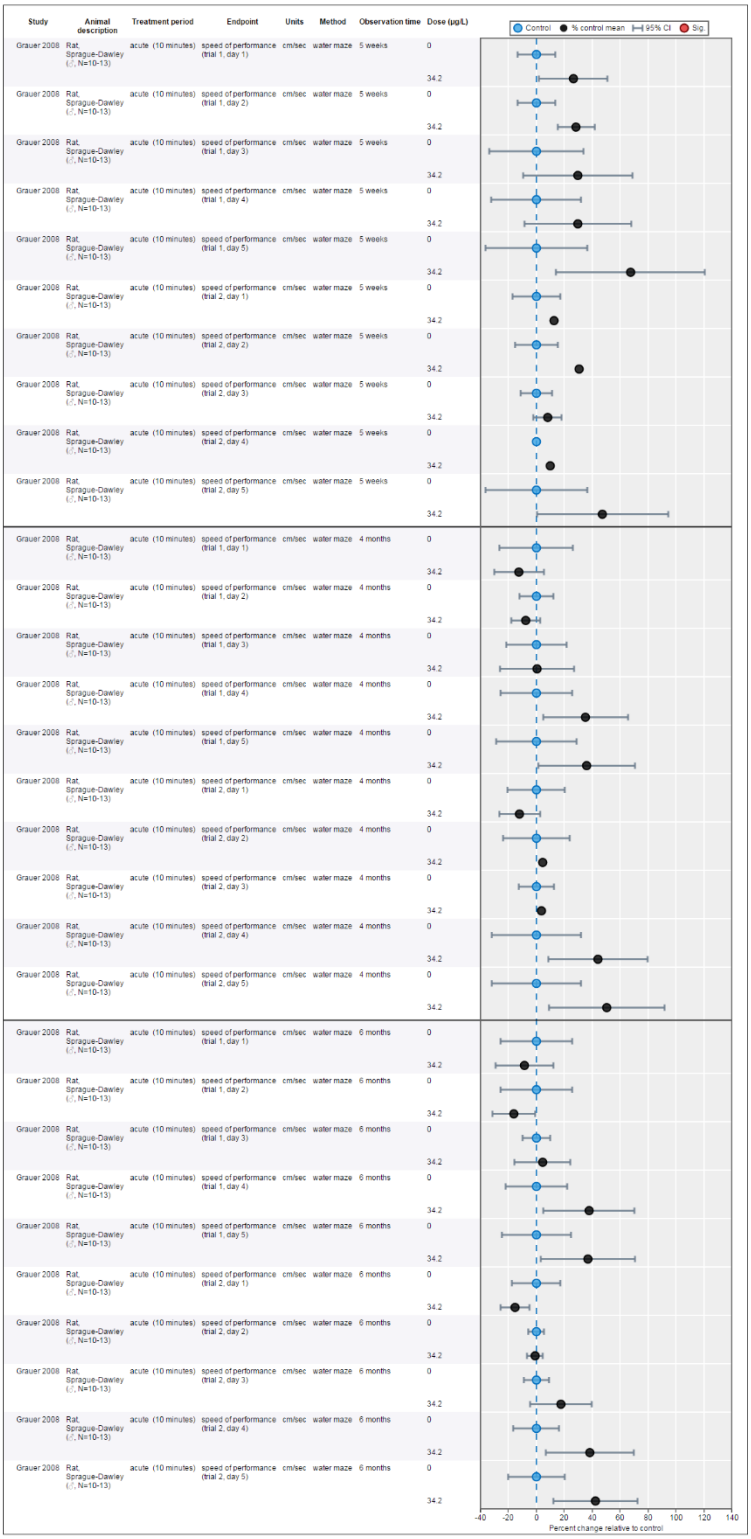
Interactive figure and additional study details in HAWC here.

Figure D23. Water Maze Latency to Reach Platform Results in Animals Following Acute Sarin Exposure



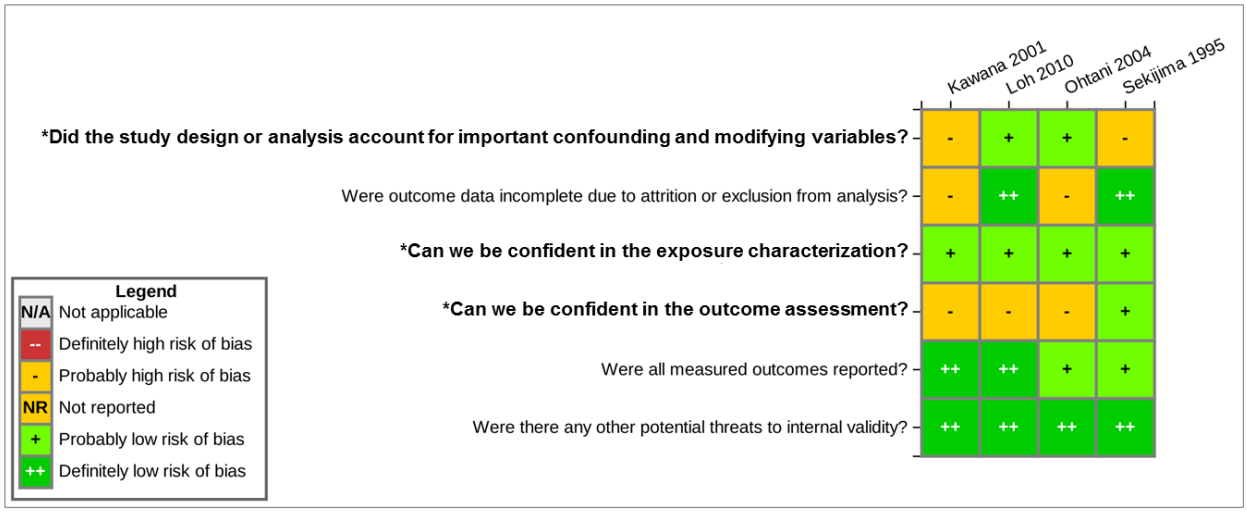
Interactive figure and additional study details in HAWC here.

Figure D24. Water Maze Speed of Performance Results in Animals Following Acute Sarin Exposure



Interactive figure and additional study details in HAWC here.

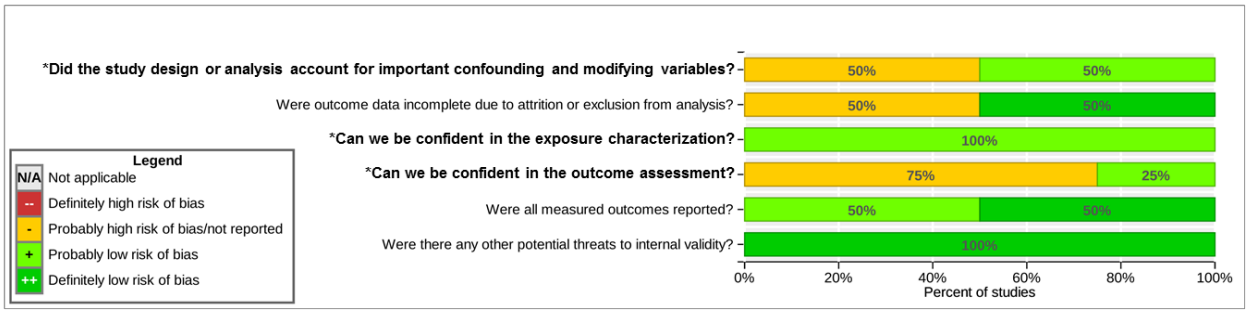
Figure D25. Risk-of-bias Heat Map for Case Reports/Series Assessing Learning, Memory, and Intelligence in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

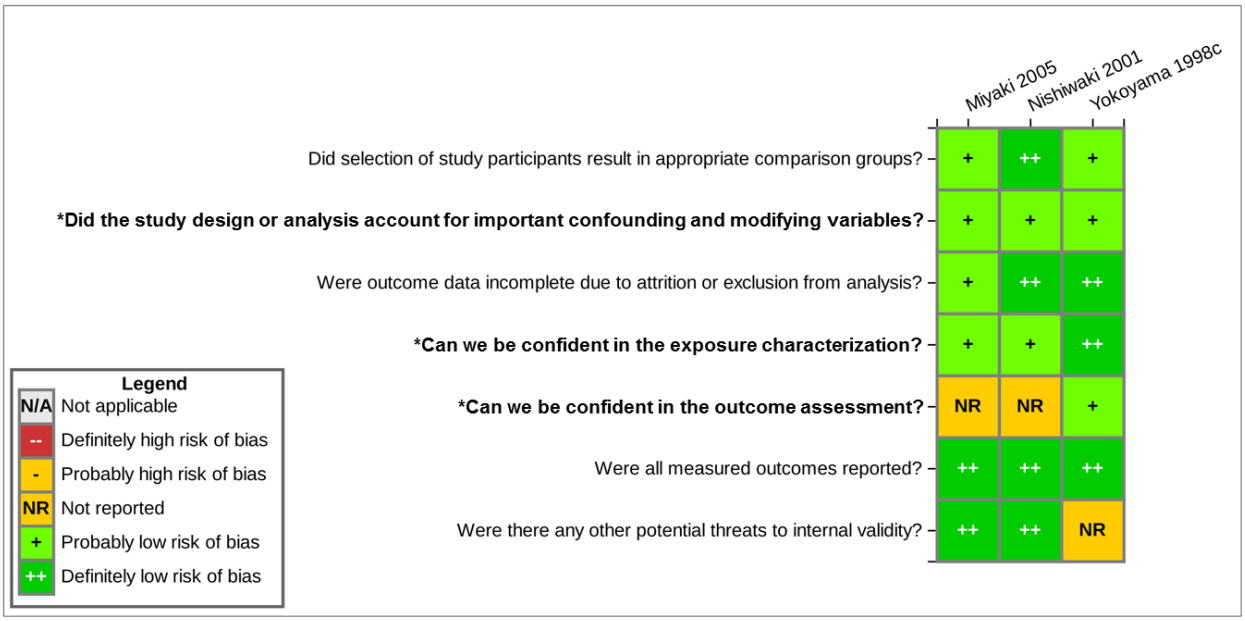
Figure D26. Risk-of-bias Bar Chart for Case Reports/Series Assessing Learning, Memory, and Intelligence in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

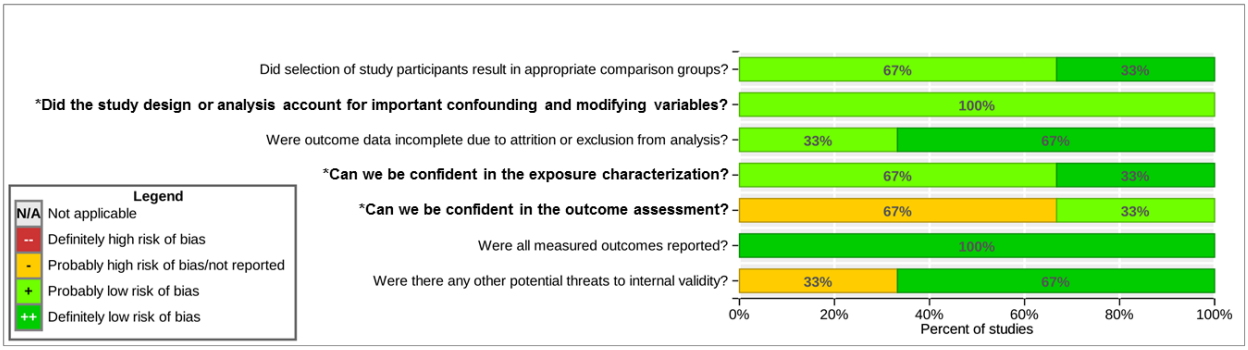
Figure D27. Risk-of-bias Heat Map for Cross-Sectional Studies Assessing Learning, Memory, and Intelligence in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human cross-sectional studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

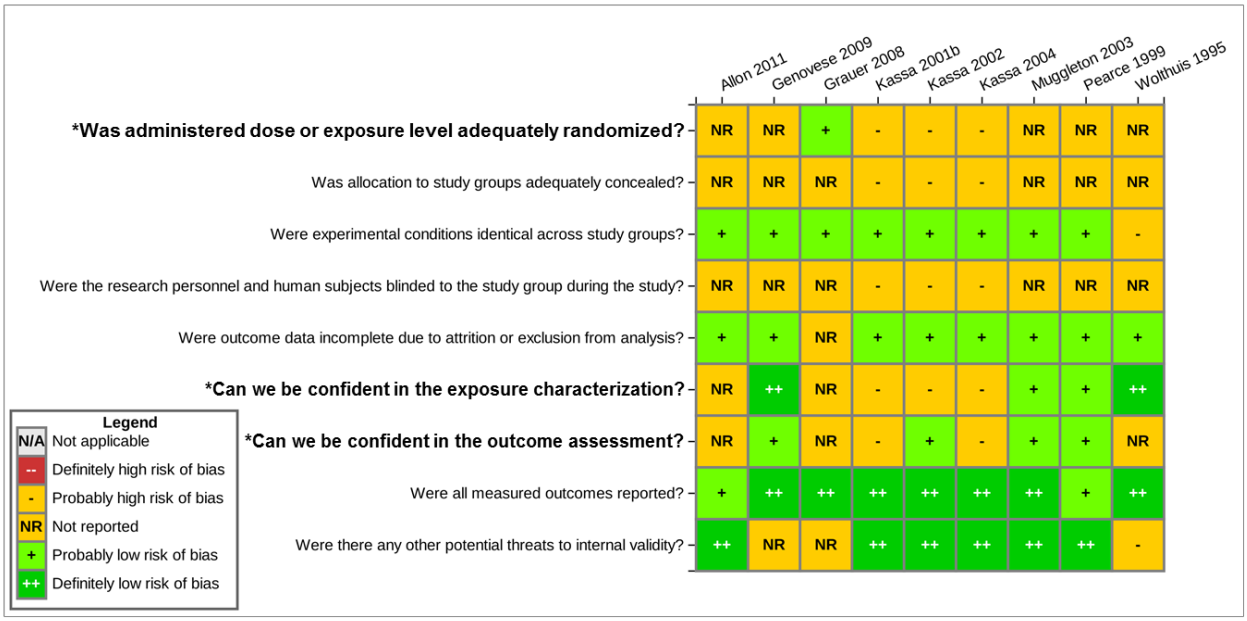
Figure D28. Risk-of-bias Bar Chart for Cross-Sectional Studies Assessing Learning, Memory, and Intelligence in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human cross-sectional studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

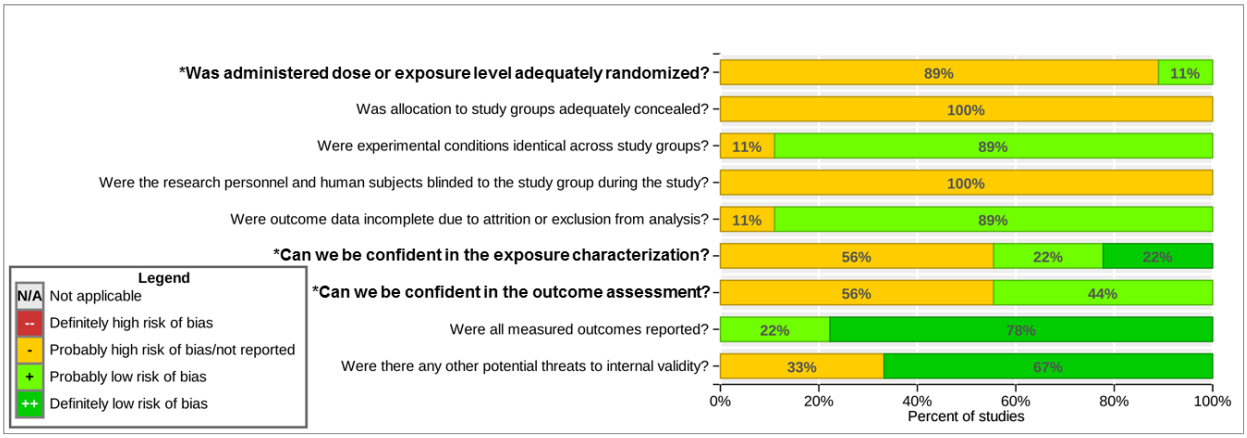
Figure D29. Risk-of-bias Heat Map for Individual Studies Assessing Learning, Memory, and Intelligence in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure D30. Risk-of-bias Bar Chart for Individual Studies Assessing Learning, Memory, and Intelligence in Animals Following Acute Sarin Exposure

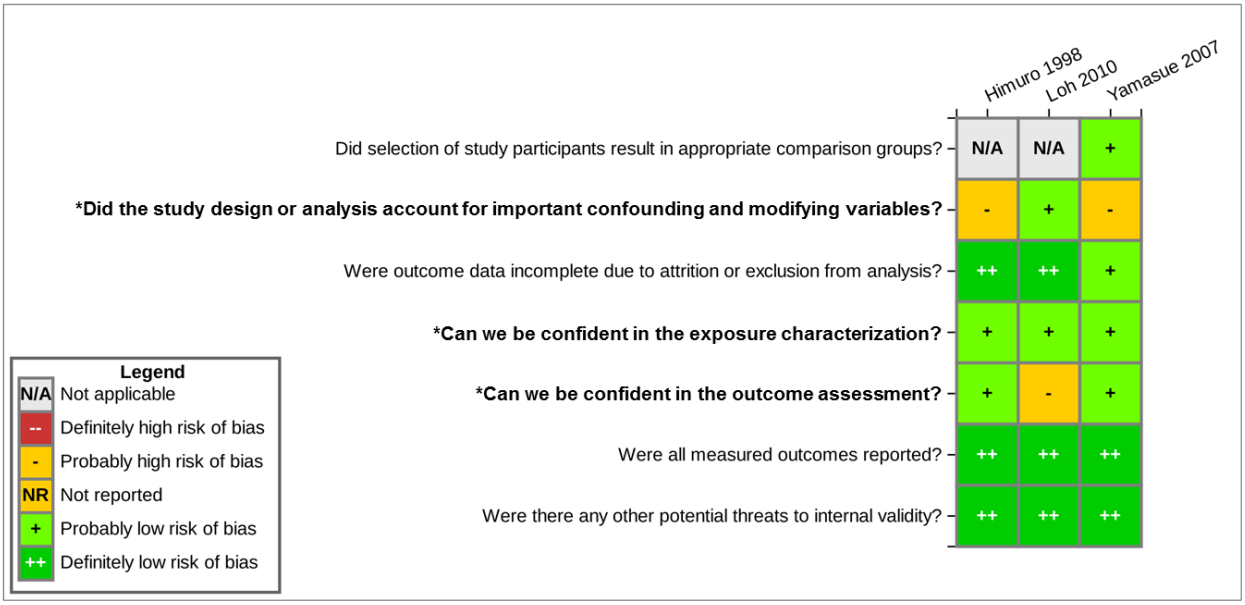


*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Morphological and Histological Changes

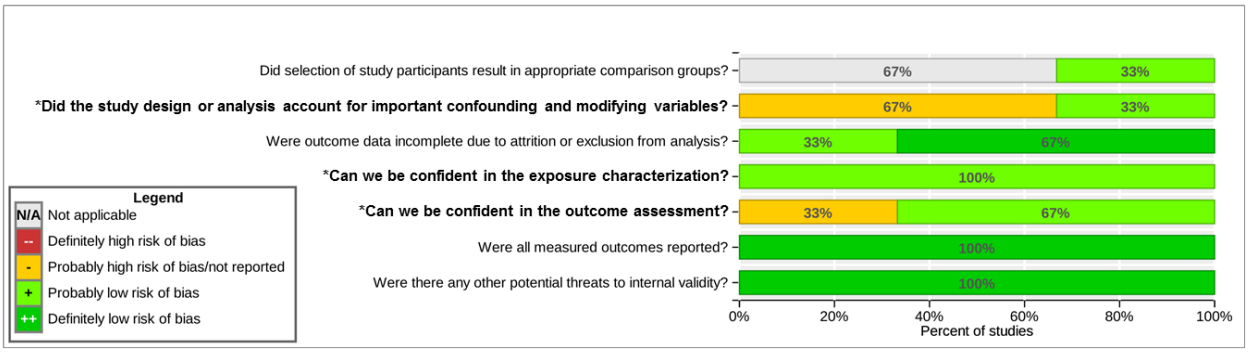
Figure D31. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Nervous System Morphology in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

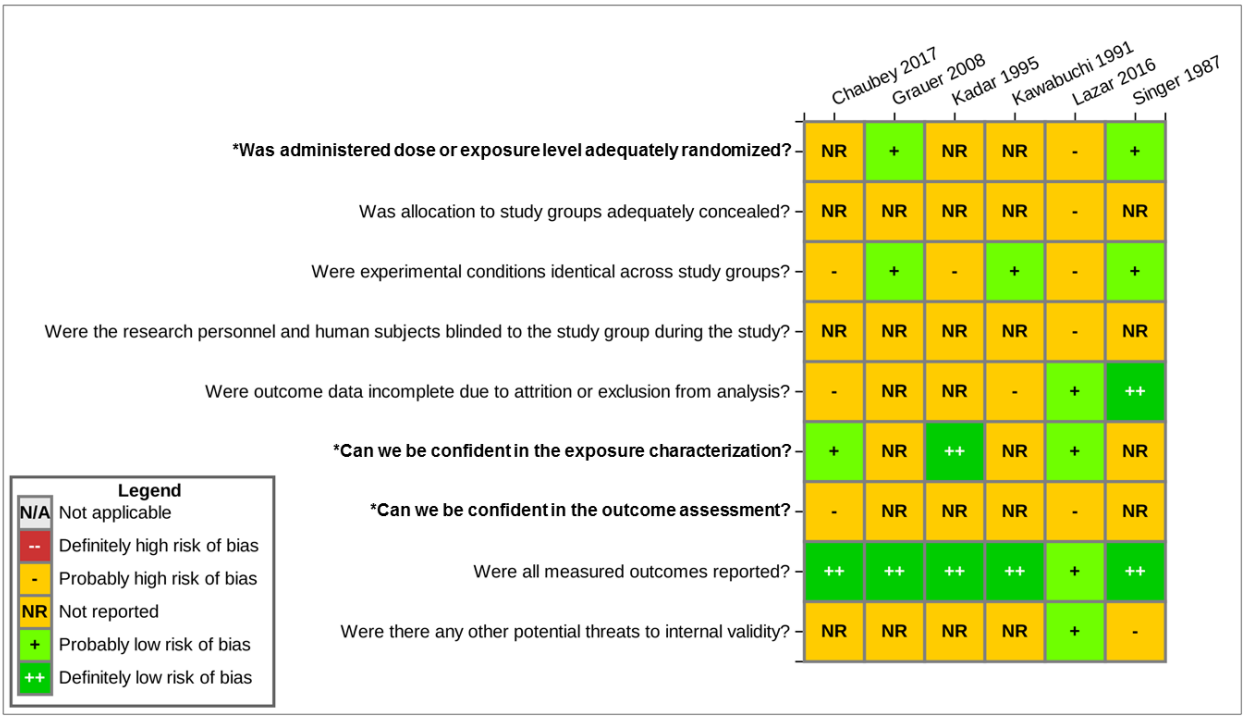
Figure D32. Risk-of-bias Bar Chart for Case Reports/Series and Standard Observational Studies Assessing Nervous System Morphology in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

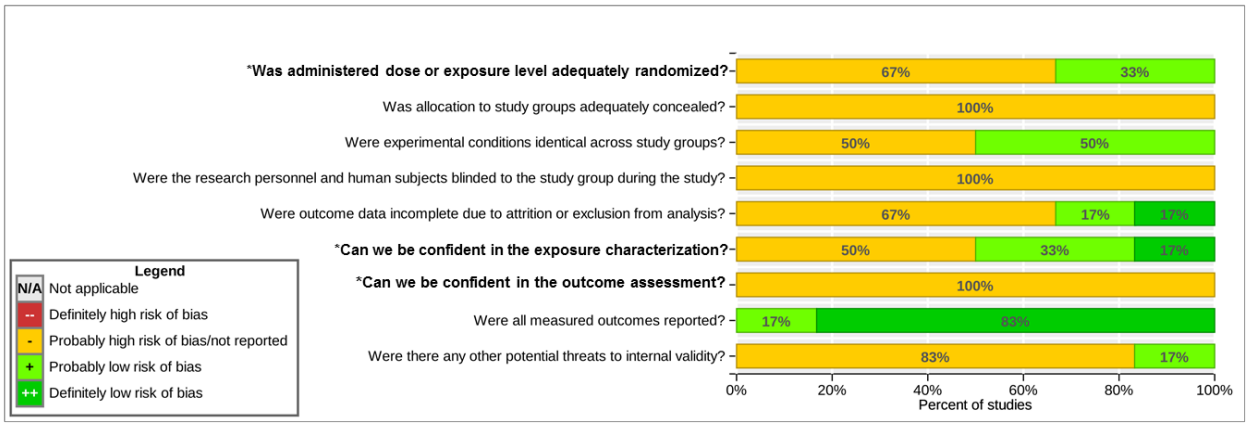
Figure D33. Risk-of-bias Heat Map for Individual Studies Assessing Nervous System Histological Changes in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure D34. Risk-of-bias Bar Chart for Individual Studies Assessing Nervous System Histological Changes in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

ABOUT THIS REVIEW

Sources of Support

National Institute of Environmental Health Sciences/Division of the National Toxicology Program

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The evaluation team is composed of federal staff and contractor staff support.

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Note: the roles of individual contractors differed: [a] indicates monograph development; [b] indicates review of data, results, and analyses; [c] indicates database and HAWC support; [d] indicates literature screening, [e] indicates data extraction, [f] indicates risk-of-bias assessment

Peer Reviewers

The peer reviewers were outside experts selected for their experience with neurotoxicity, organophosphates, and systematic review procedures. Peer reviewers were screened for conflict of interest prior to their service and did not report any conflicts of interest. Service as a peer reviewer does not necessarily indicate that the reviewer endorses the final document.

Protocol Reviewers

Name	Affiliation
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Jonathan Newmark, MD	Medical Corps, US Army (retired)

no conflicts of interest declared

Technical Review of Draft Monograph

Name	Affiliation
Jonathan Newmark, MD	Medical Corps, US Army (retired)

no conflicts of interest declared

Protocol History and Revisions

Date	Activity or revision
February 26-27, 2014:	**Problem formulation:** outcome of CounterACT workshop. Posted online and circulated internally for comment/review – impetus for this systematic review
January 5, 2016	**Draft evaluation protocol reviewed:** sent to technical advisors for peer reviewer by Drs. Madsen and Scherer
April 15, 2017	**Evaluation protocol finalized:** Review protocol finalized for use and posting

APPENDICES

Appendix 1. Literature Search Strategy

The search terms and databases searched are provided below.

Database	Search Terms
Cochrane Library	(sarin or zarin or "o Isopropylmethyl Phosphonofluoridate" or "ortho Isopropylmethyl Phosphonofluoridate" or "ortho-Isopropylmethyl Phosphonofluoridate" or "Isopropyl methylphosphonofluoridate" or "Isopropyl Methylfluorophosphonate" or "(RS)-propan-2-yl methylphosphonofluoridate" or (GB and organophos*) or (GB and nerve)):ti,ab,kw
Embase	'sarin':ab,ti OR 'sarin'/exp OR 'o isopropylmethyl phosphonofluoridate':ab,ti OR 'ortho isopropylmethyl phosphonofluoridate':ab,ti OR 'ortho-isopropylmethyl phosphonofluoridate':ab,ti OR 'isopropyl methylphosphonofluoridate'/exp OR 'isopropyl methylphosphonofluoridate':ab,ti OR 'isopropyl methylfluorophosphonate':ab,ti OR '(rs)-propan-2-yl methylphosphonofluoridate':ab,ti OR (gb:ab,ti AND organophos*:ab,ti) OR (gb:ab,ti AND nerve:ab,ti)
NIOSHTIC-2 Publications	Sarin[title] OR sarin[abstract] OR zarin[title] OR zarin[abstract]
NIOSHTIC-2 Publications	GB[abstract] AND nerve[abstract]
NIOSHTIC-2 Publications	GB[title] AND nerve[title]
NIOSHTIC-2 Publications	GB[abstract] AND organophos*[abstract]
NIOSHTIC-2 Publications	GB[title] AND organophos*[title]
PubMed/MEDLINE	(sarin[tiab] OR sarin[mesh] OR zarin[tiab] OR “o Isopropylmethyl Phosphonofluoridate”[tiab] OR “ortho Isopropylmethyl Phosphonofluoridate”[tiab] OR “ortho-Isopropylmethyl Phosphonofluoridate”[tiab] OR “Isopropyl methylphosphonofluoridate”[tiab] OR “Isopropyl Methylfluorophosphonate”[tiab] OR “(RS)-propan-2-yl methylphosphonofluoridate”[tiab] OR (GB[tiab] AND organophos*[tiab]) OR (GB[tiab] AND nerve[tiab]))
Scopus	Title-Abs-Key((sarin OR zarin OR {o Isopropylmethyl Phosphonofluoridate} OR {ortho Isopropylmethyl Phosphonofluoridate} OR {ortho-Isopropylmethyl Phosphonofluoridate} OR {Isopropyl methylphosphonofluoridate} OR {Isopropyl Methylfluorophosphonate} OR {(RS)-propan-2-yl methylphosphonofluoridate} OR (GB AND organophos*) OR (GB AND nerve)))
TOXLINE	Title: sarin[ti] OR sarin[mh] OR zarin[ti] OR “o Isopropylmethyl Phosphonofluoridate”[ti] OR “ortho Isopropylmethyl Phosphonofluoridate”[ti] OR “ortho-Isopropylmethyl

Database	Search Terms
	Phosphonofluoridate”[ti] OR “Isopropyl methylphosphonofluoridate”[ti] OR “Isopropyl Methylfluorophosphonate”[ti] OR “(RS)-propan-2-yl methylphosphonofluoridate”[ti] OR (GB[ti] AND organophos*[ti]) OR (GB[ti] AND nerve[ti])
Toxline	Abstract: sarin[ab] OR sarin[mh] OR zarin[ab] OR “o Isopropylmethyl Phosphonofluoridate”[ab] OR “ortho Isopropylmethyl Phosphonofluoridate”[ab] OR “ortho-Isopropylmethyl Phosphonofluoridate”[ab] OR “Isopropyl methylphosphonofluoridate”[ab] OR “Isopropyl Methylfluorophosphonate”[ab] OR “(RS)-propan-2-yl methylphosphonofluoridate”[ab] OR (GB[ab] AND organophos*[ab]) OR (GB[ab] AND nerve[ab])
Web of Science	TS=(sarin OR zarin OR “o Isopropylmethyl Phosphonofluoridate” OR “ortho Isopropylmethyl Phosphonofluoridate” OR “ortho-Isopropylmethyl Phosphonofluoridate” OR “Isopropyl methylphosphonofluoridate” OR “Isopropyl Methylfluorophosphonate” OR “(RS)-propan-2-yl methylphosphonofluoridate” OR (GB AND organophos*) OR (GB AND nerve))

Appendix 2. List of Included Studies

Studies in Humans

Baker D, Sedgwick E. 1996. Single fibre electromyographic changes in man after organophosphate exposure. *Hum Exp Toxicol* 15(5): 369-375.

Grob D. 1956. The manifestations and treatment of poisoning due to nerve gas and other organic phosphate anticholinesterase compounds. *AMA Arch Intern Med* 98(2): 221-239.

Grob D, Harvey J. 1958. Effects in man of the anticholinesterase compound sarin (isopropyl methyl phosphonofluoridate). *J Clin Invest* 37(3): 350-368.

Himuro K, Murayama S, Nishiyama K, Shinoe T, Iwase H, Nagao M, Takatori T, Kanazawa I. 1998. Distal sensory axonopathy after sarin intoxication. *Neurology* 51(4): 1195-1197.

Kawana N, Ishimatsu S, Kanda K. 2001. Psycho-physiological effects of the terrorist sarin attack on the Tokyo subway system. *Mil Med* 166(12 Suppl): 23-26.

Kawana N, Ishimatsu S, Matsui Y, Tamaki S, Kanda K. 2005. Chronic posttraumatic stress symptoms in victims of Tokyo subway sarin gas attack. *Traumatology* 11(2): 87-102.

Loh Y, Swanberg M, Ingram M, Newmark J. 2010. Case report: Long-term cognitive sequelae of sarin exposure. *Neurotox* 31(2): 244-246.

Miyaki K, Nishiwaki Y, Maekawa K, Ogawa Y, Asukai N, Yoshimura K, Etoh N, Matsumoto Y, Kikuchi Y, Kumagai N, Omae K. 2005. Effects of sarin on the nervous system of subway workers seven years after the Tokyo subway sarin attack. *J Occup Health* 47(4): 299-304.

Morita H, Yanagisawa N, Nakajima T, Shimizu M, Hirabayashi H, Okudera H, Nohara M, Midorikawa Y, Mimura S. 1995. Sarin poisoning in Matsumoto, Japan. *Lancet* 346(8970): 290-293.

Murata K, Araki S, Yokoyama K, Okumura T, Ishimatsu S, Takasu N, White R. 1997. Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack. *J Neurol* 244(10): 601-606.

Nakajima T, Ohta S, Morita H, Midorikawa Y, Mimura S, Yanagisawa N. 1998. Epidemiological study of sarin poisoning in Matsumoto City, Japan. *J Epidemiol* 8(1): 33-41.

Nakajima T, Ohta S, Fukushima Y, Yanagisawa N. 1999. Sequelae of sarin toxicity at one and three years after exposure in Matsumoto, Japan. *J Epidemiol* 9(5): 337-343.

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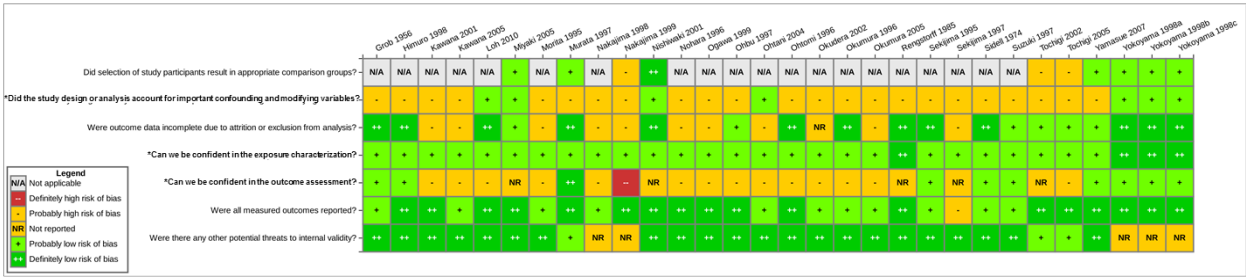
Appendix 3. Risk-of-bias Assessment for All Included Studies

Studies in Humans

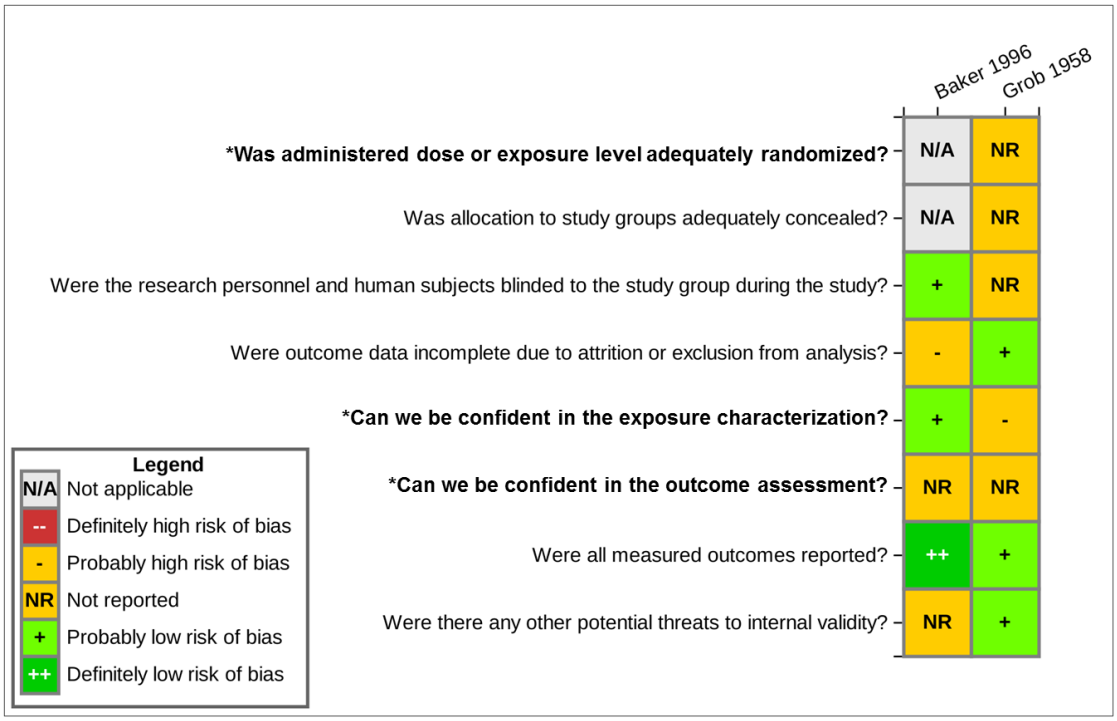
Figure A3-1. Risk-of-bias Heatmap for All Included Case Reports/Series and Standard Observational Studies in Humans Following Acute Sarin Exposure.



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure A3-2. Risk-of-bias Heatmap for All Included Controlled Trials in Humans Following Acute Sarin Exposure.

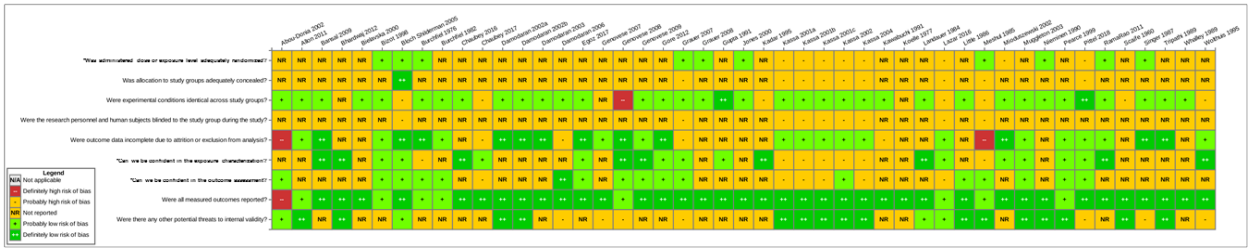


*Questions in bold are the key risk-of-bias questions for human controlled exposure studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Studies in Non-human Animals

Figure A3-3. Risk-of-bias Heatmap for All Included Studies in Animals Following Acute Sarin Exposure.



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Appendix 4. Inadequate Evidence: Evidence Synthesis and Risk-of-Bias Assessment

Sleep Disruption

Sleep disruption-related outcomes in humans included bad dreams, distressing dreams or nightmares, difficulty falling or staying asleep, insomnia, and sleep disturbance. Symptoms of sleep disruption were reported in the weeks to years following acute sarin exposure. In animals, no studies of sleep disruption-related effects were identified.

Human sleep disruption data

Based on the available studies, there is very low confidence in the body of evidence that acute sarin exposure is associated with sleep disruption in humans over all time periods after the initial exposure. Six studies reporting on sleep disruption (including bad dreams, distressing dreams or nightmares, difficulty falling or staying asleep, insomnia, and sleep disturbance) after acute sarin exposure were identified, and all of the studies are from subjects following the Matsumoto terrorist attack or the Tokyo subway terrorist attack (Ohbu *et al.* 1997, Nakajima *et al.* 1998, Nakajima *et al.* 1999, Ogawa *et al.* 1999, Kawana *et al.* 2001, Ohtani *et al.* 2004). For the initial period covering 1–7 days following acute sarin exposure, no studies were available. Subjects from three case series studies report symptoms related to sleep disturbance 3 weeks to 4 months following acute sarin exposure (Ohbu *et al.* 1997, Nakajima *et al.* 1998, Ogawa *et al.* 1999). Subjects from three case series studies and one prospective cohort study report symptoms related to sleep disruption 1–5 years following acute sarin exposure (Nakajima *et al.* 1998, Nakajima *et al.* 1999, Kawana *et al.* 2001, Ohtani *et al.* 2004). There are serious limitations in the human body of evidence to evaluate the potential association between exposure to sarin and symptoms related to sleep disruption due to risk-of-bias concerns and uncertainties related to study design for case reports/series. The case reports/series that reported effects at the intermediate period of 8 days to 1 year had an initial confidence of low and were downgraded for serious risk-of-bias concerns to support a final rating of very low confidence in the body of evidence for the intermediate period. The initial confidence of moderate for the cohort study (Nakajima *et al.* 1999) was downgraded twice for serious risk-of-bias concerns (i.e., failure to control for confounding, potential biases in outcome assessment from self-reporting of symptoms via questionnaires, and loss of subjects over time) to support a final rating of very low confidence in the body of evidence for extended period following acute sarin exposure.

The available epidemiological studies in the human body of evidence that evaluated the association between acute exposure to sarin and long-term effects on sleep were based on two terror attacks—the 1994 Matsumoto sarin attack and 1995 Tokyo subway sarin attack—and a subset of these studies followed only some of the victims over time. There is no human evidence to evaluate the potential association between sarin exposure and effects on sleep days following exposure; therefore, there is inadequate evidence in the initial days after exposure.

Three studies are available that observed sleep disruption in subjects less than a year after sarin exposure (Ohbu *et al.* 1997, Nakajima *et al.* 1998, Ogawa *et al.* 1999). At 1 month following the Tokyo subway sarin attack, Ohbu *et al.* (1997) reported that 137 of 475 hospital patients (29%) described symptoms of sleep disturbance and 48 of 475 hospital patients (10%) reported nightmares. The authors did not report on these symptoms specifically at 3 and 6 months following exposure, but noted that almost 60% of respondents still suffered from some post-incident symptoms, which can be indication of posttraumatic stress disorders (PTSD), 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident." Insomnia was self-reported in 25 (3.7%) of 681 victims of

the Tokyo subway attack surveyed 2 months after exposure (Ogawa *et al.* 1999). Nakajima *et al.* (1998) reported that 2 (<1%) of 1,743 subjects surveyed, who were inhabitants living in one of nine town districts closest to the Matsumoto city attack, reported insomnia 3 weeks following the Matsumoto attack. Four of the 105 subjects surveyed at 4 months continued to report insomnia.

Four studies are available that observed sleep disruption in subjects 1–5 years after sarin exposure (Nakajima *et al.* 1998, Nakajima *et al.* 1999, Kawana *et al.* 2001, Ohtani *et al.* 2004). At 1 year following sarin exposure, Nakajima *et al.* (1998) reported that 3 of 45 surveyed subjects continued to experience insomnia and 2 of 45 subjects were experiencing bad dreams. Although bad dreams was included in the questionnaire at 1 year, it was excluded from the questionnaires at 3 weeks and 4 months. Nakajima *et al.* (1999) reported that 8 (<1%) of 1237 surveyed participants experienced insomnia and 6 (<1%) of 1237 surveyed participants experienced bad dreams at 1 year following the Matsumoto attack. Nakajima *et al.* (1999) compared victims of the attack who were admitted to the hospital to victims who were outpatients or non-patients and did not observe any significant difference in the risk for both insomnia and bad dreams in the victims initially admitted to the hospital versus outpatients at 1 year after the exposure. At 3 years post-exposure, Nakajima *et al.* (1999) reported that no differences were found in the symptoms of those with bad dreams or insomnia between non-victims and victims. Kawana *et al.* (2001) followed victims of the Tokyo sarin attack and reported on psychological impacts, including distressing dreams or nightmares and difficulty falling or staying asleep, at 2 years (1997), 3 years (1998), and 5 years (2000) after sarin exposure. The authors then compared these symptoms of the Tokyo sarin attack victims to victims of the Matsumoto sarin attack. Distressing dreams or nightmares were reported by 26 (9.2%) of 283 victims in 1997, 19 (9.2%) of 206 victims in 1998, and 11 (5.8%) of 191 victims in 2000. In 2000, Matsumoto victims and Matsumoto controls experienced distressing dreams or nightmares at 10.3% and 2.3%, respectively. Difficulty falling or staying asleep were reported by 21 (7.4%) of 283 victims in 1997, 18 (8.7%) of 206 victims in 1998, and 15 (7.9%) of 191 victims in 2000. In 2000, Matsumoto victims and Matsumoto controls experienced difficulty falling or staying asleep at 11.4% and 6.8%, respectively. Ohtani *et al.* (2004) examined post-traumatic stress disorder symptoms 5 years after the Tokyo attack and reported that 9 of 34 victims surveyed reported nightmares (8 mild and 1 severe) and 10 of 34 victims reported insomnia (7 mild and 3 severe).

Confidence in the body of evidence for the human studies was downgraded because of serious concern for risk of bias. Risk-of-bias ratings for individual studies for all questions are available in **Figure A4-1** and **Figure A4-2**. There are a number of risk-of-bias issues in the evidence relating to design and conduct of individual studies as well as general limitations (i.e., not risk-of-bias issues) based on the case-report/series study design. Almost all studies were rated as probably high or definitely high risk of bias for two of the three key questions (i.e., confounding and outcome assessment). Only one of the six studies addressed potential confounders that may have been associated with the symptoms reported. Nakajima *et al.* (1999) was the only study that calculated odds ratios, but the authors did not account for potential confounders such as age. All outcomes were self-reported and all the subjects were aware of their exposure making the outcomes likely to be biased. The Nakajima *et al.* (1999) study included a question regarding exposure on its survey making it even more likely that the subjects were aware of the connection between exposure and symptoms. All but one study experienced attrition with only a small subset of the subjects followed through the different time points; some studies included as few as 34 of hundreds of potential subjects. This might bias the results because it is likely that only those who were concerned about exposure continued to participate in the studies.

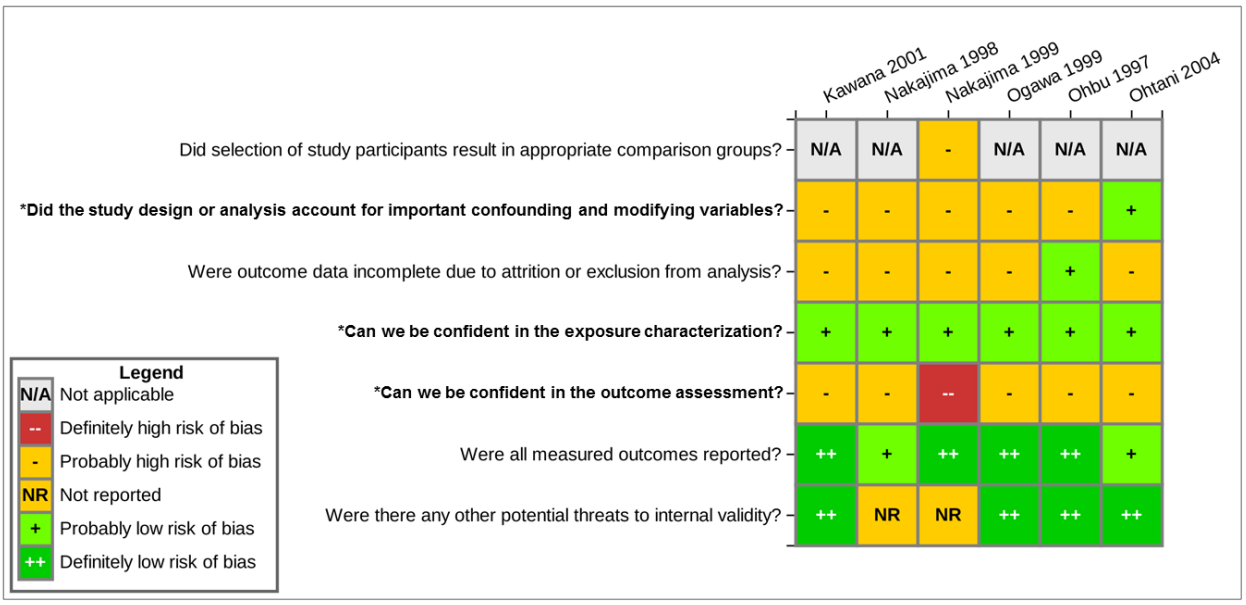
Animal sleep disruption data

No animal studies were identified on the potential association between acute sarin exposure and sleep disruption.

Integration of evidence for sleep disruption-related outcomes

There is some evidence to suggest that acute sarin exposure is associated with sleep disruption-related effects that can last for a long time after exposure; however, there is also some evidence that symptoms of sleep disruption may be an indication of psychological aftereffects of a terrorist attack or posttraumatic stress disorder. There is very low confidence in the human body of evidence that acute sarin exposure will cause long-term effects on sleep based mainly on concerns about risk of bias. The very low confidence in the human body of evidence translates into an inadequate level of evidence. There is no animal evidence to evaluate the potential association between acute exposure to sarin and sleep disruption. Based on these factors, an evidence profile or detailed discussions of the evidence synthesis were not developed for acute sarin and sleep disruption-related outcomes, and this health effect was not considered for hazard identification conclusions.

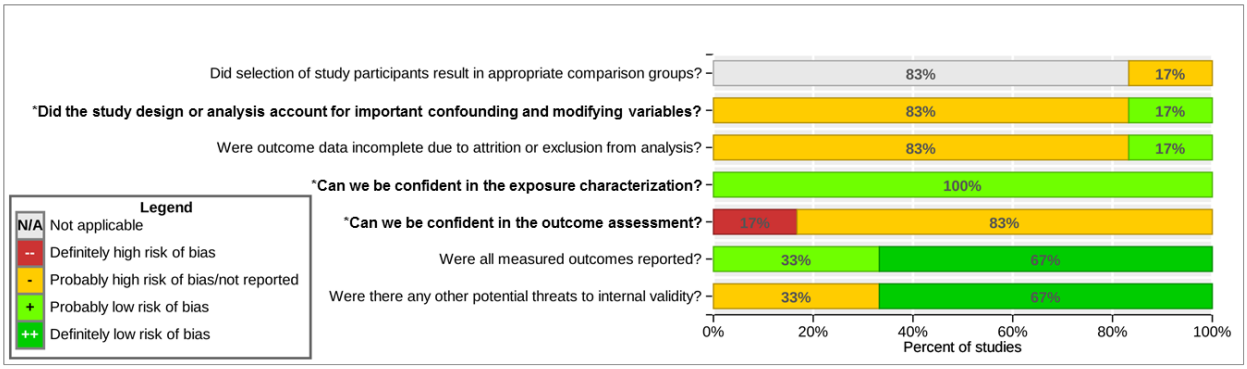
Figure A4-1. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Sleep Disruption in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure A4-2. Risk-of-bias Bar Graph for Case Reports/Series and Standard Observational Studies Assessing Sleep Disruption in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Anxiety and Fear

Endpoints related to anxiety and fear were grouped together because anxiety and fear can be related. Anxiety was measured in humans using anxiety tests scores, and fear was assessed through self-reported symptoms by study subjects who experienced a terrorist attack. In animals, anxiety and fear were measured using FOB scores for bizarre behavior, tension, tremors, urination, defecation, and vocalizations. There were many studies that specifically evaluated Post-Traumatic Stress Disorder (PTSD). This document does not specifically evaluate PTSD even in terms of anxiety and fear because PTSD cannot separate out the effects of the traumatic event (i.e., terrorist attack) from the exposure to sarin during the attack.

Human anxiety and fear data

Based on the available studies, there is very low confidence in the body of evidence that acute sarin exposure affects anxiety and fear in humans over all time periods after the initial exposure. All of the data on fear are based on symptoms reported by study subjects following the Tokyo subway terrorist attack (Ohbu *et al.* 1997, Kawana *et al.* 2001, Ohtani *et al.* 2004, Okumura *et al.* 2005), including fear in the subway or at the incident, fear concerning escape from the attack, or shaking with fear. For the initial period covering 1–7 days following acute sarin exposure, no studies were available. Subjects from one case series study report symptoms related to anxiety and fear 1 month following acute sarin exposure (Ohbu *et al.* 1997), and subjects from three case series studies report symptoms related to anxiety and fear 1–5 years following acute sarin exposure (Kawana *et al.* 2001, Ohtani *et al.* 2004, Okumura *et al.* 2005). There were three studies that evaluated anxiety (Yokoyama *et al.* 1998c, Tochigi *et al.* 2002, Tochigi *et al.* 2005). There are serious limitations in the human body of evidence to evaluate the potential association between exposure to sarin and symptoms related to anxiety and fear. Initial confidence in the evidence is moderate for cross-sectional studies and low for the case series based on study design and was downgraded twice for serious risk-of-bias concerns and uncertainties related to study design and exposure. Subjects in the case series studies also self-reported their symptoms and were aware of their exposure. Moreover, these studies do not attempt to differentiate the association between symptoms related to anxiety and fear and acute sarin exposure and symptoms related to anxiety and fear and the experience of a traumatic event (i.e., a terrorist attack) with questions based more on fear of the subway or escape. Based on the data available, there is very low confidence in the body of evidence for all time periods after the initial exposure.

The available epidemiological studies in the human body of evidence that evaluated the association between acute exposure to sarin and long-term effects on anxiety and fear were all based on two terror attacks and followed some of the victims over time. There were no studies identified that specifically evaluated symptoms related to anxiety and fear in the days following exposure. Ohbu *et al.* (1997) reported that 152 (32%) of 475 hospital patients experienced fear of using the subway, some of whom could still not use the subway, 1 month following the exposure. One year after the Tokyo attack, 39 (12.9%) of 303 subjects surveyed reported fear of the subway, and 35 (11.6%) of 303 subjects surveyed reported fear concerning escape from the attack (Okumura *et al.* 2005). Kawana *et al.* (2001) reported that 25 (8.8%) of 283 hospital patients, 18 (8.7%) of 206 hospital patients, and 21 (11.0%) of 191 hospital patients experienced fear in the subway or at the incident 2, 3, and 5 years following the exposure, respectively. Kawana *et al.* (2001) also reported that 151 (23.1%) of 655 victims of the Tokyo subway attack studied by an NGO, 7 (7.9%) of 88 victims of the 1994 Matsumoto sarin attack, and 2 (2.3%) of 87 members of a control group from Matsumoto city self-reported fear in the subway or at the incident 5 years after the exposure. Ohtani *et al.* (2004) reported that 5 years after the Tokyo attack, 5 (14.7%) of 34 subjects surveyed reported mild shaking with fear. These symptoms related to anxiety and fear were not assessed at any other time points, and no other health endpoints related to anxiety and fear were

included in study questionnaires following the terrorist attacks. Yokoyama *et al.* (1998c) did not find any significant difference in the tension-anxiety profile of mode states scores in 18 subjects 6–8 months after the Tokyo subway attack compared to 15 controls. Tochigi *et al.* (2005) evaluated state and trait anxiety, but did not really make comparisons between the Tokyo subway victims and the controls and appears to be a follow-up of the Tochigi *et al.* (2002) study. Tochigi *et al.* (2002) compared the state anxiety scores in 34 Tokyo subway victims compared to 34 controls 5 years after exposure. There was no significant difference between the groups unless the subjects identified to have PTSD were separated out and in those subjects the state anxiety score was increased.

Confidence in the body of evidence for the human studies was downgraded because of serious concern for risk of bias. Risk-of-bias ratings for individual studies for all questions are available in **Figure A4-3** through **Figure A4-6**. There are a number of risk-of-bias issues in the evidence relating to design and conduct of individual studies as well as general limitations (i.e., not risk-of-bias issues) based on the case-report/series study design. Nearly all studies were rated as probably high risk of bias for two of the three key questions (i.e., confounding and outcome assessment). All outcomes were self-reported and all the subjects were aware of their exposure making the outcomes likely to be biased. The case series studies also had probably high risk of bias due to challenges with confounding and/or attrition. None of the studies addressed potential confounders that may have been associated with the symptoms reported. For Kawana *et al.* (2001), of the 582 St. Luke patients contacted, 283 (48.6%) responded in 1997, 206 (35.3%) responded in 1998, and 191 (32.8%) responded in 2000. While the mean age and sex of the subjects over the different times were reported and were similar, there was no comparison made between those lost to follow-up and those remaining in the study. This might bias the results because it is likely that only those who were concerned about exposure continued to participate in the study. For Ohtani *et al.* (2004),Tochigi *et al.* (2002), and Yokoyama *et al.* (1998c), only 18–34 of the hundreds of potential victims participated in the study. There was no information provided on the subjects that participated compared to those who did not. Moreover, none of the studies included in the body of evidence were designed to assess symptoms related to anxiety and fear associated with acute sarin exposure separately from anxiety and fear associated with the experience of a traumatic event. Most of the questions included in questionnaires regarding anxiety and fear were tailored towards assessing fear related with the experience of the terrorist attack (e.g., fear of subway), which increases the likelihood that the reported incidence of anxiety and fear in the body of evidence is more if not mostly related to the experience of a traumatic event and not the sarin exposure alone. Studies that did conduct anxiety tests, only found significant anxiety in subjects who had PTSD.

Animal anxiety and fear data

There is low confidence in the body of evidence that acute sarin exposure affects long-term anxiety and fear in animals because of heterogeneity across endpoints studied and very serious risk-of-bias concerns. Although initial confidence in the animal data is high, confidence in the body of evidence was downgraded twice for very serious concerns for risk of bias. Health endpoints related to anxiety and fear in animal studies included FOB scores for bizarre behavior, tension, tremors, urination, defecation, and vocalizations. There were no studies identified that specifically evaluated these outcomes in the days to weeks following exposure. Kassa *et al.* (2001c) observed no effect in FOB scores for tension, tremors, and vocalizations in male albino Wistar rats 3 months after inhalation exposure to sarin (0.8–2.5 µg/L). Kassa *et al.* (2001a) also observed no effects in FOB scores for bizarre behavior, tension, tremors, or vocalizations in male albino SPF rats 6 months and 12 months after inhalation exposure to sarin (0.8–2.5 µg/L). Although Kassa *et al.* (2001a) observed no statistically significant effects in FOB scores for urination and defecation at 3 months, 6 months, and 12 months, FOB scores for urination and

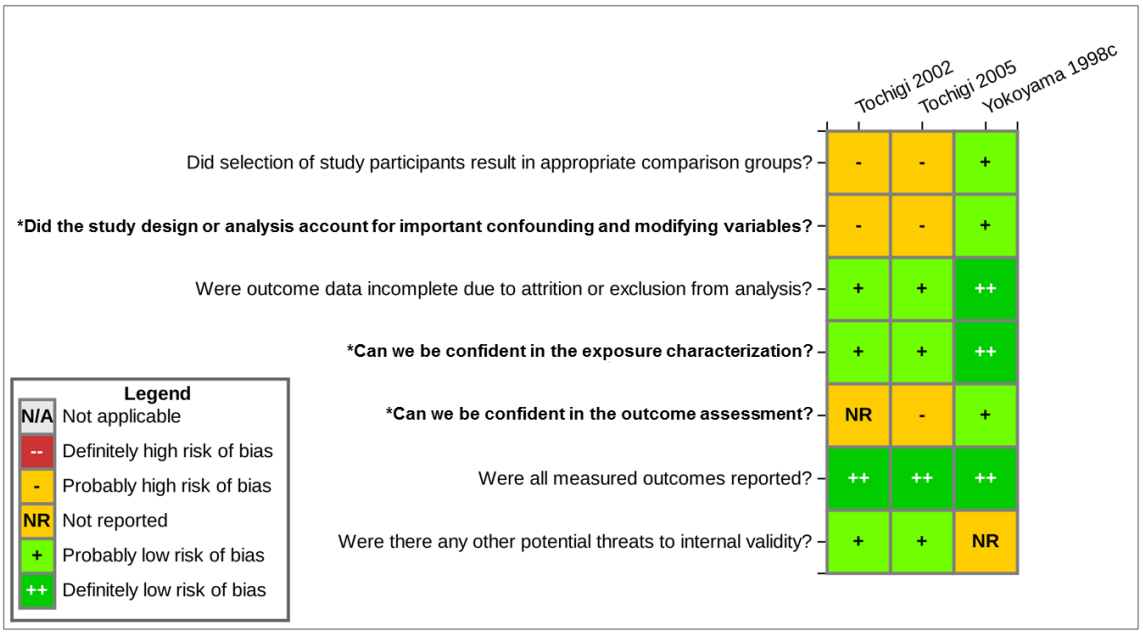
defecation had large variations with more than 15% score difference (in both directions) at all doses at 3 months, 6 months, and 12 months following sarin exposure.

There were multiple risk-of-bias concerns to support a very serious risk-of-bias rating for the animal body of evidence related to anxiety and fear. The studies in general were rated as probably high risk of bias for the 3 key questions (randomization, exposure characterization, and outcome assessment) (see **Figure A4-7** and **Figure A4-8**). Kassa *et al.* did respond to correspondence requesting information on randomization and noted that animals in their studies were not randomized to treatment. Both Kassa *et al.* studies (2001a, 2001c) administered sarin via inhalation, but none reported measuring exposure concentrations in the chambers. Correspondence with the study authors indicated that sarin concentrations in the chambers were measured, but no results were available and were only reported based on ChE depression and/or clinical symptoms. In the assessment of outcomes, none of the outcomes were measured blind and all of these studies used FOB scores to describe anxiety and fear, which increases the risk of bias due to the subjective nature of the outcome.

Integration of evidence for anxiety and fear-related outcomes

There is some human evidence to evaluate the potential association between acute exposure to sarin and increased anxiety and fear months to years after exposure; however, no epidemiological studies were identified that assessed anxiety and fear associated with acute sarin exposure separately from anxiety and fear associated with the experience of a traumatic event (i.e., a terrorist attack). There is no evidence of an association between acute sarin exposure and anxiety and fear in animals 3 months to 12 months following exposure. There is very low confidence in the human body of evidence and low confidence in the animal body of evidence that acute sarin exposure will cause long-term effects on anxiety and fear based mainly on concerns about risk of bias. The very low confidence in the human body of evidence translates into an inadequate level of evidence, and the low confidence in the animal data translates into a low level of evidence. Therefore, an evidence profile or detailed discussions of the evidence synthesis were not developed for acute sarin exposure and anxiety and fear-related outcomes, and this health effect was not considered for hazard identification conclusions.

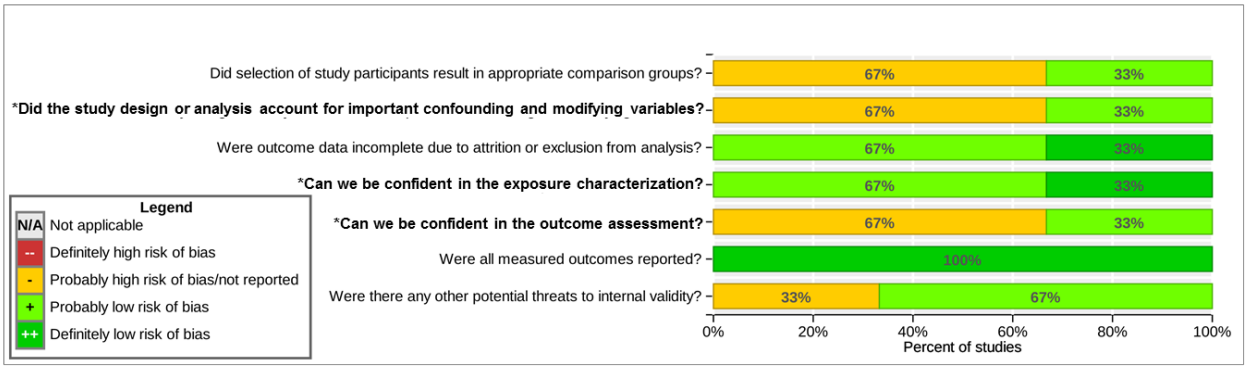
Figure A4-3. Risk-of-bias Heat Map for Standard Observational Studies Assessing Anxiety in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

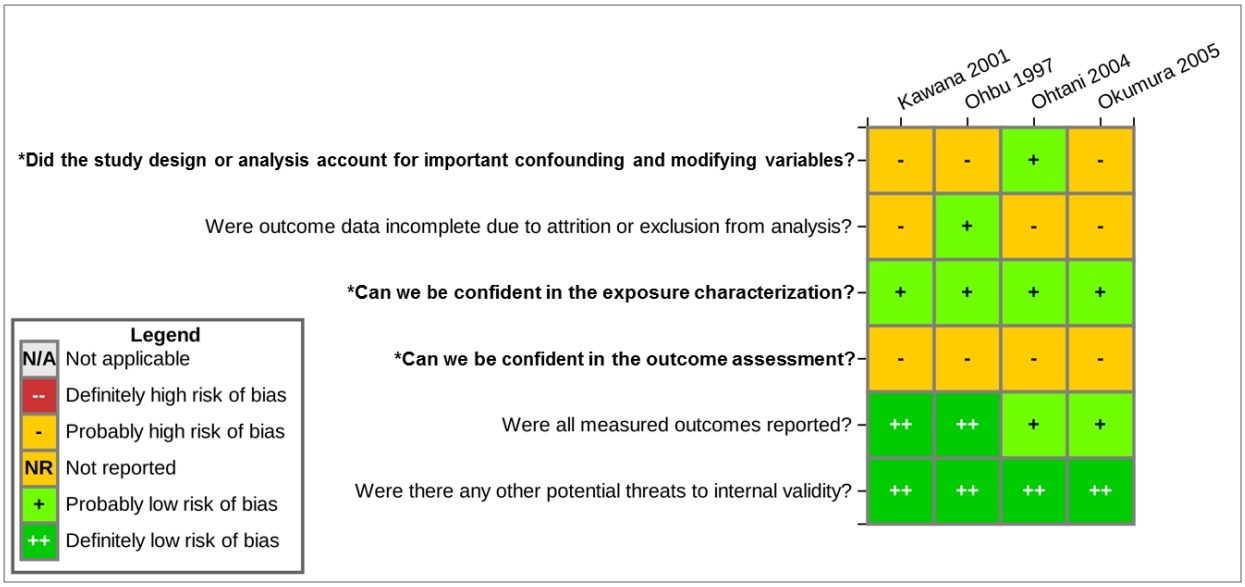
Figure A4-4. Risk-of-bias Bar Graph for Standard Observational Studies Assessing Anxiety in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

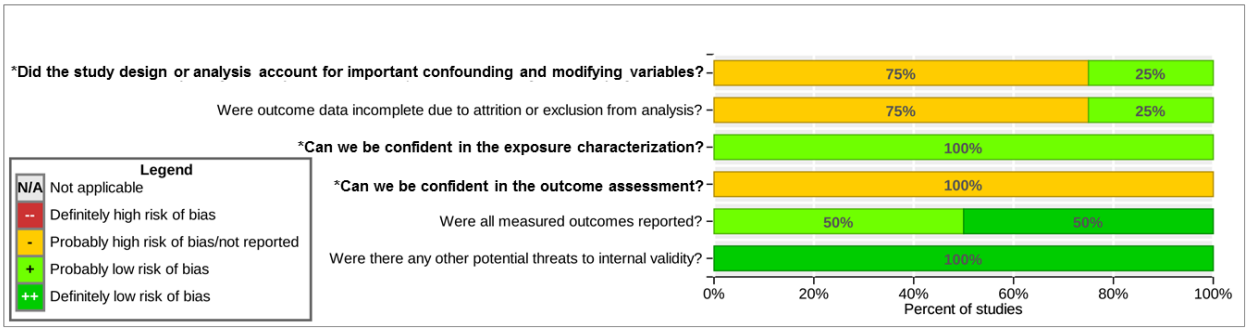
Figure A4-5. Risk-of-bias Heat Map for Case Series Assessing Fear in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case series. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

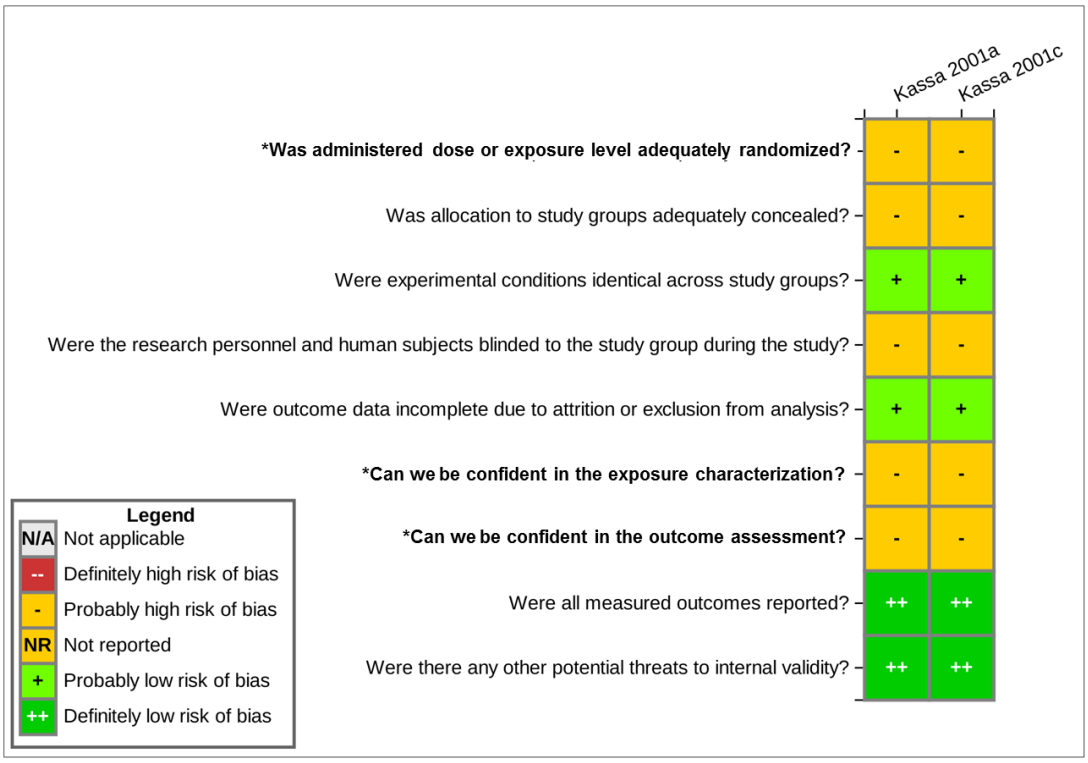
Figure A4-6. Risk-of-bias Bar Graph for Case Series Assessing Fear in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case series. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

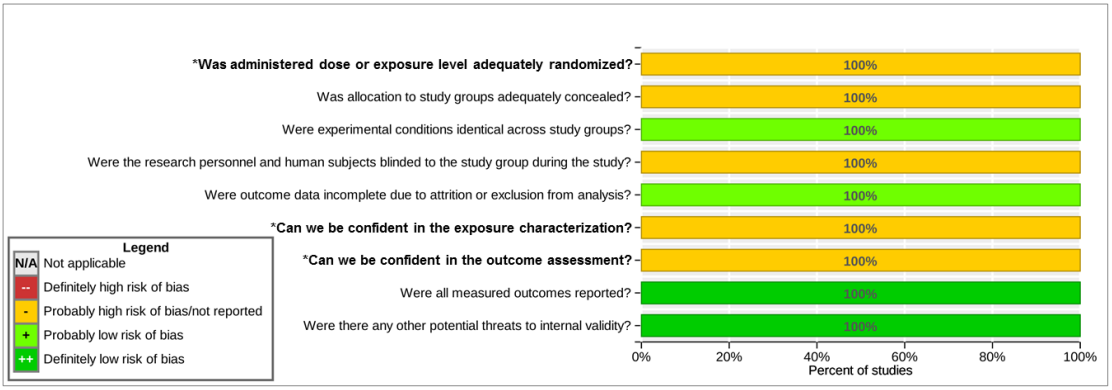
Figure A4-7. Risk-of-bias Heat Map for Individual Studies Assessing Anxiety and Fear in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure A4-8. Risk-of-bias Bar Graph for Individual Studies Assessing Anxiety and Fear in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Avoidance and Depression

Avoidance is described as behavior intended to avoid thoughts, discussion or physical locations that trigger recollections of the trauma. Depression included depressed mood/feelings, diminished interest with numbing or apathy. Symptoms of avoidance and depression were self-reported via questionnaire or recorded by a clinician as part of an evaluation of PTSD.

Human avoidance and depression data

Based on the available studies, there is very low confidence in the body of evidence that acute sarin exposure is associated with avoidance and depression in humans over all time periods after the initial exposure. The human body of evidence consists of four case series, all of which evaluated victims of the Tokyo subway system sarin attack that occurred in 1995 (Ohbu *et al.* 1997, Kawana *et al.* 2001, Ohtani *et al.* 2004, Okumura *et al.* 2005) and one cross-sectional study on the Tokyo subway attack (Yokoyama *et al.* 1998c). For the initial period covering 1–7 days following acute sarin exposure, no studies were available. One case series reported symptoms of depression (depressive mood) one month after acute sarin exposure (Ohbu *et al.* 1997). One cross-sectional study evaluated depression in profile of mood states 6–8 months after exposure and subjects from three case series studies report symptoms related to avoidance and depression 1–5 years following acute sarin exposure (Kawana *et al.* 2001, Ohtani *et al.* 2004, Okumura *et al.* 2005). There are serious limitations in the human body of evidence that evaluates the potential association between exposure to sarin and symptoms related to avoidance and depression. Initial confidence in the evidence is low for the case series based on study design and was downgraded once for serious risk-of-bias concerns. Symptoms were either self-reported and subjects were aware of their exposure or recorded by a clinician as part of criteria used to evaluate PTSD. None of these studies attempted to differentiate the cause of the reported symptoms of avoidance and depression (i.e., as due to acute sarin exposure or trauma resulting from a terrorist attack). Consequently, there is very low confidence in the body of evidence for all time periods.

The available epidemiological studies in the human body of evidence that evaluated the association between acute exposure to sarin and long-term effects on avoidance and depression were based on the Tokyo attack and followed some of the victims over time. None of the studies evaluated symptoms of avoidance and depression in the days following exposure. One case series reported symptoms of depression (depressive mood) in 74 (16%) of 475 hospital patients, one month after exposure (Ohbu *et al.* 1997). The authors noted effects at 3 and 6 months by stating that almost 60% of respondents still suffered from some post-incident symptoms, which can be indication of PTSD, 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident." Profile of mood states scores for depression were not significantly different ($p = 0.07$) in 18 subjects from the Tokyo subway attack compared to 15 control subjects 6–8 months after exposure (Yokoyama *et al.* 1998c). At one year following exposure depressive feelings were reported by 24 (7.9%) of 303 exposed subjects (Okumura *et al.* 2005). Two years after the attack depressed mood was reported in 42 (14.8%) of 283 patients; this decreased significantly one year later to 9.7% (Kawana *et al.* 2001). Subjects reporting diminished interest, numbing (a symptom of depression) also decreased after 2 years from 18/283 (6.4%) to 9/206 (4.4%) and 11/191 (5.8%), 3 and 5 years following exposure, respectively (Kawana *et al.* 2001). Avoidance of places that trigger recollections of the trauma was reported slightly less frequently at 3 years (29/206; 14.1%) after exposure compared to 2 years (42/283; 14.8%); after 5 years the frequency (30/191; 15.7%) was comparable to that at 3 years (Kawana *et al.* 2001). At 5 years following exposure 16 (47.1%) of 34 subjects self-reported avoidance behavior of places that trigger recollections of the trauma, while 11/34 reported avoidance of thoughts and conversations associated

with trauma (Ohtani *et al.* 2004). Signs of depression (diminished interest and apathy) were reported by 9/34 subjects (Ohtani *et al.* 2004). All four studies sampled from the same cohort (i.e., a total of 641 patients that were treated at St. Luke's International Hospital following the Tokyo subway attack).

Confidence in the body of evidence for the human studies was downgraded because of serious concern for risk of bias. Risk-of-bias ratings for individual studies for all questions are available in **Figure A4-9** and **Figure A4-10**. There are a number of risk-of-bias issues in the evidence relating to design and conduct of individual studies as well as general limitations (i.e., not risk-of-bias issues) based on the case-report/series study design. Nearly all studies were rated as probably high or risk of bias for two of the three key questions (i.e., confounding and outcome assessment). Outcomes were predominantly self-reported and all the subjects were aware of their exposure, which increases the likelihood of bias in the results. The case series studies probably also had a high risk of bias due to challenges with confounding and/or attrition. None of the studies addressed potential confounders that may have been associated with the symptoms that were reported. For Kawana *et al.* (2001), of the 582 St. Luke patients contacted, 283 (48.6%), 206 (35.3%) and 191 (32.8%) responded in 1997, 1998, and 2000, respectively. While the reported mean age and sex of the subjects over the different times were similar, there was no comparison made between those lost to follow-up and those remaining in the study. This might bias the results because it is likely that only those who were concerned about exposure continued to participate in the study. For Ohtani *et al.* (2004), only 34 of the same cohort of potential victims participated in the study. No information was provided on the subjects that participated compared to those who did not. Moreover, none of the studies included in the body of evidence were designed to differentiate between symptoms of avoidance and depression as a result of the long-term biological effect of acute exposure to sarin rather than as a result of the experience of a traumatic event, which could be related to PTSD. In fact, two of the studies [Kawana *et al.* (2001) and Ohtani *et al.* (2004)]considered symptoms of avoidance and depression as signs of PTSD. Ohtani *et al.* (2004) found that 32.4% of the subjects examined in their study developed PTSD over the 5 years following the attack; the authors noted that this high prevalence was likely caused by bias in the subject selection. In summary, reported symptoms of avoidance and depression in the human body of evidence could be equally related to the experience of a traumatic event or the sarin exposure.

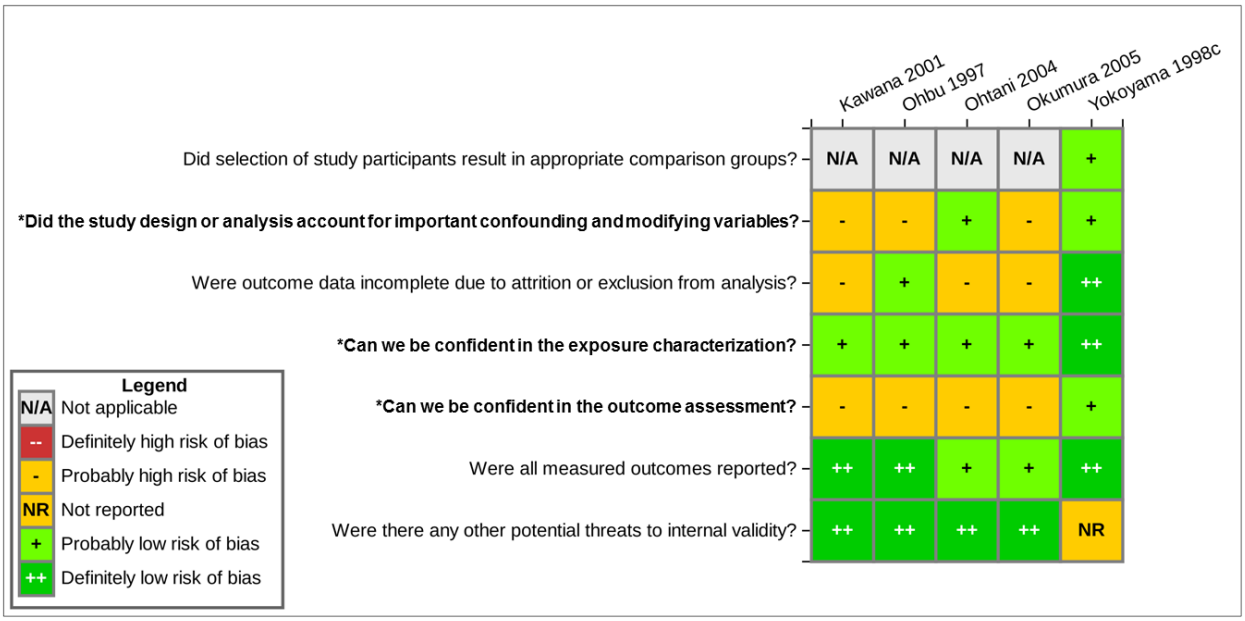
Animal avoidance and depression data

No animal studies were identified on the potential association between acute sarin exposure and avoidance and depression.

Integration of evidence for avoidance and depression-related outcomes

There is limited human evidence available to evaluate the potential association between acute exposure to sarin and increased avoidance behavior and depression months to years after exposure. Furthermore, two of the four epidemiological studies assessed these symptoms within the wider context of evaluating prevalence of PTSD. There were no studies identified that evaluated avoidance behavior and depression in animals acutely exposed to sarin. There is very low confidence in the human body of evidence that acute sarin exposure causes long-term effects on avoidance behavior and depression based mainly on concerns about risk of bias. The very low confidence in the human body of evidence translates into an inadequate level of evidence and as such, an evidence profile or detailed discussion of the evidence synthesis was not developed for acute sarin exposure and avoidance and depression-related outcomes. This health effect was not considered for hazard identification conclusions.

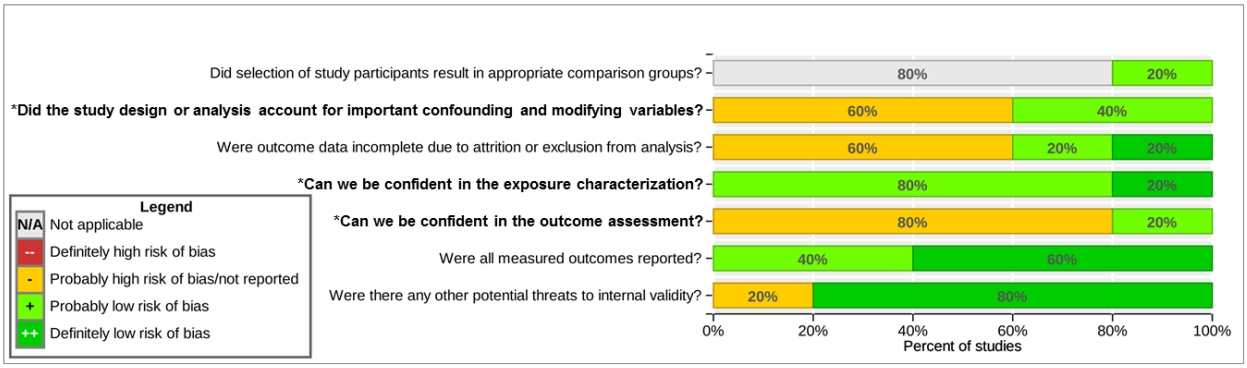
Figure A4-9. Risk-of-bias Heat Map for Case Series and Standard Observational Studies Assessing Avoidance and Depression in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure A4-10. Risk-of-bias Bar Chart for Case Series and Standard Observational Studies Assessing Avoidance and Depression in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Activity/Strength

Similar endpoints on activity and strength were grouped together due to their close relationship, such that reduced strength can result in reduced activity and reduced activity can result in reduced strength (Leblanc *et al.* 2015, Germain *et al.* 2016). Activity was not specifically measured in humans, but endpoints considered included asthenia, fatigue or tiredness, lethargy, and general weakness. In animals, activity and strength were measured using several different endpoints, including beam walking, grip strength, hand-eye coordination, incline plane slip angle, spontaneous activity, field activity, and FOB activity scores.

Human activity and strength data

Based on the available studies, there is very low confidence in the body of evidence that acute sarin exposure affects activity and strength in humans over all time periods after the initial exposure. Most of the data on health endpoints related to activity and strength (including asthenia, fatigue or tiredness, lethargy, and general weakness) are based on symptoms reported by study subjects following the Matsumoto terrorist attack or the Tokyo subway terrorist attack (Nakajima *et al.* 1998, Nakajima *et al.* 1999, Ogawa *et al.* 1999, Okudera 2002, Ohtani *et al.* 2004, Okumura *et al.* 2005). For the initial period covering 1–7 days following acute sarin exposure, no studies were available. Subjects from two case series studies report symptoms related to activity and strength 3 weeks to 4 months following acute sarin exposure (Nakajima *et al.* 1998, Ogawa *et al.* 1999). One cross-sectional study reported on profile of state mood scores related to fatigue 6–8 months following acute sarin exposure in 18 subjects from the Tokyo subway compared to 15 controls (Yokoyama *et al.* 1998a, Yokoyama *et al.* 1998c). Subjects from four case series studies and one prospective cohort study report symptoms related to activity and strength 1–5 years following acute sarin exposure (Nakajima *et al.* 1998, Nakajima *et al.* 1999, Okudera 2002, Ohtani *et al.* 2004, Okumura *et al.* 2005). There are serious limitations in the human body of evidence to evaluate the potential association between exposure to sarin and symptoms related to activity and strength. In the intermediate period of 8 days to 1 year following acute exposure, the cross-sectional study (Yokoyama *et al.* 1998a, Yokoyama *et al.* 1998c) had an initial and final confidence rating of moderate; however, no statistically significant effects were observed, and therefore the body of evidence is considered inadequate to evaluate whether sarin exposure affects long-term activity and strength in humans. The body of evidence in the intermediate period also consists of two case series with an initial confidence of low, which was downgraded once for risk-of-bias concerns. In the extended period of 1–5 years following exposure, the initial confidence of moderate for the cohort study (Nakajima *et al.* 1999) was downgraded twice for serious risk-of-bias concerns (i.e., failure to control for confounding, potential biases in outcome assessment from self-reporting of symptoms via questionnaires, and loss of subjects over time). The remaining case reports/series that reported effects in the extended period had an initial confidence of low, which was downgraded once for risk-of-bias concerns due to the self-reporting of symptoms and participants' awareness of their exposure. Based on all available data available, there is very low confidence in the body of evidence for all time periods after the initial exposure.

The available epidemiological studies in the human body of evidence that evaluated the association between acute exposure to sarin and long-term effects on activity and strength were all based on two terror attacks and followed some of the victims over time. Nakajima *et al.* (1998) reported that 39 (2.2%) of the 1,743 subjects surveyed, who were inhabitants living in one of nine town districts closest to the Matsumoto city attack, reported fatigue within the first 24 hours following the attack, of which 17 subjects (<1%) continued to report fatigue 3 weeks following the Matsumoto attack. Two of the 105 subjects surveyed at 4 months continued to report fatigue (Nakajima *et al.* 1998). Generalized weakness was self-reported in 36 (5.3%) of the 681 victims of the Tokyo subway attack surveyed 2 months after

exposure (Ogawa *et al.* 1999). Profiles of mood state scores for fatigue was not significantly different between 18 Tokyo subway victims 6–8 months after exposure compared to controls (Yokoyama *et al.* 1998a, Yokoyama *et al.* 1998c). Asthenia and fatigue were reported 1–3 years following the Matsumoto attack (Nakajima *et al.* 1998, Nakajima *et al.* 1999, Okudera 2002). Nakajima *et al.* (1999) compared victims of the attack who were admitted to the hospital to victims who were outpatients or non-patients and observed a significant increase in the risk for both asthenia and fatigue symptoms in the victims initially admitted to the hospital versus outpatients at 1 year after the exposure. Three years post-exposure, Nakajima *et al.* (1999) reported that fatigue was significantly greater in victims of the attack compared with non-victims. One year after the Tokyo attack, 36 (11.9%) of 303 subjects surveyed reported being easily fatigued (Okumura *et al.* 2005). Five years after the Tokyo attack, 12 of 34 subjects surveyed reported mild tiredness and lethargy and 14 of 34 subjects reported being easily fatigued (8 mild and 6 severe) (Ohtani *et al.* 2004). These activity/strength-related health endpoints were not assessed at any other time points, and no other health endpoints related to activity/strength were included in study questionnaires following the terrorist attacks.

Confidence in the body of evidence for the human studies was downgraded because of serious concern for risk of bias. Risk-of-bias ratings for individual studies for all questions are available in **Figure A4-11** and **Figure A4-12**. There are a number of risk-of-bias issues in the evidence relating to design and conduct of individual studies as well as general limitations (i.e., not risk-of-bias issues) based on the case-report/series study design. Almost all studies were rated as probably high or definitely high risk of bias for two of the three key questions (i.e., confounding and outcome assessment). None of the studies addressed potential confounders that may have been associated with the symptoms reported. Nakajima *et al.* (1999) was the only study that calculated odds ratios, but the authors did not account for potential confounders such as age. All outcomes were self-reported and all the subjects were aware of their exposure making the outcomes likely to be biased. The Nakajima *et al.* (1999) study included a question regarding exposure on its questionnaire making it even more likely that the subjects were aware of the connection between exposure and symptoms. Although exposure assessment was rated probably low risk of bias because subjects were recognized to be exposed, few studies evaluated exposure in terms of proximity to the release or admittance to the hospital following exposure, and the majority of studies did not consider these factors when evaluating symptoms reported years later. All studies experienced attrition with only a small subset of the subjects followed through the different time points; some studies included as few as 34 of hundreds of potential subjects. This might bias the results because it is likely that only those who were concerned about exposure continued to participate in the studies.

Animal activity and strength data

There is low confidence in the body of evidence that acute sarin exposure affects long-term activity and strength in animals because of heterogeneity across endpoints studied and very serious risk-of-bias concerns. Although initial confidence in the animal data is high, confidence in the body of evidence was downgraded twice for very serious concerns for risk of bias. Health endpoints related to activity and strength in animal studies included beam walking, grip strength, hand-eye coordination, incline plane slip angle, spontaneous activity, field activity, and FOB activity scores. The studies used different doses, species, and routes of exposure and effects were measured at various times points after exposure, leading to inconsistencies in the data. Wolthuis *et al.* (1995) observed a decrease in hand-eye coordination in marmoset monkeys 4 and 5 days after intramuscular sarin injection (12 µg/kg), but this occurred to a lesser extent at day 6. Abou Donia *et al.* (2002) observed a decrease in grip strength and beam walk score and an increase in beam walk time in male Sprague-Dawley rats at 7 and 15 days after intramuscular injection of sarin (100 µg/kg). A decrease in spontaneous activity was observed in mice 4 days after an intravenous exposure to sarin [80 µg/kg; (Little *et al.* 1986)], but open field activity was

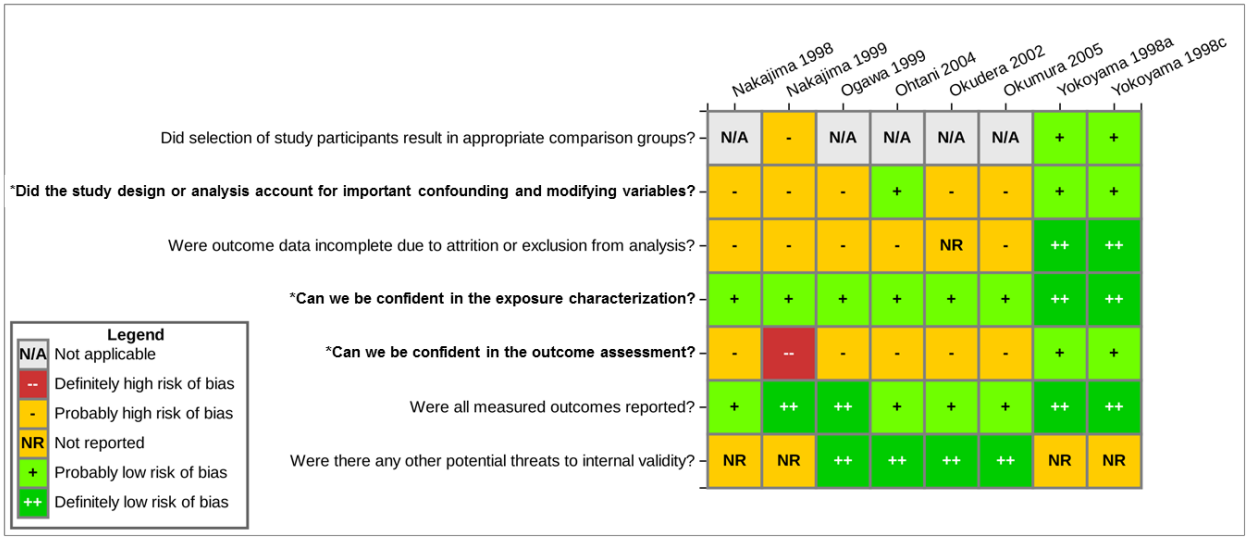
increased in male Sprague-Dawley rats through 6 months after inhalation exposure to sarin [27.2–34.2 µg/L; (Grauer *et al.* 2008, Allon *et al.* 2011)]. Grauer *et al.* (2008) also noted some changes in speed performance for sarin-exposed rats in a water maze test, but it cannot be determined if that effect was because of decreased activity or memory problems. Kassa *et al.* studies (2001a, 2001c, 2004) observed alterations in gait, mobility, and other activity FOB scores at 3 months following sarin exposure, but not at 6 or 12 months or in FOB strength scores at 3, 6, or 12 months. There were no other studies with activity FOB scores for comparison.

There were multiple risk-of-bias concerns to support a very serious risk-of-bias rating for the animal body of evidence related to activity and strength. The studies in general were rated as probably high risk of bias for the 3 key questions (randomization, exposure characterization, and blinding at outcome assessment) (see **Figure A4-13** and **Figure A4-14**). The Grauer *et al.* (2008) study was the only study where animals were known to be randomized to treatment. None of the other studies reported randomization nor did the majority of authors respond to correspondence requesting information on randomization. Kassa *et al.* did respond and noted that animals in their studies were not randomized to treatment. Only one study was known to use sarin of sufficient purity (99%) (Wolthuis *et al.* 1995); however, this study had other risk-of-bias concerns because the 5 animals used in the visual discrimination test had been trained on hand-eye coordination and had been injected once at least 2 months prior with another ChE inhibitor (stated to be highly reversible). The authors did not state into which treatment groups these animals were placed, but this indicates that the experimental conditions were not the same for all animals. All the Kassa *et al.* studies (2001a, 2001c, 2004) administered sarin via inhalation, but none reported measuring exposure concentrations in the chambers. Correspondence with the study authors indicated that sarin concentrations in the chambers were measured, but no results were available and were only reported as based on ChE depression and/or clinical symptoms. In the assessment of outcomes, Abou Donia *et al.* (2002) specified that outcomes were conducted blind, and Little *et al.* (1986) measured activity using an instrument that measured beam interruption, lowering the risk-of-bias potential. For the Kassa *et al.* studies (2001a, 2001c, 2004), none of the outcomes were measured blind and all of these studies used subjective FOB scores to describe activity and strength, which increases the potential for risk of bias. The remaining studies did not provide information on blinding (Wolthuis *et al.* 1995, Grauer *et al.* 2008, Allon *et al.* 2011). Abou Donia *et al.* (2002) had definitely high risk of bias due to attrition and lack of reporting results for tests stated to have been measured (i.e., reflexes).

Integration of evidence for activity and strength-related outcomes

There is some evidence that activity and strength are decreased after acute sarin exposure and can last for a long time after exposure; however, there is very low confidence in the human body of evidence and low confidence in the animal body of evidence that acute sarin exposure will cause long-term effects on activity and strength based mainly on concerns about risk of bias. The very low confidence in the human body of evidence translates into an inadequate level of evidence, and the low confidence in the animal data translates into a low level of evidence. Therefore, an evidence profile or detailed discussions of the evidence synthesis were not developed for acute sarin and activity and strength-related outcomes, and this health effect was not considered for hazard identification conclusions.

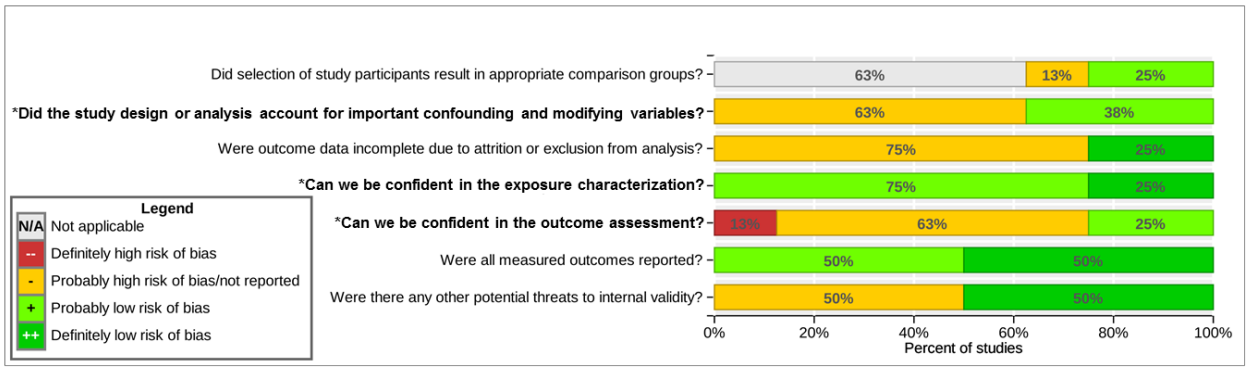
Figure A4-11. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Activity/Strength in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

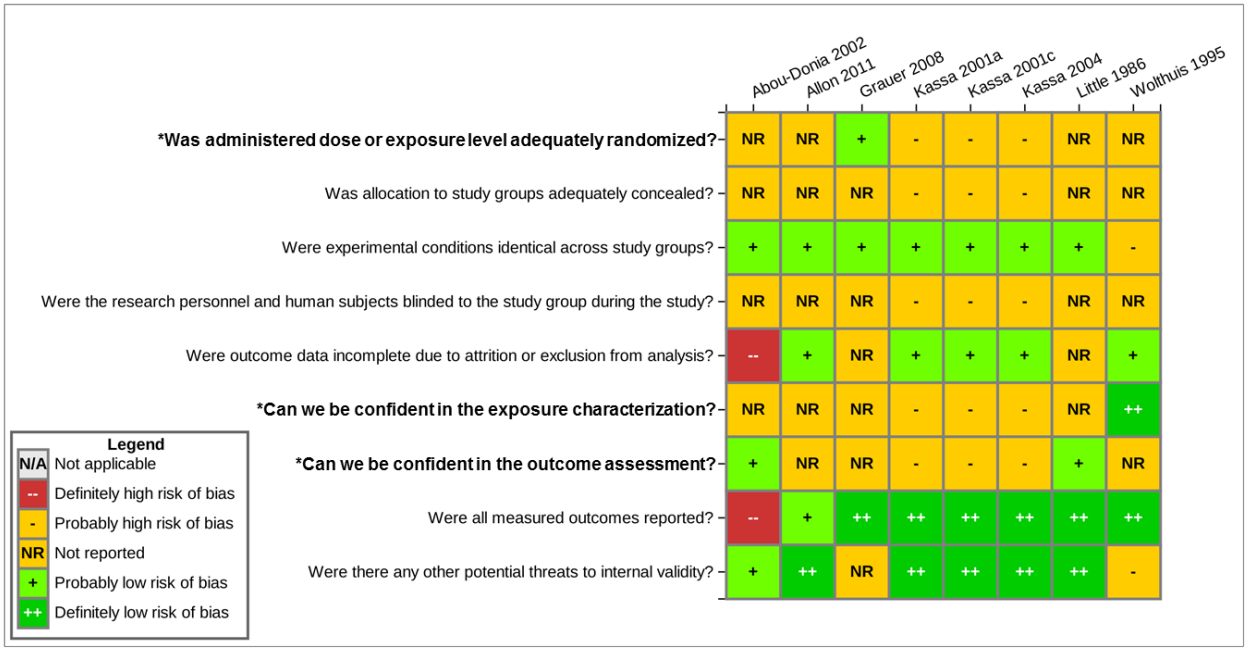
Figure A4-12. Risk-of-bias Bar Chart for Case Reports/Series and Standard Observational Studies Assessing Activity/Strength in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

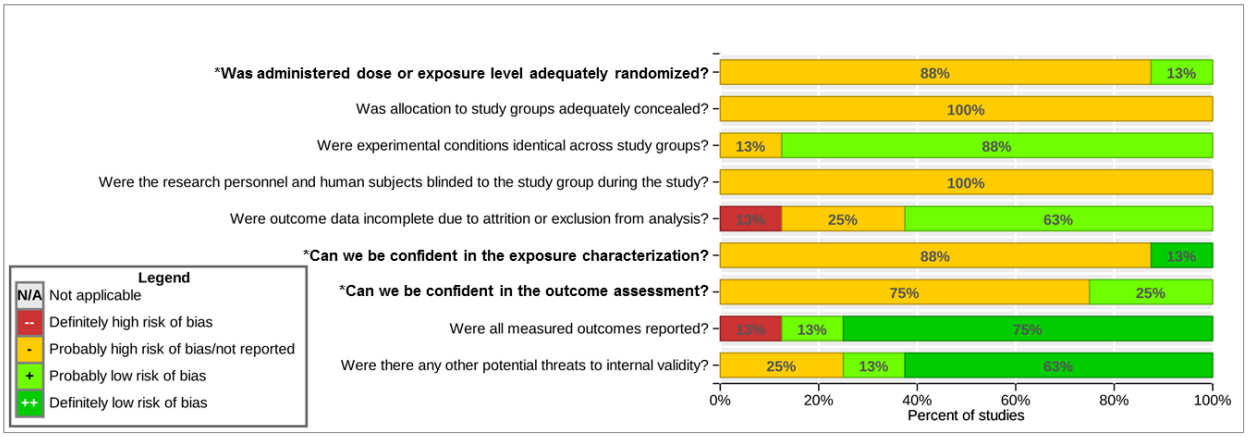
Figure A4-13. Risk-of-bias Heat Map for Individual Studies Assessing Activity/Strength in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure A4-14. Risk-of-bias Bar Chart for Individual Studies Assessing Activity/Strength in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Other Neurological Symptoms

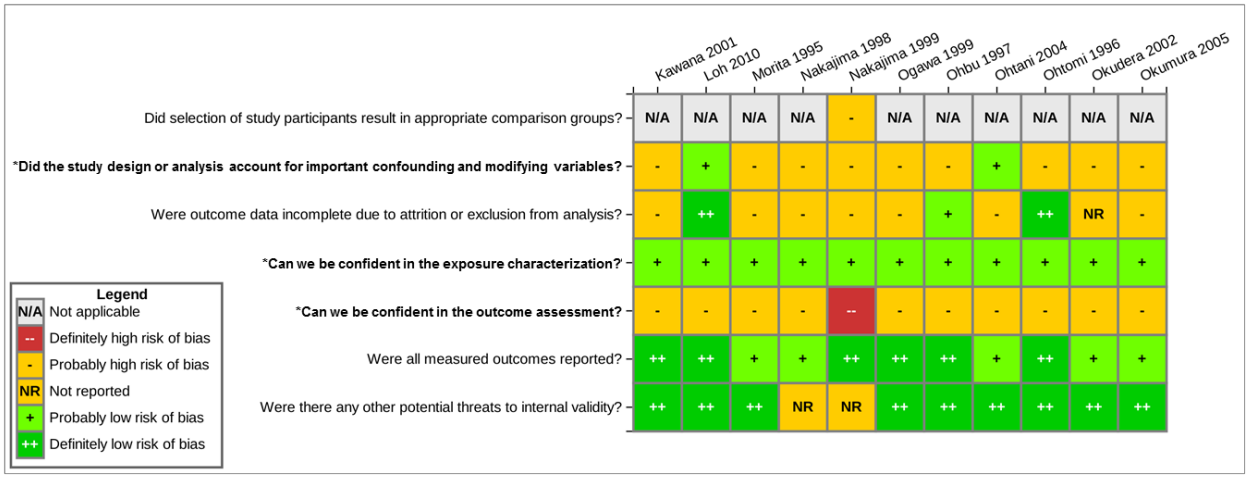
During the two terrorist attacks in Matsumoto and the Tokyo subway, hundreds of individuals were exposed to sarin. Several studies evaluated symptoms in subjects involved in these incidents for up to 5 years after the events, mostly via questionnaire. In some of these studies that evaluated symptoms over time, a symptom was added to a follow-up questionnaire because it was reported previously by some subjects. In these instances some symptoms may not have been captured early on but only at a later time point, thus influencing the results. Many of the reported symptoms have been discussed in the main document—such as visual and ocular effects—or in other sections of this appendix. The current section focuses on reported symptoms that cannot be grouped into larger categories (see **Table A5-2**). Although some of the symptoms were reported by subjects up to 5 years after the exposure, there are serious limitations in the body of evidence. The same limitations apply to other neurological symptoms discussed in this appendix. Neurological symptoms were generally evaluated as part of a case series or case report without any comparison group, with the exception of Nakajima *et al.* (1999), who conducted a prospective cohort study one and three years after the Matsumoto attack. At one year, Nakajima *et al.* (1999) compared non-patients, out patients, and admitted patients, but the exposure status was based on self-reported muscarinic and/or nicotinic symptoms that occurred within a day of the attack and self-reported hospitalization, which were reported as part of the questionnaire during the one year survey. The exposure status (i.e., victims and non-victims) of the subjects during the survey at 3 years following the exposure was based also on self-reported muscarinic and/or nicotinic symptoms that occurred within a day of the attack, but there is no indication that the exposure status was the same in the subjects during both survey. Additional concerns were that the questionnaires changed over time, attrition due to losses to follow up and in some cases due to subjects being excluded from follow-up surveys once they no longer experienced symptoms; and self-reporting of symptoms by subjects who were aware of their exposure (although only a few studies noted that they asked about the exposure and health effects in the same questionnaire). There were no tests to correlate sarin exposure to symptoms and no controls were included for comparison.

The numbers of symptoms included in the questionnaires are too numerous to list or discuss separately. Many of the symptoms occurred in the initial few days, but subsided within the first few weeks of exposure. Only potential neurological symptoms are discussed in this document, but there were many reported symptoms that related to other systems (e.g., digestive and cardiovascular effects). Headache was a symptom included in many of the studies (Morita *et al.* 1995, Ohtomi *et al.* 1996, Nakajima *et al.* 1998, Nakajima *et al.* 1999, Ogawa *et al.* 1999, Kawana *et al.* 2001, Okudera 2002, Ohtani *et al.* 2004, Okumura *et al.* 2005, Loh *et al.* 2010). Although headache is a common occurrence and may be hard to relate to a specific exposure, Ohtomi *et al.* (1996) observed a decrease in the number of subjects that reported headache over time; 40 of the 62 subjects evaluated in the study reported headache on the day of the Tokyo subway attack, while only 4 reported headache 3 months later. Okumura *et al.* (2005) found a similar effect in a larger group (316 of 627 reported headache at admission and 26 of 303 reported headache after 1 year). In a small subset of subjects (n = 34) from the Tokyo subway attack, 16 reported headache after 5 years (Ohtani *et al.* 2004). Fewer subjects reported headache in the weeks following the Matsumoto attack (i.e., 2–4 subjects at 3 weeks (Morita *et al.* 1995, Nakajima *et al.* 1998, Okudera 2002). Other symptoms were also reported to last longer than a few weeks after exposure including concentration difficulty (Kawana *et al.* 2001, Ohtani *et al.* 2004, Okumura *et al.* 2005), dizziness (Ogawa *et al.* 1999, Ohtani *et al.* 2004) (most studies found this symptom to disappear in the first few weeks), numbness or dysesthesia of extremities (Nakajima *et al.* 1998, Ogawa *et al.* 1999), and difficulty reading and writing (Nakajima *et al.* 1998).

Findings in animal studies cannot directly be compared to these symptoms in human. Many of the categories where results of animal tests could be related to a specific symptom in humans has been discussed in previous sections (e.g., anxiety and fear). There are no animal studies that could be specifically related to headache, concentration, dizziness, numbness or dysesthesia of extremities, or difficulty reading or writing. There were additional FOB scores such as ease in handling, piloerection, salivation, or muscular tonus. These either cannot be related to human effects or were not significantly changed and are not discussed.

After review of other neurological symptoms, it has been determined that the data are inadequate to reach any hazard conclusion due to the heterogeneity of the data and limitations in the human studies. The limitations include differences in the questionnaires used within and between studies (i.e., the list of symptoms potentially changing at the different time points), loss of subjects over time or sampling only a small number of the overall cohort of exposed subjects, and self-reporting of symptoms when the subjects were aware of their exposure (see **Figure A4-15** and **Figure A4-16**). In addition, these symptoms in humans cannot be replicated with animal studies.

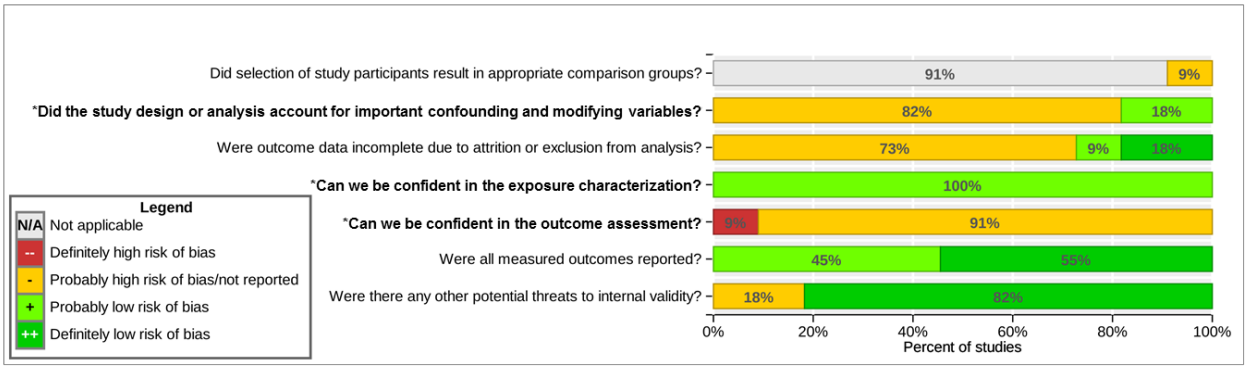
Figure A4-15. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Other Neurological Symptoms in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure A4-16. Risk-of-bias Bar Chart for Case Reports/Series and Standard Observational Studies Assessing Other Neurological Symptoms in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Electroencephalogram (EEG)

An EEG is a noninvasive way to record electrical activity in the brain by placing electrodes along the scalp. EEGs can detect abnormalities in brain waves or electrical activity of the brain that result from underlying disease or injury. Data that support persistent changes in EEGs in animals and humans exposed to sarin are limited. Human data include three case reports. Although there are five publications that report on EEG, three of the publications appear to report on the same case from the Matsumoto terrorist attack. Animal data include two studies in monkeys with inconsistent results. Because the data are limited and inconsistent, the data are inadequate for drawing any hazard conclusions. A brief discussion of the studies is provided below. Risk-of-bias results for human and animal studies are provided in **Figure A4-17** through **Figure A4-20**.

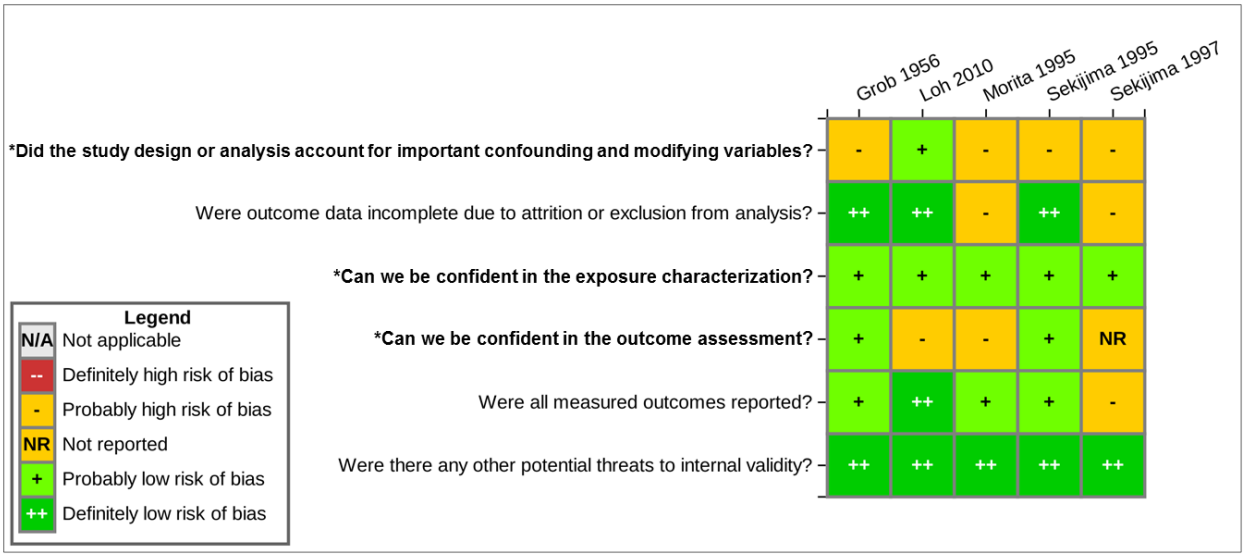
Human EEG data

An EEG of a 19-year-old male exposed to sarin during the Matsumoto attack (subject lived next to the site believed to be the target of the attack) demonstrated a frequent occurrence of high-amplitude waves in the sinciput portion of the brain and polyspike and wave complexes in the left side of the sinciput portion of the brain 30 hours after exposure (Sekijima *et al.* 1995). The EEG normalized by the 7th day of hospitalization. However, an EEG conducted on the 10th day of hospitalization demonstrated sporadic sharp waves and sigma waves when the subject was sleeping. An EEG conducted one year after exposure demonstrated sporadic sharp waves complexes in the left side of the sinciput portion of the brain when the subject was asleep even though he was experiencing no outward symptoms. Sekijima *et al.* (1997) included this subject as one of seven discussed. Morita *et al.* (1995) also appears to have included this subject in their publication, however, some details are different. As noted by Morita *et al.* (1995), EEGs conducted on day 2 demonstrated high-amplitude fast activity with frontal dominance and a 3Hz spike and wave complex in the right frontal area. The authors reported a decline in these effects over the following month. Grob (1956) provided information on three case reports, however, only one of them was given an EEG. This subject became unresponsive after oral exposure to an aqueous solution containing sarin. An EEG, obtained while the subject was unresponsive, revealed bursts of high-voltage waves in the temporofrontal leads that persisted for 6 days (no further information provided). EEG and positron emission topography (PET) were normal 8 months after exposure in a 34-year old Army sergeant exposed to sarin when disarming an IED (Loh *et al.* 2010).

Animal EEG data

Adult rhesus monkeys exposed to a single “large” dose of sarin (5 µg/kg intramuscular) exhibited a persistent increase in beta activity in the temporal lobe EEG that was still present 1 year after exposure (Burchfiel and Duffy 1982). Slight changes in EEG were observed in marmoset monkeys administered 2.5 or 3.0 µg/kg sarin for several months (Pearce *et al.* 1999). EEG measurements were collected weekly through 15 months and it was reported that no significant changes in pattern over time were observed. An increase in amplitude in the beta 2 frequency band that approached significance was attributed to a 40% increase in a single animal.

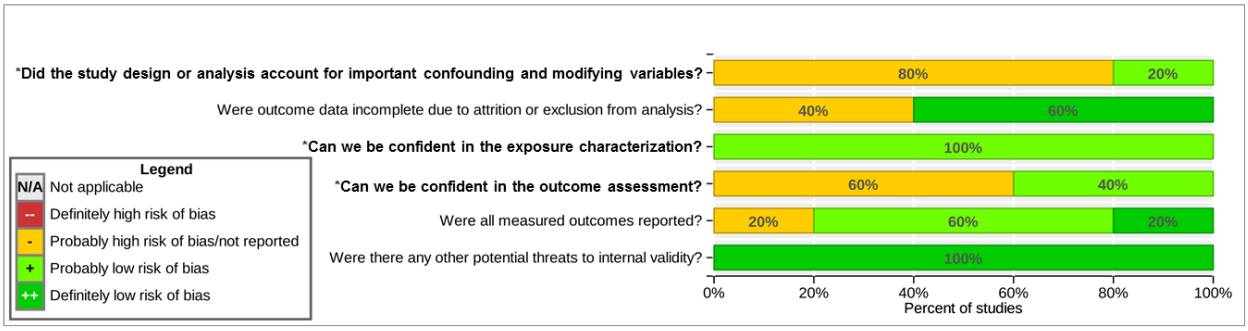
Figure A4-17. Risk-of-bias Heat Map for Case Reports/Series Assessing EEG in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

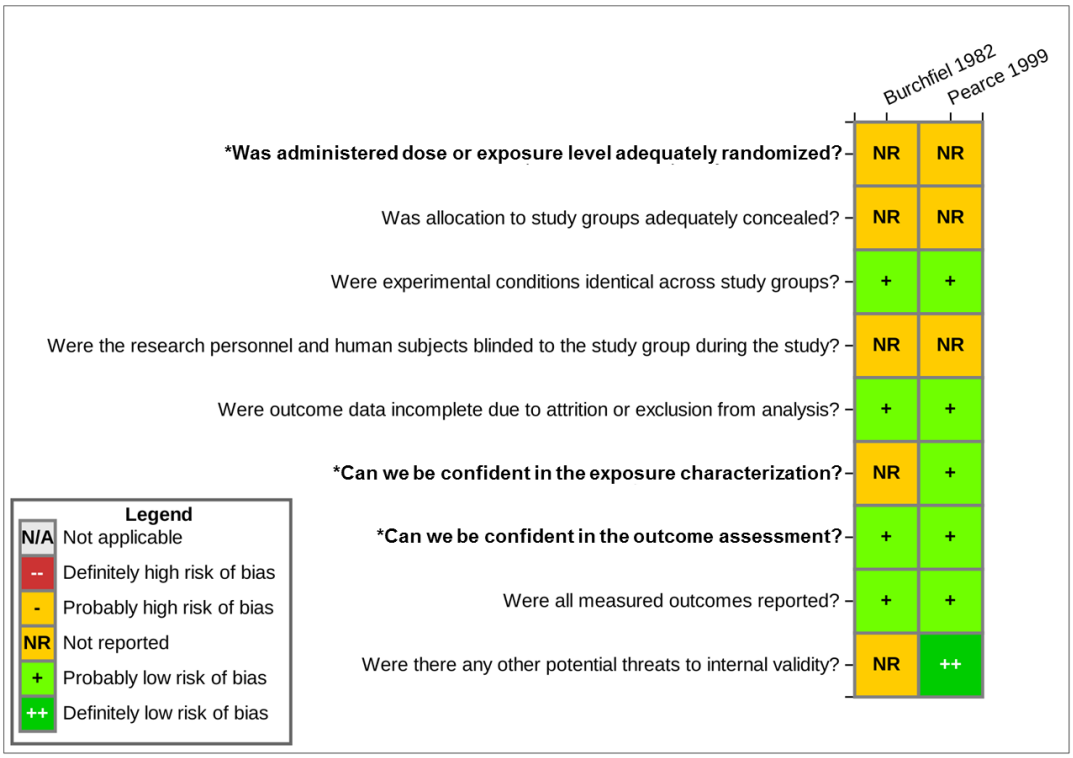
Figure A4-18. Risk-of-bias Bar Chart for Case Reports/Series Assessing EEG in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

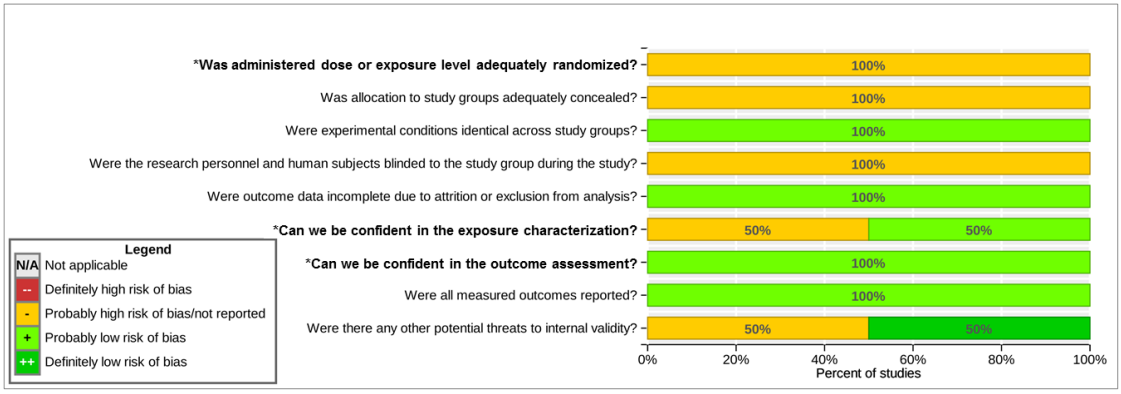
Figure A4-19. Risk-of-bias Heat Map for Individual Studies Assessing EEG in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure A4-20. Risk-of-bias Bar Chart for Individual Studies Assessing EEG in Animals Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Other Sensory Effects

Sensory effects that are not visual or ocular effects include any of the other four senses: sound, touch, taste, or smell. Visual and ocular effects are discussed separately because there were sufficient data to support a separate category. The other senses are discussed together due to the limited amount of information.

Human other sensory data

Based on the available studies, there is low confidence in the body of evidence that acute sarin exposure causes other sensory effects in humans over all time periods after the initial exposure, which is mainly due to the limited number of studies in any specific sensory effect. Four human studies were identified that evaluated effects in at least one of the four senses more than 24 hours after exposure (Murata *et al.* 1997, Sekijima *et al.* 1997, Ogawa *et al.* 1999, Nishiwaki *et al.* 2001). A survey from seven hospitals after the Tokyo subway attack included dysosmia (i.e., changes in sense of smell) in the questionnaire. ChE levels were available in the hospital records of 454 of the 681 subjects who responded to the questionnaire (1,089 were mailed questionnaires). Dysosmia was reported to still occur in 3 (0.4%) of the 681 subjects 2 months after the exposure (Ogawa *et al.* 1999). Six months after the Tokyo subway attack, Murata *et al.* (1997) did not find any significant differences in brainstem auditory evoked potential (BAEP, sense of sound) between 18 exposed subjects (exposure was related to initial serum ChE and other symptoms) and 18 controls (noted not exposed to any anticholinesterase exposure). One subject with initial severe symptoms from the Matsumoto city attack was noted to develop sensory polyneuropathy (which potentially effects sense of touch) 7 months after the exposure (Sekijima *et al.* 1997). Nishiwaki *et al.* (2001) measured vibration perception thresholds (which is considered a measure of touch sensation) 3 years after exposure in 56 male rescue team staff members and police officers working at the Tokyo subway attack compared to 52 age and occupation-matched controls. The exposed group was separated by level of exposure based on self-reported hospitalization after the exposure (high exposure group was hospitalized and low exposure group was outpatients). None of the results were found to be related to exposure. Risk-of-bias results for human studies are provided in **Figure A4-21** and **Figure A4-22**.

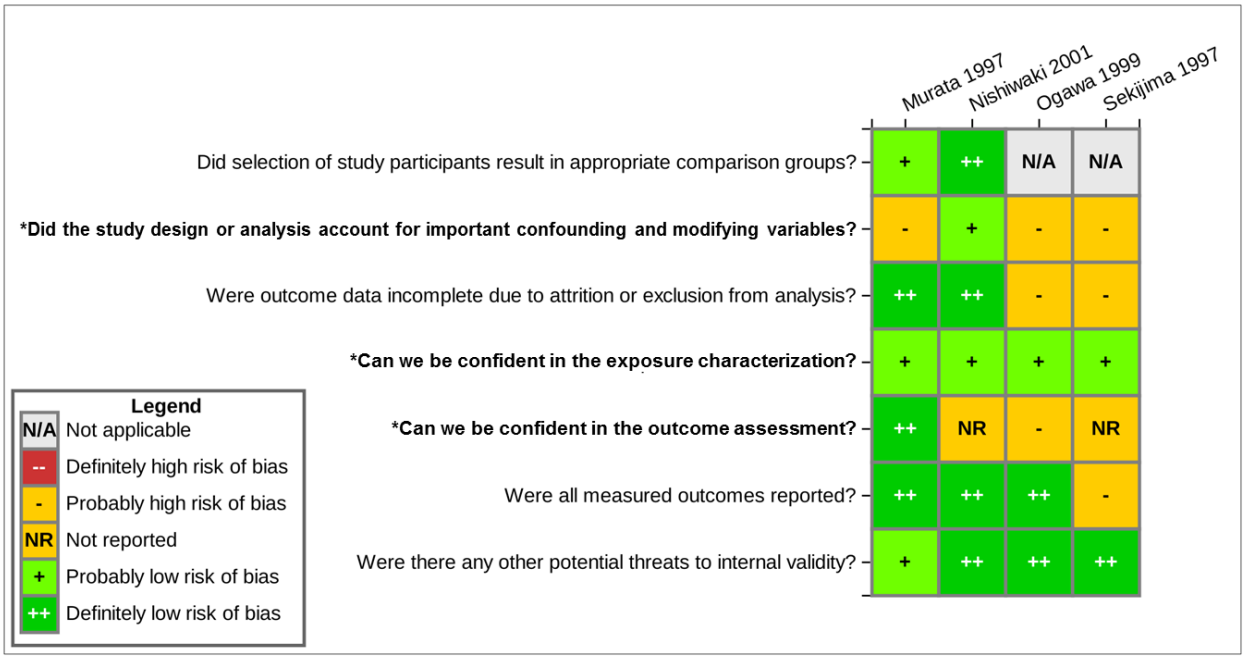
Animal other sensory data

There was a single animal study that evaluated endpoints that could be considered other sensory effects (Kassa *et al.* 2001a). This study is inadequate to evaluate this effect because all of the outcomes were FOB scores, and correspondence with the study authors indicated that the outcome assessors were not blind to the treatment. Although there were some alterations in the FOB scores for approach response, click response, tail-pinch response, and touch response, none of the results were statistically significant, and the changes were inconsistent across dose and time (see **Table A5-6**). In addition to the lack of blinding of outcome assessors, animals were not randomized to treatment.

Integration of evidence for other sensory effects for sarin

The human body of evidence is limited to a few studies that evaluated different outcomes related to different senses. Although there may have been low to moderate confidence in any specific study, the body of evidence is considered low due to the limited information available. The animal body of evidence was restricted to a single study examining the potential association between acute sarin exposure and a few FOB results at 3, 6, or 12 months after exposure. Both the human and animal evidence translate to an inadequate level of evidence. Therefore, an evidence profile table and detailed discussion of the evidence synthesis were not developed for sarin and other sensory effects, and this health effect was not considered for hazard identification conclusions.

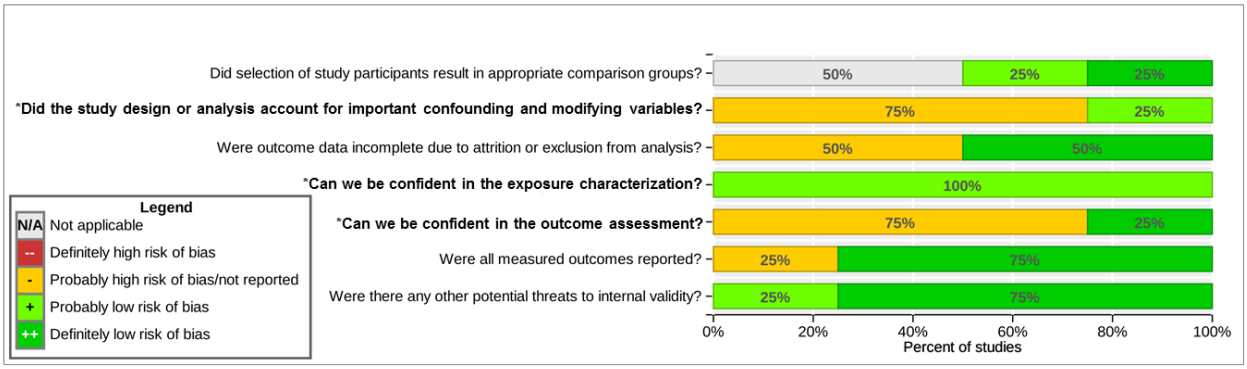
Figure A4-21. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Other Sensory Effects in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Figure A4-22. Risk-of-bias Bar Chart for Case Reports/Series and Standard Observational Studies Assessing Other Sensory Effects in Humans Following Acute Sarin Exposure



*Questions in bold are the key risk-of-bias questions for human case reports/series and standard human observational studies. These key questions relate to areas of bias which may have a greater impact on estimates of the overall effect size and are generally considered to have a greater effect on the credibility of study results in environmental health studies.

Interactive figure and additional study details in HAWC here.

Appendix 5. Additional Data Tables

Table A5-1. Summary of the Number of Studies and Time Periods Reported/Assessed in Human and Animal Studies by Endpoint Category

Neurological Endpoint Category	Summary of Endpoints Reported/Assessed (no. studies; range of timeframes)	
	Human	**Animal**
Cholinesterase	(9 studies; 1 day–5 years)	(15 studies; 1 day–45 weeks)
Visual /Ocular	(16 studies; 1 day–5 years)	(4 studies; 1 week–12 months)
Other sensory	(4 studies; 2–3 years)	(1 study; 3–12 months)
Memory	(7 studies; 10 day–7 years)	(10 studies; 1 week–6 months)
Sleep disruption	(6 studies; 3 weeks–5 years)	--
Anxiety/fear	(7 studies; 1 month–5 years)	(2 studies; 3–12 months)
Avoidance/Depression	(5 studies; 1 month–5 years)	--
Activity/strength	(7 studies; 3 weeks–5 years)	(8 studies; 4 days–12 months)
Other neurological symptoms	(11 studies; 3 weeks–5 years)	--
Morphology/histopathology	(3 studies; 8 months–5 years)	(5 studies; 2 days–90 days)
EEG	(5 studies; 30 hours–1 year)	(2 studies; 1 week–1 year)

Table A5-2. Neurological Symptom Summary for Victims of the Matsumoto and Tokyo Subway Sarin Attacks[1,2]

Symptom[3]	Study Population														Symptom References[4] (timeframes)	Reference Notes
	Matsumoto Sarin Attack (1994) *time after exposure*						Tokyo Subway Sarin Attack (1995) *time after exposure*									
	1 wk.	3 wk.	4 mo.	1 yr.	3 yr.	5 yr.	1 wk.	1 mo.	2 mo.	3 mo.	1 yr.	2 yr.	3 yr.	5 yr.		
Eye Problems																
Visual																
Asthenopia		√	√	√	√										Nakajima *et al.* (1998); Nakajima *et al.* (1999); Okudera (2002)	
Blurred vision	√	√	√	√	√		√	√	√	√				√	Nakajima *et al.* (1998); Nakajima *et al.* (1999); Ogawa *et al.* (1999); Ohtani *et al.* (2004); Ohtomi *et al.* (1996); Nohara and Segawa (1996); Okudera (2002)	
Constricted visual field									√						Ogawa *et al.* (1999)	
Darkness of visual field		√	√												Nakajima *et al.* (1998); Okudera (2002); Morita *et al.* (1995)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr.
Difficulty focusing (vision)						√						√	√	√	Kawana *et al.* (2001)	
Difficulty in seeing far														√	Ohtani *et al.* (2004)	
Difficulty in seeing nearby objects														√	Ohtani *et al.* (2004)	
Difficulty seeing close						√						√	√	√	Kawana *et al.* (2001)	
Difficulty seeing distance						√						√	√	√	Kawana *et al.* (2001)	
Dim vision	√					√	√	√	√/0	√		√	√	√	Ohtomi *et al.* (1996), Kawana *et al.* (2001); Morita *et al.* (1995), Ogawa *et al.* (1999); Nohara and Segawa (1996)	For Ohtomi *et al.* (1996), symptom was reported by two victims at 1 mo. and one victim at 3 mo., but no victims reported it at 2 mo.; symptom was reported by Ogawa *et al.* (1999) at 2 mo.
Diplopia/double vision		√	√						√						Nakajima *et al.* (1998); Ogawa *et al.* (1999)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr.
Flickering of vision		√	√												Nakajima *et al.* (1998)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr.

Symptom[3]	Study Population														Symptom References[4] (timeframes)	Reference Notes
	Matsumoto Sarin Attack (1994) *time after exposure*						Tokyo Subway Sarin Attack (1995) *time after exposure*									
	1 wk.	3 wk.	4 mo.	1 yr.	3 yr.	5 yr.	1 wk.	1 mo.	2 mo.	3 mo.	1 yr.	2 yr.	3 yr.	5 yr.		
Narrowing of visual field	√	√	√	√	√										Nakajima *et al.* (1998); Nakajima *et al.* (1999); Nohara and Segawa (1996)	
Visual field abnormalities				√			√	√	√	√					Ohtomi *et al.* (1996); Sekijima *et al.* (1997)	
Ocular																
Ciliary and conjunctival congestion							√	√	√	√					Ohtomi *et al.* (1996)	
Eye/ocular irritation									√						Ogawa *et al.* (1999)	
Eye mucus														√	Ohtani *et al.* (2004)	
Eye/ocular pain	√	√	√						√						Ogawa *et al.* (1999); Nohara and Segawa (1996); Nakajima *et al.* (1998); Okudera (2002); Morita *et al.* (1995)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr.
Eyes tend to become easily tired														√	Ohtani *et al.* (2004)	
Feeling of a foreign object in the eye														√	Ohtani *et al.* (2004)	
Increase in lacrimation		√	√						√						Nakajima *et al.* (1998); Ogawa *et al.* (1999)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr (Nakajima *et al.* 1998).
Lower intraocular pressure							√	√	√	√					Ohtomi *et al.* (1996)	
Miosis							√	√	0	0					Ohtomi *et al.* (1996)	Miosis appeared in 95% of 62 hospitalized patients but disappeared within a mo. except for 2 patients. Miosis disappeared in all patients by 2 mo. following exposure.
Ocular and periorbital pain							√	√	√	√					Ohtomi *et al.* (1996)	
Eye symptoms (general)		√				√					√	√	√	√	Kawana *et al.* (2001); Ohtani *et al.* (2004); Okumura *et al.* (2005); Okudera (2002); Morita *et al.* (1995)	
Behavioral Changes																
Avoidance																
Avoidance of places that trigger recollections of the trauma														√	Ohtani *et al.* (2004)	

Symptom[3]	Study Population														Symptom References[4] (timeframes)	Reference Notes
	Matsumoto Sarin Attack (1994) *time after exposure*						Tokyo Subway Sarin Attack (1995) *time after exposure*									
	1 wk.	3 wk.	4 mo.	1 yr.	3 yr.	5 yr.	1 wk.	1 mo.	2 mo.	3 mo.	1 yr.	2 yr.	3 yr.	5 yr.		
Avoidance of the subject of the incident						√						√	√	√	Kawana *et al.* (2001)	
Avoidance of thoughts and conversations associated with trauma														√	Ohtani *et al.* (2004)	
Concentration difficulty																
Difficulty concentrating						√						√	√	√	Kawana *et al.* (2001)	
Difficulty in focusing														√	Ohtani *et al.* (2004)	
Lack of concentration											√			√	Ohtani *et al.* (2004); Okumura *et al.* (2005)	
Depression																
Depressed mood/feelings						√		√			√	√	√	√	Kawana *et al.* (2001); Ohbu *et al.* (1997); Okumura *et al.* (2005)	Ohbu *et al.* (1997) reports that 74 of 475 hospital patients (16%) reported this symptom at 1 mo. following the exposure. The author does not report on this symptom specifically at 3 and 6 mo. following exposure, but notes that "almost 60% of respondents still suffered from some post-incident symptoms, which can be indication of PTSD, 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident."
Diminished interest, numbing						√						√	√	√	Kawana *et al.* (2001)	
Diminished interest and apathy														√	Ohtani *et al.* (2004)	
Memory																
Difficulty with memory						√						√	√	√	Kawana *et al.* (2001)	
Forgetfulness	√													√	Ohtani *et al.* (2004); Sekijima *et al.* (1995)	
Recollections of an event														√	Ohtani *et al.* (2004)	

Symptom[3]	Study Population														Symptom References[4] (timeframes)	Reference Notes
	Matsumoto Sarin Attack (1994) *time after exposure*						Tokyo Subway Sarin Attack (1995) *time after exposure*									
	1 wk.	3 wk.	4 mo.	1 yr.	3 yr.	5 yr.	1 wk.	1 mo.	2 mo.	3 mo.	1 yr.	2 yr.	3 yr.	5 yr.		
Sleep disruption																
Bad dreams				√	√										Nakajima *et al.* (1998); Nakajima *et al.* (1999);	This symptom was included in the questionnaire at 1 yr. but was excluded from the questionnaires at 3 wk. and 4 mo. (Nakajima *et al.* 1998).
Nightmares								√						√	Ohbu *et al.* (1997); Ohtani *et al.* (2004)	Ohbu *et al.* (1997) reports that 48 of 475 hospital patients (10%) reported this symptom at 1 mo. following the exposure. The author does not report on this symptom specifically at 3 and 6 mo. following exposure, but notes that "almost 60% of respondents still suffered from some post-incident symptoms, which can be indication of PTSD, 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident."
Difficulty falling or staying asleep						√						√	√	√	Kawana *et al.* (2001)	
Distressing dreams, nightmares						√						√	√	√	Kawana *et al.* (2001)	
Insomnia		√	√	√	√				√					√	Nakajima *et al.* (1998); Nakajima *et al.* (1999); Ogawa *et al.* (1999); Ohtani *et al.* (2004)	
Sleep disturbance								√							Ohbu *et al.* (1997)	Ohbu *et al.* (1997) reports that 137 of 475 hospital patients (29%) reported this symptom at 1 mo. following the exposure. The author does not report on this symptom specifically at 3 and 6 mo. following exposure, but notes that "almost 60% of respondents still suffered from some post-incident symptoms, which can be indication of PTSD, 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident."

Symptom[3]	Study Population														Symptom References[4] (timeframes)	Reference Notes
	Matsumoto Sarin Attack (1994) *time after exposure*						Tokyo Subway Sarin Attack (1995) *time after exposure*									
	1 wk.	3 wk.	4 mo.	1 yr.	3 yr.	5 yr.	1 wk.	1 mo.	2 mo.	3 mo.	1 yr.	2 yr.	3 yr.	5 yr.		
Anxiety and fear																
Fear concerning escape from the attack											√				Okumura *et al.* (2005)	
Fear in the subway or at the incident												√	√	√	Kawana *et al.* (2001)	
Fear of subway								√			√				Okumura *et al.* (2005); Ohbu *et al.* (1997)	Ohbu *et al.* (1997) reports that 152 of 475 hospital patients (32%) reported this symptom at 1 mo. following the exposure. The author does not report on this symptom specifically at 3 and 6 mo. following exposure, but notes that "almost 60% of respondents still suffered from some post-incident symptoms, which can be indication of PTSD, 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident."
Shaking with fear														√	Ohtani *et al.* (2004)	
Fatigue/lethargy/weakness																
Asthenia				√	√										Nakajima *et al.* (1998); Nakajima *et al.* (1999); Okudera (2002)	This symptom was included in the questionnaire at 1 yr. but was excluded from the questionnaires at 3 wk. and 4 mo. (Nakajima 1998).
Easily fatigued		√	√	√	√						√			√	Nakajima *et al.* (1998); Nakajima *et al.* (1999); Ohtani *et al.* (2004); Okumura *et al.* (2005); Okudera (2002); Morita *et al.* (1995)	
Tiredness														√	Ohtani *et al.* (2004)	
Lethargy														√	Ohtani *et al.* (2004)	
Weakness (general)									√						Ogawa *et al.* (1999)	

Symptom[3]	Study Population														Symptom References[4] (timeframes)	Reference Notes
	Matsumoto Sarin Attack (1994) *time after exposure*						Tokyo Subway Sarin Attack (1995) *time after exposure*									
	1 wk.	3 wk.	4 mo.	1 yr.	3 yr.	5 yr.	1 wk.	1 mo.	2 mo.	3 mo.	1 yr.	2 yr.	3 yr.	5 yr.		
Other behavior																
Astonishment								√							Ohbu *et al.* (1997)	Ohbu *et al.* (1997) reports that 52 of 475 hospital patients (11%) reported this symptom at 1 mo. following the exposure. The author does not report on this symptom specifically at 3 and 6 mo. following exposure, but notes that "almost 60% of respondents still suffered from some post-incident symptoms, which can be indication of PTSD, 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident."
Difficulty reading/writing		√	√	0											Nakajima *et al.* (1998)	
Flashbacks						√		√			√	√	√	√	Kawana *et al.* (2001); Ohbu *et al.* (1997); Okumura *et al.* (2005)	Ohbu *et al.* (1997) reports that 76 of 475 hospital patients (16%) reported this symptom at 1 mo. following the exposure. The author does not report on this symptom specifically at 3 and 6 mo. following exposure, but notes that "almost 60% of respondents still suffered from some post-incident symptoms, which can be indication of PTSD, 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident."
Hypervigilance						√						√	√	√	Kawana *et al.* (2001)	
Impact of event scale-revised (IES-R)														√	Ohtani *et al.* (2004)	
Irritability						√		√				√	√	√	Kawana *et al.* (2001); Ohbu *et al.* (1997)	Ohbu *et al.* (1997) reports that 48 of 475 hospital patients (10%) reported this symptom at 1 mo. following the exposure. The author does not report on this symptom specifically at 3 and 6 mo. following exposure, but notes that "almost 60% of respondents still

Symptom[3]	Study Population														Symptom References[4] (timeframes)	Reference Notes
	Matsumoto Sarin Attack (1994) *time after exposure*						Tokyo Subway Sarin Attack (1995) *time after exposure*									
	1 wk.	3 wk.	4 mo.	1 yr.	3 yr.	5 yr.	1 wk.	1 mo.	2 mo.	3 mo.	1 yr.	2 yr.	3 yr.	5 yr.		
																suffered from some post-incident symptoms, which can be indication of PTSD, 1 month after the incident. This percentage remained almost the same even 3 and 6 months after the incident."
Restlessness and irritability														√	Ohtani *et al.* (2004)	
Sense of suppression														√	Ohtani *et al.* (2004)	
Tension														√	Ohtani *et al.* (2004)	
Neuromuscular Effects																
Gait disturbance		**0**	**0**						√						Nakajima *et al.* (1998); Ogawa *et al.* (1999)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr. Zero patients reported this symptom at 3 wk. and 4 mo (Nakajima *et al.* 1998). For Ogawa 1999, gait disturbance persisted for 0.3% of subjects at 2 mo.
Numbness of extremities									√						Ogawa *et al.* (1999)	
Paresis of perioral muscle		√	**0**												Nakajima *et al.* (1998)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr. Zero victims reported this symptom at 4 mo.

Symptom[3]	Study Population														Symptom References[4] (timeframes)	Reference Notes
	Matsumoto Sarin Attack (1994) *time after exposure*						Tokyo Subway Sarin Attack (1995) *time after exposure*									
	1 wk.	3 wk.	4 mo.	1 yr.	3 yr.	5 yr.	1 wk.	1 mo.	2 mo.	3 mo.	1 yr.	2 yr.	3 yr.	5 yr.		
Other Neurological																
Dysomia (change in sense of smell)									√						Ogawa *et al.* (1999)	
Dizziness		√	√			√			√			√	√	√	Nakajima *et al.* (1998), Kawana *et al.* (2001); Ogawa *et al.* (1999); Ohtani *et al.* (2004)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr. (Nakajima *et al.* 1998).
Dysesthesia of extremities		√	√												Nakajima *et al.* (1998)	This symptom was included in the questionnaires at 3 wks and 4 mo but was excluded from the questionnaire at 1 yr.
Headache		√	√	√/0	√	√	√	√	√	√	√	√	√	√	Nakajima *et al.* (1998), Kawana *et al.* (2001); Nakajima *et al.* (1999); Ogawa *et al.* (1999); Ohtani *et al.* (2004); Ohtomi *et al.* (1996); Okumura *et al.* (2005); Okudera (2002); Morita *et al.* (1995)	Zero victims reported this symptom at 1 yr. (Nakajima *et al.* 1998).
Heaviness in head		√	√	0											Nakajima *et al.* (1998)	Zero victims reported this symptom at 1 yr.

Notes:

[1]This table provides a summary of neurological symptoms identified by study subjects at various timeframes after exposure. The prevalence or severity of symptoms is not indicated here.

[2]A "√" indicates the effect was reported at that time frame in at least one subject and in at least one of the studies listed. A blank indicates that no data are available for that timeframe by any of the listed studies. A "0" indicates that at least one listed study reported that the symptom had fully subsided by the timeframe.

[3]Symptoms are self-reported (via questionnaire) or based on physician observation (not determined by a specific test).

[4]All studies list this symptom for at least one of the timeframes presented, but all studies do not necessarily report symptoms for all the timeframes.

Matsumoto study timeframes: Nakajima *et al.* (1998) (3 wk., 4 mo., 1 yr.); Nakajima *et al.* (1999) (1 and 3 yr.); Kawana *et al.* (2001) (5 yr.)

Tokyo study timeframes: Ogawa *et al.* (1999) (2 mo.); Ohtomi *et al.* (1996) (1 wk., 1 mo., 2 mo., and 3 mo.);Okumura *et al.* (2005) (1 yr.); Ohtani *et al.* (2004) (5 yr.); Ohbu *et al.* (1997) (1 mo.); Kawana *et al.* (2001) (2, 3, and 5 yr.)

Table A5-3. Summary of Neurobehavioral Endpoints in Animals—Learning and Memory

	Genovese *et al.* (2008)	Genovese *et al.* (2009)	Grauer *et al.* (2008)			Kassa *et al.* (2001b)				
	Monkey	Rat	Rat			Rat				
	30 d	48 hr	5 wk	4 mo	6 mo	1 wk	2 wk	3 wk	4 wk	5 wk
Endpoint	**0, 0.701 mg/m³**	**0–4 mg/m³**	**0, 34.2 µg/L**			**0–2.5 µg/L**				
Neurological: behavior										
learning and memory										
serial probe recognition	**NS ↔**									
radial arm maze, completion time (1st 5-block session)		**NS ↑ (high dose)**								
radial arm maze, total errors (1st 5-block session)		SIG ↑ block 1								
radial arm maze, reference errors (1st 5-block session)		SIG ↑ block 1								
radial arm maze, working errors (1st 5-block session)		SIG ↑ block 1								
radial arm maze, VI56 response rate (1st session)		**NS ↕**								
radial arm maze, VI56 response rate (1st 5-block session)		**NS ↓**								
T-maze, completion						**NS ↔**	**NS ↔**	**NS ↔**	**NS ↔**	**NS ↔**
water maze, latency to reach platform (trial 1, day 1)			**NS ↔**	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 1, day 2)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 1, day 3)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 1, day 4)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 1, day 5)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 2, day 1)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 2, day 2)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 2, day 3)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 2, day 4)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					
water maze, latency to reach platform (trial 2, day 5)			↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)					

Notes:

SIG ↑ or ↓ = statistically significant increase or decrease at the dose(s) specified; if no dose is specified, it was a single-dose study or significance occurred at all doses.

NS = not significant: ↔ (≤15% change; also considered NS at ≤15% change when statistical analyses were not conducted [see Grauer *et al.* (2008)]); ↑ increased; ↓ decreased;

↕ inconsistent change; if no dose is specified, it was a single-dose study or the change occurred at all doses.

SIG UNK = level of significance unknown/unclear

Table A5-4. Summary of Neurobehavioral Endpoints in Animals—Discrimination Learning

	Kassa *et al.* (2002)					Kassa *et al.* (2004)						Muggleton *et al.* (2003)	Pearce *et al.* (1999)						Wolthuis *et al.* (1995)		
	Rat					Rat						Marmoset	Marmoset						Marmoset		
	1 wk	2 wk	3 wk	4 wk	5 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	0-12 days	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	4 d	5 d	6 d
Endpoint	0–2.5 µg/L					0–2.5 µg/L						0, 11.15 µg/kg	0, 3 µg/kg						0–12 µg/kg		
Neurological: behavior																					
discrimination learning																					
% errors; lines													**NS ↔**	**NS ↔**	**NS ↔**	**NS ↔**	**NS ↓**	**NS ↔**			
% errors; shapes													**NS ↑**	**NS ↔**	**NS ↓**	**NS ↔**	**NS ↓**	**NS ↔**			
mean errors per reversal												SIG ↓									
visual discrimination performance																			**NS ↓ (high dose)**	**NS ↓ (high dose)**	**NS ↓ (high dose)**
Y-maze, spatial discrimination (time of reaction)	SIG ↑ (high dose)	SIG ↑ (high dose)	SIG ↑ (high dose)	**NS ↑ (mid, high dose)**	**NS ↑ (mid, high dose)**	SIG ↑ (high dose)	SIG ↑ (high dose)	SIG ↑ (high dose)	**NS ↑**	**NS ↑**	**NS ↔**										

Notes:

SIG ↑ or ↓ = statistically significant increase or decrease at the dose(s) specified; if no dose is specified, it was a single-dose study or significance occurred at all doses.
NS = not significant: ↔ (≤15% change); ↑ increased; ↓ decreased; ↕ inconsistent change; if no dose is specified, it was a single-dose study or the change occurred at all doses.

Table A5-5. Summary of Neurobehavioral Endpoints in Animals—Reflexes, Motor Strength, Coordination, and Motor Activity and Memory

	Abou Donia *et al.* (2002)		Allon *et al.* (2011)		Grauer *et al.* (2008)				Kassa *et al.* (2001c)	Kassa *et al.* (2001a)			Kassa *et al.* (2004)	Little *et al.* (1986)	Wolthuis *et al.* (1995)		
	Rat		Rat		Rat				Rat	Rat			Rat	Mouse	marmosets		
	7 d	15 d	1 mo	6 mo	5 wk	6 wk	4 mo	6 mo	3 mo	3 mo	6 mo	12 mo	3 mo	4 d	4 d	5 d	6 d
Endpoint	0–100 µg/kg		0, 27.2 µg/L		0, 34.2 µg/L				0–2.5 µg/L	0–2.5 µg/L			0–2.5 µg/L	0, 80 µg/kg	0–12 µg/kg		
Neurological: behavior																	
reflexes, motor strength, coordination																	
beam walk score	SIG ↓	SIG ↓															
beam walk time	SIG ↑	SIG ↑															
grip (time to release grip)	SIG ↓	SIG ↓															
hand-eye coordination performance															**NS ↓ (high dose)**	**NS ↓ (high dose)**	**NS ↔**
incline plane (slip angle)	SIG ↓	SIG ↓															
fall from vertical position, FOB score									**NS ↔**		**NS ↔**	**NS ↔**					
fore and hindlimb grip strength, FOB score									**NS ↔**		**NS ↔**	**NS ↔**					
forelimb grip strength, FOB score									**NS ↔**		**NS ↔**	**NS altered (high dose)**					
hindlimb grip strength, FOB score									**NS ↔**		SIG altered (low, mid dose)	**NS ↔**					
landing foot splay, FOB score									**NS altered (low dose)**		**NS ↔**	**NS ↔**					
righting reflex, FOB score										**NS ↔**	**NS ↔**	**NS ↔**					
motor activity																	
spontaneous activity														↓ (SIG UNK)			
open field activity (no. of crossings)			**NS ↑**	SIG ↑													
open field activity in center (day 1)						**NS ↔**	↑ (SIG UNK)	↑ (SIG UNK)									
open field activity in center (day 2)						↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)									
open field activity in periphery (day 1)						**NS ↔**	↑ (SIG UNK)	↑ (SIG UNK)									

	Abou Donia *et al.* (2002)		Allon *et al.* (2011)		Grauer *et al.* (2008)				Kassa *et al.* (2001c)	Kassa *et al.* (2001a)			Kassa *et al.* (2004)	Little *et al.* (1986)	Wolthuis *et al.* (1995)		
	Rat		Rat		Rat				Rat	Rat			Rat	Mouse	marmosets		
	7 d	15 d	1 mo	6 mo	5 wk	6 wk	4 mo	6 mo	3 mo	3 mo	6 mo	12 mo	3 mo	4 d	4 d	5 d	6 d
Endpoint	**0–100 µg/kg**		**0, 27.2 µg/L**		**0, 34.2 µg/L**				**0–2.5 µg/L**	**0–2.5 µg/L**			**0–2.5 µg/L**	**0, 80 µg/kg**	**0–12 µg/kg**		
open field activity in periphery (day 2)						↑ (SIG UNK)	↑ (SIG UNK)	↑ (SIG UNK)									
activity, FOB score									SIG altered (high dose)		**NS ↔**	**NS ↔**	SIG altered (high dose)				
activity horizontal, FOB score									**NS ↔**		**NS altered (low, mid dose)**	**NS altered (all doses)**					
activity vertical, FOB score									**NS altered (all doses)**		**NS altered (all doses)**	**NS altered (all doses)**					
gait disorder, FOB score									SIG altered (high dose)		**NS ↔**	**NS ↔**	SIG altered (high dose)				
gait score, FOB score									SIG altered (high dose)		**NS ↔**	**NS ↔**	SIG altered (high dose)				
mobility, FOB score									SIG altered (high dose)		**NS ↔**	**NS ↔**	SIG altered (high dose)				
stereotypy, FOB score									SIG altered (mid, high dose)		**NS ↔**	**NS ↔**	SIG altered (mid, high dose)				
exploratory activity, FOB score										**NS altered (mid, high dose)**	SIG altered (low dose)	**NS altered (all doses)**					
motor activity or memory																	
water maze speed of performance (trial 1, day 1)					↑ (SIG UNK)		**NS ↔**	**NS ↔**									
water maze speed of performance (trial 1, day 2)					↑ (SIG UNK)		**NS ↔**	↓ (SIG UNK)									
water maze speed of performance (trial 1, day 3)					↑ (SIG UNK)		**NS ↔**	**NS ↔**									
water maze speed of performance (trial 1, day 4)					↑ (SIG UNK)		↑ (SIG UNK)	↑ (SIG UNK)									
water maze speed of performance (trial 1, day 5)					↑ (SIG UNK)		↑ (SIG UNK)	↑ (SIG UNK)									

	Abou Donia *et al.* (2002)		Allon *et al.* (2011)		Grauer *et al.* (2008)				Kassa *et al.* (2001c)	Kassa *et al.* (2001a)			Kassa *et al.* (2004)	Little *et al.* (1986)	Wolthuis *et al.* (1995)		
	Rat		Rat		Rat				Rat	Rat			Rat	Mouse	marmosets		
	7 d	15 d	1 mo	6 mo	5 wk	6 wk	4 mo	6 mo	3 mo	3 mo	6 mo	12 mo	3 mo	4 d	4 d	5 d	6 d
Endpoint	0–100 µg/kg		0, 27.2 µg/L		0, 34.2 µg/L				0–2.5 µg/L	0–2.5 µg/L			0–2.5 µg/L	0, 80 µg/kg	0–12 µg/kg		
water maze speed of performance (trial 2, day 1)					**NS ↔**		**NS ↔**	**NS ↔**									
water maze speed of performance (trial 2, day 2)					↑ (SIG UNK)		**NS ↔**	**NS ↔**									
water maze speed of performance (trial 2, day 3)							**NS ↔**	↑ (SIG UNK)									
water maze speed of performance (trial 2, day 4)					**NS ↔**		↑ (SIG UNK)	↑ (SIG UNK)		**NS ↔**							
water maze speed of performance (trial 2, day 5)					↑ (SIG UNK)		↑ (SIG UNK)	↑ (SIG UNK)									

Notes:

SIG ↑ or ↓ = significantly increased or decreased at the dose(s) specified; if no dose is specified, it was a single-dose study or significance occurred at all doses.

NS = not significant: ↔ (≤15% change; also considered NS at ≤15% change when statistical analyses were not conducted [see Grauer *et al.* (2008)]); ↑ increased; ↓ decreased; ↕ inconsistent change; if no dose is specified, it was a single-dose study or the change occurred at all doses.

SIG altered = significantly altered FOB score at the dose(s) specified

NS altered = not significant, but altered FOB score (≤15% change) at the dose(s) specified

SIG UNK = level of significance unknown/unclear

Table A5-6. Summary of Neurobehavioral (Sensory, Anxiety, Other), Neuromuscular, and Ocular Endpoints in Animals

Endpoint	Kassa *et al.* (2001c)	Kassa *et al.* (2001a)		
	Rat	Rat		
	3 mo	3 mo	6 mo	12 mo
	0–2.5 µg/L	0–2.5 µg/L		
Neurological: behavior				
sensory (FOB score)				
approach response		**NS altered (mid dose)**	**NS ↔**	**NS altered (all doses)**
click response		**NS altered (all doses)**	**NS altered (mid, high dose)**	**NS altered (low, high dose)**
tail-pinch response		**NS altered (all doses)**	**NS ↔**	**NS altered (low, mid dose)**
touch response		**NS altered (all doses)**	**NS ↔**	**NS altered (all doses)**
anxiety/fear (FOB score)				
bizarre behavior		**NS ↔**	**NS ↔**	**NS ↔**
Tension	**NS ↔**		**NS ↔**	**NS ↔**
Tremor	**NS ↔**		**NS ↔**	**NS ↔**
Urination		**NS altered (all doses)**	**NS altered (all doses)**	**NS altered (low, high dose)**
Defecation		**NS altered (all doses)**	**NS altered (all doses)**	**NS altered (all doses)**
Vocalizations	**NS ↔**		**NS ↔**	**NS ↔**
other neurotoxicity (FOB score)				
catch difficulty		**NS altered (low, high dose)**	**NS altered (mid, high dose)**	**NS ↔**
ease of handling		**NS altered (low, mid dose)**	**NS altered (mid, high dose)**	**NS altered (mid, high dose)**
Piloerection		**NS ↔**	**NS ↔**	**NS ↔**
Posture		**NS altered (mid dose)**	SIG altered (low, mid dose)	**NS ↔**
Salivation		**NS ↔**	**NS ↔**	**NS ↔**
Secretion		**NS ↔**	**NS ↔**	**NS ↔**
Neuromuscular (FOB score)				
muscular tonus		**NS ↔**	**NS ↔**	**NS ↔**
Ocular (FOB score)				
endo-exophthalmos		**NS ↔**	**NS ↔**	**NS ↔**
Lacrimation		**NS ↔**	**NS ↔**	**NS ↔**
palpebral closure		**NS ↔**	**NS ↔**	**NS ↔**
Pupil size		**NS ↔**	**NS ↔**	**NS ↔**
Pupil response		**NS ↔**	**NS ↔**	**NS ↔**

Notes:
NS = not significant: ↔ (≤15% change)
SIG altered = significantly altered FOB score at the dose(s) specified
NS altered = not significant, but altered FOB score (≥15% change) at the dose(s) specified

Additional Human Endpoints and Animal Biochemical Data

Additional endpoints from human studies not discussed in the main document or **Appendix 4** are listed by category in **Table A5-7**.

There were seven animal studies that included biochemical endpoint data after acute sarin exposure. Although the majority of the studies found some effect in the endpoints measured (see **Table A5-8** below), none of the studies evaluated the same endpoints. Therefore, no conclusion can be made on any potential mechanism from the available biochemical data in animals.

Table A5-7. Additional Human Endpoints

Study name	Endpoint
PTSD	
Kawana *et al.* (2001)	PTSD (DSM-IV); PTSE-Nakano; partial PTSD
Ohtani *et al.* (2004)	Impact of event scale-revised (IES-R); lifetime PTSD; current PTSD
Tochigi *et al.* (2005)	Current PTSD; PTSD; lifetime PTSD
Nishiwaki *et al.* (2001)	IES score
Murata *et al.* (1997)	PTSD
Okumura *et al.* (1996)	PTSD
Tochigi *et al.* (2002)	IES-R score
Balance	
Miyaki *et al.* (2005)	Stabilometry-x length (eyes open or closed); Stabilometry-total length (eyes open or closed); Stabilometry-y length (eyes open or closed); Stabilometry-sway area (eyes open or closed)
Yokoyama *et al.* (1998b)	Frequencies of sway (different measures, eyes open or closed); postural sway (eyes open or closed)
Nishiwaki *et al.* (2001)	Stabilometry-x length (eyes open or closed); Stabilometry-total length (eyes open or closed); Stabilometry-y length (eyes open or closed); Stabilometry-sway area (eyes open or closed)
Digit tapping	
Miyaki *et al.* (2005)	Psychomotor function-tapping (dominant); Psychomotor function-tapping (nondominant)
Loh *et al.* (2010)	Psychomotor finger tapping T-scores
Nishiwaki *et al.* (2001)	Psychomotor function-tapping (dominant); Psychomotor function-tapping (nondominant)
Neuromuscular	
Ohtomi *et al.* (1996)	Accommodative spasm
Baker and Sedgwick (1996)	Reciprocal jitter after transformation; >55 uS jitter of muscle fiber pairs
Grob (1956)	Muscle cramp; generalized muscular fasciculations
Other	
Grob (1956)	Speech difficulty
Miyaki *et al.* (2005)	Simple reaction time; Choice reaction time
Loh *et al.* (2010)	Grooved pegboard T-score; Visuoperceptual scores; seashore rhythm test; failure to maintain sets; trail making test T-scores; attention and executive function tests
Murata *et al.* (1997)	Event-related potential-P300 or N100 latency
Suzuki *et al.* (1997)	stupor
Yokoyama *et al.* (1998c)	Continuous performance test; confusion; anger-hostility; vigor
Nishiwaki *et al.* (2001)	Simple reaction time
Okumura *et al.* (2005)	Subclinical neuropsychobehavioral effects
Sekijima *et al.* (1995)	Autonomic nervous system tests-CV(R-R)

Table A5-8. Summary of Biochemical Endpoints in Animals

Brain Biochemical Endpoint	Abou Donia *et al.* (2002)	Allon *et al.* (2011)		Bhardwaj *et al.* (2012)		Bielavska and Kassa (2000)	Bloch-Shilderman *et al.* (2005)		Jones *et al.* (2000)	Lazar *et al.* (2016)
	Rat, Sprague-Dawley ♂	Rat, Sprague-Dawley ♂		Rat, Wistar ♀		Rat, Albino Spf ♂	Rat, Sprague-Dawley ♂		Rat, Sprague-Dawley ♂	Rat, Sprague-Dawley ♂
	15 days	1 month	6 months	3 days	7 days	12 months	120 hours	240 hours	90 days	48 hours
	0, 50, 75, 90, 100 µg/kg	0, 27.2 µg/L		0, 40, 80 µg/kg		0, 0.8, 1.25, 2.5 µg/L	0, 90 mg/kg		0, 1, 10, 50, 100 µg/kg	0, 80 µg/kg
Dopamine, serotonin and their metabolites										
3,4-dihydroxyphenylacetic acid (DOPAC) level						↓ 0.8 µg/L				
3-methoxytyramine hydrochloride (3-MT) level						↓ 0.8, 2.5 µg/L				
5-hydroxyindoleacetic acid (5-HIAA) level						↑ ≥0.8 µg/L				
dopamine hydrochloride (DA) level						↑ ≥1.25 µg/L				
homovanillic acid (HVA) level						↓ 0.8 µg/L				
serotonin creatinine sulfate (5-HT) level						↑ ≥0.8 µg/L				
Binding receptors										
nAChR ligand binding (cortex)									↓ ≥50 µg/kg	
nAChR ligand binding (brainstem)									↑ 100 µg/kg	
M2 mAChR ligand binding (cortex)	NS								↓ 100 µg/kg	
M2 mAChR ligand binding (brainstem)	NS								↑ 50 µg/kg	
M2 receptor binding (Bmax) (striatum)		NS	↑ 27.2 µg/L							
M2 receptor binding (Bmax) (cortex)		NS	↑ 27.2 µg/L							
M2 receptor binding (kD) (striatum)		NS	↑ 27.2 µg/L							
M2 receptor binding (kD) (cortex)		↑ 27.2 µg/L	↑ 27.2 µg/L							
Brain function markers										
apoptotic regulation (bax protein levels) (frontal cortex)										↓ (SIG UNK) 80 µg/kg
apoptotic regulation (bax protein levels) (parietal cortex)										↓ (SIG UNK) 80 µg/kg
apoptotic regulation (bax protein levels) (piriform cortex)										↓ (SIG UNK) 80 µg/kg
apoptotic regulation (bax protein levels) (hippocampus)										↓ (SIG UNK) 80 µg/kg
apoptotic regulation (bcl2 protein levels) (frontal cortex)										↓ (SIG UNK) 80 µg/kg

	Abou Donia *et al.* (2002)	Allon *et al.* (2011)		Bhardwaj *et al.* (2012)		Bielavska and Kassa (2000)	Bloch-Shilderman *et al.* (2005)		Jones *et al.* (2000)	Lazar *et al.* (2016)
	Rat, Sprague-Dawley ♂	Rat, Sprague-Dawley ♂		Rat, Wistar ♀		Rat, Albino Spf ♂	Rat, Sprague-Dawley ♂		Rat, Sprague-Dawley ♂	Rat, Sprague-Dawley ♂
	15 days	1 month	6 months	3 days	7 days	12 months	120 hours	240 hours	90 days	48 hours
Brain Biochemical Endpoint	**0, 50, 75, 90, 100 µg/kg**	**0, 27.2 µg/L**		**0, 40, 80 µg/kg**		**0, 0.8, 1.25, 2.5 µg/L**	**0, 90 mg/kg**		**0, 1, 10, 50, 100 µg/kg**	**0, 80 µg/kg**
apoptotic regulation (bcl2 protein levels) (parietal cortex)										↓ (SIG UNK) 80 µg/kg
apoptotic regulation (bcl2 protein levels) (piriform cortex)										↓ (SIG UNK) 80 µg/kg
apoptotic regulation (bcl2 protein levels) (hippocampus)										↓ (SIG UNK) 80 µg/kg
apoptotic regulation (ERK1/2 enzymatic activity) (frontal cortex)										↑ (SIG UNK) 80 µg/kg
apoptotic regulation (ERK1/2 enzymatic activity) (parietal cortex)										↑ (SIG UNK) 80 µg/kg
apoptotic regulation (ERK1/2 enzymatic activity) (piriform cortex)										↑ (SIG UNK) 80 µg/kg
apoptotic regulation (ERK1/2 enzymatic activity) (hippocampus)										↑ (SIG UNK) 80 µg/kg
apoptotic regulation (JNK enzymatic activity) (frontal cortex)										↑ (SIG UNK) 80 µg/kg
apoptotic regulation (JNK enzymatic activity) (parietal cortex)										↑ (SIG UNK) 80 µg/kg
apoptotic regulation (JNK enzymatic activity) (piriform cortex)										no change
apoptotic regulation (JNK enzymatic activity) (hippocampus)										no change
PGE2 levels		↑ 27.2 µg/L	↓ 27.2 µg/L							
TSPO binding density		NS	NS							↑ 80 µg/kg
Alterations of protein kinase C isozymes										
PKC beta II expression (cytosolic) (frontal cortex)							NS	NS		
PKC beta II expression (cytosolic) (thalamus)							↑ 90 mg/kg	↑ 90 mg/kg		
PKC beta II expression (cytosolic) (hippocampus)							↑ 90 mg/kg	↑ 90 mg/kg		

	Abou Donia *et al.* (2002)	Allon *et al.* (2011)		Bhardwaj *et al.* (2012)		Bielavska and Kassa (2000)	Bloch-Shilderman *et al.* (2005)		Jones *et al.* (2000)	Lazar *et al.* (2016)
	Rat, Sprague-Dawley ♂	Rat, Sprague-Dawley ♂		Rat, Wistar ♀		Rat, Albino Spf ♂	Rat, Sprague-Dawley ♂		Rat, Sprague-Dawley ♂	Rat, Sprague-Dawley ♂
	15 days	1 month	6 months	3 days	7 days	12 months	120 hours	240 hours	90 days	48 hours
Brain Biochemical Endpoint	**0, 50, 75, 90, 100 µg/kg**	**0, 27.2 µg/L**		**0, 40, 80 µg/kg**		**0, 0.8, 1.25, 2.5 µg/L**	**0, 90 mg/kg**		**0, 1, 10, 50, 100 µg/kg**	**0, 80 µg/kg**
PKC beta II expression (cytosolic) (striatum)							↓ 90 mg/kg	NS		
PKC beta II expression (membrane) (frontal cortex)							NS	NS		
PKC beta II expression (membrane) (thalamus)							↑ 90 mg/kg	↑ 90 mg/kg		
PKC beta II expression (membrane) (hippocampus)							↑ 90 mg/kg	↑ 90 mg/kg		
PKC beta II expression (membrane) (striatum)							↓ 90 mg/kg	NS		
PKC zeta expression (cytosolic) (frontal cortex)							↓ 90 mg/kg	↓ 90 mg/kg		
PKC zeta expression (cytosolic) (thalamus)							NS	↑ 90 mg/kg		
PKC zeta expression (cytosolic) (hippocampus)							↑ 90 mg/kg	↑ 90 mg/kg		
PKC zeta expression (cytosolic) (striatum)							NS	NS		
PKC zeta expression (membrane) (frontal cortex)							NS	NS		
PKC zeta expression (membrane) (thalamus)							↑ 90 mg/kg	↑ 90 mg/kg		
PKC zeta expression (membrane) (hippocampus)							↑ 90 mg/kg	↑ 90 mg/kg		
PKC zeta expression (membrane) (striatum)							NS	NS		
Cholinergic system										
ChAT immunoreactivity (cortex)				↓ ≥40 µg/kg	↓ 80 µg/kg					
ChAT immunoreactivity (cerebellum)				↓ ≥40 µg/kg	NS					
VAChT immunoreactivity (cortex)				NS	↓ 80 µg/kg					
VAChT immunoreactivity (cerebellum)				↓ 40 µg/kg	↓ 40 µg/kg					
Other										
blood-brain barrier permeability (cortex)									NS	

	Abou Donia *et al.* (2002)	Allon *et al.* (2011)		Bhardwaj *et al.* (2012)		Bielavska and Kassa (2000)	Bloch-Shilderman *et al.* (2005)		Jones *et al.* (2000)	Lazar *et al.* (2016)
	Rat, Sprague-Dawley ♂	Rat, Sprague-Dawley ♂		Rat, Wistar ♀		Rat, Albino Spf ♂	Rat, Sprague-Dawley ♂		Rat, Sprague-Dawley ♂	Rat, Sprague-Dawley ♂
	15 days	1 month	6 months	3 days	7 days	12 months	120 hours	240 hours	90 days	48 hours
Brain Biochemical Endpoint	**0, 50, 75, 90, 100 µg/kg**	**0, 27.2 µg/L**		**0, 40, 80 µg/kg**		**0, 0.8, 1.25, 2.5 µg/L**	**0, 90 mg/kg**		**0, 1, 10, 50, 100 µg/kg**	**0, 80 µg/kg**
blood-brain barrier permeability (midbrain)									NS	
blood-brain barrier permeability (brainstem)									↓ 100 µg/kg	
blood-brain barrier permeability (cerebellum)									NS	

Notes
↓↑ indicates statistically significant results.
↓↑ (SIG UNK) indicates a direction of effect, but significance is unknown/unclear.
NS indicates no statistically significant results.