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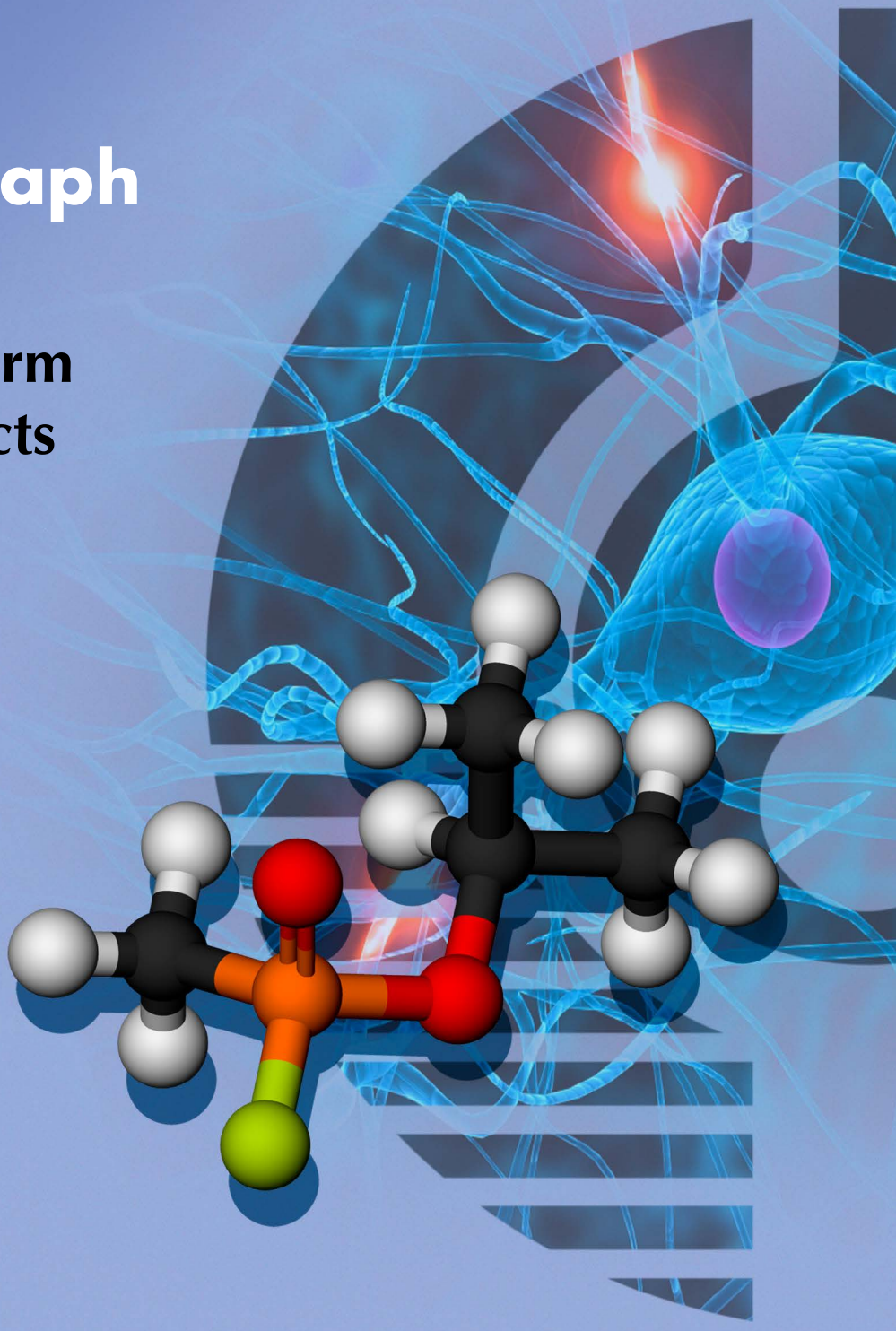
U.S. Department of Health and Human Services

NTP Monograph

on the Systematic Review of Long-term Neurological Effects Following Acute Exposure to Sarin



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NTP Monograph on the Systematic Review of Long-term Neurological Effects Following Acute Exposure to Sarin

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

NTP conducts literature-based evaluations to determine whether exposure to environmental substances (e.g., chemicals, physical agents, and mixtures) may be associated with adverse health effects. These evaluations result in hazard conclusions or characterize the extent of the evidence and are published in the NTP Monograph series, which began in 2011. NTP Monographs serve as an environmental health resource to provide information that can be used to make informed decisions about whether exposure to a substance may be of concern for human health.

NTP conducts these health effects evaluations following pre-specified protocols that apply the general methods outlined in the "Handbook for Conducting a Literature-Based Health Assessment Using the OHAT Approach for Systematic Review and Evidence Integration."[†] The protocol describes project-specific procedures tailored to each systematic review in a process that facilitates evaluation and integration of scientific evidence from published human, experimental animal, and mechanistic studies.

The key feature of the systematic review approach is the application of a transparent framework to document the evaluation methods and the basis for scientific judgements. This process includes steps to comprehensively search for studies, select relevant evidence, assess individual study quality, rate confidence in bodies of evidence across studies, and then integrate evidence to develop conclusions for the specific research question. Draft monographs undergo external peer review prior to being finalized and published.

NTP Monographs are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these evaluations are included in the Health Assessment and Workspace Collaborative.

For questions about the monographs, please email NTP or call 984-287-3211.

[†]OHAT is the abbreviation for Office of Health Assessment and Translation, which is within the Division of the National Toxicology Program at the National Institute of Environmental Health Sciences.

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Figures

This report has been reformatted to meet new NTP publishing requirements;
its content has not changed.

About This Review

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Peer Review

The National Toxicology Program (NTP) convened a virtual external ad hoc panel to peer review the *Draft NTP Monograph on Systematic Review of Long-term Neurological Effects Following Acute Exposure to the Organophosphorus Nerve Agent Sarin* on February 4, 2019. NTP announced the peer-review meeting in the Federal Register (83 FR 63662, December 11, 2018, and 84 FR 368, January 25, 2019). The public could view the proceedings online and opportunities were provided for submission of written and oral public comments. The selection of panel members and conduct of the peer review were in accordance with federal policies and regulations. The panel was charged to:

(1) Comment on whether the *Draft NTP Monograph on Systematic Review of Long-term Neurological Effects Following Acute Exposure to the Organophosphorus Nerve Agent Sarin* is technically correct, clearly stated, and objectively presented.

(2) Vote on whether the scientific evidence from animal studies and from human studies supports the level of evidence conclusions regarding health effects following acute sarin exposure.

(3) Vote on whether the scientific evidence supports NTP's policy decisions for hazard categorization on long-term neurological effects following acute sarin exposure.

NTP carefully considered the panel's recommendations in finalizing the monograph. The peer-review report is provided in Appendix G. Other meeting materials are available on the NTP website (https://ntp.niehs.nih.gov/go/meeting).

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Conflict of Interest

Individuals identified as collaborators in the About This Review section have certified that they have no known real or apparent conflict of interest related to sarin.

Abstract

Introduction: Sarin (CASRN: 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 program, conducted a systematic review to evaluate the evidence for long-term neurological effects in humans and nonhuman animals following acute exposure to sarin. (The terms “animal” and “animals” refer to nonhuman animals.)

Methods: A systematic review protocol was developed and utilized for this evaluation that followed the Office of Health Assessment and Translation 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. Because 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 (8–364 days after exposure), and extended (≥1 year after exposure) periods.

Results and Evidence Synthesis: The literature search and screening process identified 32 data sets within the 34 human studies and 47 data sets 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) effects on learning, memory, and intelligence; and (4) morphology and histopathology in nervous system tissues.

Cholinesterase levels: 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 a low 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 potential. 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 limited, with a low level of evidence in humans from one prospective study and four case reports with serious risk-of-bias concerns and an inadequate level of evidence in animals from a single study with very serious risk-of-bias concerns that did not report an effect.

Effects on learning, memory and intelligence: The majority of the human data on learning and memory evaluated potential effects in the extended period. Taken together, the human and animal bodies of evidence provide some evidence that acute exposure to sarin is associated with effects on learning and memory. There was a low level of evidence from experimental animal studies during all three time periods. Experimental studies in rats found some evidence of 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 tissues: 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, including activity and strength, anxiety and fear, avoidance and depression, electroencephalogram, sleep disruption, other neurological symptoms, and other sensory effects are included in this review. However, the evidence for these effects was not considered in reaching conclusions due to having few studies on a given outcome, inconsistency in findings, heterogeneity of the data, and study limitations.

Discussion and 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 to 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, 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 ≥1 year after exposure based on multiple effects,

including effects on learning and memory and morphological and histopathological changes in nervous system tissues.

Data Gaps: 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. Given the hazard conclusions from this review, additional research on the four 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 this report's appendices, for which there is inadequate evidence to determine whether there is an association with acute sarin exposure. Another area of research that the available data do not address is the effects of sarin on developing and aging brains. The current data are insufficient to assess if there are any susceptible populations.

Introduction

Sarin is a nerve agent developed for chemical warfare during World War II. This highly toxic nerve agent (which can cause death, seizures, and immediate cholinergic symptoms) is liquid at ambient temperatures. It is also known as GB, which is a two-character identifier assigned by the North Atlantic Treaty Organization (NATO). Sarin belongs to a chemically diverse group of organophosphorus (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. Although prohibited by international treaties, 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).

The National Toxicology Program (NTP), in partnership with 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[1] in humans and nonhuman animals[2] following acute exposure[3] to the OP nerve agent sarin (CASRN: 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)] and reports in animal studies of 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)].

Acute versus Long-term Effects

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.

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 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 occurring more than 24 hours after exposure. The 24-hour time point was

[1]Throughout this document, a "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 persist.

[2]Throughout this document, the terms "animal" and "animals" are used to refer to nonhuman animals.

[3]Throughout this document, "acute exposure" is defined as exposure to sarin occurring in a period of 24 hours or less that causes cholinergic signs and symptoms.

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 approach was followed to help determine if the long-term effects resolve or persist.

Purpose of the Review

This review critically evaluated the publicly available evidence of potential long-term neurological effects associated with acute sarin exposure to help inform the focus of treatment options for prolonged effects. Several literature reviews of the long-term neurological effects following exposure to sarin have been published (Augerson 2000; Binns et al. 2004; Brown 2009; Brown and Brix 1998; Defense Science Board 1994; IOM 2004; SIPRI 1975; White et al. 2016). Many of these reviews, however, have assessed 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. Although other OP nerve agents may cause long-term neurological effects through similar mechanisms, there may be differences in health effects, potencies, and durations of effects associated with exposures to different OP agents and mixtures of OP agents. Therefore, those data are beyond the scope of this review, although it is recognized that in a wider context the results from studies on other OP agents may support the long-term neurological effects of sarin.

This systematic review was developed to focus on a specific data set for which sarin is the only exposure. To date, a systematic review of the evidence on sarin 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 most of the evidence for potential long-term health effects of sarin addresses neurological endpoints, this review focused on neurological outcomes.

Available Data

Due to the nature of exposure to sarin (i.e., rare events that, when they occur, are most often a result of occupational accidents or terror attacks), the available studies in humans are primarily case reports, case series, or cross-sectional studies; they also include two controlled trials. The majority of human data come from individuals studied following two terrorist attacks in Japan. One attack occurred in the Tokyo subway system in 1995 (Okumura et al. 1996) and the other attack occurred in Matsumoto in 1994 in a residential area near the center of the city (Morita et al. 1995). Although it is suspected that sarin was used in recent attacks in Syria, publicly available data on long-term neurological effects from these exposures have not been identified for this review.

During the 1995 Tokyo subway attack, sarin was released in five subway cars on three separate subway lines during the morning rush hour. Eleven of the commuters died and more than 5,000 subjects required emergency medical evaluation (Okumura et al. 1996). The agent used in the

attack was quickly identified as a dilute form of sarin, and patients were treated within hours of exposure. 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 considered to be mild cases and were discharged.

The 1994 Matsumoto attack occurred in a residential area near the center of the city (Morita et al. 1995). Sarin was not immediately identified as the source of illness in residents but was detected in a city pond approximately 1 week after symptoms of poisoning were first reported. It was estimated that about 600 residents were exposed based on a survey of residents conducted 3 weeks after exposure with an 84.9% response rate (Nakajima et al. 1999; Nakajima et al. 1998). Fifty-eight people were reportedly admitted to hospitals, and seven deaths occurred (Morita et al. 1995). Although many of the subjects required hospitalization or consulted a doctor due to symptoms, treatment would have occurred for many subjects more than a week after exposure if at all.

It is acknowledged that there are likely clinical observations on individuals following acute sarin exposure that are unpublished or not publicly available due to the military and terrorism significance of sarin; however, all of the evidence in a systematic review must be fully available to support the rigor and transparency of the conclusions. This evaluation does not aim to discount the value of these clinical experiences, but for the purpose of transparency, this review only considered publicly available data.

It is also recognized that due to the hazards of working with sarin, there are only a few laboratories that are permitted to work with the substance. This limits the number of publicly available nonhuman mammalian studies. As discussed above for the clinical observations, it is likely that there are many governments that have studied neurological effects of sarin in research animals but have not made the data publicly available. NTP did not contact laboratories that worked with sarin, nor did it contact other government agencies to determine the accessibility of sarin data that are not publicly available for review.

Exposure Assessment

In experimental studies of humans and animals for which 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 of sarin at which long-term neurological effects occur is beyond the scope of this review. Most studies—with the exception of case reports on separate individuals—did not report the data in a manner that allowed for the assessment of exposure levels in relation to the results (e.g., a lack of reporting of clinical signs or severity of the cholinergic effects). It is possible that the likelihood of long-term effects may be tied to the severity of the cholinergic signs, which in turn may be related to exposure level; however, the available data do not allow for such an evaluation.

Responders to the attacks were also likely exposed to sarin to some degree depending on the timing of the response and proximity to the source of exposure. There are studies that evaluated long-term neurological effects in first responders of the Tokyo subway attack; however, none of these studies specifically addressed the exposure levels of these individuals.

Several factors may affect dose and possible long-term neurological effects. In controlled human and animal trials, 93% was the lowest percent purity that was considered sufficient to reduce potential bias of exposure. Although it is recognized that the purity of sarin may be an issue in accidental occupational exposures or during terrorist attacks, purity of the sarin used in the attack is not generally reported. Possible purity issues were only addressed when there was additional exposure that may affect treatment (e.g., sarin was not the only compound of concern).

Another factor that may affect results for long-term neurological effects of sarin is if or when a person or experimental animal receives treatment for the acute effects. After a known exposure to sarin, humans will receive treatment to alleviate the symptoms of exposure. Because the attack in Matsumoto did not immediately identify sarin as the cause of the symptoms, subjects were less likely to receive treatment or treatment was delayed compared with the Tokyo subway attack. This difference in treatment is likely to have contributed to differences in health effects reported after these two attacks. Experimental animal studies, in which the animals received treatment before or after receiving sarin, were not included in this review.

Objective and Specific Aims

Objective

The overall objective of this evaluation was 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.
- 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 predefined criteria.
- Summarize the extent and types of health effects evidence available.
- Describe limitations of the systematic review and 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 integration 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 National Institutes of Health Countermeasures Against Chemical Threats (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 and specific aims, and to develop a draft protocol for conducting the systematic review. The focus of the current project on sarin exclusively, rather than on all OP agents, was selected to aid in reaching conclusions (i.e., equivalent exposure to the same agent could be more directly compared than could exposure across multiple agents). Similarly, the focus on neurological health effects was selected as an aid in 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 2017 and used to conduct this review (Appendix I). A brief summary of the methods is presented below.

PECO Statements

PECO (**P**opulation, **E**xposure, **C**omparator, and **O**utcome) 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 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/noncombatant
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
Comparator	For controlled and uncontrolled studies, comparable populations not exposed to sarin; and for case series or case reports, no comparable populations
Outcome	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 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)
Comparator	Comparable untreated animal subjects or animals exposed to vehicle-only treatment
Outcome	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 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
Comparator	Comparable cells or tissues exposed to vehicle-only treatment or untreated controls
Outcome	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" and (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 B); the search strategy for other databases are available in the protocol (Appendix I). No language restrictions or publication year limits were imposed, and the databases were searched in April 2016, with several updated searches and a final search conducted 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 include 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 U.S. military observational studies, as well as uncontrolled studies, case series, or case reports. In all instances, the paper must: (1) document exposure to sarin and confirm both (2) 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 in Appendix I for more details).

Study Selection

Evidence Selection Criteria

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 ≥24 hours, except repeat dose studies in which 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 in which 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

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 using brackets [e.g., 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 Availability

Data extraction was completed using the Health Assessment Workspace Collaborative (HAWC), an open-source and freely available web-based interface application, for visualization and warehousing.[4] The data extraction results for included studies are publicly available (https://hawcproject.org/assessment/302/) and can be downloaded in Excel format through HAWC (NTP 2019b). Data extraction elements are listed separately for human, animal, and in vitro studies in the protocol (Appendix I) (NTP 2019c).

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 comprises 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 (Figure 1).

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 shown in Figure 2 following pre-specified criteria detailed in the protocol (Appendix I). 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 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.

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

	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

Figure 1. OHAT Risk-of-bias Questions and Applicability by Study Design

*Experimental animal studies are controlled exposure studies. Nonhuman 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 or RCTs, nonrandomized 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.

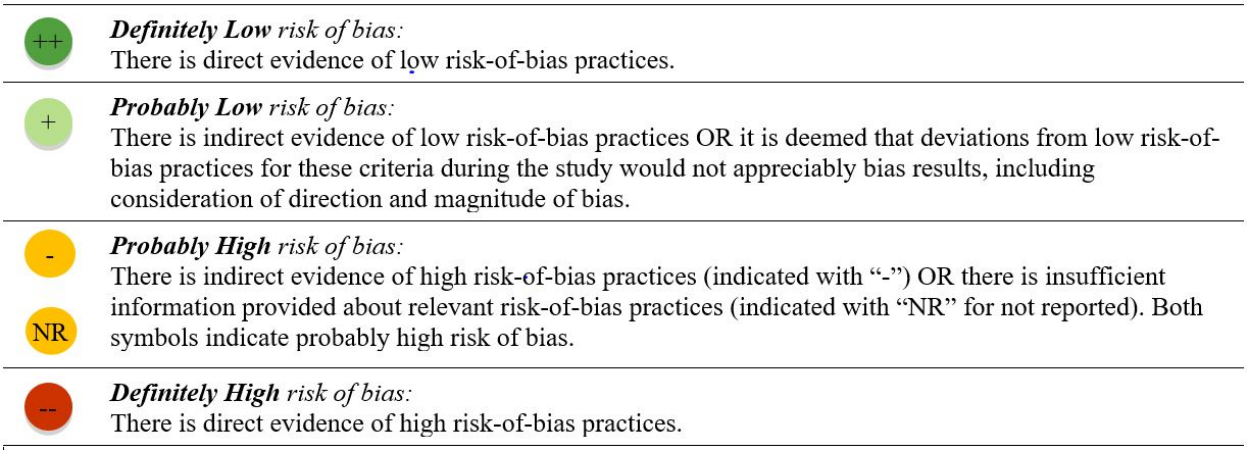

Figure 2. The Four Risk-of-bias Rating Options

Answers to the risk-of-bias questions result in one of the above four risk-of-bias ratings.

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 four main categories of neurological effects: (1) cholinesterase (ChE) levels; (2) visual and ocular effects; (3) effects on 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 are 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 a narrative approach for evidence integration. Heterogeneity within the available human and animal evidence was so high that only a narrative approach (and not a meta-analysis) was appropriate for evidence integration. Meta-analysis approaches are considered most suitable if there are at least six to 10 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 14 experimental 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 themselves 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 3), potential downgrading of the confidence rating was considered for factors that decrease confidence in the results (column 2 of Figure 3 [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 3 [large magnitude of effect, dose response, consistency across study designs/populations/animal models or species, consideration of residual confounding, and other factors that increase 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 (NTP 2015; NTP 2019a; Rooney et al. 2014).

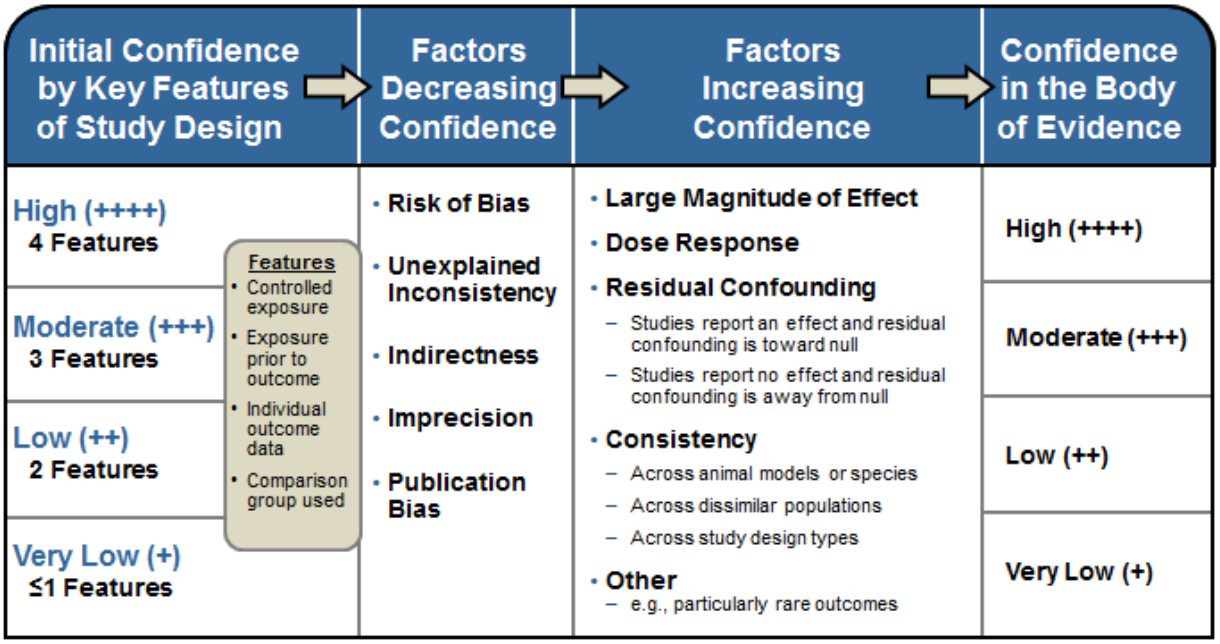

Figure 3. Assessing Confidence in the Body of Evidence

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 4 and Table 4). 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.

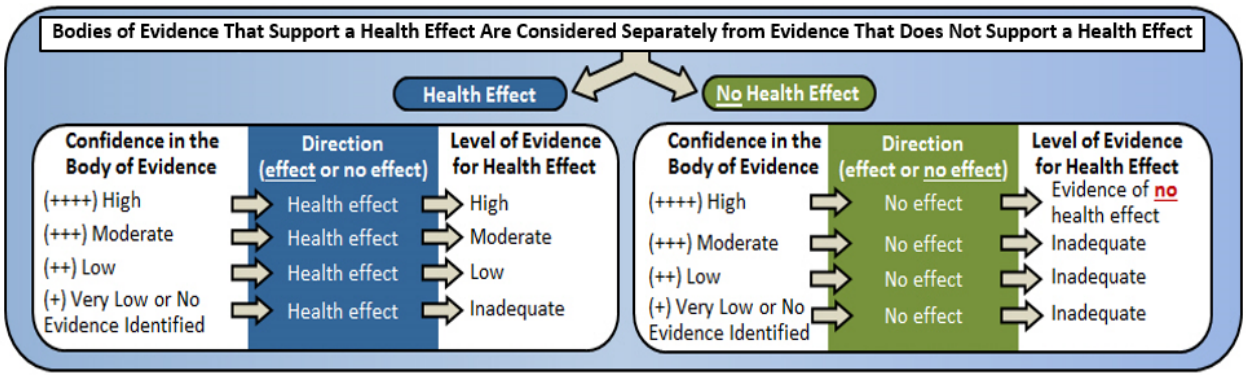

Figure 4. Translation of Confidence Ratings into Evidence of Health Effect Conclusions

Table 4. Definitions of Level-of-evidence Descriptors

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).

Integration of 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 5).

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 5).

A clarification and update to the OHAT approach for systematic review and evidence integration were posted (NTP 2019d) after the peer review draft of this systematic review was publicly available (posted December 2018). Therefore, NTP considered any potential effect of the update and determined that the 2019 update and clarification would have no effect on hazard conclusions reached in this evaluation. In brief, the update clarified how hazard conclusions are reached when there is a moderate level of evidence for human data with low or inadequate level of evidence for the animal evidence stream. In that case, a hazard identification conclusion of either "suspected to be a hazard to humans" or "presumed to be a hazard to humans" can be reached based on scientific judgement as to the robustness of the body of evidence that supports moderate confidence in the human data and consideration of the potential impact of additional studies. As presented in the Results and Evidence Synthesis section of this document, this situation applied to visual and ocular effects in the initial and intermediate time periods; learning, memory, and intelligence effects in the extended period; and morphological and histological changes in the extended period, and the 2019 clarification had no effect on hazard conclusions.

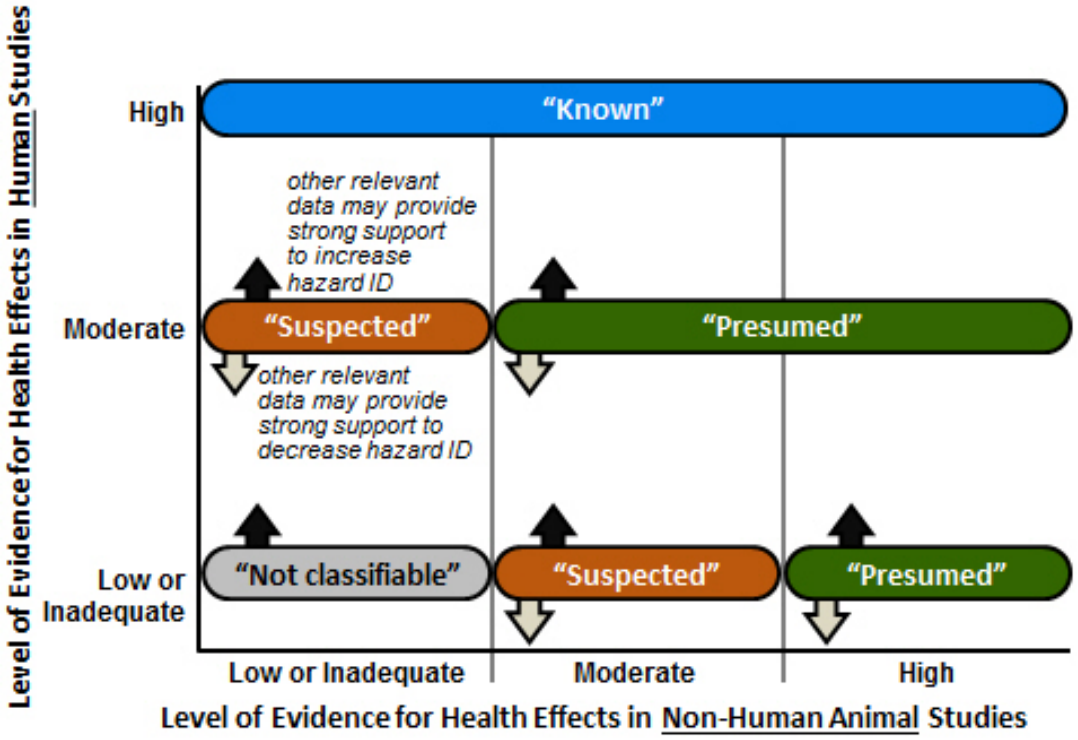

Figure 5. Hazard Identification Scheme for Long-term Neurological Effects

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 <24 hours).

Results and Evidence Synthesis

Literature Search Results

The electronic database searches retrieved 6,837 references. None of the databases searched contains confidential unpublished data; therefore, no confidential unpublished data were retrieved from these searches. Of the total references retrieved, 93% (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 6) [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, two of the human publications and four of the animal publications included data published in another study, so there were 32 human data sets within the 34 human studies and 47 animal data sets 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 Health Assessment Workspace Collaborative (HAWC) project database for this sarin evaluation (https://hawcproject.org/assessment/302/) and were reviewed to answer risk-of-bias questions regarding the data sets that were extracted (NTP 2019b). The list of included references is provided in Appendix C.

Eight studies were identified that included data that were publicly available but did not have a clear peer-review process. These studies were considered unpublished data and were reviewed for potential impact on conclusions. All eight studies were determined not to have any data that would change the hazard conclusions because the data were either subsequently published or 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, these studies were not extracted or included in the assessment.

Twenty-four studies were identified that were published in a language other than English and appeared to meet the evidence selection criteria during the title and abstract screen. The titles, abstracts (if in English), data tables and figures, and study designs for these non-English studies were reviewed and determined to have data that would potentially add to data sets already considered (e.g., additional case reports/series in humans) but were unlikely to have data that would change the hazard conclusions. Therefore, these non-English studies were not translated or included in the assessment.

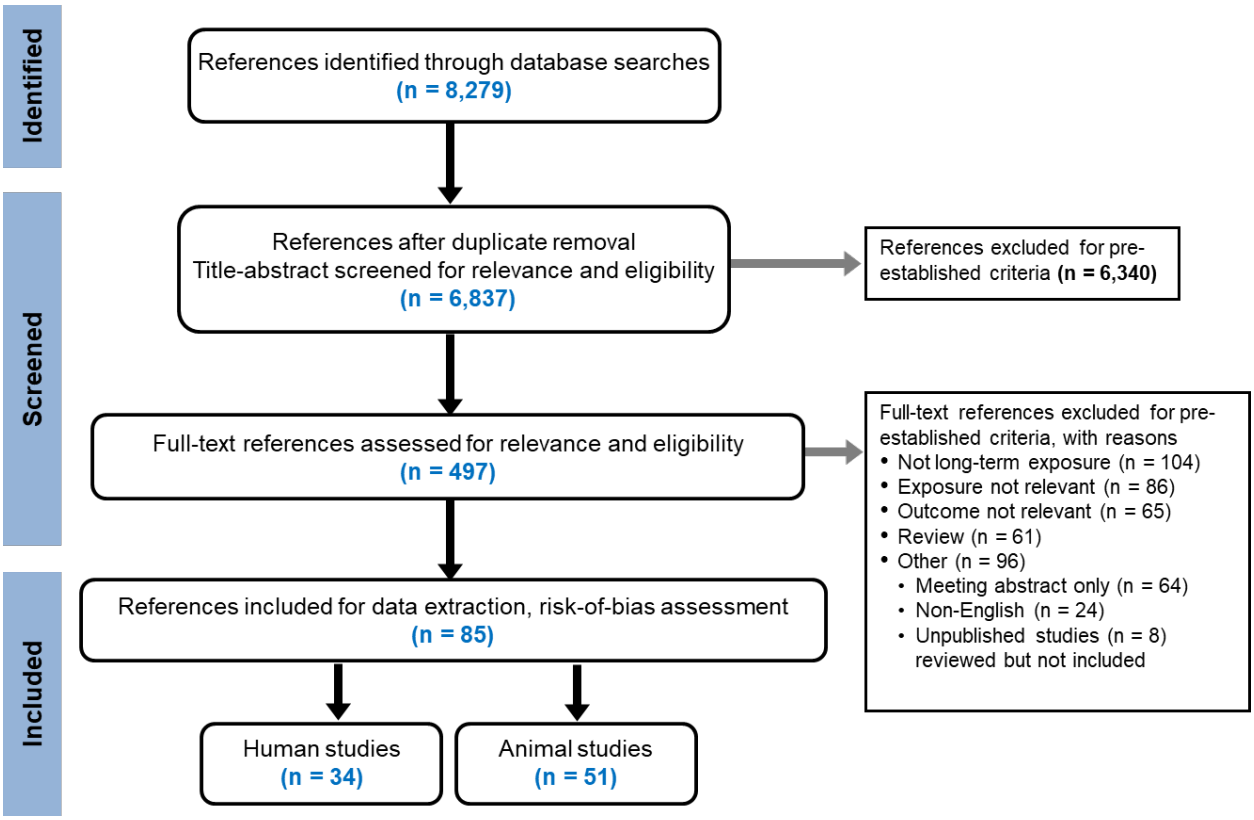

Figure 6. Study Selection Diagram

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) effects on 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 Figure F-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, a set of data had no human equivalent. Brain chemical changes in animals are presented in Table F-7, but these data are not discussed in detail because the heterogeneity of the data precludes an informative synthesis of the data.

There were many additional neurological health outcomes reported for which the evidence was inadequate to determine whether there is an association with acute sarin exposure. These outcomes included activity and strength, anxiety and fear, avoidance and depression, EEG data, sensory effects other than visual, sleep disruption, and other neurological symptoms. The body of evidence was inadequate to evaluate potential effects for these health outcomes because of 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), difficulty in separating effects associated with sarin exposure from effects associated with experiencing a traumatic event (e.g., post-traumatic stress disorder [PTSD] from a terrorist attack), or 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 E.

Due to the nature of exposure to sarin (i.e., rare events that, when they occur, are most often a result of occupational accidents or terror attacks), the majority of available studies are case reports or case series—both of which are descriptive studies that report on a group of exposed subjects followed over time but are often without known levels of exposure or controls for comparison—and 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. Regarding 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 with some standard normal value. Although 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. The inclusion of these two controlled trials in this assessment provides strong evidence of an effect in the initial time period following exposure. These controlled trials provide valuable insight that is not available in most assessments.

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 ChE inhibition is evaluated in this review as a neurological effect from acute sarin exposure, ChE can potentially be a mechanism for the other neurological effects evaluated. However, there are insufficient data to determine if the ChE 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 noncholinergic mechanisms are involved (Pope 2006). For example, disruption of the glutamatergic pathway is another potential mechanism for neurological effects of sarin; this pathway is related to seizures and neurotoxicity, possibly leading to long-term neurological effects including memory

impairment (Chen 2012; Pope 2006; Ray 1998). Other noncholinergic mechanistic pathways may include mitochondrial dysfunction and oxidative stress (leading to cell death) (Abou-Donia et al. 2016; Chen 2012; Hargreaves 2012; Ray 1998). Overall, data are insufficient to identify a clear mechanism by which sarin causes long-term neurological effects in humans.

Organization of the Results

Each section is organized to present and explain the rating of confidence in the body of evidence that sarin exposure is associated with the health effect described in that section for human and animal data separately. The confidence in the data was determined as described in Figure 3. Human data are discussed before animal data. Sections with sufficient data are structured so that the first paragraph discusses the confidence in the data, the second paragraph provides a brief overview of the available studies and of overall risk-of-bias concerns, and subsequent paragraphs summarize the data organized by time frames when appropriate.

Consideration of Health Effects by Time after Exposure

Although any effect observed more than 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. Because results may vary across these ranges of time, the evaluation of hazard conclusions was evaluated for three different time periods (see Table 5). The time periods were selected based on the available data from human studies; however, it is recognized that the selected time frames for the intermediate and extended time periods for humans are not appropriate for rodents. To address this distinction, the durations of the intermediate and extended times periods are defined differently for animals versus humans and nonhuman primates (considered more like humans in terms of life span) as shown in Table 5.

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 for humans and animals. The initial time period likely includes cholinergic effects as well as side effects related to cholinergic hyperstimulation.

The intermediate period after exposure is considered to be from 8 days through 364 days (<1 year) after exposure in humans and nonhuman 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 through 90 days. The 90-day time point was selected based on standard subchronic study dosing guidelines because of the lack of any common comparison for time-after-exposure guidelines. Rodent studies sometimes report the timing of outcome assessment in terms of months instead of days. In these cases, 3 months was considered to be the equivalent to 90 days and was therefore included as part of the intermediate period.

The extended period after exposure is considered any time ≥1 year in humans and nonhuman primates and >90 days (or 3 months) in rodents. As human studies that evaluate health effects years after exposure often define time after exposure in terms of years (i.e., 1 year, 2 years) and not a precise day (e.g., 365 days after exposure), ≥1 year after exposure for humans and nonhuman primates was considered to be the extended period.

Table 5. Definition of Time Periods after Exposure

Time Period after Exposure	Humans and Nonhuman Primates	Animals except Nonhuman Primates
Initial Time Period	>24 hours–7 days	>24 hours–7 days
Intermediate Time Period	8–364 days	8 days–90 days (or 3 months)
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 D. 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 within the consideration of the body of evidence for each health effect. They are key questions for this assessment because they address issues in study design and conduct with the potential to have the greatest impact on the results if not addressed appropriately. No study was excluded based on concerns for risk of bias, but confidence conclusions were considered, if present, with and without high risk-of-bias studies (e.g., studies rated probably high or definitely high risk of bias for two key risk-of-bias questions) to assess the effect 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 versus 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 given study, although there is good empirical evidence that particular flaws in the design, conduct, and analysis of randomized studies lead to bias. Given on these factors, it is important that all studies are evaluated for risk for bias (NTP 2015; NTP 2019a).

Cholinesterase

Sarin is an organophosphate that causes inhibition of acetylcholinesterase (AChE) (Lee 2003). This inhibition leads to an increase in acetylcholine, which leads 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. Sarin is known to inhibit AChE related to cholinergic crisis, and studies addressing effects on ChE within the first 24 hours are beyond the scope of the review and not addressed in the following text. This section provides a summary of studies addressing effects of sarin on ChE that occur more than 24 hours after exposure. Given the well-established role of AChE and ChE in cholinergic crisis, inhibition of these enzymes can be considered an effect of sarin as well as a potential mechanism for other effects; however, there are insufficient data to determine if the inhibition of AChE or ChE is 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 noncholinergic mechanisms are involved (Pope 2006). Therefore, for this evaluation, changes in AChE or ChE after sarin exposure will be considered as a health effect.

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" is the term used for all ChE measurements discussed. Although tests for ChE are considered standard, 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 with 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 with 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 *low confidence* that suppression continues over a period of months after exposure. The studies that provide data on ChE response in blood for a period of days, including two nonrandomized controlled trials (Baker and Sedgwick 1996; Grob and Harvey 1958) and two case reports (Grob 1956; Sidell 1974), reported consistent evidence of ChE inhibition following acute sarin exposure (Figure A-1). Similarly, the studies that provide data on ChE response for a period of

weeks to months, including six case reports (Grob 1956; Ohtomi et al. 1996; Okumura et al. 1996; Rengstorff 1985; Sekijima et al. 1995; Sidell 1974), showed consistent lowering of blood levels of ChE following acute sarin exposure (see Figure A-2 and Figure A-3). 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 (Abu-Qare and Abou-Donia 2002; Gupta 2015; Lee 2003; NRC 1997; Tokuda et al. 2006; Yanagisawa et al. 2006).

There is biological support for consistency of sarin-related suppression of ChE over the period of weeks 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. Although a downgrade in confidence for risk-of-bias concerns and an upgrade for magnitude of effect were considered, applying both would still result in a rating of low confidence. These studies were given a final rating of low confidence in the body of evidence for the intermediate period following sarin exposure. 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 ≥1 year after exposure due to the limited number of studies, small sample size, and risk-of-bias concerns (see Figure 7).

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 nonrandomized controlled trials and 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 6 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, whereas others specifically measured AChE in RBCs.

Table 6. 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)	Nonrandomized 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]	Nonrandomized 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 to 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 to 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 µM/mL/min	Depression of ChE activity of RBCs (4.5 µM/mL/min at 13 days vs. 13 µM/mL/min at 90 days for higher exposed individual) and plasma (4.2 µM/mL/min at 13 days vs. 5.4 µM/mL/min at 90 days for higher exposed individual); gradual increase to baseline over period of up to 90 days

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 vs. 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 are the only data that satisfy 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 in the initial and extended time periods. Risk-of-bias ratings for individual studies for all questions are available in Figure A-6 through Figure A-9. 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 for the key risk-of-bias question regarding confounding. For the controlled trials, risk-of-bias concerns were due to a lack of reporting for the key risk-of-bias questions regarding randomization, exposure assessment (for the oral study), and blinding during outcome assessment. Attempts to contact the study authors to obtain additional information when information pertinent for determining risk of bias was not reported were 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 versus higher quality studies). For the same reason, NTP was unable to compare the results of lower risk-of-bias studies with higher risk-of-bias studies as a way to assess the impact of the unreported information.

For the intermediate time period, six case reports provided consistent evidence of decreased ChE. Three of six case reports (Grob 1956; Sekijima et al. 1995; Sidell 1974) were lower risk-of-bias studies and were rated probably high risk of bias for only one key risk-of-bias question (failure to address potential confounders). The other three higher risk-of-bias studies in this time period (Ohtomi et al. 1996; Okumura et al. 1996; Rengstorff 1985) were rated probably high risk of bias for confounding and potential lack of blinding during the outcome assessment. When considered together, the confidence in the body of evidence was primarily based on the three lower risk-of-bias studies (Grob 1956; Sekijima et al. 1995; Sidell 1974) and was not downgraded for risk-of-bias concerns.

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. Most 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 addresses 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 toward 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 on 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 (three 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 1–3 days after exposure. In both controlled trials, subjects were used as their own controls (Baker and Sedgwick 1996; Grob and Harvey 1958), 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/m3 for 30 minutes and measured ChE in RBCs 3 hours and 3 days after exposure in eight 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 because 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 (Abu-Qare and Abou-Donia 2002; Gupta 2015; Lee 2003; NRC 1997; Tokuda et al. 2006; Yanagisawa et al. 2006), 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 to 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 three of the cases. The three 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 to 80% of control for plasma ChE and from 30 to 55% of control for RBC ChE. All three 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, whereas 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; Ohtomi et al. 1996; Okumura et al. 1996; Rengstorff 1985; Sekijima et al. 1995; Sidell 1974). 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 to 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, whereas the RBC ChE levels took 90 days to return to normal. The other exposed coworker 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 four of the 640 subjects who were admitted into St. Luke's International Hospital after the Tokyo subway attack. These four 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 three 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 seven 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.

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 who developed 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 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 A-4 and Figure A-5). However, there are limitations in the body of evidence, including small sample sizes (n = 2–6 for several studies) and risk-of-bias concerns. The consistent evidence supports 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). Downgrades by one or two levels were considered for the probably high risk-of-bias ratings on one key question as well as other questions. Upgrades were considered for several factors: large magnitude of effect (10–85% suppression of ChE), evidence for dose response in some studies, and consistency of effect across species (both rodents and nonhuman primates). Considering these opposing factors, the serious risk-of-bias concerns resulted in a downgrade of one level, and no upgrades were applied given the extent of the risk-of-bias concern. Therefore, 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 to support the final confidence rating of moderate (see Figure 7).

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 A-4 and Figure A-5). 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 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). Studies that measured AChE mRNA (Damodaran et al. 2003) were included in the body of evidence for completeness but did not drive the hazard conclusions.

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 A-10 and Figure A-11). 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 three 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, most 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 versus 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. Although the kinetics and re-synthesis of ChE after exposure may differ in rodents compared with humans, this was not considered a factor, as the time period for rodents was also modified.

Effects in the Initial Period after Exposure

The results from 11 experimental studies in animals demonstrated depressed ChE 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 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 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 not all studies found a decrease. 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. Scaife and Shuster (1960) observed a decrease in brain ChE in rats (90 μg/kg) and guinea pigs (30 or 35 μg/kg). The authors described the effect as reactivation (or regeneration) and also reported lower-than-normal brain ChE levels in rats at 48 hours and in guinea pigs at 50 and 150 hours. In contrast, another study 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. Koelle et al. (1977) observed a decrease in ChE in various nerves and muscle tissue in cats 2–6 days after exposure to 2 μmol/kg.

Effects in the Intermediate Period after Exposure

The nine 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 because increases were statistically significant 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). Scaife and Shuster (1960) observed lower-than-normal brain ChE (described as reactivation) in rats at 90 μg/kg at 312 hours. 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 >90 days after exposure in rodents or 1 year in nonhuman 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 concerning 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 in the 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 × 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 in 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 × 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 in the 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 × 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 well established that sarin binds to and inactivates AChE (Gunderson et al. 1992; Hargreaves 2012; Spradling and Dillman 2011).

The buildup of the acetylcholine is associated with the cholinergic effects observed with higher exposures to organophosphates including sarin. Treatment for people exposed to sarin typically includes 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 time period covered by much of the human data evaluated for this assessment. Although this ChE inhibition and regeneration may explain the ChE 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 in multiple studies at longer time periods, as it is a known acute effect of sarin, potential health effects due to prolonged ChE inhibition are unknown.

	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 – Initial High (2 Nonrandomized Controlled Trials*)[a]	↓	---	---	---	---	↑	---	---	---	**High**
Intermediate Period – Initial Low (6 Case Reports/Case Series)[b]	---	---	---	---	---	---	---	---	---	**Low**
Extended Period – Initial Moderate (1 Cross-sectional Study)[c]	↓	---	---	---	---	---	---	---	---	**Low**
Animal										
Initial Period – Initial High (11 Mammal Studies)[d]	↓	---	---	---	---	---	---	---	---	**Moderate**
Intermediate Period – Initial High (9 Mammal Studies)[e]	↓	---	---	---	---	---	---	---	---	**Moderate**
Extended Period No Studies	---	---	---	---	---	---	---	---	---	**No rating**

Figure 7. Cholinesterase Evidence Profile for Sarin

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),[d,e] Damodaran et al. (2003),** Genovese et al. (2007),[d,e] Gupta et al. (1991),[d] Jones et al. (2000),[e] Koelle et al. (1977),[d,e] Nieminen et al. (1990),[d] Pearce et al. (1999),[e] RamaRao et al. (2011),[d,e] Scaife and Shuster (1960),[d,e] Tripathi and Dewey (1989),[d,e] Whalley and Shih (1989).[d]
*There are also data from two case reports (Grob 1956; Sidell 1974), but confidence is based on the two nonrandomized 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).

Human Visual and Ocular Data

Summary

The available studies support a rating of *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 *low confidence* over the extended period of ≥1 year. The studies that provide visual or ocular data 1–7 days after acute exposure to sarin, including five case reports/series (Morita et al. 1995; Nohara and Segawa 1996; Ohtomi et al. 1996; Sekijima et al. 1995; Sidell 1974), showed consistent effects of miosis and other visual or ocular parameters (e.g., visual field abnormalities, conjunctival hyperaemia). The studies that provide data on visual or ocular effects for a period of weeks to months, including eight case series (Morita et al. 1995; Nakajima et al. 1998; Nohara and Segawa 1996; Ogawa et al. 1999; Ohtomi et al. 1996; Okudera 2002; Rengstorff 1985; Sidell 1974) 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, whereas 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 (Kawana et al. 2001; Ohtani et al. 2004; Okumura et al. 2005; Sekijima et al. 1997) and one prospective cohort study (Nakajima et al. 1999), showed evidence that other visual or ocular effects (e.g., ocular pain, blurred vision) persisted 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 ≤1 mm, and Morita et al. (1995) observed pupil diameters ≤1.5 mm 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 finding 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. 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 once for serious risk-of-bias concerns (i.e., potential biases in outcome assessment from self-reporting of symptoms via questionnaires and loss of subjects over time) to support a final rating of 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 (>24 hours to up to 5 years), the parameters that were measured, and study design (see Table 7). Most 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 were often 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 (Kawana et al. 2001; Morita et al. 1995; Nakajima et al. 1998; Nohara and Segawa 1996; Ogawa et al. 1999; Ohtani et al. 2004; Ohtomi et al. 1996; Okudera 2002; Okumura et al. 2005; Sekijima et al. 1995; Sekijima et al. 1997) or exposed accidentally in the workplace (Rengstorff 1985; Sidell 1974). Studies were all conducted in adults with a range in sample sizes of 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 7. 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) [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 <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
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); 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 ≤1 mm (right eye) as measured during medical examination	Day 1: 95% of victims had miosis, although 39% of victims had miotic pupils ≤1 mm; 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)

Study	Study Design (Location/Study) [n]	Exposure Measure Timing	Assessment Timing	Analysis	Visual/Ocular Outcome Summary
Sekijima et al. (1995)	Case report (Japan/Matsumoto) [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) [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 with matched controls
Nakajima et al. (1998)[a]	Case series (Japan/Matsumoto) [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)
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)

Study	Study Design (Location/Study) [n]	Exposure Measure Timing	Assessment Timing	Analysis	Visual/Ocular Outcome Summary
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 ≤1 mm (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) [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

Study	Study Design (Location/Study) [n]	Exposure Measure Timing	Assessment Timing	Analysis	Visual/Ocular Outcome Summary
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 2 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
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 with 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%)

Study	Study Design (Location/Study) [n]	Exposure Measure Timing	Assessment Timing	Analysis	Visual/Ocular Outcome Summary
Nakajima et al. (1999)	Prospective cohort (Japan/Matsumoto) [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 nonpatients 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) [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) [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

VEP = visual evoked potential.
[a]The data from these two studies for this effect appear to be from the same subjects.

Overall Risk-of-bias Discussion of 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 A-15 through Figure A-18. 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 most 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 risk of bias for 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 most 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. Most 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 (Morita et al. 1995; Nohara and Segawa 1996; Ohtomi et al. 1996; Sekijima et al. 1995; Sidell 1974). 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 ≤1 mm. 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 over time, pupillary diameter gradually increased through day 7 (mean 1.55 mm at 1 day [n = 13]; mean 3.9 mm at day 7 [n = 5]) (see Figure A-12). 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 <1 mm for 21 subjects, 1.5 mm for 87 subjects, 2 mm for six subjects, and 2.5 mm for 32 subjects during the first 2 days after exposure. A case series on three 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 A-13).

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) 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 with 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 because two of the five studies (Sekijima et al. 1995; Sidell 1974) were assigned a rating of probably low risk of bias for outcome assessment as 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 (Morita et al. 1995; Ohtomi et al. 1996; Rengstorff 1985; Sidell 1974). The findings on miosis from the case series include five occupationally exposed subjects, 62 individuals involved in the Tokyo sarin attack, and 4–15 subjects involved in the Matsumoto attack.

Most visual and ocular symptoms reported were generally reduced from 3 weeks to at least 4 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 accidentally 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 4 weeks; by Ohtomi et al. (1996) and Ogawa et al. (1999) in 10 of 62 subjects (16%) at 8 weeks and in 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 4 weeks; by Ohtomi et al. (1996) and Ogawa et al. (1999) in 6 of 62 subjects (9.7%) at 8 weeks and in 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 4 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 (Nakajima et al. 1998; Ogawa et al. 1999; Ohtomi et al. 1996; 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 4 months (Figure 8). In the only two studies with controls that reported effects <1 year after acute sarin exposure, VEPs were found to be significantly ($p < 0.05$) slower in sarin cases compared with 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 nonvictims (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 with 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 (Kawana et al. 2001; Ohtani et al. 2004; Okumura et al. 2005; Sekijima et al. 1997). 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 one 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 with other physical symptoms at 2, 3, and 5 years after exposure. However, the authors suggested the physical symptoms may be related to PTSD. 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 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 1–7 days after exposure with complete recovery in most cases 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. Most of the 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 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 (Egoz et al. 2017; Gore et al. 2012; Mioduszewski et al. 2002) that evaluated pupil diameter over the initial period of 1–7 days (Egoz et al. 2017; Gore et al. 2012; Mioduszewski et al. 2002) 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 did not find statistically significant sarin-related ocular effects (Egoz et al. 2017; Gore et al. 2012). 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 A-14). 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 multiple limitations in the body of evidence, including a small number of available studies and risk-of-bias concerns. As described below, the judgement was reached to downgrade the bodies of evidence for the intermediate and extended periods by three levels to reflect overall concerns across multiple downgrade factors (i.e., risk of bias and inconsistency).

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 statistically significant 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 the following key questions: 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 was 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 Figure 8).

Four animal studies in the animal body of evidence evaluated the association between acute sarin exposure and long-term ocular effects (Egoz et al. 2017; Gore et al. 2012; Kassa et al. 2001a; Mioduszewski et al. 2002). All studies were conducted in rats, but different strains were used. Three studies evaluated ocular effects over the initial period of 1–7 days following sarin exposure (Egoz et al. 2017; Gore et al. 2012; Mioduszewski et al. 2002). 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 A-19 and Figure A-20). 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 8). 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 LC_{50}, and high incidences of sublethal 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) up to 3 days after varying topical sarin exposures ranging from 0.002 to 10 μg (Gore et al. 2012) or up to 7 days after a single topical sarin exposure of 1 μg (Egoz et al. 2017). Gore et al. (2012) and Egoz et al. (2017) observed pupil constriction during the first few hours after exposure; however, neither study reported an increase in pupil size above pre-exposure levels after pupil size returned to baseline. After the initial pupil constriction in the hours following exposure, both studies continued to observe some pupil width reduction at 24 hours [statistically significant in Gore et al. (2012) at 24 hours but not in Egoz et al. (2017)], which fully returned to baseline by 48 hours with no measurement between 24 and 48 hours. Differences in exposure methods (i.e., inhalation versus direct ocular exposure) and/or animal model (Sprague Dawley versus Long-Evans rats) could contribute to the variability of results between studies although these factors alone are unlikely to account for the increase in pupil size observed in Mioduszewski et al. (2002).

Effects in the Intermediate Period after Exposure

Only one study 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

Only one study 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 *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. The animal body of evidence in the initial period following exposure is considered 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. Although a decrease in pupil diameter 1–7 days was consistently observed in the human data, this effect was harder to assess in the animal data. Two experimental animal studies found no effect on pupil diameter 48 hours or more after exposure (with no measurement available between 24 and 48 hours after exposure), and one experimental animal study observed an increase in pupil diameter 2–7 days following exposure. 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 in the Initial Period after Exposure

- **Human body of evidence:** Moderate confidence = Moderate level of evidence
- **Animal body of evidence:** Moderate confidence with no evidence of an effect that corresponds to the human data = Inadequate level of evidence
- **Initial hazard conclusion (Moderate Human × Inadequate 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 in the 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 × 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 in the Extended Period after Exposure

- **Human body of evidence:** Low confidence = Low level of evidence
- **Animal body of evidence:** Very low confidence = Inadequate level of evidence
- **Initial hazard conclusion (Low Human × Inadequate Animal)** = Not classifiable
- **Final hazard conclusion for extended period (after consideration of biological plausibility)** = Not classifiable

	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 – 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]	↓	---	---	---	---	---	---	---	---	**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**

Figure 8. Visual and Ocular Evidence Profile for Sarin

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). Acetylcholinesterase (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 (Chen 2012; Lee 2003). This inhibition leads to increased levels of synaptic acetylcholine and subsequent cholinergic hyperstimulation. Because sarin interacts with the cholinergic pathway, based on mechanism alone, it is expected that sarin may affect 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. The DRL measurement of cognition also involves vigilance, patience, time estimation, excitability of the animal, and other measures. 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 ≥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 (Loh et al. 2010; Sekijima et al. 1995), 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 (Miyaki et al. 2005; Nishiwaki et al. 2001), report evidence of 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 intelligence (see Figure 9).

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 8). There are no studies that specifically evaluated these outcomes in the initial period of 1–7 days after exposure. Most 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 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, two studies were identified that focused on subway workers and rescue personnel, which would indicate that these subjects are different from the studies evaluating the hospital victims.

Table 8. 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) [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
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)

Study	Study Design (Location/Study) [n]	Exposure Measure Timing	Assessment Timing	Analysis	Learning/Memory Outcome Summary
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
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 statistically significant; 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 backward digit span tests; dose-response increase in adjusted OR

OR = odds ratio; CI = confidence interval.

Overall Risk-of-bias Discussion of 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 A-25 through Figure A-28. 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. Most 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. Most 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) evaluated learning and memory function in a cross-sectional study 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 because only one of the three tests showed an effect. Two case reports (Loh et al. 2010; Sekijima et al. 1995) 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 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.

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 (Miyaki et al. 2005; Nishiwaki et al. 2001), which 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. Again, however, 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. Although, 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, no information was 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 two cases. In the two cross-sectional studies (Miyaki et al. 2005; Nishiwaki et al. 2001), 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 based on effects observed on the backward digit span test.

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 A-25 and Figure A-26). In addition, there were risk-of-bias concerns due to attrition in both case series, as no information was provided on the subjects who participated compared with 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 A-27 and Figure A-28).

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 *low confidence* in the animal body of evidence that acute sarin exposure affects learning and memory over all three time periods. In rats, the results show some evidence of impaired learning and memory following acute sarin exposure across multiple studies and at different time periods following exposure. The studies in monkeys showed little to no effect, but in many cases, 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) to support a final rating of low 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 Figure 9). An additional downgrade for indirectness was considered for the animal studies given that the tests used as indicators of learning and memory may not have adequately ruled out the role of impaired motor or sensory function. Although multiple downgrade factors (i.e., risk of bias, inconsistency, indirectness, and imprecision) were considered for all three time periods, the judgement was reached to downgrade each body of evidence by two levels to reflect the overall concerns.

Nine experimental studies in the animal body of evidence evaluated the association between acute exposure to sarin and effects related to learning and memory (see Table F-2 through Table F-4). 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 A-29 and Figure A-30). 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). None of the nine studies indicated that the animals were randomized to treatment, but one author responded to inquiry and indicated that animals were randomized to treatment (Grauer et al. 2008). Two of the studies discussed balancing the groups based on task performance (Genovese et al. 2009; Muggleton et al. 2003). None of the remaining six studies provided details on randomization or how animals were assigned to treatment. While most 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 five 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 most 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 most studies. Most of the endpoints can be subjective and none of the studies reported that the outcome assessors were adequately blinded to the study group; however, for eight of the nine studies, blinding was not expected to appreciably bias the results because the tests were automated or used a visual tracking system.

There is an additional consideration for animal studies of learning and memory because many of the tests rely on a motor response (e.g., latency to achieve the desired effect). Changes in motor function or activity levels associated with sarin exposure could complicate the interpretation of the results on learning and memory test performance depending on the outcome measured. These considerations are not explicitly a risk-of-bias or internal validity issue, but more appropriately addressed as indirectness. The directness of the measure as an indicator of learning and memory (i.e., the ability to rule out impaired motor or sensory function) was considered when addressing confidence in the animal data.

Effects in the Initial Period after Exposure

Experimental studies in rats (Genovese et al. 2009; Kassa et al. 2001b; Kassa et al. 2004; Kassa et al. 2002) and common marmoset (Muggleton et al. 2003; Pearce et al. 1999; Wolthuis et al. 1995) found some evidence of 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 potentially learning and memory issues associated with sarin that can occur within the first week after the acute exposure (Genovese et al. 2009; Kassa et al. 2004; Kassa et al. 2002). In monkeys, results of discrimination learning tasks within 1 week following acute sarin exposure were inconsistent. In monkeys, most 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; 2004; 2002) exposed male rats to sarin vapor and evaluated learning and spatial memory using a T-maze or Y-maze (see Figure A-21 and

Figure A-22). 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 (2004; 2002) evaluated cued discrimination (time of reaction; referred to as spatial discrimination in the study, but more likely an assessment of cued memory) 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. It was noted, however, that there were no significant differences in the number of entry errors. Kassa et al. (2004) also reported a significant alteration in motor activity (measured as mobility score and activity) at the highest dose at 3 months, which could partly explain some of the other significant results observed at this dose, although there was a dose-dependent increase in the reaction time without similar effects on motor activity at the lower concentrations.

In monkeys, results are considered inconsistent because some studies found little or no effect on learning and memory, although there was also some indication of improved function. 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. Although Pearce et al. (1999) did not find any significant learning deficits, they also noted that sarin-treated monkeys did better at shape discrimination than did the controls.

The body of evidence in rats suggests that acute sarin exposure may result 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 did not support this finding but may be of limited utility due to small sample sizes.

Effects in the Intermediate Period after Exposure

Experimental studies in the rat (Allon et al. 2011; Grauer et al. 2008; Kassa et al. 2001b; Kassa et al. 2004; Kassa et al. 2002) observed some 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 possibly learning and memory issues associated with sarin that can last for weeks after the acute exposure (Grauer et al. 2008; Kassa et al. 2004; Kassa et al. 2002) (see Figure A-21 through Figure A-24. In monkeys (Muggleton et al. 2003; Pearce et al. 1999), 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. The increase in latency could not be explained by effects on motor activity because swimming speed did not significantly change. The sarin level in the study was high enough to cause 35% mortality in the first 24 hours with overt toxicity ranging from no overt signs to severe (i.e., prolonged convulsions). The authors noted that histological brain damage correlated with the

severity of the initial symptoms but learning and memory effects were not evaluated in relation to initial severity. Kassa et al. studies (2001b; 2004; 2002) 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. (2004; 2002) 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; however, as noted previously, motor activity was affected at the highest dose at 3 months, but there was no significant change in the number of entry errors. 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 with some indication of improved discrimination of shapes compared with controls. 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 did not support the data in rats and may be of limited utility due to small sample sizes.

Effects in the Extended Period after Exposure

Results were inconsistent from the two experimental studies in the rat studies (Allon et al. 2011; Grauer et al. 2008) evaluating sarin-related effects related to working memory and reference memory 4 or 6 months after sarin exposure. 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 A-23 and Figure A-24). 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) is consistent with 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 extended-period time points (as well as during the intermediate period), sarin-exposed rats showed an increased latency to reach the platform with no effect on swimming speed, indicating that both working memory and reference memory were impaired (statistical significance not indicated). The animals in Allon et al. (2011), which found no effects on learning and memory, experienced less cholinergic symptoms than did the animals in Grauer et al. (2008), which suggests a less severe response and may be related to the difference in results from the two studies.

Integration of Evidence for Learning, Memory, and Intelligence

There is some evidence that indicates learning and memory impairments in humans and animals following acute exposure to sarin. In humans, evidence suggests 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 body of

evidence, respectively. In animals, there is *low confidence* that acute sarin exposure is associated with learning and memory effects across all time periods after exposure, with some evidence of effects in the initial and intermediate periods and inconsistent results from two studies in the extended time period. 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 in the Initial Period after Exposure

- **Human body of evidence:** No studies = Inadequate level of evidence
- **Animal body of evidence:** Low confidence = Low level of evidence
- **Initial hazard conclusion (Inadequate Human × Low Animal)** = Not classifiable
- **Final hazard conclusion for the initial period (after consideration of biological plausibility)** = Not classifiable

Effects in the Intermediate Period after Exposure

- **Human body of evidence:** Low confidence = Low level of evidence
- **Animal body of evidence:** Low confidence = Low level of evidence
- **Initial hazard conclusion (Low Human × Low Animal)** = Not classifiable
- **Final hazard conclusion for the intermediate period (after consideration of biological plausibility)** = Not classifiable

Effects in 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 × 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 some evidence that acute exposure to sarin may be 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 may 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 (Chen 2012; Gais and Schonauer 2017; Lee 2003) and could be related to secondary neuronal damage occurring in the cholinergic regions of the brain. Although 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.

	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]	↓	---	---	↓	---	---	---	---	---	**Low**
Intermediate Period – Initial High (7 Mammal Studies)[f]	↓	---	---	↓	---	---	---	---	---	**Low**
Extended Period – Initial High (2 Mammal Studies)[g]	↓	↓	---	---	---	---	---	---	---	**Low**

Figure 9. Learning and Memory Evidence Profile for Sarin

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.

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 because histopathological analyses, which can only be performed after death, are difficult to conduct in such a way as to obtain high-quality data. However, some human studies are available that examined morphological and histological changes in nervous system tissues (including brain, spine, and sural nerve) of subjects who were accidentally 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 were considered highly informative and therefore have been included and assessed in this report.

The circumstances associated with exposure to sarin (i.e., often occurring during a traumatic event, such as a terrorist attack) contribute to a "co-exposure" from the nature of the exposure event itself whereby subjects exposed to sarin might also experience PTSD due to the traumatic event, which may confound results of nervous system morphological and histological changes associated with sarin exposure. In addition, subjects could experience PTSD due to the severity of complications that occur after sarin exposure. The use of a control population (i.e., no exposure to sarin) with PTSD could allow researchers to control for PTSD as a confounder, but such controls are unlikely to be available and may be complicated by other confounders. Despite these potential limitations, the data presented in this section are still considered useful for evaluating the effects of sarin exposure. 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 9 and Figure 10) 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 ≥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 9). 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 9. 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	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

RBC = red blood cells; ChE = cholinesterase; MRI = magnetic resonance imaging.

Overall Risk-of-bias Discussion of Body of Evidence

Risk-of-bias ratings for individual studies for all questions are available in Figure A-31 and Figure A-32. 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 most potential confounders. The authors treated age, sex, socioeconomic status, and intracranial volume, as confounding factors. However, 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, few details were 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 concerning the MRI results reported in the case report, the lack of blinding would likely bias toward an effect but 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 A-31 and Figure A-32), 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 with 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 conducted 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. PTSD was not specifically addressed in this study, and the study used healthy controls. This study used 29 of the same subjects as in Tochigi et al. (2002), which reported that 8 of 34 subjects developed PTSD due to the attack with two subjects diagnosed with PTSD at the time of the study (5 years after exposure). Therefore, the current study included no more than eight subjects diagnosed with PTSD, indicating that this is unlikely to be a major contributing factor in the study.

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 A-31 and Figure A-32). 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 occur in one group at a rate that would significantly affect the brain morphology, which is assumed to likely be a result of the sarin exposure. In addition, Yamasue et al. (2007) selected subjects who had sufficient evidence of sarin exposure after the attack, and outcomes were assessed using reliable methods. As noted above, PTSD may be a potential confounder that was not addressed; however, because the PTSD may be related to severity of exposure and only a few (i.e., up to 8) subjects were diagnosed with PTSD, it is not expected to be a major contributing factor in the study.

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 Figure 10). The results provide consistent 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, although 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, suggesting a bias toward the null. Downgrades of one or two levels were considered for the risk-of-bias concerns. Downgrade considerations were also made for the opposing issue of the impact of changes in histological techniques. The decision was reached to downgrade the bodies of evidence one level to reflect the overall concerns. 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.

Six experimental studies in the animal body of evidence evaluated the association between acute exposure to sarin and morphological and histological changes in neurological tissues (Chaubey et al. 2017; Grauer et al. 2008; Kadar et al. 1995; Kawabuchi et al. 1991; Lazar et al. 2016; Singer et al. 1987). 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 A-33 and Figure A-34). 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 (Grauer et al. 2008; Singer et al. 1987). 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

Five experimental studies in rats examined sarin-related effects related to nervous tissue changes within the first 7 days after sarin exposure. Results from the five 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 six 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. Pyknotic 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 two of the three animals sacrificed at 2 days (moderate at 157 and 170 μg/kg), three of the six rats sacrificed at 6 days (moderate at 170 μg/kg and severe at 125 and 197 μg/kg), and one rat sacrificed at 7 days (severe at 170 μg/kg). Kadar et al. (1995) observed neuronal loss in the piriform cortex 1 week after a single intramuscular injection of sarin at 95 μg/kg in surviving rats. Other observations at 1 week included replacement of CA1 cells with large vacuoles in the hippocampus, expansion of lesions into the amygdaloidal nuclei, and extensive gliosis in the thalamus.

Effects in the Intermediate Period after Exposure

Three experimental studies in rats (Chaubey et al. 2017; Kadar et al. 1995; Singer et al. 1987) 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 toward 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 × 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 × 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 × 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 because 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 (Pomara et al. 2015; Raz et al. 2016; Tonni et al. 2014). 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).

	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	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 (5 Mammal Studies)[d]	↓	---	---	---	---	---	---	---	---	**Moderate**
Intermediate Period – Initial High (3 Mammal Studies)[e]	↓	---	---	---	---	---	---	---	---	**Moderate**
Extended Period	No studies available.									**No rating**

Figure 10. Morphological and Histological Changes to Nervous System Tissues Evidence Profile for Sarin

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),[d,e] Kawabuchi et al. (1991),[d] Lazar et al. (2016),[d] Singer et al. (1987).[d,e]

Discussion

The systematic review of the evidence led NTP to reach conclusions on long-term neurotoxicity following acute sarin exposure that are specific for the length of time following sarin exposure. 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 cholinesterase (ChE) in the days after exposure in humans and a moderate level of evidence in the animal studies in the same time period. 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); and morphology and histological changes (inadequate level of evidence from studies in humans and moderate level of evidence from studies in animals). 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, anxiety, and fear), and activity and strength, supported by some evidence of disruption in electroencephalograms (EEGs) (see Appendix E). 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 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 four 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) ChE levels; (2) visual and ocular effects; (3) effects on 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 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 (IOM 2004). In 2004, 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 conclusions in this systematic review (see Appendix E).

Limitations of the Evidence Base

A number of serious limitations in the body of evidence from human studies apply across the different neurological outcomes. The major limitation in the epidemiological studies is study design. Most studies followed subjects from two terrorist attacks (Matsumoto 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 International Hospital) after the attack, which only accounted for 640 of the approximate 5,000 potentially exposed subjects (Okumura et al. 1996). In addition, subjects were lost over time with no information provided on subjects lost, including the reasons why they were lost (e.g., because of death, nonparticipation in follow-up surveys) 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, most of studies 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 that 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 is 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 for which results were not self-reported, there is no indication that the outcome assessors were blind to the exposure group. Most of the studies also did not account for any potential confounders such as age. Although these limitations were true across most 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.

Many of the epidemiological studies do not fully address potential confounders and, therefore, the human evidence as a whole includes multiple challenges with confounding. As noted in the risk-of-bias sections of this report, confounders related to the outcome (e.g., age, sex, socioeconomic status) are rarely considered in case reports or case series. Although there is no evidence to suggest that the likelihood of the attack was associated with any specific confounder, most studies did not describe the subjects in terms of these potential confounders and it is possible that either attack occurred in a specific demographic (although these would be different in the different attacks). In addition, because most of studies in humans report effects following two terrorist attacks, results may be confounded by PTSD. Separating the effects of sarin exposure from the potential effects of PTSD related to a terrorist attack can be difficult, especially because subjects may also experience PTSD related to the severity of the effects from the sarin exposure. For the main endpoints examined in this review (ChE levels; visual and ocular effects; effects on learning, memory, and intelligence; and morphology and histopathology in nervous system tissues), PTSD is not considered a major confounder. For several of the other neurological effects described in Appendix E (e.g., anxiety and fear; avoidance and depression), PTSD could be a major confounder, making it difficult to separate the effects of sarin exposure from the effects due to a traumatic event.

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 set of authors 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. Most 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. (2004; 2002) responded to NTP's 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 that addresses improving human characterization of exposure with neurological tests compared to a control population, in addition to targeted research in animal models that addresses 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 (Miller and Birnbaum 2015). 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 ChE 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 ChE 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). The ability to separate the effects of the sarin exposure from the stress of the terror event would also be useful.

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 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, as well as histological changes, in different areas of the brain; however, the data are insufficient to determine if these differences correlate with the effects observed (e.g., whether the changes observed occur in areas that are known to be involved in learning and memory). 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. Studies in this area are also needed to include tests that separate out learning and memory effects from effects on motor and sensory function. Another gap in both the human and animal data is 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. Although 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. Comprehensive and rigorous

studies are also needed to characterize the neuropathy and underlying mechanisms caused by acute exposure to sarin. These are important to identify a mechanistic basis for the outcomes and drug indications being proposed for new therapeutics.

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 evaluated 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. Publicly available, unpublished data were 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. A review of the identified unpublished data led to the determination 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 sources that are not publicly available 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, data may have been available from short-term or chronic exposures that may have relevance to the findings described in this report. In addition, there is a very large literature base on the effects of other OP nerve agents and pesticides. There may be information on these other OP agents that could support the findings in this review that were not considered.

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.

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* 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, 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 ≥1 year after exposure based on 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 and key data gaps identified in this review using study design and conduct practices to minimize bias. Given the breadth of health effect data supporting the hazard conclusions, potential endpoints identified 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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Appendix A. Data Figures

Figures

A.1. Cholinesterase-related Effects and Outcomes

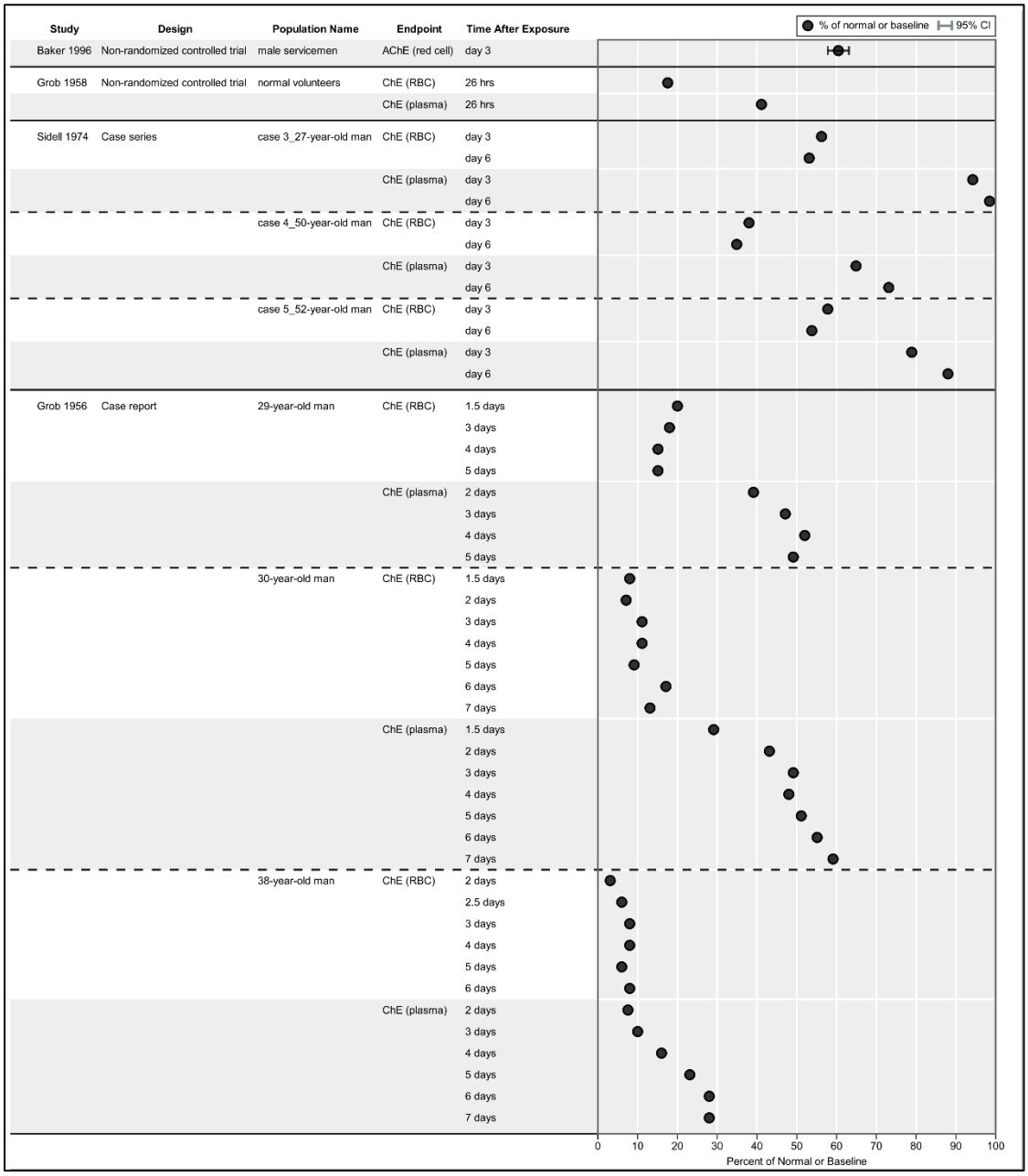

Figure A-1. ChE Levels in Blood in Humans Following Acute Sarin Exposure (Initial Period)

Interactive figure and additional study details in Health Assessment Workspace Collaborative (HAWC) (NTP, 2019b). 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.

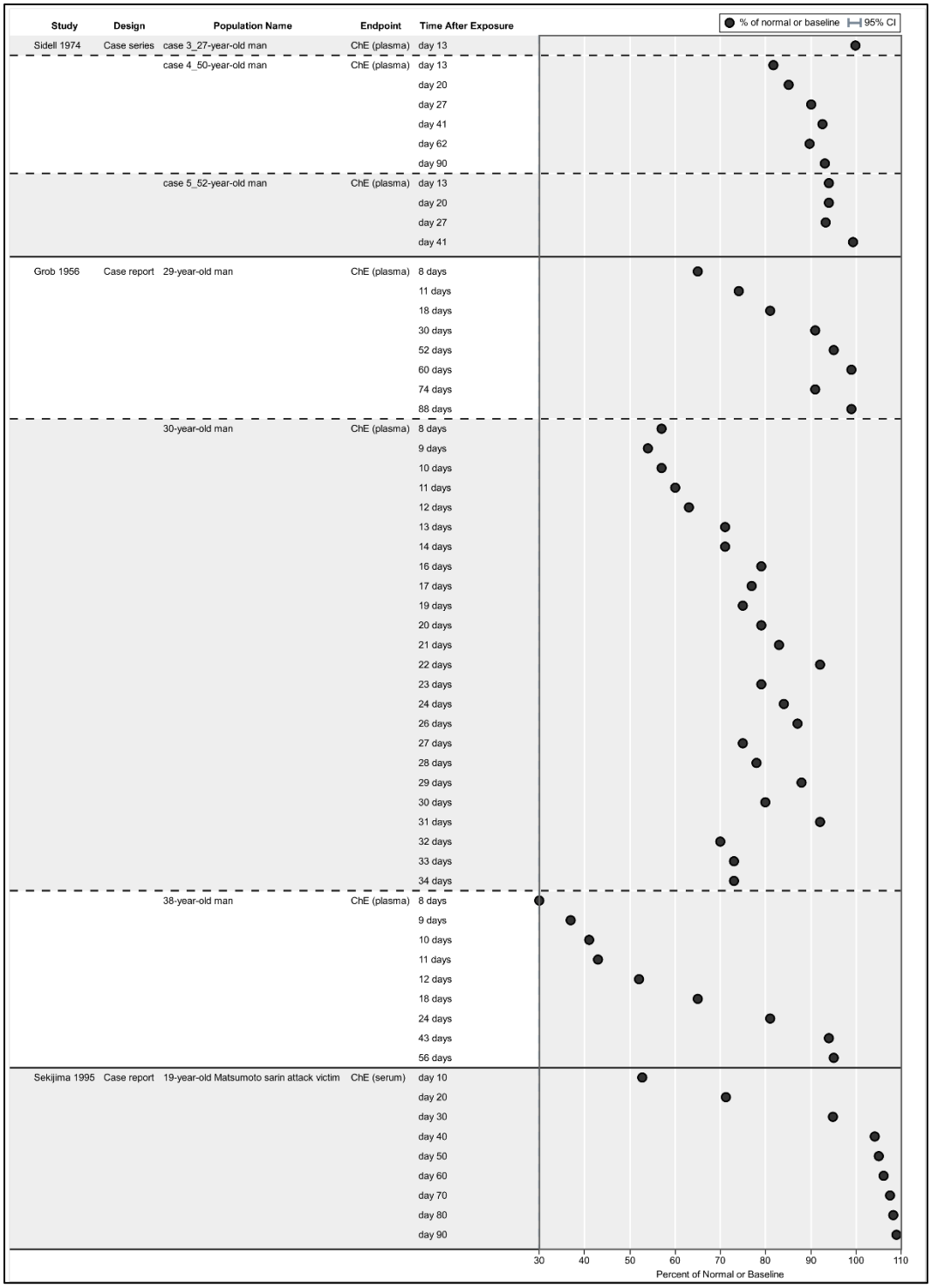

Figure A-2. ChE Levels in Blood in Humans Following Acute Sarin Exposure (Intermediate Period – Serum or Plasma)

Interactive figure and additional study details in HAWC (NTP, 2019b). 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.

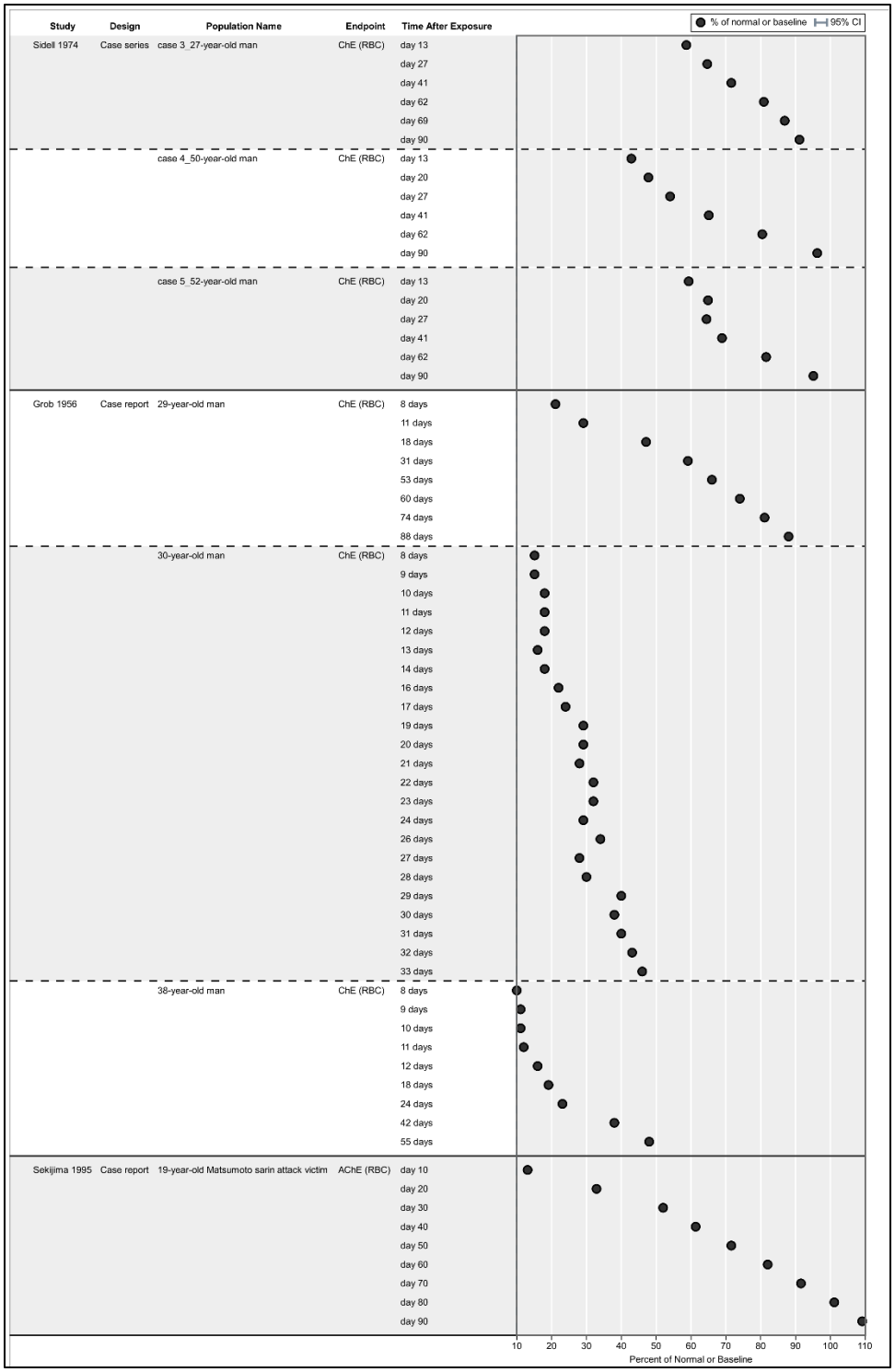

Figure A-3. ChE Levels in Blood in Humans Following Acute Sarin Exposure (Intermediate Period – Red Blood Cells)

Interactive figure and additional study details in HAWC (NTP, 2019b). 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.

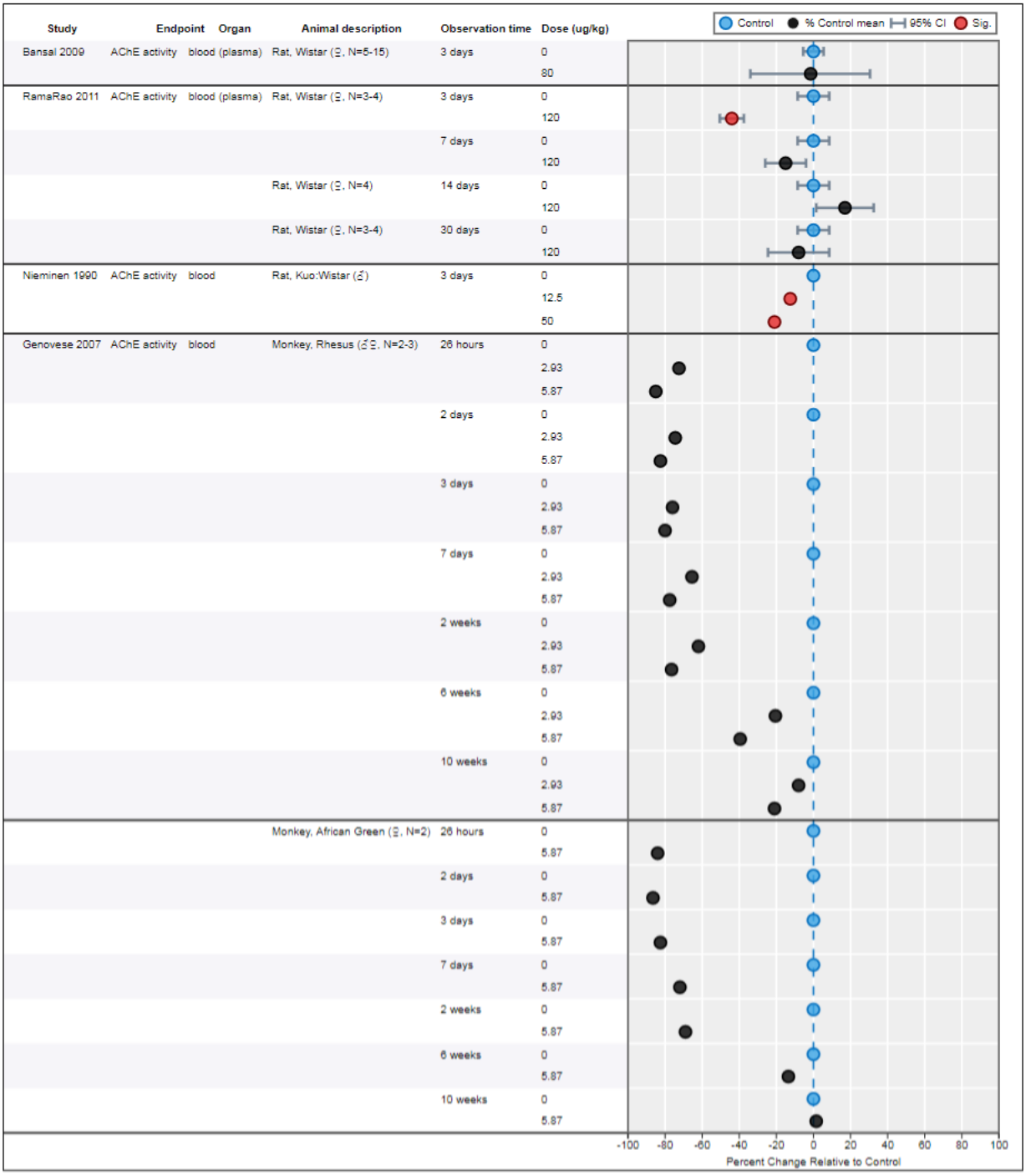

Figure A-4. AChE Levels in Blood in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b). 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 (Chaubey et al. 2017; Chaubey et al. 2016; Damodaran et al. 2003; Whalley and Shih 1989) are not included in the figure. See the text for relevant information about these studies.

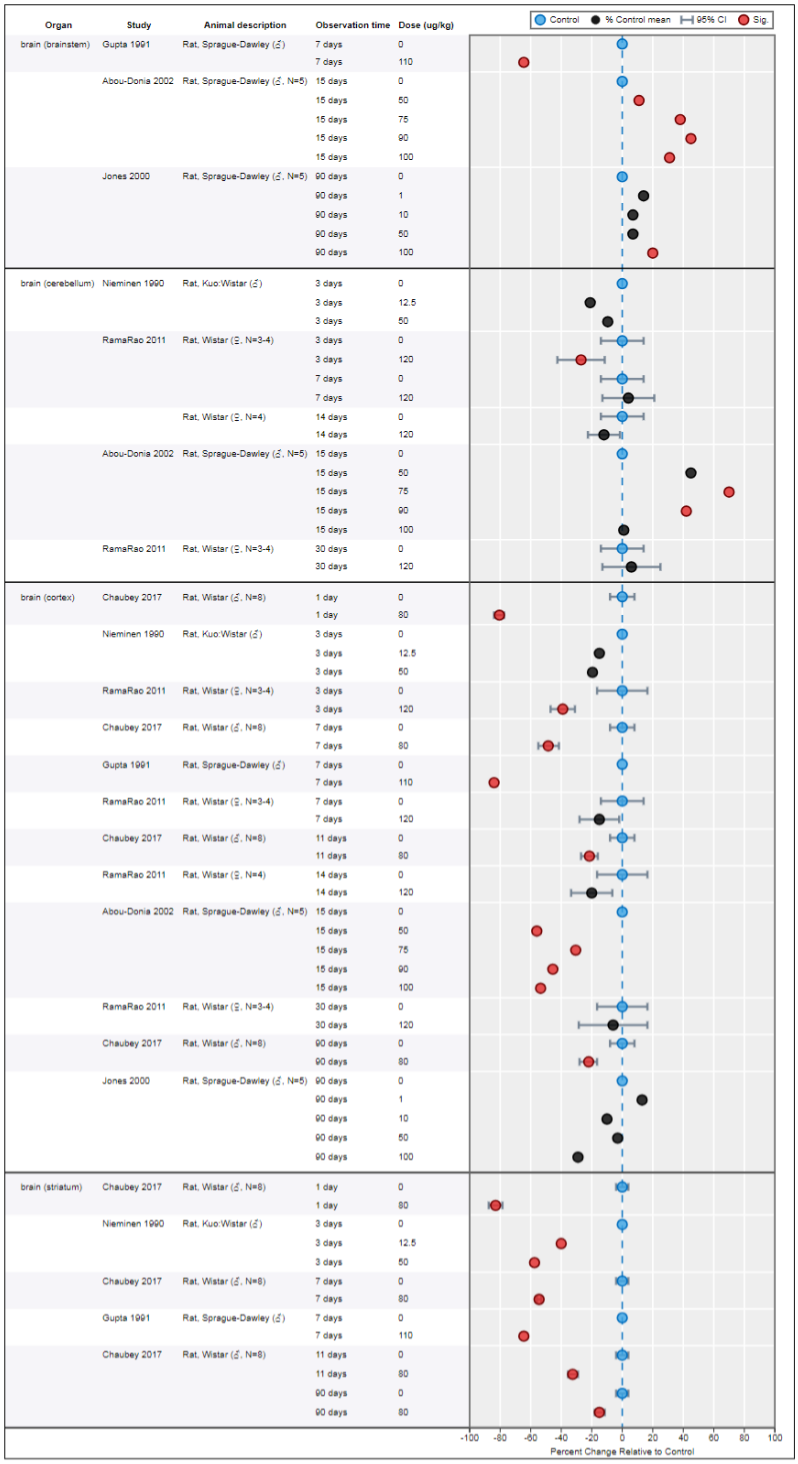

Figure A-5. AChE Levels in the Brain of Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b). This figure includes brain regions with results from more than two 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 (Chaubey et al. 2016; Damodaran et al. 2003; Whalley and Shih 1989) are not included in the figure. See the text for relevant information about these studies.

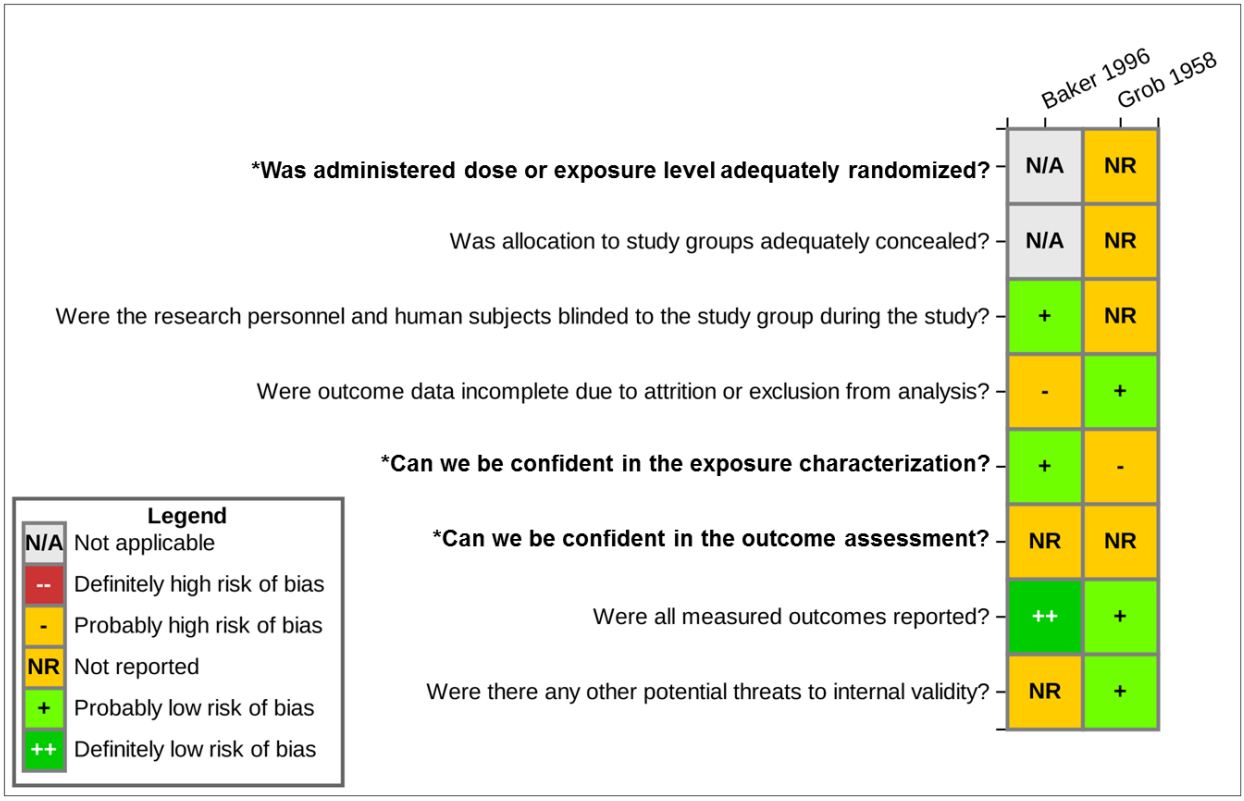

Figure A-6. Risk-of-bias Heat Map for Controlled Trials Assessing ChE Levels in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human controlled exposure studies. These key questions relate to areas of bias that 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.

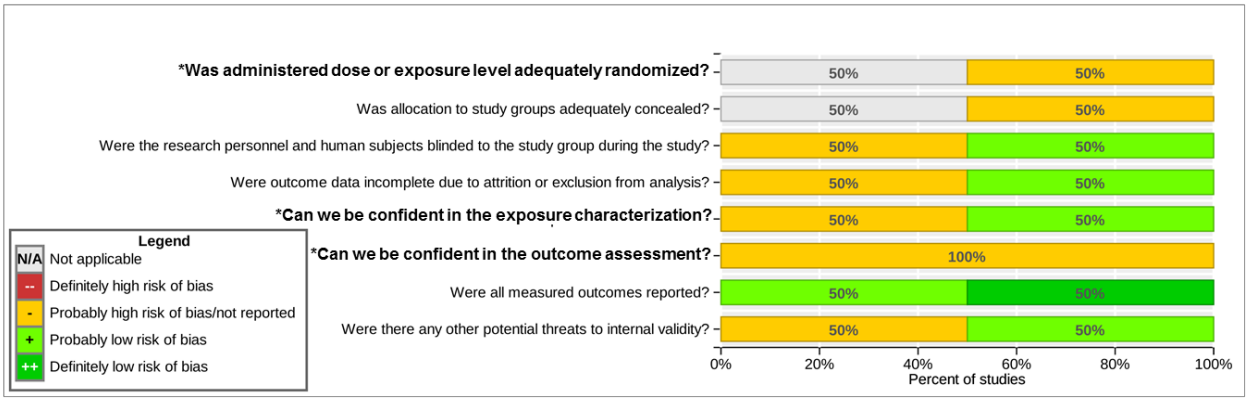

Figure A-7. Risk-of-bias Bar Chart for Controlled Trials Assessing ChE Levels in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human controlled exposure studies. These key questions relate to areas of bias that 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.

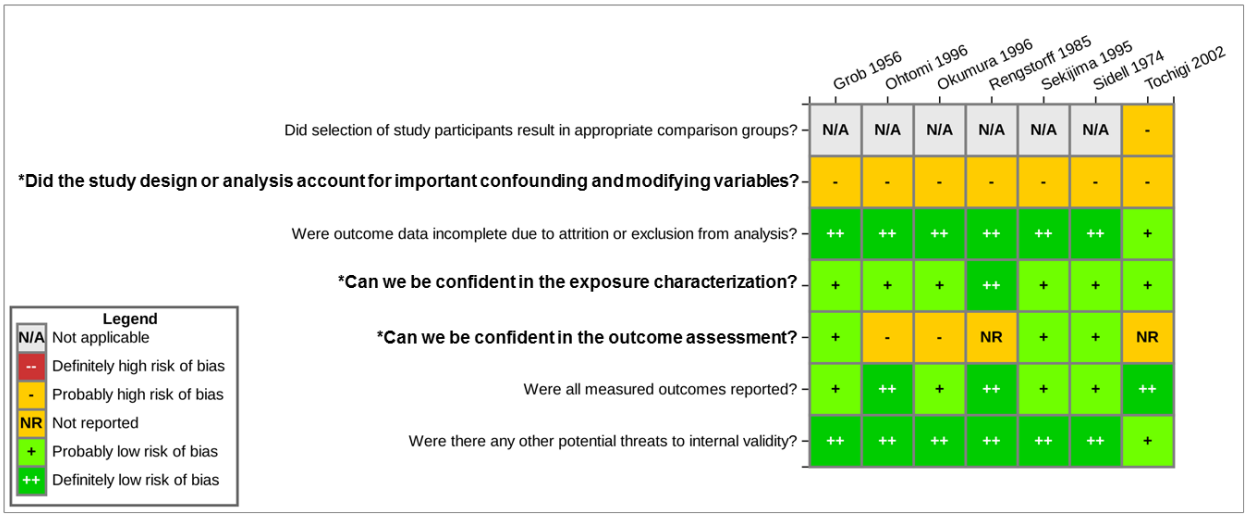

Figure A-8. Risk-of-bias Heat Map for Case Reports/Series and Cross-Sectional Studies Assessing ChE Levels in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

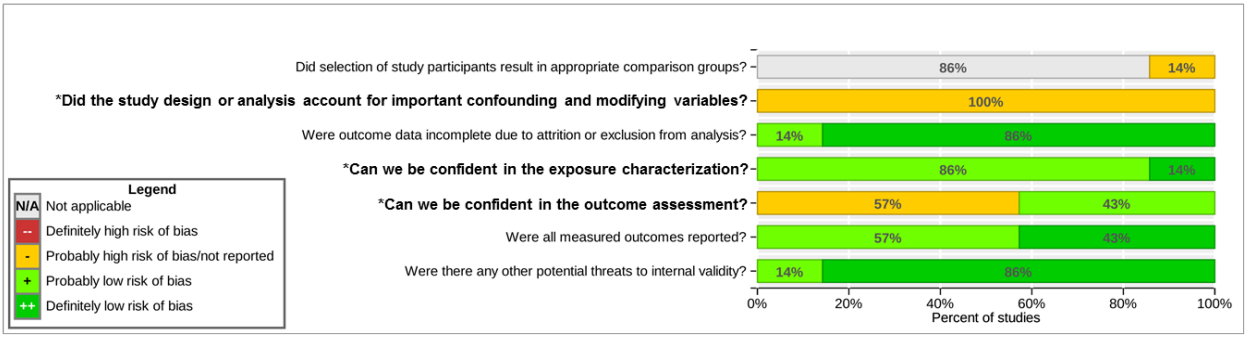

Figure A-9. Risk-of-bias Bar Chart for Case Reports/Series and Cross-Sectional Studies Assessing ChE Levels in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

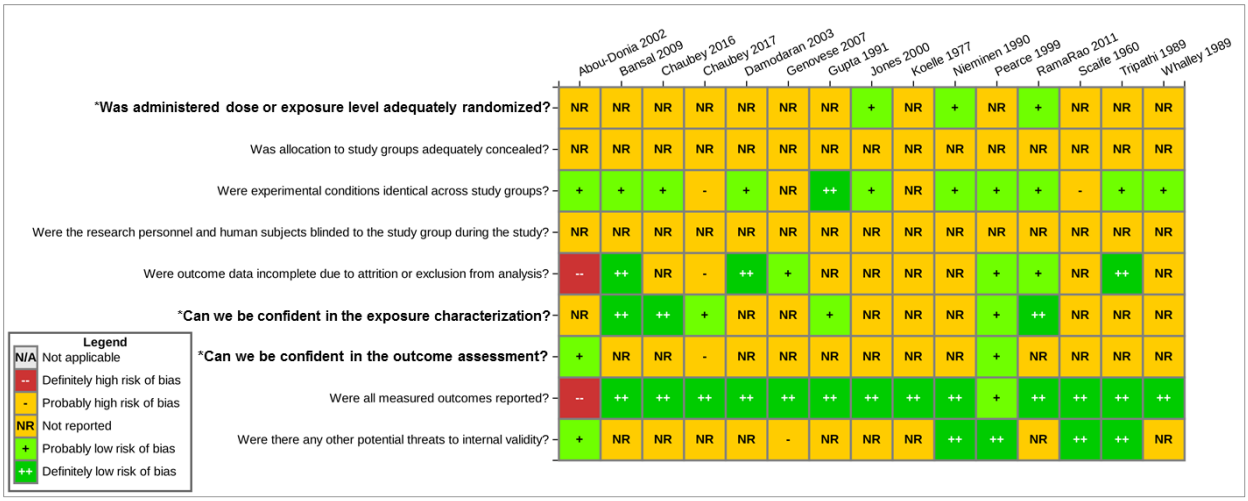

Figure A-10. Risk-of-bias Heat Map for Individual Studies Assessing AChE Levels in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

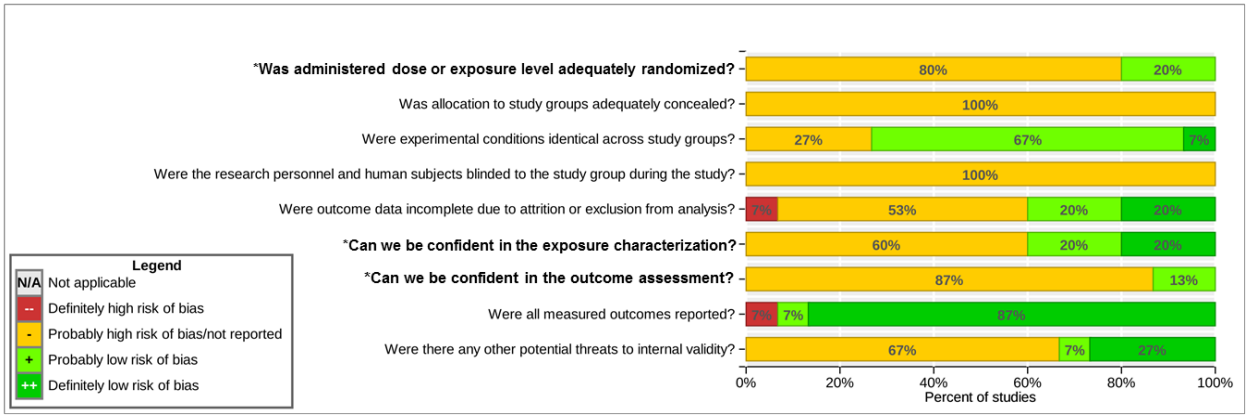

Figure A-11. Risk-of-bias Bar Chart for Individual Studies Assessing AChE Levels in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

A.2. Visual and Ocular Effects

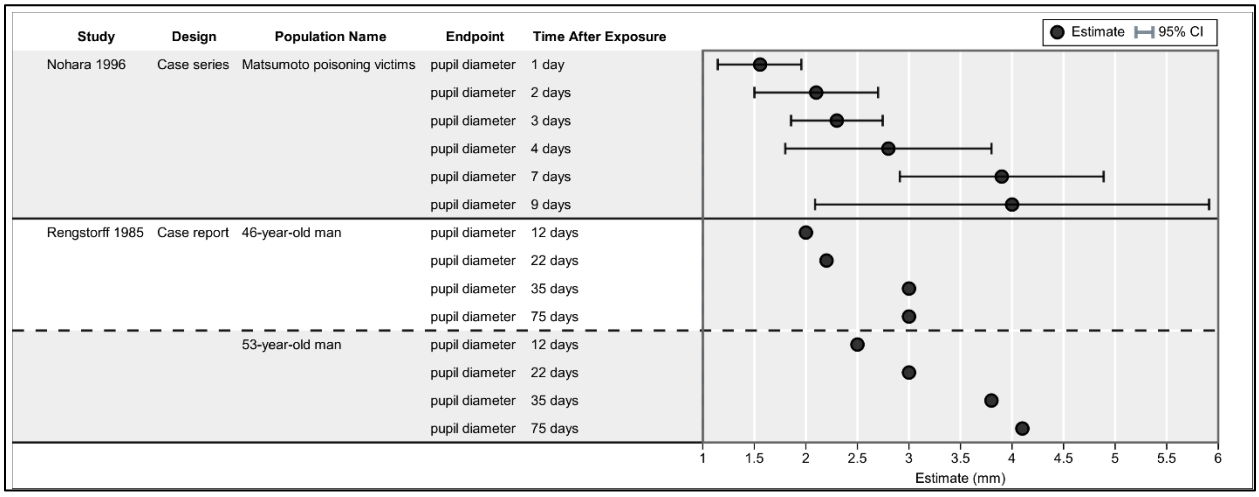

Figure A-12. Pupil Diameter in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
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.
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/).

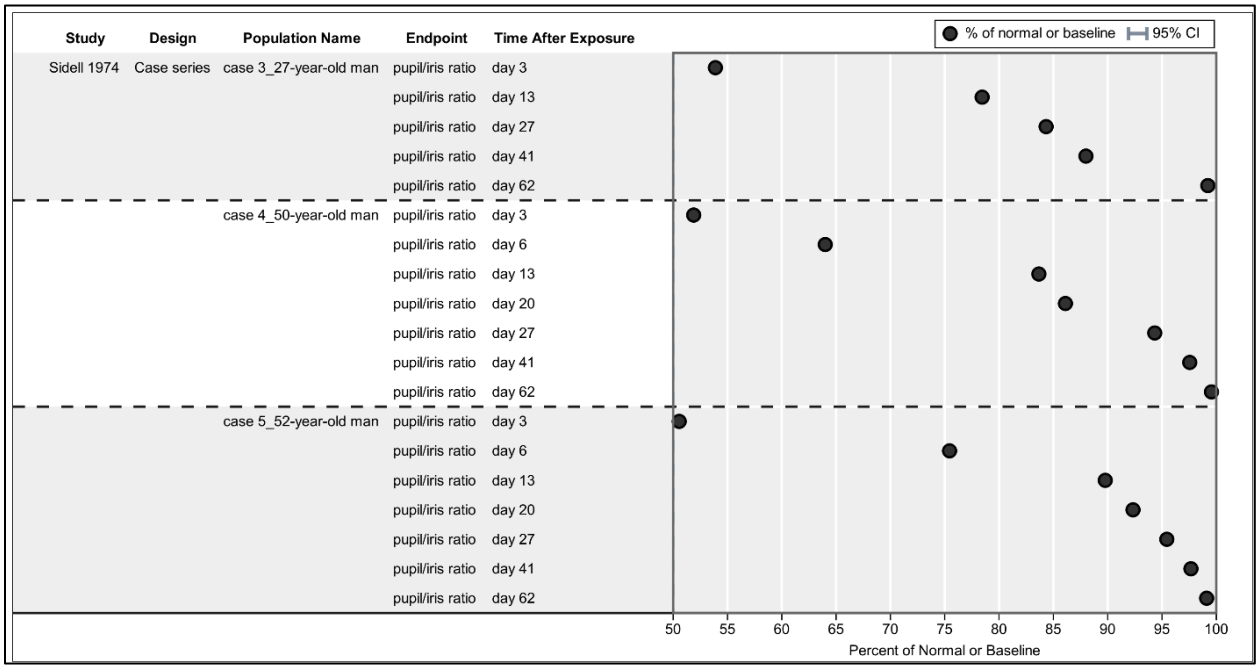

Figure A-13. Pupil/Iris Ratio in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b). 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.

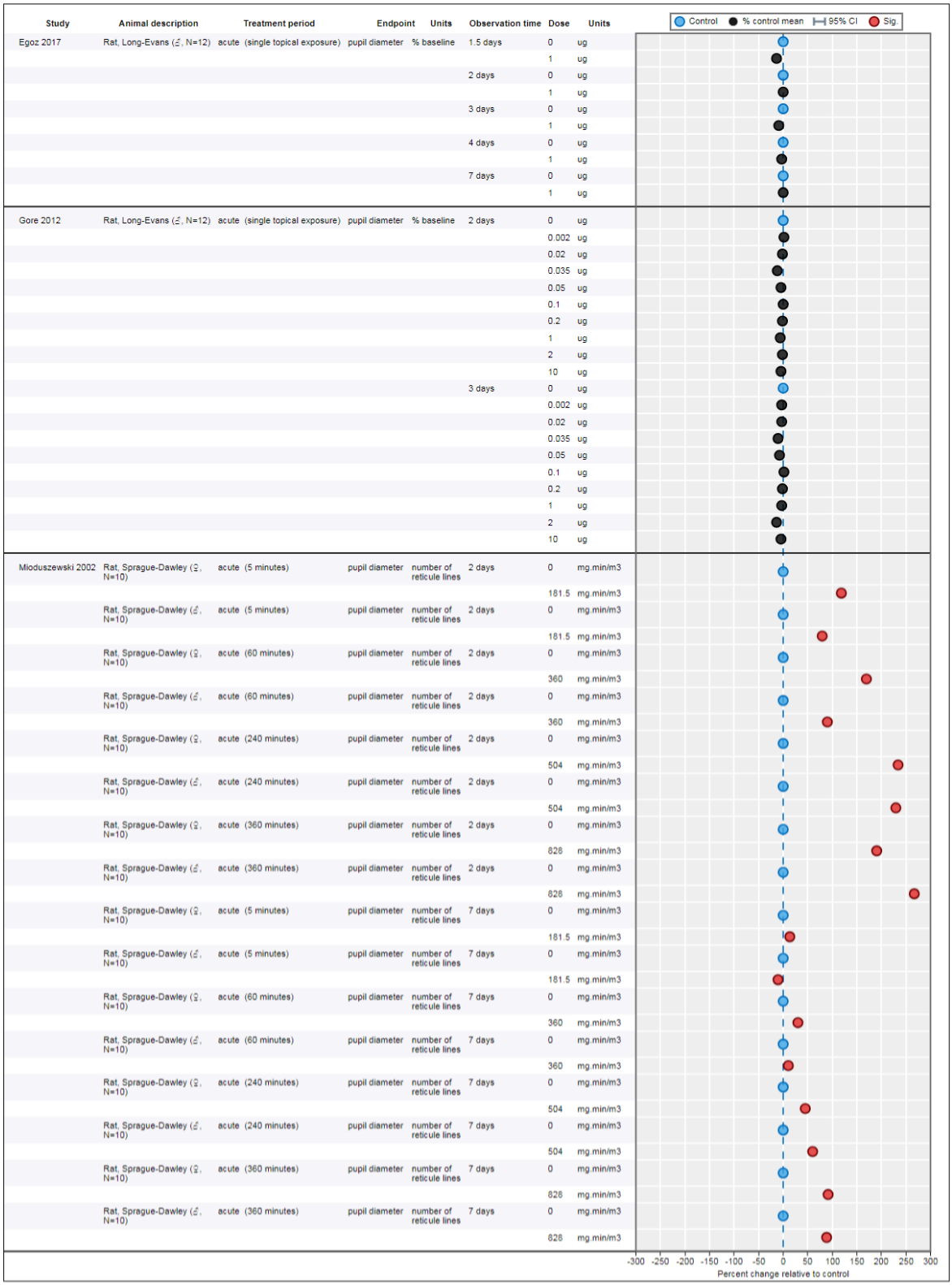

Figure A-14. Pupil Diameter in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).

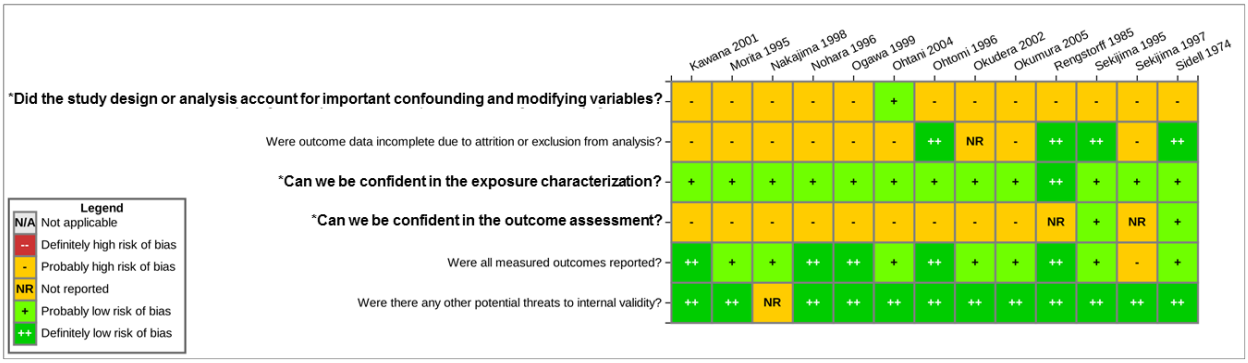

Figure A-15. Risk-of-bias Heat Map for Case Reports/Series Assessing Visual and Ocular Effects in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias that 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.

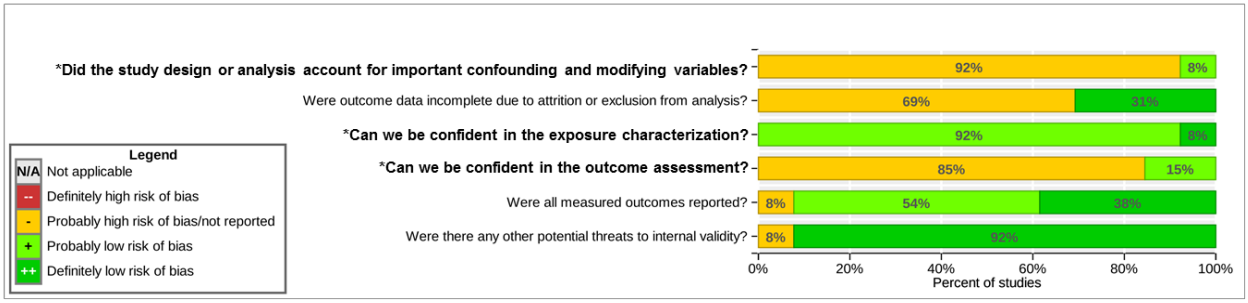

Figure A-16. Risk-of-bias Bar Chart for Case Reports/Series Assessing Visual and Ocular Effects in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias that 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.

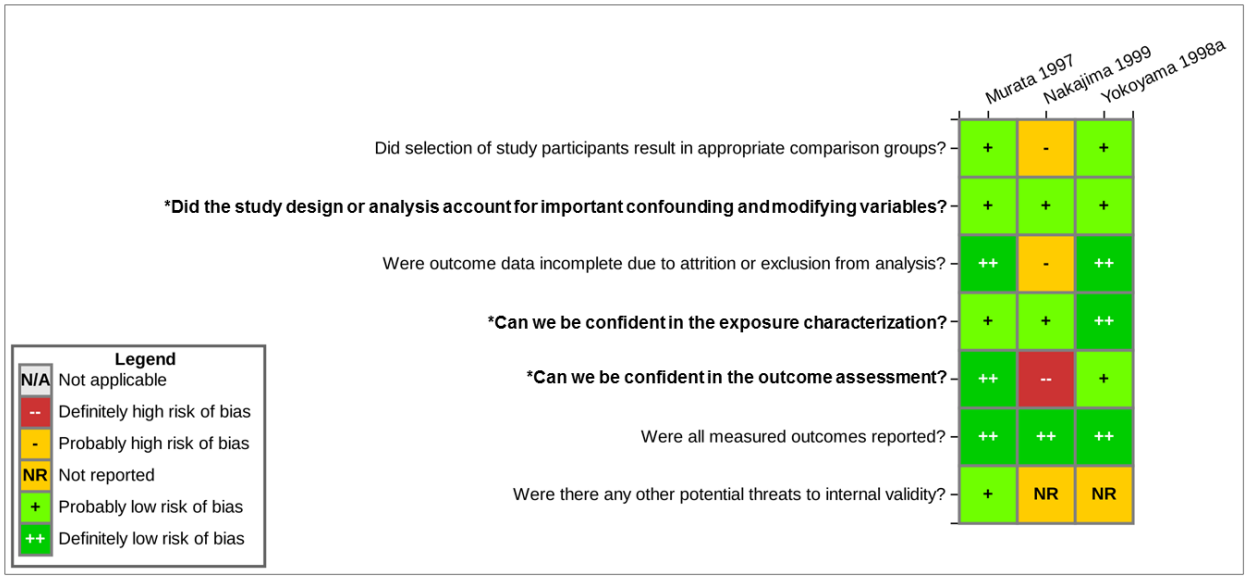

Figure A-17. Risk-of-bias Heat Map for Standard Observational Studies Assessing Visual and Ocular Effects in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for standard human observational studies. These key questions relate to areas of bias that 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.

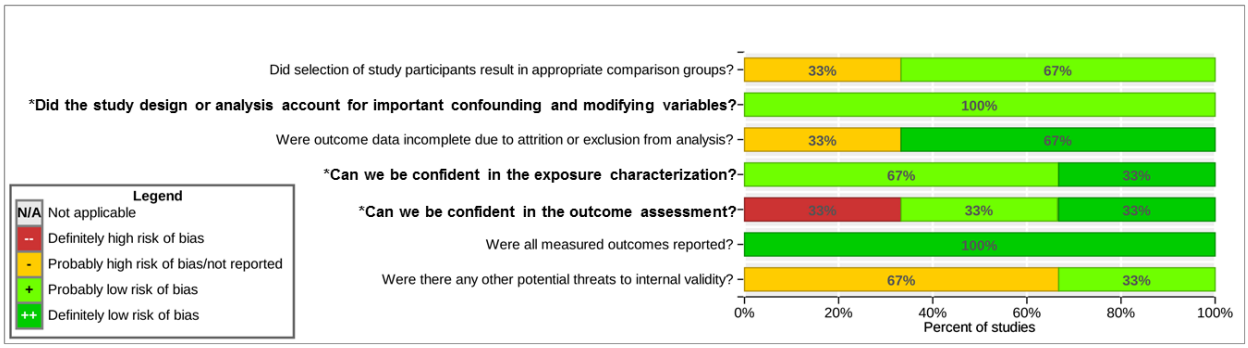

Figure A-18. Risk-of-bias Bar Chart for Standard Observational Studies Assessing Visual and Ocular Effects in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for standard human observational studies. These key questions relate to areas of bias that 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.

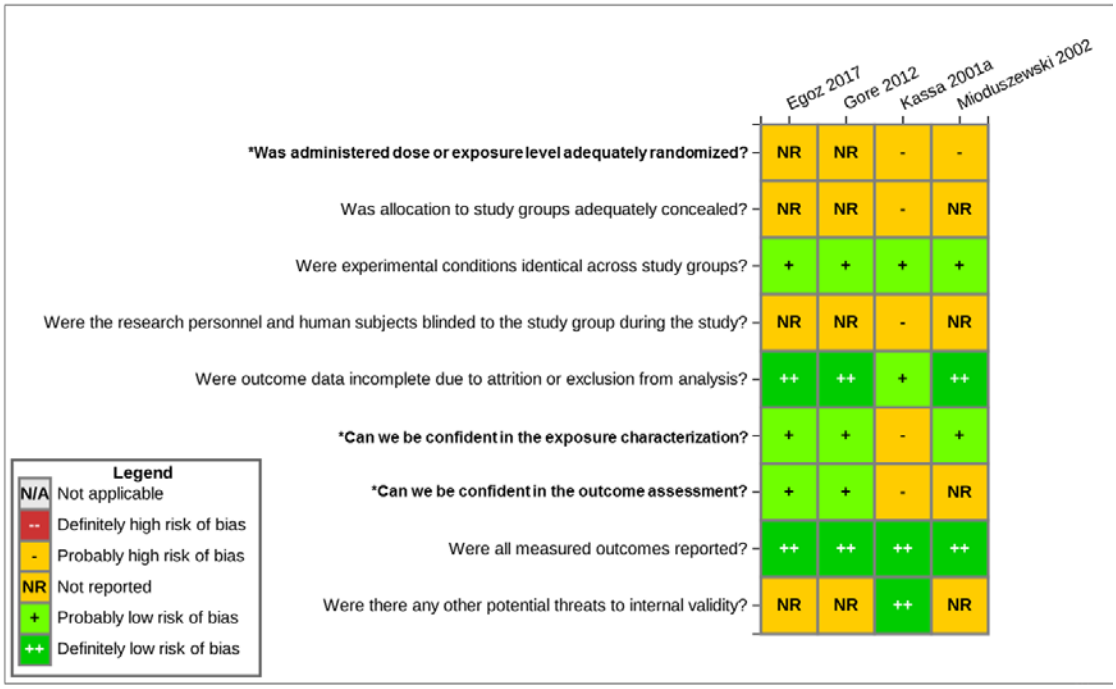

Figure A-19. Risk-of-bias Heat Map for Individual Studies Assessing Visual and Ocular Effects in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

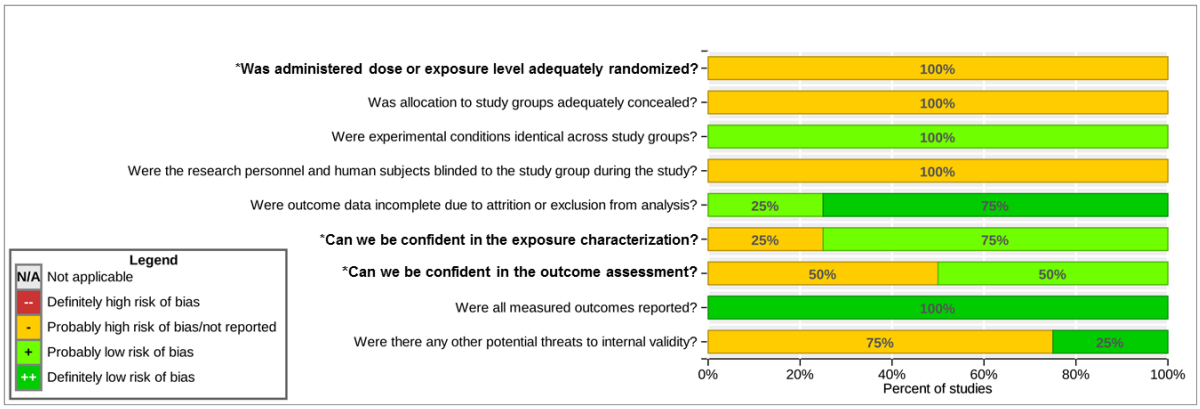

Figure A-20. Risk-of-bias Bar Chart for Individual Studies Assessing Visual and Ocular Effects in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

A.3. Effects on Learning, Memory, and Intelligence

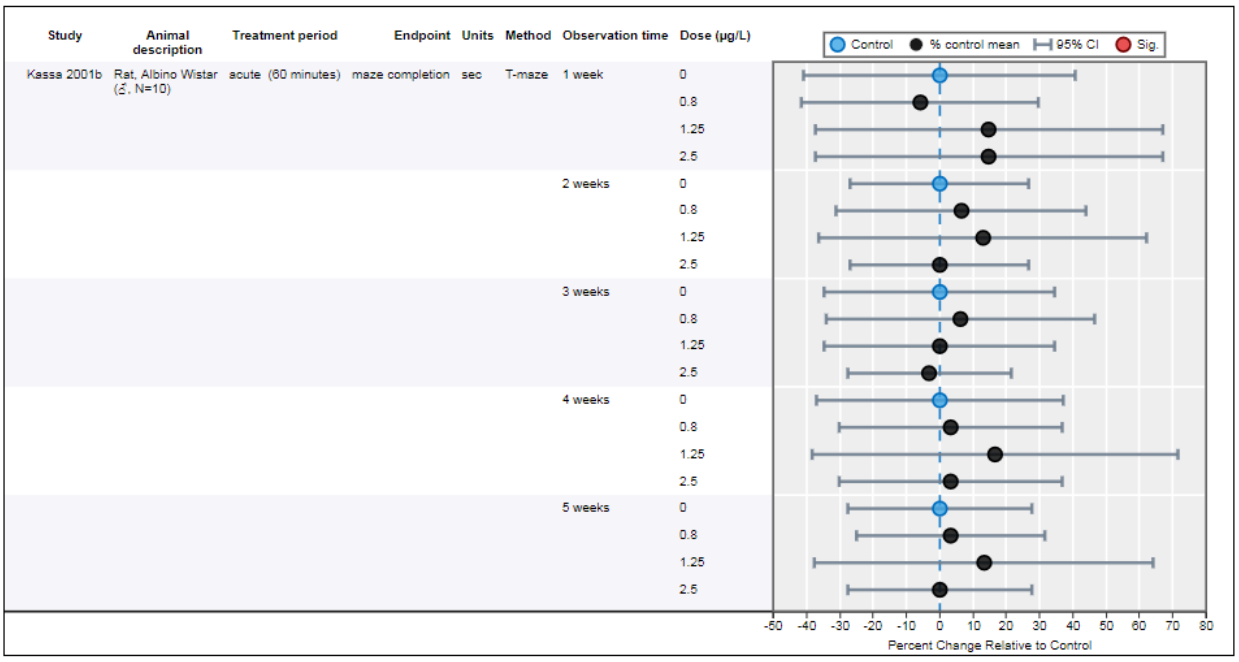

Figure A-21. T-Maze Results in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).

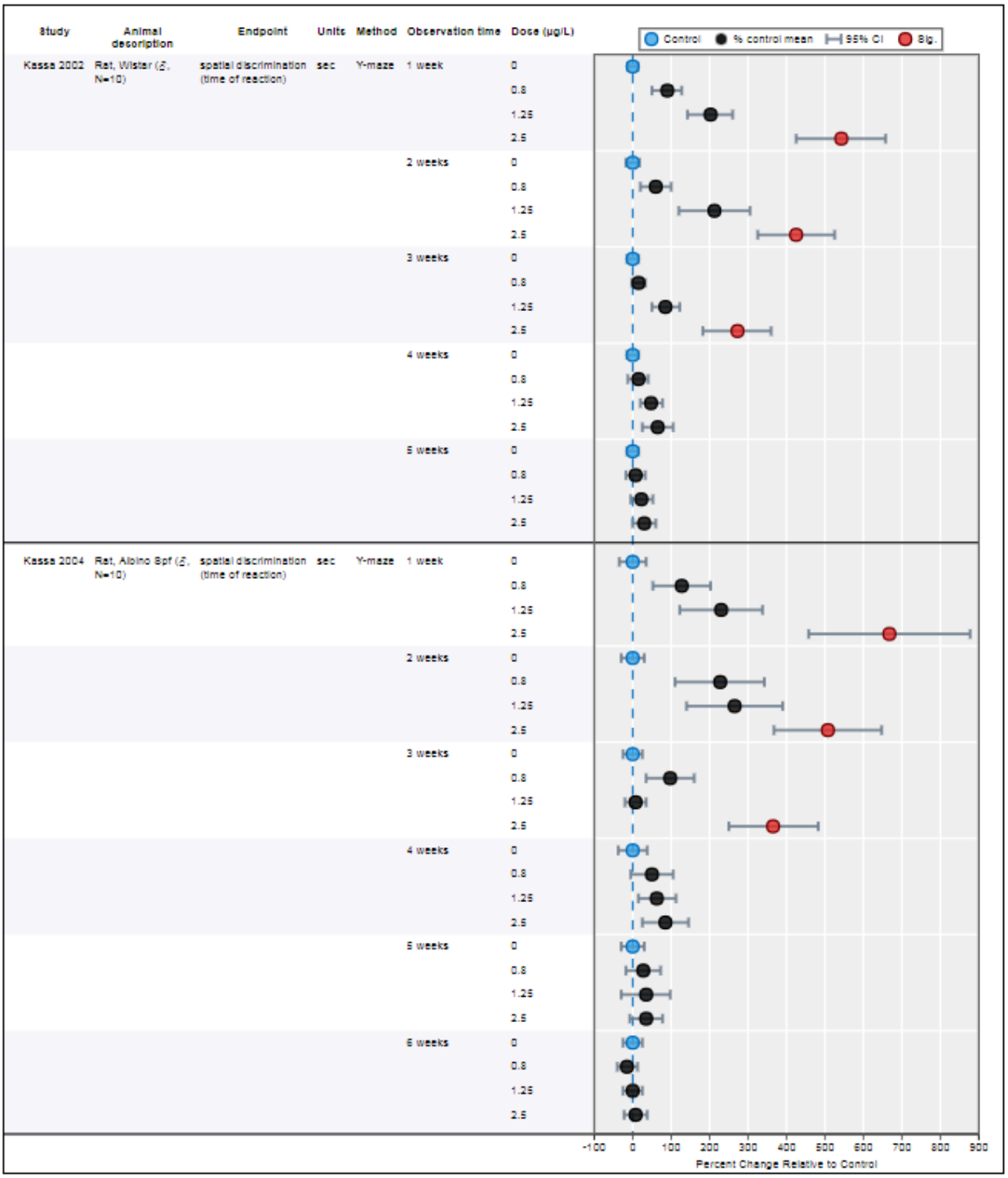

Figure A-22. Y-Maze Results in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).

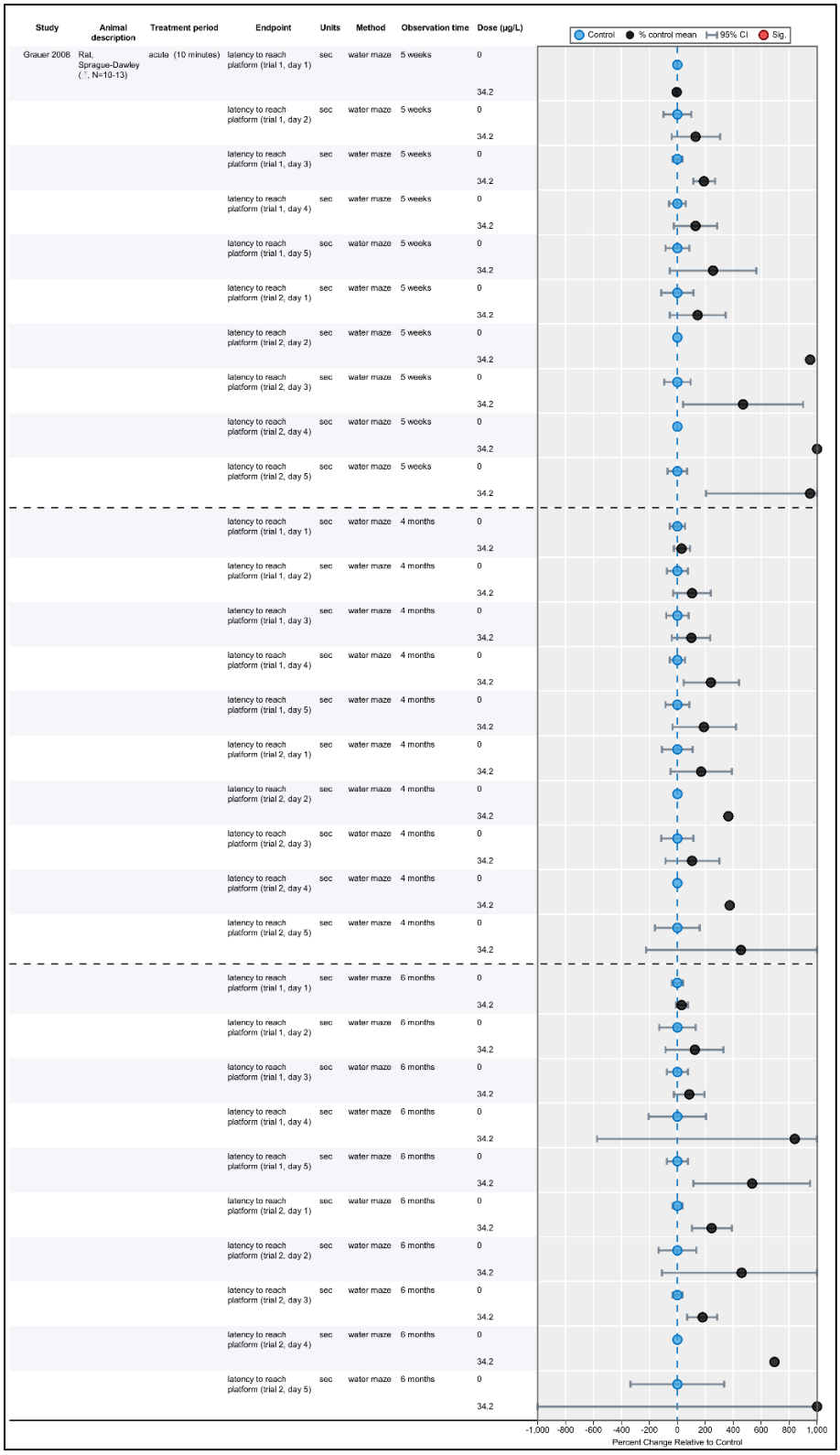

Figure A-23. Water Maze Latency to Reach Platform Results in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).

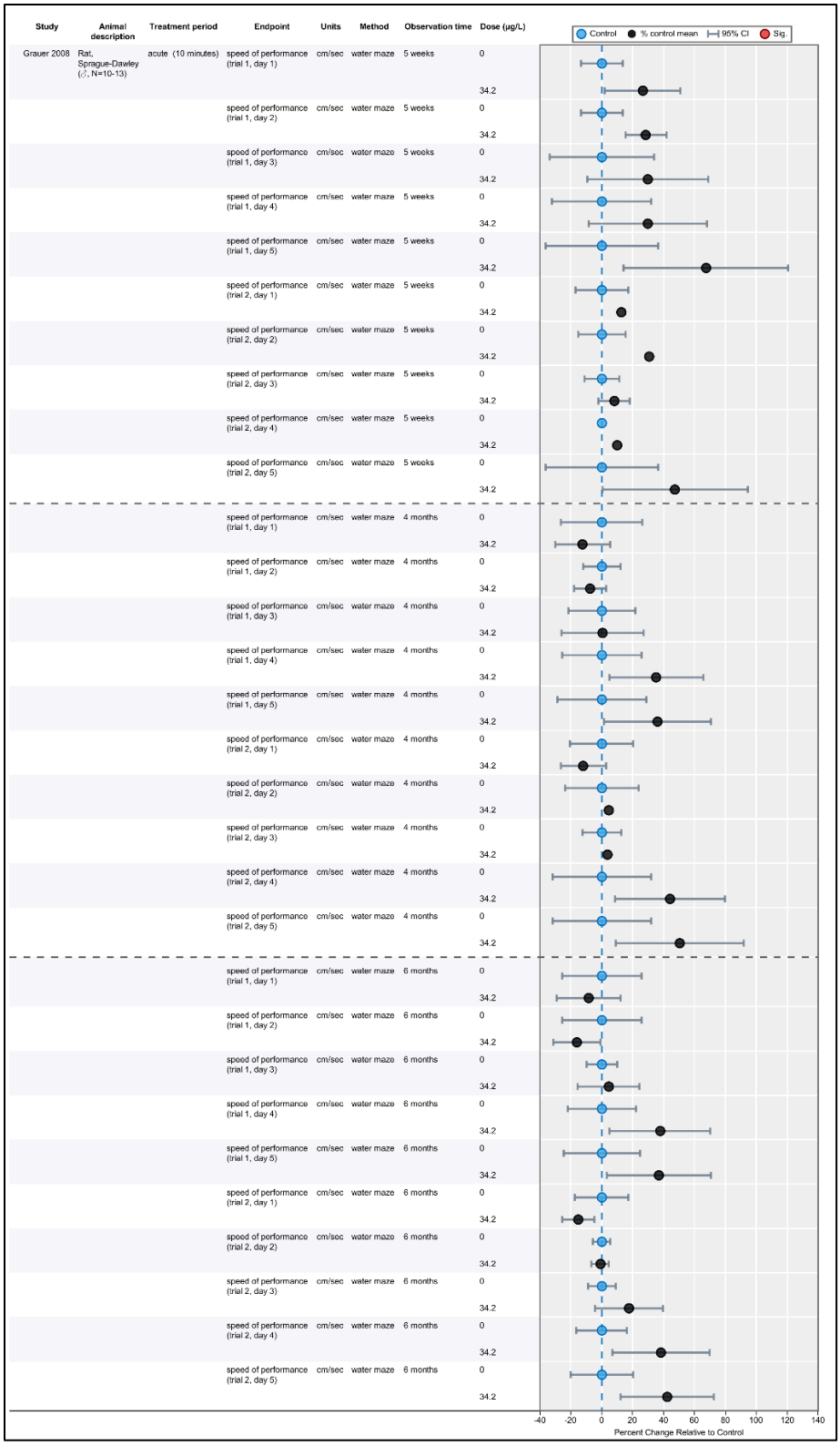

Figure A-24. Water Maze Speed of Performance Results in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).

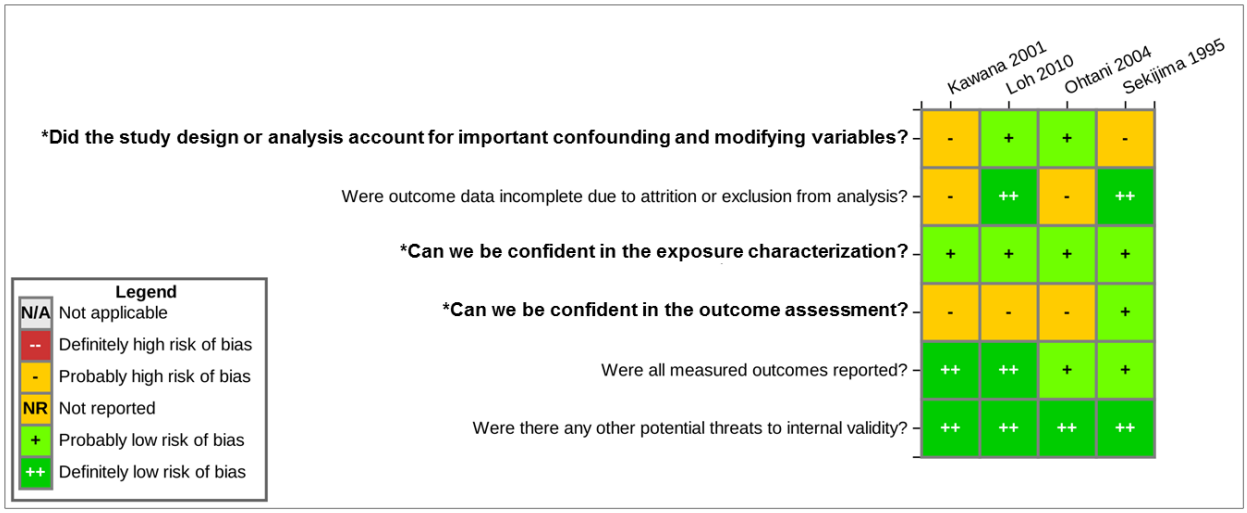

Figure A-25. Risk-of-bias Heat Map for Case Reports/Series Assessing Learning, Memory, and Intelligence in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias that 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.

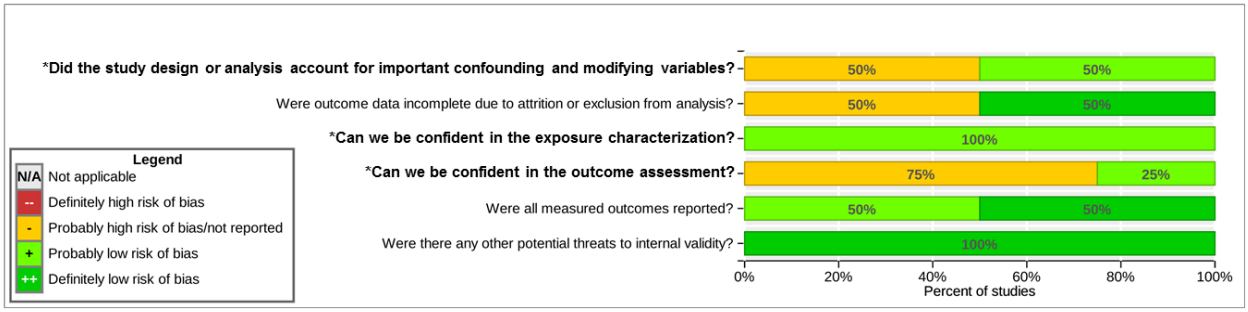

Figure A-26. Risk-of-bias Bar Chart for Case Reports/Series Assessing Learning, Memory, and Intelligence in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias that 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.

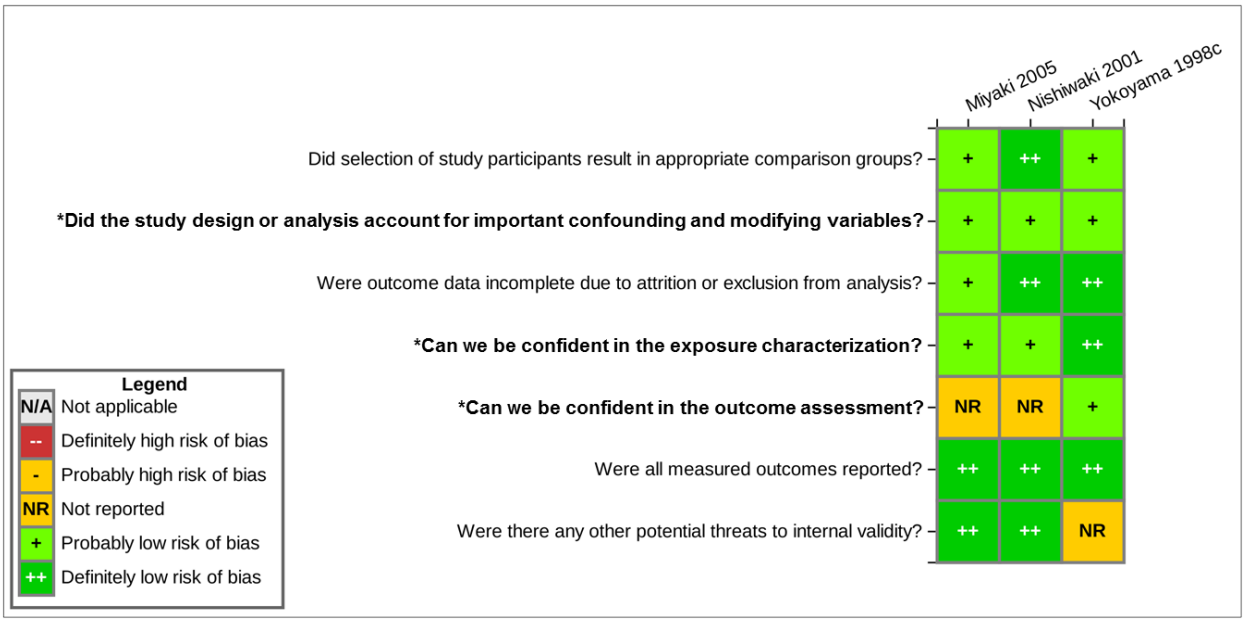

Figure A-27. Risk-of-bias Heat Map for Cross-Sectional Studies Assessing Learning, Memory, and Intelligence in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human cross-sectional studies. These key questions relate to areas of bias that 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.

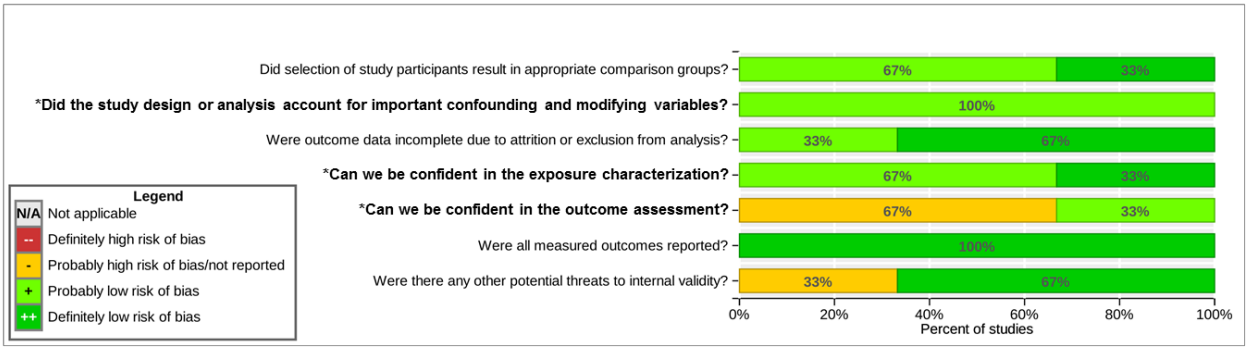

Figure A-28. Risk-of-bias Bar Chart for Cross-Sectional Studies Assessing Learning, Memory, and Intelligence in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human cross-sectional studies. These key questions relate to areas of bias that 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.

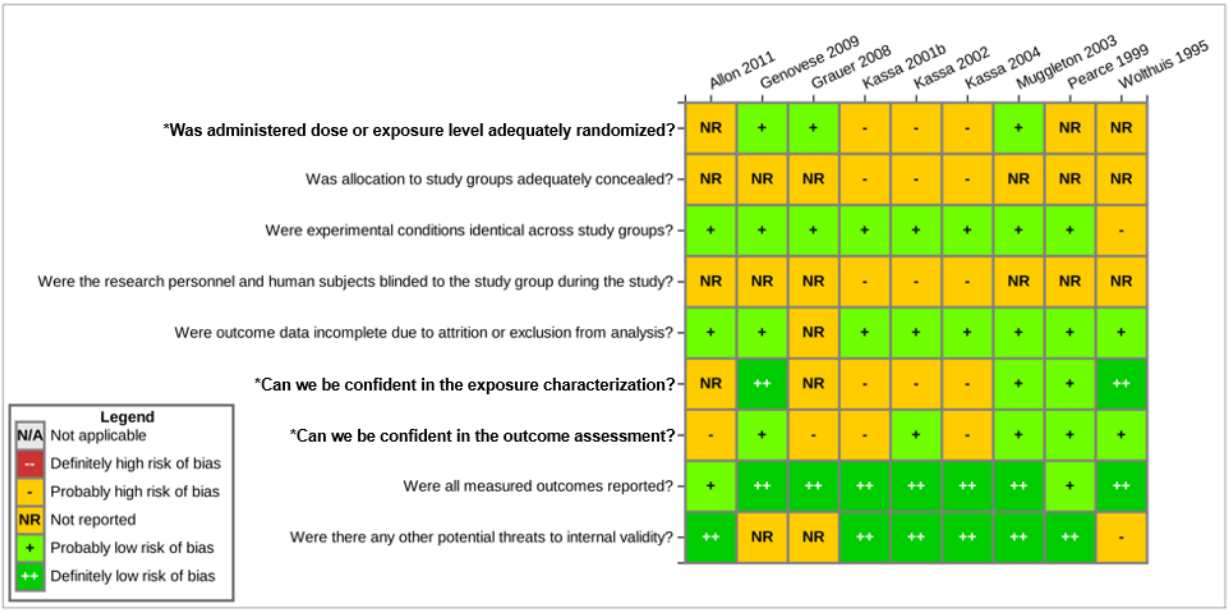

Figure A-29. Risk-of-bias Heat Map for Individual Studies Assessing Learning, Memory, and Intelligence in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

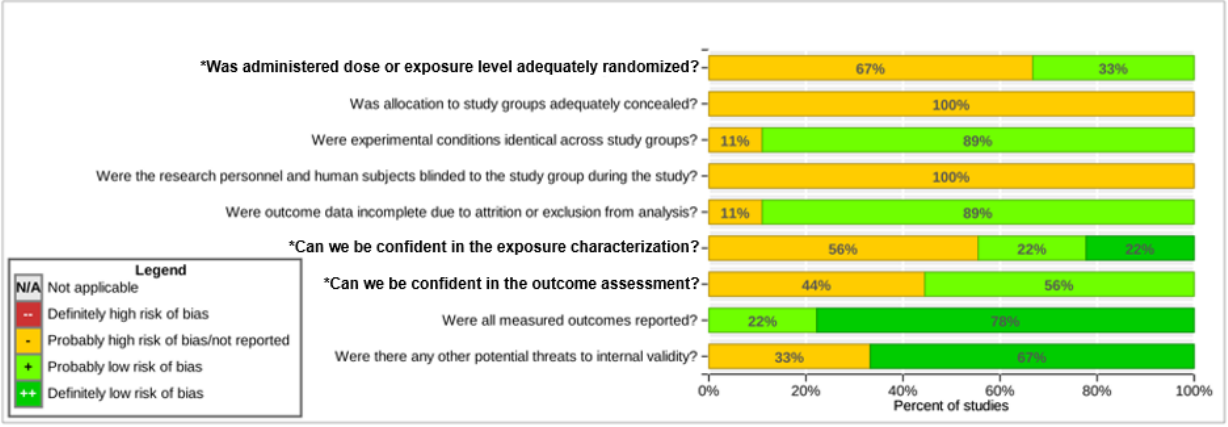

Figure A-30. Risk-of-bias Bar Chart for Individual Studies Assessing Learning, Memory, and Intelligence in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

A.4. Morphological and Histological Changes

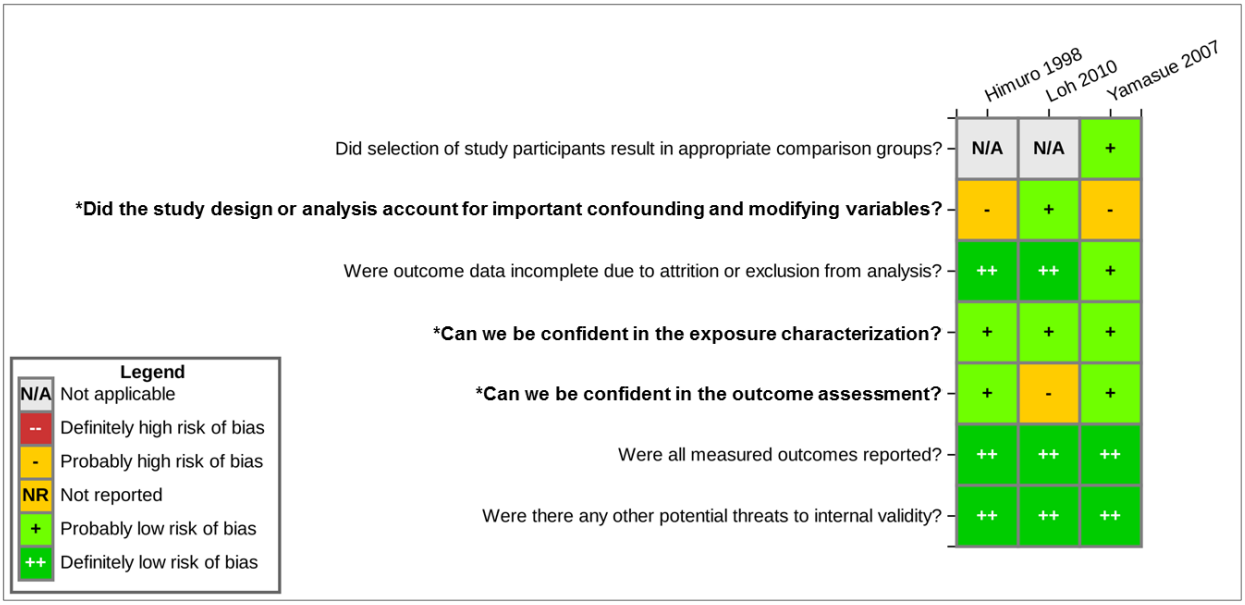

Figure A-31. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Nervous System Morphology in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

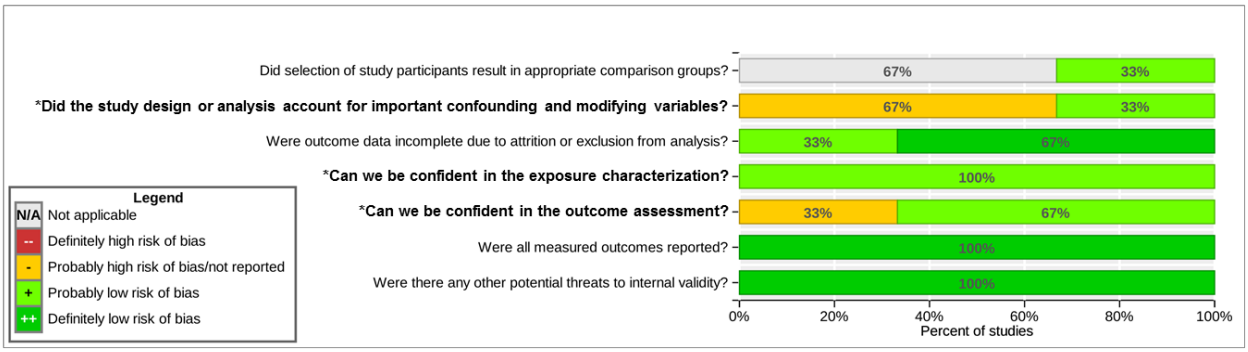

Figure A-32. Risk-of-bias Bar Chart for Case Reports/Series and Standard Observational Studies Assessing Nervous System Morphology in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

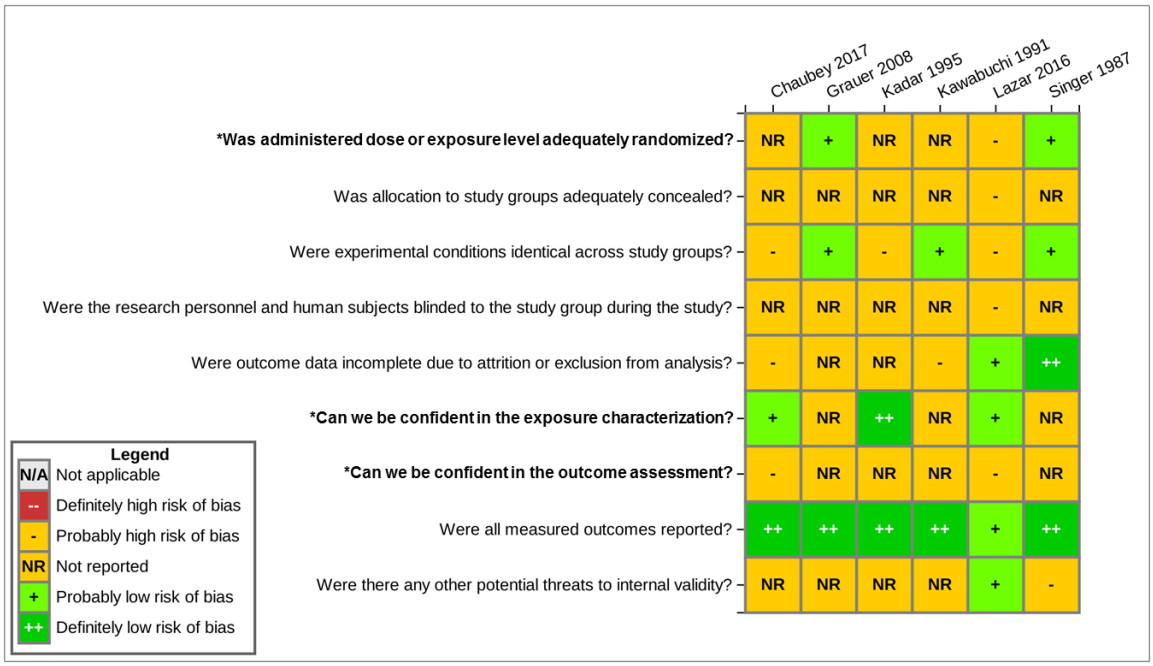

Figure A-33. Risk-of-bias Heat Map for Individual Studies Assessing Nervous System Histological Changes in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

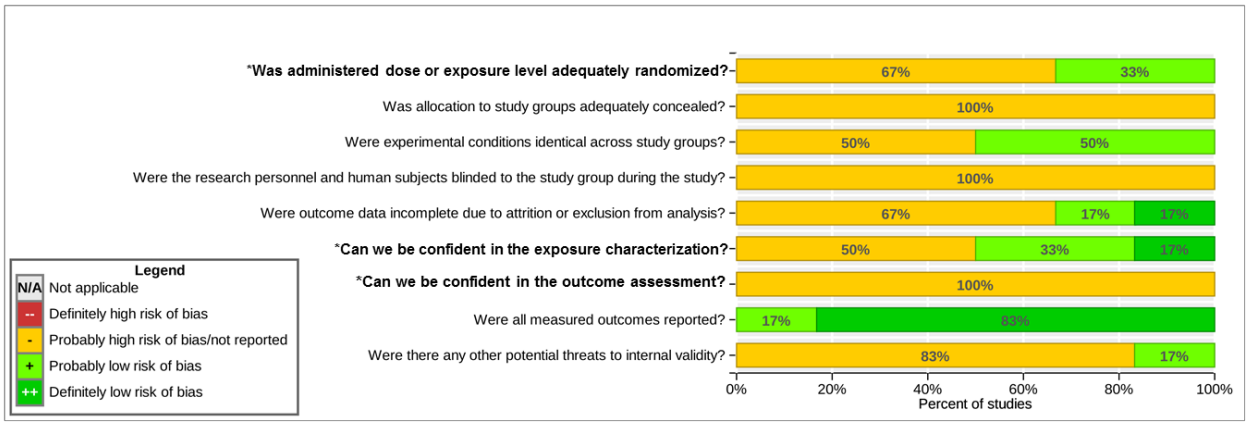

Figure A-34. Risk-of-bias Bar Chart for Individual Studies Assessing Nervous System Histological Changes in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

Appendix B. Literature Search Strategy

The search terms and databases searched are provided below.

Table B-1. Sarin Search Terms

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 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])

Database	Search Terms
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 C. List of Included Studies

C.1. Studies in Humans

Baker DJ, Sedgwick EM. 1996. Single fibre electromyographic changes in man after organophosphate exposure. Hum Exp Toxicol. 15(5):369-375. http://dx.doi.org/10.1177/096032719601500501

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. http://dx.doi.org/10.1001/archinte.1956.00250260095010

Grob D, Harvey JC. 1958. Effects in man of the anticholinesterase compound sarin (isopropyl methyl phosphonofluoridate). J Clin Invest. 37(3):350-368. http://dx.doi.org/10.1172/JCI103615

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. http://dx.doi.org/10.1212/WNL.51.4.1195

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. http://dx.doi.org/10.1093/milmed/166.suppl_2.23

Kawana N, Ishimatsu SI, Matsui Y, Tamaki S, Kanda K. 2005. Chronic posttraumatic stress symptoms in victims of Tokyo subway sarin gas attack. Traumatology. 11(2):87-102. http://dx.doi.org/10.1177/153476560501100204

Loh Y, Swanberg MM, Ingram MV, Newmark J. 2010. Case report: Long-term cognitive sequelae of sarin exposure. Neurotoxicology. 31(2):244-246. http://dx.doi.org/10.1016/j.neuro.2009.12.004

Miyaki K, Nishiwaki Y, Maekawa K, Ogawa Y, Asukai N, Yoshimura K, Etoh N, Matsumoto Y, Kikuchi Y, Kumagai N et al. 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. http://dx.doi.org/10.1539/joh.47.299

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. http://dx.doi.org/10.1016/S0140-6736(95)92170-2

Murata K, Araki S, Yokoyama K, Okumura T, Ishimatsu S, Takasu N, White RF. 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. http://dx.doi.org/10.1007/s004150050153

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. http://dx.doi.org/10.2188/jea.9.337

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. http://dx.doi.org/10.2188/jea.8.33

Nishiwaki Y, Maekawa K, Ogawa Y, Asukai N, Minami M, Omae K. 2001. Effects of sarin on the nervous system in rescue team staff members and police officers 3 years after the Tokyo subway sarin attack. Environ Health Perspect. 109(11):1169-1173. http://dx.doi.org/10.1289/ehp.011091169

Nohara M, Segawa K. 1996. Ocular symptoms due to organophosphorus gas (Sarin) poisoning in Matsumoto. Br J Ophthalmol. 80(11):1023. http://dx.doi.org/10.1136/bjo.80.11.1023

Ogawa Y, Yamamura Y, Ando H, Kadokura M, Agata T, Fukumoto M, Satake T, Machida K, Sakai O, Miyata Y et al. 1999. An attack with sarin nerve gas on the Tokyo subway system and its effects on victims. ACS Symp Ser. 745:333-355. http://dx.doi.org/10.1021/bk-2000-0745.ch022

Ohbu S, Yamashina A, Takasu N, Yamaguchi T, Murai T, Nakano K, Matsui Y, Mikami R, Sakurai K, Hinohara S. 1997. Sarin poisoning on Tokyo subway. South Med J. 90(6):587-593. http://dx.doi.org/10.1097/00007611-199706000-00002

Ohtani T, Iwanami A, Kasai K, Yamasue H, Kato T, Sasaki T, Kato N. 2004. Post-traumatic stress disorder symptoms in victims of Tokyo subway attack: A 5-year follow-up study. Psychiatry Clin Neurosci. 58(6):624-629. http://dx.doi.org/10.1111/j.1440-1819.2004.01313.x

Ohtomi S, Takase M, Kumagai F. 1996. Sarin poisoning in Japan. A clinical experience in Japan Self Defense Force (JSDF) Central Hospital. Rev Int Serv Sante Forces Armees. 69(4-6):97-102.

Okudera H. 2002. Clinical features on nerve gas terrorism in Matsumoto. J Clin Neurosci. 9(1):17-21. http://dx.doi.org/10.1054/jocn.2001.1020

Okumura T, Hisaoka T, Naito T, Isonuma H, Okumura S, Miura K, Maekawa H, Ishimatsu S, Takasu N, Suzuki K. 2005. Acute and chronic effects of sarin exposure from the Tokyo subway incident. Environ Toxicol Pharmacol. 19(3):447-450. http://dx.doi.org/10.1016/j.etap.2004.12.005

Okumura T, Takasu N, Ishimatsu S, Miyanoki S, Mitsuhashi A, Kumada K, Tanaka K, Hinohara S. 1996. Report on 640 victims of the Tokyo subway sarin attack. Ann Emerg Med. 28(2):129-135. http://dx.doi.org/10.1016/S0196-0644(96)70052-5

Rengstorff RH. 1985. Accidental exposure to sarin: Vision effects. Arch Toxicol. 56(3):201-203. http://dx.doi.org/10.1007/BF00333427

Rengstorff RH. 1994. Vision and ocular changes following accidental exposure to organophosphates. J Appl Toxicol. 14(2):115-118. http://dx.doi.org/10.1002/jat.2550140213

Sekijima Y, Morita H, Shindo M, Okudera H, Shibata T. 1995. [A case of severe sarin poisoning in the sarin attack in Matsumoto--one-year follow-up of clinical findings, and laboratory data]. Rinsho Shinkeigaku. 35(11):1241-1245.

Sekijima Y, Morita H, Yanagisawa N. 1997. Follow-up of sarin poisoning in Matsumoto. Ann Intern Med. 127(11):1042. http://dx.doi.org/10.7326/0003-4819-127-11-199712010-00028

Sidell FR. 1974. Soman and sarin: Clinical manifestations and treatment of accidental poisoning by organophosphates. Clin Toxicol. 7(1):1-17. http://dx.doi.org/10.3109/15563657408987971

Suzuki J, Kohno T, Tsukagosi M, Furuhata T, Yamazaki K. 1997. Eighteen cases exposed to sarin in Matsumoto, Japan. Intern Med. 36(7):466-470. http://dx.doi.org/10.2169/internalmedicine.36.466

Tochigi M, Otani T, Yamasue H, Kasai K, Kato T, Iwanami A, Kato N, Sasaki T. 2005. Support for relationship between serum cholinesterase and post-traumatic stress disorder; 5-year follow-ups of victims of the Tokyo subway sarin poisoning. Neurosci Res. 52(2):129-131. http://dx.doi.org/10.1016/j.neures.2005.03.012

Tochigi M, Umekage T, Otani T, Kato T, Iwanami A, Asukai N, Sasaki T, Kato N. 2002. Serum cholesterol, uric acid and cholinesterase in victims of the Tokyo subway sarin poisoning: A relation with post-traumatic stress disorder. Neurosci Res. 44(3):267-272. http://dx.doi.org/10.1016/S0168-0102(02)00146-3

Yamasue H, Abe O, Kasai K, Suga M, Iwanami A, Yamada H, Tochigi M, Ohtani T, Rogers MA, Sasaki T et al. 2007. Human brain structural change related to acute single exposure to sarin. Ann Neurol. 61(1):37-46. http://dx.doi.org/10.1002/ana.21024

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N. 1998. Chronic neurobehavioral and central and autonomic nervous system effects of Tokyo subway sarin poisoning. J Physiol Paris. 92(3-4):317-323. http://dx.doi.org/10.1016/S0928-4257(98)80040-5

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N. 1998. A preliminary study on delayed vestibulo-cerebellar effects of Tokyo Subway Sarin Poisoning in relation to gender difference: Frequency analysis of postural sway. J Occup Environ Med. 40(1):17-21. http://dx.doi.org/10.1097/00043764-199801000-00006

Yokoyama K, Araki S, Murata K, Nishikitani M, Okumura T, Ishimatsu S, Takasu N, White RF. 1998. Chronic neurobehavioral effects of Tokyo subway sarin poisoning in relation to posttraumatic stress disorder. Arch Environ Health. 53(4):249-256. http://dx.doi.org/10.1080/00039899809605705

Yokoyama K, Araki S, Nishikitani M, Sato H. 2002. Computerized posturography with sway frequency analysis: Application in occupational and environmental health. Ind Health. 40(1):14-22. http://dx.doi.org/10.2486/indhealth.40.14

C.2. Studies in Nonhuman Animals

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Bansal I, Waghmare CK, Anand T, Gupta AK, Bhattacharya BK. 2009. Differential mRNA expression of acetylcholinesterase in the central nervous system of rats with acute and chronic exposure of sarin & physostigmine. J Appl Toxicol. 29(5):386-394. http://dx.doi.org/10.1002/jat.1424

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

Figures

D.1. Studies in Humans

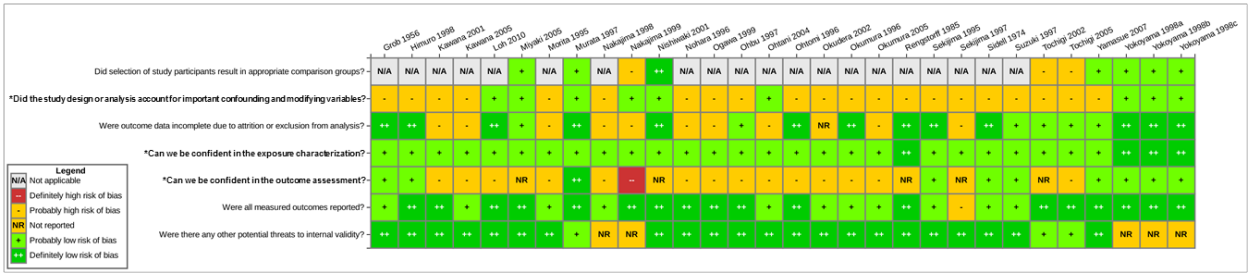

Figure D-1. Risk-of-bias Heatmap for All Included Case Reports/Series and Standard Observational Studies in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in Health Assessment Workspace Collaborative (HAWC) (NTP, 2019b).
*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 that 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.

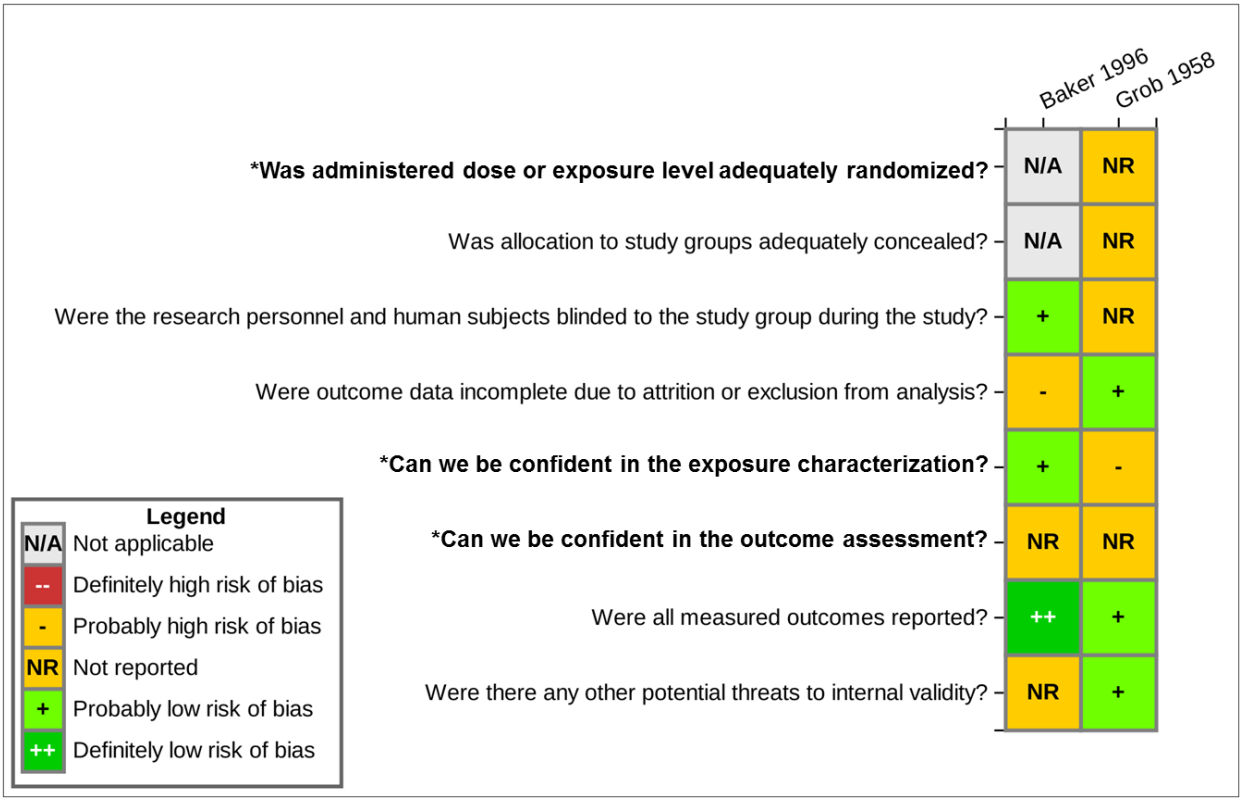

Figure D-2. Risk-of-bias Heatmap for All Included Controlled Trials in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human controlled exposure studies. These key questions relate to areas of bias that 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.

D.2. Studies in Nonhuman Animals

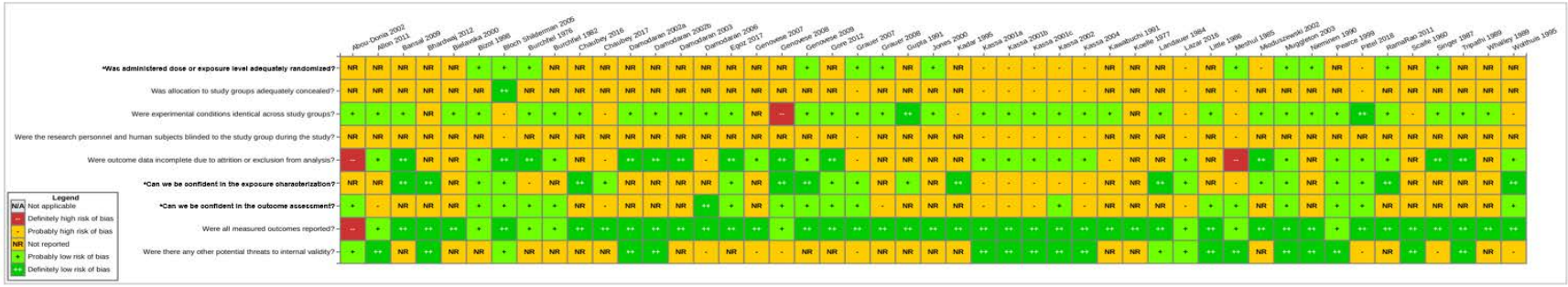

Figure D-3. Risk-of-bias Heatmap for All Included Studies in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

Appendix E. Inadequate Evidence: Evidence Synthesis and Risk-of-bias Assessment

Table of Contents

Figures

E.1. 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.

E.1.1. Human Sleep Disruption Data

The available studies support a rating of *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 (Kawana et al. 2001; Nakajima et al. 1999; Nakajima et al. 1998; Ogawa et al. 1999; Ohbu et al. 1997; 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 (Nakajima et al. 1998; Ogawa et al. 1999; Ohbu et al. 1997). Subjects from three case series studies and one prospective cohort study report symptoms related to sleep disruption 1–5 years following acute sarin exposure (Kawana et al. 2001; Nakajima et al. 1999; Nakajima et al. 1998; 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 PTSD as a confounder, 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 <1 year after sarin exposure (Nakajima et al. 1998; Ogawa et al. 1999; Ohbu et al. 1997). 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 post-traumatic 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 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 (Kawana et al. 2001; Nakajima et al. 1999; Nakajima et al. 1998; 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 two of 45 subjects were experiencing bad dreams. Although bad dreams were included in the questionnaire at 1 year, they were 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 nonpatients 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 nonvictims and victims. Kawana et al. (2001) followed victims of the Tokyo sarin attack and reported on psychological effects, 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 nine of 34 victims surveyed reported nightmares (eight mild and one severe) and 10 of 34 victims reported insomnia (seven mild and three 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 E-1 and Figure E-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). Two 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. 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.

E.1.2. Animal Sleep Disruption Data

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

E.1.3. 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 post-traumatic 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. An evidence profile or detailed discussions of the evidence synthesis were not developed because of the limitations of the bodies of evidence for acute sarin and sleep disruption-related outcomes, and this health effect was not considered for hazard identification conclusions.

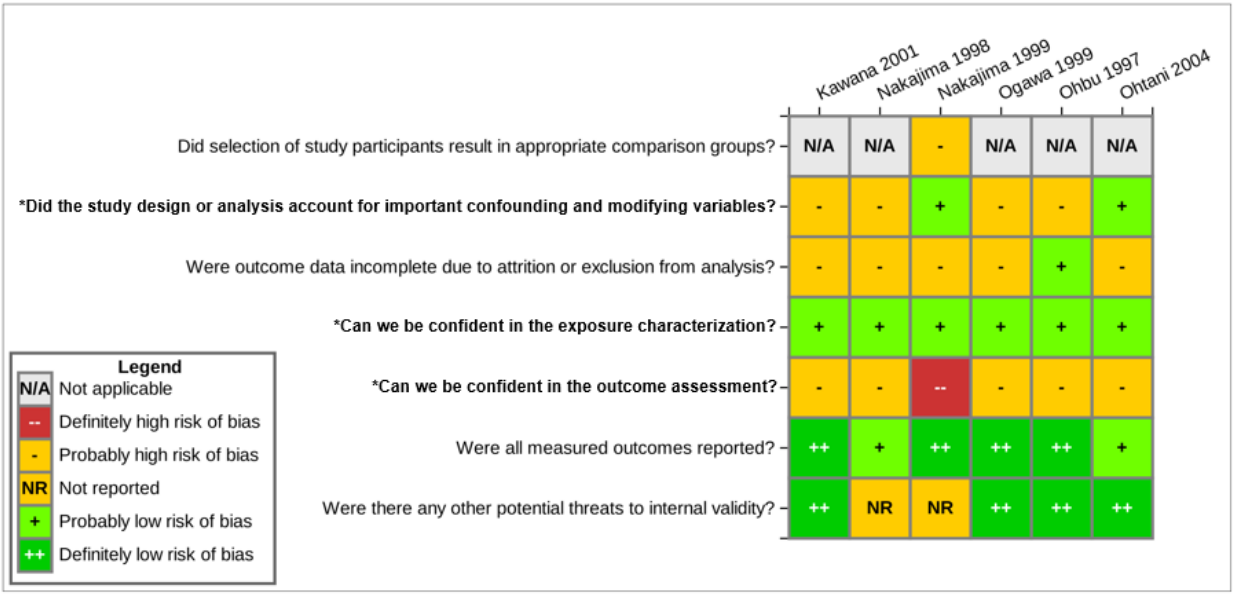

Figure E-1. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Sleep Disruption in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in Health Assessment Workspace Collaborative (HAWC) (NTP, 2019b).
*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 that 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.

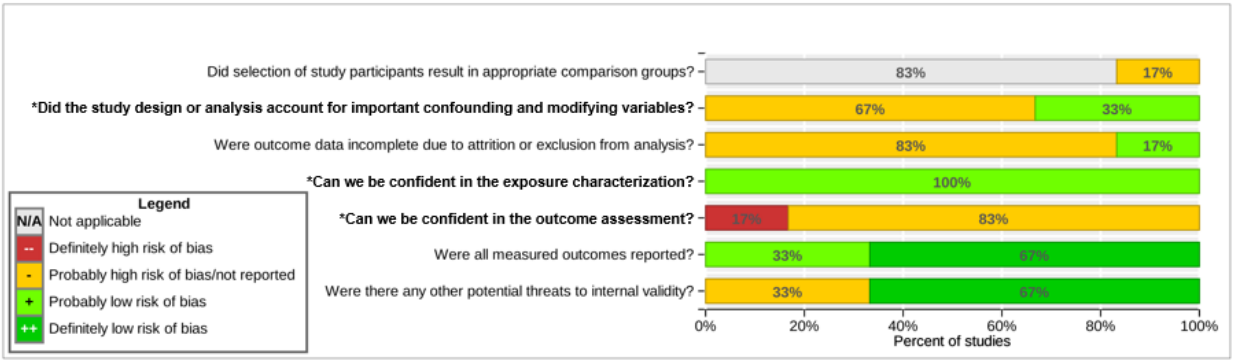

Figure E-2. Risk-of-bias Bar Graph for Case Reports/Series and Standard Observational Studies Assessing Sleep Disruption in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

E.2. 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. Many studies specifically evaluated PTSD. This document does not specifically evaluate PTSD, even in terms of anxiety and fear, because of the inability to separate the effects of the traumatic event (i.e., terrorist attack) from the effects related to exposure to sarin during the attack.

E.2.1. Human Anxiety and Fear Data

The available studies support a rating of *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 (Kawana et al. 2001; Ohbu et al. 1997; 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). Three studies evaluated anxiety (Tochigi et al. 2005; Tochigi et al. 2002; Yokoyama et al. 1998c). 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. The data available support a rating of 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. No studies were 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 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 E-3 through Figure E-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. No information was provided on the subjects who participated compared with 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 toward 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.

E.2.2. 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. No studies were 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 three key questions (randomization, exposure characterization, and outcome assessment) (see Figure E-7 and Figure E-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.

E.2.3. 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.

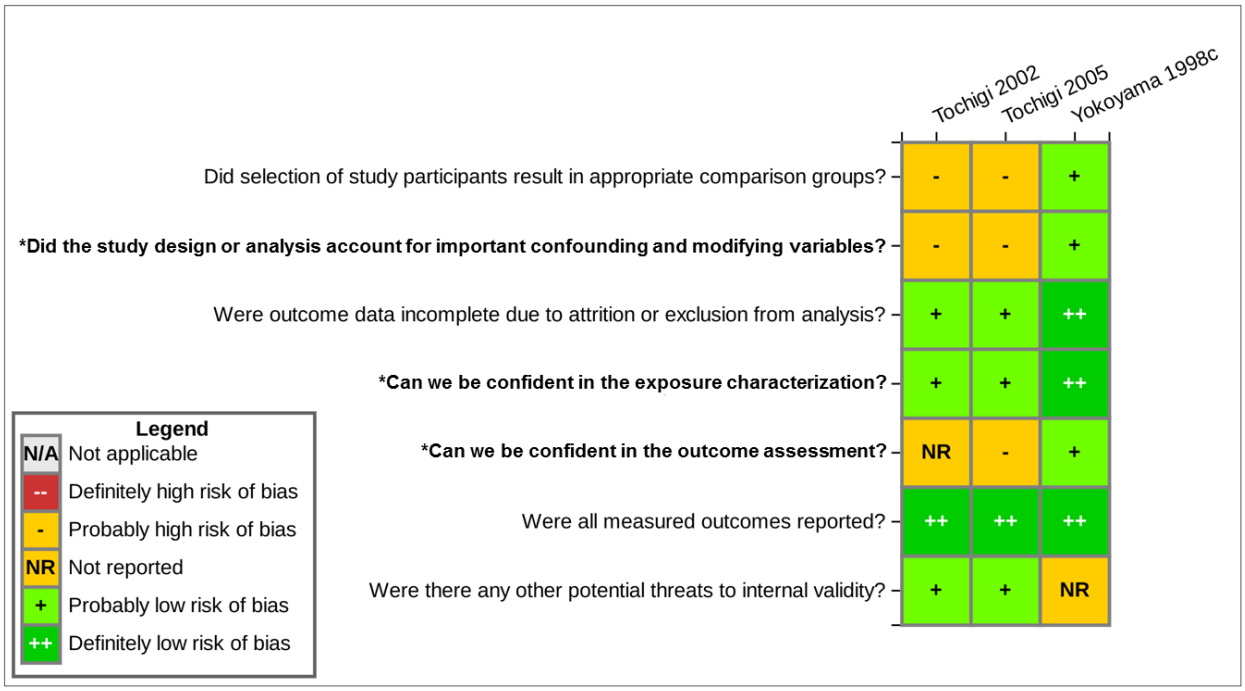

Figure E-3. Risk-of-bias Heat Map for Standard Observational Studies Assessing Anxiety in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for standard human observational studies. These key questions relate to areas of bias that 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.

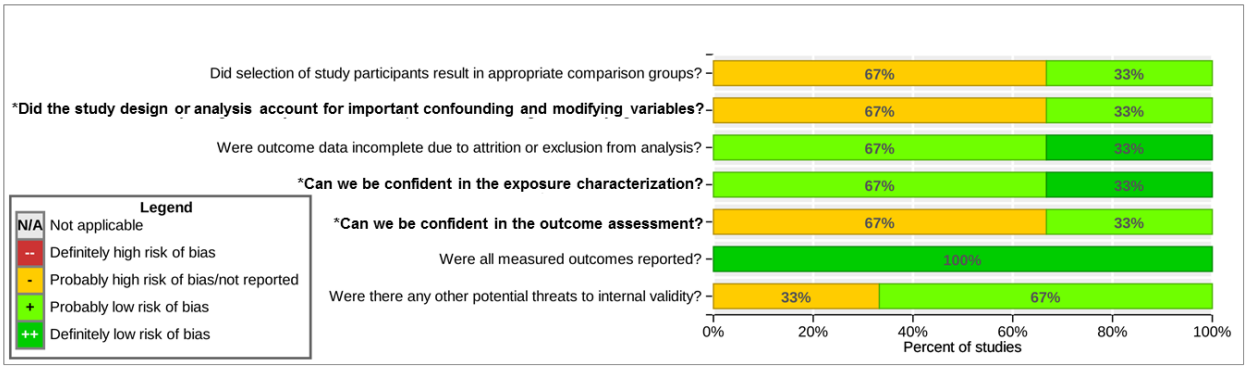

Figure E-4. Risk-of-bias Bar Graph for Standard Observational Studies Assessing Anxiety in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for standard human observational studies. These key questions relate to areas of bias that 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.

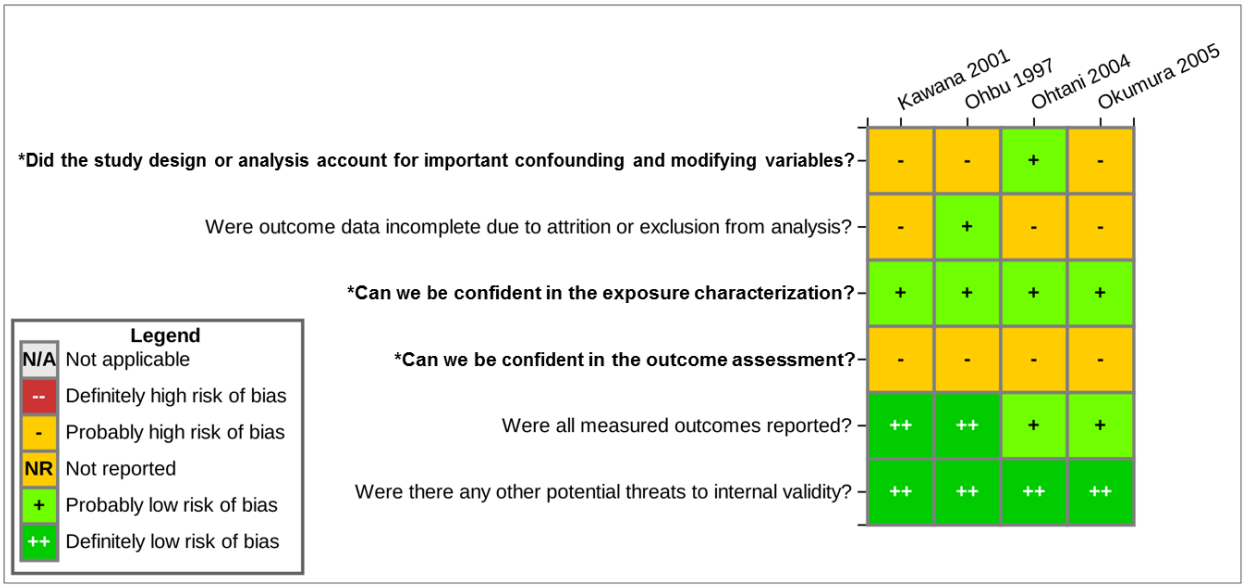

Figure E-5. Risk-of-bias Heat Map for Case Series Assessing Fear in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human case series. These key questions relate to areas of bias that 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.

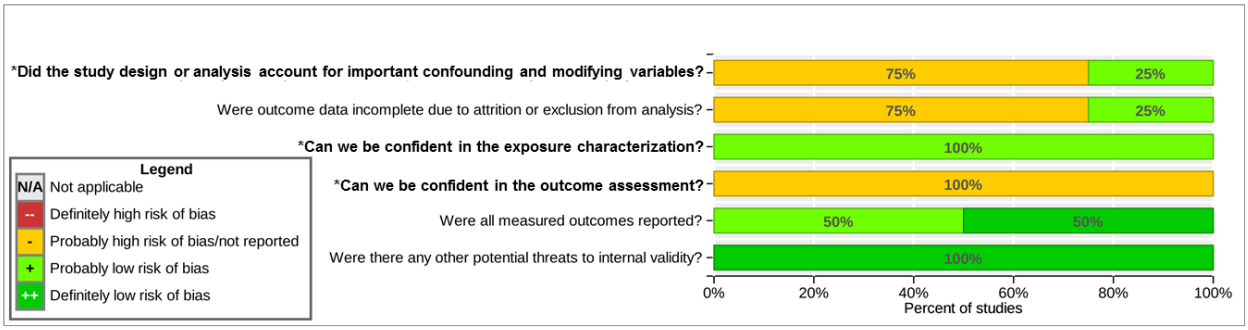

Figure E-6. Risk-of-bias Bar Graph for Case Series Assessing Fear in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human case series. These key questions relate to areas of bias that 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.

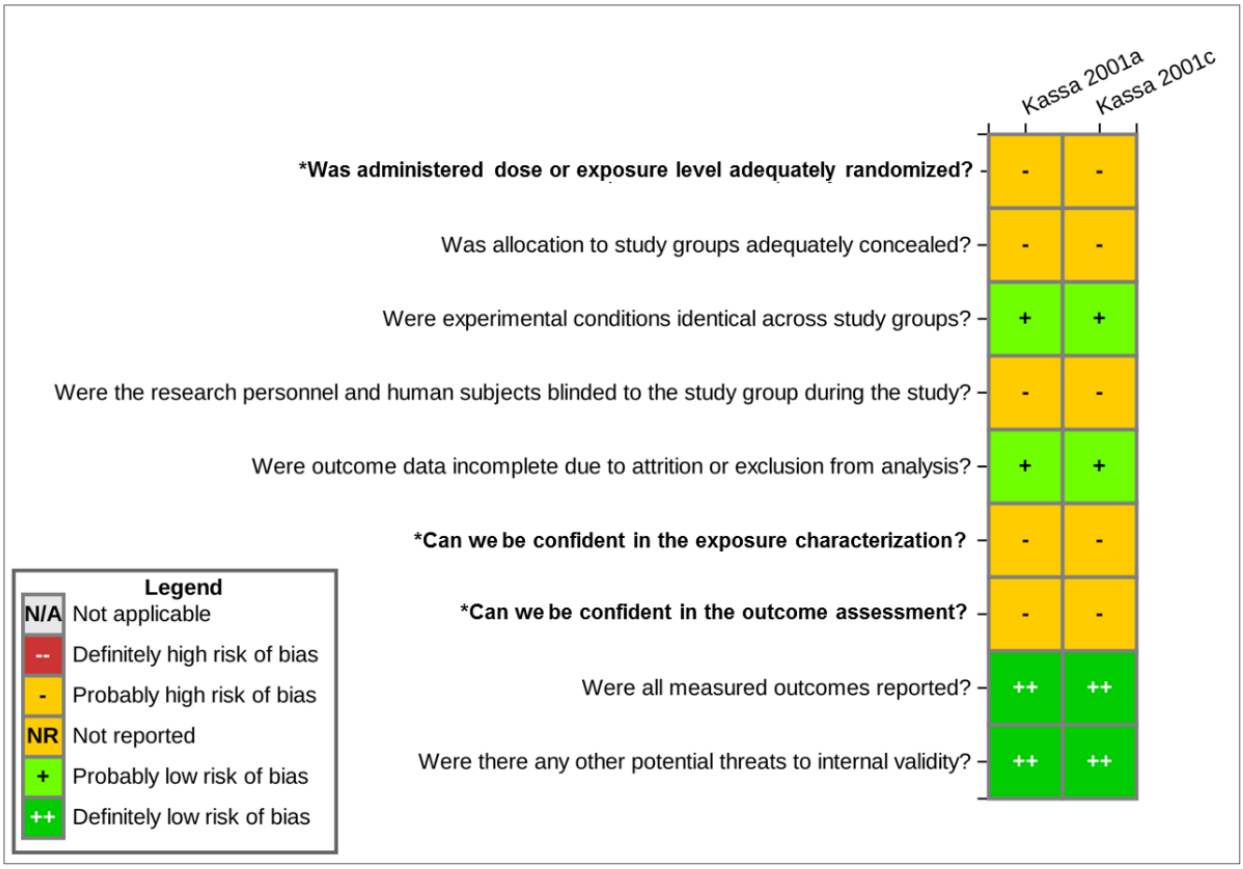

Figure E-7. Risk-of-bias Heat Map for Individual Studies Assessing Anxiety and Fear in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

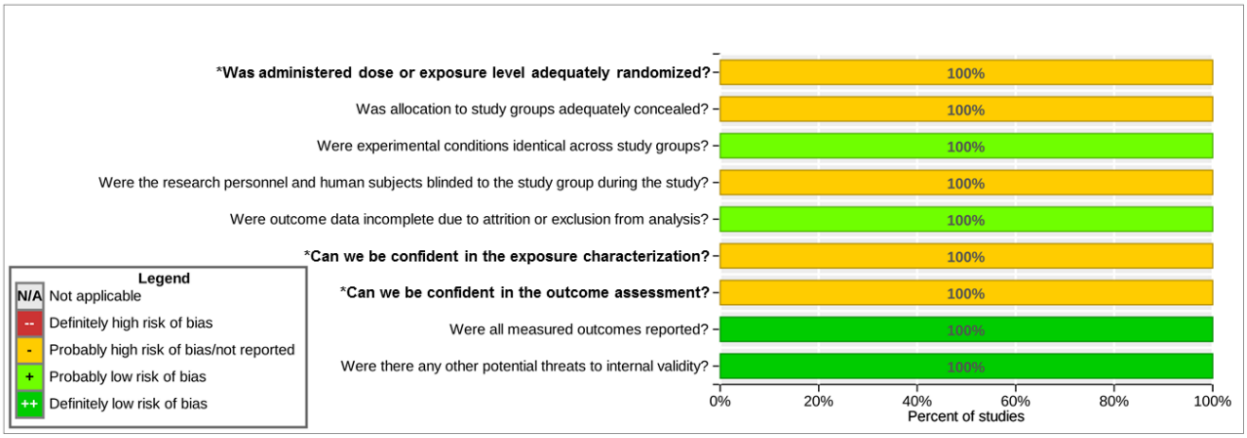

Figure E-8. Risk-of-bias Bar Graph for Individual Studies Assessing Anxiety and Fear in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

E.3. 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.

E.3.1. Human Avoidance and Depression Data

The available studies support a rating of *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 (Kawana et al. 2001; Ohbu et al. 1997; 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) 1 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 (Yokoyama et al. 1998c) 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, 1 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. Profiles 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 1 year following exposure depressive feelings were reported by 24 of 303 (7.9%) exposed subjects (Okumura et al. 2005). Two years after the attack depressed mood was reported in 42 of 283 (14.8%) patients; this decreased significantly 1 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 with 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 of 34 (47.1%) subjects self-reported avoidance behavior of places that trigger recollections of the trauma, whereas 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 E-9 and Figure E-10. There are a number of risk-of-bias issues in the evidence that relate 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 who 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 to the sarin exposure.

E.3.2. Animal Avoidance and Depression Data

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

E.3.3. 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. No studies were 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.

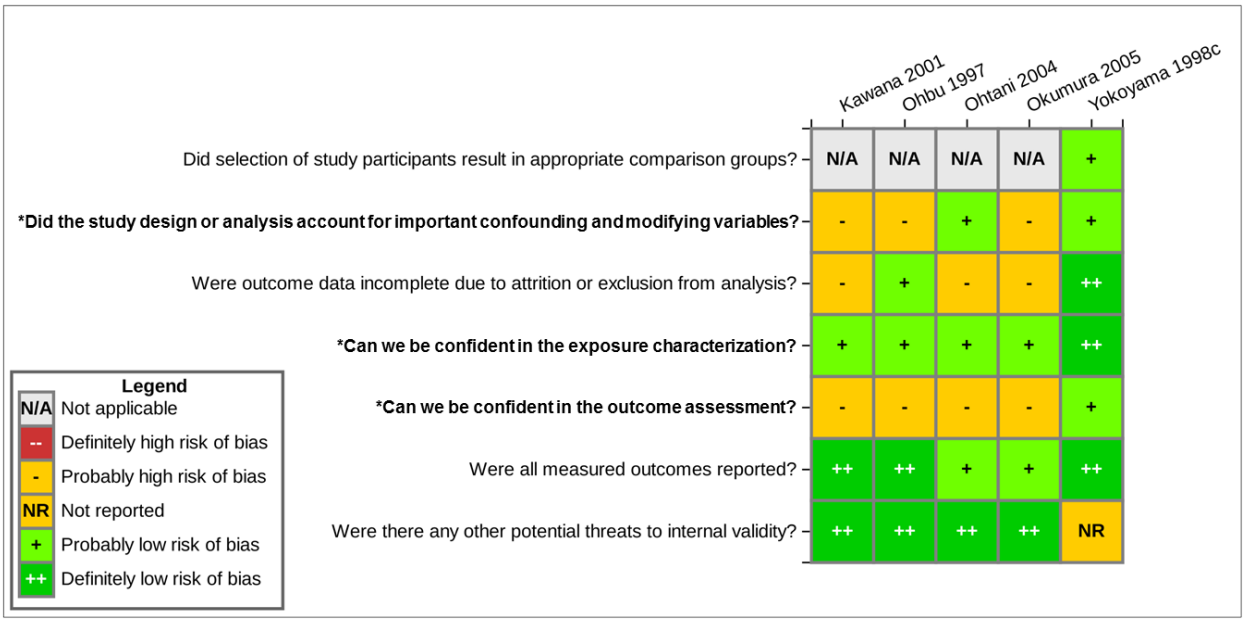

Figure E-9. Risk-of-bias Heat Map for Case Series and Standard Observational Studies Assessing Avoidance and Depression in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

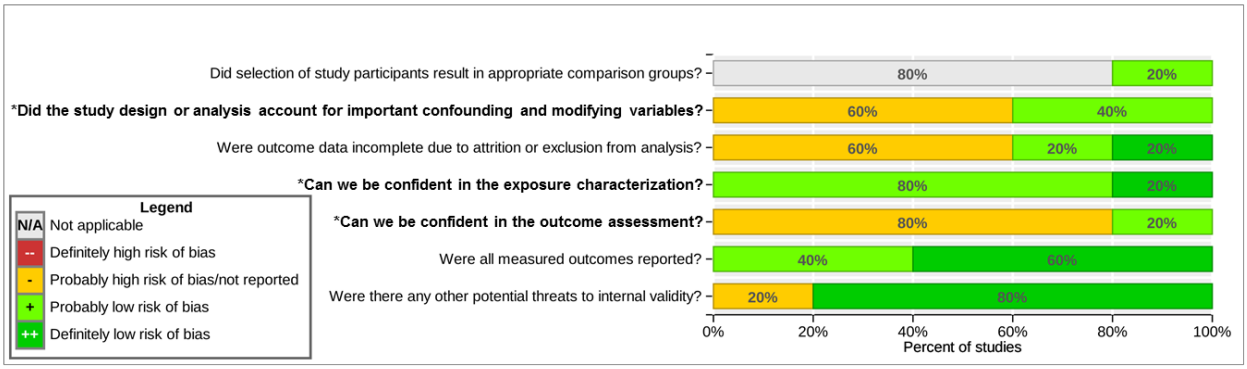

Figure E-10. Risk-of-bias Bar Chart for Case Series and Standard Observational Studies Assessing Avoidance and Depression in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

E.4. Activity and 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 (Germain et al. 2016; Leblanc et al. 2015). 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.

E.4.1. Human Activity and Strength Data

The available studies support a rating of *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. 1999; Nakajima et al. 1998; Ogawa et al. 1999; Ohtani et al. 2004; Okudera 2002; Okumura et al. 2005). For the initial period covering 1–7 days following acute sarin exposure, no studies were available. Subjects from four case series studies report symptoms related to activity and strength 3 weeks to 4 months following acute sarin exposure (Morita et al. 1995; Nakajima et al. 1998; Ogawa et al. 1999; Okudera 2002). 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. 1999; Nakajima et al. 1998; Ohtani et al. 2004; Okudera 2002; 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 four 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 PTSD or PTSD-related effects [e.g., sleep disruption] as confounders, 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. The available data support a rating of 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 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). Easy fatigue was also reported during an examination 3 weeks following the Matsumoto attack in 12 (7.7%) of the 155 subjects examined (Morita et al. 1995; Okudera 2002). 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 with controls (Yokoyama et al. 1998a; Yokoyama et al. 1998c). Asthenia and fatigue were reported 1–3 years following the Matsumoto attack (Nakajima et al. 1999; Nakajima et al. 1998; Okudera 2002). Nakajima et al. (1999) compared victims of the attack who were admitted to the hospital to victims who were outpatients or nonpatients 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 nonvictims. 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 (eight mild and six severe) (Ohtani et al. 2004). These activity and strength-related health endpoints were not assessed at any other time points, and no other health endpoints related to activity and 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 E-11 and Figure E-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). Only one 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. 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 most 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.

E.4.2. 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 a single marmoset monkey (only one was tested) 2–4 days after an intramuscular sarin injection (12 μg/kg). 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; (Allon et al. 2011; Grauer et al. 2008)]. Grauer et al. (2008) also noted some changes in speed performance for sarin-exposed rats in a water maze test that were stated to correspond with the increased activity observed. Kassa et al. studies (2001a; 2004; 2001c) 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 [outcomes assessed in all three studies at 3 months but only in Kassa et al. (2001a) at 6 and 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 three key questions (randomization, exposure characterization, and blinding at outcome assessment) (see Figure E-13 and Figure E-14). The Grauer et al. (2008) study was the only study in which animals were known to be randomized to treatment. None of the other studies reported randomization nor did most 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 five 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; 2004; 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 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; 2004; 2001c), 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, but appear to have used either automated tests or a video tracking system (Allon et al. 2011; Grauer et al. 2008; Wolthuis et al. 1995). Allon et al. (2011) and Grauer et al. (2008) did not use standard methods in the maze test, and the methods used had potential for bias. 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).

E.4.3. 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.

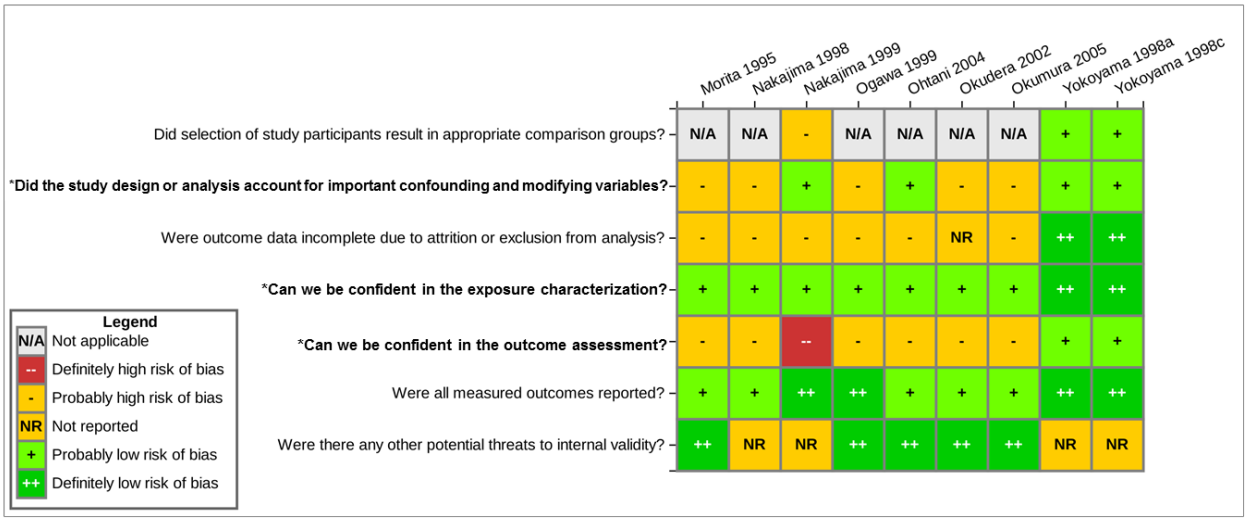

Figure E-11. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Activity and Strength in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

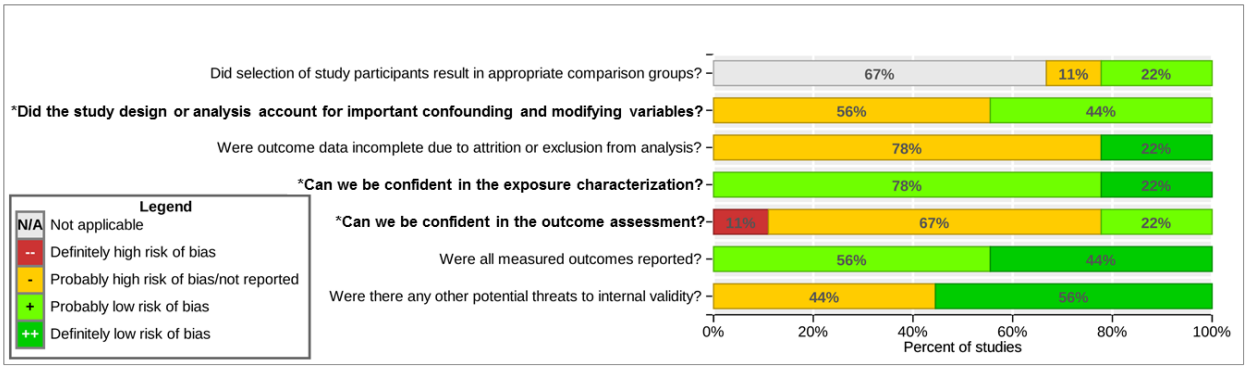

Figure E-12. Risk-of-bias Bar Chart for Case Reports/Series and Standard Observational Studies Assessing Activity and Strength in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

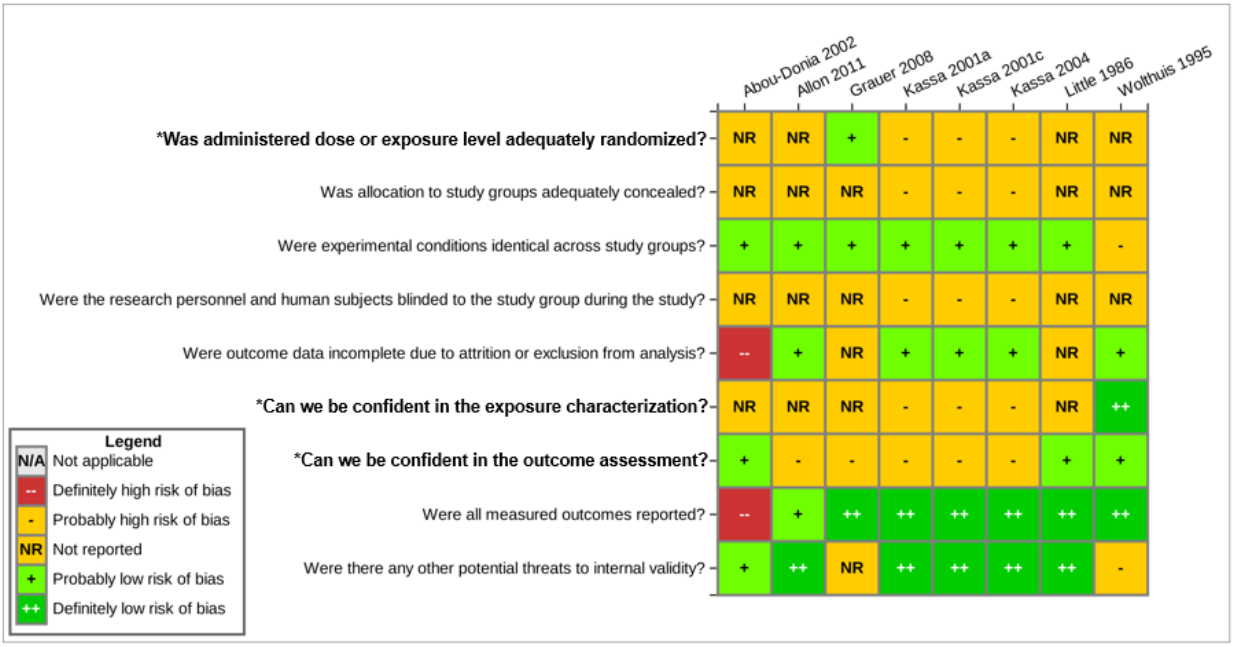

Figure E-13. Risk-of-bias Heat Map for Individual Studies Assessing Activity and Strength in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

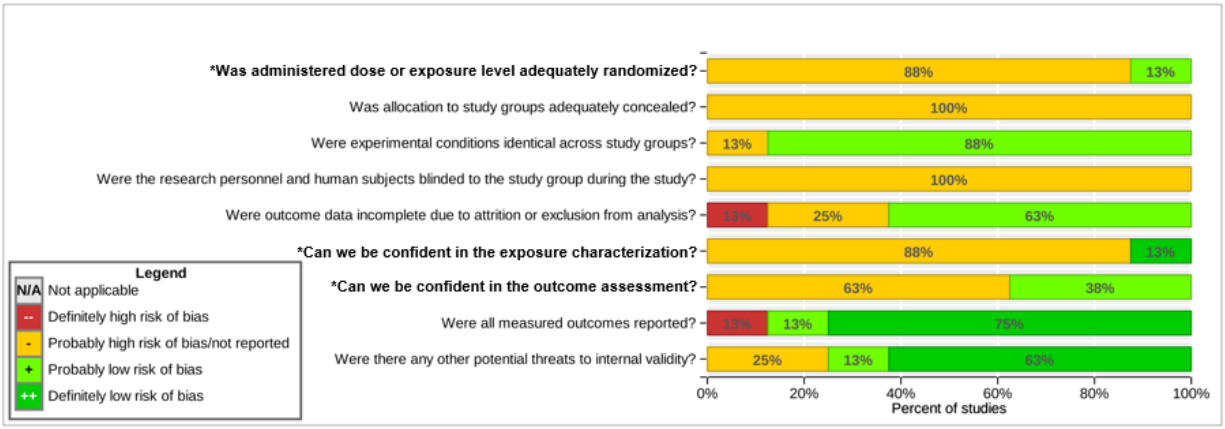

Figure E-14. Risk-of-bias Bar Chart for Individual Studies Assessing Activity and Strength in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

E.5. 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 F-1). 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 1 and 3 years after the Matsumoto attack. At 1 year, Nakajima et al. (1999) compared nonpatients, outpatients, 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 1-year survey. The exposure status (i.e., victims and nonvictims) 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 surveys. Additional concerns were that the questionnaires changed over time; attrition because of 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 many reported symptoms were related to other systems (e.g., digestive and cardiovascular effects). Headache was a symptom included in many of the studies (Kawana et al. 2001; Loh et al. 2010; Morita et al. 1995; Nakajima et al. 1999; Nakajima et al. 1998; Ogawa et al. 1999; Ohtani et al. 2004; Ohtomi et al. 1996; Okudera 2002; Okumura et al. 2005). 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 who reported headache over time; 40 of the 62 subjects evaluated in the study reported headache on the day of the Tokyo subway attack, whereas only four 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 humans. Many of the categories for which results of animal tests could be related to a specific symptom in humans have been discussed in previous sections (e.g., anxiety and fear). No animal studies 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 E-15 and Figure E-16). In addition, these symptoms in humans cannot be replicated with animal studies.

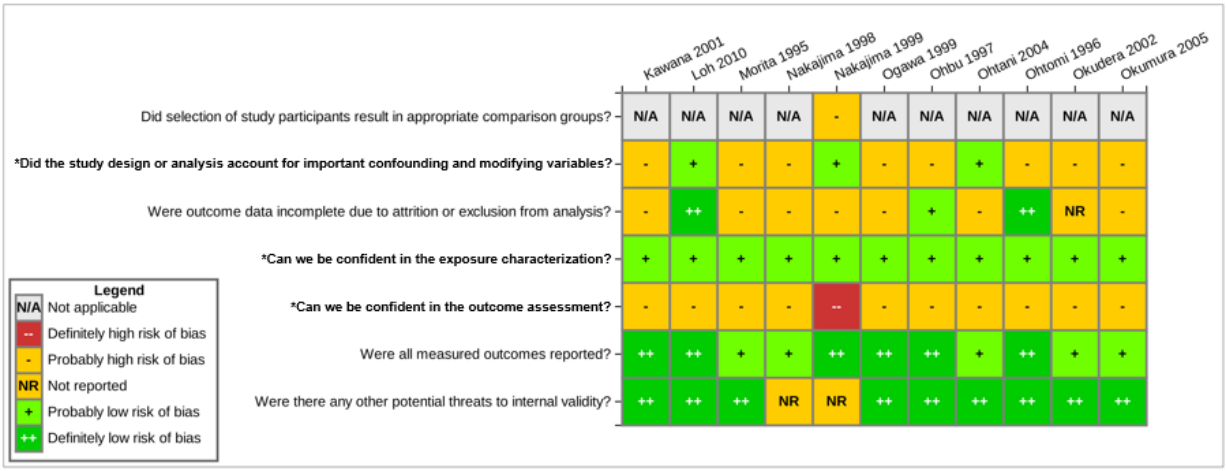

Figure E-15. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Other Neurological Symptoms in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

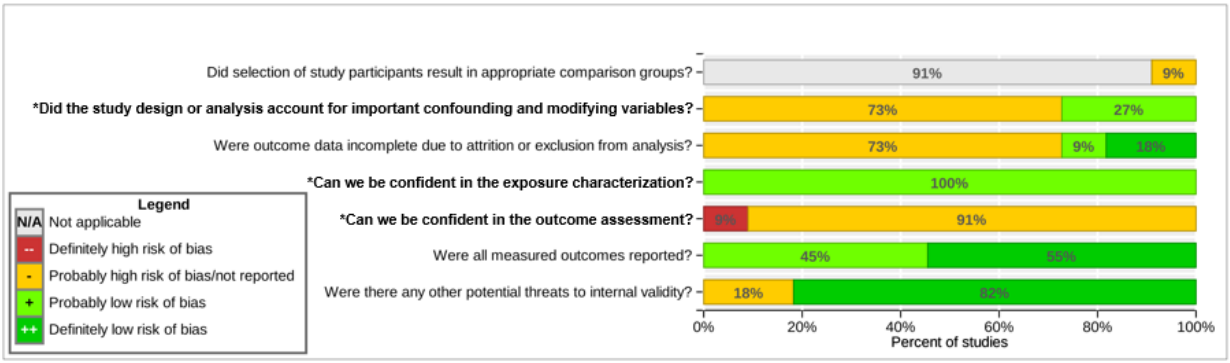

Figure E-16. Risk-of-bias Bar Chart for Case Reports/Series and Standard Observational Studies Assessing Other Neurological Symptoms in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

E.6. Electroencephalogram

An electroencephalogram (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 five publications 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 E-17 through Figure E-20.

E.6.1. 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 day 7 of hospitalization. However, an EEG conducted on day 10 of hospitalization demonstrated sporadic sharp waves and sigma waves when the subject was sleeping. An EEG conducted 1 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).

E.6.2. 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.

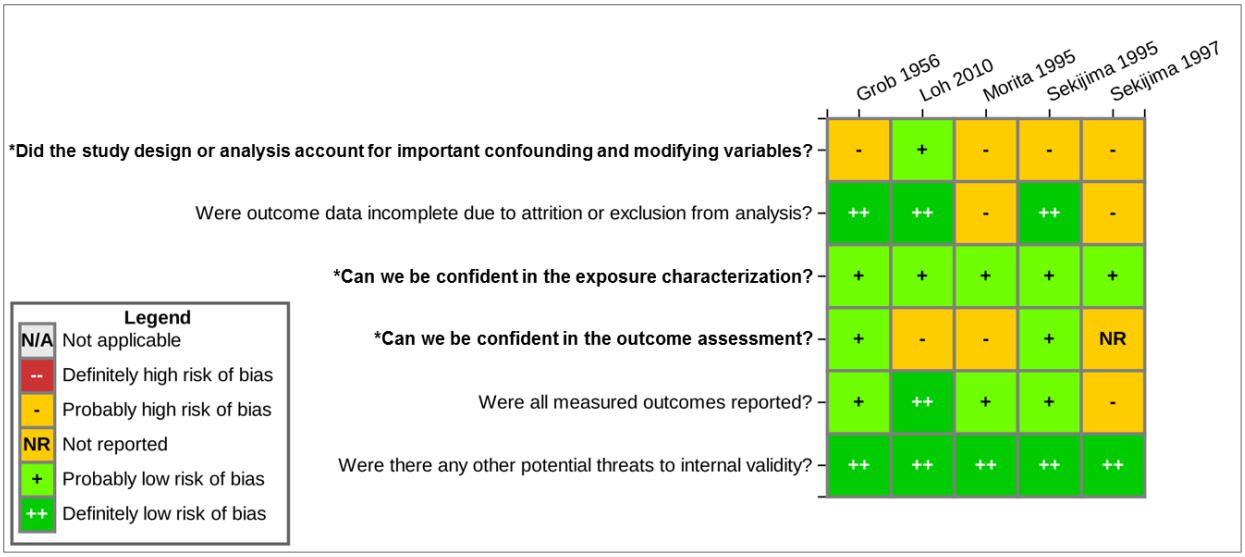

Figure E-17. Risk-of-bias Heat Map for Case Reports/Series Assessing Electroencephalogram Data in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias that 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.

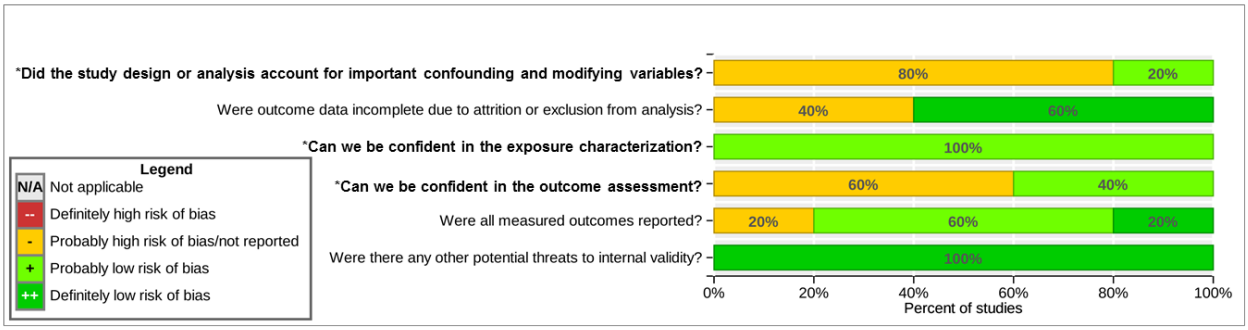

Figure E-18. Risk-of-bias Bar Chart for Case Reports/Series Assessing Electroencephalogram Data in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for human case reports/series. These key questions relate to areas of bias that 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.

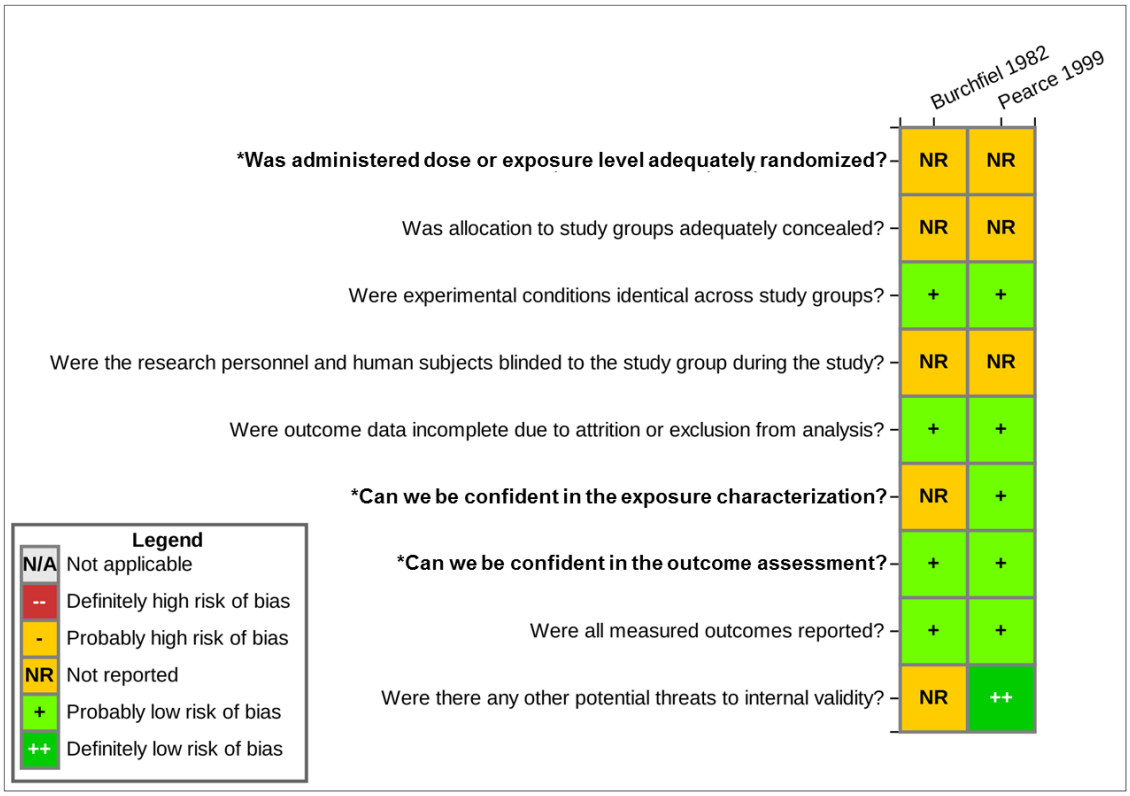

Figure E-19. Risk-of-bias Heat Map for Individual Studies Assessing Electroencephalogram Data in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

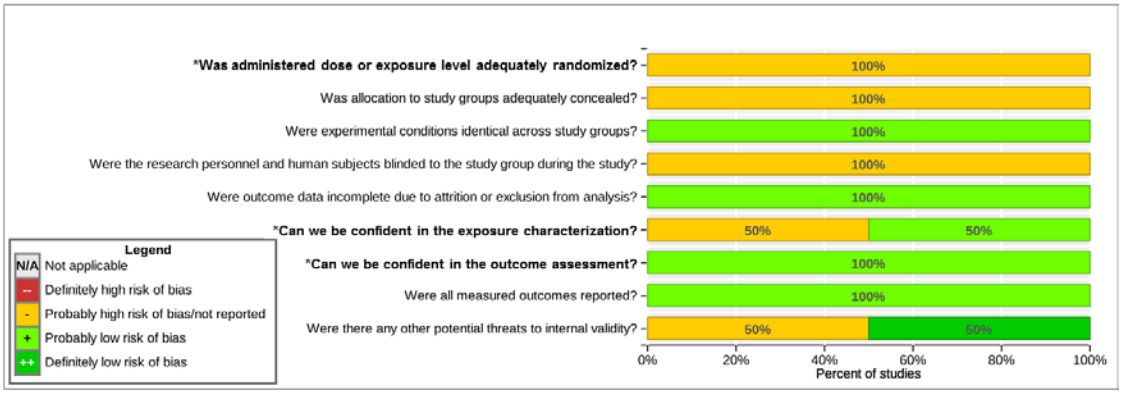

Figure E-20. Risk-of-bias Bar Chart for Individual Studies Assessing Electroencephalogram Data in Animals Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*Questions in bold are the key risk-of-bias questions for experimental animal studies. These key questions relate to areas of bias that 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.

E.7. 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.

E.7.1. Human Other Sensory Data

The available studies support a rating of *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; Nishiwaki et al. 2001; Ogawa et al. 1999; Sekijima et al. 1997). 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 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 with 52 age and occupation-matched controls. The exposed group was separated by level of exposure based on self-reported hospitalization after the exposure (the 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 E-21 and Figure E-22.

E.7.2. Animal Other Sensory Data

A single animal study 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 F-5). In addition to the lack of blinding of outcome assessors, animals were not randomized to treatment.

E.7.3. 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.

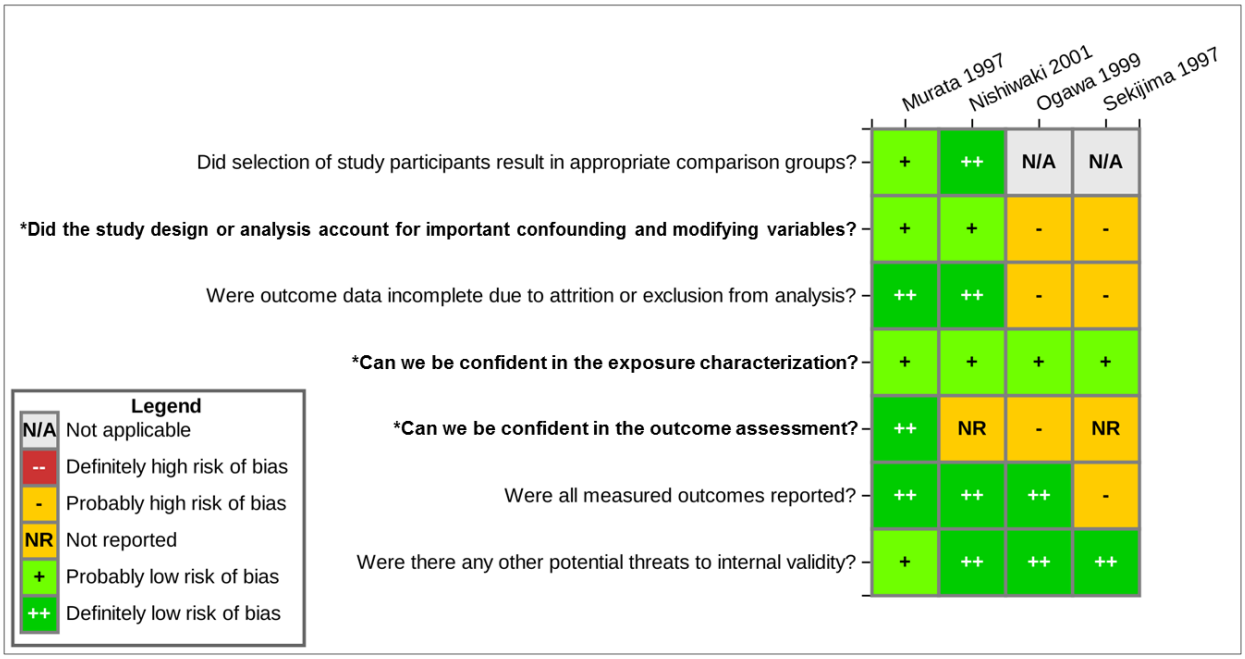

Figure E-21. Risk-of-bias Heat Map for Case Reports/Series and Standard Observational Studies Assessing Other Sensory Effects in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

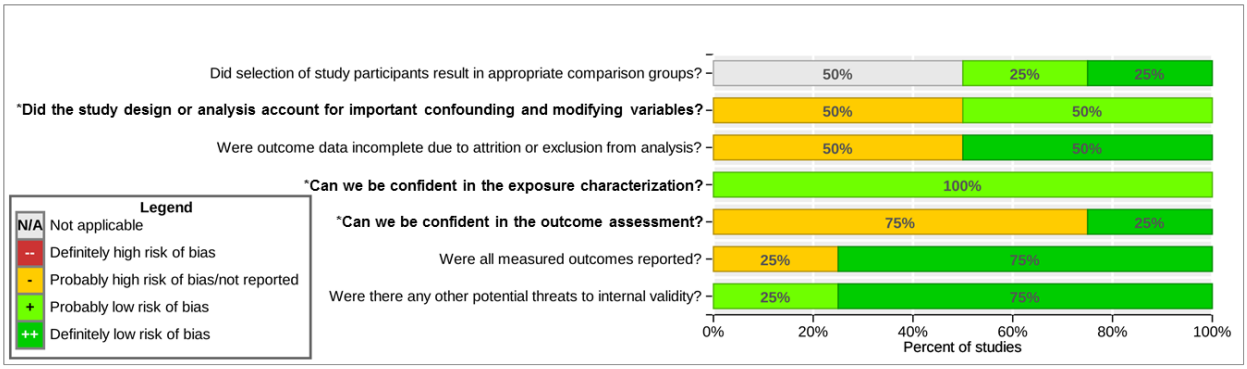

Figure E-22. Risk-of-bias Bar Chart for Case Reports/Series and Standard Observational Studies Assessing Other Sensory Effects in Humans Following Acute Sarin Exposure

Interactive figure and additional study details in HAWC (NTP, 2019b).
*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 that 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.

Appendix F. Additional Data Tables

Tables

Figures

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

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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 wk. 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)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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.		
Dim vision	√	–	–	–	–	√	√	√	√/0	√	–	√	√	√	Kawana et al. (2001); Ohtomi et al. (1996); 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 wk. 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 wk. and 4 mo. but was excluded from the questionnaire at 1 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)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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/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 wk. 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 wk. 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 1 mo. except for two 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)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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.		
Behavioral Changes																
Avoidance																
Avoidance of places that trigger recollections of the trauma	–	–	–	–	–	–	–	–	–	–	–	–	–	√	Ohtani et al. (2004)	
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)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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.		
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 mo. after the incident. This percentage remained almost the same even 3 and 6 mo. 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[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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 mo. after the incident. This percentage remained almost the same even 3 and 6 mo. after the incident."
Difficulty falling or staying asleep	–	–	–	–	–	√	–	–	–	–	–	√	√	√	Kawana et al. (2001)	
Distressing dreams, nightmares	–	–	–	–	–	√	–	–	–	–	–	√	√	√	Kawana et al. (2001)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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.		
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 mo. after the incident. This percentage remained almost the same even 3 and 6 mo. after the incident."
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)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]							Symptom References[f] (Time Frames)	Reference Notes
	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.	
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 mo. after the incident. This percentage remained almost the same even 3 and 6 mo. 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 et al. 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)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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.		
Lethargy	–	–	–	–	–	–	–	–	–	–	–	–	–	√	Ohtani et al. (2004)	
Weakness (general)	–	–	–	–	–	–	–	–	√	–	–	–	–	–	Ogawa et al. (1999)	
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 mo. after the incident. This percentage remained almost the same even 3 and 6 mo. after the incident."
Difficulty reading/writing	–	√	√	0	–	–	–	–	–	–	–	–	–	–	Nakajima et al. (1998)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]							Symptom References[f] (Time Frames)	Reference Notes
	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.	
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 mo. after the incident. This percentage remained almost the same even 3 and 6 mo. after the incident."
Hypervigilance	–	–	–	–	–	√	–	–	–	–	–	√	√	√	Kawana et al. (2001)	
Effect of event scale-revised (IES-R)	–	–	–	–	–	–	–	–	–	–	–	–	–	√	Ohtani et al. (2004)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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.		
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 suffered from some post-incident symptoms, which can be indication of PTSD, 1 mo. after the incident. This percentage remained almost the same even 3 and 6 mo. after the incident."
Restlessness and irritability	–	–	–	–	–	–	–	–	–	–	–	–	–	√	Ohtani et al. (2004)	
Sense of suppression	–	–	–	–	–	–	–	–	–	–	–	–	–	√	Ohtani et al. (2004)	
Tension	–	–	–	–	–	–	–	–	–	–	–	–	–	√	Ohtani et al. (2004)	

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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.		
Neuromuscular Effects																
Gait disturbance	–	0	0	–	–	–	–	–	√	–	–	–	–	–	Nakajima et al. (1998); Ogawa et al. (1999)	This symptom was included in the questionnaires at 3 wk. 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 wk. and 4 mo. but was excluded from the questionnaire at 1 yr. Zero victims reported this symptom at 4 mo.
Other Neurological																
Dysomia (change in sense of smell)	–	–	–	–	–	–	–	–	√	–	–	–	–	–	Ogawa et al. (1999)	
Dizziness	–	√	√	–	–	√	–	–	√	–	–	√	√	√	Kawana et al. (2001); Nakajima et al. (1998); Ogawa et al. (1999); Ohtani et al. (2004)	This symptom was included in the questionnaires at 3 wk. and 4 mo. but was excluded from the questionnaire at 1 yr. (Nakajima et al. 1998).

Symptom[c]	Study Population: Matsumoto Sarin Attack (1994) *Time after Exposure*[d]						Study Population: Tokyo Subway Sarin Attack (1995) *Time after Exposure*[e]								Symptom References[f] (Time Frames)	Reference Notes
	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.		
Dysesthesia of extremities	–	√	√	–	–	–	–	–	–	–	–	–	–	–	Nakajima et al. (1998)	This symptom was included in the questionnaires at 3 wk. and 4 mo. but was excluded from the questionnaire at 1 yr.
Headache	–	√	√	√/0	√	√	√	√	√	√	√	√	√	√	Kawana et al. (2001); Nakajima et al. (1998); 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.

[a]This table provides a summary of neurological symptoms identified by study subjects at various time frames after exposure. The prevalence or severity of symptoms is not indicated here.
[b]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 dash indicates that no data are available for that time frame by any of the listed studies. A "0" indicates that at least one listed study reported that the symptom had fully subsided by the time frame.
[c]Symptoms are self-reported (via questionnaire) or based on physician observation (not determined by a specific test).
[d]Matsumoto study time frames: Nohara and Segawa (1996) (1 wk.); Morita et al. (1995) (1 wk.); Sekijima et al. (1995) (1 wk.); Nakajima et al. (1998) (3 wk., 4 mo., 1 yr.); Nakajima et al. (1999) (1 and 3 yr.); Kawana et al. (2001) (5 yr.).
[e]Tokyo study time frames: 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.).
[f]All studies list this symptom for at least one of the time frames presented, but all studies do not necessarily report symptoms for all the time frames.

Table F-2. Summary of Neurobehavioral Endpoints in Animals – Learning and Memory

Endpoint	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.
	0, 0.701 mg/m^3	0–4 mg/m^3	0, 34.2 µg/L			0–2.5 µg/L				
Neurological: Behavior										
Learning and Memory										
Serial probe recognition	**NS ↔**	–	–	–	–	–	–	–	–	–
Radial-arm maze, completion time (first 5-block session)	–	**NS ↑ (high dose)**	–	–	–	–	–	–	–	–
Radial-arm maze, total errors (first 5-block session)	–	SIG ↑ block 1	–	–	–	–	–	–	–	–
Radial-arm maze, reference errors (first 5-block session)	–	SIG ↑ block 1	–	–	–	–	–	–	–	–
Radial-arm maze, working errors (first 5-block session)	–	SIG ↑ block 1	–	–	–	–	–	–	–	–
Radial-arm maze, VI56 response rate (first session)	–	**NS ↕**	–	–	–	–	–	–	–	–
Radial-arm maze, VI56 response rate (first 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)	–	–	–	–	–

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 F-3. Summary of Neurobehavioral Endpoints in Animals – Discrimination Learning

Endpoint	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 d.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	2 d.	3 d.	4 d.
	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 ↔**	–	–	–	–	–	–	–	–	–	–

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 F-4. Summary of Neurobehavioral Endpoints in Animals – Reflexes, Motor Strength, Coordination, and Motor Activity and Memory

Endpoint	Abou-Donia et al. (2002)		Allon et al. (2011)		Grauer et al. (2008)				Kassa et al. (2001a)			Kassa et al. (2004)	Little et al. (1986)	Wolthuis et al. (1995)		
	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.[a]	6 mo.	12 mo.	3 mo.	4 d	4 d	5 d	6 d
	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, 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 ↔**	–	–	–	–	–

Endpoint	Abou-Donia et al. (2002)		Allon et al. (2011)		Grauer et al. (2008)				Kassa et al. (2001a)			Kassa et al. (2004)	Little et al. (1986)	Wolthuis et al. (1995)		
	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.[a]	6 mo.	12 mo.	3 mo.	4 d	4 d	5 d	6 d
	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, 80 µg/kg	0–12 µg/kg		
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)	–	–	–	–	–	–	–	–
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)	–	–	–	–

Endpoint	Abou-Donia et al. (2002)		Allon et al. (2011)		Grauer et al. (2008)				Kassa et al. (2001a)			Kassa et al. (2004)	Little et al. (1986)	Wolthuis et al. (1995)		
	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.[a]	6 mo.	12 mo.	3 mo.	4 d	4 d	5 d	6 d
	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, 80 µg/kg	0–12 µg/kg		
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)	–	–	–	–	–	–	–	–
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 ↔**	–	–	–	–	–	–	–	–

Endpoint	Abou-Donia et al. (2002)		Allon et al. (2011)		Grauer et al. (2008)				Kassa et al. (2001a)			Kassa et al. (2004)	Little et al. (1986)	Wolthuis et al. (1995)		
	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.[a]	6 mo.	12 mo.	3 mo.	4 d	4 d	5 d	6 d
	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, 80 µg/kg	0–12 µg/kg		
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)	–	–	–	–	–	–	–	–
Water maze speed of performance (trial 2, day 5)	–	–	–	–	↑ (SIG UNK)	–	↑ (SIG UNK)	↑ (SIG UNK)	–	–	–	–	–	–	–	–

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.
[a]Except for righting reflex and exploratory activity, these 3-month data were also presented in Kassa et al. 2001c.

Table F-5. 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.[a]	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 and 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 ↔**

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.
[a]The 3-month data reported for Kassa et al. 2001a were also reported in Kassa et al. 2001c.

F.1. Additional Human Endpoints and Animal Biochemical Data

Additional endpoints from human studies not discussed in the main document or Appendix E are listed by category in Table F-6.

Seven animal studies included biochemical endpoint data after acute sarin exposure. Although most of the studies found some effect in the endpoints measured (see Table F-7 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 F-6. Additional Human Endpoints

Study Name	Endpoint
PTSD	
Kawana et al. (2001)	PTSD (DSM-IV); PTSE-Nakano; partial PTSD
Ohtani et al. (2004)	Effect 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 μs jitter of muscle fiber pairs
Grob (1956)	Muscle cramp; generalized muscular fasciculations
Other	
Grob (1956)	Speech difficulty

Study Name	Endpoint
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 F-7. 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 d.	1 mo.	6 mo.	3 d.	7 d.	12 mo.	120 hr.	240 hr.	90 d.	48 hr.
	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	–

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 d.	1 mo.	6 mo.	3 d.	7 d.	12 mo.	120 hr.	240 hr.	90 d.	48 hr.
	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
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

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 d.	1 mo.	6 mo.	3 d.	7 d.	12 mo.	120 hr.	240 hr.	90 d.	48 hr.
	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

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 d.	1 mo.	6 mo.	3 d.	7 d.	12 mo.	120 hr.	240 hr.	90 d.	48 hr.
	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 (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	–	–
PKC beta II expression (cytosolic) (striatum)	–	–	–	–	–	–	↓ 90 mg/kg	NS	–	–
PKC beta II expression (membrane) (frontal cortex)	–	–	–	–	–	–	NS	NS	–	–

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 d.	1 mo.	6 mo.	3 d.	7 d.	12 mo.	120 hr.	240 hr.	90 d.	48 hr.
	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 (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	–	–

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 d.	1 mo.	6 mo.	3 d.	7 d.	12 mo.	120 hr.	240 hr.	90 d.	48 hr.
	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 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	–
Blood-brain barrier permeability (midbrain)	–	–	–	–	–	–	–	–	NS	–
Blood-brain barrier permeability (brainstem)	–	–	–	–	–	–	–	–	↓ 100 µg/kg	–
Blood-brain barrier permeability (cerebellum)	–	–	–	–	–	–	–	–	NS	–

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

Neurological Endpoint Category	Human by Time Period				Animal by Time Period			
	Initial	Intermediate	Extended	Total	Initial	Intermediate	Extended	Total
Activity and Strength		6	5	9	3	6	3	8
Anxiety and Fear		2	5	7		2	1	2
Avoidance and Depression		2	3	5				
Cholinesterase	4	6	1	9	12	9		15
EEG	3	5	1	5		1	2	2
Learning, Memory, and Intelligence		3	4	7	7	7	2	9
Morphological and Histological		1	2	3	5	3		6
Other Neurological Symptoms	1	7	5	11				
Other Sensory Effects		3	1	4		1	1	1
Sleep Disruption		3	4	6				
Visual and Ocular	5	10	5	16	3	1	1	4

Figure F-1. Number of Studies by Endpoint Category, Evidence Stream, and Time Period

Interactive figure and additional study details in Tableau®.

Appendix G. Peer-review Report

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G.1. Attendees[5]

Peer Review Panel

Chair: Pam Factor-Litvak, Columbia University Medical Center

Frédéric Baud, Université Paris Diderot, Assistance Publique – Hôpitaux de Paris (by WebEx)

John Beard, Brigham Young University (by WebEx)

Peter Blain, Newcastle University (by WebEx)

Michelle Block, Indiana University School of Medicine (by WebEx)

Arik Eisenkraft, The Hebrew University Faculty of Medicine (by WebEx)

Lawrence Engel, University of North Carolina at Chapel Hill (by WebEx)

Virginia Moser, Private Neurotoxicology Consultant (by WebEx)

National Toxicology Program Board of Scientific Counselors Liaison

Kenneth McMartin, Louisiana State University Health Sciences Center (by WebEx)

National Institute of Environmental Health Sciences Staff

Brian Berridge

John Bucher

Elizabeth Maull

Andrew Rooney

Mary Wolfe

Other Federal Agency Staff

David Jett, National Institute of Neurological Disorders and Stroke (by WebEx)

Contract Support Staff

Robyn Blain, ICF (by WebEx)

Canden Byrd, ICF

Pamela Hartman, ICF

Ernie Hood, Bridport Services

Chris Sibrizzi, ICF

Catherine Smith, ICF

G.2. Introductions and Welcome

The National Toxicology Program (NTP) convened a peer-review panel for the *Draft NTP Monograph on Systematic Review of Long-term Neurological Effects Following Acute Exposure to the Organophosphorus Nerve Agent Sarin* on February 4, 2019 via webcast. Dr. Pam Factor-Litvak served as chair. Dr. Kenneth McMartin viewed the webcast as the NTP Board of Scientific Counselors liaison. Representing NTP were Drs. Brian Berridge, John Bucher, Elizabeth Maull, Andrew Rooney, and Mary Wolfe. Dr. Maull served as the Designated Federal Official.

Dr. Factor-Litvak called the meeting to order at 9:00 a.m., welcomed everyone to the meeting, and asked all attendees to introduce themselves. Dr. David Jett from the National Institute of Neurological Disorders and Stroke, Director of the National Institutes of Health Countermeasures Against Chemical Threats (CounterACT) program added his welcome and provided background information about CounterACT. Dr. Berridge welcomed all participants to

[5]The meeting was webcast. Individuals who viewed the webcast are not listed except as noted.

the meeting. Dr. Maull read the conflict of interest policy statement and briefed the attendees on meeting logistics. Dr. Factor-Litvak informed the panel and the audience of the format for the peer review.

G.3. Public Comments

G.3.1. Written Public Comments

No written public comments on the draft monograph were received.

G.3.2. Oral Public Comments

No requests for oral public comments on the draft monograph were received.

G.4. Peer Review of the Draft NTP Monograph on Systematic Review of Long-term Neurological Effects Following Acute Exposure to the Organophosphorus Nerve Agent Sarin

G.4.1. Introduction to the Draft NTP Monograph

G.4.1.1. Presentation

Dr. Andrew Rooney, Acting Director of the Division of the NTP Office of Health Assessment and Translation (OHAT), presented an introduction to the monograph. Background information on sarin included well known short-term health effects of acute exposure as well as less well characterized long-term neurological effects of human sarin exposure. Sarin was nominated by CounterACT, which requested that NTP conduct a systematic review of the evidence for long-term neurological effects of exposure to sarin to inform the need to develop therapeutics.

Dr. Rooney described the OHAT approach to conducting systematic reviews and the specific process used in the sarin systematic review. The stepwise methods identify, evaluate, and integrate evidence from animal and human studies to reach hazard conclusions on whether sarin is associated with long-term neurological effects. NTP's confidence ratings translated directly into level of evidence conclusions that also considers the direction of the effect (i.e., confidence that the evidence supports a health effect or no effect). The highest level of evidence conclusions for each time period was used to develop initial hazard identification conclusions. Hazard identification conclusions were developed for three post-exposure time periods: initial (>24 hours to 7 days after exposure), intermediate (8 days to 1 year after exposure), and extended (>1 year after exposure). Four main health effect categories were identified: changes in cholinesterase levels; visual and ocular effects; learning, memory, and intelligence effects; and nervous system morphological and histological changes. Other outcomes were considered, with data included in Appendix 4 of the monograph.

G.4.1.2. Peer-Review Comments and Panel Discussion on Introduction to the Draft NTP Monograph

Dr. Arik Eisenkraft said that he appreciated NTP's thorough work. He felt that data on health effects from other organophosphates should have been included. Although the focus on cholinesterase was appropriate, he observed that other neurotransmitter pathways were ignored. Dr. Rooney responded that the process of problem formulation was challenging, and in order to meet the needs of CounterACT, it was decided to focus on sarin only.

Dr. John Beard appreciated Dr. Rooney's clarification of the treatment of non-English studies.

Dr. Frédéric Baud questioned whether the time periods in the experimental animal studies translated well to human time periods. Dr. Rooney noted that for the initial time period, NTP used equivalent time periods between the human and non-human animals. However, for the intermediate time period, animal studies with time periods up to 90 days were considered equivalent to a human time period up to 1 year given the shorter life span of rodents compared to humans. He said NTP would appreciate suggestions for any modification of the approach and would attempt to clarify the text regarding animal time periods in the monograph.

Dr. Peter Blain noted the difficulty of conducting a review on a nerve agent due to the limited number of studies in the public arena. He wondered if there was any way to liaise with appropriate agencies to access classified data relevant to the monograph, or to have agencies comment on the monograph. Dr. Rooney indicated that NTP shared the monograph on an inter-

agency level, including the Departments of Defense and Homeland Security and other agencies with access to classified data.

Dr. Rooney presented the peer review panel's charge to:

1. Comment on whether the *Draft NTP Monograph on Systematic Review of Long-term Neurological Effects Following Acute Exposure to the Organophosphorus Nerve Agent Sarin* is technically correct, clearly stated, and objectively presented.
2. Vote on whether the scientific evidence from animal studies and from human studies supports the level of evidence conclusions regarding health effects following acute sarin exposure.
3. Vote on whether the scientific evidence supports NTP's policy decisions for hazard categorization on long-term neurological effects following acute sarin exposure.

G.5. Peer Review of Health Effect Areas

G.5.1. Changes in Cholinesterase Levels

G.5.1.1. Presentation

Dr. Rooney presented the draft monograph information on changes in cholinesterase levels.

Dr. Rooney described the body of evidence as well as factors that increased or decreased NTP's confidence considerations for both the animal and human studies. The confidence ratings and corresponding level of evidence conclusions in the body of evidence for animal studies were:

- Initial time period (>24 hours to 7 days): moderate confidence, moderate level of evidence
- Intermediate time period (8 days to 1 year): moderate confidence, moderate level of evidence
- Extended time period (>1 year): no confidence rating, inadequate level of evidence

The confidence ratings and corresponding level of evidence conclusions on the body of evidence for human studies were:

- Initial time period (>24 hours to 7 days): high confidence, high level of evidence
- Intermediate time period (8 days to 1 year): very low confidence, inadequate level of evidence
- Extended time period (>1 year): low confidence, inadequate level of evidence

G.5.1.2. Peer-Review Comments and Panel Discussion on Changes in Cholinesterase Levels, Animal Data

Dr. Peter Blain, first reviewer, noted the small number of studies involved, but said the review of the studies was reasonable and the conclusions were justified. He suggested adding differentiation between butyrylcholinesterase and acetylcholinesterase, as the two enzymes have different kinetics for breakdown of sarin and re-synthesis. He added that the focus on blood did not necessarily reflect the kinetics in the nervous system tissues such as the brain. The monograph could acknowledge that changes in cholinesterase are sometimes viewed as a surrogate marker of exposure and recovery. With those caveats delineated in the monograph, he opined that the conclusions reached were perfectly reasonable. Dr. Peter Blain also noted that decreases in cholinesterase brain levels in the initial time period may be relevant to memory and cognition symptoms in human cases.

Dr. Virginia Moser, second reviewer, indicated that the Damodoran 2003 was not included or described in the discussion on cholinesterase inhibition; if it was not discussed, it should not be listed as one of the studies. She disagreed with downgrading some studies based on sample size or failure to blind assessors to treatments. Dr. Moser indicated that the sample sizes for the rodent studies were actually quite high for cholinesterase studies. For those studies where the assessments were the actual cholinesterase assays themselves, she suggested that blinding of the technician would not be of concern. In the initial time period, while NTP concluded that only a moderate confidence level was appropriate due to differences across studies, Dr. Moser noted strikingly similar cholinesterase data across the various experiments. Of the nine rodent studies that measured cholinesterase in the initial time period, eight of them recorded decreases at one, two, or three days. The only exception was the Bansal 2009 study, which was the only study to

use isopropanol as the vehicle; she was not convinced that isopropanol would not alter sarin absorption. Dr. Moser noted the only two studies that did not report cholinesterase inhibition at seven days was Bansal 2009 and RamaRao 2011, which used female rats. Even with the lack of information on the risk of bias issues of randomization and chemical purity, she felt that downgrading for risk of bias was unwarranted. Dr. Moser suggested NTP should consider a high level of evidence conclusion in the context of the consistent evidence of effects seen during the initial time period. For the intermediate time period, she agreed with moderate confidence in the database. Citing dose response limitations in the two studies discussed at the longer time points, she stated that upregulation should be downplayed as a factor in the intermediate exposure. She noted that there were no animal studies in the extended time period, and thus had no comments on NTP's level of evidence conclusion.

Dr. Rooney appreciated Dr. Peter Blain's thoughts about adding data on the re-synthesis kinetics and linking cholinesterase effects on different brain regions to the other monograph sections such as morphology. Responding to Dr. Moser's comments, Dr. Rooney indicated that he would check on the use of isopropanol as a vehicle and review the upregulation issue. He noted that risk-of-bias assessment is used in systematic reviews to address study quality and transparently report where there might be concerns in study design or conduct that could impact the results. With regard to blinding, he noted that even in more equipment-based procedures, there remains the potential for researchers to intentionally or unintentionally bias the results and therefore there is greater certainty in results that are measured where researchers are blind to treatment level. He noted that the panel is free to disagree with NTP's assessments of risk of bias and provide alternate study quality ratings.

Dr. Eisenkraft agreed with Dr. Moser on her assessment of the body of evidence for the initial time period. He suggested inclusion of descriptions of which studies were performed on rats, and which were performed on non-rodent animals, to define how animal model may impact risk-of-bias ratings.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the initial time period in animal studies. Dr. Peter Blain moved to accept the level of evidence conclusion as written, Dr. Michelle Block seconded. The panel voted 5 yes, 2 no, 0 abstentions. Drs. Eisenkraft and Moser explained that their no votes ensued from their belief that the conclusion should have been a high level of evidence.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the intermediate time period in animal studies. Dr. Moser moved to accept the level of evidence conclusion as written, Dr. Peter Blain seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the extended time period in animal studies. Dr. Block moved to accept the level of evidence conclusion as written, Dr. Peter Blain seconded. The panel voted 7 yes, 0 no, 0 abstentions.

G.5.1.3. Peer-Review Comments and Panel Discussion on Changes in Cholinesterase Levels, Human Data

Dr. Eisenkraft, first reviewer, thought it would be important to add the Tokyo, Japan, events, where sarin of relatively low quality was deployed. He thought that a group of experts needed to be involved in the research, especially given the current use of sarin in the Middle East. Including "time-to-treatment" data should be considered for the monograph. Dr. Eisenkraft agreed with the level-of-evidence conclusions.

Dr. Lawrence Engel, second reviewer, noted that it is nearly impossible to accurately assess the level of exposure. While the best exposure surrogate is often cholinesterase, cholinesterase per se is not a measure of exposure. Recognizing that as a limitation, he agreed with the assessment in the early time period. However, Dr. Engel disagreed with the very low confidence rating for the intermediate time period. The strong effects of butyrylcholinesterase and acetylcholinesterase inhibition in the blood should offset some of the limiting factors.

Dr. Rooney said he appreciated the thoughtful comments from both reviewers. He said he would edit the text to address Dr. Eisenkraft's comments. He thanked Dr. Engel for his suggestion to upgrade the intermediate time period conclusion from very low to low.

Dr. Moser agreed with Dr. Engel's suggestion regarding the confidence rating for the intermediate time period.

Dr. Beard commented that “case series” should be identified as “cohort studies” since humans are identified by cholinergic signs and symptoms (exposure versus the outcome). Dr. Beard added that a confounder should be defined as a third variable that is associated with the exposure and the outcome, and it cannot be affected by a prior exposure. Regarding the Tokyo and Matsumoto attacks in Japan, terrorist attacks seem like random events, and Dr. Beard struggled to identify determinants of the exposure and, therefore, likely confounders. He questioned whether downgrading level of evidence because of the risk of bias in terms of confounding was warranted in some cases, as it would be difficult to determine what to adjust for as the confounder.

Dr. Factor-Litvak indicated that confounding is easily confused with effect modification, which means that the reported associations might differ by some characteristic. Thus, what to control for can be very tricky, particularly in considering factors such as sex, which should be considered an effect modification variable. She also commented that treatment for the acute episode might reduce the later outcomes, or bias results toward the null. The issue is how the direction of bias comes in play with treatment.

Dr. Baud noticed six or seven studies referencing cholinesterase inhibition at 13 days following exposure, and therefore thought that an inadequate level of evidence for the intermediate time period was not adapted to the data.

Based on the discussion, Dr. Rooney indicated that he would examine the issue of confounding versus effect modification and re-examine the risk-of-bias ratings to see how treatment effects were addressed. Cholinesterase levels were not the only indicator of exposure; individuals were assumed exposed due to their presence at the attack sites. Confidence ratings were not influenced by how studies were described (i.e., if a study was described as “case-series”) but rather on the presence of study design characteristics such as whether exposure preceded outcome assessment.

Dr. Peter Blain noted that in one study, effects were seen long after cholinesterase levels had returned to normal, so cholinesterase is just an effect marker, and not necessarily a direct marker of an adverse effect. Dr. Factor-Litvak suggested that perhaps cholinesterase is an intermediate marker on a pathway to another outcome.

Dr. Rooney asked the panel for clarification: should cholinesterase be considered as only a biomarker of exposure? If so, does that preclude its use in the conclusions? Or can NTP provide additional language in the text to better describe the situation?

Dr. Engel commented that cholinesterase should be listed and interpreted as an effect. However, what is being measured in the human studies is a surrogate of the effect and should be clearly communicated as such. Dr. Factor-Litvak agreed that intermediate markers are important.

Dr. Factor-Litvak called for a motion on the conclusion of a high level of evidence for the initial time period in human studies. Dr. Engel moved to accept the level of evidence conclusion as written, Dr. Peter Blain seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the intermediate time period in human studies. Dr. Engel moved to change the level of evidence conclusion to low, Dr. Moser seconded. The panel voted 7 yes, 0 no, 0 abstentions to change the level of evidence conclusion to “low.”

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the extended time period in human studies. Dr. Peter Blain moved to accept the level of evidence conclusion as written, Dr. Engel seconded. The panel voted 7 yes, 0 no, 0 abstentions.

G.5.2. Visual and Ocular Effects

G.5.2.1. Presentation

Mr. Chris Sibrizzi from ICF presented the draft monograph information on visual and ocular effects.

Mr. Sibrizzi described the body of evidence as well as factors that increased or decreased NTP’s confidence considerations for both the animal and the human studies. The confidence ratings and corresponding level of evidence conclusions on the body of evidence for animal studies were:

- Initial time period (>24 hours to 7 days): moderate confidence, inadequate level of evidence

- Intermediate time period (8 days to 1 year): very low confidence, inadequate level of evidence
- Extended time period (>1 year): very low confidence, inadequate level of evidence

The confidence ratings and corresponding level of evidence conclusions for the body of evidence for human studies were:

- Initial time period (>24 hours to 7 days): moderate confidence, moderate level of evidence
- Intermediate time period (8 days to 1 year): moderate confidence for 2 cross-sectional studies and very low confidence for 8 case reports/series, moderate level of evidence
- Extended time period (>1 year): very low confidence, inadequate level of evidence

Dr. Factor-Litvak asked if it is possible to misclassify a poorly done study with some evidence for an association, or no evidence for an association as "inadequate" rather than "low" level of evidence. Dr. Rooney indicated that the NTP methodology would specifically indicate that the level of evidence was inadequate to determine if there was an effect rather that stating that nothing was reported.

G.5.2.2. Peer-Review Comments and Panel Discussion on Visual and Ocular Effects, Animal Data

Dr. Block, first reviewer, commented that the analysis was appropriate and agreed with the final level of evidence conclusions. However, she commented that the entry of "unexplained inconsistency" (Table 10 in the draft monograph) for an initial study was more a reflection of insufficient data. Dr. Block did not consider the confidence conclusion for the initial time period to be moderate but agreed that the level of evidence conclusion was inadequate because of the insufficient data. In addition, she thought that one of the studies included in the extended time period should technically be classified as belonging to the intermediate period.

Dr. Moser, second reviewer, concurred with Dr. Block's analysis and had nothing to add.

Dr. Rooney responded that NTP would consider a change in the confidence call from moderate to low for the initial time period. Regarding time period classifications, animal studies of >90 days were included in the extended time period. Clarification would be provided in the revised monograph.

Dr. Eisenkraft pointed out that the 2017 Egoz et al. study was mischaracterized in the monograph. Dr. Rooney agreed and indicated that the text would be revised to reflect his comment.

Dr. Baud suggested that an inadequate level of evidence for the initial period was too conservative and should be upgraded to low.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the initial time period in animal studies. Dr. Baud moved to change the level of evidence conclusion to low, and no one seconded. Dr. Block moved to accept the level of evidence conclusion as inadequate, Dr. Moser seconded. The panel voted 5 yes, 2 no, 0 abstentions. Drs. Baud and Eisenkraft both considered the inadequate level of evidence too conservative.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the intermediate time period in animal studies. Dr. Block moved to accept the level of evidence conclusion as written, Dr. Moser seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the extended time period in animal studies. Dr. Engel moved to accept the level of evidence conclusion as written, Dr. Peter Blain seconded. The panel voted 7 yes, 0 no, 0 abstentions.

G.5.2.3. Peer-Review Comments and Panel Discussion on Visual and Ocular Effects, Human Data

Dr. Baud, first reviewer, mentioned a difference in the number of cases in the different time periods as a cause for concern.

Dr. Beard, second reviewer, reiterated his earlier comment about case series versus cohorts, although it would not affect the level of evidence conclusion. Referencing Dr. Factor-Litvak's previous comments on confounders and effect measure modifiers, Dr. Beard recommended upgrading the level of evidence from inadequate to low for the extended time period, as the Nakajime 1999 study had controlled for confounding.

Dr. Rooney asked Dr. Baud to elaborate on his concern about the number of cases in the different time periods. Dr. Baud said he was concerned that the initial time period only had about 300 patients, whereas the intermediate and extended time periods had closer to 3,000. Dr. Rooney said NTP would go back and look at the studies involved.

Referring to the visual and ocular responses, Dr. Peter Blain said there was a common thread running through the monograph suggesting an effect following exposure.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the initial time period in human studies. Dr. Beard moved to accept the level of evidence conclusion as written, Dr. Peter Blain seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the intermediate time period in human studies. Dr. Engel moved to accept the level of evidence conclusion as written, Dr. Block seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the extended time period in human studies. Dr. Beard moved to change the level of evidence conclusion to “low.” Dr. Engel seconded. The panel voted 7 yes, 0 no, 0 abstentions to change the level of evidence conclusion from inadequate to low for the extended time period.

G.5.3. Learning, Memory, and Intelligence Effects

G.5.3.1. Presentation

Dr. Robyn Blain from ICF presented the draft monograph information on learning, memory, and intelligence effects.

Dr. Robyn Blain and Mr. Sibrizzi described the body of evidence as well as factors that increased or decreased NTP’s confidence considerations for the animal and human studies, respectively. The confidence ratings and corresponding level of evidence conclusions for the body of evidence for animal studies were:

- Initial time period (>24 hours to 7 days): moderate confidence, moderate level of evidence
- Intermediate time period (8 days to 1 year): moderate confidence, moderate level of evidence
- Extended time period (>1 year): low confidence, low level of evidence

The confidence ratings and corresponding level of evidence conclusions for the body of evidence for human studies were:

- Initial time period (>24 hours to 7 days): no confidence rating, inadequate level of evidence
- Intermediate time period (8 days to 1 year): low confidence for 1 cross-sectional study and low confidence for 2 case reports, low level of evidence
- Extended time period (>1 year): moderate confidence for 2 cross-sectional studies and very low confidence for 2 case series, moderate level of evidence

Responding to a question posed by Dr. Factor-Litvak, Dr. Robyn Blain indicated that the results from human studies in the monograph were not stratified by sex. Dr. Factor-Litvak further asked if intelligence was considered or if memory and executive function were measured in any of the human studies. Dr. Robyn Blain cited one case report of an Army sergeant exposed to sarin who underwent IQ testing. He had difficulty remembering numbers, a memory effect.

Dr. Peter Blain was concerned about translating neurobehavioral animal studies to humans as done in the monograph. He pointed out that humans have higher executive function than what is achievable in non-humans. He asked if the most common change between the animal and human studies was memory. Dr. Robyn Blain confirmed that the most common neurobehavioral effect observed in the animal and human studies involved memory.

G.5.3.2. Peer-Review Comments and Panel Discussion on Learning, Memory, and Intelligence Effects, Animal Data

Dr. Block, first reviewer, agreed with most of the animal data. However, she was somewhat concerned about the need to combine several different types of tests into one overarching category. She thought the confidence rating for the intermediate time period was low but could

be convinced that it should be moderate. She noted conflicting analyses, particularly in the rat discussion.

Dr. Moser, second reviewer, was concerned about the assessments and interpretations of the studies. She felt that in terms of the methods description and evaluation, the data presentation and study quality factors need to be looked at again. Dr. Moser agreed with a downgrade in confidence rating. However, she thought that some of the information was incorrect. While NTP stated that only the Grauer 2008 paper reported how the animals were randomized to treatment groups, Dr. Moser was unable to find that information in the paper. More importantly, three other studies (Genovese 2009, Pearce 1999, and Muggleton 2003) clearly assigned the animals to the treatment groups to balance performance factors – which she suggested is a standard and appropriate way to assign pre-trained animal to different groups to protect against pre-existing differences in their performance. Regarding outcome assessments, Dr. Moser indicated that eight of nine studies used automated equipment, while the monograph stated that only five studies used automated equipment. An automated visual tracking system that collects and analyzes all data was used for both water maze studies (Allon 2011 and Grauer 2008). Noting a comment in the heat map stating that it was unclear if a particular system analyzed the data, Dr. Moser confirmed that it did, citing her 20 years of experience. She indicated that the Wolthuis 1995 study also used a completely automated computer system. However, there was no information on how the data were collected for the Kassa 2001b T-maze method. It can be assumed that the data were collected by the observer, but it is unknown if the observer was blinded.

The summary for the overall discussion states that all the learning and memory tests used acceptable methods, but Dr. Moser suggested this is not the case. According to Dr. Moser, the water maze procedure used by Allon 2011 and Grauer 2008 studies failed to conform to the standard method originally described by Morris for the Morris Water Maze. Additionally, the Grauer 2008 study used a non-standard approach for putting the rats on the platform and changed the position of the platform daily to test reference memory. While Grauer 2008 stated that their procedure did not impact the outcome, Dr. Moser stated that many other studies have shown otherwise. The cues can alter the cognitive processes that are used by the animals to complete the task. So, this cannot be considered a true assessment of reference memory. The only dependent variable in the Kassa study (Kassa 2001b) with the T-maze was time to reach the goal box, and this was greatly impacted by motor changes. Without any other data on motor functioning, the increased latency cannot be considered a clear cognitive effect. Furthermore, T-mazes are typically used for positional discrimination studies, either using a spontaneous or delayed alternation method, which was not the case in the Kassa study (Kassa 2001b). Dr. Moser indicated that in fact, the Kassa 2001b study used arms that were different colors, so instead of evaluating positional or spatial discrimination, it is actually evaluating cued discrimination. She suggested that these points are all very important for understanding the data obtained from these studies.

In the initial time period, Dr. Moser continued, NTP concluded that the seven studies represented consistent effects. She concluded that these studies do not actually represent evidence of cognitive effects in rats and marmosets, but instead provide inadequate evidence of effects, or possibly even evidence of no effect. The Kassa studies (Kassa 2001b; Kassa 2002; Kassa 2004) used the T-maze and the Y-maze. One study reported an effect on latency, which is not a clear cognitive effect. The 2002 and 2004 Kassa studies used a Y-maze and reported increased latency to the goal box. The studies, which also measured arm entries, reported no increase in the entry error, that is, entering into the wrong arm. She indicated that the error rate is a better measure of memory compared to latency, which is affected by motor function. For this reason, Dr. Moser said she interpreted the data on latency as a measure of motor function, not a specific cognitive effect. She noted that the 2002 and 2004 Kassa studies both report data that are almost exactly the same, although the figures are graphed differently and with different scales.

Dr. Moser considered the Genovese 2009 study misrepresented in the monograph. The statistical analysis in the paper does not support the monograph statement that total errors were significantly altered in the first block, and that there were no overall significant dose effects and no dose-by-block interactions. She indicated that no further analyses should be conducted in the absence of an overall significant effect; however, the Genovese 2009 author proceeded with the step-down analysis and found effects in the first block. NTP's assumption that this was meaningful directly contradicts the authors' summary, who stated in their discussion that no differences existed on measures of accuracy (working and reference errors) and no difference existed in completion time between rats in the first single exposure.

Dr. Moser continued, and indicated that although the monograph summary describes the results of the monkey studies as inconsistent, the studies very consistently found no effect. The Wolthuis 1995 and Muggleton 2003 studies showed acute effects of treatment that did not persist past the day of exposure, and the Pearce 1999 study reported no negative effects on behavior. On the other hand, both the Muggleton 2003 and Pearce 1999 studies reported improved error rates and improvement on certain components of discrimination sequences in the days after dosing, so there was consistent evidence of no impairment to learning or memory, and some suggestion of improved performance in the initial time period.

These studies consistently demonstrate a lack of effects, except for endpoints that are based on motor functions rather than specifically learning and memory, as well as potentially improved function on some measures. None of the data showed a true dose response. Given these questionable findings, Dr. Moser rated these studies with a low final confidence rating.

The intermediate time period effects are based mostly on some of the same papers that provided the data for the initial time period, so Dr. Moser indicated she would not go over those data again. Two additional papers (Allon 2011 and Grauer 2008) relevant for this time period came from the same laboratory. Both studies used the same non-standard procedure for the water maze with only the Grauer 2008 paper reporting little improvement in latency between trials on the same day and across days in treated rats. While this clearly suggests effects on learning and memory, severe toxicity was also observed, including convulsions and high mortality after exposure. Convulsions produced by a variety insults can cause hippocampal damage, which, in turn, can impact maze performance. Therefore, Dr. Moser stated it is unclear if the water maze effects were due to the sarin or if they were the result of the sarin-induced convulsions. The Allon 2011 paper, using a lower, non-lethal, exposure level which produced limited toxicity (10%) in the exposure group, reported no change in water maze behavior, supporting Dr. Moser's assertion that the observed learning and memory effects in Grauer 2008 are more a consequence of the debilitating toxicity, including convulsions. Taken together, Dr. Moser thought there was little evidence of impaired cognitive function in the intermediate time period. The inconsistency of these findings, with the effects on motor function and improved performance, warrant another downgrade. Also, upgrading based on the dose-response is not appropriate in studies where it looks like the data were based on motor function, not cognition.

Dr. Moser's comments on the studies in the extended time period were the same as her comments on the studies in the intermediate time period, as the water maze studies and a single the monkey study were the only ones that evaluated the longer time period. She saw these papers as supporting no effect or being inadequate to support any kind of effect on learning and memory in animals.

Dr. Rooney said he appreciated Dr. Moser's comments and expertise. OHAT's risk of bias procedure calls for contacting study authors for additional details not included in a publication. Based on her comments, NTP would reconsider the risk-of-bias evaluations. He noted that they have tried to separate out motor activity effects from behavioral effects in previous evaluations and would consider edits to the text to address this issue.

Dr. Eisenkraft added that it is sometimes difficult to discriminate between the toxic effects of exposure and hypoxic or convulsion-related effects in the longer time periods. More clinical context should be provided when learning and memory deficits are considered.

Dr. Peter Blain agreed with Dr. Eisenkraft's comments. Higher-level effects are likely to be the result of damage to the hippocampus and limbic structures due to excitotoxic or hypoxic effects, and not necessarily a direct toxic effect of exposure to sarin.

Dr. Factor-Litvak brought up the potential impact of survivor effects as a potential source of bias in the animal studies. Dr. Moser said that learning and memory studies generally avoid consideration of survivor effects; they are not typically included in statistical analysis of the results.

Dr. Block suggested that the response mechanisms would be different for lower dose versus a higher dose in an acute exposure scenario. Longer-term effects could lead to persistent, chronic neuropathological effects.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the initial time period in animal studies. Dr. Moser moved to change the level of evidence

conclusion to “low.” Dr. Block seconded. The panel voted 7 yes, 0 no, 0 abstentions to change the level of evidence conclusion to “low.”

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the intermediate time period in animal studies. Dr. Moser moved to change the level of evidence conclusion to “low.” Dr. Block seconded. The panel voted 7 yes, 0 no, 0 abstentions to change the level of evidence conclusion to “low.”

Dr. Factor-Litvak called for a motion on the conclusion of a low level of evidence for the extended time period in animal studies. Dr. Block moved to accept the level of evidence conclusion as written. Dr. Moser seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Rooney asked the panel to comment on whether the learning and memory testing seen in the studies would be useful in testing therapeutics, given the changes across the board to a “low” level of evidence. Dr. Eisenkraft said that because of the low level of evidence, there must be more studies. Dr. Moser agreed that more studies are needed, with improved methods. Dr. Peter Blain said that higher-animal studies, such as in monkeys and non-human primates, would be more valuable in neurobehavioral assessments than studies in rodents. Dr. Factor-Litvak suggested that NTP recommend a standard protocol for testing, including rodent studies. Dr. Eisenkraft pointed out that some of the studies are deemed to be equivalent to clinical studies that cannot be performed in humans by the U.S. Food and Drug Administration (FDA); the FDA should be involved in helping with study design in such cases.

G.5.3.3. Peer-Review Comments and Panel Discussion on Learning, Memory, and Intelligence Effects, Human Data

Dr. Beard, first reviewer, repeated his earlier comments about case series versus cohort, and confounding. While he agreed with the NTP’s level of evidence conclusion, he disagreed with the learning and memory outcomes summaries in Table 11 (in the draft monograph) for the studies conducted by Miyaki in 2005 and Nishiwaki in 2001 and took issue with the treatment of small sample size. Dr. Beard suggested wording changes for both summaries for NTP’s consideration. He also disagreed with the inadequate level of evidence characterization for the intermediate period, as the statement contrasts with other sentences stating that human data provide low evidence.

Dr. Eisenkraft, second reviewer, reiterated the issues regarding long-term psychological effects, which cannot be differentiated between direct sarin exposure and stress from the sarin exposure event.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the initial time period in human studies. Dr. Engel moved to accept the level of evidence conclusion as written. Dr. Beard seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of a low level of evidence for the intermediate time period in human studies. Dr. Beard moved to accept the level of evidence conclusion as written. Dr. Engel seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the extended time period in human studies. Dr. Beard moved to accept the level of evidence conclusion as written. Dr. Eisenkraft seconded. The panel voted 7 yes, 0 no, 0 abstentions.

G.5.4. Nervous System Morphological and Histological Changes

G.5.4.1. Presentation

Dr. Robyn Blain from ICF presented the draft monograph information on nervous system morphological and histological effects.

Dr. Robyn Blain and Mr. Sibrizzi described the body of evidence as well as factors that increased or decreased NTP’s confidence considerations for animal and human studies, respectively. The confidence ratings and corresponding level of evidence conclusions for the body of evidence for animal studies were:

- Initial time period (>24 hours to 7 days): moderate confidence, moderate level of evidence
- Intermediate time period (8 days to 1 year): moderate confidence, moderate level of evidence

- Extended time period (>1 year): no confidence rating, inadequate level of evidence

The confidence ratings and corresponding level of evidence conclusions for the body of evidence for human studies were:

- Initial time period (>24 hours to 7 days): no confidence rating, inadequate level of evidence
- Intermediate time period (8 days to 1 year): low confidence, inadequate level of evidence
- Extended time period (>1 year): moderate confidence for 1 cross-sectional study and low confidence for 1 cast report, moderate level of evidence

Dr. Factor-Litvak asked if the morphological changes seen in the human data were definitively attributed to sarin exposure. Dr. Robyn Blain explained that while an MRI in the single case report showed changes in the brain, definitive attributions to sarin exposure could not be done with any certainty. That case report would have risk-of-bias concerns. She added that the moderate confidence level was based more on the cross-sectional study.

Dr. Eisenkraft requested that clinical assessments, such as evidence of seizures, be added to the monograph. As Dr. Robyn Blain did not recall reports of seizures, she indicated that she would review the studies.

G.5.4.2. Peer-Review Comments and Panel Discussion on Nervous System Morphological and Histological Changes, Animal Data

Dr. Peter Blain, first reviewer, noted that few animal studies met the criteria for inclusion. However, there were consistent findings within those studies, particularly regarding limbic structures in the brain and those findings provided evidence for long-term effects. While there were reports of nerve fiber degeneration, fitting with the electrophysiology of the peripheral nervous system, it cannot be determined if this is a direct effect of sarin exposure or due to either a respiratory effect (hypoxia), or to cytotoxic effect resulting from seizure activity.

Dr. Block, second reviewer, opined that even if the effects resulted from an indirect mechanism leading to neurotoxicity, it is still an effect of sarin exposure. She was less enthusiastic about the animal evidence and would downgrade both the initial and intermediate level of evidence conclusions to “low.” There needs to be some method of quantification, even if it is not the same as current methods. Some of the studies do not hit that bar, she observed, and she cited several examples of studies that did not quantify appropriately. Nonetheless, Dr. Block believed that there was evidence to suggest that something is occurring, but only at a gross level. She noted that neuropathology would get worse over time, and there would be ongoing pathology. Dr. Block recommended downgrading the level of evidence rating based on low confidence in the evidence based on low quality of the studies.

In response to a question posed by Dr. Rooney, Dr. Block replied that there was not any specific aspect of the OHAT method that caused her to downgrade the level of evidence; rather the methods used in the studies had been modified over time because they had much bias. Dr. Block indicated that it is important to note the level of assay stringency reflects the time when the study was conducted.

Dr. Peter Blain, speaking from clinical experience, cited organophosphate-related hypoxia and seizure activity causing long-term brain damage. Quantification does not necessarily conform with clinical experience as the effects are not necessarily the result of a direct toxic mechanism of sarin itself; it is the secondary effects, such as damage to the brain, that are of the most concern.

In response to a question posed by Dr. Factor-Litvak, Dr. Robyn Blain indicated that none of the investigators had stratified by levels of hypoxia or convulsions in the animals.

Dr. Eisenkraft stated that most of the models did not represent a realistic scenario, as neither oxygen nor ventilation was provided to the animals, which would be highly unacceptable in humans. Also, the exposure levels in many of the studies were extremely high. Dr. Eisenkraft was unsure if the long-term effects observed were direct or indirect effects of sarin, although it does not matter much, because the effects must be treated. He hoped that one of the things to emerge from the review would be protocol guidelines for future animal studies.

Dr. Peter Blain suggested that a higher animal model would be more advantageous to use, for a more realistic outcome.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the initial time period in animal studies. Dr. Eisenkraft moved to accept the level of evidence conclusion as written. Dr. Peter Blain seconded. The panel voted 5 yes, 2 no, 0 abstentions. Dr. Block explained her no vote as stemming from her belief that the conclusion should have been "low," based on the fact that the studies were not stringent. Dr. Moser also voted no, for similar reasons.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the intermediate time period in animal studies. Dr. Eisenkraft moved to accept the level of evidence conclusion as written. Dr. Peter Blain seconded. The panel voted 5 yes, 2 no, 0 abstentions. Dr. Block explained her no vote, reiterating the conclusion should have been "low," because the studies were not stringent. Dr. Moser also voted no, for similar reasons.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the extended time period in animal studies. Dr. Block moved to accept the level of evidence conclusion as written. Dr. Peter Blain seconded. The panel voted 7 yes, 0 no, 0 abstentions.

G.5.4.3. Peer-Review Comments and Panel Discussion on Nervous System Morphological and Histological Changes, Human Data

Dr. Baud, first reviewer, indicated that there is a very limited body of knowledge, with no studies in the initial time period and only one case report for a single U.S. military member for the intermediate time period. That patient underwent MRI and PET scans, both of which were normal for the brain and the spine. There were two studies in the extended time period: one case report and one cross-sectional study, both stemming from the Tokyo, Japan, subway attack. Dr. Baud summarized the two studies and indicated that it was quite difficult to characterize the conclusion for the extended time period as moderate or low.

Dr. Engel, second reviewer, agreed that there was a very limited amount of evidence, with two case reports of one person each and one cross-sectional study of small-to-moderate size. He found it interesting that in the Loh study, the patient showed no effect on the MRI, but did have a substantial decrement in cholinesterase levels. He said he would score that (the intermediate time period) as a very low level of evidence. As to the extended time period, he was curious whether some of the reported effects could be post-traumatic stress disorder (PTSD)-related. However, he agreed with an earlier comment that it should not matter whether the effect is a direct sarin insult or an indirect effect mediated through some other biological mechanism. A different study design may have been able to address that issue. He agreed with the conclusion of moderate level of evidence for the extended time period.

Dr. Rooney indicated that Dr. Baud's conclusions were clear. He asked Dr. Baud to submit his specific comments on the two case reports and one cross-sectional study in writing so they could be considered in revisions, because his audio was poor during his comments.

Dr. Robyn Blain said that the PTSD issue would arise later in some of the other studies to be considered. It is difficult to tease out what caused the PTSD. Few sarin studies specifically considered PTSD.

Dr. Eisenkraft indicated that there are many similarities between the long-term neurobehavioral and morphological effects of PTSD and either sarin or other organophosphates. He disagreed with a sentence on page 60 of the monograph saying that "given the subject's symptoms after the exposure, it is likely that the effects are related to the sarin exposure." He felt that the symptoms were more of an anoxic event. In his opinion, the Himuro 1998 study looked like hypoxic anoxia not directly related to sarin. He said that it can be seen that sarin exposure causes long-term damage, with a moderate to high level of confidence. It should be decided if the discussion should address direct or indirect effects.

Dr. Peter Blain said that in recent clinical experience with sarin and other organophosphate nerve agents, for patients that survived, their physiology supported evidence that hypoxia and seizure activity were prevented. Thus, these patients can survive with minimal residual damage by controlling hypoxia and seizure activity.

Dr. Factor-Litvak asked if the evidence suggests a dose-response relationship. Dr. Peter Blain replied that people with low-level organophosphate exposures can and do survive. Dr. Eisenkraft indicated that if the seizures are not stopped as early as possible, they will become more severe and more treatment-resistant, citing evidence from both sarin and other organophosphate nerve

agents. He said he was not aware of an accurate dose-response curve with nerve agents for humans.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the initial time period in human studies. Dr. Engel moved to accept the level of evidence conclusion as written. Dr. Eisenkraft seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of an inadequate level of evidence for the intermediate time period in human studies. Dr. Baud moved to accept the level of evidence conclusion as written. Dr. Engel seconded. The panel voted 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak called for a motion on the conclusion of a moderate level of evidence for the extended time period in human studies. Dr. Eisenkraft moved to accept the level of evidence conclusion as written. Dr. Baud seconded. The panel voted 7 yes, 0 no, 0 abstentions.

G.5.5. Other Outcomes that Did Not Reach Hazard Conclusions

G.5.5.1. Presentation

Dr. Rooney presented material to the panel regarding other outcomes that did not reach hazard conclusions.

There was inadequate evidence to determine whether there is an association with acute sarin exposure for the following outcomes:

- Sleep disruption
- Anxiety and fear
- Avoidance and depression
- Activity and strength
- Other neurological symptoms
- Electroencephalogram (EEG) data
- Other sensory effects

The studies that failed to reach a level of evidence rating that would support a hazard conclusion were placed in Appendix 4 of the monograph. The confidence ratings were low, very low, or single studies reporting no evidence of health effects, and for some time periods there were no data. Some of the studies had very serious risk-of-bias concerns. In general, there were very few overlapping endpoints considered across studies.

G.5.5.2. Peer-Review Comments and Panel Discussion on Other Outcomes that Did Not Reach Hazard Conclusions

Dr. Factor-Litvak opened the discussion for comments from panel members.

Dr. Eisenkraft noted that the first four bullets on the slide (sleep disruption, anxiety and fear, avoidance and depression, and activity and strength) could be combined into a PTSD endpoint. Dr. Rooney said that PTSD had initially been considered as a potential outcome. Dr. Robyn Blain indicated that it was not included as few studies had specifically considered PTSD, and it was not possible to determine if symptoms were caused by PTSD from the stress of the attack or were directly sarin-related.

Dr. Beard said it seemed that in most sections of the report, standard observational studies were separated from case series studies. He cited the Yokoyama 1998c study as an example and indicated that a low level of evidence was as high as he would go given risk-of-bias concerns.

Dr. Baud suggested that if the anoxic brain damage is considered to be somewhat related, even indirectly, to sarin, then perhaps it should also be considered that sarin may indirectly cause PTSD.

Dr. Factor-Litvak rephrased the question: are the PTSD symptoms more directly related to the event than to sarin? Could PTSD symptoms be considered to be adverse associations due to sarin, including the event and the actual exposure to the compound?

Dr. Baud suggested that the anoxic brain damage be accepted as a downstream effect related to sarin exposure. Dr. Rooney said he had difficulty with the idea that the data could separate whether the sarin exposure resulted in brain damage directly or if sarin would lead to anoxia which would result in brain damage. Similarly, he noted that the attack had two different aspects,

one being sarin-related, the other being the attack itself, which is not specific to the chemical exposure; this was the portion that was difficult to separate out to account for PTSD. Dr. Rooney said he would welcome suggestions from the panel on how to communicate that aspect in the monograph.

Dr. Engel commented that distinguishing the effects of events-induced PTSD versus sarin-induced PTSD was an important issue and suggested several potential approaches to studying the problem. He noted that it is an issue of concern that warrants further investigation.

Dr. Factor-Litvak suggested looking at the evidence from the studies that captured sarin plus the attack, and then to compare them to other studies that had attack but without sarin, to see if there were differences in some of the outcomes. Dr. Rooney appreciated the suggestion.

Dr. Eisenkraft raised the issue of cultural differences between nations and peoples.

Dr. Moser commented that the animal studies were even more minimal than described in the monograph, and there is not much data on the endpoints.

Dr. Peter Blain pointed out that sleep disruption has been a feature of anecdotal reports of acute exposures to organophosphates.

In response to a question posed by Dr. Baud, Dr. Rooney replied that there was no particular ranking for the signs and symptoms listed on the slide.

Dr. Factor-Litvak summarized the discussion, indicating general agreement among the panel that the outcomes at present do not reach the level that would warrant a hazard conclusion. With no hazard conclusions, there would be no vote for the section. She asked for any dissent from panel members. Dr. Eisenkraft said he did not disagree but indicated that it would be important to state the identified issues in order to study them in future investigation. Dr. Rooney said that the identified signs and symptoms would be acknowledged in the monograph.

Dr. Peter Blain requested that data from single fiber electromyography, used to biomonitor patient recovery, be included in the monograph as well.

G.5.6. Integration of Animal and Human Evidence for Reaching Hazard Categorization

Dr. Rooney presented material to the panel on the process of evidence integration to reach overall hazard conclusions.

He noted that the process includes two stages: an initial hazard conclusion, the result of considering human and animal evidence together, and a final hazard conclusion, which considers the impact of other data such as relevant mechanistic data and biological plausibility of effect. The final hazard conclusions consider whether there is strong support to increase the hazard identification conclusion, or strong opposition to decrease the hazard identification, or no impact on the hazard identification conclusion.

The final hazard conclusions for acute sarin exposure were as follows:

- Initial time period: **Known to be a neurological hazard to humans** based on suppression of cholinesterase.
- Intermediate time period: **Suspected to be a neurological hazard to humans** based on multiple health effects.
- Extended time period: **Suspected to be a neurological hazard to humans** based on multiple health effects.

Dr. Factor-Litvak opened the floor for comments from panel members.

Dr. Eisenkraft indicated that he could not accept the “suspected” hazard conclusion for the intermediate and extended time periods and recommended that the conclusion be changed to “presumed.” Asked by Dr. Factor-Litvak to elaborate, Dr. Eisenkraft indicated that his suggestion was based on his working experience with other organophosphates, as well as the devastating effects resulting from sarin exposure. Dr. Rooney asked Eisenkraft if he could identify specific evidence that would support a conclusion of “presumed.”

Dr. Factor-Litvak noted that because the evaluation is meant to be transparent to both the scientific and non-scientific communities, and reproducible, the hazard conclusions must be totally based on the published evidence presented during the review.

Dr. Peter Blain suggested including a reference to the relationship between dose and long-term outcomes in the monograph. Dr. Rooney considered that an excellent communication point, such a message would be included.

Since some of the individual health effects for the extended time period were non-classifiable, Dr. Moser asked how a hazard conclusion of "suspected" was determined. Dr. Rooney indicated that any of the time periods could be supported by the body of evidence for a single health effect.

Dr. Baud said that he did not feel that there was sufficient data to support raising the hazard conclusion to a higher level.

Dr. Factor-Litvak said there would be three votes, one for each time period. She called for a vote on the hazard conclusion ("known") for the initial time period. Dr. Baud moved to accept the conclusion as written, Dr. Engel seconded. The vote was 7 yes, 0 no, 0 abstentions.

Dr. Factor-Litvak asked for a motion to accept the hazard conclusion ("suspected") for the intermediate time period. Dr. Baud so moved; Dr. Moser seconded. The vote was 6 yes, 1 no, 0 abstentions. Dr. Eisenkraft explained his no vote based on his prior remarks (prior experience with organophosphates, severity of sarin-induced effects).

Dr. Factor-Litvak asked for a motion to accept the hazard conclusion ("suspected") for the extended time period. Dr. Engel so moved; Dr. Baud seconded. The vote was 6 yes, 1 no, 0 abstentions. Dr. Eisenkraft said he voted no based on the same concerns he had previously expressed.

G.6. Closing Remarks on the Draft NTP Monograph

Dr. Factor-Litvak asked the panel to comment on the overall organization of the monograph, particularly in terms of clarity and coherent presentation of the information and its synthesis.

Dr. Engel indicated that within the limit of the published data, the conclusion in the monograph is the strongest statement that can be made. Following up on Dr. Eisenkraft's comments, there would be a case for a stronger case on sarin in the intermediate and extended time periods, based on anecdotal evidence. The biggest issue in reaching a stronger conclusion is the lack of data.

Dr. Factor-Litvak said that it needs to be clear that there is a substantial amount of classified evidence that could not be incorporated in the monograph.

Dr. Eisenkraft thought that the inclusion of raw data from studies would enable better analysis.

Dr. Baud agreed with Dr. Engel that the body of knowledge did not allow the conclusions to go further. There is value in identifying gaps in current knowledge; it would be helpful to highlight them in the monograph.

Dr. Beard asked if NTP could recommend that future studies should include an actual screener for PTSD, so that the issue could be better teased out. Dr. Factor-Litvak felt that that could probably be included as a future recommendation.

Closing the meeting, Dr. Factor-Litvak thanked the reviewers for their hard work and excellent comments. She also thanked the NTP staff and ICF staff for preparing an excellent monograph and a productive meeting. Dr. Rooney thanked Dr. Factor-Litvak for her efforts in chairing the meeting.

Dr. Maull added her thanks to everyone.

Dr. Factor-Litvak adjourned the meeting at 3:00 p.m. EST on February 4, 2019.

Appendix H. Protocol History

The protocol is available in Appendix I.

Table H-1. 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.

Appendix I. Supplemental Files

The following supplemental file is available at https://doi.org/10.22427/NTP-DATA-MGRAPH-6.

I.1. Protocol Information

Protocol
Sarin_protcol.pdf

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