



## REGION 8

DENVER, CO 80202

April 13, 2026

### **MEMORANDUM**

**SUBJECT:** Technical Memorandum on Updating the Libby Amphibole Asbestos Literature Review for Characterization of Potential Autoimmune Health Outcomes

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#### **1.0 Executive Summary**

Concerns and uncertainty have been expressed by the affected community about the public health protectiveness of EPA's remedial response at the Libby Amphibole Asbestos (LAA) Superfund Site in and around Libby, Montana, specifically regarding new research on outcomes relevant to autoimmune diseases associated with LAA exposures (LASOC, 2025). In response, EPA Region 8 conducted a review of published scientific literature identifying human and experimental animal studies evaluating LAA exposure and autoimmune outcomes that were not previously evaluated in the 2014 Integrated Risk Information System (IRIS) Toxicological Review of LAA cancer and noncancer hazard (USEPA, 2014), which provided the scientific basis for the risk-informed response actions taken. This newly available scientific information was evaluated to understand the relationship between LAA exposure and markers of systemic autoinflammatory diseases (SAID), which can indicate underlying autoimmune disease. This assessment also evaluated the relationship between SAID outcomes and the pulmonary and/or pleural hazards previously described by USEPA (2014).

Data from these new studies continue to support the conclusion that SAID endpoints are potential adverse health outcomes in humans following exposure to LAA, consistent with previous observations (USEPA, 2014). However, there remains a lack of quantitative exposure data paired with validated autoimmune endpoints in either human epidemiological or experimental animal inhalation studies, as noted previously (USEPA, 2014). The available evidence from human and experimental animal studies further suggests that autoimmune outcomes may be associated with pulmonary and/or pleural effects of LAA exposure.

#### **2.0 Experimental Approach**

A systematic literature search and review was conducted to identify human and experimental animal studies evaluating exposure to LAA and reporting SAID-relevant endpoints, which were not previously evaluated by USEPA (2014). At the time, limitations in the number, scope, and design of available studies precluded any quantitative estimates associating LAA exposure with autoimmune disease outcomes (USEPA, 2014). The

methodology, results, and conclusions from the current literature review update are detailed in Appendix A. Briefly, the initial literature search identified 264 potential references as being relevant to the primary objectives of this review and not previously evaluated by USEPA (2014). Following screening and full-text review, only 15 references remained which evaluated health effects outcomes in full-length study reports, consisting of 6 human studies of LAA-exposed persons and 9 experimental animal studies, although only 1 of the 9 experimental animal studies evaluated inhalation exposures.

No quantitative exposure-response data were available in human studies; mortality from SAIDs was significantly increased in an update from a large community-based health-screening program from Libby, Montana, and higher anti-fibroblast antibodies (a marker of potential autoimmune disease) were reported in a separate study of LAA-exposed subjects. Across 8 experimental animal studies, a variety of markers of autoimmune disease were elevated based on administration of LAA via non-physiological routes of exposure (e.g. intratracheal instillation, gavage, or intraperitoneal injection, which are less directly relevant to human inhalation exposures). The single experimental animal inhalation study identified did not report fiber counts (which is how human exposures are determined), did not evaluate endpoints directly relevant to SAID (e.g., serum autoantibodies), and reported only a transient increase in a serum marker of acute inflammation associated with the highest concentration of LAA evaluated.

### **3.0 Conclusions**

The newly available data, not considered in the previous IRIS Toxicological Review (USEPA, 2014), provides suggestive evidence that autoimmune effects relevant to SAID may be associated with pulmonary and/or pleural effects of LAA exposure. Data from these studies support the conclusion that SAID endpoints are potential hazards of concern in humans following exposure to LAA, consistent with the conclusions noted previously by USEPA (2014). However, a lack of quantitative exposure information necessary to conduct a dose-response evaluation of LAA exposures and SAID outcomes remains, as noted previously by USEPA (2014). Due to this lack of quantitative exposure data in human studies and lack of validated autoimmune endpoints in the only animal inhalation study identified, available data are still inadequate to derive a reference concentration (RfC) value based upon autoimmune effects. Until sufficient data are collected and made available, it cannot be determined how a RfC value derived for autoimmune effects would differ from the existing RfC based upon pleural outcomes in humans exposed to LAA.

A quantitative Uncertainty Factor adjustment of 3x was specifically included in the current LAA RfC to account for uncertainties regarding the relationship between exposures and autoimmune disease outcomes (USEPA, 2014). This remains the best scientifically supported approach to protect public health.

### **4.0 References**

LASOC, (2025). Libby Asbestos Superfund Site Five Year Review, Delisting Sitewide- Public Health Emergency. Letter from Libby Asbestos Superfund Oversight Committee (LASOC), addressed to Cyrus Western, Administrator, EPA Region 8. 27 June.

USEPA, (2014). Toxicological review of libby amphibole asbestos: In support of summary information on the Integrated Risk Information System (IRIS) [EPA Report]. (EPA/635/R-11/002F). Washington, DC: Integrated Risk Information System, National Center for Environmental Assessment, Office of Research and Development. <https://iris.epa.gov/static/pdfs/1026tr.pdf/>

### **5.0 Appendix A**

Libby Amphibole Asbestos (LAA) Literature Review Technical Memorandum

## Appendix A

### LIBBY AMPHIBOLE ASBESTOS (LAA) LITERATURE REVIEW TECHNICAL MEMORANDUM

#### EXECUTIVE SUMMARY

Human and animal studies of LAA exposure and autoimmune outcomes that were not evaluated in the 2014 Integrated Risk Information System (IRIS) assessment of LAA ([U.S. EPA, 2014](#)) were reviewed to determine whether the reference concentration (RfC) value is protective of systemic autoinflammatory disease (SAID) endpoints. Data from these studies support the conclusion that SAID endpoints are potential hazards of concern in humans following exposure to LAA. However, due to lack of quantitative exposure data in human studies and lack of validated autoimmune endpoints (e.g., autoantibodies) in the only animal inhalation study identified, available data are not adequate to determine whether a RfC value derived for autoimmune effects would be lower or higher than the existing RfC. Available data provide suggestive evidence that autoimmune effects may be associated with pulmonary and/or pleural effects of LAA.

#### ACRONYMS

|            |                                                        |
|------------|--------------------------------------------------------|
| AFA        | anti-fibroblast antibodies                             |
| ANA        | antinuclear antibodies                                 |
| ANAA       | antinuclear auto-antibodies                            |
| ANCA       | anti-neutrophil cytoplasmic antibodies                 |
| ARD        | asbestos-related disease                               |
| ATSDR      | Agency for Toxic Substances and Disease Registry       |
| auto-dsDNA | double-stranded DNA autoantibodies                     |
| CARD       | Center for Asbestos Related Diseases                   |
| CCP        | cyclic citrullinated peptide                           |
| CI         | confidence interval                                    |
| DPPC       | 1,2-dipalmitoyl- <i>sn</i> -glycero-3-phosphocholine   |
| dsDNA      | double-stranded DNA                                    |
| DSPC       | distearoylphosphatidylcholine                          |
| ELISA      | enzyme-linked immunosorbent assay                      |
| ENA        | extractable nuclear antigens                           |
| HERO       | Health and Environmental Research Online               |
| Ig         | immunoglobins                                          |
| IL         | interleukins                                           |
| IRIS       | Integrated Risk Information System                     |
| KC/GRO     | keratinocyte chemoattractant/growth-regulated protein  |
| LAA        | Libby amphibole asbestos                               |
| LERP       | Libby Epidemiology Research Program                    |
| LOD        | limit of detection                                     |
| LPT        | lamellar pleural thickening                            |
| MCAA       | mesothelial cell antibodies                            |
| MCP-1      | monocyte chemoattractant protein-1                     |
| MDPHHS     | Montana Department of Public Health and Human Services |
| MeSH       | Medical Subject Heading                                |
| MHC II     | major histocompatibility complex class II              |
| NE         | not evaluated                                          |

|                   |                                                                    |
|-------------------|--------------------------------------------------------------------|
| NS                | not specified                                                      |
| PBS               | phosphate-buffered saline                                          |
| PCNA              | proliferating cell nuclear antigen                                 |
| PECO              | Population, Exposure, Comparator, and Outcome                      |
| PMN               | polymorphonuclear cell                                             |
| POD               | point of departure                                                 |
| QC                | quality control                                                    |
| RA                | rheumatoid arthritis                                               |
| RF                | rheumatoid factor                                                  |
| RfC               | reference concentration                                            |
| RNP               | ribonucleoprotein P                                                |
| SAID              | systemic autoinflammatory disease                                  |
| SD                | standard deviation                                                 |
| SE                | standard error                                                     |
| SMR               | standardized mortality ratio                                       |
| SSc               | systemic sclerosis                                                 |
| SLE               | systemic lupus erythematosus                                       |
| Th                | T helper                                                           |
| T <sub>H</sub> 1  | cytokine profile including IL-2, IFN- $\gamma$ , and TNF- $\alpha$ |
| T <sub>H</sub> 2  | cytokine profile including IL-4, IL-5, and IL-10                   |
| T <sub>H</sub> 17 | cytokine profile including IL-6 and IL-7                           |
| TNF               | tumor necrosis factor                                              |
| U.S. EPA          | U.S. Environmental Protection Agency                               |
| WBC               | white blood cell                                                   |
| WOS               | Web of Science                                                     |
| WRG               | W.R. Grace & Co.                                                   |

## BACKGROUND

The purpose of the LAA literature review was to identify and review studies of exposure to LAA and SAID endpoints that were not evaluated in the 2014 IRIS assessment of LAA ([U.S. EPA, 2014](#)). The IRIS assessment ([U.S. EPA, 2014](#)) reviewed epidemiology and animal studies and concluded that limitations in the number, scope, and design of these studies make it difficult to reach conclusions on the role of LAA exposure in autoimmune disease. Although epidemiology studies demonstrated an association between exposure to LAA asbestos and autoimmune diseases, including systemic lupus erythematosus (SLE), scleroderma, and rheumatoid arthritis (RA), they did not provide exposure-response information. Therefore, it was not known whether a lower point of departure (POD) or RfC value would be derived for autoimmune effects.

An updated literature search was performed to obtain new studies on LAA exposure and SAID endpoints including autoimmune diseases, autoantibodies, and other endpoints that may be related to the development or manifestation of SAIDs, with the primary goal to determine whether the LAA RfC is protective of autoimmune effects. Any information regarding a possible relationship between SAID and pleural effects of LAA was also noted.

## METHODS

### *Literature Search*

SRC conducted non-date-limited PubMed and Web of Science (WOS) database searches of LAA and autoimmune disease and outcomes, which were uploaded to a project page<sup>1</sup> on the U.S. Environmental Protection Agency (U.S. EPA) Health and Environmental Research Online (HERO) database on January 16, 2026. Search query strings are presented in Appendix A. Preliminary testing of search terms related to LAA exposure in PubMed and WOS indicated that date limits and inclusion of health effect terms were not needed to reduce the number of hits for screening. An additional strategy targeting amphibole asbestos in combination with specified autoimmune terms in PubMed (both Medical Subject Heading [MeSH] indexed terms as well as text words) captured articles related to autoimmunity that do not include “Libby” in the indexed terms or abstracts of the records. SRC tested the autoimmune terms provided by EPA Region 8 in the 2025\_Nov\_Lit-Search-Strings Word file and revised the query strings to ensure that all relevant citations were captured in the search.

### *Literature Screening*

Title/abstract and full-text screening was completed independently by two senior toxicologists to identify references that met the Population, Exposure, Comparator, and Outcome (PECO) criteria (see Table 1), and any conflicts between the toxicologists were resolved by discussion.

| Table 1. PECO Criteria for the LAA Literature Review |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PECO Element                                         | Evidence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <b>P</b> opulation                                   | Human studies and experimental animal studies; <u>excluding</u> any <i>in vitro</i> or non-whole mammalian system                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>E</b> xposure                                     | LAA only; other asbestos forms excluded<br><b>Human:</b> Quantitative measures of exposure <u>not</u> required                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>C</b> omparator                                   | <b>Human and Animal:</b> Any reasonable comparison, including vehicle controls, lower exposures, etc.; general population presumably unexposed<br><b>Human:</b> Also include case reports and ecological studies                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>O</b> utcome                                      | <b>Human and Animal:</b><br><u>SAID endpoints</u> , including (but not limited to) scleroderma, SLE, RA, systemic sclerosis (SSc), Sjögren Syndrome, sarcoidosis, and mixed connective tissue disease<br><u>Markers of autoimmune response</u> , including (but not limited to) antinuclear antibodies (ANA), antinuclear auto-antibodies (ANAA), extractable nuclear antigens (ENA), mesothelial cell autoantibodies (MCAA), anti-plasminogen antibodies, double-stranded DNA autoantibodies (auto-dsDNA), rheumatoid factor (RF), anti-neutrophil cytoplasmic antibodies (ANCA), serum immunoglobulins (Ig), anti-histone, anti-chromatin, anti-Ro52, anti-Ro60, immune complexes, Th17 cytokines, and anti-fibroblast antibodies (AFA) |

### *Study Evaluation of Key Studies*

A full study quality/risk-of-bias assessment was not performed for this project; however, a streamlined list of *a priori* criteria was developed for evaluating whether each study was adequate or deficient in key domains identified by [U.S. EPA \(2022\)](#), with core questions revised to be specific to LAA and

<sup>1</sup>Libby Amphibole Asbestos Literature Review (2026): <https://hero.epa.gov/project/4960/>.

autoimmune outcomes (where appropriate). Evaluation criteria were developed separately for epidemiology and animal studies and are presented in Appendix B. Two senior toxicologists independently performed study quality evaluation, and any conflicts were resolved via discussion to reach a final determination for each study.

#### *Data Extraction of Key Studies*

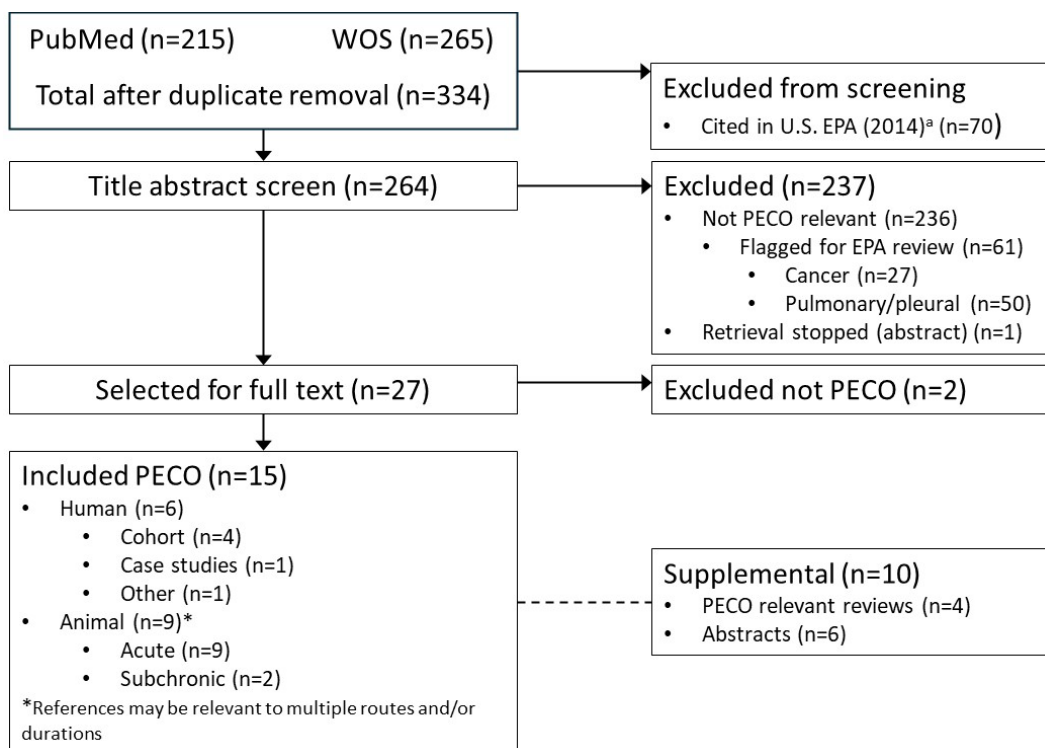
Tabular data extraction was performed by a senior toxicologist for all epidemiology and experimental animal studies and a second senior toxicologist performed quality control (QC) review to ensure accuracy and completion of all data tables. For epidemiological studies, data extractions included study design; subject number, sex, age, and inclusion and/or exclusion criteria; exposure metric and reported levels (or exposure categories); analysis details; and results. For animal studies, data extractions included species, strain, number and sex per group, age (if relevant), exposure route, vehicle (if relevant), duration, doses or concentrations, fiber type and characteristics, endpoints examined, and effects. An endpoint was extracted if: (1) it was an outcome mentioned in the PECO statement (Table 1) or (2) it was identified by study authors as relevant to assessing the potential development of autoimmune disease, despite lacking specificity for SAID (e.g., markers of inflammation, spleen weight, etc.). The specificity of extracted endpoints is discussed in the results section.

## **RESULTS**

#### *Literature Search and Screening*

The literature search resulted in 334 citations following duplicate removal across the PubMed and WOS databases. References that were cited in the LAA IRIS assessment ([U.S. EPA, 2014](#)) were removed prior to screening (n=70) resulting in 264 titles and abstracts for screening. Title and abstract screening excluded 237 references as not relevant: 236 references that did not meet PECO criteria and 1 reference for which retrieval was stopped (abstract-only not available in HERO). Of the references that did not meet PECO criteria, 61 were flagged for further U.S. EPA review due to relevance to known health effects associated with exposure to LAA (27 references evaluating exposure to LAA and cancer; 50 references evaluating exposure to LAA and pulmonary or pleural effects). After removing these references, the remaining 27 items proceeded to full-text review. An additional 2 references were excluded during full-text screening (did not meet PECO criteria) and the remaining 25 references were identified as PECO-relevant: 15 references included primary health effects data from full-length study reports, and 10 references contained other supporting data (4 reviews, 6 abstracts). A summary of the results of the literature search and screening is presented in Figure 1.

**Figure 1. Literature Flow Diagram**



<sup>a</sup>[U.S. EPA \(2014\)](#)

The included references (n=15) consisted of 6 human references and 9 animal references that contained primary health effects data. Human references include four cohort studies, one case-series study, and one study using a non-traditional study design (compared binding of plasminogen in serum from LAA-exposed persons [with and without mesothelial cell autoantibodies] with binding in serum from healthy controls without mesothelial cell autoantibodies).

Animal references (n=9) include 2 subchronic studies and 9 acute studies (one reference evaluated both subchronic and acute durations and one reference evaluated acute exposure via two different routes). There was only one inhalation study. The majority of animal studies (6/11) were acute instillation studies (intratracheal, oropharyngeal, or endotracheal), with 3 acute injection studies, 1 subchronic instillation study, and 1 subchronic inhalation study. Figure 2 displays a heatmap visualizing the distribution of available primary health effects studies. The identified PECO-relevant primary health effects studies were moved on to study evaluation.

**Figure 2. Heatmap of PECO Relevant Primary Health Effect Studies**

| Species | Duration   | Study type  |        |       |            |           |              |
|---------|------------|-------------|--------|-------|------------|-----------|--------------|
|         |            | Case-Series | Cohort | Other | Inhalation | Injection | Instillation |
| Human   | Chronic    | 1           | 4      | 1     |            |           |              |
| Rat     | Acute      |             |        |       |            |           | 1            |
|         | Subchronic |             |        |       | 1          |           |              |
| Mouse   | Acute      |             |        |       |            | 3         | 5            |
|         | Subchronic |             |        |       |            |           | 1            |

### *Study Evaluation*

Study evaluations for the six identified epidemiological studies are shown in Figure 3 (refer to Supplemental File 1 for detailed evaluations containing study-specific justifications for all deficient ratings). Critically, none of the identified studies contained adequate exposure information, with most studies providing only qualitative determinations of exposure to LAA. Two studies provided unitless exposure scores for different groups ([Pfau et al., 2019](#); [Pfau et al., 2018](#)). The majority of identified studies (4/6) also did not adequately adjust for confounders, and 3/6 studies did not include unexposed referents. All studies were rated adequate for the following domains: outcome ascertainment, participant selection, and selective reporting, and most studies (5/6) were rated adequate for sensitivity.

Study evaluations for the 11 identified animal studies from the 9 PECO-relevant references are shown in Figure 4 (refer to Supplemental File 1 for detailed evaluations containing study-specific justifications for all deficient ratings). Only two studies were considered adequate across all domains ([Badger et al., 2021](#); [Kodavanti et al., 2014 \[acute\]](#)). The most common deficiency was that 9/11 studies did not report the method of animal allocation to animal treatment groups; this is considered a minor deficiency with regard to interpreting study results. Four studies did not provide adequate characterization of the LAA fibers utilized in the study and 3/11 studies had inadequate results presentation due to pooling of data across sexes. Two studies were rated deficient for confounding ([Pfau et al., 2017](#); [Ferro et al., 2014](#)) due to severe barbering among the animals that resulted in high numbers of humane euthanasia in both control and treatment groups. Because excessive barbering can be a sign of stress, and barbering incidence in surviving animals was not reported, this may have impacted immune responses in all groups (including controls). Lastly, two studies that had 7-month observation periods post-exposure were rated deficient for the attrition domain because they did not report initial and/or final animal numbers ([Gilmer et al., 2016](#); [Zebedeo et al., 2014](#)) and one study was rated deficient for sensitivity due to a small sample size ([Brown et al., 2016](#)). All studies were rated adequate for observational bias/blinding (most endpoints were quantitative) and selective reporting.

**Figure 3. Heatmap of Study Evaluation Results for Epidemiological Studies<sup>a</sup>**

| Reference                            | Exposure Measurement | Outcome Ascertainment | Participant Selection | Confounding | Analysis  | Selective Reporting | Sensitivity |
|--------------------------------------|----------------------|-----------------------|-----------------------|-------------|-----------|---------------------|-------------|
| <a href="#">Diegel et al. (2018)</a> | Deficient            | Adequate              | Adequate              | Deficient   | Deficient | Adequate            | Deficient   |
| <a href="#">Hanson et al. (2016)</a> | Deficient            | Adequate              | Adequate              | Deficient   | Adequate  | Adequate            | Adequate    |
| <a href="#">Larson et al. (2020)</a> | Deficient            | Adequate              | Adequate              | Adequate    | Adequate  | Adequate            | Adequate    |
| <a href="#">Pfau et al. (2009)</a>   | Deficient            | Adequate              | Adequate              | Adequate    | Adequate  | Adequate            | Adequate    |
| <a href="#">Pfau et al. (2018)</a>   | Deficient            | Adequate              | Adequate              | Deficient   | Deficient | Adequate            | Adequate    |
| <a href="#">Pfau et al. (2019)</a>   | Deficient            | Adequate              | Adequate              | Deficient   | Deficient | Adequate            | Adequate    |

■ Adequate
 ■ Deficient

<sup>a</sup>Study-specific justifications for all deficient ratings are provided in Supplement 1.

Figure 4. Heatmap of Study Evaluation Results for Animal Studies<sup>a</sup>

| Reference                                            | Allocation | Observational Bias/Blinding | Confounding | Attrition | Chemical Administration and Characterization | Endpoint Measurement | Results Presentation | Selective Reporting | Sensitivity |
|------------------------------------------------------|------------|-----------------------------|-------------|-----------|----------------------------------------------|----------------------|----------------------|---------------------|-------------|
| <a href="#">Badger et al. (2021)</a>                 | Adequate   | Adequate                    | Adequate    | Adequate  | Adequate                                     | Adequate             | Adequate             | Adequate            | Adequate    |
| <a href="#">Brown et al. (2016)</a>                  | Deficient  | Adequate                    | Adequate    | Adequate  | Adequate                                     | Adequate             | Deficient            | Adequate            | Deficient   |
| <a href="#">Christofidou-Solomidou et al. (2019)</a> | Deficient  | Adequate                    | Adequate    | Adequate  | Adequate                                     | Adequate             | Adequate             | Adequate            | Adequate    |
| <a href="#">Ferro et al. (2014)</a>                  | Deficient  | Adequate                    | Deficient   | Adequate  | Adequate                                     | Adequate             | Adequate             | Adequate            | Adequate    |
| <a href="#">Gilmer et al. (2016)</a>                 | Deficient  | Adequate                    | Adequate    | Deficient | Deficient                                    | Adequate             | Adequate             | Adequate            | Adequate    |
| <a href="#">Kodavanti et al. (2014) (acute)</a>      | Adequate   | Adequate                    | Adequate    | Adequate  | Adequate                                     | Adequate             | Adequate             | Adequate            | Adequate    |
| <a href="#">Kodavanti et al. (2014) (subchronic)</a> | Deficient  | Adequate                    | Adequate    | Adequate  | Deficient                                    | Adequate             | Adequate             | Adequate            | Adequate    |
| <a href="#">Pfau et al. (2017)</a>                   | Deficient  | Adequate                    | Deficient   | Adequate  | Adequate                                     | Adequate             | Adequate             | Adequate            | Adequate    |
| <a href="#">Pfau et al. (2014) (instillation)</a>    | Deficient  | Adequate                    | Adequate    | Adequate  | Deficient                                    | Adequate             | Deficient            | Adequate            | Adequate    |
| <a href="#">Pfau et al. (2014) (injection)</a>       | Deficient  | Adequate                    | Adequate    | Adequate  | Deficient                                    | Adequate             | Deficient            | Adequate            | Adequate    |
| <a href="#">Zebedeo et al. (2014)</a>                | Deficient  | Adequate                    | Adequate    | Deficient | Adequate                                     | Adequate             | Adequate             | Adequate            | Adequate    |

■ Adequate 
 ■ Deficient

<sup>a</sup>Study-specific justifications for all deficient ratings are provided in Supplement 1.

## Data Extraction

Data extraction tables providing details on study methodology and results for human and animal studies are presented in Appendix C. Epidemiology studies are described in Table C-1 and animal studies are described in Table C-2. The outcomes that were examined in this literature review include autoimmune disease outcomes and measured endpoints that may be associated with SAID. Autoimmune responses to asbestos exposure have generally included positive autoantibody tests, increased immunoglobulins, and immune complex deposition ([Pfau et al., 2009](#)); however, other tests of immune function may also be relevant to autoimmunity, as described in Table 2.

| Table 2. Description of Measured Endpoints and Their Relationship to SAID                                                         |                                                                                                                                                                                                                                                                            |
|-----------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Endpoint                                                                                                                          | Relationship to SAID                                                                                                                                                                                                                                                       |
| Autoantibodies<br>(including ANA, ANAA, ENA, MCAA, auto-dsDNA, ANCA, AFA, and anti-plasminogen antibodies)                        | Prevalence of autoantibodies is associated with SAID in humans ( <a href="#">Pfau et al., 2009</a> ).                                                                                                                                                                      |
| Ig levels (measured in serum or peritoneal lavage fluid)                                                                          | IgM, IgA, and IgG antibodies are associated with autoimmunity.                                                                                                                                                                                                             |
| B1a B-cells                                                                                                                       | Implicated in autoimmune disease due to their production of IgM (considered to be innate autoantibodies), reactivity to self-antigen, and elevated levels in humans with autoimmune disease and animal models of autoimmune disease ( <a href="#">Pfau et al., 2014</a> ). |
| Other immune cells<br>(including other B-cells, T-cells, lymphocytes, neutrophils, and macrophages)                               | Associated with a general immune response; non-specific for autoimmune dysfunction.                                                                                                                                                                                        |
| T <sub>H</sub> 17 cytokines<br>(including interleukins [IL] IL-17, and IL-6 and tumor necrosis factor [TNF]-α)                    | T <sub>H</sub> 17 cytokines have been associated with autoimmune disease ( <a href="#">Badger et al., 2021</a> ; <a href="#">Pfau et al., 2017</a> ; <a href="#">Brown et al., 2016</a> ; <a href="#">Ferro et al., 2014</a> ).                                            |
| Other cytokines<br>(including IL-1β, IL-2, IL-4, IL-5, IL-10, interferon [IFN]-γ, and monocyte chemoattractant protein-1 [MCP-1]) | Not specific for autoimmune dysfunction.                                                                                                                                                                                                                                   |
| Other biomarkers<br>(including serum α1-acid glycoprotein, α2-macroglobulin, osteopontin, and leucine-rich α2-glycoprotein)       | Biomarkers of acute-phase response or inflammation; not specific for autoimmune dysfunction ( <a href="#">Kodavanti et al., 2014</a> ).                                                                                                                                    |

## Association Between LAA Exposure and SAID or Related Endpoints

A synthesis of the significant findings from inhalation studies is provided in Table 3. No exposure-response data are available in human studies, and limited data are available comparing exposed populations from Libby, Montana to unexposed referents (see Table C-1 for details). The most direct evidence of an association between exposure to LAA and SAID is from a 17-year mortality follow-up of a

large community-based health-screening program from Libby, Montana, which reported a significant increase in mortality from SAIDs (SLE, RA, scleroderma combined) in LAA-exposed subjects compared to calendar-specific U.S. mortality rates after adjustment for confounders ([Larson et al., 2020](#)). In a small prospective cohort study, LAA-exposed subjects had significantly higher anti-fibroblast antibodies than unexposed age- and sex-matched referents ([Pfau et al., 2009](#)). Other prospective cohort studies also reported LAA-exposed subjects with serum samples positive for autoantibodies, including MCAA, ANA, and/or anti-plasminogen autoantibodies; however, findings were not compared to unexposed referents and thus it is unclear if the prevalence of autoantibodies is related to exposure ([Pfau et al., 2019](#); [Pfau et al., 2018](#); [Pfau et al., 2009](#)). In a case-series report of 10 subjects exposed to “high” levels of LAA with clinical presentation of severe autoimmune disease, 7/10 were ANA+ ([Diegel et al., 2018](#)).

| <b>Table 3. Summary of New Literature Evaluating Association Between Inhalation Exposure to LAA and SAID or Related Endpoints<sup>a</sup></b> |                                                                                                                                                                                               |                                                                                                                                                                                                    |             |
|-----------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| <b>Outcome</b>                                                                                                                                | <b>Human Evidence</b>                                                                                                                                                                         | <b>Animal Evidence</b>                                                                                                                                                                             |             |
|                                                                                                                                               |                                                                                                                                                                                               | <b>Rats</b>                                                                                                                                                                                        | <b>Mice</b> |
| SAID                                                                                                                                          | ↑ mortality from SLE, RA, and scleroderma combined ( <a href="#">Larson et al., 2020</a> )                                                                                                    | NE                                                                                                                                                                                                 | NE          |
| Autoantibodies                                                                                                                                | ↑ anti-fibroblast antibodies ( <a href="#">Pfau et al., 2009</a> )<br>Higher exposure score among subjects with anti-topo-1 autoantibodies than without ( <a href="#">Pfau et al., 2009</a> ) | NE                                                                                                                                                                                                 | NE          |
| Ig levels                                                                                                                                     | NE                                                                                                                                                                                            | NE                                                                                                                                                                                                 | NE          |
| B1a B-cells                                                                                                                                   | NE                                                                                                                                                                                            | NE                                                                                                                                                                                                 | NE          |
| Other immune cells                                                                                                                            | NE                                                                                                                                                                                            | NE                                                                                                                                                                                                 | NE          |
| T <sub>H</sub> 17 cytokines                                                                                                                   | NE                                                                                                                                                                                            | ↔ IL-6 in serum                                                                                                                                                                                    | NE          |
| Other cytokines                                                                                                                               | NE                                                                                                                                                                                            | NE                                                                                                                                                                                                 | NE          |
| Other biomarkers                                                                                                                              | NE                                                                                                                                                                                            | ↑ α-2-macroglobulin in serum (after 1 day; ↔ after 1, 3, or 18 months)<br>↔ α1-acid glycoprotein, osteopontin, or leucine-rich α2-glycoprotein in serum ( <a href="#">Kodavanti et al., 2014</a> ) | NE          |

<sup>a</sup>Additional study details are provided in Appendix C (Tables C-1 and C-2)

↑ = statistically significant increase as reported by the study authors; ↓ = statistically significant decrease as reported by the study authors; ↔ = no statistically significant difference; NE = not evaluated

The inhalation route was evaluated in only one animal study; details are shown in Table C-2. Endpoints that were evaluated in the 13-week inhalation study in rats [Kodavanti et al. \(2014\)](#) were limited to a single T<sub>H</sub>17 cytokine (IL-6), biomarkers of an acute-phase response or inflammation (serum α1-acid glycoprotein, α2-macroglobulin), and biomarkers of immune dysfunction, inflammation and/or cancer (osteopontin and leucine-rich α2-glycoprotein). The only exposure-related finding was a significant increase in α2-macroglobulin at the highest concentration evaluated (10 mg/m<sup>3</sup>) 1 day after exposure; this finding did not persist when rats were evaluated 1 month or later post-exposure. [Kodavanti et al.](#)

(2014) did not report LAA exposure in terms of fiber counts, which is the dosimetric used to evaluate health hazards from LAA in humans (U.S. EPA, 2014). Reliable methods to convert from gravimetric exposure levels (mg/m<sup>3</sup>) to fiber counts have not been developed.

A synthesis of the supporting findings from animal studies using non-physiological routes of exposure (i.e., those less directly relevant to human exposures, which are almost entirely via inhalation) is provided in Table 4, and additional details are available in Table C-2. Fiber distribution from non-physiological routes is anticipated to differ from inhalation exposure. Dose-response data are limited to the study by Kodavanti et al. (2014), which evaluated rats following a single intratracheal installation. Serum  $\alpha$ 1-acid glycoprotein and  $\alpha$ 2-macroglobulin were significantly elevated at  $\geq 0.5$  mg/rat, and IL-6 was significantly elevated at 1.5 mg/rat 1 day post-exposure; these findings did not persist when rats were evaluated 3 months or later post-exposure. All other animal studies were conducted in mice, evaluated only one dose level, exposed animals only once or twice, and administered LAA via instillation (intra/endotracheal or oropharyngeal) or intraperitoneal injection. Several studies reported elevated incidences of mice positive for autoantibodies (ANA+, MCAA+) 7-8 months following LAA exposure via intratracheal instillation (Gilmer et al., 2016; Ferro et al., 2014; Zebedeo et al., 2014). No significant change in the incidence of ANA+ or MCAA+ mice was observed 7 months after oropharyngeal instillation (Pfau et al., 2017). Autoantibodies were not evaluated in intraperitoneal injection studies.

| Table 4. Summary of New Literature Evaluating Association Between Exposure to LAA by Non-Physiological Routes and SAID or Related Endpoints <sup>a</sup> |      |                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Outcome                                                                                                                                                  | Rats | Mice <sup>b</sup>                                                                                                                                                                                                                                                                                                                                               |
| Endotracheal, intratracheal, and oropharyngeal instillation                                                                                              |      |                                                                                                                                                                                                                                                                                                                                                                 |
| SAID                                                                                                                                                     | NE   | NE                                                                                                                                                                                                                                                                                                                                                              |
| Autoantibodies                                                                                                                                           | NE   | ↑ ANA+ incidence (Ferro et al., 2014)<br>↑ ANA+ incidence (Zebedeo et al., 2014)<br>↑ MCAA+ incidence (Gilmer et al., 2016)<br>↔ ANA+, MCAA+ incidence (Pfau et al., 2017)<br>↔ anti-histone or anti-chromatin antibody incidence (Ferro et al., 2014)                                                                                                          |
| Ig levels                                                                                                                                                | NE   | ↓ total IgG <sub>1</sub> in serum (Ferro et al., 2014)<br>↔ IgM or IgA in serum (Ferro et al., 2014)                                                                                                                                                                                                                                                            |
| B1a B-cells                                                                                                                                              | NE   | ↑ B1a B-cells in spleen (Pfau et al., 2017; Ferro et al., 2014)<br>↓ % B1a B-cells in pleural cavity (Pfau et al., 2014)<br>↓ B1a B-cells in peritoneum (Pfau et al., 2017)<br>↔ B1a B-cells in lung (Ferro et al., 2014)                                                                                                                                       |
| Other immune cells                                                                                                                                       | NE   | ↑ B-cells in spleen and peritoneum (Pfau et al., 2017)<br>↓ T-cells in peritoneum (Pfau et al., 2017)<br>↓ macrophages and ↑ neutrophils in whole lung lavage fluid (Brown et al., 2016)<br>↔ overall % B-cells or T-cells in the spleen or lungs (Ferro et al., 2014)<br>↔ T-cells in the spleen or monocytes or lymphocytes in peritoneum (Pfau et al., 2017) |

| Table 4. Summary of New Literature Evaluating Association Between Exposure to LAA by Non-Physiological Routes and SAID or Related Endpoints <sup>a</sup> |                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Outcome                                                                                                                                                  | Rats                                                                                                                                                                                                    | Mice <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| T <sub>H</sub> 17 cytokines                                                                                                                              | ↑ IL-6 in serum ( <a href="#">Kodavanti et al., 2014</a> )                                                                                                                                              | ↑ IL-6, IL-17 in serum ( <a href="#">Ferro et al., 2014</a> )<br>↑ TNF-α, IL-6 in whole lung lavage fluid ( <a href="#">Brown et al., 2016</a> )<br>↑ TNF-α, IL-6, IL-17 in serum ( <a href="#">Pfau et al., 2017</a> )                                                                                                                                                                                                                                         |
| Other cytokines                                                                                                                                          | NE                                                                                                                                                                                                      | ↑ IL-2, IFN-γ in serum ( <a href="#">Ferro et al., 2014</a> )<br>↑ IL-1β, IL-5 in whole lung lavage fluid ( <a href="#">Brown et al., 2016</a> )<br>↑ IFN-γ, IL-10 in serum ( <a href="#">Pfau et al., 2017</a> )<br>↔ IL-10 in serum ( <a href="#">Ferro et al., 2014</a> )<br>↔ IL-2, IL-4 in serum ( <a href="#">Pfau et al., 2017</a> )<br>↔ IL-10 or KC/GRO in whole lung lavage fluid ( <a href="#">Brown et al., 2016</a> )                              |
| Other biomarkers                                                                                                                                         | ↑ serum α1-acid glycoprotein and α2-macroglobulin in serum ( <a href="#">Kodavanti et al., 2014</a> )<br>↔ serum osteopontin or leucine-rich α2-glycoprotein ( <a href="#">Kodavanti et al., 2014</a> ) | NE                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Intraperitoneal injection                                                                                                                                |                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| SAID                                                                                                                                                     | NE                                                                                                                                                                                                      | NE                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Autoantibodies                                                                                                                                           | NE                                                                                                                                                                                                      | NE                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Ig levels                                                                                                                                                | NE                                                                                                                                                                                                      | ↓ IgG <sub>1</sub> and IgG <sub>2b</sub> in peritoneal lavage fluid ( <a href="#">Badger et al., 2021</a> )<br>↑ IgA in peritoneal lavage fluid ( <a href="#">Badger et al., 2021</a> )<br>↑ IgM in peritoneal fluid ( <a href="#">Pfau et al., 2014</a> )<br>↔ IgG or IgA in peritoneal fluid ( <a href="#">Pfau et al., 2014</a> )<br>↔ IgG <sub>2a</sub> , IgG <sub>3</sub> , IgM, or IgE in peritoneal lavage fluid ( <a href="#">Badger et al., 2021</a> ) |
| B1a B-cells                                                                                                                                              | NE                                                                                                                                                                                                      | ↑ % B1a B-cells in spleen and peritoneum ( <a href="#">Badger et al., 2021</a> )<br>↓ B1a B-cells in peritoneal cavity ( <a href="#">Pfau et al., 2014</a> )<br>↓ B1a B-cells in peritoneum ( <a href="#">Christofidou-Solomidou et al., 2019</a> )                                                                                                                                                                                                             |
| Other immune cells                                                                                                                                       | NE                                                                                                                                                                                                      | ↓ Th cells in peritoneum ( <a href="#">Christofidou-Solomidou et al., 2019</a> )<br>↔ Th cells in peritoneum ( <a href="#">Badger et al., 2021</a> )<br>↓ % B-cells and T-cells in peritoneum ( <a href="#">Badger et al., 2021</a> )<br>↔ % B-cells and T-cells in peritoneum ( <a href="#">Christofidou-Solomidou et al., 2019</a> )<br>↔ B1b, B2b B-cells or T-cells in peritoneal cavity ( <a href="#">Pfau et al., 2014</a> )                              |

| Table 4. Summary of New Literature Evaluating Association Between Exposure to LAA by Non-Physiological Routes and SAID or Related Endpoints <sup>a</sup> |      |                                                                                                                                                                                                                                                                                                                                                                                                        |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Outcome                                                                                                                                                  | Rats | Mice <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                      |
|                                                                                                                                                          |      | ↑ WBCs and PMNs in peritoneum ( <a href="#">Badger et al., 2021</a> ; <a href="#">Christofidou-Solomidou et al., 2019</a> )<br>↑ macrophages in peritoneum ( <a href="#">Christofidou-Solomidou et al., 2019</a> )<br>↓ % Lymphocytes in peritoneum ( <a href="#">Christofidou-Solomidou et al., 2019</a> )<br>↔ WBCs, B-cells, T-cells, or Th cells in spleen ( <a href="#">Badger et al., 2021</a> ) |
| TH17 cytokines                                                                                                                                           | NE   | ↑ IL-6 in peritoneal lavage fluid and serum ( <a href="#">Badger et al., 2021</a> )<br>↔ TNF-α in peritoneal lavage fluid or serum ( <a href="#">Badger et al., 2021</a> )                                                                                                                                                                                                                             |
| Other cytokines                                                                                                                                          | NE   | ↑ MCP-1 in peritoneal lavage fluid ( <a href="#">Badger et al., 2021</a> )<br>↔ MCP-1 in serum ( <a href="#">Badger et al., 2021</a> )<br>↔ IL-10, IFN-γ, or IL 12p70 in peritoneal lavage fluid or serum ( <a href="#">Badger et al., 2021</a> )                                                                                                                                                      |
| Other biomarkers                                                                                                                                         | NE   | NE                                                                                                                                                                                                                                                                                                                                                                                                     |

<sup>a</sup>Additional study details are provided in Appendix C (Table C-2)

Results presented in *italicized gray font* are from studies confounded by barbering ([Ferro et al., 2014](#)) and ([Pfau et al., 2017](#)).

↑ = statistically significant increase compared to control as reported by the study authors; ↓ = statistically significant decrease as reported by the study authors; ↔ = no statistically significant difference;  
 KC/GRO = keratinocyte chemoattractant/growth-regulated protein; NE = not evaluated;  
 PMN = polymorphonuclear cell; Th = T helper; WBC = white blood cell

Some mouse studies reported altered Ig levels in mice exposed to LAA; however, no consistent patterns were observed. Following intratracheal instillations, mice showed decreased serum IgG<sub>1</sub> after 8 months, with no significant change in the IgG<sub>1</sub>:IgG<sub>2</sub> ratio or change in IgM or IgA levels ([Ferro et al., 2014](#)). Severe barbering leading to humane euthanasia occurred in both control and treatment groups of this study, resulting in confounding of the study results and uncertainty in the interpretation of these findings. Findings in intraperitoneal studies were mixed; however, studies are not directly comparable because evaluation timing post-exposure differed. One study reported decreased IgG<sub>1</sub> and IgG<sub>2b</sub> and increased IgA, relative to control, in peritoneal lavage fluid 14 days post-exposure with no changes in IgG<sub>2a</sub>, IgG<sub>3</sub>, IgM, or IgE ([Badger et al., 2021](#)). When evaluation was 3 days post-exposure, peritoneal fluid levels of IgM were elevated with no changes in IgA or IgG ([Pfau et al., 2014](#)).

Changes in B1a B-cell levels and/or trafficking were observed in several mouse studies following exposure to LAA. B1a B-cells are implicated in autoimmune disease due to their production of IgM (considered to be innate autoantibodies), reactivity to self-antigen, and elevated levels in humans with autoimmune disease and animal models of autoimmune disease ([Pfau et al., 2014](#)). B1a B-cells were

elevated in the spleen, but not the lung, 8 months following intratracheal instillation ([Ferro et al., 2014](#)). Similarly, B1a B-cells were elevated in the spleen (sexes combined) and peritoneum (males only) 7 months following oropharyngeal instillation; peritoneal B1a B-cells in females did not differ from control despite a decrease in B suppressor cells ([Pfau et al., 2017](#)). However, B1a B-cells were decreased in the pleural cavity 4 days following endotracheal instillation, with no change from control 8 days post-exposure ([Pfau et al., 2014](#)). In intraperitoneal injection studies, B1a B-cells were decreased in the peritoneum 3-7 days following exposure ([Christofidou-Solomidou et al., 2019](#); [Pfau et al., 2014](#)); B suppressor cells were also decreased by 3 days post-exposure ([Christofidou-Solomidou et al., 2019](#)). The decreased number (or percentage) of B1a B-cells observed at the site of exposure (i.e., pleural cavity with lung exposure and peritoneum with intraperitoneal injection) and increased number (or percentage) of B1a B-cells at other sites may be due to altered B1a B-cell trafficking following LAA-exposure. In an intraperitoneal injection study in mice, [Pfau et al. \(2014\)](#) demonstrated that exposure to LAA increased B1a B-cell trafficking to the spleen and mesenteric lymph nodes. Migration was tracked following adoptive transfer of CD45.1+ B1 cells injected into the peritoneal cavity. Increased spleen cellularity is supported by elevated spleen weight ([Pfau et al., 2014](#)). Elevated spleen weight was also reported in the other intraperitoneal injection study reporting decreased peritoneal B1a B-cells ([Christofidou-Solomidou et al., 2019](#)), although no change in spleen weight or overall cellularity was observed 7 months following oropharyngeal instillation despite elevated splenic B1a B-cells ([Pfau et al., 2017](#)). Other immune cell alterations were reported in mice exposed to LAA: however, these cell functions are associated with general inflammation or adaptive immunity rather than autoimmune function (see Tables 4 and C-2). For two of the three instillation studies that evaluated immune cell alterations in mice, severe barbering leading to humane euthanasia occurred in both control and treatment groups ([Pfau et al., 2017](#); [Ferro et al., 2014](#)). This results in significant confounding of the study results and introduces uncertainty in the interpretation of study findings.

Several studies reported that one or more  $T_H17$  profile cytokines, or cytokines involved in  $T_H17$  cell differentiation, were elevated following LAA-exposure and these cytokines have been associated with autoimmune disease ([Badger et al., 2021](#); [Pfau et al., 2017](#); [Brown et al., 2016](#); [Ferro et al., 2014](#)). In instillation studies, findings included increased serum IL-6 and IL-17 levels 7- 8 months post-exposure ([Pfau et al., 2017](#); [Ferro et al., 2014](#)) and increased whole-lung lavage IL-6 levels 24 hours post-exposure ([Brown et al., 2016](#)); IL-6 levels were also increased in peritoneal lavage 14 days after intraperitoneal injection ([Badger et al., 2021](#)). While TNF- $\alpha$  is classified as a  $T_H1$  cytokine ([Ferro et al., 2014](#)), it promotes  $T_H17$  differentiation via IL-6 and IL-1 $\beta$  production ([Zheng et al., 2014](#)). Both elevated TNF- $\alpha$  and IL-1 $\beta$  levels were reported in serum or whole-lung lavage following LAA instillation, but not intraperitoneal injection ([Pfau et al., 2017](#); [Brown et al., 2016](#)). Other  $T_H1$  and  $T_H2$  inflammatory cytokines, which are non-specific for autoimmunity, were also altered in several studies (see Tables 4 and C-2). For two of the three instillation studies that evaluated cytokine profiles in mice, severe barbering leading to humane euthanasia occurred in both control and treatment groups ([Pfau et al., 2017](#); [Ferro et al., 2014](#)). This results in significant confounding of the study results and introduces uncertainty in the interpretation of study findings.

#### *Association Between Autoimmune Endpoints and Pleural Effects of LAA*

Human and animal studies provide suggestive evidence that autoimmune effects may be associated with classic asbestos disease. [Pfau et al. \(2009\)](#) characterized the specific target antigens of the autoantibodies identified in the Libby cohort. The most common were chromatin and Ro52, which are

associated with SLE, and anti-topo I (also known as Scl-70), which is associated with scleroderma. When autoantigens were evaluated based on relative exposure level, ranked on a scale of 0-4 based on the duration of exposure and the number of known routes of exposure, only anti-topo I was significantly correlated with asbestos-related disease (interstitial fibrosis, pleural plaques, and mesothelioma). In an additional prospective cohort study, the frequency of ANA-positive autoantibodies was higher in subjects with progressive lamellar pleural thickening (LPT) than those with stable LPT or no LPT ([Pfau et al., 2019](#)).

Instillation studies in mice reported pulmonary and/or pleural fibrosis in LAA-exposed mice associated with findings suggestive of autoimmune reactivity ([Pfau et al., 2017](#); [Brown et al., 2016](#)). [Gilmer et al. \(2016\)](#) evaluated the role of MCAA+ autoantibodies in progressive lamellar pleural fibrosis by injecting MCAA+ serum from LAA-exposed mice into the peritoneum of mice and evaluating collagen formation. Findings showed that mice injected with MCAA+ serum had significantly higher fibrotic responses (measured as collagen deposition the peritoneal cavity) compared to mice injected with control serum or serum cleared of IgG.

## CONCLUSIONS

**Primary Study Question: Is the LAA reference concentration (RfC) still protective? Specifically, is there any new evidence on SAID and antibody outcomes that pertains to this question?**

Human and animal studies of LAA exposure and autoimmune outcomes that were not evaluated in the 2014 IRIS assessment of LAA ([U.S. EPA, 2014](#)) were reviewed. Data from these studies support the conclusion that SAID endpoints are potential hazards of concern in humans following exposure to LAA. However, due to lack of quantitative exposure data in human studies and lack of validated autoimmune endpoints (e.g., autoantibodies) in the only animal inhalation study identified, available data are not adequate to determine whether a RfC value derived for autoimmune effects would be lower or higher than the existing RfC. This conclusion is consistent with [U.S. EPA \(2014\)](#). In order to adequately address this study question, human studies with adequate quantitative exposure data and/or animal dose-response data evaluating validated and specific biomarkers of autoimmune dysfunction are needed.

**Secondary Study Question: What is the relationship of SAID to classic asbestos associated disease?**

Available data provide suggestive evidence that autoimmune effects may be associated with pulmonary and/or pleural effects of LAA. One human study reported an association between autoantibodies to a single antigen (anti-topo I, which is associated with scleroderma) and asbestos-related disease. An additional study in humans correlated the presence of ANA autoantibodies with progressive LPT. Two animal studies provided suggestive evidence that autoimmune dysregulation and classic asbestos-associated disease occur after the same exposure paradigm, and a third animal study demonstrated that intraperitoneal injection of MCAA+ serum from LAA-exposed mice can trigger fibrosis in an unexposed animal. No data were available to inform the temporality or mechanism of the association between pulmonary and/or pleural fibrosis and generation of autoantibodies.

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## APPENDIX A. LITERATURE SEARCH QUERY STRINGS

Preliminary testing of search terms related to LAA exposure in PubMed and WOS indicated that date limits and inclusion of health effect terms were not needed. An additional strategy targeting amphibole asbestos in combination with specified autoimmune terms in PubMed (both MeSH indexed terms as well as text words) captured articles related to autoimmunity that do not include “Libby” in the indexed terms or abstracts of the records. SRC tested the autoimmune terms provided by U.S. EPA Region 8 in the 2025\_Nov\_Lit-Search-Strings Word file and revised the query strings to ensure that all relevant citations were captured in the search.

| Table A-1. Literature Search Query Strings – 01/16/2026 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|---------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Database                                                | Query                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| PubMed                                                  | (Libby[tw] AND (amphibole[tw] OR asbestos[tw] OR fiber[tw] OR fibers[tw] OR vermiculite[tw] OR (Montana[tw] OR MT[tw] OR Mont[tw]))) OR (("Asbestos, Amphibole"[nm] OR (asbestos[tw] AND amphibole[tw])) AND ("Autoimmune Diseases"[mh] OR "Autoimmunity"[mh] OR "Autoantibodies"[mh] OR "B-Lymphocytes"[mh] OR "T-Lymphocytes"[mh] OR "Immunoglobulin G"[mh] OR "Integrin alpha4"[mh] OR "Interleukin-17"[mh] OR "Interleukin-6"[mh] OR "Macrophages"[mh] OR "Th1 Cells"[mh] OR "Th17 Cells"[mh] OR "Th2 Cells"[mh] OR "autoimmune"[tw] OR "autoimmunity"[tw] OR "autoantibodies"[tw] OR "autoantibody"[tw] OR "auto-antibody"[tw] OR "auto-antibodies"[tw] OR "extractable nuclear antigen"[tw] OR "dsDNA"[tw] OR "anti-dsDNA"[tw] OR "immunoglobulin"[tw] OR "immunoglobulins"[tw])) |
| WOS                                                     | TS=Libby AND TS=(amphibole OR asbestos)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

## **APPENDIX B. STUDY EVALUATION CRITERIA**

Streamlined study quality/risk-of-bias criteria for human (Table B-1) and animal (Table B-2) studies are presented below. Criteria were developed using the domains and core questions defined in [U.S. EPA \(2022\)](#). However, to streamline rating efforts, each domain received a single “adequate” or “deficient” rating based on rating criteria defined in Tables B-1 and B-2. These rating criteria are modifications of general considerations outlined for each domain in [U.S. EPA \(2022\)](#), revised to be fit-for-purpose to evaluate autoimmune outcomes following exposure to LAA.

Study evaluations were conducted by two independent senior toxicologists using Microsoft Excel templates. Any deficient ratings were explained in brief comments providing justification. Conflicts between the toxicologists were resolved by discussion. Individual study evaluations are provided as a Supplemental File 1 and the ratings for each study are presented for each domain in Figures 3 and 4 (above).

| Table B-1. Epidemiology Evaluation Criteria |                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domain <sup>a</sup>                         | Core Question <sup>b</sup>                                                                                                                                                                      | Rating <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Exposure measurement                        | Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of autoimmune diseases? | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>Valid exposure assessment methods used, and quantitative individual exposure estimates were made.</li> <li>Exposure was measured prior to outcome.</li> <li>Exposure misclassification is expected to be minimal OR misclassification might exist but is not expected to greatly change the effect estimate.</li> </ul> <p>Deficient (one or more):</p> <ul style="list-style-type: none"> <li>Exposure estimated qualitatively (e.g., exposed vs. unexposed).</li> <li>Valid exposure assessment methods are used, but there are concerns about or evidence for reverse causality.</li> <li>There is evidence for exposure misclassification that is expected to influence effect estimate and/or exposure measurement was not independent of outcome status.</li> </ul> |
| Outcome ascertainment                       | Does the outcome measure reliably distinguish the presence or absence of autoimmune disease?                                                                                                    | <p>Adequate:</p> <ul style="list-style-type: none"> <li>High-to-moderate confidence in the outcome definition (i.e., specificity and sensitivity), with minimal concerns with respect to misclassification OR some uncertainty with respect to misclassification but not expected to greatly change the effect estimate.</li> </ul> <p>Deficient:</p> <ul style="list-style-type: none"> <li>Outcome definition was not specific/sensitive or was an invalid/insensitive marker of outcome (e.g., measured biomarker has not been established as specific/reliable biomarker for autoimmune disease).</li> </ul>                                                                                                                                                                                                                             |
| Participant selection                       | Is there evidence that selection into or out of the study (or analysis sample) was jointly related to exposure and to autoimmune outcome?                                                       | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>Minimal concern for selection bias based on description of recruitment process and follow-up OR enough of a description of the recruitment process to be comfortable that there is no serious risk of bias.</li> <li>Exclusion and inclusion criteria for participants specified and would not induce bias.</li> <li>Participation rate is reported at all steps of study OR incompletely reported but available information indicates participation is unlikely to be related to exposure.</li> </ul> <p>Deficient:</p>                                                                                                                                                                                                                                                  |

| Table B-1. Epidemiology Evaluation Criteria |                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------------------------------------------|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domain <sup>a</sup>                         | Core Question <sup>b</sup>                                                                                  | Rating <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                             |                                                                                                             | <ul style="list-style-type: none"> <li>• Little information on recruitment process, selection strategy, sampling framework or participation. OR</li> <li>• Aspects of these processes raises the potential for bias (e.g., healthy worker effect, survivor bias) or show clear evidence of bias (e.g., convenience sample with no information about recruitment and selection, cases and controls are recruited from different sources with different likelihood of exposure, recruitment materials stated outcome of interest and potential participants are aware of or are concerned about specific exposures).</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Confounding                                 | Is confounding of the effect of the exposure likely?                                                        | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>• Conveys strategy for identifying all or most key confounders, including coexposures.</li> <li>• Inclusion of potential confounders in statistical models.</li> <li>• Does not include variables in the models likely to be influential colliders or intermediates on the causal pathway.</li> <li>• Key confounders are evaluated appropriately and documented as unlikely sources of substantial confounding where appropriate.</li> </ul> <p>Deficient (one or more):</p> <ul style="list-style-type: none"> <li>• The potential for bias to explain some of the results is high based on an inability to rule out residual confounding OR confounding is present and not accounted for.</li> <li>• Descriptive information on key confounders (e.g., their relationship relative to the outcomes and exposure levels) is not presented.</li> <li>• Strategy of evaluating confounding is unclear or is not recommended.</li> <li>• Includes variables in the models that are colliders or intermediates in the causal pathway, indicating that substantial bias is likely from this adjustment.</li> </ul> |
| Analysis                                    | Does the analysis strategy and presentation convey the necessary familiarity with the data and assumptions? | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>• No or minimal deficiencies in analysis evident; discussion of some details may be missing (e.g., examination of outliers).</li> <li>• Use of an optimal characterization of the outcome variable.</li> <li>• Quantitative results presented.</li> <li>• Where applicable, for exposure, includes limit of detection (LOD, and percentage below the LOD), and decision to use log transformation.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

| Table B-1. Epidemiology Evaluation Criteria |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domain <sup>a</sup>                         | Core Question <sup>b</sup>                                                                       | Rating <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                             |                                                                                                  | <ul style="list-style-type: none"> <li>Includes analyses that address robustness of findings; must include examination of exposure-response and/or appropriate referent/control group.</li> </ul> <p>Deficient (one or more):</p> <ul style="list-style-type: none"> <li>Does not conduct analysis using optimal characterization of the outcome variable OR analysis methods are not appropriate for design or data of the study.</li> <li>Effect estimates and p-value presented without standard error or confidence interval.</li> <li>Results presented as statistically “significant”/“not significant” without additional data.</li> </ul>                                                                                                                                                             |
| Selective reporting                         | Is there reason to be concerned about selective reporting of autoimmune outcomes?                | <p>Adequate:</p> <ul style="list-style-type: none"> <li>The autoimmune results reported by study authors are consistent with the primary (and secondary) autoimmune analyses described in a registered protocol or methods section.</li> </ul> <p>Deficient (one or more):</p> <ul style="list-style-type: none"> <li>Concerns were raised based on previous publications, a methods paper, or a registered protocol indicating that autoimmune analyses were planned or conducted that were not reported, or that hypotheses originally considered to be secondary were represented as primary in the reviewed paper.</li> <li>Only subgroup analyses were reported, suggesting that results for the entire group were omitted.</li> <li>Only statistically significant results were reported.</li> </ul>    |
| Sensitivity                                 | Is there a concern that sensitivity of the study is not adequate to detect an autoimmune effect? | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>There are no notable concerns raised regarding study sensitivity OR there might be issues identified that could reduce sensitivity, but they are considered unlikely to substantially impact the overall findings of the study. It is deemed: <ul style="list-style-type: none"> <li>There is sufficient variability/contrast in exposure to evaluate primary hypotheses.</li> <li>The study population was sensitive to the development of the outcomes of interest (e.g., ages, lifestage, sex).</li> <li>The study was considered adequately powered to detect an effect (based on factors such as sample size [overall and across subgroups], precision, prevalence of outcome, number of covariates in model).</li> </ul> </li> </ul> |

| Table B-1. Epidemiology Evaluation Criteria |                            |                                                                                                                                                                                                                                                                                                 |
|---------------------------------------------|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domain <sup>a</sup>                         | Core Question <sup>b</sup> | Rating <sup>b</sup>                                                                                                                                                                                                                                                                             |
|                                             |                            | Deficient: <ul style="list-style-type: none"> <li>Concerns were raised about the considerations described for “Adequate” that are expected to notably decrease the sensitivity of the study to detect associations for the outcome OR a true association is unlikely to be detected.</li> </ul> |

<sup>a</sup>Domains based on [U.S. EPA \(2022\)](#), revised to be specific to LAA and autoimmune outcomes (where appropriate).

<sup>b</sup>Core questions and rating scheme developed specifically to determine if studies are adequate for dose-response assessment of the potential relationship between exposure to LAA and autoimmune disease, in consultation with core and prompting questions in [U.S. EPA \(2022\)](#).

| Table B-2. Animal Evaluation Criteria |                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domain <sup>a</sup>                   | Core Question <sup>b</sup>                                                                                                                                                                                                                                                                   | Rating <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Allocation                            | Were animals assigned to experimental groups using a method that minimizes selection bias?                                                                                                                                                                                                   | <p>Adequate:</p> <ul style="list-style-type: none"> <li>Animals were allocated randomly into experimental groups OR</li> <li>Authors used a nonrandom method to control for important modifying factors across experimental groups (e.g., body-weight normalization).</li> </ul> <p>Deficient:</p> <ul style="list-style-type: none"> <li>Method of animal allocation is not reported OR</li> <li>Bias in the animal allocations was reported or inferable.</li> </ul>                                                                                                                                                                                                                                                                                            |
| Observational bias/blinding           | Did the study implement measures to reduce observational bias for autoimmune outcomes?                                                                                                                                                                                                       | <p>Adequate:</p> <ul style="list-style-type: none"> <li>Measures to reduce observational bias (e.g., blinding) were described or inferred OR</li> <li>Measures to reduce observations were not described/inferred BUT the potential concern for bias was mitigated by type of evaluation (e.g., automated/computer driven systems, standard laboratory kits, objective measures, screening level histopathology).</li> </ul> <p>Deficient:</p> <ul style="list-style-type: none"> <li>Measures to reduce observations were not described/inferred AND the potential impact on the results is major (e.g., outcome measures are highly subjective) OR</li> <li>Strong evidence for observational bias that impacted the results.</li> </ul>                        |
| Confounding                           | <p>Are variables with the potential to confound or modify autoimmune results controlled for and consistent across experimental groups?</p> <p><i>Note: Consideration of overt toxicity (possibly masking more specific effects) is addressed under endpoint measurement reliability.</i></p> | <p>Adequate:</p> <ul style="list-style-type: none"> <li>Outside of the exposure of interest, variables likely to confound or modify results appear to be controlled for and consistent across experimental groups (e.g., consistent animal handling across groups to avoid differences stress-induced immune responses) OR</li> <li>Some concern that variables likely to confound or modify results were uncontrolled or inconsistent across groups but are expected to have a minimal impact on the results.</li> </ul> <p>Deficient:</p> <ul style="list-style-type: none"> <li>Notable concern that potential or known confounding variables were uncontrolled or inconsistent across groups and are expected to substantially impact the results.</li> </ul> |

| Table B-2. Animal Evaluation Criteria        |                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domain <sup>a</sup>                          | Core Question <sup>b</sup>                                                                                                                                                                                                                               | Rating <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Attrition                                    | Did the study report autoimmune results for all tested animals?                                                                                                                                                                                          | <p>Adequate:</p> <ul style="list-style-type: none"> <li>Results were reported for all or most animals AND</li> <li>If animal attrition is identified, the authors provide an explanation, and attrition is not expected to impact the interpretation of the results OR attrition is not explained but this is not expected to significantly impact the interpretation of the results.</li> </ul> <p>Deficient:</p> <ul style="list-style-type: none"> <li>Moderate to high level of animal attrition that is not explained and could significantly impact the interpretation of the results OR</li> <li>Extensive animal attrition that prevents comparisons of results across treatment groups.</li> </ul>      |
| Chemical administration and characterization | <p>Did the study adequately characterize exposure to Libby amphibole asbestos and the exposure administration methods?</p> <p><i>Note: Considerations pertaining to selection of route and exposure levels are not evaluated under risk of bias.</i></p> | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>Description of chemical administration and characterization is complete OR any uncertainties regarding chemical administration and characterization are expected to have minimal impact on interpretation of the results.</li> <li>Exposure levels are verified using reliable analytical methods.</li> </ul> <p>Deficient:</p> <ul style="list-style-type: none"> <li>Uncertainties in the exposure characterization are identified and expected to substantially impact the results OR there is reasonable certainty that the study results are largely attributable to factors other than exposure to the chemical of interest.</li> </ul> |
| Endpoint measurement                         | Are the selected procedures, protocols, and animal models adequately described and appropriate for the autoimmune endpoint(s)/ outcome(s) of interest?                                                                                                   | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>Adequate description of methods and animal models.</li> <li>Use of generally accepted and reliable endpoint methods.</li> <li>Adequate sample sizes were used.</li> <li>Appropriate control groups were used.</li> <li>Any issues that could affect endpoint measurement were identified and considered unlikely to substantially impact overall findings or the ability to reliably interpret those findings.</li> </ul>                                                                                                                                                                                                                     |

| Table B-2. Animal Evaluation Criteria |                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domain <sup>a</sup>                   | Core Question <sup>b</sup>                                                                               | Rating <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                       |                                                                                                          | <p>Deficient (one or more):</p> <ul style="list-style-type: none"> <li>• Study report lacks key details necessary to evaluate the appropriateness of study design (e.g., description of the assays or protocols; information on the strain, sex, or lifestage of the animals).</li> <li>• Study protocol/endpoint selection is nonpreferred or lacks specificity for evaluating autoimmune disease.</li> <li>• Appropriate control group(s) were not used.</li> <li>• Sample sizes are too small to adequately evaluate whether or not effects related to exposure occurred.</li> <li>• Overt toxicity (e.g., mortality, extreme weight loss) is observed and might mask interpretation of outcome(s) of interest.</li> </ul>                                                                                                                                                                                                                                                                                 |
| Results presentation                  | Are the autoimmune endpoint results presented and compared in a way that is appropriate and transparent? | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>• No concerns with how the data are reported or the completeness of data reporting OR identified concerns are considered unlikely to substantially impact the overall findings or the ability to reliably interpret those findings.</li> <li>• Results are quantified or otherwise presented in a manner that allows for independent consideration of the data.</li> </ul> <p>Deficient (one or more):</p> <ul style="list-style-type: none"> <li>• Nonpreferred presentation of data (e.g., only absolute organ weight data reported).</li> <li>• Pooling of data across sexes or ages, when responses are known or expected to differ.</li> <li>• Incomplete presentation of the data (e.g., presentation of mean without variance data; concurrent control data are not presented; dichotomizing or truncating continuous data) or concerns about results presentation that substantially impact the results interpretation.</li> </ul> |

| Table B-2. Animal Evaluation Criteria |                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------------------------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Domain <sup>a</sup>                   | Core Question <sup>b</sup>                                                                  | Rating <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Selective reporting                   | Did the study report results for all prespecified autoimmune outcomes?                      | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>Quantitative or qualitative results were reported for all or most prespecified autoimmune outcomes, exposure groups, and evaluation time points.</li> <li>Any data omission is not expected to impact interpretation of the results.</li> </ul> <p>Deficient (one or more):</p> <ul style="list-style-type: none"> <li>Quantitative or qualitative data are missing for many or most prespecified autoimmune outcomes, omissions that are not explained and could significantly impact interpretation of results.</li> <li>Extensive results omissions prevents comparison of results across treatment groups.</li> </ul>                                                                                                                                                                                                                                                                                                                                                             |
| Sensitivity                           | Are there concerns sensitivity in the study is not adequate to detect an autoimmune effect? | <p>Adequate (need all):</p> <ul style="list-style-type: none"> <li>Experimental design (e.g., exposure period, timing, frequency, duration) is appropriate and sensitive for evaluating the autoimmune outcomes of interest.</li> <li>The selected animal model (species, strain, sex, lifestage) is known or assumed to be appropriate and sensitive for evaluating the autoimmune outcomes of interest.</li> <li>If potential issues were identified that could reduce sensitivity (related to first 2 bullets), they are unlikely to impact the overall findings of the study.</li> </ul> <p>Deficient (one or more):</p> <ul style="list-style-type: none"> <li>Concerns were identified regarding experimental design and/or animal model that are expected to notably decrease the sensitivity of the study to detect an autoimmune response in the exposed group(s).</li> <li>Severe concerns were raised about the sensitivity of the study and experimental design such that any observed associations are likely explained by bias.</li> </ul> |

<sup>a</sup>Domains based on [U.S. EPA \(2022\)](#), revised to be specific to LAA and autoimmune outcomes (where appropriate).

<sup>b</sup>Core questions and rating scheme developed specifically to determine if studies are adequate for dose-response assessment of the potential relationship between exposure to LAA and autoimmune disease, in consultation with core and prompting questions in [U.S. EPA \(2022\)](#).

## APPENDIX C. DATA EXTRACTION TABLES

| Table C-1. Epidemiological Study Data Extraction                                               |                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                            |                                                                                                                                                                                                                |
|------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reference and Study Design                                                                     | Subjects                                                                                                                                                                                                                                                                                                                   | Exposure Metric and Reported Levels Analysis                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                            | Results                                                                                                                                                                                                        |
| Prospective cohort studies                                                                     |                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                            |                                                                                                                                                                                                                |
| <a href="#">Larson et al. (2020)</a><br><br>Prospective cohort;<br>17-year mortality follow-up | 8,043 subjects (3,958 males; 4,085 females); 10-90 years old at baseline.<br><br><u>Inclusion criteria:</u><br>Subjects were participants in ATSDR (2000-2001) and MDPHHS (2003-2008) community-based health screening programs who lived, worked, or played in Libby, Montana at least 6 months prior to the end of 1990. | Subjects were assigned to one of five mutually-exclusive qualitative exposure categories (ranked from presumed highest to lowest exposure):<br>1) Workers employed by the W.R. Grace & Co. vermiculite operation (WRG workers)<br>2) Secondary contract workers to the vermiculite operation<br>3) Persons reporting other potential occupational asbestos or LAA exposure<br>4) Household contacts of WRG workers<br>5) Residents not falling into another category (i.e., residents without occupational asbestos exposure) | SMR<br><br><u>Stratifications/adjustments:</u><br>Age, sex, race, and calendar year (the standardizing variables), and smoking status, LAA exposure category, and radiographic (pleural and parenchymal) abnormality.      | Mortality in all asbestos-exposed groups through December 31, 2016 from systemic autoimmune disease, combined (SLE, RA, scleroderma):<br>SMR (95% CI): 4.30 (2.19, 6.40)                                       |
| <a href="#">Pfau et al. (2009)</a><br><br>Prospective cohort                                   | 70 subjects from ATSDR screening/CARD programs (former or current residents of Libby, Montana exposed to LAA for at least 6 months before December 1990) and                                                                                                                                                               | Libby residents were ranked on a scale of 0-4, where:<br>0: No known exposure<br>1: One to two routes of exposure for <5 years<br>2: Three or more routes of exposure for <5 years                                                                                                                                                                                                                                                                                                                                            | The presence, frequency, and/or quantification of ANA, ENAs (Sm, RNP, Ro52, SS-A60, SS-B, topo-I, Jo-1, ribosomal P, and chromatin), IgM RF, anti-CCP, and anti-fibroblast autoantibodies in serum, and the correlation of | Endpoints reported in LAA-exposed subjects and referents:<br><ul style="list-style-type: none"><li>LAA-exposed subjects had significantly higher anti-fibroblast antibodies than Missoula referents.</li></ul> |

| Table C-1. Epidemiological Study Data Extraction                    |                                                                                                                                                                                                                                                                    |                                                                                                                            |                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|---------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reference and Study Design                                          | Subjects                                                                                                                                                                                                                                                           | Exposure Metric and Reported Levels<br>Analysis                                                                            |                                                                                                                                                                                                                 | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|                                                                     | <p>unexposed age- and sex-matched referents (residents of Missoula, Montana).</p> <p><u>Exclusion criteria:</u><br/>Participants were excluded if they were currently using medications known to be associated with positive ANA tests and drug-induced lupus.</p> | <p>3: One to two routes of exposure for ≥5 years<br/>4: Three or more routes of exposure for ≥5 years</p>                  | <p>autoantibodies with the extent of exposure and ARD.</p> <p><u>Stratifications/adjustments:</u><br/>Statistical analyses were not adjusted for confounders; however, referents were age- and sex-matched.</p> | <p>Endpoint reported in LAA-exposed subjects only</p> <p><u>Autoantibodies:</u></p> <ul style="list-style-type: none"> <li>• ANA-positive: 50/70 (70%)</li> <li>• ENA-reactive: 16/70 (23%)</li> <li>• Chromatin: 7/16 (44%) (5/7 [71%] histone)</li> <li>• Rho52: 3/16 (19%)</li> <li>• Topo-I (Scl-70), RNP: 2/16 (13%)</li> <li>• SS-A60, SS-B: 1/16 (6%)</li> <li>• Jo1, Sm, ribo P: 0/16 (0%)</li> <li>• Topo-I-positive (ELISA): 8/70 (11%)</li> <li>• IgM RF-positive: 24/70 (34%)</li> <li>• Anti-CCP-positive: 3/45 (6.6%)</li> </ul> <p>Correlation with extent of exposure and ARD:</p> <ul style="list-style-type: none"> <li>• LAA subjects with anti-topo I autoantibodies more likely to have ARD and higher exposure scores than those without the autoantibodies</li> </ul> |
| <p><a href="#">Pfau et al. (2018)</a></p> <p>Prospective cohort</p> | <p>397 subjects who lived or worked in the Libby area (239 males, mean age 61.8 years; 158 females, mean age 59.2 years); recruited</p>                                                                                                                            | <p>Range of exposure for jobs reported in the cohort: 2.2-182.1 fibers/cc</p> <p>Mean exposure scores ± SD (unitless):</p> | <p>Serum was measured for the following targets:</p> <p>ENAs: dsDNA, Sm, RNP, ribosomal P protein, PCNA, SSA/Ro60, SSB/La,</p>                                                                                  | <p>Based on microarray analysis of the LAA cohort:</p> <ul style="list-style-type: none"> <li>• 43% ANA-positive</li> <li>• Autoantibodies highly expressed in the cohort included those for histone, ribosomal P protein,</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

| Table C-1. Epidemiological Study Data Extraction                    |                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reference and Study Design                                          | Subjects                                                                                                                                                                                                                                                                                                | Exposure Metric and Reported Levels Analysis                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                          | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                                     | <p>through the LERP and CARD programs. Subjects exposed mostly occupationally (53.3%) or mostly environmentally/residentially (46.7%).</p> <p><u>Exclusion criteria:</u> Participants were excluded if they were taking known lupus-inducing prescription medications or if samples were hemolyzed.</p> | <p>Occupational: 9.3±12.4<br/>Environ/Resident: 2.9±4.5</p> <p>Relative exposure scores were provided based on primary exposure path and the duration (number of years exposures occurred), developed by <a href="#">Noonan et al. (2006)</a>.</p>                                                                    | <p>Ro52/TRIM21, Scl-70 (topoisomerase I), and Jo-1.</p> <p>Myositis: Jo-1, Mi-2, Mi2α, MDA5, NXP2, TIF1γ, PL7, PL12, PM/Scl. K, SRP EJ, OJ; antibodies to chromatin and histones.</p> <p><u>Stratifications/adjustments:</u> None.</p>                                   | <p>Sm/RNP, Jo-1 (ENA and myositis), PM100, NXP2, and Mi2α.</p> <ul style="list-style-type: none"> <li>• Anti-histone antibodies were more prevalent in the cohort of LAA subjects than in a group of 87 steamfitters with exposure to other or unknown forms of asbestos.</li> </ul>                                                                                                                                                                                                               |
| <p><a href="#">Pfau et al. (2019)</a></p> <p>Prospective cohort</p> | <p>95 subjects who worked or lived in Libby, Montana for at least 6 months at least 10 years ago (85 males, 10 females; mean age 60.7-61.9 years); recruited through the CARD program. Subjects were assigned to one of the following three study groups based on diagnostic criteria for LPT:</p>      | <p>Mean exposure scores ± SD (unitless):</p> <p>Group 1: 42±29.9<br/>Group 2: 56.6±39.7<br/>Group 3: 48.1±35.8</p> <p>Relative exposure scores were calculated based on primary exposure path reported by subjects (occupational, household, environmental) multiplied by the duration (number of years exposures</p> | <p>The frequency of ANA, MCAA, and anti-plasminogen antibodies in serum; quantification of serum IL-1 beta, and IL-17.</p> <p><u>Stratifications/adjustments:</u> Statistical analyses were not adjusted for confounders; however, groups were age- and sex-matched.</p> | <p>Data were presented graphically:</p> <ul style="list-style-type: none"> <li>• The frequency of ANA positives was significantly higher in Group 1 than in Groups 2 or 3 (overall and in male and females evaluated separately).</li> <li>• For both MCAA and anti-plasminogen endpoints: The frequency of positives was significantly higher in Group 1 than in Group 3. Based on scores (tabulated as the number of SDs above the control mean), the Group 1 score was significantly</li> </ul> |

| Table C-1. Epidemiological Study Data Extraction        |                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reference and Study Design                              | Subjects                                                                                                                                                                                                                                                                                                                   | Exposure Metric and Reported Levels Analysis                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                | Results                                                                                                                                                                                                                                                                                                                                                                                            |
|                                                         | <p>Group 1: Progressive LPT<br/>Group 2: Stable LPT<br/>Group 3: No LPT</p> <p><u>Inclusion criteria:</u><br/>Subjects had a CT scan within the last 2 years and a serum sample within 2 years of their scan.</p>                                                                                                          | occurred), modified from <a href="#">Noonan et al. (2006)</a> .                                                                                                                                                                                                               |                                                                                                                                                                                                                                | <p>higher than Groups 2 or 3 (this effect persisted for males only when the sexes were evaluated separately).</p> <ul style="list-style-type: none"> <li>• There were no significant effects on the levels of IL-1 beta or IL-17 among groups.</li> </ul>                                                                                                                                          |
| Other study types                                       |                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                    |
| <a href="#">Diegel et al. (2018)</a><br><br>Case-series | <p>10 subjects (9 males and 1 female); aged 50-77 years</p> <p><u>Inclusion criteria:</u><br/>Subjects consisted of current or former residents of Libby and Troy, Montana in the LERP and/or CARD programs. Cases were selected by a research team of physicians based on presentations of severe autoimmune disease.</p> | <p>Qualitatively, all cases were exposed to “high” levels of LAA; exposure was “lifelong” in 7/10 cases. Determinations of high exposure were based on an exposure matrix as described in <a href="#">Noonan et al. (2015)</a>; and <a href="#">Noonan et al. (2006)</a>.</p> | <p>No quantitative analysis. Cases were qualitatively described with respect to autoantibody testing results and symptoms/diagnoses of autoimmune diseases.</p> <p><u>Stratifications/adjustments:</u><br/>Not applicable.</p> | <ul style="list-style-type: none"> <li>• 7/10 cases ANA+, 1/10 cases ANA-.</li> <li>• Reported symptoms included, but were not limited to, joint swelling, fatigue, ulcers, and arthralgia.</li> <li>• Rheumatology revealed 5/10 with RA; other diagnoses included (but were not limited to) lupus or lupus-like, polymyalgia, psoriatic arthritis, and urticarial vasculitis disease.</li> </ul> |

| Table C-1. Epidemiological Study Data Extraction                         |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reference and Study Design                                               | Subjects                                                                                                                                                                                                                                                                                                                                                                                                           | Exposure Metric and Reported Levels Analysis                        |                                                                                                                                                                                                                                                                                                                                                                                                            | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <a href="#">Hanson et al. (2016)</a><br><br>Non-traditional study design | 107 LAA-exposed subjects exposed to from the CARD program in Libby, Montana (63 males, 44 females; mean age 59.2-61.0 years); exposed subjects included MCAA+ (33 males, 23 females) and MCAA- (30 males, 21 females) serum samples. Forty normal human serum samples, matched for age and sex (MCAA-; 25 males, 15 females; mean age 58.7 years), were used as unexposed referents (obtained from Precision Med). | Qualitative exposure measures only (i.e., exposed vs. non-exposed). | Absorbance of binding by pooled serum samples to plasminogen were expressed as the number of SD above the mean absorbance for negative control sera; subsets of serum samples were evaluated as the percent positive for anti-plasminogen autoantibodies.<br><br><u>Stratifications/adjustments:</u> Statistical analyses were not adjusted for confounders; however, referents were age- and sex-matched. | Data were presented graphically: <ul style="list-style-type: none"> <li>• The mean absorbance of binding by pooled MCAA+ serum was 3 times higher (<math>p&lt;0.01</math>) than the absorbance of MCAA- samples or normal human serum.</li> <li>• 88% of the MCAA+ samples showed binding to plasminogen (i.e., had anti-plasminogen autoantibodies; <math>p&lt;0.01</math>) compared to about 30-40% of MCAA- and normal human sera samples.</li> </ul> |

ARD = asbestos-related disease; ATSDR = Agency for Toxic Substances and Disease Registry; CARD = Center for Asbestos Related Diseases; CCP = cyclic citrullinated peptide; CI = confidence interval; dsDNA = double-stranded deoxyribonucleic acid; ELISA = enzyme-linked immunosorbent assay; LERP = Libby Epidemiology Research Program; MDPHHS = Montana Department of Public Health and Human Services; PCNA = proliferating cell nuclear antigen; RNP = ribonucleoprotein P; SD = standard deviation; SMR = standardized mortality ratio

| Table C-2. Animal Study Data Extraction |                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                    |                                                                                                                                                                                                                                  |                                         |
|-----------------------------------------|------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| Species (sex)                           | Exposure and Study Design                                                                            | Fiber Type and Characteristics                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Endpoints Examined                                                 | Effects <sup>a</sup>                                                                                                                                                                                                             | Reference                               |
| Inhalation studies                      |                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                    |                                                                                                                                                                                                                                  |                                         |
| F344 rat;<br>6-8 males/group            | Inhalation exposure (nose-only); endpoints evaluated at 1 day and 1, 3, and 18 months after exposure | LAA from Rainy Creek Complex near Libby, Montana (2007); characterized in <a href="#">Cyphert et al. (2012)</a><br><br><u>All particles:</u><br>Fiber length:<br>Range of means:<br>2.6-3.7 µm<br>Fiber width:<br>Range of means:<br>0.38-0.44 µm<br>Aspect ratio:<br>Range of means:<br>7.0-9.5<br><br><u>Fibers with aspect ratio ≥5:</u><br>Fiber length:<br>Range of means:<br>4.8-5.4 µm<br>Fiber width:<br>Range of means:<br>0.38-0.41 µm<br>Aspect ratio:<br>Range of means:<br>13.5-14.3 | Cytokines                                                          | No effect on serum IL-6.                                                                                                                                                                                                         | <a href="#">Kodavanti et al. (2014)</a> |
|                                         |                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Candidate biomarkers of inflammation and/or autoimmune dysfunction | Significantly increased α-2-macroglobulin at 10.0 mg/m <sup>3</sup> (1 day after exposure, with no effects at later time points).<br><br>No effects on serum α1-acid glycoprotein, osteopontin, or leucine-rich α2-glycoprotein. |                                         |

| Table C-2. Animal Study Data Extraction     |                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                               |                                                                    |                                                                                                                                                                                                                                       |                                         |
|---------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| Species (sex)                               | Exposure and Study Design                                                                                                                                                                                                                                                                    | Fiber Type and Characteristics                                                                                                                                                                                                                                | Endpoints Examined                                                 | Effects <sup>a</sup>                                                                                                                                                                                                                  | Reference                               |
| Intra- or endotracheal instillation studies |                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                               |                                                                    |                                                                                                                                                                                                                                       |                                         |
| F344 rat;<br>6-8 males/group                | Intratracheal instillation (250 µL); single exposure; endpoints evaluated at 1 day and 3 and 15 months after exposure<br><br>0, 0.5, or 1.5 mg/rat in dispersion medium (5.5 mM <i>d</i> -glucose, 0.6 mg/mL rat serum, and 0.01 mg/mL DPPC in Ca <sup>2+</sup> /Mg <sup>2+</sup> -free PBS) | LAA from Rainy Creek Complex near Libby, Montana (2007); characterized in <a href="#">Cyphert et al. (2012)</a>                                                                                                                                               | Cytokines                                                          | Significantly increased serum IL-6 at 1.5 mg/rat (1 day after exposure, with no effects at later time points).                                                                                                                        | <a href="#">Kodavanti et al. (2014)</a> |
|                                             |                                                                                                                                                                                                                                                                                              | <u>All particles:</u><br>Fiber length:<br>Mean: 1.9 µm<br>Fiber width:<br>Mean: 0.39 µm<br>Aspect ratio:<br>Mean: 6.4<br><u>Fibers with aspect ratio ≥5:</u><br>Fiber length:<br>Mean: 5.0 µm<br>Fiber width:<br>Mean: 0.29 µm<br>Aspect ratio:<br>Mean: 19.5 | Candidate biomarkers of inflammation and/or autoimmune dysfunction | Significantly increased serum α1-acid glycoprotein and α2-macroglobulin at ≥0.5 mg/rat (1 day after exposure, with no effects at later time points).<br><br>No effect on serum levels of osteopontin or leucine-rich α2-glycoprotein. |                                         |

| Table C-2. Animal Study Data Extraction |                                                                                                                                                                                |                                                                                                     |                    |                                                                                                                                                                                                                                             |                                     |
|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| Species (sex)                           | Exposure and Study Design                                                                                                                                                      | Fiber Type and Characteristics                                                                      | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                                                                        | Reference                           |
| C57BL/6 mice, 7-11 females/group        | Intratracheal instillation (30 µL); two instillations 3-4 weeks apart; endpoints examined 8 months after exposure<br><br>0 (saline) or 1 mg/mL (30 µg) fiber suspension in PBS | LAA (Six Mix)<br>Fiber length: Mean: 7.21 µm<br>Fiber diameter: Mean: 0.61 µm<br>Aspect ratio: 11.8 | Autoantibodies     | ANA+ incidence<br>Control: 1/7<br>LAA: 8/11*<br><br>ENA testing suggested Sm and Jo-1 as the most frequent antigen targets.<br><br>No significant difference in anti-histone or anti-chromatin autoantibodies.                              | <a href="#">Ferro et al. (2014)</a> |
|                                         |                                                                                                                                                                                |                                                                                                     | Immunoglobulins    | Significantly decreased total IgG <sub>1</sub> subtype antibodies in the serum of LAA-exposed mice (without significant change in IgG <sub>2</sub> /IgG <sub>1</sub> ratio). No significant changes in IgM or IgA.                          |                                     |
|                                         |                                                                                                                                                                                |                                                                                                     | Cytokines          | Significantly increased production of serum cytokines in the T <sub>H</sub> 1 (IL-2, IFN-γ) and T <sub>H</sub> 17 (IL-6, IL-17) pathways.<br><br>Trend for increased serum IL-10 (T <sub>H</sub> 2 pathway; not statistically significant). |                                     |
|                                         |                                                                                                                                                                                |                                                                                                     | Immune cells       | B1a B-cells in the spleen (but not the lungs) were significantly increased in LAA-exposed mice.<br><br>No changes in overall percentages in B- or T-cells in the spleen or lungs.                                                           |                                     |

| Table C-2. Animal Study Data Extraction              |                                                                                                                                                              |                                                                                            |                    |                                                                                                                                                                                                                                               |                                      |
|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| Species (sex)                                        | Exposure and Study Design                                                                                                                                    | Fiber Type and Characteristics                                                             | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                                                                          | Reference                            |
| C57BL/6 mice, NS control and 11 exposed (sex NS)     | Intratracheal instillation (30 µL); single exposure; endpoints examined 7 months after exposure<br><br>0 (saline) or 1 mg/mL (30 µg) fiber suspension in PBS | LAA from U.S. Geological Survey (not further specified)                                    | Autoantibodies     | MCAA+ incidence<br>Control: None (incidence not reported)<br>LAA: 7/11* (p = 0.03)<br><br>Significantly increased MCAA binding when pooled serum of LAA-treated mice was cultured with mouse primary mesothelial cells, compared to controls. | <a href="#">Gilmer et al. (2016)</a> |
| C57BL/6 mice, 5/group per timepoint (sexes combined) | Endotracheal instillation (60 µL), single instillation, endpoints examined 1-8 days after exposure<br><br>0 or 1 mg/mL (60 µg) fiber suspension in PBS       | LAA (Six Mix) provided by the U.S. Geological Survey (fiber characterization not reported) | Immune cells       | Significantly decreased percentage of B1a B-cells in the pleural cavity of LAA-exposed mice compared to controls 4 days post-exposure; percentage comparable to control by 8 days post-exposure.                                              | <a href="#">Pfau et al. (2014)</a>   |

| Table C-2. Animal Study Data Extraction |                                                                                                                                                                                                                                                        |                                                                                                              |                    |                                                                                                                                                                                          |                                       |
|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|
| Species (sex)                           | Exposure and Study Design                                                                                                                                                                                                                              | Fiber Type and Characteristics                                                                               | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                     | Reference                             |
| C57BL/6 mice, 11-18 females/group       | Intratracheal instillation (30 µL); two instillations 1 week apart; endpoints examined 7 months after exposure<br><br>0 (saline) or 1 mg/mL (30 µg) fiber suspension in PBS                                                                            | LAA (Six Mix)<br>Fiber length:<br>Mean: 7.21 µm<br>Fiber diameter:<br>Mean: 0.61 µm<br>Aspect ratio:<br>11.8 | Autoantibodies     | ANA+ incidence<br>Control: 4/18<br>LAA: 8/11*                                                                                                                                            | <a href="#">Zebedeo et al. (2014)</a> |
| Oropharyngeal instillation studies      |                                                                                                                                                                                                                                                        |                                                                                                              |                    |                                                                                                                                                                                          |                                       |
| C57BL/6 mice, 3/sex/group               | Oropharyngeal instillation (25 µL); single dose administered to pups 12 weeks of age; endpoints evaluated 24 hours after exposure<br><br>0 or 2 mg/mL (50 µg) particle suspension in dispersion medium (60% PBS, 39% mouse serum albumin, and 1% DSPC) | LAA (Six Mix), as described in <a href="#">Gunter et al. (2003)</a> ; <a href="#">Meeker et al. (2003)</a>   | Cytokines          | <b>Whole-lung lavage fluid:</b><br>Significantly increased IL-1β, TNF-α, IL-6, and IL-5 in exposed pups (sexes combined).<br><br>No change in IL-10 or KC/GRO.                           | <a href="#">Brown et al. (2016)</a>   |
|                                         |                                                                                                                                                                                                                                                        |                                                                                                              | Immune cells       | <b>Whole-lung lavage fluid:</b><br>Significantly decreased macrophages and significantly increased neutrophils in exposed pups (sexes combined).<br><br>No effect on overall cell count. |                                       |

| Table C-2. Animal Study Data Extraction |                                                                                                                                                                                |                                                                                                      |                    |                                                                                                                                                                                                                                                                                                                                                                                              |                                    |  |         |     |       |  |  |         |
|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|--|---------|-----|-------|--|--|---------|
| Species (sex)                           | Exposure and Study Design                                                                                                                                                      | Fiber Type and Characteristics                                                                       | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                                         | Reference                          |  |         |     |       |  |  |         |
| C57BL/6 mice, 4–9/sex/group             | Oropharyngeal instillation (15 µL) two instillations 2 weeks apart; endpoints examined 4 and 7 months after exposure<br><br>0 or 0.1 mg/mL (1.5 µg) fiber suspension in saline | LAA (Six Mix)<br>Fiber length: Mean: 2.3 µm<br>Fiber width: Mean: 0.36 µm<br>Aspect ratio: Mean: 8.4 | Autoantibodies     | Nonsignificant (p=0.1) increase in ANA+ mice in LAA-exposed group, compared to control, at 7 months (sexes combined); most positives were identified in females. No change at 4 months.<br><br>No increase in MCAA+ mice in LAA-exposed group, compared to control (time evaluated NS).                                                                                                      | <a href="#">Pfau et al. (2017)</a> |  |         |     |       |  |  |         |
|                                         |                                                                                                                                                                                |                                                                                                      | Immune cells       | <b>Spleen (7 months):</b><br>Significantly increased number of B-cells and B1a B-cells (percent of parent population) in LAA-exposed mice (sexes combined). No change in B suppressor cells (percent of parent population). No significant change in number or percentage of B-cells when stratified by sex.<br><br>No significant change in number or percent of T-cells or subpopulations. |                                    |  |         |     |       |  |  |         |
|                                         |                                                                                                                                                                                |                                                                                                      |                    | <b>Peritoneum (7 months):</b><br>Significantly increased B-cells and decreased B1a B-cells (percent of parent population), with sexes combined. <table><tr><td></td><td>Control</td><td>LAA</td></tr><tr><td colspan="3">Males</td></tr><tr><td>B-cells</td><td>49.2±9.4<sup>a</sup></td><td>63.2±2.5*</td></tr><tr><td>B1a B-cells</td><td>26.1±7.1</td><td>12.5±3.5*</td></tr></table>     |                                    |  | Control | LAA | Males |  |  | B-cells |
|                                         | Control                                                                                                                                                                        | LAA                                                                                                  |                    |                                                                                                                                                                                                                                                                                                                                                                                              |                                    |  |         |     |       |  |  |         |
| Males                                   |                                                                                                                                                                                |                                                                                                      |                    |                                                                                                                                                                                                                                                                                                                                                                                              |                                    |  |         |     |       |  |  |         |
| B-cells                                 | 49.2±9.4 <sup>a</sup>                                                                                                                                                          | 63.2±2.5*                                                                                            |                    |                                                                                                                                                                                                                                                                                                                                                                                              |                                    |  |         |     |       |  |  |         |
| B1a B-cells                             | 26.1±7.1                                                                                                                                                                       | 12.5±3.5*                                                                                            |                    |                                                                                                                                                                                                                                                                                                                                                                                              |                                    |  |         |     |       |  |  |         |

| Table C-2. Animal Study Data Extraction |                           |                                |                    |                                                                                                                              |                       |             |           |
|-----------------------------------------|---------------------------|--------------------------------|--------------------|------------------------------------------------------------------------------------------------------------------------------|-----------------------|-------------|-----------|
| Species (sex)                           | Exposure and Study Design | Fiber Type and Characteristics | Endpoints Examined | Effects <sup>a</sup>                                                                                                         |                       |             | Reference |
|                                         |                           |                                |                    | B suppressor                                                                                                                 | 4.9±1.7               | 6.0±3.1     |           |
|                                         |                           |                                |                    | Females                                                                                                                      |                       |             |           |
|                                         |                           |                                |                    | B-cells                                                                                                                      | 59.3±6.6              | 66.9±10.7   |           |
|                                         |                           |                                |                    | B1a B-cells                                                                                                                  | 19.9±5.0              | 13.6±5.5    |           |
|                                         |                           |                                |                    | B suppressor                                                                                                                 | 6.9±3.2               | 3.3±1.5*    |           |
|                                         |                           |                                |                    | <sup>a</sup> Values are percent of parent population ± SD.<br>*Statistically significant as determined by the study authors. |                       |             |           |
|                                         |                           |                                | Cytokines          | Significantly decreased T-cells (percent of parent population) when sexes were combined, but not when stratified by sex.     |                       |             |           |
|                                         |                           |                                |                    | No significant changes in monocytes or lymphocytes (percent of total cells), with sexes combined.                            |                       |             |           |
|                                         |                           |                                |                    | Serum (7 months):                                                                                                            |                       |             |           |
|                                         |                           |                                |                    | Cytokine                                                                                                                     | Control               | LAA         |           |
|                                         |                           |                                |                    | IFN-γ (T <sub>H</sub> 1)                                                                                                     | 4.0±0.77 <sup>a</sup> | 9.1±5.2     |           |
|                                         |                           |                                |                    | Males                                                                                                                        | 3.8±0.64              | 14.1±2.4*   |           |
|                                         |                           |                                |                    | Females                                                                                                                      | 4.3±0.96              | 5.0±0.98    |           |
|                                         |                           |                                |                    | IL-10 (T <sub>H</sub> 2)                                                                                                     | 69.0±8.6              | 117.6±54.5* |           |
|                                         |                           |                                |                    | Males                                                                                                                        | 65.2±8.0              | 163.6±11.5* |           |
|                                         |                           |                                |                    | Females                                                                                                                      | 76.0±4.0              | 80.8±11.5   |           |
|                                         |                           |                                |                    | TNF-α (T <sub>H</sub> 1)                                                                                                     | 25.6±2.2              | 41.6±18.2*  |           |

| Table C-2. Animal Study Data Extraction |                           |                                |                                     |                                                                                                              |          |            |           |
|-----------------------------------------|---------------------------|--------------------------------|-------------------------------------|--------------------------------------------------------------------------------------------------------------|----------|------------|-----------|
| Species (sex)                           | Exposure and Study Design | Fiber Type and Characteristics | Endpoints Examined                  | Effects <sup>a</sup>                                                                                         |          |            | Reference |
|                                         |                           |                                |                                     | Males                                                                                                        | 25.1±1.8 | 56.8±18.0* |           |
|                                         |                           |                                |                                     | Females                                                                                                      | 26.6±2.7 | 29.4±2.4   |           |
|                                         |                           |                                |                                     | IL-6 (T <sub>H</sub> 17)                                                                                     | 8.3±1.1  | 10.5±2.6*  |           |
|                                         |                           |                                |                                     | Males                                                                                                        | 7.9±0.96 | 12.2±3.1*  |           |
|                                         |                           |                                |                                     | Females                                                                                                      | 8.9±1.1  | 9.2±1.1    |           |
|                                         |                           |                                |                                     | IL-17 (T <sub>H</sub> 17)                                                                                    | 1.4±0.95 | 12.6±2.5*  |           |
|                                         |                           |                                |                                     | Males                                                                                                        | 1.1±0.88 | 10.8±2.6*  |           |
|                                         |                           |                                |                                     | Females                                                                                                      | 1.9±0.94 | 14.0±1.4*  |           |
|                                         |                           |                                |                                     | <sup>a</sup> Values are mean±SD, in pg/mL.<br>*Statistically significant as determined by the study authors. |          |            |           |
|                                         |                           |                                |                                     | No change in IL-2 (T <sub>H</sub> 1) or IL-4 (T <sub>H</sub> 2) in males, females, or sexes combined.        |          |            |           |
|                                         |                           |                                | Spleen weight and splenocyte counts | No effect on absolute spleen weight (or splenocyte count) based on data for the combined sexes.              |          |            |           |

| Table C-2. Animal Study Data Extraction |                                                                                                                                                                             |                                                                                                                                                                                            |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                      |         |         |
|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|---------|---------|
| Species (sex)                           | Exposure and Study Design                                                                                                                                                   | Fiber Type and Characteristics                                                                                                                                                             | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                         | Reference                            |         |         |
| Intraperitoneal injection studies       |                                                                                                                                                                             |                                                                                                                                                                                            |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                      |         |         |
| C57BL/6 mice, 6/sex/group               | Intraperitoneal injection (200 µL); one injection on study day 0; endpoints evaluated 14 days after treatment<br><br>0 (saline) or 1 mg/mL (200 µg) fiber suspension in PBS | LAA, as described in <a href="#">Duncan et al. (2014)</a><br><br>Fiber length:<br>Range of means: 2.3-3.7 µm<br>Fiber width:<br>Mean: 0.36 µm<br>Aspect ratio:<br>Range of means: 8.4-12.8 | Immune cells       | <b>Peritoneum:</b><br>Data for combined sexes: <ul style="list-style-type: none"><li>Significantly increased PMN cells and WBCs (as percent of total cells).</li><li>Significantly increased B1a B-cells (as percent of total lymphocytes).</li><li>Significantly decreased total B-cells and T-cells (as percent of total cells and as percent of lymphocytes).</li><li>Trend for reduced Th cells (non-significant).</li></ul><br><u>Sex effect:</u> None. | <a href="#">Badger et al. (2021)</a> |         |         |
|                                         |                                                                                                                                                                             |                                                                                                                                                                                            |                    | <b>Spleen:</b><br>Data for combined sexes: <ul style="list-style-type: none"><li>Significantly increased B1a B-cells (as percent of total lymphocytes).</li><li>No effect on total WBC numbers.</li><li>No effect on total B-cells, total T-cells, or Th cells.</li></ul><br><u>Sex effect:</u> Significantly increased B1a B-cells in females compared to males, unrelated to treatment.                                                                    |                                      |         |         |
|                                         |                                                                                                                                                                             |                                                                                                                                                                                            | Immunoglobulins    | <b>Peritoneal lavage fluid:</b><br>Data for combined sexes: <table><tr><td>Ig type</td><td>Control</td><td>LAA</td></tr><tr><td>IgG<sub>1</sub></td><td>1.00±0.10<sup>a</sup></td><td>0.53±0.08*</td></tr></table>                                                                                                                                                                                                                                           |                                      | Ig type | Control |
| Ig type                                 | Control                                                                                                                                                                     | LAA                                                                                                                                                                                        |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                      |         |         |
| IgG <sub>1</sub>                        | 1.00±0.10 <sup>a</sup>                                                                                                                                                      | 0.53±0.08*                                                                                                                                                                                 |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                      |         |         |

| Table C-2. Animal Study Data Extraction                                           |                           |                                |                    |                                                                                        |                        |                         |           |  |  |  |
|-----------------------------------------------------------------------------------|---------------------------|--------------------------------|--------------------|----------------------------------------------------------------------------------------|------------------------|-------------------------|-----------|--|--|--|
| Species (sex)                                                                     | Exposure and Study Design | Fiber Type and Characteristics | Endpoints Examined | Effects <sup>a</sup>                                                                   |                        |                         | Reference |  |  |  |
|                                                                                   |                           |                                |                    | IgG <sub>2b</sub>                                                                      | 1.00±0.08              | 0.53±0.04*              |           |  |  |  |
|                                                                                   |                           |                                |                    | IgA                                                                                    | 1.00±0.14              | 1.68±0.26*              |           |  |  |  |
|                                                                                   |                           |                                |                    | <sup>a</sup> Values are relative concentration ± SE, using saline control as standard. |                        |                         |           |  |  |  |
|                                                                                   |                           |                                |                    | *Statistically significant as determined by the study authors.                         |                        |                         |           |  |  |  |
|                                                                                   |                           |                                |                    | • No effect on relative concentrations of IgG2a, IgG3, IgM, or IgE.                    |                        |                         |           |  |  |  |
|                                                                                   |                           |                                |                    | Sex effect: Significantly increased IgA in control males compared to control females.  |                        |                         |           |  |  |  |
|                                                                                   |                           |                                | Cytokines          | Peritoneal lavage fluid:                                                               |                        |                         |           |  |  |  |
|                                                                                   |                           |                                |                    | Cytokine                                                                               | Control                | LAA                     |           |  |  |  |
|                                                                                   |                           |                                |                    | IL-6                                                                                   | 0.36±0.17 <sup>a</sup> | 16.84±4.16*             |           |  |  |  |
|                                                                                   |                           |                                |                    | Males                                                                                  | 0.46±0.22              | 18.03±5.89 <sup>#</sup> |           |  |  |  |
|                                                                                   |                           |                                |                    | Females                                                                                | 0.26±0.12              | 12.86±1.77 <sup>#</sup> |           |  |  |  |
|                                                                                   |                           |                                |                    | MCP-1                                                                                  | 0.77±0.2               | 1.88±0.29*              |           |  |  |  |
|                                                                                   |                           |                                |                    | Males                                                                                  | 0.90±0.22              | 1.63±0.29               |           |  |  |  |
|                                                                                   |                           |                                |                    | Females                                                                                | 0.65±0.18              | 1.90±0.32 <sup>#</sup>  |           |  |  |  |
|                                                                                   |                           |                                |                    | <sup>a</sup> Values are means ± SE in pg/mL.                                           |                        |                         |           |  |  |  |
|                                                                                   |                           |                                |                    | *Statistically significant as determined by the study authors.                         |                        |                         |           |  |  |  |
| <sup>#</sup> Statistically significant based on t-test performed for this review. |                           |                                |                    |                                                                                        |                        |                         |           |  |  |  |

| Table C-2. Animal Study Data Extraction |                                                                                                                                                                                                                                                                        |                                                                                |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
|-----------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------------|---------|-------|------|------------------------------|--------------------------|------------------------------|-------------------|------------------------------------------------------|---------|-----------------|-----------------|--|
| Species (sex)                           | Exposure and Study Design                                                                                                                                                                                                                                              | Fiber Type and Characteristics                                                 | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Reference |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
|                                         |                                                                                                                                                                                                                                                                        |                                                                                |                    | <ul style="list-style-type: none"><li>No effect on IL-10, TNF-<math>\alpha</math>, IFN-<math>\gamma</math>, or IL-12p70.</li></ul> <p><u>Sex effect:</u> None.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |           |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
|                                         |                                                                                                                                                                                                                                                                        |                                                                                |                    | <p><b>Serum:</b></p> <table><tr><td>Cytokine</td><td>Control</td><td>LAA</td></tr><tr><td>IL-6</td><td>5.47<math>\pm</math>0.82<sup>a</sup></td><td>7.87<math>\pm</math>1.06*</td></tr><tr><td>Males</td><td>5.66<math>\pm</math>0.97</td><td>8.71<math>\pm</math>1.73</td></tr><tr><td>Females</td><td>5.28<math>\pm</math>1.42</td><td>7.04<math>\pm</math>1.30</td></tr></table> <p><sup>a</sup>Values are means <math>\pm</math> SE in pg/mL.<br/>*Statistically significant as determined by the study authors.</p> <ul style="list-style-type: none"><li>No effect on MCP-1, IL-10, TNF-<math>\alpha</math>, IFN-<math>\gamma</math>, or IL-12p70.</li></ul> <p><u>Sex effect:</u> None.</p> |           | Cytokine         | Control | LAA   | IL-6 | 5.47 $\pm$ 0.82 <sup>a</sup> | 7.87 $\pm$ 1.06*         | Males                        | 5.66 $\pm$ 0.97   | 8.71 $\pm$ 1.73                                      | Females | 5.28 $\pm$ 1.42 | 7.04 $\pm$ 1.30 |  |
|                                         |                                                                                                                                                                                                                                                                        |                                                                                | Cytokine           | Control                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |           | LAA              |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
|                                         |                                                                                                                                                                                                                                                                        |                                                                                | IL-6               | 5.47 $\pm$ 0.82 <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |           | 7.87 $\pm$ 1.06* |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
| Males                                   | 5.66 $\pm$ 0.97                                                                                                                                                                                                                                                        | 8.71 $\pm$ 1.73                                                                |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
| Females                                 | 5.28 $\pm$ 1.42                                                                                                                                                                                                                                                        | 7.04 $\pm$ 1.30                                                                |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
| Spleen weight                           | <p><u>Data for combined sexes:</u><br/>Significantly increased relative spleen weight, with no body weight effects (absolute spleen weights not reported).</p> <p><u>Sex effect:</u> Significantly increased in females compared to males, unrelated to treatment.</p> |                                                                                |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
| C57BL/6 mice, 7-10/ sex/group           | Intraperitoneal injection (200 $\mu$ L); one injection on study day 0;                                                                                                                                                                                                 | LAA, as described in <a href="#">Duncan et al. (2014)</a><br><br>Fiber length: | Immune cells       | <p><b>Peritoneum:</b></p> <table><tr><td>Endpoint</td><td>Control</td><td>LAA</td></tr><tr><td>Males</td><td></td><td></td></tr><tr><td>WBCs (x10<sup>6</sup>)</td><td>5.87<math>\pm</math>0.65<sup>a</sup></td><td>22.46<math>\pm</math>4.61*</td></tr></table>                                                                                                                                                                                                                                                                                                                                                                                                                                   | Endpoint  | Control          | LAA     | Males |      |                              | WBCs (x10 <sup>6</sup> ) | 5.87 $\pm$ 0.65 <sup>a</sup> | 22.46 $\pm$ 4.61* | <a href="#">Christofidou-Solomidou et al. (2019)</a> |         |                 |                 |  |
| Endpoint                                | Control                                                                                                                                                                                                                                                                | LAA                                                                            |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
| Males                                   |                                                                                                                                                                                                                                                                        |                                                                                |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |
| WBCs (x10 <sup>6</sup> )                | 5.87 $\pm$ 0.65 <sup>a</sup>                                                                                                                                                                                                                                           | 22.46 $\pm$ 4.61*                                                              |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |                  |         |       |      |                              |                          |                              |                   |                                                      |         |                 |                 |  |

| Table C-2. Animal Study Data Extraction |                                                                                                          |                                                                                                    |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |            |           |
|-----------------------------------------|----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------|-----------|
| Species (sex)                           | Exposure and Study Design                                                                                | Fiber Type and Characteristics                                                                     | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                                                                               |           |            | Reference |
|                                         | endpoints evaluated 3 days after treatment<br><br>0 (saline) or 1 mg/mL (200 µg) fiber suspension in PBS | Range of means: 2.3-3.7 µm<br>Fiber width: Mean: 0.36 µm<br>Aspect ratio: Range of means: 8.4-12.8 |                    | Lymphocytes (%)                                                                                                                                                                                                                                                                                                                                                                                                                    | 47.2±5.68 | 11.7±5.82* |           |
|                                         |                                                                                                          |                                                                                                    |                    | PMNs (%)                                                                                                                                                                                                                                                                                                                                                                                                                           | 1.1±1.89  | 12.2±2.39* |           |
|                                         |                                                                                                          |                                                                                                    |                    | Females                                                                                                                                                                                                                                                                                                                                                                                                                            |           |            |           |
|                                         |                                                                                                          |                                                                                                    |                    | WBCs (x10 <sup>6</sup> )                                                                                                                                                                                                                                                                                                                                                                                                           | 2.03±0.56 | 6.29±1.41* |           |
|                                         |                                                                                                          |                                                                                                    |                    | Lymphocytes (%)                                                                                                                                                                                                                                                                                                                                                                                                                    | 22.3±3.10 | 8.3±4.21*  |           |
|                                         |                                                                                                          |                                                                                                    |                    | PMNs (%)                                                                                                                                                                                                                                                                                                                                                                                                                           | 12.2±2.00 | 32.9±4.13* |           |
|                                         |                                                                                                          |                                                                                                    |                    | <sup>a</sup> Values are means ± SD.<br>*Statistically significant as determined by the study authors.                                                                                                                                                                                                                                                                                                                              |           |            |           |
|                                         |                                                                                                          |                                                                                                    |                    | <ul style="list-style-type: none"><li>Significantly increased percent macrophages (males and combined sexes).</li><li>Significantly decreased percent Th cells (combined sexes).</li><li>No effect on percent B-cells or T-cells (combined sexes).</li><li>Significantly decreased percent B1a B-cells, alpha 4-integrin expression on B1a B-cells, and percent B suppressor cells (males, females, and combined sexes).</li></ul> |           |            |           |
|                                         |                                                                                                          |                                                                                                    |                    | <u>Sex effect:</u> Significantly increased WBCs in males compared to females, unrelated to treatment. Significantly decreased percent PMN neutrophils in males                                                                                                                                                                                                                                                                     |           |            |           |
|                                         |                                                                                                          |                                                                                                    |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                    |           |            |           |

| Table C-2. Animal Study Data Extraction |                           |                                |                    |                                                                                                                                                                                                        |                       |  |                         |
|-----------------------------------------|---------------------------|--------------------------------|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|--|-------------------------|
| Species (sex)                           | Exposure and Study Design | Fiber Type and Characteristics | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                                   | Reference             |  |                         |
|                                         |                           |                                |                    | compared to females, unrelated to treatment. Significantly increased percent lymphocytes in control males compared to control females.                                                                 |                       |  |                         |
|                                         |                           |                                | Spleen weight      | Endpoint                                                                                                                                                                                               | Control               |  | LAA                     |
|                                         |                           |                                |                    | Males                                                                                                                                                                                                  |                       |  |                         |
|                                         |                           |                                |                    | Body weight (g)                                                                                                                                                                                        | 26.7±1.4 <sup>a</sup> |  | 27.1±1.8                |
|                                         |                           |                                |                    | Spleen (mg)                                                                                                                                                                                            | 77.7±8.3              |  | 131.1±10.2 <sup>#</sup> |
|                                         |                           |                                |                    | Relative spleen (%)                                                                                                                                                                                    | 0.29±0.02             |  | 0.48±0.01 <sup>*</sup>  |
|                                         |                           |                                |                    | Females                                                                                                                                                                                                |                       |  |                         |
|                                         |                           |                                |                    | Body weight (g)                                                                                                                                                                                        | 19.3±1.5              |  | 18.0±1.2                |
|                                         |                           |                                |                    | Spleen (mg)                                                                                                                                                                                            | 92.0±12.8             |  | 122.6±11.3 <sup>#</sup> |
|                                         |                           |                                |                    | Relative spleen (%)                                                                                                                                                                                    | 0.48±0.07             |  | 0.68±0.06 <sup>*</sup>  |
|                                         |                           |                                |                    | <sup>a</sup> Values are means ± SD.<br><sup>*</sup> Statistically significant as determined by the study authors.<br><sup>#</sup> Statistically significant based on t-test performed for this review. |                       |  |                         |
|                                         |                           |                                |                    | <u>Sex effect</u> : Significantly increased relative spleen weight in females compared to males, unrelated to treatment.                                                                               |                       |  |                         |

| Table C-2. Animal Study Data Extraction                |                                                                                                                                                   |                                                                                                                                                                                                                                                                                              |                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                    |
|--------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Species (sex)                                          | Exposure and Study Design                                                                                                                         | Fiber Type and Characteristics                                                                                                                                                                                                                                                               | Endpoints Examined | Effects <sup>a</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Reference                          |
| C57BL/6 mice, 3-5/group per timepoint (sexes combined) | Intraperitoneal injection (100 µL), once, endpoints examined 1, 3, and 7 days after exposure<br><br>0 or 1 mg/mL (100 µg) fiber suspension in PBS | LAA (Six Mix) provided by the U.S. Geological Survey (fiber characterization not reported)<br><br>Mice injected with lymphocytes from CD445.1 transgenic mice; cells fractions measured in the peritoneal cavity, spleen, and mesenteric lymph nodes using flow cytometry after 3 and 6 days | Immune cells       | <b>Peritoneal cavity:</b> <ul style="list-style-type: none"> <li>Significantly decreased percent B1a B-cells (of peritoneal B-cells) 7 days post-exposure.</li> <li>Significantly decreased number of B1a B-cells 3 days post-exposure.</li> <li>Significantly decreased expression of α4 integrin and MHC II on B1a B-cells 3-7 days post-exposure.</li> <li>No significant change in B1b B-cells, B2 B-cells, or T-cells.</li> </ul> <b>B-cell trafficking:</b> <ul style="list-style-type: none"> <li>B1a B-cells decreased in the peritoneal cavity and increased in the spleen and mesenteric lymph nodes</li> <li>Increased spleen cellularity is supported by elevated spleen weight</li> </ul> | <a href="#">Pfau et al. (2014)</a> |
|                                                        |                                                                                                                                                   |                                                                                                                                                                                                                                                                                              | Immunoglobulins    | Significant increase in IgM levels in peritoneal fluid of LAA-exposed mice compared to controls 3 days post-exposure. No changes in IgA or IgG.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                    |

<sup>a</sup>Quantitative data (e.g., means and SDs) reported for adverse effects when available. Graphically presented data and non-adverse findings summarized qualitatively.

DPPC = 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC = distearoylphosphatidylcholine; MHC II = major histocompatibility complex class II; NS = not specified; PBS = phosphate-buffered saline; SE = standard error; T<sub>H</sub>1 = cytokine profile including IL-2, IFN-γ, and TNF-α; T<sub>H</sub>2 = cytokine profile including IL-4, IL-5, and IL-10; T<sub>H</sub>17 = cytokine profile including IL-6 and IL-17

|                         |           |                                                              |           |                                                                                              |           |                                                                                                                                                                               |
|-------------------------|-----------|--------------------------------------------------------------|-----------|----------------------------------------------------------------------------------------------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Supplemental File 1     |           | Human Studies                                                |           |                                                                                              |           |                                                                                                                                                                               |
| Citation (Author, year) |           | Diegel et al. 2018 (hero.6859866)                            |           | Hanson et al. 2016 (hero.3530939)                                                            |           | Larson et al. 2020 (hero.6868484)                                                                                                                                             |
| DOMAIN                  | RATING    | COMMENT                                                      | RATING    | COMMENT                                                                                      | RATING    | COMMENT                                                                                                                                                                       |
| Exposure measurement    | Deficient | Exposure was measured qualitatively only.                    | Deficient | Exposure was measured qualitatively only.                                                    | Deficient | Exposure was measured qualitatively only.                                                                                                                                     |
| Outcome ascertainment   | Adequate  |                                                              | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |
| Participant Selection   | Adequate  |                                                              | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |
| Confounding             | Deficient | Information on potential confounders was not described.      | Deficient | Matched on age and sex, no confounding included in statistical analyses.                     | Adequate  |                                                                                                                                                                               |
| Analysis                | Deficient | Case-series, no control group was used.                      | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |
| Selective Reporting     | Adequate  |                                                              | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |
| Sensitivity             | Deficient | Quantitative results were not presented (case-series report) | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |
|                         |           |                                                              |           |                                                                                              |           |                                                                                                                                                                               |
|                         |           |                                                              |           |                                                                                              |           |                                                                                                                                                                               |
| Citation (Author, year) |           | Pfau et al. 2009 (hero.3092696)                              |           | Pfau et al 2018 (hero.6869521)                                                               |           | Pfau et al 2019 (hero.6875447)                                                                                                                                                |
| DOMAIN                  | RATING    | COMMENT                                                      | RATING    | COMMENT                                                                                      | RATING    | COMMENT                                                                                                                                                                       |
| Exposure measurement    | Deficient | Exposure was measured qualitatively only.                    | Deficient | No quantitative individual exposure estimates were provided.                                 | Deficient | Exposure was measured qualitatively only.                                                                                                                                     |
| Outcome ascertainment   | Adequate  |                                                              | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |
| Participant Selection   | Adequate  |                                                              | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |
| Confounding             | Adequate  |                                                              | Deficient | No mention of adjustment for confounders in statistical analyses.                            | Deficient | Matched on age and sex, no confounding included in statistical analyses. No mention of excluding subjects based on medications known to be associated with positive ANA tests |
| Analysis                | Adequate  |                                                              | Deficient | No referent control group (results compared to workers exposed to predominantly chrysotile). | Deficient | No referent control; outcomes were compared among groups with varying levels of pleural fibrosis.                                                                             |
| Selective Reporting     | Adequate  |                                                              | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |
| Sensitivity             | Adequate  |                                                              | Adequate  |                                                                                              | Adequate  |                                                                                                                                                                               |

| Supplemental Files 1                         |          | Animal Studies                                                                                                                                                                                                                                                                |            | Brown et al. 2016 (hero.3520487)                                                                                                                                                                                                                                    |            | Christoforou-Solomidou et al. 2021 (hero.6874711)                                                                                                                                                                                                                                                                                                                                                                                                                                                               |            | Ferre et al. 2014 (hero.3078982)                                                                                                                                                                                                                                                                                                                                                                |            | Glimmer et al. 2016 (hero.3530998)                                                                                                                                                                                      |            |                                                                                                                                                                                                           |
|----------------------------------------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Citation (Author, year)                      |          |                                                                                                                                                                                                                                                                               |            |                                                                                                                                                                                                                                                                     |            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |            |                                                                                                                                                                                                                                                                                                                                                                                                 |            |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
| DOMAIN                                       | RATING   | COMMENT                                                                                                                                                                                                                                                                       | RATING     | COMMENT                                                                                                                                                                                                                                                             | RATING     | COMMENT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | RATING     | COMMENT                                                                                                                                                                                                                                                                                                                                                                                         | RATING     | COMMENT                                                                                                                                                                                                                 |            |                                                                                                                                                                                                           |
| Allocation                                   | Adequate |                                                                                                                                                                                                                                                                               | Inadequate | Method of dam allocation (filtered air or tobacco smoke) is not reported. The study indicated that pups from each group were selected randomly with the same sex ratio, but how pups were allocated into asbestos vs dispersion media (DM) groups was not reported. | Inadequate | Method of animal allocation is not reported.                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Inadequate | Method of animal allocation is not reported.                                                                                                                                                                                                                                                                                                                                                    | Inadequate | Method of animal allocation is not reported.                                                                                                                                                                            |            |                                                                                                                                                                                                           |
| Observational bias/blinding                  | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
| Confounding                                  | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Inadequate | Concerns were identified based on barbering. Animals from all groups were subjected to euthanasia due to excess barbering (including a disproportionate number of controls). Barbering incidence in surviving animals was not reported. Excessive barbering could be indicative of stress in colony (PMID 40033013), and may have impacted immune responses in all groups (including controls). | Adequate   |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
| Attrition                                    | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Inadequate | The numbers of animals per group (and/or the number that died) was not explicitly stated, and given that outcomes were measured 7 mos after exposure, this had the potential to substantially impact the study results. |            |                                                                                                                                                                                                           |
| Chemical administration and characterization | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Inadequate | The LA used in the experiment was not fully characterized (only specified that the asbestos sample was obtained from the US Geological Survey).                                                                         |            |                                                                                                                                                                                                           |
| Endpoint measurement                         | Adequate |                                                                                                                                                                                                                                                                               | Inadequate | Atypical biomarker of autoimmune disease (shift from Th1 to Th2 cytokine response)                                                                                                                                                                                  | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
| Results presentation                         | Adequate |                                                                                                                                                                                                                                                                               | Inadequate | Data for pups were pooled across sexes.                                                                                                                                                                                                                             | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
| Selective reporting                          | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
| Sensitivity                                  | Adequate |                                                                                                                                                                                                                                                                               | Inadequate | Detection of significant effects (e.g., differences between males and females) was precluded by the small sample sizes used in the study.                                                                                                                           | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
|                                              |          |                                                                                                                                                                                                                                                                               |            |                                                                                                                                                                                                                                                                     |            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |            |                                                                                                                                                                                                                                                                                                                                                                                                 |            |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
|                                              |          |                                                                                                                                                                                                                                                                               |            |                                                                                                                                                                                                                                                                     |            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |            |                                                                                                                                                                                                                                                                                                                                                                                                 |            |                                                                                                                                                                                                                         |            |                                                                                                                                                                                                           |
| Citation (Author, year)                      |          | Kodavanti et al. 2014 (hero.2671447) (acute)                                                                                                                                                                                                                                  |            | Kodavanti et al. 2014 (hero.2671447) (subchronic)                                                                                                                                                                                                                   |            | Pfau et al. 2017 (hero.6869425)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |            | Pfau et al. 2014 (hero.3078201) (instillation)                                                                                                                                                                                                                                                                                                                                                  |            | Pfau et al. 2014 (hero.3078201) (injection)                                                                                                                                                                             |            | Zelbedea et al. 2014 (hero.3078202)                                                                                                                                                                       |
| DOMAIN                                       | RATING   | COMMENT                                                                                                                                                                                                                                                                       | RATING     | COMMENT                                                                                                                                                                                                                                                             | RATING     | COMMENT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | RATING     | COMMENT                                                                                                                                                                                                                                                                                                                                                                                         | RATING     | COMMENT                                                                                                                                                                                                                 | RATING     | COMMENT                                                                                                                                                                                                   |
| Allocation                                   | Adequate | Method of animal allocation not described in Kodavanti et al. (2014); however, this is re-examination of serum samples from previously reported studies (Oyphert et al. 2012 Toxicological sciences 130(2), 405-415). As per that study, rats were randomized by body weight. | Inadequate | Method of animal allocation not described; study authors noted this is a re-examination of serum samples from a prior inhalation study but no citation for the prior study was provided, so no further details on animal allocation could be ascertained.           | Inadequate | Method of animal allocation is not reported.                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Inadequate | Method of animal allocation is not reported.                                                                                                                                                                                                                                                                                                                                                    | Inadequate | Method of animal allocation is not reported.                                                                                                                                                                            | Inadequate | Method of animal allocation is not reported.                                                                                                                                                              |
| Observational bias/blinding                  | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         | Adequate   |                                                                                                                                                                                                           |
| Confounding                                  | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Inadequate | Concerns were identified based on barbering. Animals from all groups were subjected to euthanasia due to excess barbering. Study authors note that barbering is not unusual in this mouse strain; however, to the extent that it requires euthanasia seems extreme for such a common strain. Barbering incidence in surviving animals was not reported. Excessive barbering could be indicative of stress in colony (PMID 40033013), and may have impacted immune responses in all groups (including controls). | Adequate   | Method of animal allocation is not reported.                                                                                                                                                                                                                                                                                                                                                    | Adequate   |                                                                                                                                                                                                                         | Adequate   |                                                                                                                                                                                                           |
| Attrition                                    | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         | Inadequate | The numbers of animals per group (and/or the number that died) was not explicitly stated, and given that outcomes were measured 7 mos after exposure, this had the potential to impact the study results. |
| Chemical administration and characterization | Adequate |                                                                                                                                                                                                                                                                               | Inadequate | Inhalation exposure levels were not verified analytically.                                                                                                                                                                                                          | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Inadequate | The test substance was not adequately characterized; only referred to as "S-Mx."                                                                                                                                                                                                                                                                                                                | Inadequate | The test substance was not adequately characterized; only referred to as "S-Mx."                                                                                                                                        | Adequate   |                                                                                                                                                                                                           |
| Endpoint measurement                         | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         | Adequate   |                                                                                                                                                                                                           |
| Results presentation                         | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Inadequate | Data provided for the sexes combined.                                                                                                                                                                                                                                                                                                                                                           | Adequate   | Data provided for the sexes combined.                                                                                                                                                                                   | Adequate   |                                                                                                                                                                                                           |
| Selective reporting                          | Adequate |                                                                                                                                                                                                                                                                               | Adequate   |                                                                                                                                                                                                                                                                     | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                                                                                                                                                                                                 | Adequate   |                                                                                                                                                                                                                         | Adequate   |                                                                                                                                                                                                           |