Environmental Libby Amphibole Asbestos:

Potential Risk of Injury to U.S. Fish and Wildlife Service's Trust Resources and Their Habitat

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Pre-Assessment Screen (PAS) Report for a Natural Resource Damage Assessment (NRDA)

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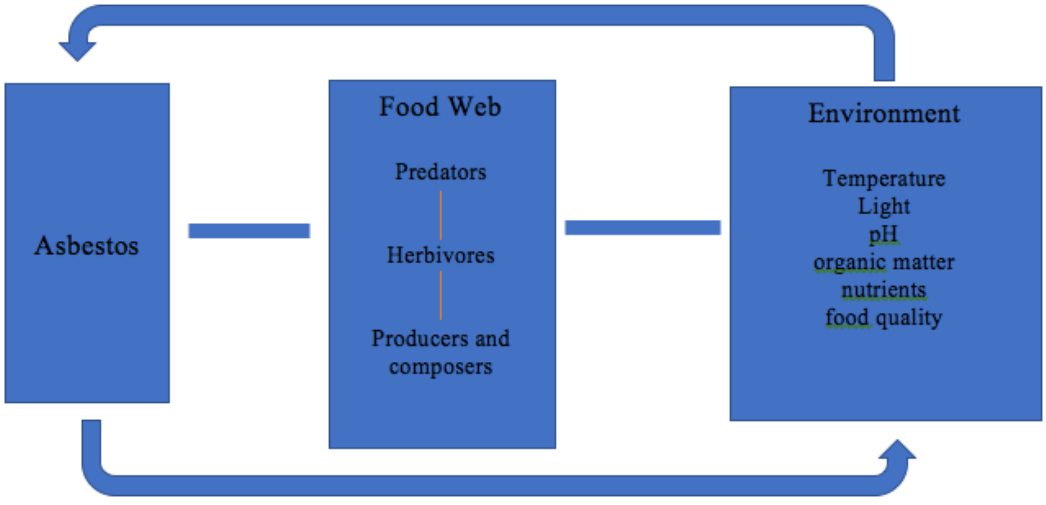

Schematic representing relationships with an environmental contaminant and possible impacts on the food web via direct and indirect mechanisms. Adopted from Freshwater Biology (2016), 61:1991-2001.

1.0 Introduction

The Federal Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) establishes the authority to develop claims for natural resource injury, destruction, or loss of natural resource services due to release of hazardous substances into the environment. An assessment performed to support such claims is referred to as a Natural Resource Damage Assessment (NRDA). The purpose of NRDA is to determine and quantify the extent of the injury, destruction or loss of services; to calculate and recover the damages needed to compensate for the injury, destruction or loss; and to use the recovered damages to restore, replace or acquire the equivalent of the injured natural resources or resource services. The first step of this process requires a pre-assessment screen (PAS). The PAS provides the basis for NRDA Trustees' determination if further investigation and assessment efforts are warranted, and if there is a reasonable probability of making a successful claim against a party or parties responsible for the release of hazardous substances. The following report provides background information for a PAS relevant to the Libby Asbestos Superfund Site, with an emphasis on Operable Unit 3 (OU3). This review of reports and published materials allows examination of the potential for harm to natural resources and natural resource services by environmental asbestos contamination of the site.

1.1 Description of the Affected Environment

The Libby Asbestos Superfund Site is 482.6 acres located in the northwestern region of Montana [1]. It was established due to adverse human health effects related to exposure to asbestiform fibers, components of the vermiculite mined there starting around 1920. There are multiple operable units (OU) in this Superfund site, including the mine area itself, the export and screening plants for the mine, railroad properties used by the mine, specific contamination sites, and the towns of Libby and Troy. A primary site of concern is the Zonolite vermiculite mine and surrounding property. This land includes the outer edges of the Kootenai National Forest, the Kootenai river and sediments, and the Rainy Creek Watershed. The land provides habitats to migratory birds and other trust resources of concern. Due to tailings run-off, wind, widespread use of the vermiculite product, and distribution of ore along roads and railroads as the material was transported, all of the operable units contained measurable asbestos in the soil, tree bark, and waterways [1-3]. This Superfund site was included in the National Priorities List, and the Environmental Protection Agency (EPA) began its clean-up operations in 2002. Remedial activities have been completed on seven of the eight Libby Asbestos OUs, with ongoing operation and maintenance activities by EPA, state, and landowners to ensure that the remedies remain protective. A remedial investigation of the mine and associated lands of OU3 was completed in 2016 and completion of a feasibility study for the site cleanup is anticipated in 2021 [1].

A description of the natural resources of the Libby Asbestos Superfund Site can be found in the Site-Wide Baseline Ecological Risk Assessment (BERA), which was finalized for the Environmental Protection Agency (EPA) in 2014 [2]. The populations considered potentially at risk included fish, benthic macroinvertebrates, amphibians, mammals and birds. The majority of the BERA focused on OU3, which includes the mine site, surrounding forest and watershed lands, and waterways (primarily Rainy Creek and the Kootenai River). The BERA evaluated Libby Amphibole (LA) fiber concentrations in water, air, mine tailings, sediments of streams and ponds, forest soil/duff, and tree bark. While concentrations decrease with distance from the mine, LA fibers were found throughout OU3 in all sample types, in all

directions from the mine. Distribution of the fibers is assumed to have been by roads, wind, and erosion by precipitation draining in all directions away from the mine on Zonolite Mountain.

1.2 Hazardous Material of Interest

Asbestos is the generic name of a group of naturally occurring, heat-resistant, silicate minerals that form long thin fibers when crystalized (See [86] for greater mineralogical detail). Asbestos types are divided into two basic mineral families by morphology and structure of the fibers, serpentine and amphibole. Serpentine asbestos has long, curly fibers, while amphibole fibers are brittle and needle-like. Chrysotile asbestos, the single form in the serpentine family, is associated with many manufacturing uses such as insulation, brake linings, etc., and has been regulated. Five forms of amphibole asbestos (actinolite, tremolite, amosite, crocidolite, and anthophyllite) have also been commercialized and subsequently regulated. In addition to these six commercialized and regulated forms of asbestos, the remaining asbestiform mineral fibers found in the environment are not generally included in asbestos definitions. Since the asbestiform amphibole minerals in the Libby vermiculite contain only a small amount of tremolite (6%), and primarily the non-regulated amphiboles, winchite (84%) and richterite (11%), this combination is more appropriately termed Libby Asbestiform Amphibole (LAA) or Libby Amphibole (LA). Like regulated forms of asbestos, LA causes all the asbestos-related diseases in humans, and most of those effects have been reproduced in laboratory rodents. This report will use the general term asbestos to describe LA, and it assumes (based on many published studies, cited below) that health impacts of asbestos on animals and cells will be mirrored by LA.

1.3 Human Health Concerns - Origins and Current Status

1.3.1 History of Disease Recognition.

The literature of asbestos toxicology is heavily rooted in the clinical and epidemiological studies of how these minerals impact human health, and in assessments of laboratory species studied to help interpret the mechanisms by which human health is impacted. The limited availability of information on the toxic effects of asbestos in fish and wildlife species has led to a dependence on the nature and occurrence of effects seen in humans and their laboratory surrogates. To best understand asbestos toxicity, it is useful to examine the evolution of our knowledge of diseases experienced by exposed humans.

Awareness of the toxicity and resulting risk of asbestos exposure was developed in the early 20th century as the asbestos mining and manufacturing industries born in the previous century matured and exposure occurred to large numbers of workers [87]. Early observations of unhealthful conditions associated with asbestos industry gained scrutiny and by the late 1920s, asbestosis was first being recognized when found in autopsies of asbestos workers. Characterized as the occurrence of “asbestos bodies” within lung tissues and composed of fibrous tissue containing embedded asbestos crystals, this pulmonary fibrosis could be debilitating and lead to lethal respiratory effects due to loss of lung elasticity and oxygen exchange capacity. Soon thereafter, thickenings of horn-like calcareous and collagenous plaques were noticed on the pleura, the surface covering of the lungs and surrounding chest cavity, in autopsies of asbestos workers, often co-occurring with asbestosis. With sufficient development, these pleural plaques were the cause of painful respiration and sometimes death due to impaired pulmonary function.

By the mid-20th century, it was noted in some studies of asbestosis mortalities that up to 16% of the cases also reported concurrent carcinoma of the lung, occurring frequently in the deeper tissue portions of the lung. In the 1960s, the recognition of that pleural mesothelioma was tied to asbestos exposure marked the second form of cancer to be associated with asbestos and the fourth manifestation of asbestos toxicity in the respiratory system [87]. Though asbestos control technologies in the workplace were thought to have controlled asbestosis in the early years after its description, it became evident by mid-century that asbestos-associated disease in its variety of forms continued to plague those who were exposed. Further, by this time it was well recognized that mining and manufacturing workers were not alone in their exposure and risk from asbestos toxicity, but were joined by their families, by populations in the vicinity of mines and production facilities, and by those that worked with and lived in the presence of asbestos-containing products.

The 21st century brought with it new understandings of asbestos impacts on those exposed. In particular, investigations of its interaction with and effects on the immune system demonstrate pathways that go beyond previously conceived toxicity mechanisms associated with the direct interactions of asbestos with target tissues. Inhibition of immune responses to invading pathogens and cancerous tissue occurs with exposures to asbestos, allowing a less hindered progression of these diseases. Conversely, asbestos, particularly amphibole forms, triggers formation of autoantibodies in exposed individuals that can lead to increased incidence of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and scleroderma. For over one hundred years, the understanding of asbestos toxicity has progressed episodically, with sporadic discoveries of previously unknown diseases, their effects, and their causation. This progression has continued up to the present, as research begins to explain the occurrence and ramifications of immunotoxicity and autoimmune diseases triggered by exposure to asbestos.

1.3.2. Current Understanding of Mechanisms and Processes

Libby amphibole is known to cause disease states including asbestosis, pleural fibrosis, mesothelioma, immunotoxicity, and systemic autoimmune diseases. In Libby, hundreds of people have died while thousands of others have been, and continue to be, diagnosed with some sort of asbestos-related disease. In humans, the mechanisms of disease caused by the commercial/regulated classes of asbestos fibers have been well studied. Those mechanisms of fibrogenic, carcinogenic, and immunotoxic effects are mimicked by the unregulated amphiboles mined and disbursed around Libby [4-9].

Mucosal and epithelial tissues in the lungs are vulnerable to the inhalation of asbestos fibers. Macrophages attempt to digest the fibers as foreign bodies. This initiates a release of cytokines and induces an inflammatory response including release of oxygen radicals, hydrogen peroxide, and superoxide by immune cells, which can damage DNA and membranes leading to lung damage and eventually fibrosis, cancer, or inflammatory/immune diseases. Furthermore, recent studies have demonstrated that exposure to amphibole asbestos (including LA) leads to immune dysfunction including systemic inflammation and an increased risk for systemic autoimmune diseases such as systemic lupus erythematosus in people and laboratory mice [5, 10, 11].

The mammalian immune system has the ability to modify the ways in which it responds to challenges by invading pathogens, and this allows the immune cells, using different types of cells and chemical signals, to specialize their attack depending on whether viruses, bacteria, larger parasites, or even internal cancer

cells are the target. Researchers describe the various response types by "Th" nomenclature which refers to the type of T “helper” cell that is most efficient at leading the attack on particular pathogens. The impact of LA (and other asbestos forms) on the immune system is of critical importance because of its ability to tip the balance of Th function [7]. Cancer, for example, occurs when at least one of two conditions exists: a) presence of a carcinogen, such as asbestos and other mineral fibers, and b) the inability of the immune system to destroy the cancer cells. Several studies have demonstrated that exposure to asbestos in humans and mice drives an immune dysfunction that detracts from its cancer surveillance function (known as a Th1 response) and turns its attention toward inflammatory and autoimmune responses (Th17 responses) [7, 12-15]. Reduced Th1 responses are also known to inhibit anti-viral immune responses, leaving the host susceptible to infection [16], and respiratory infections are common in asbestos-exposed humans [17, 18]. Th17 responses, however, support parasitic infections such as the common mouse pathogen, pinworms, by reducing the Th9 mechanisms required for parasite expulsion from the intestines [19]. There are no studies of risk of infections in mice exposed to asbestos, likely because laboratory studies of asbestos typically evaluate chronic disease outcomes. Any mouse that gets sick early in a chronic disease study either dies or is removed from the study and euthanized because it becomes a “confounder” for the chronic disease development.

1.3.3 Autoimmune Disease with LA

As mentioned above, studies in humans and mice exposed to LA have revealed an increased susceptibility to autoimmune dysfunction and systemic autoimmune diseases (SAID) such as systemic lupus erythematosus (SLE). SAID are linked mechanistically to chronic, unresolved inflammation and chronic tissue damage [7], Both oxygen radicals and chronic tissue damage caused by LA can alter tissues normally seen as “self” by the immune system to appear foreign, and therefore to trigger autoimmune responses. Such a response has been demonstrated for exposure of mice to silica dust, which is well-known to cause SAID [81]. In humans, LA exposure increases the risk of several autoimmune diseases as well as autoimmune syndromes that do not meet diagnostic criteria for a specific disease, but yet are symptomatically severe and disabling [5]. There is no way to detect such diseases except by analyzing serum antinuclear autoantibodies (ANA), which has never been done for wild animals exposed to LA, but is highly indicative of LA exposure in laboratory mice [10, 11]. In addition to ANA, LA exposure induces antibodies to mesothelial cells in both mice and humans [82, 84], likely due to chronic inflammation of pleural membranes. These mesothelial cell autoantibodies (MCAA) may be markers of progressive pleural disease in humans, and can drive serous fibrosis in laboratory mice [83, 84, 85], and may be another important marker that should be explored in LA-exposed wild mice.

While health effects of LA on humans and laboratory mice are well known and documented, the same is not true for wildlife and aquatic species. Montana has diverse biological communities and little is known about the effects of asbestos on the native flora and fauna of the area. Effects of these toxic industrially-concentrated fibers on fragile ecosystems could impact the ecological diversity of the area.

This report compiles the available research on the effects of asbestos on plants and animals with a focus on the ecosystem (including plants, animals, and soils). This compilation provides the background to understand the potential effects of LA in the ecosystems and hence injury to its resident resources, of the Libby area.

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2.0 The Libby Asbestos Superfund Site

2.1 Potentially Responsible Parties

The Zonolite Mines were owned and operated by W.R Grace and its subsidiary, Kootenai Development Corporation (KDC).

2.2 Site History

Vermiculite was first found in 1881 in Libby, Montana. Vermiculite's lightweight and fire-resistant properties were desirable for use in industry. The Zonolite company based in Libby, MT was purchased by the W.R. Grace Corporation in 1963, the same year that vermiculite samples were found to contain high concentrations of "tremolite" asbestos (22.5 percent), now known to be a mixture of fibrous amphiboles. The mine went on to provide the majority of the vermiculite produced worldwide, and was in operation from the 1920's until 1990.

An Industrial Hygiene Engineer for the Division of Disease Control for the State of Montana, Ben Wake, conducted a study evaluating the working environment at the Zonolite Company in 1956 [20]. The concentration of dust in the air varied, but it did surpass the established healthy limit. While he did not yet have a reliable method to determine asbestos in samples, the company records reported an asbestos dust concentration of 30 million parts per cubic foot (mppcf). This is substantially higher than the established (at the time) healthy limit of 5 mppcf. Structural changes to the mine operations were recommended to decrease local emission concentrations. A follow-up study by Wake in 1962 concluded that there was no progress made in reducing hazardous dust concentrations at the dry mill. Due to unhealthy conditions at the Zonolite operation, the local Union 361 wrote to Ben Wake in 1964, requesting assistance. Mr. Wake followed up with another inspection of the operation and found that few improvements had been made. In 1973, Federal Asbestos Regulations, the Clean Air Act, was adopted, and focused on demolition and renovation of sites containing asbestos as a commercial product. Montana Department of Health and Environmental Sciences (DHES) inspected the Zonolite plant in 1974 and discussed ways to reduce occupational exposures to LA. The Environmental Protection Agency (EPA) issued an enforcement action in 1979 for an Air Quality Violation measurement of emissions from the plant. W. R. Grace awarded a research grant to McGill University to study the health of current and former Libby mine and mill workers. They reported that workers employed in the period from 1940 through 1960 had an increase in deaths, compared to the general public, that were lung related and that current workers had a 5% to 10% increase in the risk of developing lung lesions.

On September 30, 1990, W. R. Grace ceased operations and all structures and equipment were removed and dismantled. March 31, 1992, due to a citizen complaint, an asbestos demolition and renovation inspection was conducted by the DHES which found that proper abatement or demolition had not been done for five buildings that contained asbestos material. In 1993, the EPA took civil action against W.R. Grace because of violations of the National Emission Standards for Hazardous Air Pollutants (NESHAP), and in 2009, for the first time in history, the EPA declared Libby to be a Public Health Emergency to provide federal health care for victims of asbestos exposure. By November of 2018, the cleanup of the town of Libby (OU4) was completed at all public areas including parks, schools, and the vermiculite processing plants; however, it did not include the former vermiculite mine or forested areas [1].

The Libby mine was in operation from 1919-1990, during this time it was releasing toxic asbestos fibers into the surrounding area. The asbestos levels in the air were above the federal regulation of 5 mppcf, at about 20 mppcf. An emergency response team was sent in 1999, and in 2002 Libby was designated as a National Priority List site. The EPA has worked with the residents to clean up and reduce the asbestos residue left in Libby and Troy. However, significant amounts of asbestos continue to contaminate the environment adjacent to the mine and mill sites.

2.3 Exposures in OU3

The amounts of LA fibers in soils, air, sediments and tree bark in Libby and surrounding areas have been studied over the years. An extensive evaluation of LA fibers in the BERA study area was performed using standardized testing/counting methods. Water and air samples were analyzed by transmission electron microscopy (TEM) using the counting and recording rules from the International Organization of Standardization (ISO) method 10312:1995 [21]. While recommended for detecting the presence of asbestos and well-accepted at the time of the study, this method has limitations and has since been revised and updated as ISO 10312:2019 [22]. In addition, ISO 10312 stipulates counting of fibers must satisfy 3 parameters, including detecting amphibole asbestos by xray diffraction. Since LA has only a very small proportion of defined, regulated amphibole asbestos (tremolite) and mostly unregulated amphibole fibers, it is not clear from the BERA how well their method picked up LA fibers. For soil and sediment samples, the BERA used polarized light microscopy (PLM) in accordance with EPA recommendations for the Libby site. Unfortunately, this method, as with all light microscopes, is unable to distinguish very small fibers (shorter than 5 microns, thinner than about 0.4 microns). LA fibers in tree bark, having impacted tree bark via the wind, are predominantly < 5 microns in length and < 0.4 microns in diameter [23]. These fibers can then re-enter the air or soil when bark or trees fall. Small fibers are now known to have pathogenic potential, and are being considered in health risk studies [24, 25]. The PLM method, while it may be the best for bulk materials like soil for determining the presence of asbestos, would miss large numbers of small fibers and therefore the soil and sediment counts are likely underestimated. Despite these limitations, the BERA study acknowledges the imprecision of asbestos detection and counting, and nevertheless provides data regarding relative amounts of LA at different sites. The primary caution is that sites deemed non-detect or trace by these methods may actually contain large numbers of pathogenic small fibers. Thus, the area of exposure may be much larger, may contain more fibers, and may pose a higher risk to animals than what is reported in the BERA. This is supported by data reported in Revision #4 of the LA Superfund Site Data Summary Report [26], in that while sediment values for Mill Pond and LRC-1 of OU3 had trace to 1% LA by PLM, fish from Mill Pond had tissue burden levels averaging 1.6 x 10^6 fibers/g wet weight. Importantly, these fibers were found in the fish muscle (‘filet”), which would not be expected to contain fibers, according to the BERA. Organs from the 7 fish collected for tissue burden analysis were kept, but not analyzed. Organ tissues may have even higher fiber burdens. As discussed below, this tissue burden is much higher than those reported in humans and animals with asbestos-related diseases.

There is no known “safe” level of exposure to asbestos or to LA [27, 28], especially for species for which few or no toxicological studies have been done. Also, most recommended guidelines focus on cancer as the health outcome, while we now know that pleural fibrosis and autoimmunity are far more common in humans exposed to LA and occur at very low exposure levels. Importantly, LA is the only asbestiform mineral for which a reference concentration (a level below which disease may not occur, RfC) has been

calculated based on a non-cancer outcome (pleural fibrosis), and the results show that disease can occur at exposure levels far below levels at which OSHA and EPA regulate asbestos [29]. In addition, this concentration, although known to cause health effects, is so low that standard asbestos counting rules would generally define this as "non-detect".

In addition to the fish fiber burden studies mentioned above, Revision #4 of the LA Superfund Site Data Summary Report [26] also reports fiber burden studies in deer. Unfortunately, only one deer was tested, and no fibers were detected in 2 samples from the lungs of this deer. Although tissues were preserved from deer mice in OU3 and reference sites, tissue burden studies were not done. Studies evaluating wild or domestic animals as sentinels of exposure have clearly shown that histological detection of fibers or "asbestos bodies" is not an acceptable methodology, since many fibers were detected by scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) in animals that tested negative for "asbestos bodies" [30, 31]. Therefore, it would be essential to determine the organ to test for asbestos burden (likely the lung), and perform SEM/EDS in order to optimally calculate fiber burden. Work has shown that lung fiber burden in rodents gives reasonably good correlation with fiber exposure levels [31], and inhalation studies in mice demonstrate that at moderate (occupational) inhalation exposure levels (13.6 mg/m^3) 6 hr/day for 5 days/week, fiber burdens after 1 month in lung ranged from 3 $x10^5$ to 1.3 x 10^6 fibers/mg dry lung by TEM [32]. Using these available data, a calculation could be made on the fiber burden to expect in rodents depending on fiber numbers in soil and air at the site where the animals were caught. In the Ingravalle study, rats living in areas with environmental levels of soil fibers (amounts not given) had lung fiber burdens from 20,000 – 70,000 fibers/gram dry weight (ff/gdw). In comparison, a patient with lung cancer from environmental exposure to LA had very similar lung fiber burden at approximately 40,000 fibers/gram [33]. In Capella et al., 2017 [34], lung fiber burdens averaging 50,000 ff/gdw were detected by SEM/EDS in animals (cows, deer, chamois) living in areas of northwestern Italy that are known to have environmental (rock and soils) asbestos as well as old quarries that closed in the 1980's. They also tested animals from a region devoid of environmental or building-material asbestos, and fibers were rarely detected in the lungs of those animals (approximately 130 ff/gdw). Thus rodents and other animals living where soil fiber concentrations are due to environmental materials and anthropogenic activities (similar to the OU3) would be expected to have lung fiber burdens in the range at which human health issues arise. Laboratory experiments in rats demonstrate inflammatory, fibrogenic and tumorigenic effects of LA in animals with lung fiber burdens much lower than the values reported above for sentinel animals in northwestern Italy.

3.0 Potential Ecological Impacts of LA

This section summarizes the literature and findings regarding the impact of LA (or other asbestos) on the at-risk populations of the Libby Asbestos Superfund Site. The Baseline Ecological Risk Assessment (BERA) of the Libby Asbestos Superfund Site (work done years prior to 2014) provides the results of extensive evaluation of the ecological impacts of LA, primarily in the OU3, and is the only such study that has been done. Because decisions regarding remediation or monitoring of any potential ecological damage have been made based on that study, it is a critical piece of this report. Considering the difficulty of working in a hazardous site, trying to evaluate multiple species for multiple health outcomes, the normal challenges of field work, and the complexity and ruggedness of the landscape, the BERA was a substantial undertaking. The study recognizes and discusses many of the "weaknesses" and complexities in the data that make interpretation difficult, and admits in each section that confidence in the conclusions

is not consistently high. In the sections below, the BERA is evaluated in view of our understanding of human health effects, cellular toxicity of LA, and alternative interpretations and hypotheses regarding the conclusions made in the BERA. Our understanding of the health risks of exposure to LA has grown tremendously since the research was done on the BERA, and it is likely that the study would be designed differently if done 10 years later than it was, simply because of new knowledge.

In the BERA, in the section on Data Evaluation, the authors explain the ways in which they determined whether a difference between the OU3 site and the reference site was due to LA exposure. This section clearly elucidates the complexity in these analyses, and that having a conceptual model was essential. There are, however, weaknesses in these criteria. It is stated in the BERA that in order to be attributed to LA, the following 3 criteria were considered:

a. Was the observed difference characteristic of the known effects of asbestos on the exposed organisms?
b. Does the magnitude or severity of the effect appear to depend on the level of LA?
c. Are there other recognizable differences (e.g., habitat factors) that might explain some or all of the observed difference?

For (a), the criterion is too narrow for two reasons. One is that the effects of asbestos are largely unknown in most of the species studied, so there is no way to satisfy the criterion. And second, known asbestos effects mentioned in their section on “reported effects” in mammals include cancers and fibrosis, neither of which is expected to occur prior to at least 7-8 months of age in mice, and the average age of mice in this study was less than 6 months. The study was too short in duration for gross and histopathological lesions related to cancer or fibrosis to develop in mice. Other sensitive parameters indicating exposure and effects, such as autoantibodies, cytokine profiles, and gene expression changes, were overlooked. Therefore, the BERA may not have incorporated sensitive enough measurement endpoints to identify asbestos risk to a variety of organisms. The other known health effect of LA is immune dysfunction, which can increase susceptibility to parasites and other infections and occurs with much shorter latency, and that was not considered in the BERA. For criterion (b), health effects in humans and mice are observed at extremely low exposure levels, so a clear dose-response relationship is not expected, especially for immunological effects. In addition, no proof of exposure (fiber burden) studies were done, so this criterion also cannot be met. For criterion (c), although it is very possible that other habitat factors contributed to the health differences seen in OU3 compared to reference sites, this is NOT a reason to summarily dismiss the possibility that those differences are indeed due to LA until experiments are done to tease apart the etiology. Rather, differences in habitat suspected of contributing to outcomes would need to be tested in a controlled laboratory experiment, and few such studies were done in the BERA. This data gap adds uncertainty to the BERA results and therefore negates the conclusions drawn. Finally, a criterion is missing that was not adequately evaluated in the BERA, which is actual evidence that the test animals had been exposed. Without more fiber burden analysis, it is impossible to know which animals were actually exposed and estimate their exposure. Nevertheless, fiber burden analyses in fish in the OU3 are evidence of significant exposures. Searching OSHA or NIOSH websites yields statements such as, "all levels of asbestos exposures studied to date have demonstrated asbestos-related disease", and "there is no level of exposure below which clinical effects do not occur." (https://www.osha.gov/SLTC/asbestos/; https://www.cdc.gov/niosh/nioshtic-2/00186632.html). Since there is no known “safe” exposure level to any form of asbestos, including LA, then demonstration of

exposure using fiber burden studies, plus the evidence provided in this report, would strongly suggest that the biological communities in OU3 are indeed being affected.

Based on studies reviewed in this report regarding asbestos (including LA) exposure, it is likely that within the lifespans of wild animals, the following outcomes would occur:

a) respiratory inflammation and scarring, even mild/subclinical, resulting in slowed activity levels, less foraging. Healthier animals would be the ones most likely to be caught when foraging, so this effect is unlikely to be detected in trapped animals.
b) increased infections of all kinds, but especially viruses and parasites
c) effects on nutrition when activity levels are reduced.
d) effects on reproduction when activity levels are reduced, leading to reduced population numbers, narrowing the range of ages in the population
e) effects on ability to care for young when activity levels and nutrition are reduced
f) shortened life spans due to increased infections, reduced nutrition, poor evasion of predators; also would narrow the age range in populations
g) overall effects on populations, including impacts on predators that rely on affected prey animals
h) impacts on food webs

3.1 Routes of Exposure

Ingestion/Inhalation

Due to proximity to the mine and the access road to the mine, the Rainy Creek watershed accumulated large amounts of ore and fibers by air and run-off. This led to LA contamination of Rainy Creek and the Kootenai River. Recent research in Libby, MT found that disturbance of soils with less than 1% asbestos could result in airborne concentrations of asbestos that are a potential human health concern [35]. A similar estimate is extended to mammals because of numerous laboratory studies in rodents using similar exposure levels and having similar lung fiber burdens as is seen in humans with disease. The goal of the EPA was to reduce soil contamination to trace levels (around 0.2% or below), wherever the soil could be accessed. However, a clean-up of the entire forested watershed area was impossible. This means that there is still a potential that soil within the Rainy Creek watershed contains enough asbestos to cause harm to humans, plants, and animals.

There are 2600 contaminated properties within Libby and Troy and 7600 properties within the Superfund site. To date, the EPA has removed more than 1 million cubic yards of contaminated soil and 30,000 cubic yards of contaminated building material [1]. The contaminated soils are disposed of at the former vermiculite mine (thereby adding to potential environmental contamination by wind and runoff), and contaminated construction debris was placed in a specially designed landfill offsite. Coverage of the mine with the soil from contaminated properties in Libby helped reduce levels of fibers at the surface since that material had far lower levels of contamination than at the mine site. Currently grass and trees are growing in some areas, suggesting some success in remediating the site to reduce erosion. However, the vertical lift edges remain exposed to wind and water run-off.

For humans, the greatest concern for exposure has been by inhalation of airborne dust containing LA fibers. This is also a concern for air-breathing organisms whenever contaminated soil is disturbed by

human or animal activities or by wind. In addition, any animals eating at or near ground level would be expected to ingest some soil along with plants or prey being eaten. Fibers would also be ingested and inhaled during grooming by mammals and preening by birds. Asbestos fibers are dense enough to settle in water, so they become a layer at the bottom of streams, ponds, lakes and rivers so that aquatic species are also exposed when they contact or disturb the sediment. Alteration of water and soil pH by the presence of asbestos can also affect plants (see below, section 3.4).

3.2 Mammals

3.2.1 Background on Studies in Rodents

The health effects of LA in humans and laboratory mice have been extensively studied, as described in section 1.3. Intratracheal exposure to LA led to immune changes consistent with autoimmune dysfunction and lupus-like autoimmune disease in both species [10, 11]. Rats exposed to LA by inhalation exhibit significant inflammatory, fibrogenic and tumorigenic effects even at lung fiber burdens much lower than those reported in sentinel animals in Section 2.3 as early as 10 days after exposure and out to 18 months [36]. Mice were also shown to have significant increases in interstitial and pleural fibrosis seven months after very low dose exposure [10]. In laboratory mice, 7 months is about half the normal life-span, suggesting a long latency for development of fibrotic disease, similar to humans. Cancers in mice have even longer latency, as much as a year [37]. This makes studying health effects in wildlife extremely difficult, since predation and disease often lead to shortened average life-spans, particularly in small mammals. Nevertheless, LA is carcinogenic, fibrogenic, and immunotoxic in laboratory mice and humans [7]. The latter effect is critical since immune dysfunction occurs very soon after exposure (short latency to effects) and can then lead to susceptibility to cancer or infection by bacteria or other parasites, and potentially susceptibility to toxins and other stressors.

Very little research has been done on the effect of asbestos on living organisms other than humans and laboratory rodents. However, mesothelioma is known to occur in dogs and cats following exposure. It is assumed that once fibers are in tissues, they will cause cellular damage no matter what the organism is. A recent study of wild mice around a factory in Israel tested the genetic diversity of *Mus musculus domesticus* living in an asbestos-contaminated area where the soil had high concentrations of a mixture of amphibole and chrysotile fibers [38]. The mice from contaminated soil had significantly elevated levels of somatic mutations and increased homozygosity among alleles. Homozygosity generally occurs due to inbreeding, possibly due to a reduction in population size or geographic isolation. It tends to reduce the adaptability of a population and is therefore detrimental. Overall, if asbestos in soil can impact the genetic diversity and mutation rates of natural populations, it follows that LA could do the same. Asbestos is also known to cause changes in DNA methylation and other epigenetic effects that can impact gene expression and are associated with health outcomes [39-41]. There is also strong evidence for its ability to cause oxidative stress in cells, which is a strong mediator of genetic damage [42].

A great deal is now known about the effects of asbestos and LA on gene expression in humans and rodents, providing huge databases of that serve as the basis for determining health outcomes [43-46]. Many genes and pathways have been associated with health effects making them markers that predict ultimate outcomes of exposure [36, 43, 45]. Unfortunately, despite these valuable resources, no studies have evaluated gene expression changes in wild animals exposed to LA.

3.2.2 Effect of LA on Small Mammals in OU3

The BERA studied deer mice as the only mammal. Mice are likely to have high exposure to LA due to living in the soil and duff of the forest, which is known to contain fairly high numbers of LA fibers. In addition, they receive exposure by both inhalation and ingestion (food and grooming), and are relatively numerous compared to other mammals in the area. The population studied consisted only of deer mice, including 38 animals from OU3 and 34 from reference sites. It is not clear why the study was unable to reach its target of 120 animals from OU3, although this was a time-consuming and likely costly endeavor due to human health risks involved in the collections. Although voles were also considered to be at risk, the collection of these rodents was unsuccessful. This in itself could indicate effects of exposure since voles in Montana can range up to hundreds to thousands per acre, and they are active day and night through all seasons [47]. No population studies of density or diversity of mammals was performed, and no controlled laboratory toxicity studies were done. Despite these limitations, the BERA concluded that "LA exposures in OU3 are not causing any ecologically significant effects on populations of small mammals residing in the forest areas of OU3." In this report, the BERA study is revisited, providing evidence that paradigm shifts in our understanding of LA health effects reduce the confidence level for this conclusion.

Gross tissue observations were recorded for 34 mice collected in reference sites and 38 collected in OU3. However, in the Table 11.2 of the Data Summary Report, only 3 mice were actually necropsied. This is too few animals from which to derive any conclusions [26]. No cancers were seen, but they would not be expected, since laboratory mice do not develop cancers, even in the presence of carcinogens, until nearly a year [37, 48]. Since the maximum ages of the studied mice was closer to 6 months, no cancers would be expected. No significant lung lesions or fibrosis were seen, but again this was only gross observation, not microscopy or collagen analysis. In addition, visible fibrosis also would not be expected in mice younger than 8-10 months, based on laboratory studies of normal (non-genetically modified) mice [10, 49]. The only outcome measures that would indicate asbestos impacts in mice of the age studied in the BERA would be a) overt immunological changes (measures of antibodies, T or B cells, serum cytokines), b) evidence of excess infection indicative of immune dysfunction, or c) changes in the structure of populations indicative of impacts on reproduction or sex-specific changes. The BERA did not test any immunological markers, which still needs to be done. However, they did have findings in the areas of b and c, described below.

There was an increase in the prevalence or mean severity of lesions in mice from the OU3 compared to reference sites. Statistically significant differences in numbers of lesions were detected in the larynx, bronchus, duodenum and jejunum. These sites would be consistent with inhalation or ingestion exposure. Significant increases in the severity of lesions in OU3 mice was seen for larynx, bronchus and stomach, again consistent with exposures expected for LA. However, these lesions were dismissed in the BERA because they were attributed to parasites, and not to LA. For LA, the researchers only considered known effects seen with LA in humans and experimental mice, primarily cancers or fibrosis; however, this type of lesion would not be expected in a population whose average age was less than 6 months. Other types of lesions, such as inflammatory or necrotic lesions were assumed to be due to parasites or other infections, although only a few apparent parasitic lesions were actually sent out for analysis, and no lesions were tested for the presence of fibers. No consideration was given to the possibility that LA caused immune dysfunction that increased the susceptibility to infections, and no immunological assessments were

performed that could have given a better evaluation of health impacts. This is a data gap that led to flawed interpretation of the findings, and that gap remains to be filled.

A striking and significant difference was seen between OU3 and reference sites regarding population sex ratios. Female to male ratio of reference site mice was 1.8 and was 0.8 for OU3 mice. With the assumption that unhealthy mice would remain in the nest or at least forage less, the captured mice were likely relatively healthy in that they were above ground and foraging. Although there is no way at this time to determine the cause of this sex ratio difference, it is a key difference that cannot be dismissed until further studies reveal contributing factors. One hypothesis that could be tested is that female mice are more susceptible to types of immune dysfunction, as has been demonstrated in laboratory mice [50], such that they ended up more susceptible to infection and died, or were sick enough not to be actively foraging at the time of collection.

3.2.3 Larger mammals

No study of any large animals was performed in the BERA. Despite the potential for high exposure in other, larger, longer-lived mammals, the result in mice was extrapolated in the BERA to include larger animals, stating that, "there is no compelling evidence to presume that mammals with longer life spans than mice would likely be more at risk than mice." Nevertheless, it might also be said that because there were indeed effects in mice, there is no compelling evidence to suggest that larger animals would NOT be at risk for health impacts. There is no good case for extrapolating from the small mammal study of the BERA due to its noted weaknesses and invalid conclusions. Like humans, animals including cats and dogs develop mesothelioma when exposed to asbestos [51-54]. One case of mesothelioma in a dog occurred apparently due to rummaging through bushes and sniffing in the woods near her home (https://www.dailymail.co.uk/news/article-3106466/The-dog-killed-asbestos-Pet-dies-rare-lung-cancer-despite-20-000-treatment-sniffing-toxic-substance-rummaged-woodland.html.) This therefore was a soil/inhalation exposure. Finally, as described above in Section 3.2.1, large sentinel animals (cows, deer, chamois) living on asbestos-containing soils in Northwestern Italy had lung fiber burdens as high or higher than those seen in rodents and humans with asbestos health effects [34]. Mesothelioma, a cancer ascribed to exposure to asbestos, have been demonstrated in cows, horses, and goats [55-58], and a wild boar living in the same asbestos-contaminated region in northwestern Italy described above in which soils contain asbestos fibers from old, closed rock quarries [59].

3.3 Amphibians

3.3.1 Background Literature

Crocidolite (an amphibole asbestos) has the ability to disrupt the membrane properties of African clawed frog (*Xenopus laevis)* oocytes, measured as the resting membrane potential (RP) and membrane resistance (Rm). Using a standard two- microelectrode voltage clamp technique, crocidolite exposure caused a reduction in voltage, over time, within a murine macrophage cell line and *Xenopus* oocytes [60]. These observed electrophysiological effects on the RP and Rm show asbestos' ability to propagate an unnatural change to the normal membrane state, thus affecting the overall ionic permeability of the organism's membrane. Similarly, asbestos- exposed oocytes released significantly more reactive oxygen species (ROS) than unexposed oocytes [61]. Therefore, similar to mammalian cells, asbestos influences the release of ROS from *Xenopus* oocytes. Reactive Oxygen Species (ROS) are vital to organism survival, but

the overproduction can create a toxic environment, leading to lipid, protein and DNA damage. Taken together, these asbestos fiber effects not only change the physical characteristics of the oocyte membrane itself, but also affect the cell's ability to persist at all, and therefore could lead to reduced reproduction of amphibians.

3.3.2 Effect of LA on Amphibians

The BERA performed site-specific laboratory-based sediment toxicity tests, and did field surveys of gross and histologic lesion frequency and severity in amphibians from OU3 and reference sites. Although there were no apparent differences regarding histological lesions in organisms from the OU3 compared to reference sites, the laboratory studies did reveal a developmental delay in reaching metamorphosis when amphibians were exposed to OU3 sediments. It was nevertheless deemed "not ecologically meaningful" because it was only a lag, rather than cessation of development. The authors did allow that the delay could lead to high rates of death if ponds dried up before metamorphosis could occur. This could occur as a result of climate change or drought. Since no further studies were done, this question remains unanswered, and therefore the impact of LA on amphibians in the OU3 remains unknown.

No studies were done on reptiles in the BERA because, "..reptilian skin is covered in scales that would be expected to decrease exposure from direct contact pathways", so amphibians were considered more at risk. This ignores the differences in physiology, life-expectancy, and other differences between these groups, leaving a gap in knowledge regarding turtles and snakes known to reside in the OU3 area.

3.4 Soil and Soil Organisms

The BERA study states (p. 42) that "…asbestos is not expected to be of concern for aquatic or terrestrial plants.", and therefore no studies on plants were performed. These researchers are correct that soil asbestos may not affect plants directly [62], but they are incorrect about asbestos not affecting plant growth. In fact, asbestos in soil can affect soil pH and alter soil quality in other ways [62, 63]. Asbestos mine tailings and other asbestos-containing soils often lack vegetation, or the vegetation is sparse. Altered soil pH can stunt plant growth by altering nutrient mobility [62]. This can occur with either chrysotile or amphibole fibers, and in fact one site (Nottingham Park, PA) was a chromium mine and stone quarry with some amphibole content in the rock. So, similar to the vermiculite mine in Montana, the asbestos was simply a natural contaminant. The grassland soils surrounding the mine site remain contaminated with anthophyllite asbestos (1-2% by PLM), and vegetation is stunted. These soils inhibited germination up to 35% and root growth by 30% for native species, leading to significantly reduced biomass compared to the control site [62]. The other site in this study contained primarily chrysotile asbestos, and the effects on plant growth were similarly inhibitory, although to a lesser degree. Therefore, studies on chrysotile may reveal more information about effects on plants.

A study of crop plants (wheat, pea and mustard) grown in chrysotile asbestos-contaminated soil (10 km from an asbestos cement factory) demonstrated that exposed plants had significant decreases in height of the shoot (up to 48%), length of the root (up to 52%), biomass (up to 49%), chlorophyll content (up to 38%), and a decline in seed germination. (up to 42%), and leaf protein content (up to 44%) [64]. This is important because asbestos fibers degrade at an incredibly slow rate. As these fibers slowly degrade, they

release ions and elements into the soil that cause growth limitations. Any effect on plant growth would reduce habitat quality for all species inhabiting the affected area.

Due to the focus only on direct exposure to fibers, many asbestos effect pathways are not evaluated by the BERA study. Indirect effects of asbestos, by affecting soil quality may have many subsequent effects on plants and the animals that depend on them. These questions remain unanswered for the Libby Superfund Site.

3.4.1 Earthworms

Soils rich in asbestos not only provide a poor medium for the growth of plants, but also a detrimental habitat for earthworms. The standard lifespan of an earthworm was found to be 297 days; however, in asbestos-rich soil the earthworms only survived 21 to 30 days [65]. The authors concluded that this may have been because of the alkalinity that the asbestos contamination caused in the soil. They also found that there was mineral accumulation- Mg and Ni- in the worms that were living in asbestos soil, compared to the control worms [65]. Minerals like nickel and magnesium are likely to cause tissue damage when accumulated in earthworm specimens. Effects on earthworms could further affect plant growth and nutrient cycling in the soil. No studies have been done on earthworms in OU3, leaving a significant gap in the overall knowledge of the ecological impact of LA, since damage to earthworm populations could reduce the overall habitat quality by impacting plant growth and nutrition.

3.5 Water: Plants, Fish and Benthic Invertebrates

3.5.1 Background Literature

Several studies have been performed to examine toxicity of asbestos in water-dwellers. These are nicely reviewed in the BERA. Mortality was observed in larvae of *Artemia salina,* a filter-feeding planktonic crustacean, when asbestos (either chrysotile or amphibole) concentrations were 10-100 million fibers/liter (MFL) [66]. These concentrations are closer to those seen in the Upper Rainy Creek (URC, considered a control site in the BERA), and are far below many of the fiber concentrations seen in Lower Rainy Creek (LRC).

The effects of chrysotile exposure were investigated on larval, juvenile, and adult Asiatic clams [67, 68]. Exposures at or below 1 million fibers per liter (MFL) caused a significant reduction in release of larva by brooding adults as well as increased mortality in larvae. Effects were seen in adult clams and juveniles in terms of siphoning (feeding) behavior and shell growth after 30 days of exposure. These data are relevant for species of mussels in Montana that are important sources of food for racoons, muskrats, otters and other animals living near mussel habitat. For example, the Western Pearlshell mussel is known to inhabit the Kootenai and Yaak River watershed areas (https://pnwmussels.org/wp-content/uploads/2016/07/Fa088ea0-bcf4-4547-9816-e496cc105be6.pdf).

Belanger et al. [69] studied the effects of chrysotile asbestos on eggs and larvae of Japanese Medaka fish (*Oryzias latipes*). Eggs were exposed to chrysotile until hatching (13-21 days) and larvae were exposed for thirteen weeks. At 1 MFL or higher, asbestos tended to delay hatching, but egg survival (hatching success) was not grossly or significantly impaired. Larval Medaka experienced reduced growth at

concentrations of 1 MFL or higher. High concentrations (10,000 MFL) led to 100% mortality by 56 days. Concentrations of chrysotile as low as 0.01 MFL reduced the number of successful spawns per female and eggs per female.

Additionally, in salmonids, asbestos fibers accumulated in muscle, kidney, and liver tissues [69]. Coho salmon larvae (*Oncorhynchus kisutch)*, and juvenile green sunfish (*Lepomis cyanellus)*, expressed erratic swimming behaviors and loss of rheotaxic positioning when exposed to asbestos in water [69]. A mild stress challenge revealed that Coho salmon larvae, when exposed to low levels of asbestos, were significantly affected. Belanger et al. [41] concluded that "asbestos-stressed fish may display increased susceptibly to other waterborne pollutants", making them susceptible to disease and behavioral changes. Because these effects did not result in mortality directly, however, these behavior studies were critical in discovering these important effects of asbestos in fish in the absence of obvious overt toxicity.

Unlike soil plants, water plants are directly affected by the presence of asbestos. Two studies examined the effects of asbestos on duckweed. One focused on asbestos effects on growth and physiology [70], and the other determined that the mechanism is likely cellular oxidative stress [71]. Although these studies used chrysotile, the mechanism of action in terms of the plant damage was shown to be oxidative stress, such that both chrysotile and amphibole fibers would have the same effects in terms of reactive oxygen species. Duckweed, grown in water containing environmental concentrations of fibers (0.1 g/L) in petri dishes, exhibited reduced number of fronds, root length, and biomass. It also reduced content of chlorophyll, protein and carbohydrates. Increases in ROS and superoxide dismutase activity all indicated oxidative stress as the causative mechanism for the phytotoxicty of asbestos.

Finally, there is evidence of an indirect effect on water quality due to the presence of asbestos in soils through which water drains. Well and surface water from asbestos-rich soil areas and control areas were tested for asbestos and metals [72]. Significantly elevated levels of asbestos fibers were found in the water fed by asbestos-rich soils, as expected, but also metals in the water were significantly altered by the presence of asbestos in the soils and rocks. Whether or not this could contribute to toxicity has not been studied, but sensitive species might be at risk, leaving a potentially important data gap for OU3 where soils have high levels of contamination of LA.

3.5.2 Effects of LA in Fish

The BERA concluded that, although there was evidence of decreased fish density, increased average fish size (consistent with fewer small fish), decreased hatching success, swimming abnormalities, and decreased biomass in the Lower Rainy Creek (LRC), there was not sufficient evidence that LA was the cause. One of the reasons given was that the LRC has differences in gravel, debris, water temperature, and pool availability compared to the reference sites used in that study. In fact, however, this suggests that the reference sites were not appropriate comparison sites if they were so different, and therefore no conclusion as to the impact of asbestos can be made. However, the inability to determine whether LA was the cause is not a valid reason to conclude that it was not the cause of any of these effects, especially in view of large numbers of LA fibers in the tissues of fish in OU3.

Because no further studies were done, it appears that due diligence was not performed regarding the potential risk of LA to fish in the LRC. Further, the BERA concluded that their LRC results could be extended (without any testing) to the Kootenai River and its resident white sturgeon and bull trout to say that there is no risk there either. The authors admitted that their exposure studies may have missed higher exposure levels at different times. Therefore, the conclusion of no risk to fish in the Kootenai River was based on questionable conclusions regarding trout in the LRC and questionable concentration/exposure data. Since no studies were performed regarding the health of fish in the Kootenai River, due diligence again was not done. At the very least, it will be critical to measure sediment LA concentrations in the Kootenai River and how they change over time, since sturgeon are bottom feeders.

3.5.3 Effects of LA on Benthic Macroinvertebrates

The BERA reported on laboratory-based site-specific sediment testing and site-specific community population studies for two test organisms: *Hyalella azteca* and *Chironomus tentans. H. Azteca* is a small (~ 0.5-1cm) crustacean that is very common in fresh waters, and serves as a critical food source for waterfowl and fish. *C. tentans* is a flying midge that is an important food for fish. Even though they may be less prevalent in mountain streams than other native species, they are commonly used to test water-borne toxicity. In the study, both species showed effects of site sediment on survival, including up to 25% decrease in *C. tentans*. These effects were dismissed because "the applicability of the results to other species, and hence the potential magnitude of effects on the benthic invertebrate community as a whole, are difficult to judge from this line of evidence alone.." However, since these two species represent important food sources, this conclusion does not satisfactorily determine potential impacts on the benthic community as a whole nor the populations that depend on them.

Site-specific population studies in the LRC also showed effects on benthic communities, but this was dismissed since the populations were similar to upper Rainy Creek. By using upper Rainy Creek as their comparison site (which also contains asbestos, albeit small amounts), no conclusions can be made as to the effects of asbestos. Nevertheless, these effects were dismissed as "habitat quality" (no details given), but that since estimates of LA concentrations were uncertain, it was concluded that "LA contamination in LRC may be causing small to moderate effects on survival of some species, but the overall benthic macroinvertebrate community is not substantially impacted." From the evidence given, it is not clear how this overall conclusion was reached.

3.6 Birds

3.6.1 Background Literature

Only one study was found regarding asbestos exposure and birds, although there are anecdotal reports of pet birds suffering from ill health from asbestos. *Gallus gallus domesticus,* also known as white leghorn chicken fowl, exhibited inflammation when asbestos fibers were administered into the lumen of the air sacs [73]. Tumors were present around the lung, carina, esophagus, and proventriculus. *Gallus gallus domesticus* exposed to crocidolite asbestos developed granulomatous tumors with asbestos fibers present. For four years, the asbestos fibers remained unchanged and asbestos bodies were found in the interalveolar septa of the lung [73]. These results are very consistent with studies in mammals, so it seems reasonable to expect that health effects would occur in any exposed birds.

LA exposure in birds could occur via collection of nesting material from the forest floor, from predation of LA-containing food sources (mice, fish, insects), and ground-foraging birds would have high fiber exposures similar to mice. However, at this time, very little is known about asbestos exposure in birds, and birds were not studied in the BERA.

3.6.2 Effects of LA on Birds

The BERA did not study birds. Instead, it was assumed that there would not be any effects in birds because they had concluded that there were no effects in mice. They also assumed that primary exposure to asbestos by birds would be by inhalation, so significant effects were not expected because their avian respiratory physiology expert consultant indicated that it was his opinion that birds are not more susceptible than mammals to inhaled particulates. No reference for this statement was provided, and there was no consideration of life-expectancy differences. In fact, however, mice are primary consumers and are prey for birds such as hawks and eagles. Asbestos, being non-biodegradable, could accumulate up the food chain if animals were eaten that contained asbestos. Asbestos is listed as a persistent, bioaccumulative, toxic chemical (PBT) by the SaferChemicals/HealthyFamilies Foundation [74]. The assumption that asbestos would not bioaccumulate, as stated in the BERA report (p. 41), is not based on any data; rather the BERA only lists websites stating that since no fibers were demonstrated in muscle, fibers would not be eaten [75]. These sites refer to people eating meat from wild game, not about the health of the wild predators. Even if muscle tissue does not contain asbestos, predatory birds do not selectively eat muscle of their prey, so the assumption of no bioaccumulation in the food chain seems to have no basis. In addition, more current studies have demonstrated that asbestos fibers migrate throughout the body, and can be found in virtually all tissues [76-79]. In fact, one study showed that asbestos fibers bioaccumulated in the livers and other organs of mice exposed to drinking water containing asbestos [80].

4.0 Summary and Conclusions

Very little research has been done on effects of asbestos exposures in the ecological environment. Only a few studies were found that explored effects of asbestos on wild populations of fish, crustaceans, reptiles/amphibians, small mammals and birds. However, all of the studies that were found, including the BERA, did suggest at least the potential for impacts on individual members or entire communities. This report further suggests that the BERA, while providing extensive and useful information, should not be considered due diligence in the evaluation of the potential ecologic impact of LA. Rather, it can be used as a baseline and a tutorial to tailor future studies. Studies are needed now, ten years later, to evaluate populations, immune impacts and other short-term outcomes in view of the BERA results, new knowledge about LA and disease latency, and continuing exposures in OU3. The situation is unique in the Libby Asbestos Superfund Site compared to many other spill sites in that massive amounts of LA-containing material remain on the mountain, continuously blowing in the wind and washing down into the watershed lands, renewing and extending the exposures. In order to fully understand the ecological impacts of LA, the environmental concentrations (and therefore potential exposures) need to be re-evaluated to determine how they have changed over time.

To date, the OU3 has had inadequate testing and inappropriate extrapolations from the small amount of data available, leading to unsupported conclusions. The lack of fiber burden analyses in mice, particularly in the lesions that were attributed to parasites, prevents confirmation of exposure in animals with and without apparent health effects. The complete lack of data regarding immunological impacts is inconsistent with our current understandings of the health effects of LA. Simple blood collection would allow testing of serum cytokines and antibodies, a much more sensitive measure of health outcomes in animals than gross observation.

Other species that the BERA was not able to evaluate should be included in future studies, including larger, longer-lived animals. At a minimum, fiber burden should be evaluated in at least a few individuals to demonstrate exposure. Genetic studies can reveal changes in allele frequencies, changes in DNA methylation, and other epigenetic modifications that can lead to abnormal gene expression, all of which occur with asbestos exposure. These are easily measurable markers for health outcomes that do not require death of the animal. Such studies can be done with small samples from live animals. A tiered and targeted approach is needed, where fiber measurements at key sites (reference compared to animal collection sites) are followed up with optimal methods. For example, polarized light microscopy (PLM) can be used as a soil screening tool, but it should be followed up with Transmission Electron Microscopy (TEM) to provide more sensitive evaluation of concentrations. Population studies should follow up those done in the BERA plus key large animal populations, and then studies could be performed to detect immunological and genomic impacts, which are far more sensitive measures of health impacts than gross lesions and histology, occur within the normal life-span of animals in the wild, and do not require killing the animals.

With all of the data reviewed here, it is more likely than not that natural resources in the OU3 are being impacted by the presence of LA, and that the damage to those populations is being felt throughout the food and support webs even beyond the edges of OU3 due to mobile carnivorous populations such as migratory birds, birds of prey, and far-ranging mammalian predators that are dependent on food resources in the OU3. Damage to the resource organisms discussed in this report include direct health effects such as infections and respiratory dysfunction, but damage to their ecological niches, plants and other food sources will have severe impacts on populations indirectly as well. A clearer evaluation of these impacts should include the following. Comparison of OU3 populations with a true control area would be necessary, and that in itself would be extremely difficult.

1. Fiber burden studies of a broad range of species throughout OU3.There is no known concentration of LA below which health effects do not occur. Therefore, if exposure is confirmed, the scientifically sound conclusion would be that health effects are occurring.
2. Catch & release population counts, activity mapping, sex, age and weight in OU3 and true control sites.
3. Serum sampling of small mammals for autoantibody and cytokine measurements for immune dysfunction/inflammation
4. Blood sampling of mammals, fish and birds in OU3 and control sites for genetic/epigenetic studies
5. Tissue sample of a small number of animals for genetic/epigenetic studies (lung)

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