

## Risk Assessment for Arthropod Vectors

**T**HE INTENT OF this section is to provide guidance and to establish a framework for selecting the appropriate arthropod containment level (facilities, equipment, and practices) that reduce risks of release and exposure of laboratory workers and the public to a vector and associated agents.

“Risk” implies the probability that harm, injury, or disease will occur among laboratorians or the general public because of accidental release of a competent disease vector and/or associated agents. In the context of vector research laboratories, risk assessment considers two kinds of effects: direct effects, such as biting, infestations, and myiasis, and indirect morbidity and mortality due to the pathogens transmitted. The latter is by far of higher concern, and direct effects will not be considered here. Therefore, in this document, arthropod containment levels are directly correlated with the appropriate BSL levels of the agents with which they are naturally or experimentally infected or may transmit in the event of accidental release (see U.S. Dept. of Health and Human Services, 1999, Section VI).

While the focus of this document is public health risk, effects on animals because of arthropods known to transmit animal disease are to be considered. Researchers are encouraged to consult with the U.S. Fish and Wildlife Service and USDA-APHIS regarding risks and regulation before completing a risk assessment.

The laboratory director or principal investigator has primary responsibility for assessing risks in order to set the appropriate biosafety level for the work. This is done in close collaboration with the Institutional Biosafety Committee (IBC) to ensure compliance with established guidelines and regulations. Development and review of the risk assessment and the planned safety precautions by consultation with experts in the biology and public health significance of the arthropod is essential.

In performing a qualitative risk assessment, all the risk factors are first identified and explored considering related information available such as BMBL, the NIH Recombinant DNA Guidelines, the Canadian Laboratory Biosafety Guidelines, the WHO Biosafety Guidelines, and the ACAV Catalogue of Arboviruses. In many cases, one must rely on other sources of information such as field data, the literature concerning aspects of vector competence, and environmental requirements through consultation with recognized experts in arthropod and pathogen relationships.

The greatest challenge of risk assessment lies in those cases where complete information on these factors is unavailable. A conservative approach is advisable when insufficient information forces subjective judgment.

### PRINCIPLES OF RISK ASSESSMENT

Arthropod risk assessment is primarily a qualitative judgment that cannot be based on a prescribed algorithm. Several factors must be considered in combination: the agents transmitted, whether the arthropod is or may be infected, the mobility and longevity of the arthropod, its reproductive potential, biological containment, and epidemiological factors influencing transmission in the proposed location or region at risk.

Arthropod vectors of infectious agents can be assigned to the following discrete categories. Each category has a range of risks that need to be assessed.

*Arthropods known to be free of specific pathogens*

Risk from these materials to laboratorians is similar to that experienced by the general public: nuisance due to consequences of escape and temporary or permanent establishment. Consequently the public health risk is likely to be low unless epidemiological conditions exist that could reasonably be expected to result in an increase in transmission of an endemic disease in that particular region, or establishment of the released vector leads to significant risk of future transmission potential for an exotic pathogen. In the event that establishment is likely, the arthropod must be handled under more stringent containment conditions.

If an accidental release occurs, followed by even transient establishment of an uninfected arthropod, the probability of increased transmission must be considered in the context of the location in which the work will be performed or in regions to which escaped arthropods could likely migrate. For example, escape of an exotic malaria vector in a malarious region has significantly higher probability of increasing transmission and therefore higher risk than escape in a non-malarious region. The pathogenicity of the agent and availability of treatments and drugs should also be considered.

Answers to the following questions will affect the level of risk due to accidental escape of uninfected arthropods:

- Is the arthropod species already established in the locale?
- If the arthropod is exotic, is it likely that the arthropod would become temporarily or permanently established in the event of accidental escape?
- Are the agents that the arthropod is known to transmit cycling in the locale, or has the agent been present in the past?
- Are agents that the arthropod could reasonably be expected to transmit to animals present in the locale?
- Would accidental release of the arthropod significantly increase the risk to humans and animals above that already in existence in the event of introduction of exotic pathogens in the area?
- In the case of zoonotic diseases, does the animal reservoir exist in the locale, and, if so, what is its infection status?
- Could the arthropod be controlled or locally eradicated by traditional methods (e.g. spraying, trapping) in the event of escape?
- Was the exotic arthropod derived from a subpopulation (strain, geographically distinct form) whose phenotype is known or suspected to vary in ways that could reasonably be expected to significantly increase its vector competence? If so, it should be handled under the more stringent conditions within ACL-2 (described below) even if uninfected.
- Are disabled strains available whose viability after escape would be limited (e.g. eye-color mutants, cold-sensitive)?

*Arthropods known to contain specific pathogens*

Arthropods that are known to be, or suspected of being, infected with infectious agents always have risks that must be identified, and appropriate precautions must be taken for worker and public health safety. The characteristics of most known infectious agents have been well defined and are the starting point for determining risk from these arthropods.

Information useful to risk assessment can be obtained from laboratory investigations, disease surveillance, and epidemiological studies. Infectious agents known to have caused laboratory-associated infections are included in the BMBL agent summary statements (Section VII). Other sources include the American Public Health Association's manual, *Control of Communicable Diseases* (Chin, 1995). Literature reviews on laboratory acquired infections also may be helpful (Sewell, 1995; Collins, 1983; Herwalt, 1949).

The pathogenicity of the infectious or suspected infectious agent, including disease incidence and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease) is the most important consideration in assessing the risk due to accidental exposure to an infected arthropod vector. As the initial criterion, it is clear that the more severe the potentially acquired disease, the higher the risk.

Readers will observe that the Arthropod Containment Level 2 (ACL-2) level has broad latitude in the specific practices. This reflects, in part, the widely differing degrees of effects of arthropod-borne agents, many of which fall within the BSL2 level. Considerable variation in morbidity and mortality exists within the level 2 classification. For example, level 2 arboviruses range from La Crosse virus with a 1% or less mortality rate and limited, mild neurological sequelae to Eastern Equine Encephalitis (EEE) with a mortality rate that approaches 50% in clinical cases and survivors frequently suffer long term or permanent neurological deficits. Higher containment levels are recommended for agents that cause disease in humans that are considered potentially severe, life threatening, or cause residual damage. Our general approach in formulating these guidelines has been to include a wide range of ACL-2 features that reflect this broad range of agent potency. Moreover, the possible natural and artificial modes of infection (e.g., parenteral, airborne, ingestion) of the agent are considered. This is essential to prevent infections in laboratorians.

The established availability of an effective prophylaxis or therapeutic intervention is another essential factor to be considered. The most common form of prophylaxis is immunization with an effective vaccine. Considering the example above, while EEE carries intrinsically higher risk than La Crosse virus to laboratory workers who become infected, a vaccine is available for the former. In some instances therefore, immunization may affect the biosafety level or ACL. However important, the availability of therapeutics and vaccines only serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment. Occasionally, immunization or therapeutic intervention (antibiotic or antiviral therapy) may be particularly important in field conditions. The offer of immunizations is part of risk management to protect laboratory workers. For example, vaccination may be demanded, as a condition of employment, for any laboratory worker working with yellow fever virus, or any pathogens for which an efficacious vaccine is available.

Medical surveillance is encouraged to ensure that the instituted safeguards provide the expected health outcomes. Surveillance may include serum banking, monitoring employee health status, and participating in post-exposure management. In the arthropod vector laboratory, this must be combined with regular monitoring for escaped arthropods, e.g., through direct counting of infected arthropods, an effective arthropod trapping program, and regular inspection of the facilities for disrepair that could result in escape.

Risk assessment must also include an evaluation of the experience and skill level of at-risk personnel such as laboratorians, maintenance, housekeeping, and animal care personnel. Additional education may be necessary to ensure the safety of persons working at each biosafety level.

*Arthropods containing unknown infectious agents or whose status is uncertain*

The challenge here is to establish the most appropriate containment level with the limited information available. Some questions that may help in this risk assessment include:

- Why is an infectious agent suspected?
- What route of transmission is indicated?
- Are agents that the arthropod transmits transferred horizontally?
- Are there reasons to believe that a novel or unknown agent is present?
- What epidemiologic data are available?
- What is the morbidity or mortality rate associated with the agent?

The responses to these questions may identify the agent or a surrogate agent whose existing agent summary statement can be used to determine a biosafety level. In the absence of hard data, a conservative approach is advisable, and stringent precautions are indicated. For example, collections of vectors, particularly adults, from disease-endemic regions must always be treated with the suspicion that they may contain individuals carrying infectious agents.

Similarly, researchers working in field sites often handle arthropods of unknown infection status under conditions that do not allow implementation of typical laboratory precautions. However, an effort should be made to define the probable risks that personnel will encounter and protective measures should be taken. Answers to the questions above will assist researchers in determining potential risks and reasonable solutions.

*Vector arthropods containing recombinant DNA molecules*

The purpose of this section is to present principles of risk assessment of vector arthropods that have been genetically modified, typically via recombinant DNA technology. This includes both vector arthropods that contain modified microbes or which themselves are genetically modified. These principles primarily address the public health significance of the modified organisms rather than environmental concerns. These technologies continue to evolve rapidly, and experimental procedures designed to derive novel modified symbionts and recombinant arthropods are becoming commonplace. The National Institutes of Health publication, *Guidelines for Research Involving Recombinant DNA Molecules* (9), is a key reference in establishing an appropriate biosafety level for work involving recombinant organisms including microorganisms for use in arthropods and genetically modified arthropods themselves.

In selecting an appropriate arthropod containment level for such work, the greatest challenge is to evaluate the potential biohazard *change* resulting from a particular genetic modification relative to the unmodified arthropod. In the context of public health, the selection of an appropriate level begins by establishing the phenotypic change in the arthropod and/or microorganism due to the DNA manipulation, and potential impact of escaped arthropods containing the modification. Among the points to consider in work with recombinant arthropod vectors and those containing recombinant microbes are:

- Does the inserted gene encode a product known or likely to alter the vector capacity or competence for pathogens it is known to transmit?
- Does the inserted gene cause phenotypic changes that could significantly affect the ability to control the arthropod if there were an accidental escape, e.g., an insecticide resistance marker?

- Does the modification have the potential to alter the range or seasonal abundance of the arthropod?
- If so, would the new range increase the likelihood that the vector could transmit new pathogens?
- Is the modified strain disabled in a way that viability after escape would be limited (e.g., eye-color mutants, cold-sensitive)?
- Does the modification have the potential to increase the reproductive capacity of the arthropod that carries it?
- Is the phenotype conferred by the modification, including its marker and other expressed genes, if any, consistently expressed after numerous generations of propagation?
- Is the modification undergoing rearrangement or other mutation at a measurable rate?
- Can the DNA transgene vector be mobilized in natural populations?
- Is the host range of the symbiont known?
- Would the modified symbiont pose increased risk to immunocompromised persons relative to the native symbiont?
- Is the entire sequence of the DNA insertion known, and are the coding sequences defined?
- Is horizontal transfer of the transgene to other microbes with which the modified microbe is likely to come into contact possible?
- Is the original insertion site known so that stability can be assessed later?

This list of questions is not meant to be exhaustive. Rather, it illustrates the information needed to provide an accurate and conservative assessment of risk to judge the appropriate containment level. Since in many cases the answers to the above questions will not be definitive, it is important that the organization have a properly constituted and informed IBC, as outlined in the NIH guidelines, to evaluate the risk assessment and provide prudent adherence to the appropriate safety guidelines for the assigned risk.