Arthropod Containment Evaluation in an Academic Institution

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President and Managing Director

Objectives

- Understanding the issues and challenges of arthropod research in academic settings and in particular, what was found at Arizona State University (ASU);
- Understanding the regulatory guidance and recommenced best practices in design and operation of ACL-1 ACL-2 laboratories;
- Recommendations for the management of arthropod research and containment (ACL-1 ACL-2) in academic institutions;

Arthropod Research in Academic Institutions

- The amount of arthropod research is increasing
- Emerging and re-emerging vector-borne diseases
- Looking at the impacts of climate change and huge declines in arthropod species
- Funding for research into genetically modified insects as alternatives to pesticides and to combat vector-borne diseases

Arthropod Research in Academic Institutions

- Challenges for already over-burdened and short staffed biosafety officers to keep track of all research going on campus-wide when it is not required to be reviewed by the IBC
- Lack of guidance to the research community regarding what is required to perform arthropod work
- Lack of appropriate facilities to perform the work

Arthropod Research Guidance and Containment Requirements in the US

- Arthropod research increased significantly in the late 90's. In 2000 the entire genome of the fruit fly *Drosophila melanogaster* was sequenced
- Prior to that insect containment guidance was provided recommending BMBL's containment levels (Higgs and Beaty1996)
- *Guidelines for Research Involving Recombinant DNA Molecules* (NIH 1999) is a basic reference for assessing risk and assigning containment for genetically modified arthropod vectors and micro-organisms in vectors. Requires BSL-2 for genetically modified insects

Arthropod Containment Guidelines (ACG 1)

- Approved by ACME (American Committee of Medical Entomology) at the 2002 annual meeting of the ASTMH (American Society of Tropical Medicine and Hygiene) and published in a special issue of *Vector-Borne and Zoonotic Diseases* (Benedict et al. 2003)VER 3.1
- The most recent version 3.2 was published in January 2018, still a draft

Major Changes between Version 3.1 and 3.2

- References to the Select Agent Rule as it pertains to work with arthropods.
- Recommendations added to better address nonflying arthropod vectors (ticks, fleas, mites)
- Language has been added to reemphasize that the ACL guidelines are recommendations and not regulations and that site and task specific modifications are based on a risk assessment.
- Language has been added to address diagnostic samples and guidance for clinical diagnostic laboratories.
- Requirements identifying separation of infected and non-infected arthropods
- Inventory requirements for ACL-3 arthropods

Major Changes between Version 3.1 and 3.2

- Stronger language regarding killing arthropods prior to disposal
- Container-within-container approach to housing and containment of arthropods
- Stronger PI directed risk assessment requirements
- Stronger medical surveillance requirements

Do I Have A Problem?

- July 2017 Sharpe Solutions International (SSI) worked with ASU EHS Biosafety staff to conduct a third-party review of all "known" arthropod laboratories.
- Labs were selected according to the following:
  - Based on IBC registration,
  - Known by the biosafety staff that a lab been modified to house insects,
  - Completion of Responsible Party Information sheet (RPI).

Arizona State University

Environmental Health and Safety

Responsible Party Information (RPI) Sheet – Submit Annually Date

Instructions – Each lab room must have a separate RPI. Please fill in the blue shaded fields. Save the form for each room separately before beginning a new form for a new room. Please submit completed forms via email to ASU EH&S Department through EHSregistration@asu.edu or campus mail at 6412.

General Information

Building		Room No.		Campus		Department		Mail Code	
Principal Investigator (PI)						ASU Affiliate ID (10 digit ID #)	
Location of Material Safety Data Sheets (Bldg. & Room)							

Emergency Contact Information

Emergency Contact	Title	ASU Affiliate ID (10 digit ID #)	ASU Phone	Emergency Phone

Biosafety (Check all that apply)

	Biological hazards, pathogens, or infectious materials		Animals (including transgenic animals)
	Human specimens (e.g., blood, cells, tissues, urine)		Animal specimens (e.g., blood, cells, tissues)
	Microorganisms		Arthropods (e.g., insects, arachnids)
	Recombinant or synthetic nucleic acids		Plants or seeds (including genetically modified)
	CRISPR, TALENs, ZFNs or other genome editing tools		Autoclaves
	Gene drives		Biological safety cabinets, laminar flow cabinets
	Environmental samples (e.g., soil, water, wastewater)		Centrifuges, flow cytometers, or other aerosol producing devices
	Toxins of biological origin (e.g., venom, tetrodotoxin)		Large scale biological research (>10 liters)
	Archaeological samples (e.g., bones, clothing fragments)		CDC/APHIS Select Agents or Toxins

Hazards or Special Concerns (Check all that apply)

	Carcinogens		Ionizing radiation / radioactive materials
	Compressed gas		Lasers - Indicate highest laser class:
	Corrosive liquids (acids or strong base)		Magnetic field generator
	Cryogenics		Pyrophorics
	Flammable liquids		X-rays
	High voltage equipment (>600 volts)		Oxidizers
	Hydrofluoric acid		Designated hot work area

OSHA Carcinogens - Does this location contain any amount of the following chemicals? (Check all that apply)

	Acrylonitrile		1,2-Dibromo-3-Chloropropane		Vinyl Chloride		beta-Propiolactone
	Asbestos		Ethylene Oxide		2 - Acetylaminofluorene		bis-Chloromethyl ether
	Benzene		Formaldehyde		alpha-Naphthylamine		3,3'-Dichlorobenzidine (and its salts)
	1,3-Butadiene		Inorganic Arsenic		4-Aminodiphenyl		
	Cadmium		Methylene Chloride		Benzidine		
	Chromium (VI)		Methylenedianiline		beta-Naphthylamine		

ACL-1 and ACL-2 labs

#	PI	BSL	rDNA?	Insect	Locations			
11-377		1	Y	transgenic honey bee	ISTB1: 330, 332, 334, 336, 338, 340			
15-569		1	Y	transgenic drosophila melanogaster	LSA: L1-67			
16-654		1	Y	transgenic drosophila melanogaster	CLCC: 310			
16-655		1	Y	transgenic drosophila melanogaster	ISTB1: 353, 360			
16-689		1	Y	termite	LSE: 604, 625			
16-697		1	N	ants: Pogonomyrmex barbatus; P. californicus; P. rugosus' P. anergism; P. colei; P. pencosensis; P. mayri; P. huachucanus; Messor pergandei; Mymecocustus spp.; Nasonia, spp.;	ISTB1: 340, 341, 342, 381			
17-722		1	Y	transgenic Apis mellifera	ISTB1: 330; Polytechnic Bee Facility			
17-744		2	Y	ticks, varroa mites, aphids, leafhoppers, mosquitoes and whiteflies	BDA: 339, 369A			
17-750		1	Y	transgenic drosophila melanogaster	ISTB1: 353, 360			
No disclosure		1	N	locust and grasshoppers	Life Sciences D			
No disclosure		1	N	Spiders, crickets	ISTB1 Basement			
No disclosure		1	N	locust pest species	LSA L1-95 and L1-99			
No disclosure		1	N	ants, cockroaches	ISTB1 3rd floor			

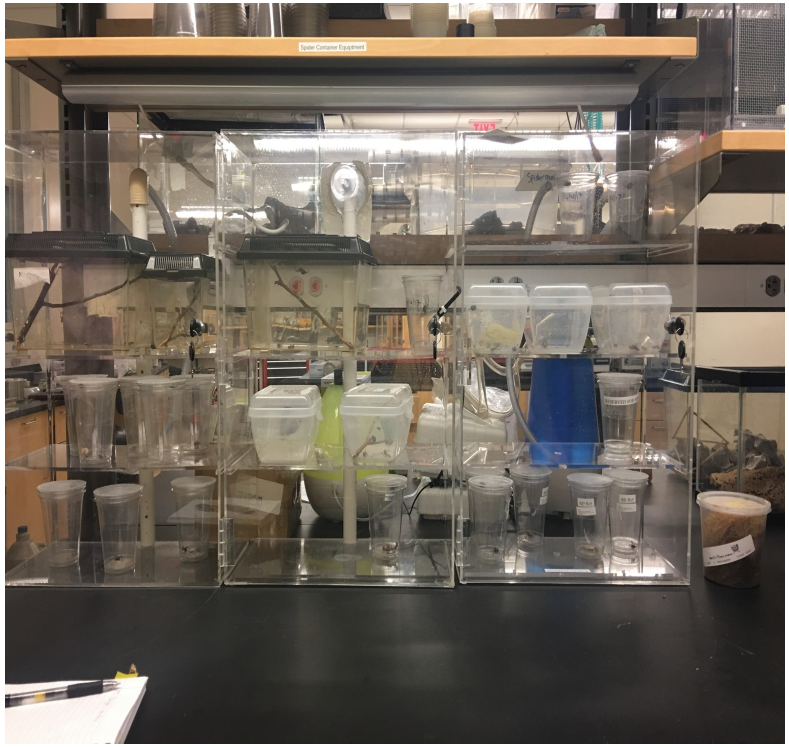
Research with Native Tarantulas, Black Widows, Golden Orbs ISTB1 L2 63B

- Operational/Management Issues:
  - No documentation of who was working on the project
  - Site-specific safety manual and SOPs should be written and all staff and students should have documented training to these SOP's. The SOP should include information such as what to do in the in the event of a sting, escape, feeding, research procedures, waste disposal etc.
  - No PPE requirements
  - No documented training
  - No medical surveillance to determine if the students had allergies or sensitivities to insect venom
  - No guidance to students if bitten (other than to report to the medical clinic)
  - No inventories of the larger spiders

Containment/Design Issues

- No door signage
- No door sweeps
- No security minimizing access only to those working on the project
- No sticky traps and sticky paper by the doors
- No drain covers

Rearing and Housing of Spiders



ISTB1 360 ISTB1 353, 353F, ISTB1 353B

- Large shared lab space where work was occurring with mosquitoes, bees, transgenic fruit flies, and Hissing Cockroaches.
- Operational/Management Issues:
  - There was evidence of escaped fruit flies in the public spaces, in addition the kitchen area had a fruit fly trap
  - Lack of primary container labeling of the species and stage of development
  - No cage-within-a-cage to compensate for the lack of ante-rooms
  - No documentation of who was working on the projects

Operational/Management Issues Cont.

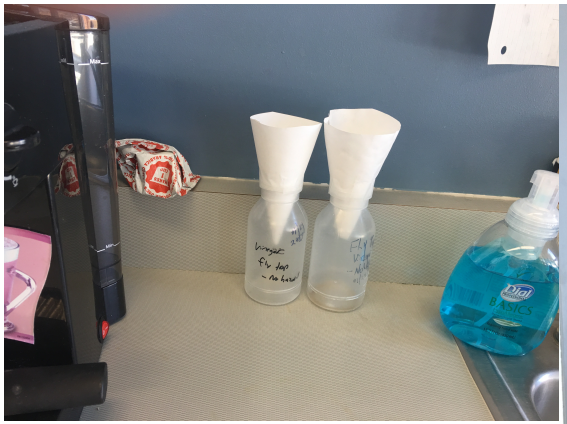
- Site-specific safety manual and SOPs should be written and all staff and students should have documented training to these SOP's. The SOP should include information such as what to do in the in the event of a sting, escape, feeding, research procedures, waste disposal etc.
- Medical surveillance questionnaires should be completed for all students and staff working with these insects. Forms should request information regarding potential allergies or sensitivities to venom or the insects themselves.

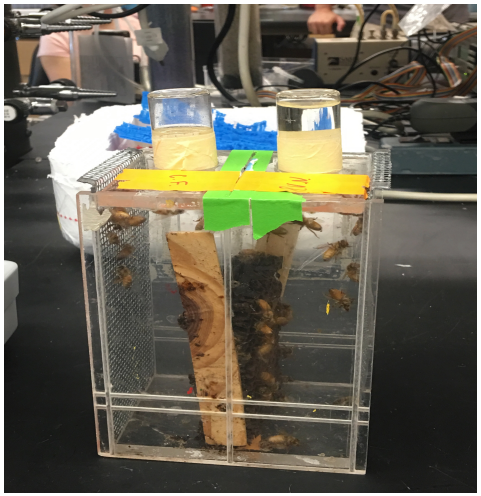
ACL-2 Design Issues

- Door signs should be placed on all entrances to include PPE and organisms,
- Door sweeps should be installed on all exterior doors,
- Double self closing doors should be on the entrance to the lab due to the transgenic fruit flies regulated as ACL-2,
- Security on the doors limiting access to only trained individuals,
- Screens on drains and supply and exhaust ducts if air curtains are not used
- Charging of drains
- Use of sticky traps and paper on the floor near doors

Bee hives on the roof with unlimited access

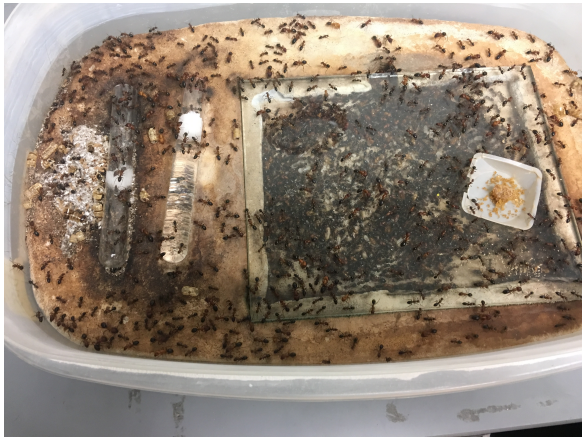
Traps in breakroom and several insect species being used in one laboratory

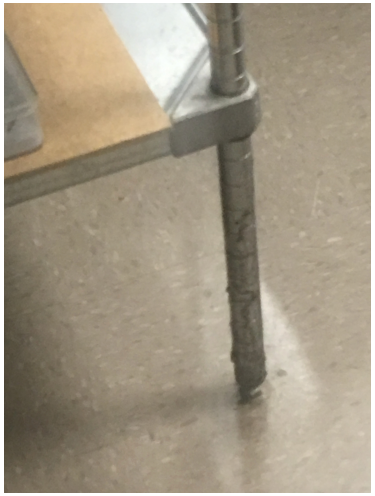


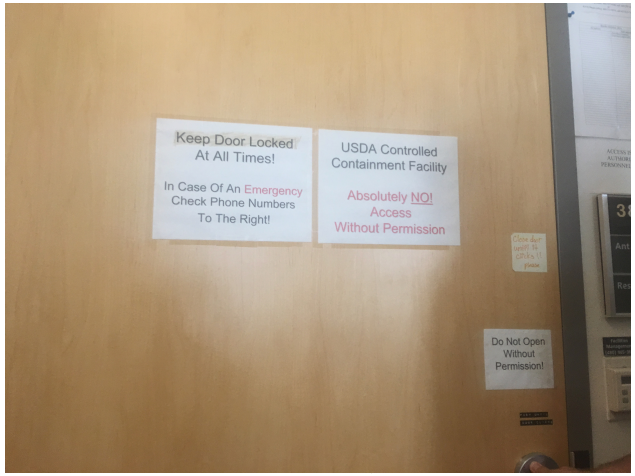


ISTB1 381, 385, 385A, 385B, 364, USDA Research with both native and non-native species of ants, termites, and mealworms.

- These were the most compliant labs. Likely due to USDA permit requirements and USDA inspections.
- Had lab-specific SOPs and procedures
- Physical barriers preventing escapes and security was adequate



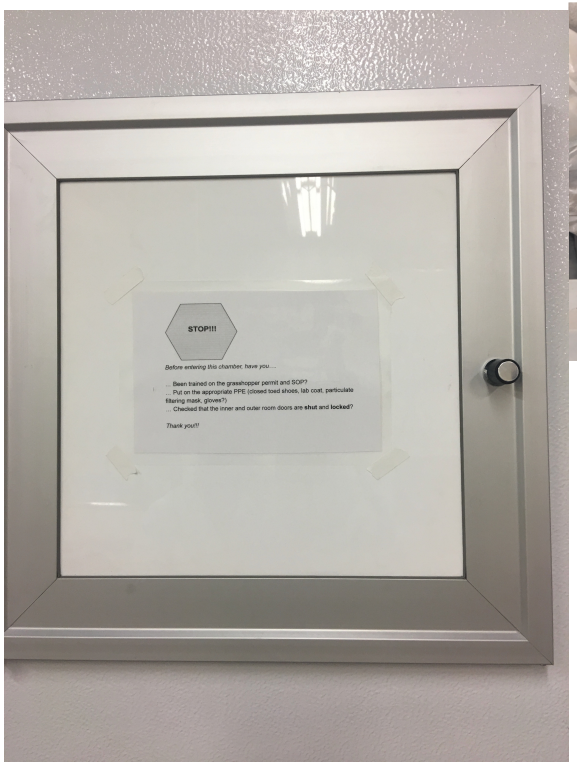


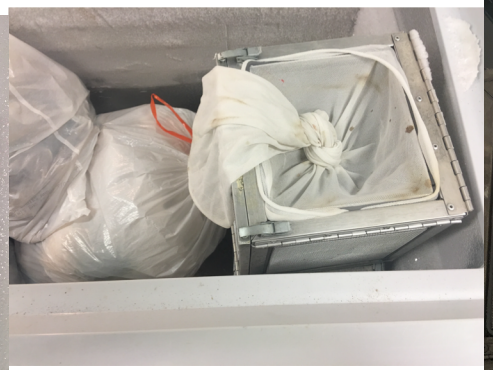

Keep Door Locked
At All Times!
In Case Of An Emergency
Check Phone Numbers
To The Right!
USDA Controlled
Containment Facility
Absolutely NO!
Access
Without Permission
Do Not Open
Without
Permission!

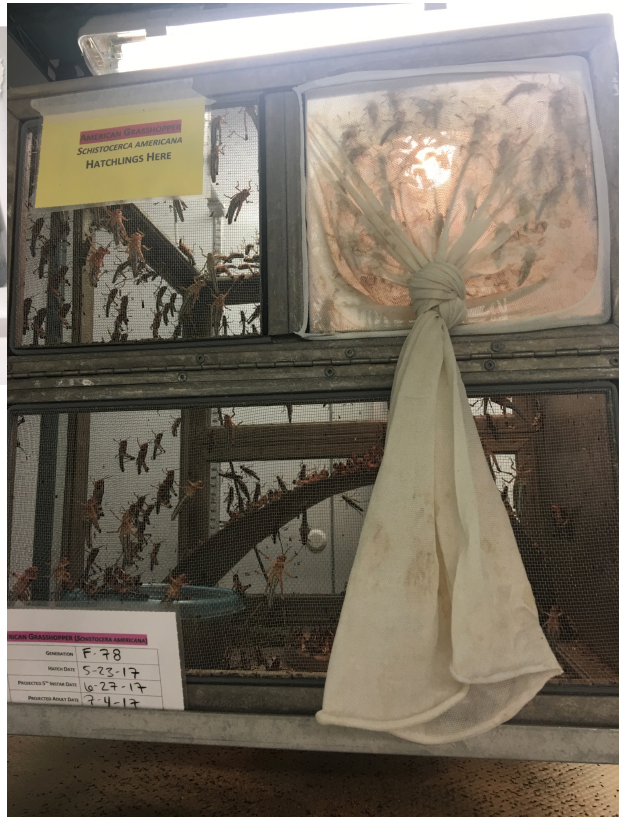
Life Sciences A L1 95-99 Non-native Grasshoppers and Locusts

- USDA permitted work in a purpose built laboratory. All requirements were in place.

Entry requirements, freezing dead insects, labeling life stages







ASU Bee Annex

- Maintains native and non-native bees species
  - No formal bee-keeper training
  - No medical surveillance or screening for allergies
  - Although no work was going on with the transgenic bees the enclosure was not ready for use due to holes in the netting
  - Other hazards including snake bites and heat exhaustion should be addressed


088
065
ASU


027
108
ASU

Sharpe Solutions International

Arizona State University

ASU Insectary Evaluation

PI	Location	Insect Transgenic or Non-native*	Containment Issues	SOPs	Training	Medical Surveillance	Waste Managed Per ACL	Signage	Security
	ISTB1 L2 63B	Tarantulas, Black widows, Golden orb; crickets	Yes, gaps under doors	No	No	No	No	No	No
	ISTB1 Terrace	Honey bees	Yes, bees can get into building	No	No	No	No	No	Door was locked
	ISTB1 381, 385 385A, 385B 364	N-ants*, N-Termites* mealworms	No but sticky paper is a suggestion	Yes	?	No	Yes	Yes	Keyed access.
	ISTB1 353, 360 353E, 353F,	Honey bees, T-fruit flies*, mosquitoes, Hissing Cockroach	Yes, escaped insects are found in public hallways, gaps under doors	No	No	No	No	No	No
	Life Sciences D 101C	N-Grass-hoppers and locusts*	Should have 2 self closing door for non-natives	Yes	Yes	No	Yes	Yes	Yes
	Life Sciences (LS) D 101	crickets, mealworms (feeders)	NA	No	?	No	?	?	?
	Life Sciences 103	Isopods	No	No	No	No	?	?	No
	Life Sciences A L1-95	N-Grass hoppers, Locusts	No	Yes	Yes	No	Yes	Yes	Yes
	Life Sciences A L1-99	N-Grass hoppers, Locusts	No	Yes	Yes	No	Yes	Yes	Yes

Sharpe Solutions International

ASU Arizona State University

ASU Insectary Evaluation

PI	Location	Insect Transgenic or Non-native*	Containment Issues	SOPs	Training	Medical Surveillance	Waste Managed Per ACL	Signage	Security
	Life Sciences E 625	N-Termites*	Yes	No	No	No	No	No	No
	Life Sciences L2-52	RG-3 Infected Mosquitoes proposed	Yes, significant containment issues	No	No	No	No	No	No
	Life Sciences E 342	Proposed fruit flies	Yes	No	No	No	No	No	No
	Life Sciences A L1-67	T-Fruit Flies	Yes, should have double doors w/sweeps	No	No	No	No	No	No
	Bee Lab, Poly	Honey bees, T-Honey Bees	Yes if working with trans-genics	No	No	No	?	Yes	No
	CLCC 310 W	T-Fruit Flies	Yes, should have double doors w/sweeps	Yes	Yes	No	Yes	Yes	No
	CLCC 316 W	Spiders, B. Widow	Yes, gaps under doors	Yes, but unknown to staff	No	No	No	No	No
	CLCC 338B W	Crickets (feeder)	NA	?	?	No	NA	No	No

T=Transgenic species *N=Non-native species

ASU Recommendations

- Develop a University-wide arthropod research requirements manual
- Require review and/or approval for all arthropod research
- EHS should share more information, perform joint inspections between general laboratory safety and biosafety inspections
- Implement a medical surveillance program for all staff involved in arthropod research, it can be as simple as a questionnaire
- Require PI's to perform a risk assessment
- Labeling of arthropod containers

ASU Recommendations

- Develop lab specific safety manuals and SOPs, ensure staff have been trained to these documents
- Ensure PPE is appropriate and laundered or disposed on-site to prevent escapes and minimize allergies
- Arthropod Containment Level 2 (ACL-2) must be practiced if working with exotic and indigenous arthropods infected with BSL-2 agents or that are suspected of being infected with such agents.
- Arthropod Containment Level 2 (ACL-2) must be practiced if working with genetically modified arthropods

ACL-2 Facility Design Recommendations

- Recommended entrance to the insectary is via a double-door, self closing vestibule that prevents flying and crawling arthropod escape
- Sealed windows
- Sealed or screened drains, charge traps
- Filters on house vacuum systems
- Sealed, light colored, easy to clean surfaces with few place for insects to hide

ACL-2 Facility Design Recommendations

- Inward directional air, progressively negative where arthropods are stored. Air-curtains or glove boxes may be required based on risk assessment
- Light fixture flush with ceiling
- Conveniently located autoclaves
- Lab is inspected at least annually

New Signs Developed by Biosafety Staff

Arthropod Containment Level One (ACL - 1)

New Signs Developed by ASU Biosafety Staff

Arthropod Risk Assessment

- 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?
- Does the arthropod have a known or characterized insecticide resistant genotype or phenotype?
- Could the arthropod be realistically controlled or locally eradicated by traditional methods (e.g. spraying, trapping) in the event of escape?
- Are the agents that the arthropod is known to transmit cycling in the locale, or has the agent been present in the past?

Arthropod Risk Assessment

- 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 it infection status?
- 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 even if uninfected.

Genetically Modified Arthropods

- 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?

Genetically Modified Arthropods

- 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?

Genetically Modified Arthropods

- 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?

Useful References

- *Recommendations for Laboratory Containment and Management of Gene Drive Systems in Arthropods* (Benedict and Capuuro October 2017) https://www.researchgate.net/publication/320463331_Recommendations_for_Laboratory_Containment_and_Management_of_Gene_Drive_Systems_in_Arthropods

- Version 3.2 of the Arthropod Containment Guidelines https://www.astmh.org/getattachment/Subgroups/ACME/Arthropod-Containment-Guidelines-For-Website-3-2018.pdf

Managing for IGF (Invasive genetic factors)

- There are essentially three ways that accidental escape can occur: (1) any living stage, including immatures in solid and liquid waste; (2) transport on equipment or staff; and (3) penetrations of the containment zones, including doors, windows, and ventilation.

Special thanks to the ASU biosafety safety staff for all their help and support!

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Questions or Comments

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