Lab Notes

Summer 2010

Unclutter Your Path to Safety

By: K. Lee Stone, M.S., MT (ASCP), NRCC-CHO

Have you looked around your lab lately? Is it a cluttered mess? Does it resemble a teenager's bedroom? Would your mother be proud of your neatness? Poor housekeeping increases the chance for slips, trips, falls and fires. Proper housekeeping techniques can eliminate conditions that could cause accidents and serious injuries.

Housekeeping isn't difficult if done regularly. The following are some simple steps that can be taken to make your laboratory space a safer one:

Keep floors and work surfaces clear of unnecessary objects.
Keep walkways free from objects that could trip someone. Common trip hazards include boxes, chairs, drawers and electric cords.

Don't block aisles, exits, or emergency equipment.
Blocking aisles and exits with clutter and/or equipment will impede your ability to exit in an emergency. Blocking emergency equipment such as emergency showers, eyewashes and fire extinguishers can prevent you from accessing them when needed during an emergency.

Don't block sprinkler heads.
Never store items within 18 inches of the ceiling in laboratories. Items stored within 18 inches of the ceiling will inhibit the ability of the fire suppression system to function properly by obstructing the flow of water from the sprinkler heads.

Routinely check floor surfaces.
If you find loose floor tiles, rough spots or other flooring surface problems, report it to Campus Facility Services at 278-1900.

Put it away when you are done.
You can greatly reduce the clutter by simply returning your equipment, glassware, supplies, chemicals etc... to proper storage after use. Make it a habit to never leave things lying around that can be simply returned to proper storage when you are finished. It does not take long for a little clutter to turn into a big mess.

Clean work surfaces regularly.
Routinely clean your work areas as appropriate to inhibit the spread of infectious diseases and help prevent chemical exposure.

When you finish reading this newsletter, take a look around your lab. Can you improve the housekeeping in your lab and make it a safer place? Don't allow clutter to endanger or contaminate your research.

Hazardous Materials Training Updates

By: Amanda Stinnett, CHMM

Hazardous materials are chemicals in various forms that have the potential to cause serious damage to life, health, or property. These products are shipped daily on the nation's highways, railroads, waterways, and pipelines. The U.S. Department of Transportation (DOT) regulates hazardous materials in transport, and requires that those individuals involved in the transportation process receive training. This would include individuals who receive, package, represent packages by signing airway bills, or load packages onto vehicles. There are a number of IUPUI personnel who perform one or more of these regulated activities. For this reason, Environmental Health and Safety has developed two training programs to facilitate both compliance and safety on our campus.

One training program is for those individuals who package and ship biological materials. The Shipment of Biological Materials classroom training is available for all individuals needing first time training. Once you have received training, you are required to complete refresher training every two years. I am happy to announce that this refresher course is now available through OnCourse!

A second new OnCourse training is available to those individuals who minimally handle hazardous materials packages, but do not need extensive training. This training is titled Hazardous Materials Package Receiving and General Awareness. This training module covers general information regarding the nine Department of Transportation hazard classes, how to safely handle packages containing hazardous materials, explains shipping documents, and outlines general emergency and security procedures. I recommend this training to anyone working with hazardous materials, but individuals who receive or sign (including on-line submissions) airway bills for hazardous materials packages meets the DOT definition of a hazmat employee and should take this training.

If you have questions about your individual training needs, please call our office at 274-2005. To see a complete list of all of our online courses, visit our website **Here**.

Prevent Heat Illness

Heat Index	Category	Dangers
80-90	Caution	Fatigue possible with prolonged exposure and/or physical activity.
91-105	Extreme caution	Sunstroke, heat cramps and heat exhaustion possible with prolonged exposure and/or physical activity.
106-129	Danger	Sunstroke, heat cramps or heat exhaustion likely, and heat stroke possible with prolonged exposure and/or physical activity.
130 or Higher	Extreme Danger	Heatstroke/sunstroke highly likely with continued exposure.

An Ounce of Prevention

How often do we take the time, even when we have recognized a potentially hazardous situation, to alleviate the risk and prevent an accident from happening? Proactive actions often require some time and planning to help eliminate being in the reactive mode when things don't go as planned. Accidents can happen in any laboratory, sometimes with disastrous results. But every accident has a cause and most accidents can be prevented if you know how to recognize a potential accident and take steps to prevent it.

Be on the lookout for these "Accidents Waiting to Happen":

- No eye protection or the wrong type of eye protection
- No gloves, improper gloves or gloves that are damaged or contaminated from previous use
- Not knowing what to do in an emergency
- Not knowing the signs and symptoms of overexposure and what to do if overexposed
- Improper use of fume hood or not verifying air flow
- Storing food in lab refrigerator, eating in the lab or using lab equipment in place of cups or plates
- Frayed or damaged electrical cords
- Flammable liquids not stored in appropriate cabinets or stored in household-type refrigerators
- Unsecured compressed gas cylinders
- Incompatible chemicals stored together
- Aisles in the lab or storeroom blocked by equipment or chemicals
- Safety showers or eyewash stations that are blocked by equipment or supplies
- Physical hazards, such as unguarded moving belts or pulleys or unattended heat sources such as open flames
- Believing that 'just this once' is a justifiable reason for unsafe behavior

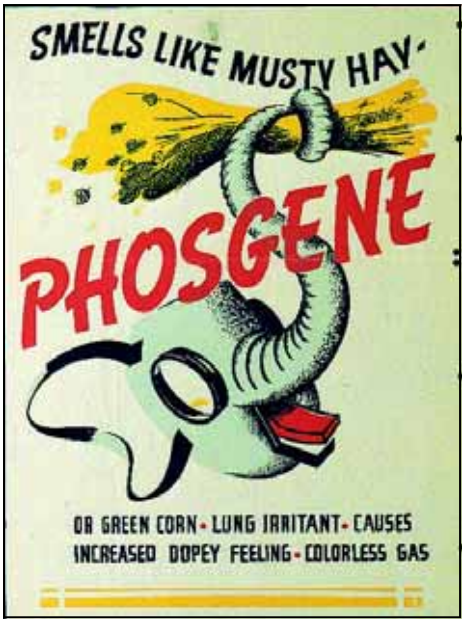
Adapted from Improving Safety in the Chemical Laboratory, Jay A. Young, Ed.

New Employee Training Schedule

Training	Time	2010 Dates	Building	Room
Laboratory Safety- REQUIRED for all new employees working in laboratories with hazardous chemicals.	9:30 AM-Noon	July 12 August 9 September 13 October 11	Union Building	Roof Lounge-6th Floor
Bloodborne Pathogens- REQUIRED for all employees working with human blood, body fluids or tissues.	8:30 AM-9:30 AM	July 12, 26 August 9, 23 September 13, 27 October 11, 25	Union Building	Roof Lounge-6th Floor
New Employee Orientation- REQUIRED for all new employees.	1:30 PM- 4:00 PM	August 3, 10, 17, 24, 31 September 7, 21, 28 September 14 October 5, 12, 19, 26	Campus Center Campus Center Union Building Campus Center	305 305 Roof Lounge-6th Floor 305
Biosafety Training- All employees who work with biohazardous materials are encouraged to attend.	9:30 AM-11:30 AM	July 26 August 27 September 23 October 25	Union Building	Roof Lounge-6th Floor

Phosgene-Chloroform's Deadly Little Secret

By: K. Lee Stone, M.S., MT (ASCP), NRCC-CHO



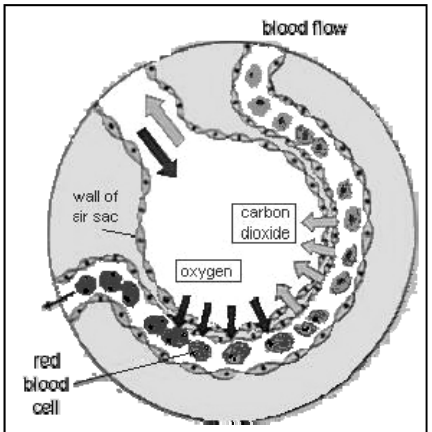
Phosgene is a highly toxic chemical that was used as a chemical weapon in WWI. This lethal compound can be generated from chloroform as it ages. Researchers at UCLA found this out the hard way when they were using a three-year-old bottle of chloroform on the bench top and suddenly became violently ill. After the exposure incident analysis of the chloroform container revealed concentrations of 15,000 ppm of phosgene in the head space of the bottle and a 1.1% concentration of phosgene in the bulk solution. The chloroform was stored properly and was stabilized with amylene. (1)

The odor threshold for phosgene is about 0.5 to 1 ppm. This level will vary with the individual and is usually higher following exposure because of olfactory fatigue. The odor of phosgene at 0.5 ppm has been described as pleasant and similar to new-mown hay or cut-green corn. At higher levels, the odor may be strong, stifling and unpleasant.(2)

The lowest concentration that will cause immediate throat irritation is 3 ppm. A level of 4 ppm will cause immediate eye irritation, 4.8 ppm causes coughing and brief exposure to 50 ppm may be fatal. (3) During or immediately after exposure to dangerous concentrations of phosgene, the following signs and symptoms may develop: Coughing; Burning sensation in the throat and eyes; Watery eyes; Blurred vision; Difficulty breathing or shortness of breath; Nausea and vomiting. Skin contact can result in lesions similar to those from frostbite or burns.

Following exposure to high concentrations of phosgene, a person may develop fluid in the lungs (pulmonary edema) within 2 to 6 hours. Exposure to phosgene may cause delayed effects that may not be apparent for up to 48 hours after exposure, even if the person feels better or appears well following removal from exposure. Therefore, people who have been exposed to phosgene should be monitored for 48 hours afterward. Delayed effects that can appear for up to 48 hours include the following: Difficulty breathing; Coughing up white to pink-tinged fluid (a sign of pulmonary edema); Low blood pressure; Heart failure. (4)

When inhaled phosgene quickly travels to the bronchioles, and causes damage to these airways. The chemical also causes damage to the capillaries and alveoli of the lung by reacting with the proteins and enzymes in these membranes. The primary function of these membranes is to keep the blood in the capillaries separated from the air in the alveoli where the oxygen exchange is taking place in the hemoglobin of the red blood cells. Once these membranes are damaged by phosgene, the plasma, which is the liquid part of the blood, leaks through the damaged membrane into the alveoli and fills them full of fluid so air cannot enter them. In essence this is what happens when an individual drowns in water, in that the alveoli fill up with water. Since the fluid



Oxygen andCO2 exchange in alveoli

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build up in the lungs from phosgene exposure is from the fluid in the blood and not from an external source, phosgene poisoning is often described as "dry land drowning."(5)

The following are some basic recommendations for laboratories that store and/or use chloroform:

Only Chloroform that is stabilized with alcohol should be purchased.

Chloroform comes in three basic varieties: a) no stabilizer present, b) stabilized with amylene, and c) stabilized with an alcohol such as ethanol. Alcohol is usually added in greater concentrations than amylene so it provides better protection from phosgene generation. Also, there is evidence that amylene may not prevent phosgene generation.

Chloroform should be treated as a time-sensitive chemical.

This is particularly true of chloroform that is either not stabilized or is stabilized with amylene. Date when received; date when opened; and discard within one year.

Open chloroform containers in a chemical fume hood.

By opening the chloroform containers inside of a fume hood you are ensuring that the vapors released will be contained inside of the hood and away from your breathing zone. You should also make every attempt to also use the chloroform in the fume hood once it has been removed from the stock container.

Chloroform should always be stored in a dark place.

The 1995 edition of Prudent Practices in the Laboratory states (p. 283): "In the presence of light, chloroform undergoes autoxidation to generate phosgene; this can be minimized by storing this substance in the dark under nitrogen.

Dispose of old or unused chloroform.

If you have opened unstabilized chloroform or chloroform stabilized with amylene that has been in the laboratory for more than one year, eliminate it as hazardous waste using our online hazardous waste manifest form found Here. If you have chloroform in your lab and you are not actively using this material, it would also be wise to eliminate it as hazardous waste.

Order minimal amounts.

If you do need chloroform for a protocol in use, stock only what you can use in three months or less. Set up a "just-in-time" purchasing policy and purchase the smallest container size that is practical.

Ensure chloroform containers are tightly capped when not in use.

Keep all chloroform bottles tightly capped and stored in a dark ventilated storage cabinet or, at last resort, in a fume hood.

Substitute.

Use a less hazardous substitute for chloroform, if possible.

Please practice safe science by following the above recommendations and give chloroform the respect it deserves. Don't let chloroform release its deadly little secret in your laboratory.

References:

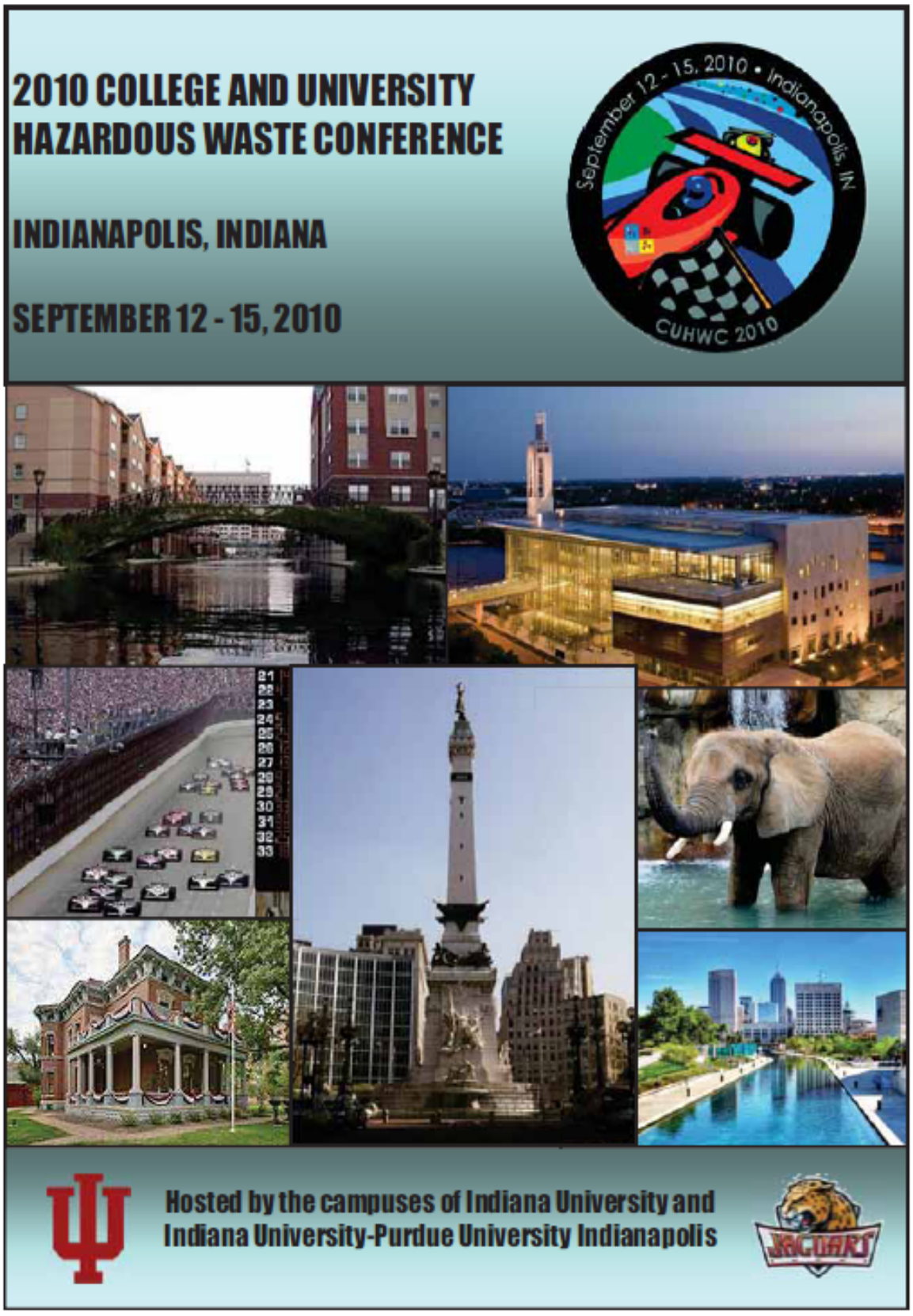
1) Chemical & Engineering News, March 2, 1998

2) Hardy, E. In "Kirk-Othmer Encyclopedia of Chemical Technology"; Grayson, Martin, Ed.; John Wiley & Sons: New York, 1982; Vol. 17 416-425.

3) Proctor, N.; Hughes, G. "Chemical Hazards of the Workplace"; J. B. Lippincott Company: Philadelphia, 1978; 414-415.

4) CDC; Emergency Preparedness & Response. Facts About Phosgene. Available from, as of July 17, 2007: http://www.bt.cdc.gov/agent/phosgene/basics/facts.asp.

5) Chemical Casualty Care Division/USAMRICD; Field Management of Chemical Casualties Handbook, 2nd ed. (July 2000).


2010 COLLEGE AND UNIVERSITY
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2010 College and University Hazardous Waste Conference Programs

General Environmental Compliance Track

Air Pollution Requirements for Colleges and Universities: Greenhouse Gases Rule and Emission Standard for Stationary Diesel Engines (Emergency Generators) Paul Dubenetzky, Keramida Environmental Services, Inc.

Assessing Response: A Critical Look at What Hazmat Response is Prudent Considering the Regulations and Financial Commitment Undertaken Kevin Kinast, Stanford University

Biodiesel Production at Louiana State University: How It Relates to Waste Management and Campus Sustainability Michael Hooks, LSU

Campus Flooding: Issues and Challenges Paul Hoza, University of Louisville

Chemical Suicides: A Case Study Rex Howard, Indiana University

A Common Sense Approach to Site Characterization, Remediation and Closure Jennifer Bush, Aegis Environmental Services, Inc.

Environmental Self Audit and Self Disclosure Policies: What You Don't Know Can Hurt You Dan Derheimer, Indiana University

Environmental Updates: PCB in Building Materials & Lead-Based Paint Standard. Dr. Jack Leonard, Environmental Management Institute

Globally Harmonized System for the Classification and Labeling of Chemicals – It's Coming Sooner than You May Think… Are You Prepared? Melissa McAffrey, MSDS-Online

The Gulf Oil Disaster: LSU's Involvement From the Perspective of Research and Emergency Management Michael Hooks, LSU

High Performance (Low-Flow) Fume Hoods, Do They Work?- A Discussion of IUPUI's Modified ASHRAE Test Results on High Performance Hoods Lee Stone, IUPUI

Tracing and Eliminating Mercury in Waste Water: Finding the Proverbial Needle in the Haystack Alan Call, US EPA

Hazardous Waste Track

Case Study Follow-Up: University of Arizona New Hazardous Waste Facility Jeff Christensen, University of Arizona

Development of Columbia University's Enhanced Radioactive Program Over Four Campuses with One Department Lauren Kelly, Columbia University

Haz Waste Disposal – It Pays to Do Some of it Yourself Curtis Plotkin, California State University Fullerton

Implementation and Enforcement of the Lab Rule (Back to Back Sessions) Kristen Fitzgerald, Jessica Young, US EPA

Environmental Stewardship – The Life Cycle of Waste Management Christine R. LeBlanc, Harvard Uni-versity, Jeffrey Sacre, CHWMEG, Inc.

Pharmaceutical Waste Handling and Disposal Christina Bailey, Heritage Environmental Services, Inc.

The Pros and Cons of Self-Packing Lab Pack Chemical Wastes Adam Peters, University of Virginia and Frank Imperatore, Virginia Tech University

Registering, Use and Disposal of DEA Controlled Substances: It's a Whole Different Animal Nikki Ad-ler, University of Utah

The RFP Process: A Winning Combination Andrea Antell, PSC Environmental

School Horrors: What Can Happen When you Ignore Lab Safety Dan Klein, Disposal Consultant Services, Inc.

Subpart K: One Year After Implementation (What We Are Learning and Still Learning) Rebecca Steiner, Milwaukee School of Engineering

What is Your Lab GPA? – A Unique Approach to Lab Safety Inspections and Increased Hazardous Waste Compliance Lee Stone, Indiana University

Glove Disposal in Research

By: K. Lee Stone, M.S., MT (ASCP), NRCC-CHO

Appropriate gloves are required PPE when handling hazardous materials. The proper disposal of used gloves in academic research areas and/or laboratories is also essential. Recently a policy has been passed by the IUPUI Laboratory Safety Committee to provide guidance for the proper disposal of gloves in the laboratory.

The following summarizes the Glove Disposal in Research policy which will hopefully clear up some of the confusion surrounding disposal of gloves in the laboratory:

1) Gloves used when handling the following material must be autoclaved prior to disposal if they have not been used in combination with hazardous agents such as mutagens, teratogens, carcinogens, radioactive materials or chemicals with a high level of acute toxicity:
   a) Any material containing or contaminated with human pathogens
   b) Any material containing or contaminated with animal pathogens
   c) Any material containing or contaminated with plant pathogens
   d) Any material containing or contaminated with recombinant DNA or recombinant organism: NIH Guidelines Appendix G-II-A-1-c (BL1), G-II-B-1-c (BL2), G-II-C-1-b (BL3). All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
   e) Any material containing or contaminated with any other potentially infectious material: IC 16 -41-16 – Communicable Disease: Treatment of Infectious Waste; Biosafety in Microbiological and Biomedical Laboratories, 5th Ed., Section IV-C-4-c (BL1, BL2, BL2), Dispose of used gloves with other contaminated laboratory waste.

   f) Gloves worn while working inside of a biosafety cabinet.
2) Gloves used in laboratories while handling mixed wastes such as hazardous chemicals or radioactive materials in combination with the materials listed above, or any other material that is not listed above must be disposed of without autoclaving.
3) All gloves must be disposed of prior to leaving the laboratory if no materials are being transported outside of the laboratory. If materials requiring gloves are transported outside of the laboratory then a glove may be worn on the hand carrying the material and the other hand shall be glove free to open doors or touch areas outside of the laboratory with the ungloved hand.
4) A thorough hand washing shall be performed once gloves are removed before personnel leave the laboratory.

Please remember that gloves used for chemical manipulations are disposed in a different manner than gloves used for manipulation of biological agents. Feel free to contact me at 278-6150 if you have any questions concerning the disposal of gloves in your laboratory.

IUPUI ENVIRONMENTAL HEALTH AND SAFETY
SUMMER 2010

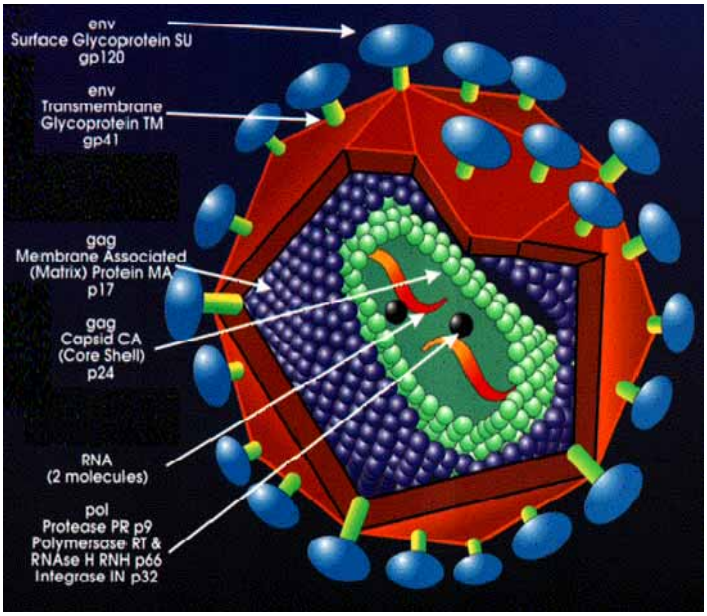
Biosafety Issues of Lentiviral Vectors Used in Research

By: James Klenner, MSc, MPH, MPA, RBP, CBSP

Lentiviruses represent a subset of retroviruses, e.g., simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV), and have the ability to integrate into host cell chromosomes. They are also unique in that they can infect non-dividing cells due to their preintegration complex being able to bypass the nuclear envelope. Wildtype lentiviruses are infectious particles that can result in progeny virus.

The distinction must be made between lentiviruses and lentiviral vectors. A lentiviral vector has the ability to infect cells and integrate a research gene into a target cell, but under ideal situations will not result in the generation of replication competent lentiviruses (RCL). This is from the development of a 4 plasmid system used to assemble lentiviral particles. By using 4 plasmids the vector and packaging functions are separated. Several essential genes needed for replication and Tat synthesis have been deleted from commercial packaging systems which increase the safety of using these vectors while further diminishing the likelihood of an intracellular recombination event. In order to increase the range of target cells in research, i.e., viral tropism, a vesicular stomatitis virus (VSV-G) coat protein is incorporated (pseudotyped) into the packaging system. This increase in host cell types that can be infected by lentiviral vectors does add to the biosafety containment required in research labs.

Some of the determining factors outlined in research proposals that affect any risk assessment may include some or all of the following; whether or not the packaging system is a 4 plasmid or older 2-3 plasmid system, if a pseudotyping system is used, the nature of the transgene (oncogenes, toxic products, etc.), laboratory or large scale (>10 liters) production, the use of sharps, permissive hosts, and animals engrafted with permissive cells that allow for HIV replication (xenografts). Most in vitro work with lentiviral vectors can safely be done under Biosafety Level 2 (BL2) conditions. However, BL2 + BL3 Precautions, aka BL2 Enhanced, is called for when the vector is injected using a syringe, if the transgene is an oncogene, or produced in amounts > 10 liters and anytime human cells are transfected with a lentiviral vector prior to grafting into another animal like a mouse or rat. The direct injection of lentiviral vectors directly into nonpermissive animals such as rodents do allow for a reduction of housing biosafety levels after a few days.



Commercial vendors like to tout the safety of lentiviral vector systems based on the lack of RCL production, but the testing occurs in vitro and does not take into account what recombination events may occur if target cells are infected with HIV (known or unknown). Additionally it is estimated that 10% of the human genome is comprised of endogenous retroviruses (HERVs) obtained through the centuries. The opportunity for the role of HERV sequences in the generation of RCLs may not be fully understood and as few as 10 base pairs of homology resulted in replication

(Continued on page 10)

(Continued from page 9)

competent retrovirus generation. Otto, E., et al, Hum Gene Ther. 1994;5:567. In summary, these vectors are advertised as safe reagents for use in research projects but like anything else in biosafety reviews a conservative approach to risk assessment is prudent as one "never knows" what the outcome may be. While the likelihood of RCL generation is minimal (some would debate as zero), exposure to mucosal membranes or accidental exposure by accidents like needlesticks do pose their own particular risks. Keep in mind that these vectors are amphotropic and designed to permanently insert foreign gene sequences into target cells. Following accidental exposure – your cells are the target cells and there are examples of leukemia development from insertional mutagenesis during gene therapy trials.

The IUPUI Biosafety staff in the Office of EHS are always available for questions related to lentiviral vector use so please do not hesitate to contact Amanda Snyder (274-2103), Rachel Bennett (274-2056), or myself (274-2830).

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"Don't learn safety by accident."