



# Reporting An Untoward Event



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# Introduction

The National Institutes of Health (NIH), *Guidelines for the Use of Recombinant DNA Molecules*, requires that incidents involving a spill or release of recombinant DNA molecules are reported to the Office of Biotechnology Activities (OBA) within 24 hours in some cases or 30 days.

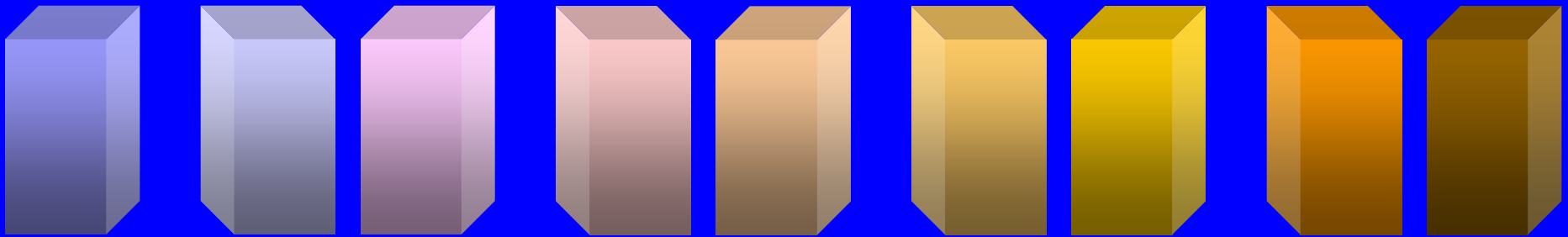


# Introduction

This presentation will discuss the observations associated with an unexpected outcome in an experiment and the reporting of this incident in accordance with the NIH Recombinant DNA Guidelines.



# The Experiment As Designed



Concept  
Risks  
Regulatory Basis

# Purpose of the Experiment

- The experiment was part of a project that involved the immortalization of bone marrow macrophages.
- It was approved by the University of California-Berkeley (UCB) Institutional Biosafety Committee (IBC), known locally as CLEB (the Committee for Laboratory and Environmental Biosafety) at Biosafety Level 2.

# The Experiment

- The investigator's research goals were to determine the molecular basis of the immunological response to pathogens.
- One phase of the experiment involved the production of immortalized mouse bone marrow macrophage cell lines by introducing mouse primary bone marrow cells to a retrovirus, called J2.

# The Experiment

- This would result in the expression of v-raf/myc fusion oncogene.
- The virus was supposed to be replication deficient and ecotropic, infecting only mouse cells. However, the investigator noticed some activity suggesting otherwise.
- The concern was that was there an event that now allowed these viruses to infect mammalian cells.

# Validation

- The investigator performed a validation test to verify if viral DNA was present.
  - With supernatant from the immortalized cell line, he filtered it through a 2 micron filter and then introduced it to fresh mouse bone marrow.
  - Result: the fresh bone marrow was immortalized which indicated that the cell line was producing infectious particles.



# Validation

- The investigator performed a validation test to see if the supernatant could infect human cells.
- With supernatant from another J2 immortalized cell line, he introduced it to HEK 293 cells.
  - Result: Inconclusive-viral DNA was not observed in the human cells but sensitivity in detection may account for this.

# Interpretation

- The J2 retrovirus had a viral RNA genome-the presence of any viral DNA would suggest the virus had entered the cells and reverse transcribed its genome.
- Was it a new viral genome generated by recombination? Unlikely-because the size of the v-raf/myc, gag and pol would exceed the size that could be efficiently packaged.

# Interpretation

- What could have attributed to this observation?
  - Activation of endogenous helper virus through the expression of the raf/myc oncogene?
  - Cross-Contamination with viruses providing gag, pol for the retrovirus?
- Was the contaminant amphotrophic to allow infection of mammalian and possibly human cells.

# Interpretation-worst case

- Since the J2 virus may have the ability to infect human cells, the original packaging cell line used to generate the virus may have been contaminated with amphotrophic helper virus.
- Less likely was the probability that murine bone marrow inherently had helper virus present.



# The National Institutes of Health Requirements



The NIH Guidelines

# Approval by the IBC

## Condition of Approval

- UC Berkeley's biosafety applications have a condition of approval that all accidents and incidents involving recombinant DNA must be reported to CLEB must be reported within 8 working hours.

# Approval by the IBC

## Condition of Approval

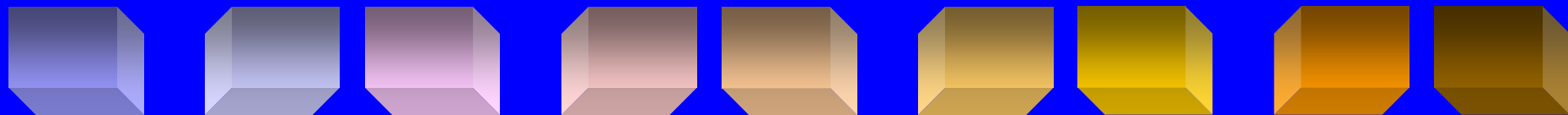
- Any accidents must be reported:
  - National Institutes of Health, Guidelines for the Use of Recombinant DNA Molecules, Section IV-D-7-e-2

# Approval by the IBC

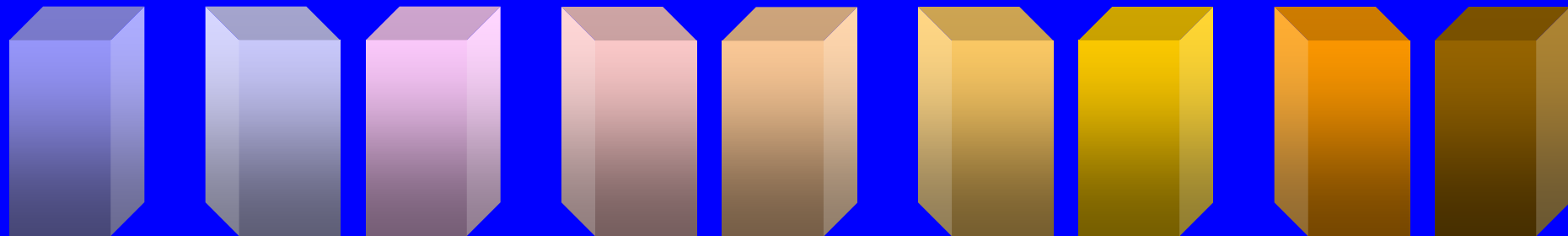
## Principal Investigator's Notification

- After observing the unaccounted for contamination, the investigator notified and filed a report with the Biosafety Officer.
- The Biosafety Officer and the Chair of CLEB recommended that the PI complete the NIH OBA incident reporting template and the Chair would send it to the NIH.





# The Response By The NIH



# What Should Your Institution Do?

- This incident did not involve an exposure to laboratory personnel nor did it result in an environmental release of recombinant DNA.
- The NIH Guidelines, Section IV-B-7-e-(2): Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities.

# The NIH Response

- UC Berkeley received a letter from NIH OBA. In this correspondence:
  - Acknowledgement of the submitted report
  - Scientific interpretation of the report
  - Assessment of institutional actions
  - Recommendation for biological containment level
- Notification that the experiment can proceed.

# Conclusion

## Things to Remember

- Untoward research outcomes are an undesired event in any experiment-if one involves a potential safety risk, the IBC and Biosafety Officer should examine the data to determine if the experiment needs to be performed at a higher level of containment.
- If the experiment involves recombinant DNA, determine if a report to NIH/OBA is necessary.