GRYPHON SCIENTIFIC

Applied Biosafety Research:
Human Reliability and Source Terms

Southern Biosafety Assn Spring Symposium

Rocco Casagrande, PhD

May, 2023

Sponsored by the Open Philanthropy Project

Motivation

- Over the past 15 years, Gryphon and colleagues performed critical studies in biosafety and biosecurity
- We developed approaches that leverage existing data to support critical decisions
  - Most data available were dose-response data or stability data
  - Data on accident causes and source terms were lacking in the life sciences
    - We analogized from other industrial accidents to inform our estimates

A Lack of Biosafety Scholarship Hampers Biosafety Risk Management

- We lack data on how accidents occur in biological laboratories
  - Most Lab Acquired Infections cannot be associated with a particular accident
  - What drives most exposures/Loss Of Containment incidents?
    - Fine motor mistakes (tripping a pipette tip over a well)
    - Major motor mistakes (dropping a flask or rotor)
    - Protocol mistakes (choosing the wrong rotor tube for a centrifuge)
    - Protocol violations/ignorance (ignoring an alarm on a PAPR or BSC)

A Lack of Biosafety Scholarship Hampers Biosafety Risk Management

- We lack data on how accidents occur in biological laboratories
  - Most Lab Acquired Infections cannot be associated with a particular accident
  - What drives most exposures/LOC incidents?
    - Fine motor mistakes (tripping a pipette tip over a well)
    - Major motor mistakes (dropping a flask or rotor)
    - Protocol mistakes (choosing the wrong rotor tube for a centrifuge)
    - Protocol violations/ignorance (ignoring an alarm on a PAPR or hood)

Training Valuable

A Lack of Biosafety Scholarship Hampers Biosafety Risk Management

- We lack data on how accidents occur in biological laboratories
  - Most Lab Acquired Infections cannot be associated with a particular accident
  - What drives most exposures/LOC incidents?
    - Fine motor mistakes (tripping a pipette tip over a well)
    - Major motor mistakes (dropping a flask or rotor)
    - Protocol mistakes (choosing the wrong rotor tube for a centrifuge)
    - Protocol violations/ignorance (ignoring an alarm on a PAPR or hood)

Equipment Valuable

GRYPHON SCIENTIFIC

5

A Transformative Grant

- To start to address this gap, we received a grant from the Open Philanthropy Project to undertake critical research in biosafety
- There are three lines of research:
  - **Conduct failure analysis** to determine how laboratory accidents generate hazards
  - **Conduct human reliability research** to determine how/how frequently researchers create incidents
  - **Gather data on innovations in biosafety** to learn from the measures that have already been implemented but are not widely known
- At Gryphon, this work was conducted along with Kelly Kim and Henry Wyneken

Open Philanthropy Project

GRYPHON SCIENTIFIC

FAILURE ANALYSIS AND SOURCE TERMS:

CHARACTERIZE THE HAZARD CREATED BY AN ACCIDENT
UNDERSTAND PROBABILITIES AND MECHANISMS

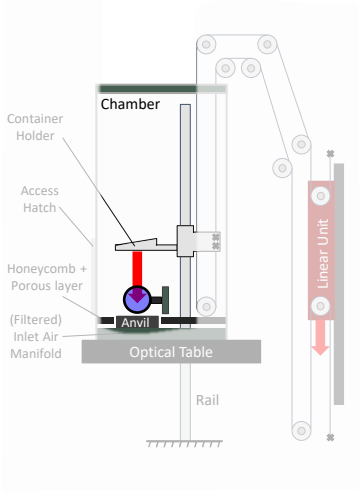
This work was done in the FLOW Lab in the Dept of Mechanical Engineering, UC Berkeley by:
Sungkyu Kim
Benoit Lebon
Chelsea Preble
Simo A. Mäkiharju, Principle Investigator

These physical science researchers have never before considered laboratory safety as an area of research

GRYPHON SCIENTIFIC

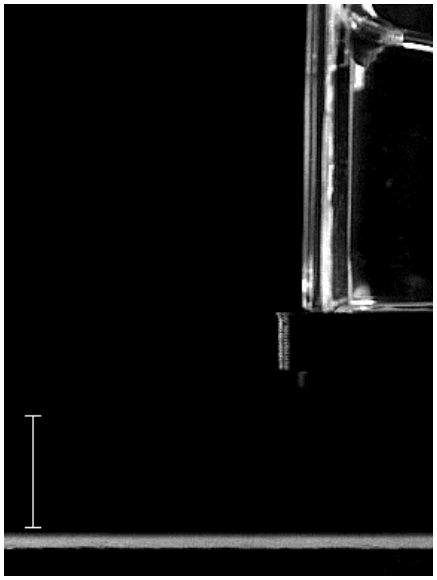
Experimental Set Up: Repeatable Accident INitiator (RAIN)

- Real drops are highly variable, must control many parameters to investigate one factor at a time
- We created RAIN:
  - Impact speed controlled by a pulley system driven by a linear drive
  - Impact angle controlled by a 'Forklift-like' container holder
  - Air-tight chamber with anti-static wall and inlet air filtered
  - High-speed Imaging (Phantom v1210—70k+ FPS) & Digital In-Line Holography



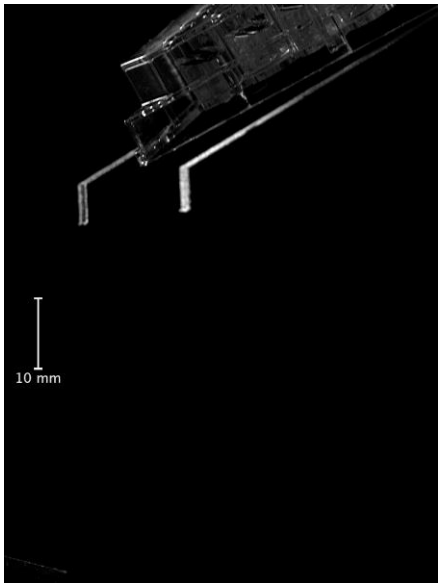
Images Captured During Simulated Laboratory Accidents

- At a visceral level, these images show how hazardous common laboratory accidents can be
- They are very convincing pieces of data that demonstrate why respiratory protection may be valuable even when not performing aerosol generating procedures



Images Captured During Simulated Laboratory Accidents

- At a visceral level, these images show how hazardous common laboratory accidents can be
- They are very convincing pieces of data that demonstrate why respiratory protection may be valuable even when not performing aerosol generating procedures



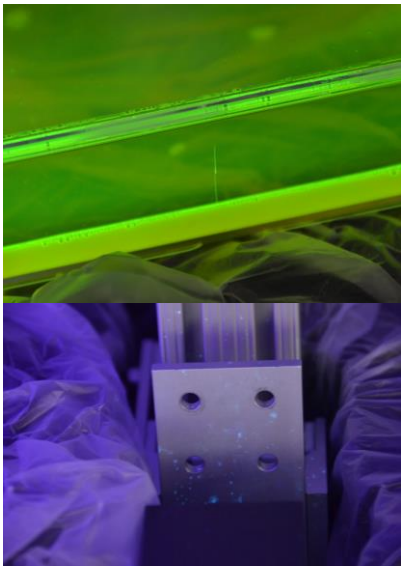
Flask Failures

20 drops:

1. 10 x distilled water, 100 mL
2. 10 x fluorescein solution, 100 mL

Result:

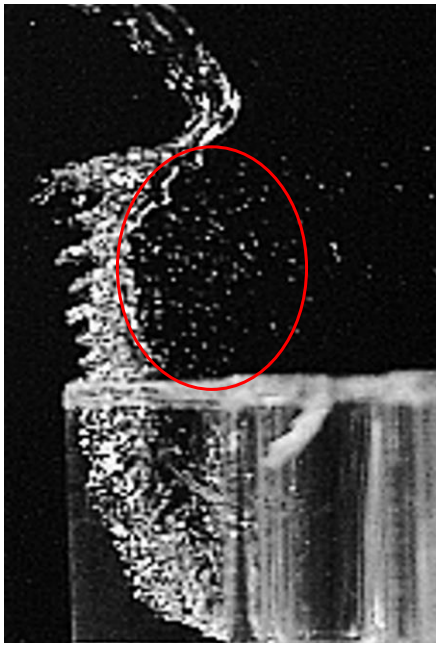
- 5/20 flasks failed with visible leakage
- 2/10 flasks spilled small droplets only detectable under UV light
- 1/20 flasks had a crack
- Some flasks have shown nearly undetectable leaks.



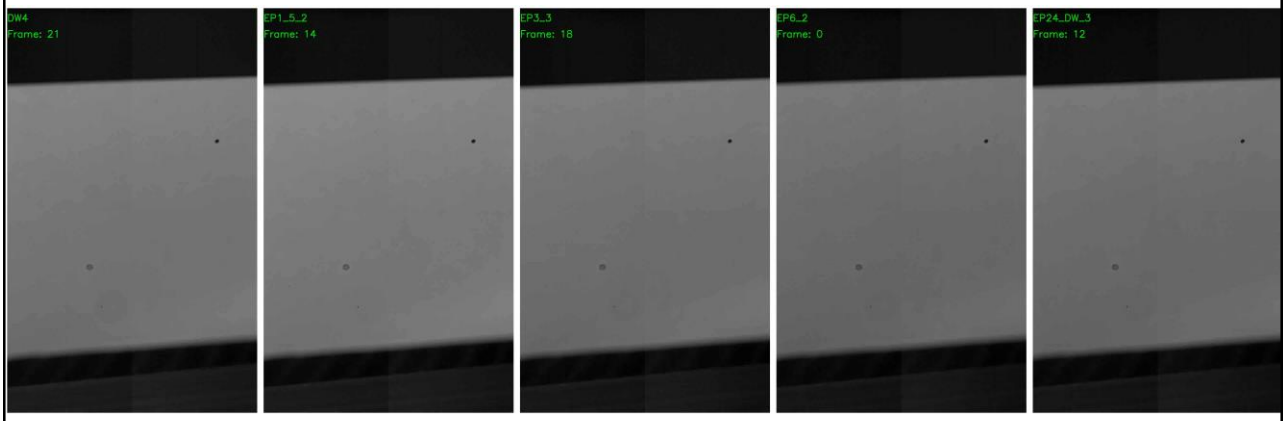
11

Following Interesting Observations

- In this research thrust, we set out to better characterize the hazard created when common labware is dropped
  - Due to the incredible complexity of the problem, we have not generated enough data to accomplish this goal (yet)
  - We have created a methodology and an experimental set up that will enable us to generate these data with statistically significant sample sizes
  - We have begun to understand influence of fluid properties on hazard (viscosity, surface tension)
- However, our observations have suggested that vibrations established upon impact are critical for changing a splash into an aerosol



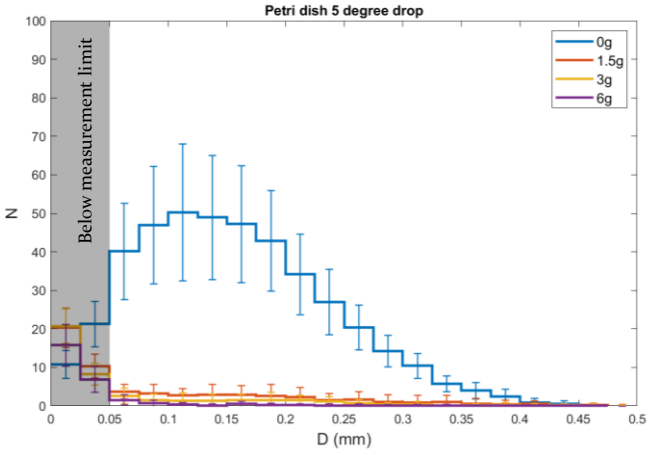
Engineering Inherently Safer Labware



Increasing mass of epoxy on underside of plate, changing resonance frequency

Engineering Inherently Safer Labware

- Adding mass by adding epoxy to the Petri dish significantly changed the resonance of the plastic, and thus the aerosols produced.
  - Data on large (50 micron and larger) droplets is significant
  - Data on aerosols in the respirable range is not reliably measured by this method
    - Awaiting holography data



Failure Analysis and Source Terms:

Characterize the hazard created by an accident

Understand probabilities and mechanisms

This work was done in the laboratory of Rebecca Roberts at Ursinus College

These experiments are simple yet fill critical data gaps in biosafety
These experiments are suitable to conduct at small, liberal arts colleges
These researchers had never before considered biosafety research

GRYPHON SCIENTIFIC

Conduct Failure Analysis

- How frequently do centrifuge tubes leak when inverted?
  - Filled thousands of centrifuge tubes with tracer and checked for leaks using a wipe
  - Determining modes of failure
    - Leakage when tube is closed
    - Leakage onto threads of tube when opened
  - 15ml conical centrifuge tubes leak (TTL) 0.5-1.5% of the time
  - 50ml conical centrifuge tubes leak (TTL) 0.2-0.8% of the time
  - Liquid gets on the threads of either tube 11-26% of the time
  - Brand is not a statistically significant factor in leak rate
  - Extra ring in cap does not affect leak rate

Summary of Leaks from Conical Tubes EMM with (Unadjusted Averages)

15 mL			50 mL		
Brand	P(TTL)	P(Thread)	Brand	P(TTL)	P(Thread)
E-15	0.015 (0.015)	0.26 (0.21)	E-50	0.008 (0.021)	0.12 (0.14)
B-15	0.004 (0.004)	0.21 (0.17)	D-50	0.006 (0.014)	0.13 (0.14)
D-15	0.01 (0.01)	0.18 (0.14)	C-50	0.002 (0.006)	0.11 (0.12)
A-15	0.005 (0.005)	0.11 (0.15)	B-50	0.003 (0.008)	0.20 (0.17)
C-15	0.012 (0.012)	0.25 (0.22)	A-50	0.001 (0.002)	0.16 (0.12)

GRYPHON SCIENTIFIC

16

HUMAN RELIABILITY:

RATE OF MISTAKES WHILE PIPETTING
ABILITY TO RECOGNIZE MISTAKES
PRELIMINARY DATA ON EFFECT OF TRAINING ON MISTAKE RATE

UFCSPA: Ana Gorini da Veiga and Aline Gehlen Dall Bello

IPT: Anissa Chouikha and Sana Masmoudi

RSS Jordan: Nesreen Alhmoud

CSU: Rebecca Moritz, Joanie Ryan and Jessie Harrell for empirical studies
Peter Justice for classroom observational studies

UMD: Sherry Supernavage Bohn and Sylvia Costa

GRYPHON SCIENTIFIC

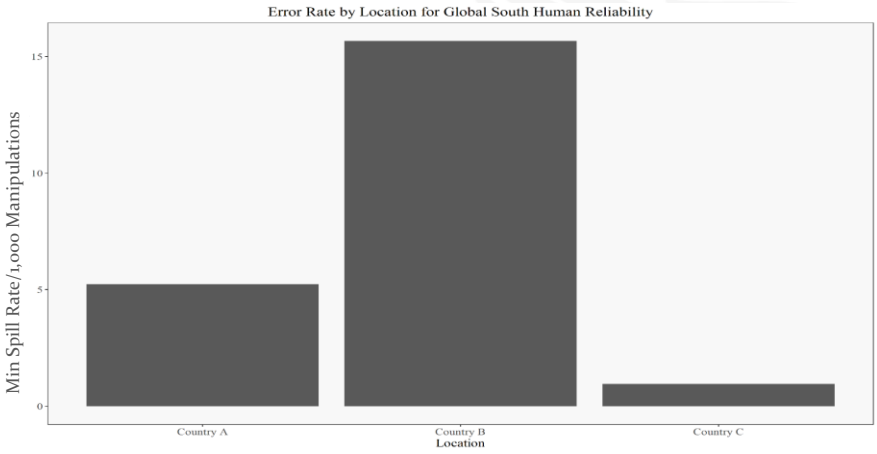
Conduct Human Reliability Research

- We performed two sets of experiments to determine human error rate
  - In clinical labs overseas, used dummy clinical samples in blinded experiments with tracer dye
  - In US institutions, used volunteers who knew they were working with non-hazardous materials.
- This work should help build a community of practice by demonstrating that researchers in low resource settings can contribute essential data
  - Key experiments do not rely on expensive capital equipment or reagents
  - The amount of worker time needed places a premium on low-cost settings
    - Overseas locales and locales with student volunteers

GRYPHON SCIENTIFIC

Error Rate in Clinical Labs

- Error rate was at least 1-15 spills per 1,000 manipulations for blinded dummy samples in clinical labs
  - Errors were rare, so we had good confidence that days with multiple spots of GloGerm were due to a single incident



Error Rate in Volunteer Studies

- In volunteer studies, subjects filled 96 well-plates from a weigh boat of GloGerm to examine rates of errors
  - A blacklight was used to identify contamination after each plate
  - High opportunity for error in between checks necessitated a statistical method of determining a likely error rate
  - That is, when more than one spot of contamination was observed, was it due to more than one error or a single error that created multiple spots of contamination?
  - Assuming independence of the errors, we found that the data suggested at least some of the errors led to multiple spots
    - Created a Poisson model to approximate the true number of errors that led to the spots and fit this to the observed and expected data

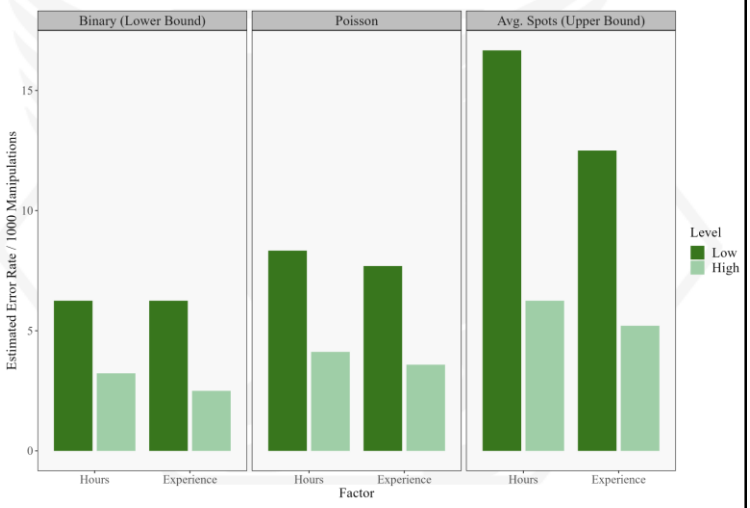
Error Rate in Volunteer Studies

- Error rate was at least 4.5 spills per 1,000 manipulations and most likely 6.5 per 1,000
- Importantly, these data validate the use of volunteers using simulants as a good proxy for real staff working with samples they think are pathogenic in their normal work environment
  - Recall that the rate in clinical labs was 1-15 spills per 1,000 depending on location
  - In the GoF Risk/Benefit Assessment, analogizing from data in other industries, we estimated the fine motor skill error rate was estimated to be 0.05-5 spills per 1,000 manipulations
    - Overlaps with but slightly exceeds high end of range

Location	Replicates	Min Error/ 1,000 manips.	Modeled error/ 1,000 manips.	Max error/ 1,000 manips.
A	36,000	4.6	6.1	15.5
B	16,000	5.3	7.4	24.6
Combined	52,000	4.8	6.5	18.3

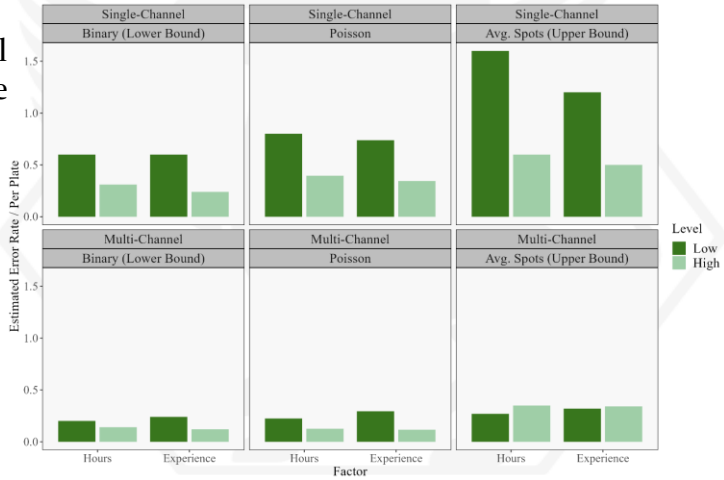
Effect of Experience on Error Rate

- Meta-data collected on each subject included:
  - How many years they have worked in a laboratory
  - How many hours a week they work in a laboratory
- Those who work at least a full day a week in a laboratory have a lower error rate than those who work less
- Those with more than 4 years of experience have a lower error rate than those with less



Effect of a Multi-Channel Pipette on Error Rate

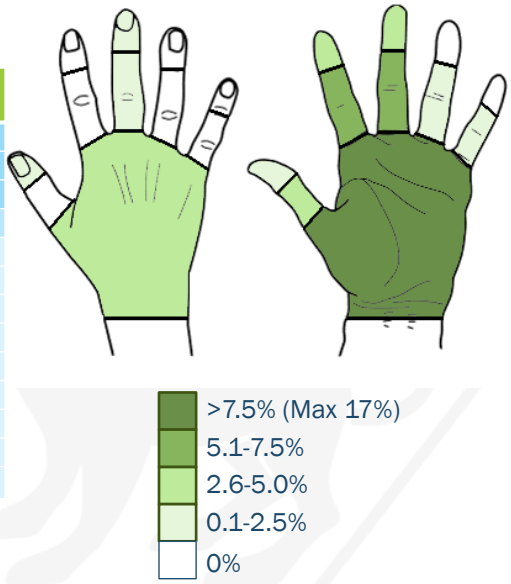
- Even inexperienced users make fewer errors when filling a 96-well plate with a multi-channel pipette than with a single-channel



Location of Contamination

- We also tracked where contamination landed
- Most frequently found on the plate and around it
- Almost all contamination was found near the pipetting error
- Some contamination was likely due to transfer from contaminated gloves

Location	% of Plates w/ Contam	% of total Contam Spots
96 well plate	67	27
Bench pad	65	39
Waste container	35	16
Gloves	22	6
Micropipette	20	3
Well Plate Lid	13	2
Tip box	9	4
Interior BSC surface	6	1
Exterior BSC surface	2	0
Goggles	2	0
Lab coat	2	1
Reagent reservoir	2	0
Volunteer Hands	2	0



Ability to Recognize Mistakes

- Volunteers were asked to "call out" when they thought they made a mistake
  - N=540 plates
  - There was poor correlation between errors that were called out by volunteers and location of contamination
    - Just under half of contamination events were not noticed associated with a declared error
- Preliminary conclusions:
  - Decontamination should occur regularly and in the general area worked, and not just where a mistake was thought to be

True Positive 27%	False Positive 15%
False Negative 20%	True Negative 38%

Location Agnostic

True Positive 1.4%	False Positive 2.4%
False Negative 1.9%	True Negative 94.5%

Location Specific

GRYPHON SCIENTIFIC

25

Conduct Human Reliability Research

- Working with CSU we also collected observational data
- TAs and instructors in the intro microbiology lab course were given a data collection instrument to guide them on which incidents we wanted recorded
- A total of 4,500 student-lab-hours were observed
- The following incidents RATES were observed
  - 17 incidents of microbial stain on the body per 1,000 lab hours
  - 3.5 major motor mistakes per 1,000 lab hours (half of which required a spill response)
  - 39 incidents of gloves thrown in regular waste per 1,000 lab hours

GRYPHON SCIENTIFIC

26

EXPLOITING EXISTING DATA:

Much of the data in this section are from the U of Chicago:
Sa-Lin Bernstein
Jay Schroeder
Joe Kanabrocki

GRYPHON SCIENTIFIC

Rate of Needlesticks

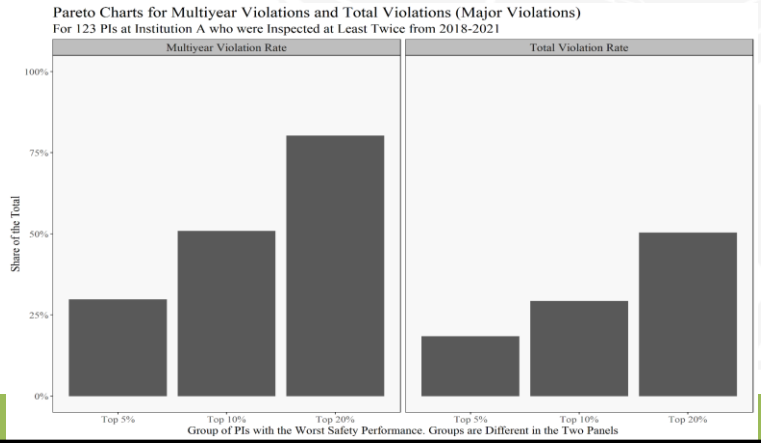
- Calculated a rate of needlestick in labs by comparing reported needlesticks to needles purchased
- Rate of reported needlesticks is 1.4 per 10,000 needles purchased

GRYPHON SCIENTIFIC

28

Variance in Safety Violations

- To explore how the lab leadership influences the culture of biosafety, we investigated the distribution of biosafety violations and repeat violation by PI
- Repeated violations were highly unequally distributed—the top 20% of PIs were responsible for about 80% of repeat major violations
- The top 13% of labs were responsible for 80% of failures to recertify a BSC before the deadline



GRYPHON SCIENTIFIC

29

Best Practices and Innovations in Biosafety

- To identify potential innovations and best practices we held discussions with more than 100 thought leaders in biorisk mgt
  - In the past, we've found that those with new practices didn't know they were being innovative
    - Many folks we contacted tried to suggest they didn't have anything worth talking about
  - To draw out innovations, we focused on the process of biorisk management
    - How did they manage risks that they encountered while assessing risks
- More than 500 practices were identified
- We discussed these practices at two workshops
  - One in Washington DC and one in Malta (focused on low-resource settings)
- We are in the process of writing up our findings in a free publication

GRYPHON SCIENTIFIC

Gryphon Scientific's Innovations and Best Practices in Biosafety Workshop Interviewees

GRYPH SCIENTI

31

Developing an International Biorisk Research Agenda

- Gryphon received a grant from the US Dept of State to help develop an international biorisk research agenda over the next three years
  - Soliciting input in discussions and workshops from researchers and biosafety professionals in countries of all resource levels
  - Seeking to characterize key knowledge gaps in biorisk management and identify research needs
- The final year will be dedicated to review by researchers, policy makers and regulators to guide the implementation of new research projects to:
  - Inform biorisk management
  - Strengthen international networks
  - Foster sustainable global expertise
- We will work closely with the RAV3N network as they pursue a common goal with funding from the USDA

U.S. DEPARTMENT *of* STATE

32

Thank you

- Thanks again to Open Philanthropy for funding all of the work described in this talk
- We hope the government will begin to fund follow on research
- Please reach out to me if you are interested in participating in the development of the biosafety research agenda
- Contact me at Rocco@gryphonscientific.com