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TODAY'S TOPIC:

Overview of Research for Neutralization and Attenuation of Ricin Toxin

November 13, 2019

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Overview of Research for Neutralization and Attenuation of Ricin Toxin



Homeland Security Research Program

Webinar Series

Joseph Wood

November 13, 2019



Presentation Overview

- Background, toxicity for ricin
- Incidents of ricin contamination across the country
- EPA's response role
- Why we're doing this research
- EPA research for ricin
 - Sampling and analysis for ricin
 - Decontamination options for ricin
 - Details of ricin attenuation study
- Next steps for ricin decontamination research

- Ricin toxin is a highly toxic protein produced within the beans of the *Ricinus communis* (castor bean) plant.
- One of the most lethal natural poisons
- Ricin may be disseminated as an aerosol or through addition to food and water
- Powders sent through US mail



Ricinus communis



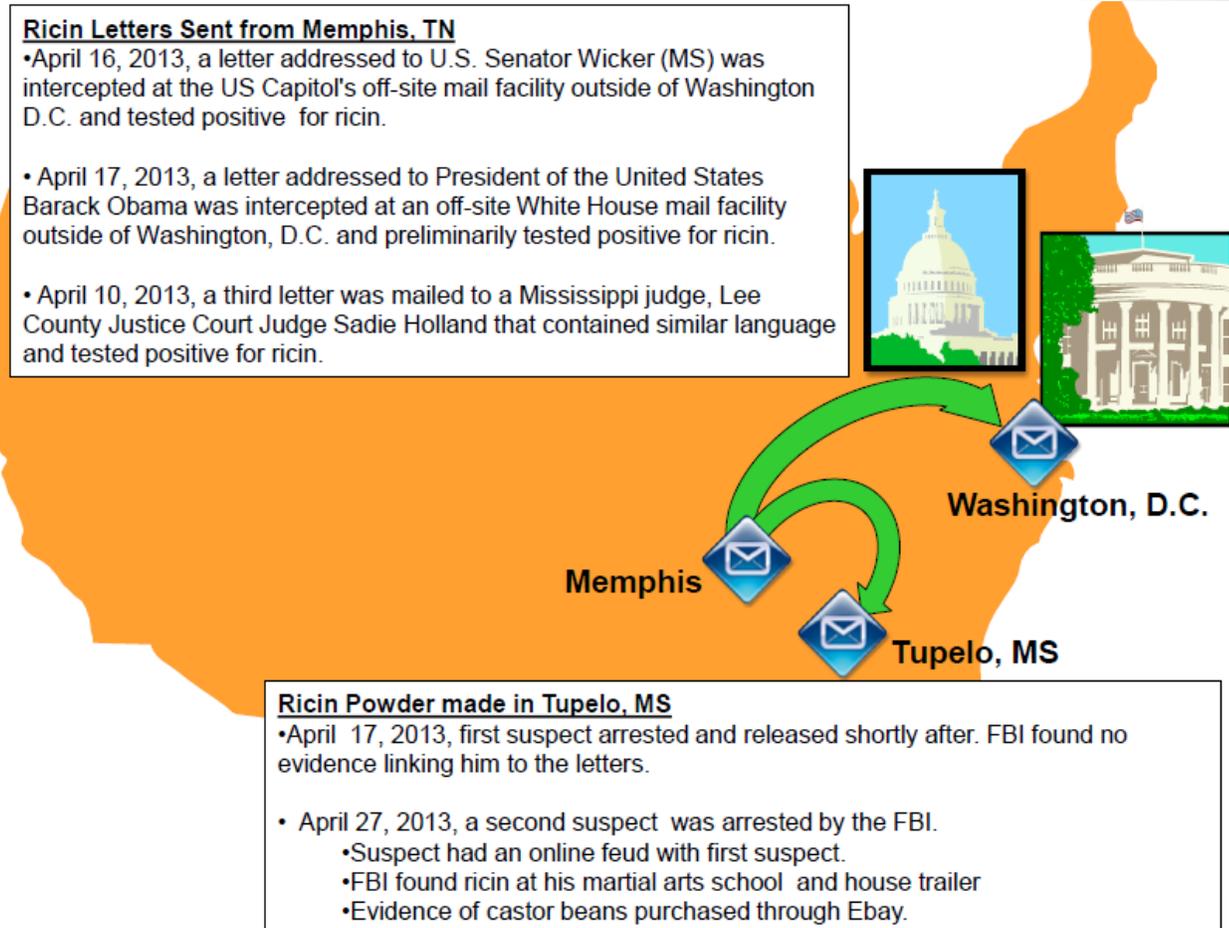
Background, Toxicity

- The median lethal dose (LD₅₀) in mice is 5 micrograms per kilogram (μg/kg) body weight via intravenous (IV) injection.
- Ricin aerosol LD₅₀ for nonhuman primates is estimated to be 10-15 μg/kg body weight.
- Human LD₅₀ is estimated to be 1-5 milligrams per kg IV.
- Contamination can occur from ricin production and/or dissemination



Notable Ricin Incidents

- Capitol Hill, 2004
 - Ricin found in mail room attached to a Senator's office
- Tupelo MS; Memphis TN; National Capital Region; 2013
 - Several letters that contained ricin toxin were sent from Memphis to various locations, including the White House and the office of the NYC mayor
 - Ricin powder made in Tupelo





Other Ricin Incidents with EPA Involvement

- Everett, Washington 2009
- Seattle, Washington 2005
- Kirkland, Washington 2004

Most sites, if found positive for ricin and decontaminated, were done so with diluted bleach or pH adjusted bleach



Tupelo decon
lay down table



More Recent Ricin Incidents with EPA Involvement

- Pelican Bay, CA, 2019
Advisory role
- Boulder, CO Apartment Nov 2017
- Shelburne VT Nov 2017
- Oklahoma City, OK 2015
- Oshkosh, WI 2014



Sample collection Oshkosh, WI
Photos from OSC webpage

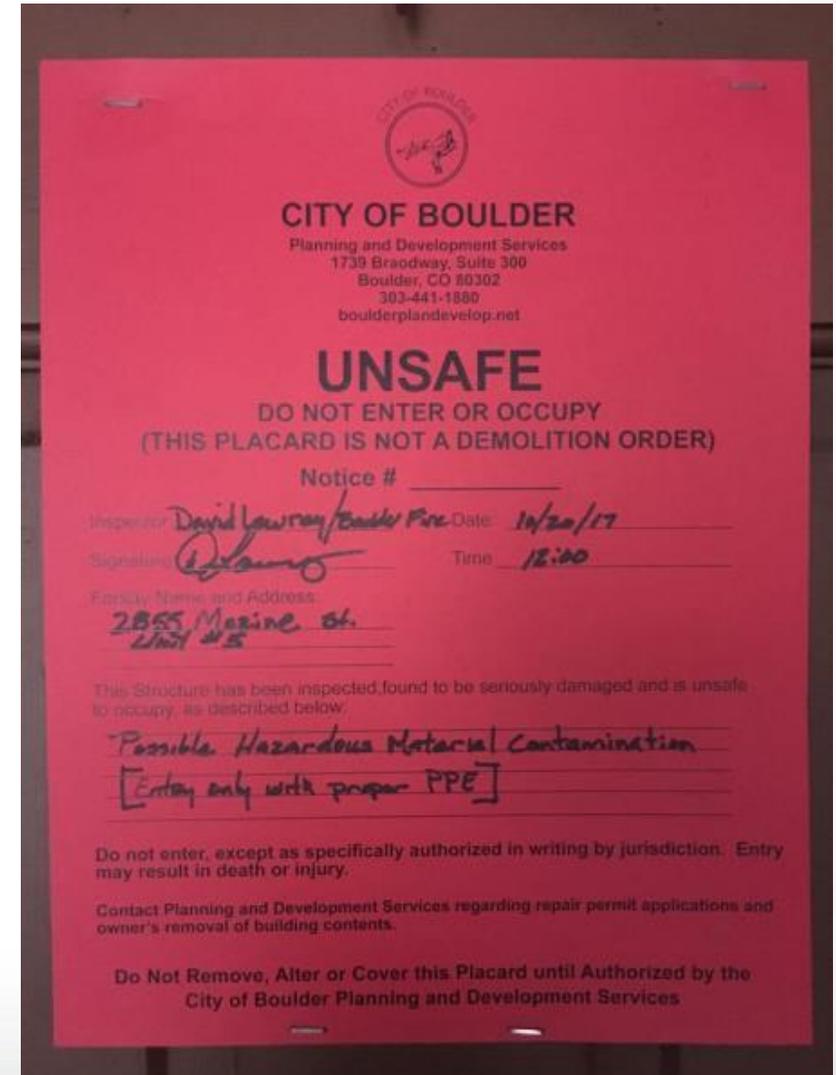


Image from EPA OSC Boulder ricin response site



EPA's Response Role



Assist water utilities in protecting their systems and responding to contamination incidents

Respond to environmental contamination incidents

Regulate pollutant discharge into waters

Register antimicrobials

Control hazardous wastes from cradle to grave



Lead the protection of water infrastructure

Conduct biological and chemical agent cleanup

Develop capable laboratory network and surveillance/monitoring systems



Why are we doing this research?

- Based on some of the ricin incidents discussed, several gaps were encountered, related to challenges with sampling/analysis and decontamination
- In working with our partners at the state and local level, as well as with EPA On-Scene Coordinator and EPA's CBRN Consequence Management Advisory Div (CMAD), issues were identified and solutions developed, including formulating research plans





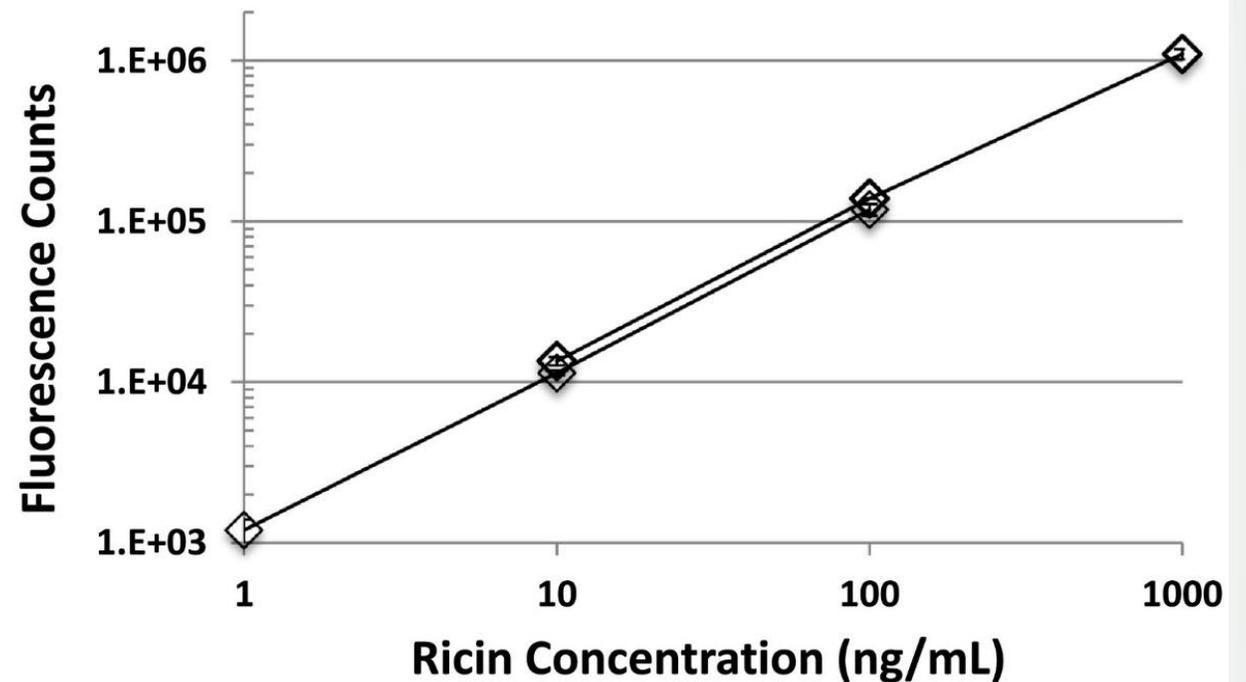
Impact from Ricin Studies

- US EPA research conducted and presented here on ricin will assist responders in their decision making for the following:
 - Whether attenuation of ricin, with or without additional heating, may be an option for ricin neutralization for your case
 - What other decontamination options are available
 - What analytical methods are available to quantify ricin in environmental samples



Sampling and Analysis for Ricin

- The time-resolved fluorescence (TRF) immunoassay is one of the primary screening methods for ricin in environmental samples.
- It is also a method used by the Laboratory Response Network of the U.S. Centers for Disease Control and Prevention (CDC).
- **US EPA's Homeland Security Research Program with Lawrence Livermore National Lab developed a sample processing method to enhance performance and sensitivity of the TRF assay**



From: Kane et al. Sample Processing Approach for Detection of Ricin in Surface Samples. J. Immunol. Methods 451:54-60



Sampling and Analysis for Ricin

- Utilizes prefilters and centrifugal ultrafiltration devices to clean up and concentrate the ricin solution extracted from samples/materials to minimize false positives and negatives.
 - Could be used during sample analysis to remove many aqueous phase soluble assay interferences.
- From the tests, TRF had no issues with interferences from chlorine bleach, which may be used to decontaminate areas suspected of ricin.
- A technical brief has been developed for this sample processing method for ricin analysis



Decontamination Options for Ricin

- Diluted bleach or pH-adjusted bleach has been commonly used in ricin remediation efforts, but few lab data available to confirm efficacy on surfaces
- No reason to think bleach would not be efficacious, although we know it does lose efficacy (against microorganisms) in presence of organic matter/materials
- Removal of visible, gross contamination and cleaning of surfaces recommended prior to decon



Titration for bleach concentration analysis



Decontamination Options for Ricin

- HSRP studies have also shown that chlorine dioxide gas and hydrogen peroxide vapor effective volumetric decontaminants
- 200 parts per million (ppm) chlorine dioxide for 30 minutes achieved 93 to $\geq 99\%$ reduction of pure ricin, depending on material (seven materials tested).
- 240 – 400 ppm for 8 to 16 hours hydrogen peroxide vapor were required to achieved $> 99\%$ reduction on all materials tested
 - Investigated different generation techniques
 - Crude and pure ricin, different levels



Hydrogen peroxide vapor system



Initial HSRP Ricin Attenuation Study

- Initial study conducted to determine if attenuation would be a viable option for decontamination
 - A screening study conducted in 2006 using only a few materials and test conditions
- Test conducted using galvanized metal and painted concrete
- Assessed effect of temperature and humidity on bioactivity





Previous NHSRC Ricin Attenuation Study

- Results: Ricin was still recovered and detected after 14 days at 30 degrees C, low relative humidity (RH; < 40%) on both materials
- >99% reduction on galvanized metal at 14 days at ambient or elevated RH
- Ricin more persistent on painted concrete, with only 72% reduction at 14 d at 30 °C and > 70% RH
- Results indicate that elevated RH may be useful for neutralizing the ricin



Rationale for More Recent Attenuation Study

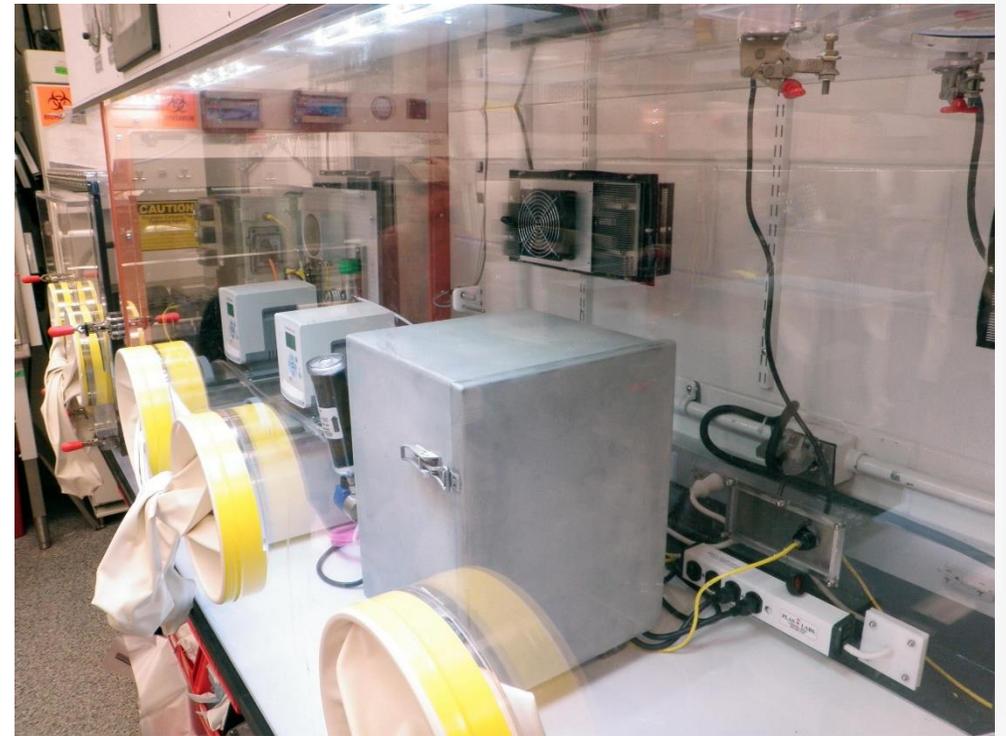
- Minimal data available for attenuation of ricin on solid surfaces at air temperatures that could be used in a building
- US Army reports ricin stable for one hr at pH 7.8, 50 °C.
 - No data provided; pH indicates test in liquid
- Other research (from Food and Drug Admin) on ricin stability in food or drinks (baby formula, juice), based on boiling or autoclaving





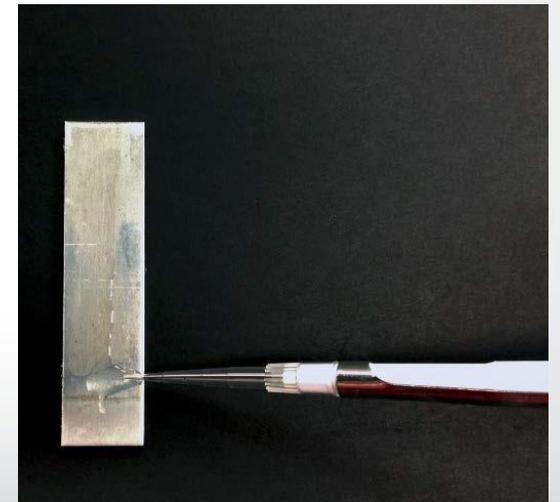
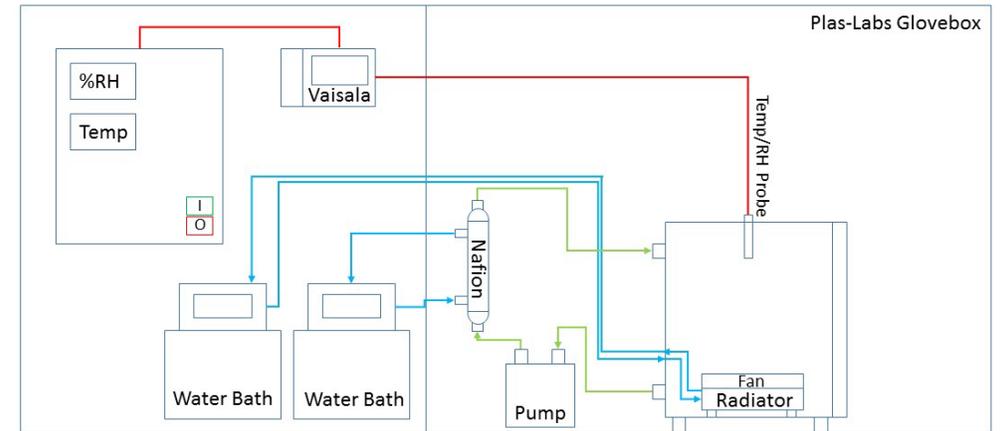
Rationale for Recent Attenuation Study

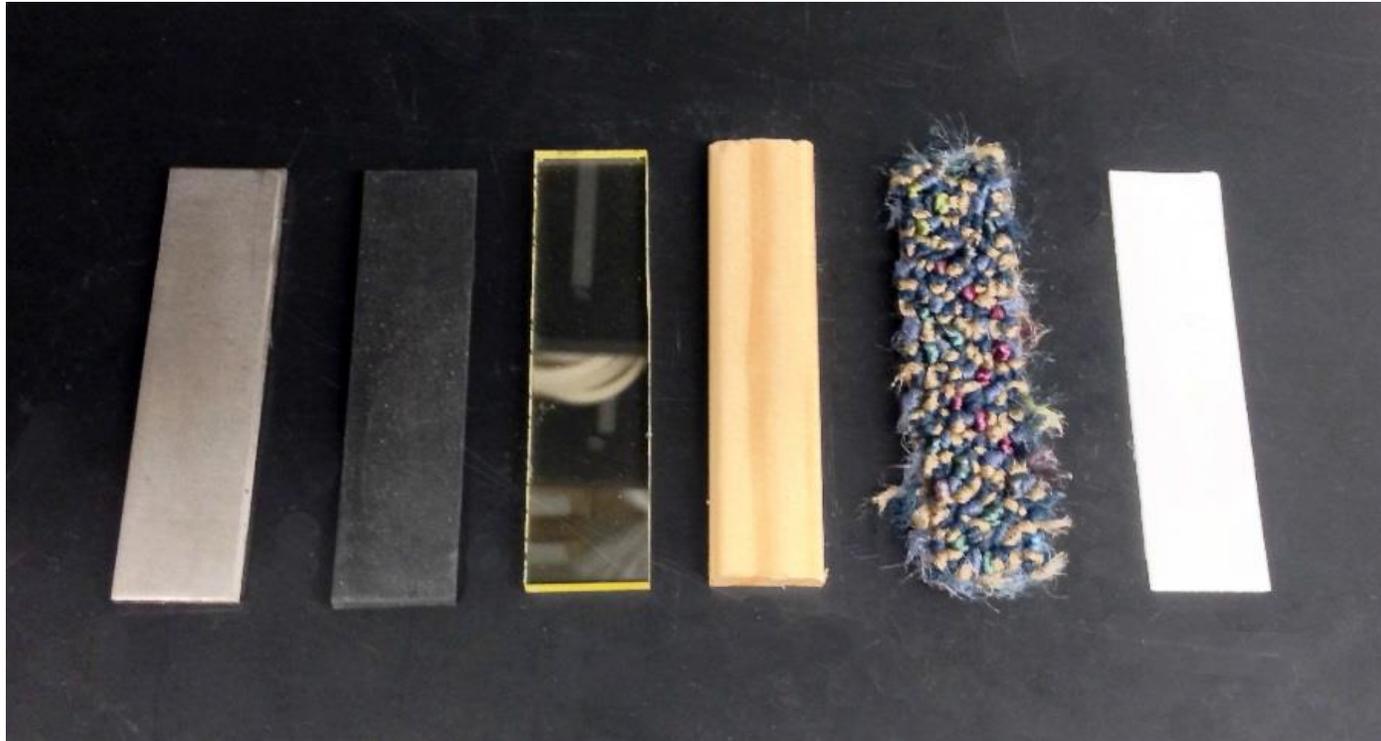
- This study builds on the previous HSRP work, but filling in the gaps with using more materials, temps, RH levels, etc.
- Higher temperatures selected for testing based on what is used for bed bug thermal treatments



Test chamber

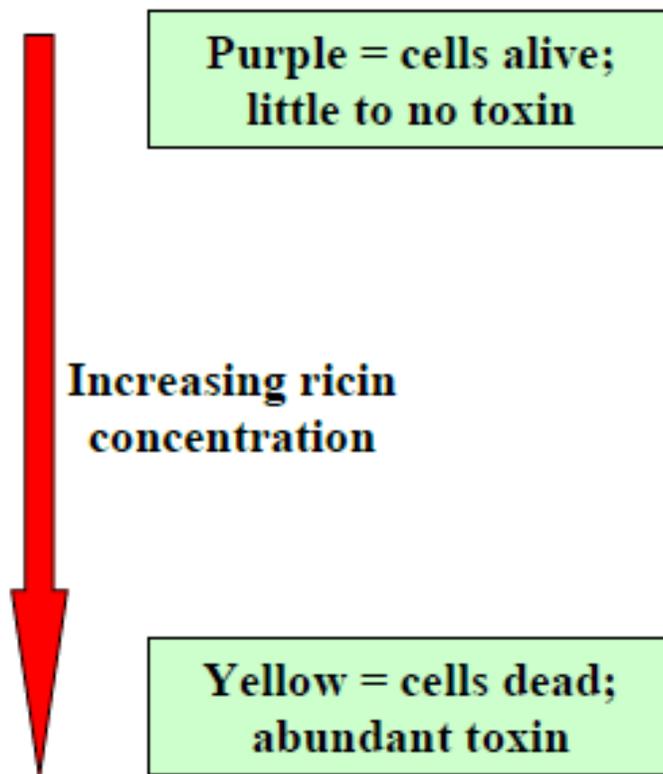
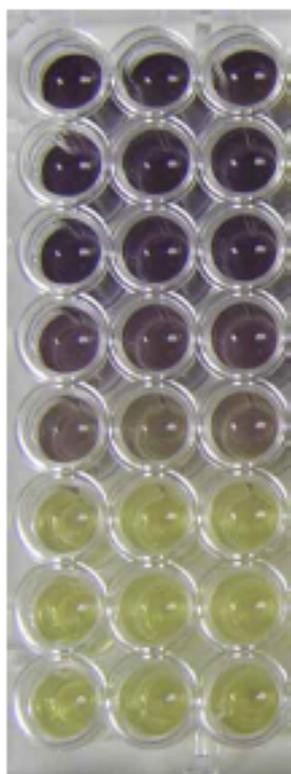
- Test variables/matrix included the following:
 - Prep of ricin: crude and pure
 - Ambient temperatures: 20, 25 and 30 °C
 - Heated temperatures: 40 and 50 °C
 - Relative humidity 40 or 75%
 - Contact time: < 1 day to weeks
- **Crude ricin more realistic of what would be found in a contamination incident**





Coupon Materials from Left to Right: Mild Steel, Neoprene Rubber, Optical Plastic, Pine Wood, Industrial Carpet, Paper

- 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay used to measure toxicity against Vero cells





Methods – Attenuation Calculation

Attenuation presented in terms of percent reduction

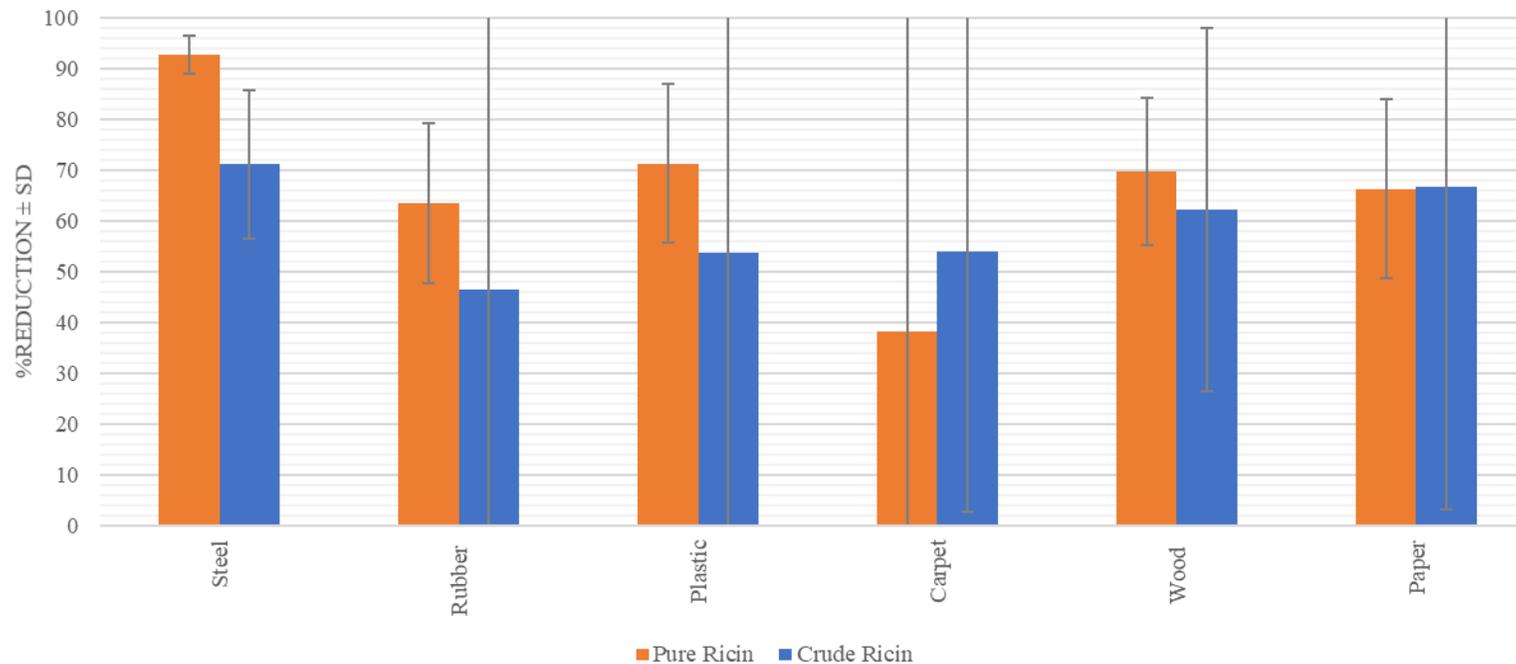
$$\frac{\text{Avg. mass ricin recovered from positive controls} - \text{Avg. mass ricin from test coupons}}{\text{Avg. mass ricin recovered from positive controls}} \times 100$$



Study Test Matrix Overview

Target Temperatures °C	Test Materials	Target %RH	Contact Times (Days)
20, 25, 30	Mild Steel, Neoprene Rubber, Optical Plastic, Pine Wood, Industrial Carpet, Paper	45, 75	7, 14, 21, 28
40, 50	Mild Steel	uncontrolled	0.25, 1, 1.25, 2, 3, 4, 5, 6, 7

Pure vs. Crude Ricin Attenuation by Material



Overall averages for 20-30 °C tests, Tests 1-14.

Crude ricin results more variable and less attenuated most likely due to extraneous material from castor beans, not found in lab grade pure form



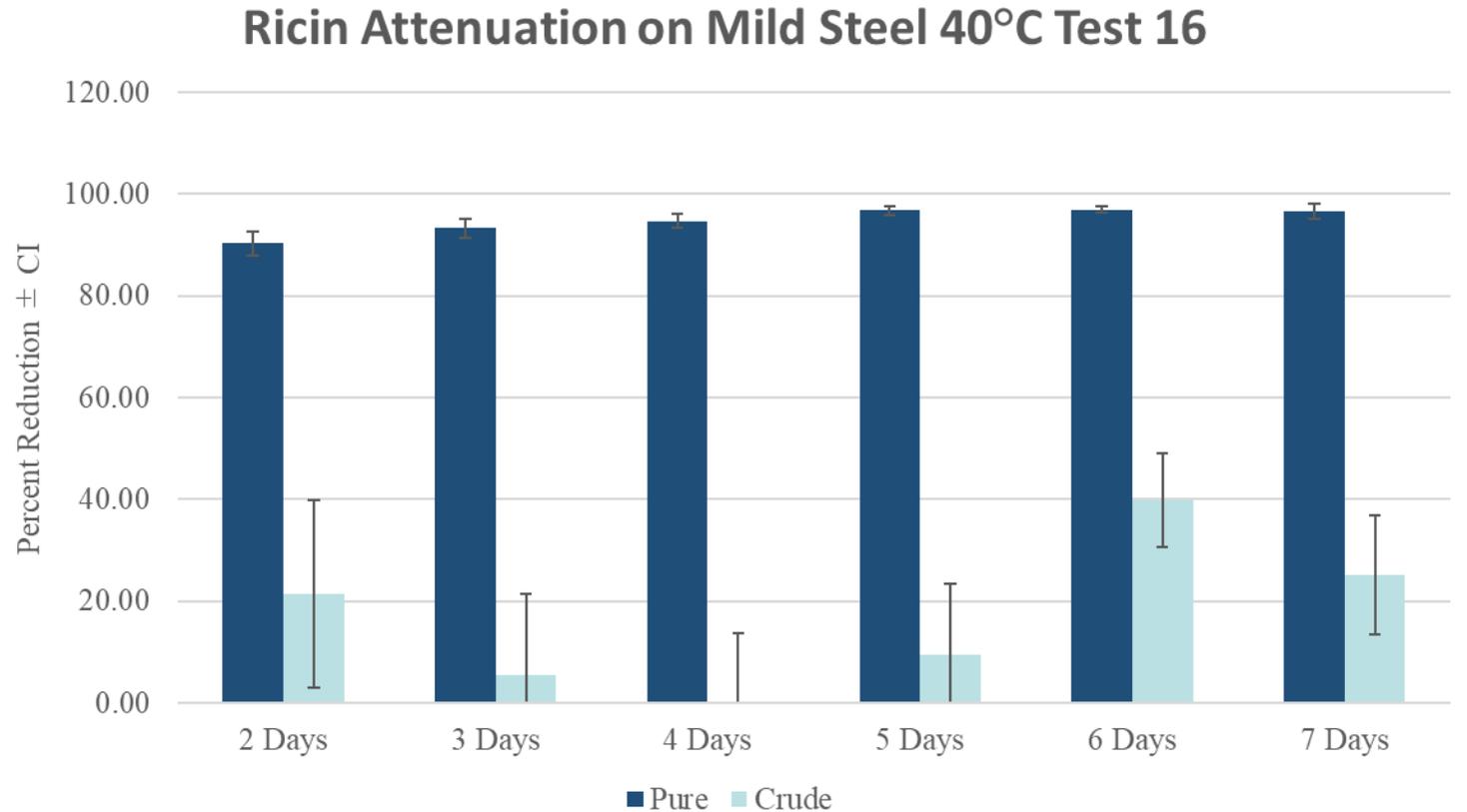
Results – Average Percent Attenuation for Each Environmental Condition Tested

Temp °C/%RH	Test duration (days)	Average % Attenuation for Pure Ricin*	Average % Attenuation for Crude Ricin
20/45	14	63%	7%
20/75	14	58%	56%
25/45	14	88%	73%
25/75	14	88%	51%
30/45	14	75%	81%
30/75	14	63%	39%
20/45	28	80%	77%



Results – 40 degrees °C Heat Treatment

This chart describes treatment tests done only on mild steel to allow multiple time points

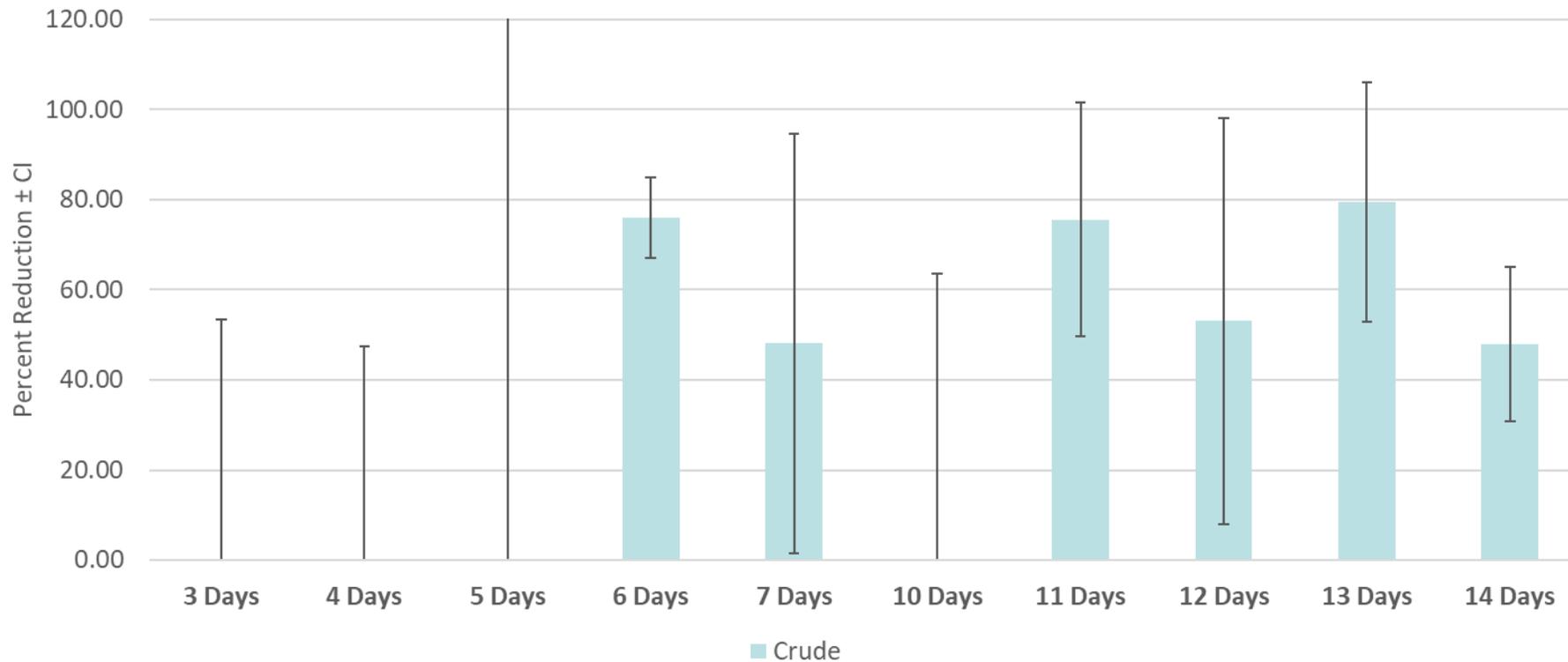


At 40 ° C, minimal attenuation of crude ricin at 7 days



Results – 40 ° C Heat Treatment

Ricin Attenuation on Mild Steel 40°C Test 18

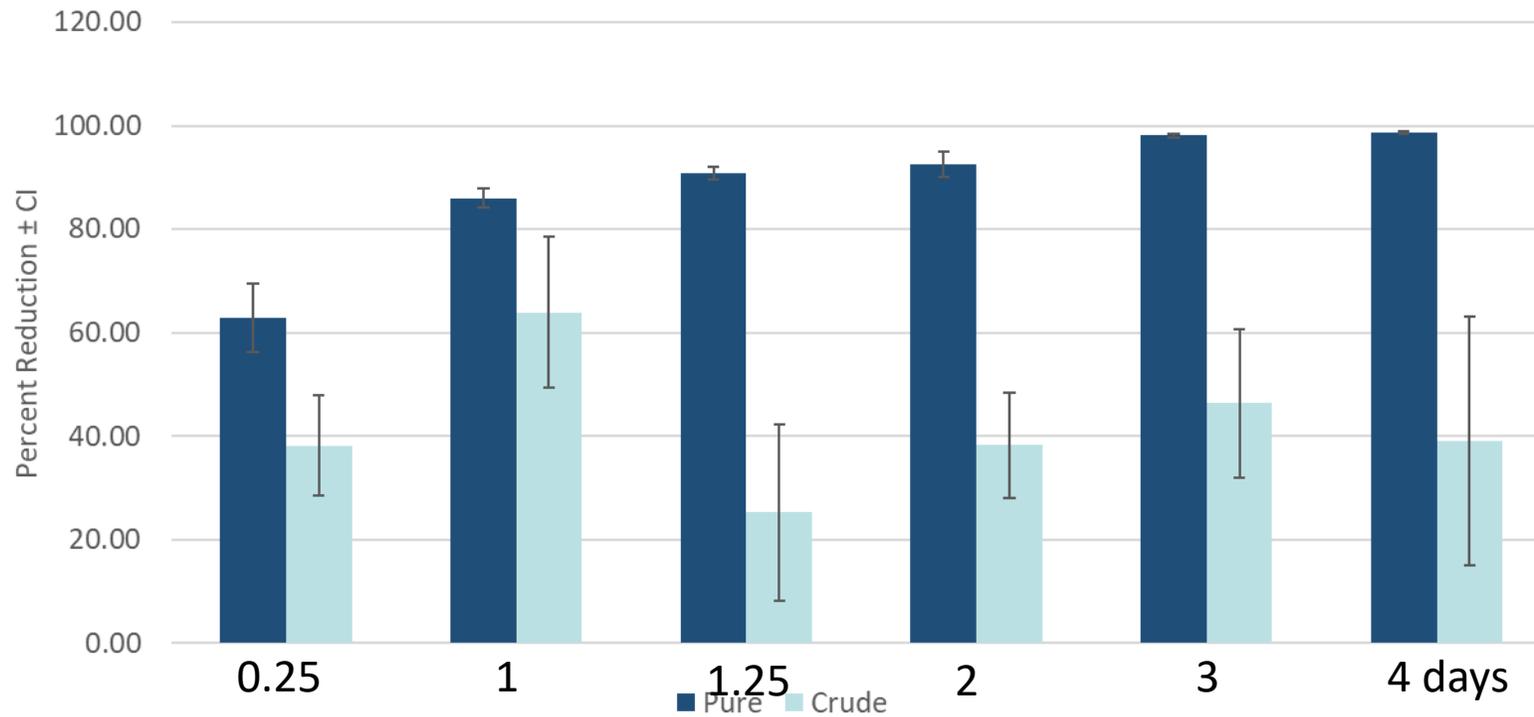


Even out to 14 days, still inadequate attenuation of crude ricin at 40 ° C



Results – 50 ° C Heat Treatment

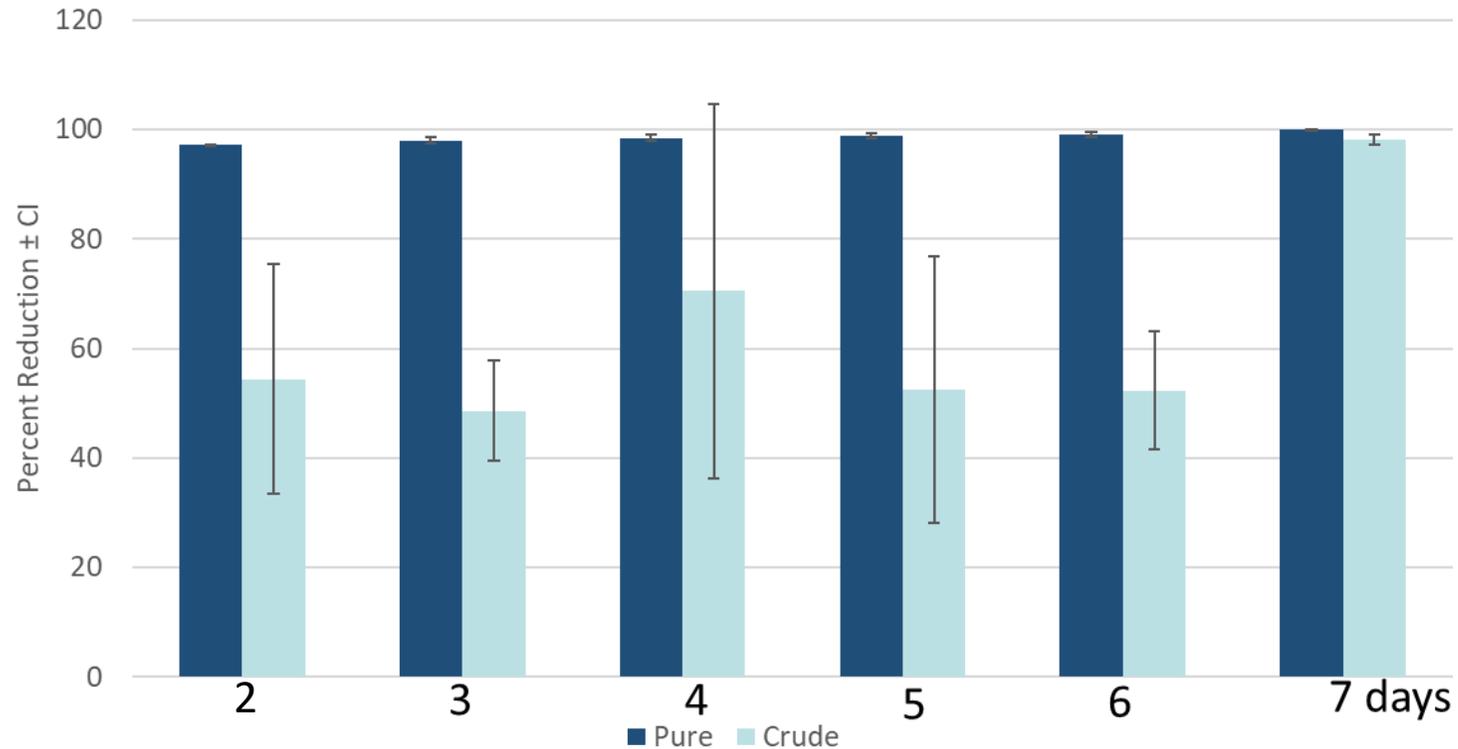
Ricin Attenuation on Mild Steel 50°C Test 15





Results – 50 ° C Heat Treatment

Ricin Attenuation on Mild Steel 50°C Test 17



98% attenuation of crude ricin at 7 days



Results

Test parameter combinations demonstrating over 99% attenuation*

Ricin Form	Temperature °C	RH %	Contact time (d)	Material
Pure	25	75	7	Mild steel
Crude	25	75	7	Paper
Pure	25	75	14	Mild steel
Pure	30	45	7	Wood, paper
Pure	50	20	6	Mild steel

***out of over 200 test parameter combinations**



Summary/Highlights of Attenuation Study

- Crude ricin was less attenuated, and had more variable results, than pure form
- Essentially zero attenuation of crude ricin at 14 days at room ambient conditions, except on mild steel
- RH did not affect attenuation



Image from EPA OSC Boulder ricin response site



Summary/Highlights of Attenuation Study (Continued)

- Increasing temperature generally increased attenuation, but not always
- Greatest attenuation occurred on mild steel, but in general material effects were minor
- 7 day heat treatment at 50 °C required to achieve 98% attenuation of crude



Publications from Ricin Studies

• Reports

- EPA/600/R-16/061, 2016
- EPA/600/R-15/131, 2015
- EPA/600/R-11/076, 2011
- EPA/600/R-10/097, 2010
- EPA/600/R-08/002, 2006
- EPA/600/S-18/160, 2018

• Journal Article in PLOS One

- Wood JP, Richter W, Smiley MA, Rogers JV. Influence of environmental conditions on the attenuation of ricin toxin on surfaces. PloS one. 2018 Aug 8;13(8):e0201857.





Next Steps for Ricin Decontamination Research

- Confirm efficacy of liquid decontaminants
 - Diluted bleach, pH adjusted bleach, peracetic acid et al.
 - Continue to evaluate effect of materials
- Evaluate use of low concentration hydrogen peroxide vapor



Acknowledgements and Disclaimer

- Attenuation study US EPA project team members:
 - Larry Kaelin, Consequence Management Advisory Division
 - Rich Rupert On-Scene Coordinator Region 3
- Other EPA researchers, CMAD, and support contractors having conducted ricin research:
 - Shawn Ryan, Shannon Serre, Sanjiv Shah, Lawrence Livermore Natl Lab, Battelle Memorial Institute



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