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Attenuation of Ricin Toxin under Ambient Conditions and Elevated Temperature and Humidity

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REPORT

Attenuation of Ricin Toxin under Ambient Conditions and Elevated Temperature and Humidity

U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

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Executive Summary

The U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), Homeland Security Research Program (HSRP) is striving to protect human health and the environment from adverse impacts resulting from acts of terror by investigating the effectiveness and applicability of technologies for homeland security-related applications. This report presents the results of an investigation to evaluate the attenuation of ricin toxicity on indoor materials as a function of environmental conditions.

This study focused on the attenuation of ricin toxin on six types of materials representative of a mail sorting facility and/or indoor building materials. Attenuation tests were conducted under various combinations of temperature, relative humidity (RH), and contact time, using two forms of ricin toxin: a commercially-available "pure" preparation and a "crude" form of the toxic material prepared in the laboratory from castor beans.

Summary of Major Findings

Overall, the crude ricin was more persistent than the pure form of the toxin. Average two-week attenuation of the crude ricin at temperatures between 20–30 °C (temperatures expected to be achievable with the HVAC system of the building) ranged from 7% (at 20 °C/45% RH) to 81% (at 30 °C/45% RH). After 28 days at 20 °C/45% RH, the crude ricin averaged 77% attenuation across the six materials tested.

For the pure ricin, heat treatments at the elevated temperatures of 40 °C for 5 days and 50 °C for 2-3 days achieved greater than 96% attenuation on mild steel. For the crude ricin preparation, appreciable recovery of the ricin still occurred at 40 °C after two weeks. A seven-day heat treatment at 50 °C was required to achieve greater than 98% attenuation of the crude ricin on mild steel.

From the statistical analyses, increasing temperature either had no significant effect on attenuation, or there was an increase in attenuation associated with increasing temperature. The effect of increasing temperature on attenuation was more pronounced when comparing results with a 10 °C temperature difference. Under the environmental condition most resembling the indoor environment (20 °C, 45% RH), there was no significant attenuation of the crude ricin at 14 days on any of the materials except mild steel. At 30 °C, the average ricin attenuation across all materials at 14 days ranged from 39-81%.

Table ES-1 provides another perspective of the study results in terms of highlighting the fact that there were only seven cases (out of over 200 test combinations of ricin type, temperature, RH, material, and contact time) in which we observed greater than 99% attenuation of ricin. As seen in this table, in general, elevated temperature or RH was required to achieve greater than 99% reduction of pure or crude ricin toxin. More specifically, there were no cases in which any form of ricin was attenuated more than 99% at 20 °C or at 25 °C/45% RH. There was only one case where the crude ricin preparation was attenuated more than 99%. Attenuation of more than 99% occurred most often on the mild steel and paper materials.

While in many of the tests there was no significant effect of material on the attenuation of ricin, in the cases where there was a significant effect, ricin was generally attenuated most on mild steel and the least on wood.

In general, increasing the RH level from 45 to 75% did not significantly affect attenuation of ricin. The effect of RH (or lack thereof) was similar for both ricin types, while the effect of RH appeared to be somewhat dependent on the material. For example, for both the mild steel and wood materials, there was either no effect of RH, or an increase in RH significantly increased the ricin attenuation in nearly all of the comparisons. The opposite effect of RH occurred with the plastic and rubber materials.

The results showed that the crude ricin was more stable, i.e., more difficult to attenuate, than the pure ricin in most of the tests conducted in this study. The attenuation results for the crude ricin also exhibited more variability than the attenuation results for the pure ricin. The higher variability of results and stability of the crude ricin may be due to the presence of additional proteins and other organic materials in the crude suspension. These potentially extraneous proteins and other materials (e.g., carbohydrates, fatty acids, ash) could have mitigated the effects of the environmental conditions, as well as interfered in the quantitation assay, and would be absent in the commercially available pure material. The use of a biological system (a cell-based assay) to quantitate ricin toxicity, regardless of ricin type, may also have contributed to variability in results.

Table ES-1. Test Parameter Combinations Demonstrating Over 99% Attenuation of Ricin

Test	Ricin Form/Target Mass	Temp °C %RH	Contact Time (Days)	Material	% Reduction ± 95% Confidence Interval
4	Pure/250 µg	25 °C 75%	7	Mild Steel	99.87 ± 0.11
	Crude/320 µg		7	Paper	99.54 ± 0.12
5	Pure/250 µg	25 °C 75%	14	Mild Steel	99.95 ± 0.03
6	Pure/250 µg	30 °C 45%	7	Pine Wood	99.38 ± 0.47
			7	Paper	99.83 ± 0.24
17[a]	Pure/250 µg	50 °C 20%	6	Mild Steel	99.05 ± 0.48
			7	Mild Steel	99.92 ± 0.02

[a] Detailed data from each test number can be referenced in Appendix A.

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Abbreviations/Acronyms

4-PL	four-parameter logistic
ANOVA	Analysis of variance
ASTM	American Society of Testing and Materials
BSC	biological safety cabinet
CI	confidence interval
cm	centimeter(s)
°C	degree(s) Celsius
E-beam	electron beam
EPA	U.S. Environmental Protection Agency
h	hour
HSRP	Homeland Security Research Program
HVAC	Heating, ventilation, and air conditioning
IV	intravenous
kg	kilogram(s)
kGy	kilogray(s)
L	liter(s)
LD_{50}	median lethal dose; individual dose required to kill 50 percent of a population of test animals
LOD	limit of detection
µg	microgram(s)
µL	microliter(s)
mg	milligram(s)
mL	milliliter(s)
mil	thousandth of an inch
min	minute(s)
MTT	3-(4,5-dimethlythiazol-2-yl)-2,5-diphenyltetrazolium bromide
NA	not applicable
ng	nanogram(s)
nm	nanometer(s)
NHSRC	National Homeland Security Research Center
ORD	Office of Research and Development
PBS	phosphate buffered saline
QA	quality assurance
QAPP	Quality Assurance Project Plan
QC	quality control
QMP	Quality Management Plan
RH	relative humidity
rpm	revolution(s) per minute
SD	standard deviation

SE	standard error
SFW	sterile filtered water (cell-culture grade)
STREAMS	Scientific, Technology, Research, Engineering, and Modeling Support
T0	time zero
TSA	technical systems audit
USAMRIID	United States Army Medical Research Institute of Infectious Diseases

1.0 Introduction

The U.S. Environmental Protection Agency Office of Research and Development's (ORD's) Homeland Security Research Program (HSRP) is helping protect human health and the environment from adverse impacts resulting from the release of chemical, biological, or radiological agents. With an emphasis on decontamination and consequence management, water infrastructure protection, and threat and consequence assessment, the HSRP is working to develop technology and information that will help detect the intentional introduction of chemical or biological contaminants in buildings or water systems; contain these contaminants; decontaminate buildings, water systems, or other infrastructure; and facilitate the disposal of material resulting from restoration activities.

In 2013, several letters that contained ricin toxin were sent to various locations, including the White House and the office of the New York City mayor (according to the U.S. Attorney's Office in a memorandum dated June 28, 2013). These contaminated letters had the potential to contaminate the corresponding mail-sorting facilities and equipment, creating an exposure risk for those working in the area. Ricin toxin is a highly toxic protein produced within the beans of the *Ricinus communis* (castor bean) plant. The median lethal dose (LD_{50}) in mice is 5 micrograms per kilogram (µg/kg) via intravenous (IV) injection.[1] Extrapolations have been made that indicate a human LD_{50} exposure could be ~1 to 5 milligrams per kg (mg/kg) IV. The ricin aerosol LD_{50} for nonhuman primates is estimated to be 10-15 µg/kg.

In this investigation, the attenuation of ricin toxin activity (pure and crude preparations) over time was evaluated under varying environmental conditions to help determine conditions under which further decontamination may be needed. The majority of the tests were conducted with multiple materials under temperature conditions relatively easily obtainable (20-30 degrees Celsius [°C]) with a building's heating, ventilation, and air conditioning (HVAC) system. Relative humidity (RH) levels in these tests were controlled to either 40 or 75%.

In the last four experiments of the study, we assessed the attenuation of ricin at elevated temperatures (with no RH control) where additional heating equipment would probably be needed (40 and 50 °C), but the elevated temperatures would not be expected to be overly detrimental to the interior materials. In these last four tests, we used just one material (mild steel), which allowed us to assess multiple time points in one experiment.

The present study discussed in this report builds on an earlier EPA HSRP study[2] in which the attenuation of pure ricin was assessed for just a few materials and a few environmental conditions. We were unable to identify any other scientific literature describing the attenuation of ricin on material surfaces for the more benign air temperatures we studied. While the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) reports that ricin is stable for only an hour at 50 °C at a pH of 7.8[3], no data or references were provided for this assertion. Also, since pH was reported, it is assumed the USAMRIID information is applicable only to ricin stability in aqueous liquids. Other researchers have similarly reported on the persistence of ricin in foods or liquids at elevated temperature treatments achieved through means such as boiling or autoclaving.[4, 5] Lastly, another source mentioned that pure and crude ricin on materials could be effectively denatured at 82-88 °C, 80-85% RH, for 24 hours.[6] None of the aforementioned research investigated the attenuation of ricin on solid surfaces at air temperatures that could be used in a building.

The results of this investigation attempt to fill some of the data gaps discussed above, by providing stakeholders with high quality, peer-reviewed data on how ricin may attenuate over time as a function of the type of ricin preparation, the material the ricin is associated with, temperature, and RH. The materials used in this study include those associated with mail sorting equipment such as mild steel, neoprene rubber, paper, and optical grade plastic, while bare pine wood and industrial carpet were used to represent building materials.

2.0 Procedures

This section provides an overview of the procedures used for the evaluation of controlled and ambient environmental parameters to naturally attenuate both pure and crude forms of ricin toxin on six different materials. Testing was performed in accordance with the peer-reviewed and EPA-approved *Quality Assurance Project Plan (QAPP) for the Neutralization of Ricin Toxicity Using Elevated Temperature and Humidity*.[7] The QAPP provides additional procedural details that are not included in this report. Procedures were also consistent with a previous study examining the efficacy of hydrogen peroxide vapor to neutralize ricin.[8]

2.1 Test Matrix

The test matrix for the attenuation tests is shown in Table 2-1. Tests 1 -14 used the full set of materials included in this study while Tests 15 through 18 were evaluated utilizing a downselected material (mild steel). We chose to use mild steel in the last four tests since this hard nonporous material exhibited less variability in ricin recoveries among its replicates then the other materials. The use of only one material in these last four trials also allowed us to assess ricin recovery at multiple timepoints, with all test coupons using the same positive controls.

Table 2-1. Attenuation Test Matrix

<table>
<tr><th>Test Number</th><th>Materials</th><th>Target Ricin Mass (µg)</th><th>Target Temp °C</th><th>Target %RH</th><th>Time (Days)</th></tr>
<tr><td>1</td><td rowspan="14">Mild Steel
Rubber
Plastic
Wood
Carpet
Paper</td><td rowspan="16">Pure 250
Crude 250</td><td>30</td><td>75</td><td>7</td></tr>
<tr><td>2</td><td rowspan="2">25</td><td rowspan="2">45</td><td>7</td></tr>
<tr><td>3</td><td>14</td></tr>
<tr><td>4</td><td rowspan="2">25</td><td rowspan="2">75</td><td>7</td></tr>
<tr><td>5</td><td>14</td></tr>
<tr><td>6</td><td rowspan="2">30</td><td rowspan="2">45</td><td>7</td></tr>
<tr><td>7</td><td>14</td></tr>
<tr><td>8</td><td>30</td><td>75</td><td>14</td></tr>
<tr><td>9</td><td rowspan="2">20</td><td rowspan="2">45</td><td>7</td></tr>
<tr><td>10</td><td>14</td></tr>
<tr><td>11</td><td rowspan="2">20</td><td rowspan="2">75</td><td>7</td></tr>
<tr><td>12</td><td>14</td></tr>
<tr><td>13</td><td rowspan="2">20</td><td rowspan="2">45</td><td>21</td></tr>
<tr><td>14</td><td>28</td></tr>
<tr><td>15</td><td rowspan="4">Mild Steel*</td><td>50</td><td rowspan="4">20**</td><td>6,24,30,48,72, and 96 hours (h)</td></tr>
<tr><td>16</td><td>40</td><td>48,72,96,120,144, and 168 h</td></tr>
<tr><td>17</td><td>50</td><td>48,72,96,120,144, and 168 h</td></tr>
<tr><td>18</td><td>Crude 250</td><td>40</td><td>3,4,5,6,7,10,11,12,13,14 days</td></tr>
</table>

*Only one material tested to allow for multiple time points per test.

**RH monitored but not controlled, average value shown.

2.2 Ricin Toxin

Testing was conducted with a commercially-available preparation of pure ricin toxin (Cat. No. L-1090: *Ricin communis* agglutinin II, 5 mg per milliliter [mg/mL] protein concentration, Vector Laboratories, Burlingame, CA), which was stored at 2 to 8 °C and used as received. In addition, a crude preparation of the toxin was extracted from whole castor beans obtained from Vector Laboratories (Vector Laboratories, Inc.). The crude preparation of ricin toxin was performed in the laboratory using methods derived from the scientific literature,[9] and this batch was used throughout the study. Briefly, whole castor beans were de-husked and homogenized into a slurry, precipitated from the solution, dialyzed, and rinsed with sterile phosphate buffered saline (PBS [Cat #D8537 Sigma-Aldrich, St. Louis, MO]). The final crude ricin toxin was prepared in sterile PBS and stored at 2 to 8 °C. While our target ricin titer for the crude preparation was 5 mg/mL, the actual titer averaged 6.4 mg/mL based on post-test statistical analyses.

2.3 Test Materials

The test materials included mild steel, neoprene rubber, optical plastic, pine wood, industrial carpet, and paper. Information on these materials is presented in Table 2-2, and a picture of each is presented in Figure 2-1. Material coupons were cut to uniform length and width (Table 2-2) from larger pieces of stock material. Materials were prepared for testing by either sterilization via electron beam (E-beam) irradiation at ~200 kilograys (kGy; E-beam Services Inc., Lebanon, OH) or autoclaved at 121 °C for 15 minutes (min). E-beam-irradiated material coupons were sealed in 6 mil (0.006 inch) Uline Poly Tubing (Cat. No. S-2940, Uline, Chicago, IL), and autoclaved coupons were sealed in sterilization pouches (Cat. No. 01-812-50, Fisher, Pittsburgh, PA) to preserve sterility until the coupons were ready for use. Sterilization was intended to eliminate contamination by microorganisms that might interfere with the cell-based assay used to assess ricin bioactivity.

Table 2-2. Test Materials

Material	Lot, Batch, ASTM No., or Observation	Manufacturer/ Supplier Name Location	Approximate Coupon Size, Width x Length x Thickness	Material Preparation
Mild (Carbon) Steel	Gauge 12	Adept Products, West Jefferson, OH	1.9 centimeters (cm) x 7.5 cm x 0.2 cm	Autoclave
Neoprene Rubber	Nonmarking Neoprene Rubber Part # 8837K214	McMaster Carr Aurora, OH	1.9 cm x 7.5 cm x 0.3 cm	E-Beam
Optical Grade Plastic	Optically Clear Cast Acrylic Sheet McMaster Item #8560K263	McMaster Carr Aurora, OH	1.9 cm x 7.5 cm x 0.3 cm	E-Beam
Pine Wood	Item #: 3542 Model #: 142 8PINE	Lowes Hilliard, OH	1.9 cm x 7.5 cm x 0.3 cm	E-Beam
Carpet	Shaw Swizzle EcoWorx, Style: 10401 Color: Jacks	Shaw Industries Dalton, GA	1.9 cm x 7.5 cm x 0.7 cm	E-Beam
Paper	Boise Aspen Laser Paper 24 pounds Part #BPL-2411-RC	Office MaxC Hilliard, OH	1.9 cm x 7.5 cm x 0.3 cm	E-Beam

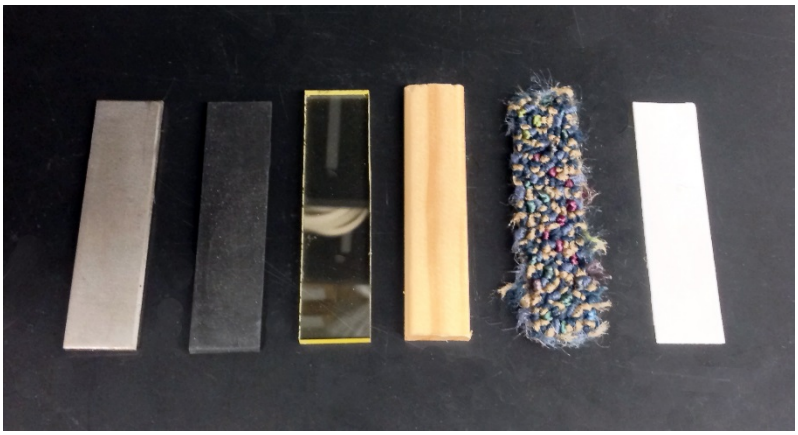

Figure 2-1. Coupon Types from Left to Right: Mild Steel, Neoprene Rubber, Optical Plastic, Pine Wood, Industrial Carpet, Paper

2.4 Inoculation of Coupons

Test and positive control coupons were placed on a flat surface within a Class II biological safety cabinet (BSC) and inoculated individually with a target mass of approximately 250 µg of either the purified or crude ricin toxin. The mass quantity of pure ricin toxin inoculated was 250 µg per material based on certified titer of stock material as received from the vendor. Actual delivered mass of crude ricin toxin per coupon material was determined using a cell-based bioassay (see Section 2.6) and averaged approximately 320 µg per coupon for the study. While this average actual quantity of crude ricin applied to each coupon was greater than our target of 250 µg, ricin attenuation was calculated based on the actual recovery of mass from positive controls from each test. The higher than expected crude ricin inoculum levels may be attributed to the variability of the cell-based assay at the higher dilutions, the variability of ricin content associated with using actual castor beans, and potential bias from additional proteins in the crude suspension.

A 50 microliter (µL) inoculum of either the purified (5 mg/mL) or crude ricin (6.4 mg/mL) toxin stock suspension was dispensed using a micropipette and applied as a single streak across the coupon surface (Figure 2-2). This technique provided decreased drying times and enabled greater distribution of toxin across the coupon surface as compared to a single drop of the suspension. After inoculation, the coupons were transferred to a Class III BSC and left undisturbed to dry for approximately one hour (h) (or until visually dry) under ambient conditions, ~22 °C and 40% RH.

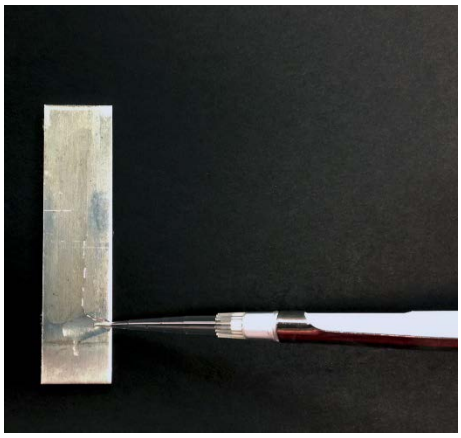

Figure 2-2. Liquid Inoculation of Coupon Using a Micropipette

The number and type of replicate coupons used for each combination of material and environmental condition included were:

- Five test coupons (inoculated with ricin toxin and exposed to experimental temperature/RH for the test duration)
- Five positive controls (inoculated with ricin toxin and extracted after 1 h drying time)
- One laboratory blank (not inoculated and not exposed to experimental temperature/RH)
- One procedural blank (not inoculated and exposed to experimental temperature/RH).

Approximately 1 h post-inoculation (or until materials were visibly dry), coupons intended for attenuation testing (including blanks) were transferred into the test chamber and exposed to the environmental conditions using the environmental test chamber and application conditions specified in Section 2.5. Positive controls were then extracted and analyzed.

2.5 Environmental Test Chamber and Procedures

Figure 2-3 shows a schematic diagram of the bench-scale exposure chamber. Attenuation testing was conducted inside the approximately 38 liter (L) stainless steel chamber. The chamber was insulated to prevent condensation on the inside walls. As a means of secondary containment and considering laboratory personnel safety, this test chamber was housed inside a custom acrylic compact glove box (Plas Labs, Inc., Lansing, MI) that was hard-ducted to the facility exhaust system to maintain negative pressure.

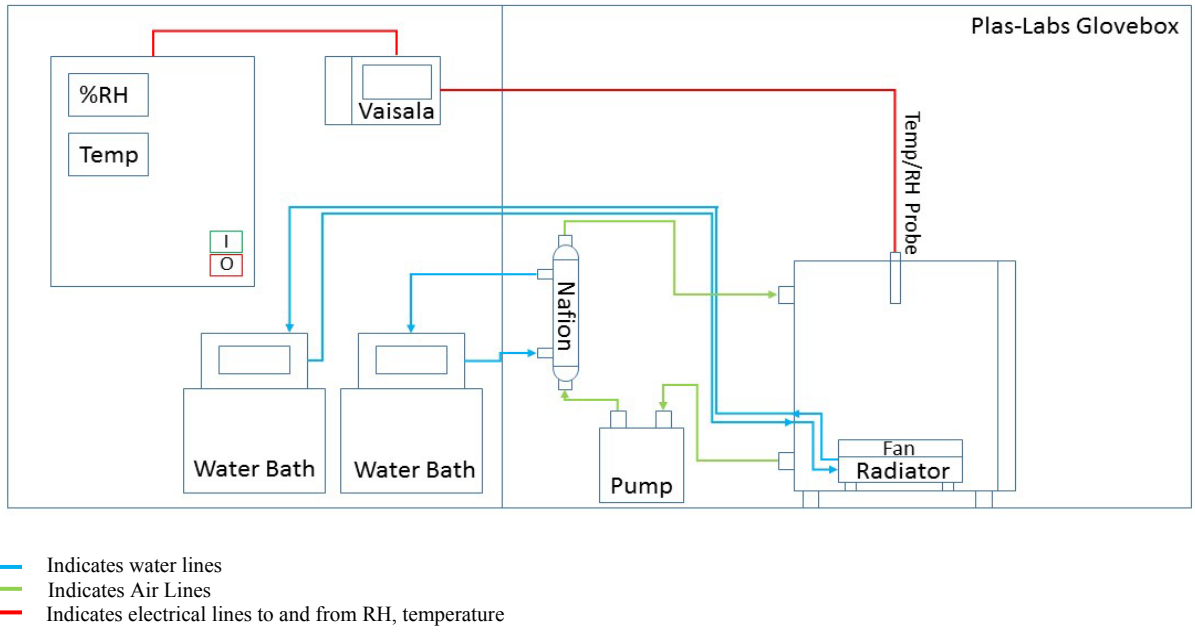

Figure 2-3. Schematic Diagram of Environmental Exposure Chamber

Temperature was controlled using an external water bath connected to a heat exchanger within the test chamber. RH was controlled using an external water bath connected to a Nafion tube pervaporation system (Perma Pure; Lakewood, NJ) and through the use of fixed humidity point salts (Sigma-Aldrich; St. Louis, MO).[10] Temperature and RH in the test chamber were measured using an HMT368 temperature and humidity probe (Vaisala, Inc., Woburn, MA) and controlled with a CNI-822 controller (Omega Engineering, Stamford, CT). Data were recorded every minute during the experimental temperature/RH exposure time using the controller-associated Omega Engineering iLOG software. A typical representative graph of the environmental conditions (Test 10) data collection can be seen in Figure 2-4.

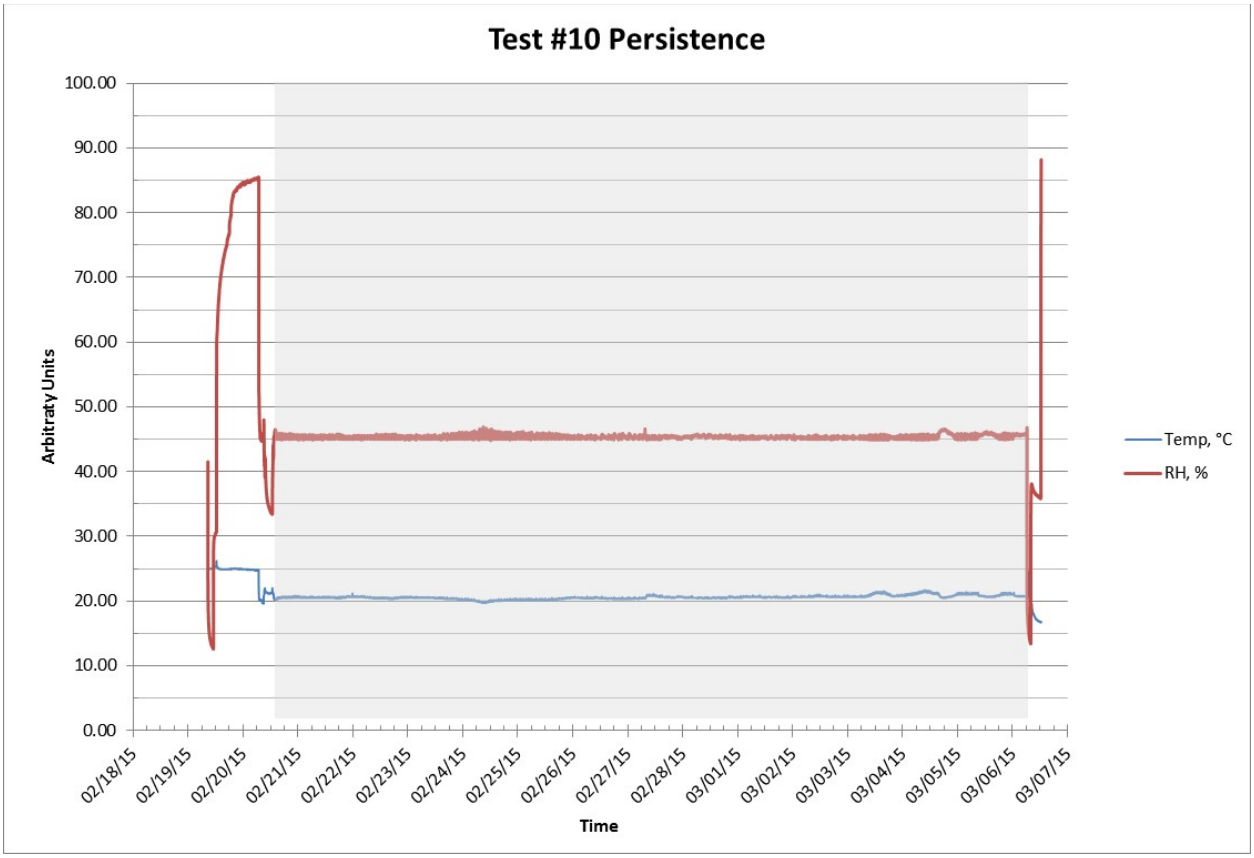

Figure 2-4. Representative Graph of Temperature and RH Stability (Test 10; shaded portion of graph shows time period for test)

2.6 Coupon Extraction and Ricin Toxin Quantification

At time zero (T0) (positive controls and blanks) and each non-zero time point, the coupons and blanks were individually placed in 50 mL conical tubes containing 10 mL of sterile PBS for extraction. The vials were capped, placed on their sides and agitated on an orbital shaker for 15 min at approximately 200 revolutions per minute (rpm) at room temperature. The presence of residual active toxin in the test and control coupon extracts was determined using the bioassay described below.

The mechanism of action by which ricin toxin exerts its toxic effect is through inhibition of protein synthesis within cells. Such inhibition of protein production leads to cell death. Therefore, an *in vitro* cytotoxicity assay was used to evaluate the level of bioactive ricin toxin extracted from both attenuated and positive control material coupons. The bioassay used in this evaluation for determining the cytotoxicity (concentration) of bioactive ricin toxin is based on the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay developed by Mosmann.[11] Cytotoxicity is reported as mass of bioactive toxin as determined using a reference standard prepared from the purified form of ricin toxin.

To conduct this MTT assay, Vero cells (ATCC; Manassas, VA; kidney epithelial cells from the African green monkey) were seeded in wells of a 96-well microplate at a density of approximately 2×10^4 cells/well. Cells were then incubated for approximately 18 to 30 h at 37 ± 2 ºC under 95% air and 5% carbon dioxide and exposed to the various coupon extracts (test, positive controls and blank controls) by adding 100 µL extract or test dilution to each well and performing a series of two-fold dilutions down each plate. Following 48 to 72 h exposure to sample extracts, the cells were incubated in the presence of MTT, where mitochondrial enzymes convert the yellow MTT to a purple formazan salt. The absorbance of this purple reaction product, read at 570 nanometers (nm) using a SPECTRAmax PLUS 384 microplate reader (Molecular Devices, Sunnyvale, CA), is directly proportional to the number of living cells and inversely proportional to the cytotoxic potential of ricin toxin (Figures 2-5 and 2-6). For all dilutions and sample transfers into the individual wells of a 96-well plate (Fisher Scientific; Pittsburgh, PA), a micropipette (Mettler-Toledo Rainin; Oakland, CA) was used with the pipette tip was placed between wells to ensure that cross contamination did not occur.

To determine the concentration of ricin toxin from each test sample, a pure ricin toxin standard (Vector Laboratories, Inc.) was prepared from the commercially-available stock solution and assayed in parallel on each test plate. The pure ricin toxin stock solution was used to prepare a seven point-standard curve of absorbance versus calculated mass of ricin toxin protein. For each standard and test sample, absorbance values of the reference wavelength (630 nm) were subtracted from the absorbance values at 570 nm for each well. For each point used in generating the standard curve, the mean absorbance values (Y-axis) were plotted against the concentration in nanograms (ng)/mL, and a four-parameter logistic (4-PL) curve was generated by the SoftMax Pro Version 4.7 software included in the SPECTRAmax PLUS 384 microplate reader using the equation:

$$Y = \min + \frac{(\max - \min)}{1 + (X / C)^B} \qquad (1)$$

where:

Y = absorbance %;
X = concentration of ricin ng/mL;
max = Y-value of the asymptote at the low values of X % absorbance;
min = Y-value of the asymptote at the high values of X % absorbance;
B = value related to the slope of the curve between the asymptotes;
C = X-value of the midpoint between max and min ng/mL

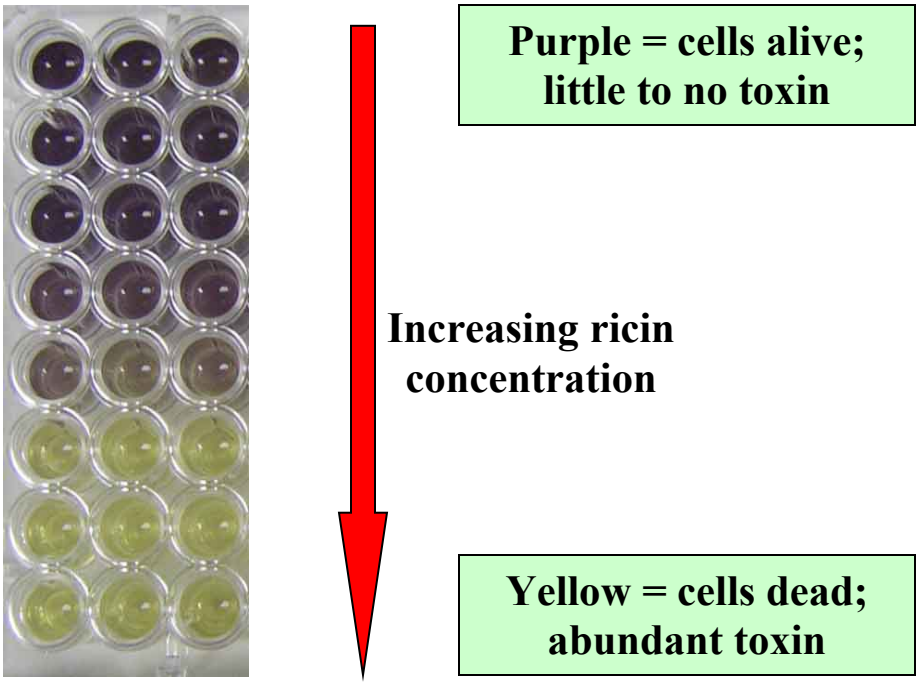

Figure 2-5. Visual Demonstration of MTT Assay on a Microplate

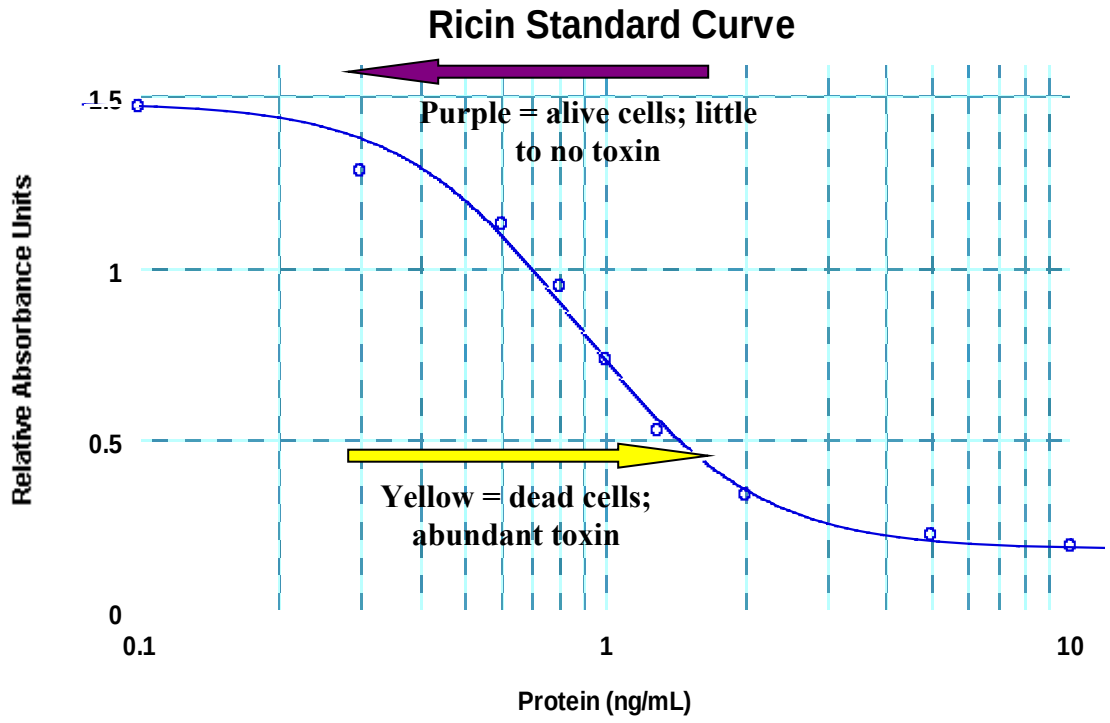

Figure 2-6. Example of Ricin Cytotoxic Profile with Corresponding Absorbance Measured Using a Microplate Reader

Throughout the study, the inherent cytotoxicity of material coupon extracts from laboratory and procedural blank coupons was assessed to determine a starting dilution that could mitigate any potential confounding cytotoxic effects observed in the ricin bioassay. To account for this potential for coupon extract-induced cytotoxicity in the ricin bioassay, the dilution factor of coupon extracts exhibiting cytotoxicity of less than 20%, when compared to negative controls (cell culture medium only), were selected as the starting dilution for all test samples. The average dilution schemes that effectively baselined the cytotoxicity of the test coupons are shown in Table 2-3.

Table 2-3. Average Dilution Factors per Coupon Material

Material	Dilution Factors Required to "Zero Out" Coupon Cytotoxicity
Mild Steel	1:10
Neoprene Rubber	1:25
Optical Grade Plastic	1:8
Pine Wood	1:7
Industrial Carpet	1:135
Paper	1:8

2.7 Attenuation Calculation

The attenuation of ricin was assessed by determining the mass of bioactive toxin extracted from each test coupon subjected to specified environmental conditions as compared to the average mass of bioactive toxin extracted from the 1 h (T0) positive control coupons.

Attenuation in terms of percent reduction for a given environmental condition, material, and ricin type, was calculated as the difference between the mean control mass values and the mean test mass values, divided by the mean control mass values, i.e.:

$$\frac{\overline{Massc}_{ij} - \overline{Masst}_{ij}}{\overline{Massc}_{ij}} * 100\,\% = \left(1 - \frac{\overline{Masst}_{ij}}{\overline{Massc}_{ij}}\right) * 100\,\%. \quad (2)$$

where $Massc_{ij}$ refers to the j individual mass values obtained from the positive control coupons, $Masst_{ij}$ refers to the j individual mass values obtained from the corresponding test coupons, and the overbar designates a mean value. In this study, there were five positive controls and five corresponding test coupons (*i.e.*, $j = 5$) for each coupon material.

In samples where no bioactive toxin was observed in any of the five test coupon extracts after attenuation, an adjusted limit of detection (LOD) value for that material was assigned. The adjusted LOD was defined as mass of ricin toxin that corresponded to the lowest dilution factor in the standard curve.

The variance of the mean percent reduction was estimated through propagation of error using Taylor series approximation. Let ^{2} be the variance of the five positive control coupons, and let ^{2} be the variance of the five test coupons. Then the estimated standard error (SE) of percent reduction is:

$$\sqrt{\frac{\overline{Masst_i}^2}{\overline{Massc_i}^2}\left(\frac{S^2 t_i}{\overline{Masst_i}^2}+\frac{S^2 c_i}{\overline{Massc_i}^2}\right)}{5} * 100\%. \quad (3)$$

where the number 5 represents the number j of coupons in both the control and test data sets. Each attenuation result is reported as a mass value with an associated 95% CI, calculated as follows:

$$95\ \%\ \text{CI} = \textit{Attenuation (\% Mass Reduction)} \pm (1.96 \times \text{SE}) \quad (4)$$

Significant differences in attenuation for the different test conditions and toxin types may be assessed visually in some of the figures presented in Section 4, based on whether or not the 95% CI values for each attenuation result overlapped. However, significant effects of test variables were more robustly analyzed using the statistical procedures described below.

2.8 Statistical Analysis

The assumption of normality for the data set was more reasonable for the log-transformed attenuation (percent reduction) values than the untransformed values. This assessment was based on comparison of the histogram and normal probability plots that were created for the residuals from the ANOVA models, for both the log-transformed and untransformed values.

Thus, all models were fitted to the log-transformed values. In addition, since the primary interest was in percent reduction from time zero, the ratio of values at each time point was taken relative to the baseline (Day 0) mean prior to analysis. The log-transformed ratio was analyzed, but results were transformed so that interpretation could be in terms of the percent reduction. A smaller ratio is associated with a larger percent reduction, and a ratio greater than one is associated with a negative percent reduction. All statistical analysis was performed using SAS (Version 9.4; Cary, NC, USA).

Analysis of variance (ANOVA) models were fitted to the log-transformed ratios for each combination of temperature and humidity. The models included main effects for time, material, and ricin preparation (pure or crude). The models also included all pairwise interactions and the three-way interaction. The following three-way ANOVA model was fitted to the base-10 log-transformed ratio response, separately for each combination of temperature and humidity:

$$Y_{ijkn} = \mu + \text{time}_i + \text{material}_j + \text{type}_k + (\text{time*material})_{ij} + (\text{time*type})_{ik} + (\text{material*type})_{jk} + (\text{time*material*type})_{ijk} + \varepsilon_{ijkn}$$

where Y_{ijkn} is the observed log-transformed, baseline-adjusted value of the n^{th} replicate for time_i, material_j, and type_k. The parameter μ is an overall constant, time_i is the effect of time i, material_j is the effect of material j, type_k is the effect of ricin type k, $(\text{time*material})_{ij}$ is the interaction effect between time i and material j, $(\text{time*type})_{ik}$ is the interaction effect between time i and

ricin type k, (material*type)$_{jk}$ is the interaction effect between material j and ricin type k, (time*material*type)$_{ijk}$ is the interaction effect between time i, material j, and ricin type k, and ε_{ijkn} is the random error unexplained by the model. Baseline (Day 0) results were not included in the model. The three-way interaction was significant in all of the models, and thus the effect of each factor (time, material and preparation) had to be interpreted separately at each combination of the other two factors. The models were used to estimate the percent reduction with 95 percent confidence for each combination of time, material and ricin type. In addition, pairwise comparisons were performed to test for significant differences between each combination of time, material and ricin type that differed in only one parameter. For these comparisons, unadjusted and Tukey-adjusted p–values were reported. P-values below 0.05 indicate a significant effect.

ANOVA models were also fitted separately for each combination of ricin type and material. The models included main effects for temperature, humidity, and time. The models also included all pairwise interactions and the three-way interaction. The following three-way ANOVA model was fitted to the base-10 log-transformed ratio response, separately for each combination of ricin type and material:

$$Y_{ijkn} = \mu + \text{time}_i + \text{temperature}_j + \text{humidity}_k + (\text{time*temperature})_{ij} + (\text{time*humidity})_{ik} + (\text{temperature*humidity})_{jk} + (\text{time*temperature*humidity})_{ijk} + \varepsilon_{ijkn}$$

where Y_{ijkn} is the observed log-transformed baseline-adjusted value for the n^{th} replicate for time$_i$, temperature$_j$, and humidity$_k$. The parameter μ is an overall constant, time$_i$ is the effect of time i, temperature$_j$ is the effect of temperature j, humidity$_k$ is the effect of humidity k, (time*temperature)$_{ij}$ is the interaction effect between time i and temperature j, (time*humidity)$_{ik}$ is the interaction effect between time i and humidity k, (temperature*humidity)$_{jk}$ is the interaction effect between temperature j and humidity k, (time*temperature*humidity)$_{ijk}$ is the interaction effect between time i, temperature j, and humidity k, and ε_{ijkn} is the random error unexplained by the model. Only results at study day 7 and study day 14 were included in the model with the exception of steel that included all available time points. The three-way interaction was significant in most of the models, and thus the effect of temperature and humidity had to be interpreted separately at each combination of the other two factors. The models were used to perform pairwise comparisons to test for significant differences between each combination of time, temperature and humidity that differed in only one parameter. For these comparisons, unadjusted and Tukey-adjusted p–values were reported. For the purposes of this report, the effects of test variables were reported as significant if the Tukey-adjusted P-values were less than or equal to 0.05. The detailed results of these statistical analyses are presented in Appendix B.

2.9 Surface Damage

The physical effect of environmental parameters on the materials was qualitatively monitored during the evaluation. This approach provided a gross visual assessment of whether the environmental state changed the appearance of the test materials. The procedural blank (coupon that is exposed to environmental conditions, but has no toxin applied) was visually compared to a laboratory blank coupon (a coupon not exposed to the environmental conditions and having no

toxin applied). No obvious visible damage was observed even at temperatures of 40 and 50°C (mild steel was only material tested at 40 and 50 °C tests), which might include structural damage, surface degradation, discoloration, or other aesthetic impacts.

3.0 Quality Assurance/Quality Control

Quality assurance (QA)/quality control (QC) procedures were performed in accordance with the Scientific, Technology, Research, Engineering, and Modeling Support (STREAMS II) Program Quality Management Plan (QMP), Version 3 and the QAPP.[7] The QA/QC procedures and results are summarized below.

3.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, microplate reader, biological safety cabinets) and monitoring devices (e.g., thermometer, hygrometer) used at the time of the evaluation were verified as being certified, calibrated, or validated.

3.2 QC Results

QC efforts conducted during testing included positive control samples (inoculated, dried for ~one h, then recovered), procedural blanks (not inoculated, attenuation), laboratory blanks (not inoculated, no attenuation), and inoculation control samples (analysis of the stock toxin suspension).

Positive control samples were run at the beginning of each test to determine the loss of cytotoxicity over the ~one h drying period. The amount of ricin recovered from these positive controls was sufficient to determine percent reduction due to the cytotoxicity assay standard range of 0.1 to 10 ng.

All procedural and laboratory blanks met the acceptance criteria by the use of dilution to mitigate inherent material specific cytotoxicity, as previously discussed. Inoculation control samples were taken from the purified and crude stock toxin suspension each day of testing and assayed against the 4-PL standard curve. Using a Grubbs outlier test, the control samples were assessed, and no outliers were found.

3.3 Audits

3.3.1 Performance Evaluation Audit

Performance evaluation audits were conducted to assess the quality of the results obtained during these experiments. Table 3-1 summarizes the performance evaluation audits that were performed.

Table 3-1. Performance Evaluation Audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
Volume of liquid from micropipettes	Gravimetric evaluation	± 10%	± 0.07% to 5.97%
Time	Compared to independent clock	± 2 seconds/hour	0 seconds/hour
Temperature	Compared to independent calibrated thermometer	± 2 °C	± 0.1 to 0.2 °C
Relative Humidity	Compare to independent calibrated hygrometer	± 10%	± 0.3 to 1.7%

3.3.2 Technical Systems Audit

Observations and findings from technical systems audits (TSAs) were documented and submitted to the laboratory technical lead for response. TSAs were conducted on December 2 and 5, 2014, to ensure that tests were being conducted in accordance with the appropriate QAPP and QMP. As part of the audit, test procedures were compared to those specified in the QAPP, and data acquisition and handling procedures were reviewed. One deviation addressing the use of sterile filtered water was noted during the TSA (see below).

3.3.3 Deviations

Two deviations occurred during this study. The first, a QAPP deviation, addressed the inadvertent addition of language to the QAPP stating that sterile filtered water (SFW) would be applied to the blank test coupons. These coupons were not nor were they intended to be inoculated with SFW. The blank test coupons were used to determine background cytotoxicity of each material type per test, and the addition of other materials such as SFW (if used) may have changed cytotoxic effects.

The second deviation was prepared to address the failure of the iLOG software described in Section 2.5. This failure occurred during Test 17, when a site-wide software update was applied to the computer running the data collection software. Application of this update caused the computer to restart, resulting in the loss of approximately one day of temperature and RH readings. The impact of this deviation was considered minimal since the RH remained running under operational conditions, and it takes at least 24 h for those to equilibrate after a cold start of the system. Corrective actions were taken to remove the computer from the automatic update list.

3.3.4 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. A QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were verified. Only minor issues were noted with the data, mostly manual data transcription errors that were corrected.

3.4 QA/QC Reporting

Each assessment and audit was documented in accordance with the QAPP and QMP. For these tests, findings were noted (none significant) in the data quality audit, and no follow-up corrective action was necessary. The findings were mostly minor data transcription errors requiring some recalculation of attenuation results, but none were gross errors in recording. QA/QC procedures were performed in accordance with the QAPP.

3.5 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in this report.

4.0 Summary of Results and Discussion

The attenuation of purified and crude forms of ricin toxin inoculated onto porous and nonporous material coupons was evaluated under various controlled environmental conditions and elapsed times. For the eighteen tests in this evaluation, the environmental conditions ranged from 20-50 °C and 20-75% RH for durations of 6 h to 28 days. Tests 1 through 14 examined six different material types while Tests 15 through 18 examined mild steel only, but with an increased number of timed collection points at elevated temperatures. Test 18 included crude ricin only to further increase the number of collection points evaluated.

4.1 Test Environmental Conditions

The temperature and RH during Tests 1-14 were controlled as described in Section 2.0. Temperature and RH readings were taken once every minute for the duration of each test. The actual environmental conditions for each test are shown in Table 4-1 and reported as the average value ± standard deviation (SD). In Tests 15-18, temperature was controlled to either 40 or 50 °C, but RH was not controlled and averaged 20-27% RH. Actual temperatures were within ±1 °C of target, while RH was within ±3% of target RH.

Table 4-1. Actual Attenuation Conditions for Environmental Chamber Tests

Test	Target Temperature °C	Actual Temperature °C	Target %RH	Actual %RH	Contact Time (Days)
1	30	30.09±0.30	75	73.60±2.49	7
2	25	25.01±0.09	45	47.03±0.30	7
3	25	24.99±0.22	45	46.39±1.06	14
4	25	25.95±1.35	75	72.43±7.20	7
5	25	25.58±1.07	75	73.93±5.50	14
6	30	29.70±0.16	45	48.09±1.73	7
7	30	30.03±0.42	45	45.61±3.52	14
8	30	30.31±0.21	75	72.96±1.16	14
9	20	20.41±0.23	45	45.22±1.47	7
10	20	20.59±0.29	45	45.26±1.40	14
11	20	20.80±0.55	75	75.28±1.05	7
12	20	20.84±0.80	75	72.43±5.14	14
13	20	19.80±0.53	45	44.81±4.03	21
14	20	19.82±0.47	45	45.06±4.18	28
15	50	50.26±0.24	uncontrolled	21.05±2.67	6, 24, 30, 48, 72, and 96 h
16	40	39.95±0.43	uncontrolled	26.62±3.31	2, 3, 4, 5, 6, 7
17	50	50.41±0.72	uncontrolled	19.79±2.20	2, 3, 4, 5, 6, 7
18	40	40.37±0.49	uncontrolled	21.56±2.48	3, 4, 5, 6, 7, 10, 11, 12, 13, 14

4.2 Recovery of Ricin from Positive Controls

The average percent recoveries for the pure and crude ricin from the positive control test materials are shown in Figure 4-1. These are the study-wide averages of the percent ricin recovered one hour after the coupons were inoculated. The percent recoveries were calculated based on a 250 µg pure ricin inoculum and an average of 320 µg inoculum of the crude form. Average positive control recoveries by material ranged from 3 to 90% for pure ricin and 17 to 127% for the crude ricin. The positive control percent recoveries were generally higher for the crude form, although the average recoveries were not significantly different for the two preparations. Wood had the lowest average recovery at 3 and 17% for pure and crude ricin, respectively, while carpet had the highest average recovery at 90 and 127% for pure and crude, respectively. Note the wider variability (as standard deviation error bars) in recovery from the crude ricin positive controls as compared to the pure ricin.

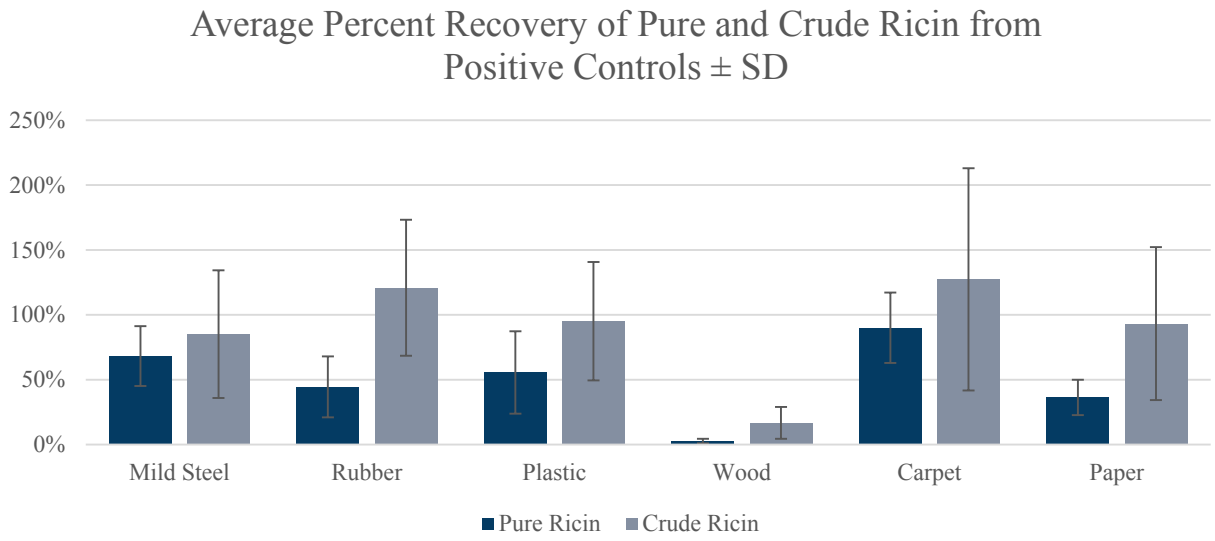

Figure 4-1. Summary of Average Percent Recovery from Positive Controls for Pure Ricin and Crude Ricin by Material Type, ± Standard Deviation

4.3 Environmental Conditions Required for 99% Attenuation

Table 4-2 provides an overview of the study results in terms of presenting the few (seven) cases where we observed >99% reduction on any of the material types tested (out of over 200 test combinations of ricin type, temperature, RH, material, and contact time). As seen in Table 4-2, in general, elevated temperature or RH was required to achieve greater than 99% reduction of pure or crude ricin toxin. More specifically, there were no cases in which any form of ricin was attenuated to a greater extent than 99% at 20 °C (either RH, up to 28 days) or at 25 °C/45% RH. There was only one case where the crude ricin preparation was attenuated more than 99%. Attenuation of more than 99% occurred most often on the mild steel and paper materials.

The detailed attenuation results for each test and for each material are provided in Appendix A. For each test and material, the results are presented in terms of the ricin recovered from each replicate positive control and test coupon and the average percent reduction.

Table 4-2. Test Parameter Combinations Demonstrating Greater than 99% Reduction of Ricin

Test*	Ricin Form/Target Mass	Temp °C %RH	Contact Time (Days)	Material	% Reduction	±CI
4	Pure/250 µg	25 °C 75%	7	Mild Steel	99.87	0.11
	Crude/320 µg		7	Paper	99.54	0.12
5	Pure/250 µg	25 °C 75%	14	Mild Steel	99.95	0.03
6	Pure/250 µg	30 °C 45%	7	Pine Wood	99.38	0.47
			7	Paper	99.83	0.24
17[a]	Pure/250 µg	50 °C 20%	6	Mild Steel	99.05	0.48
			7	Mild Steel	99.92	0.02

* Detailed data from each test number can be referenced in Appendix A.
[a]The only material tested was mild steel.

4.4 Attenuation Results for Tests at 20-30 °C

The tests conducted at 20-30 °C are representative of the environmental conditions that would be expected to be achieved using the heating system of a building without any additional equipment. For each of the tests conducted at 20-30 °C (1-14), percent reduction results by material are shown in Figures 4-2 through 4-4. Pure and crude ricin extracts were recovered on all material types tested for at least 28 days at 20 °C and 45% RH, and for at least 14 days for all other environmental conditions tested. For crude ricin exposed to 20 °C/45% RH, recovery of the toxin at 14 days was not significantly different from the positive control recoveries for most of the materials (see Table 1a in Appendix B). Detailed values for the attenuation results for each test are provided in Appendix A. See also Table 1 in Appendix B, which provides the detailed numerical attenuation results by environmental condition, elapsed time, material, and ricin form.

Significant differences in attenuation between the two ricin forms, as a function of elapsed time, or between environmental conditions on a specified material may be assessed visually via Figures 4-2 through 4-4, i.e., depending on whether the 95% CIs of the two attenuation results overlap. Generally, attenuation increased over time and at higher temperatures. However, because there was a considerable amount of variability in the results (especially with the crude ricin), in some cases attenuation may appear to have decreased over time or at higher temperatures. Because of this variability, additional multiple statistical pair-wise comparisons of the data were conducted using ANOVA models, the results of which are detailed in Section 4.6 and in Appendix B. These more robust statistical approaches allow us to better assess the effect

of the environmental conditions and materials on attenuation, and these results are summarized in Section 4.6.

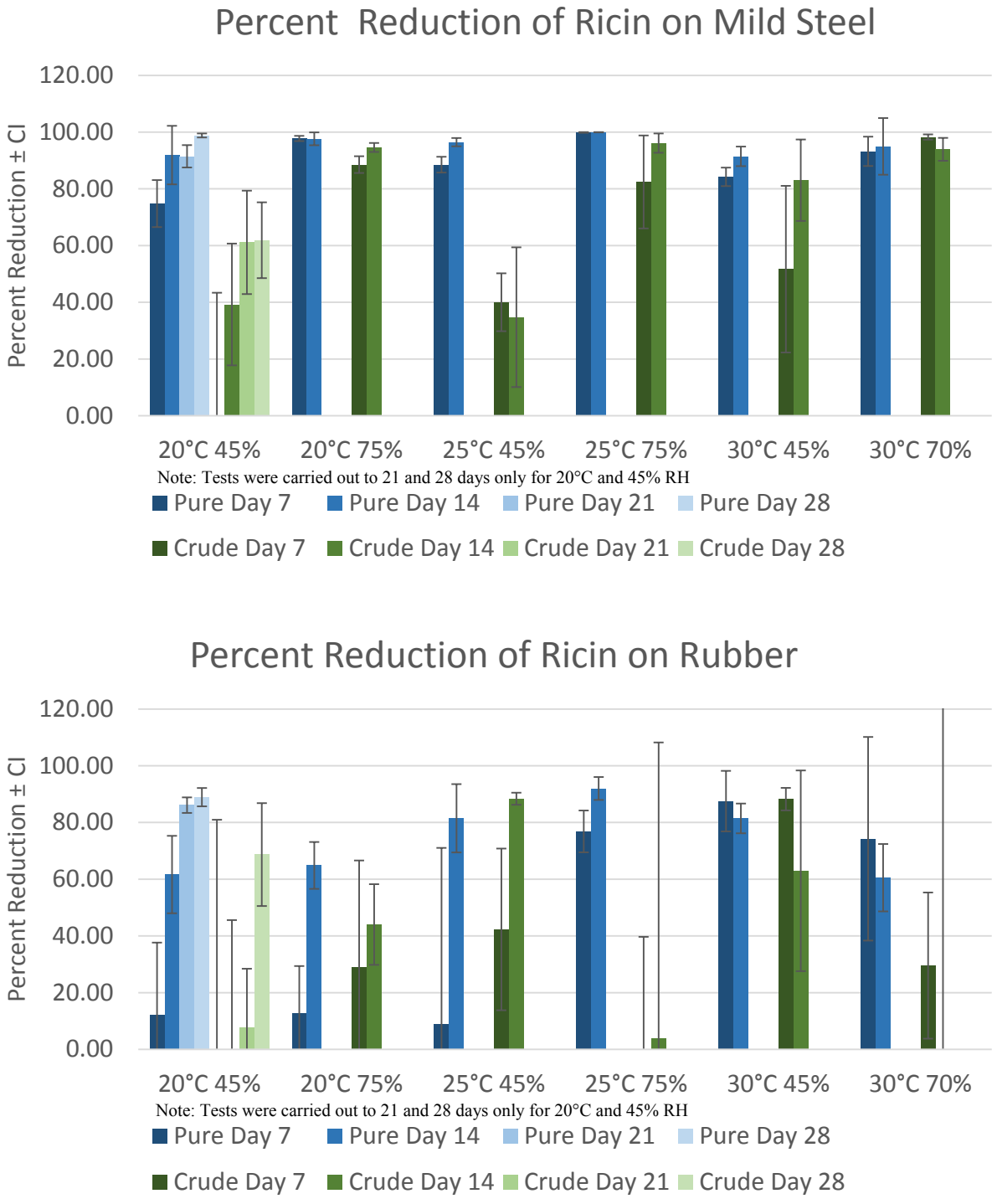

Figure 4-2. Summary of Percent Reduction (Tests 1-14) Results for Steel and Rubber, by Environmental Condition, Comparing Pure and Crude Ricin ± 95% Confidence Interval

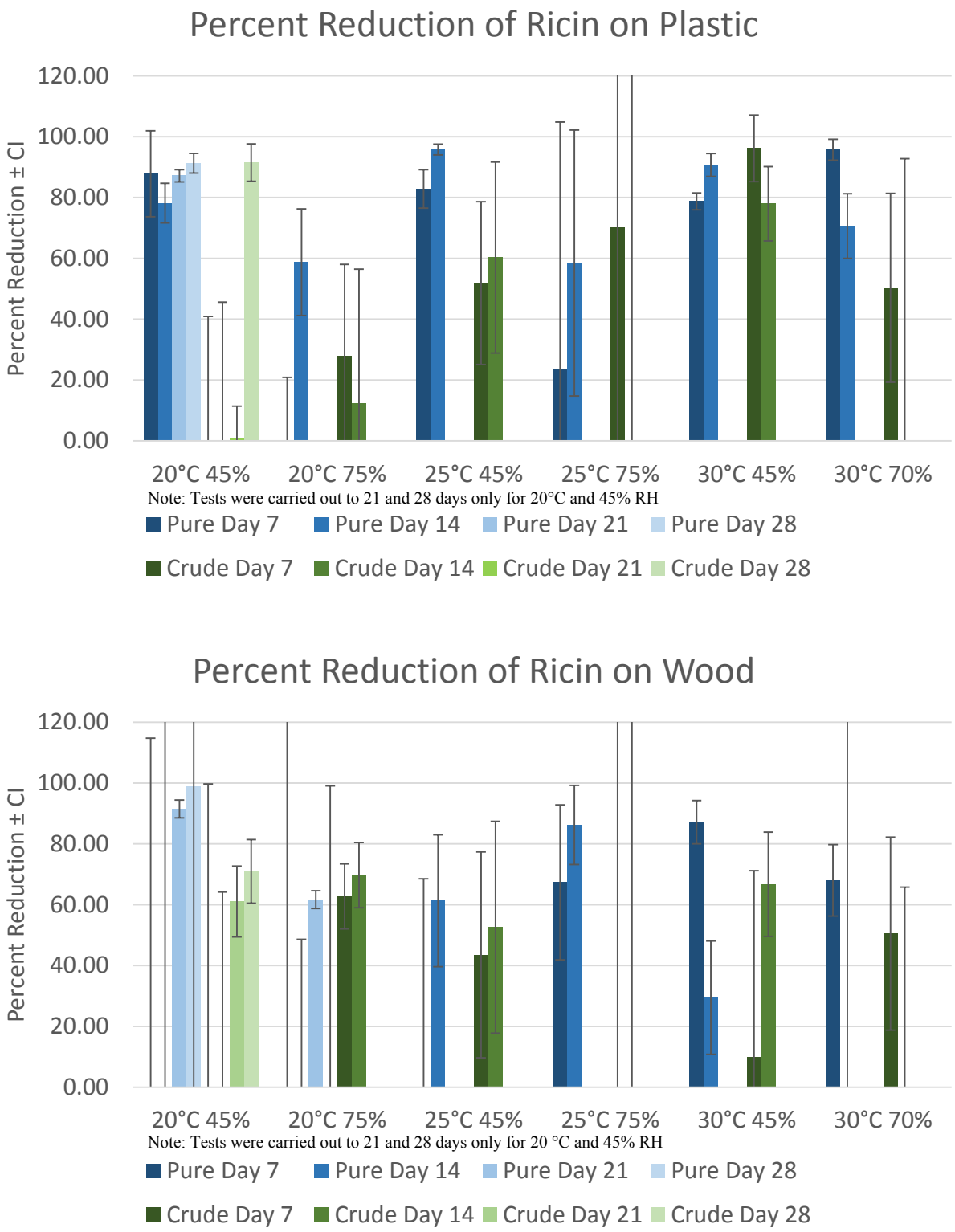

Figure 4-3. Summary of Percent Reduction (Tests 1-14) Results for Plastic and Wood, by Environmental Condition, Comparing Pure and Crude Ricin ± 95% Confidence Interval

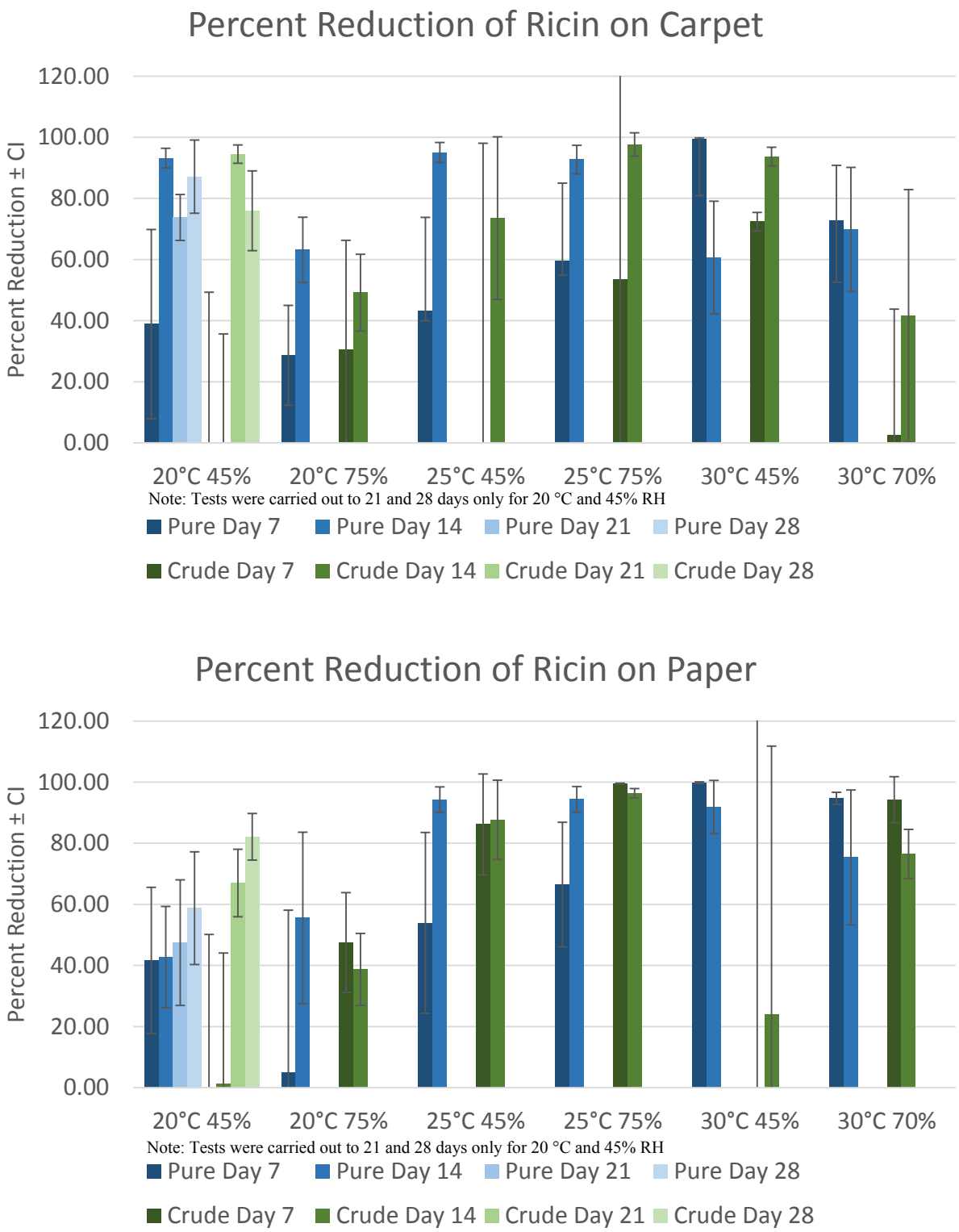

Figure 4-4. Summary of Percent Reduction (Tests 1-14) Results on Carpet and Paper, by Environmental Condition, Comparing Pure and Crude Ricin ± 95% Confidence Interval

In Table 4-3, we provide a summary of the attenuation results, by environmental condition, for the tests conducted at 20-30 °C. To allow for simple comparisons, the percent reduction results were averaged across all materials for the 14-day elapsed time period, since all six environmental conditions were evaluated at this time point. Fourteen days was the longest test duration investigated for the study, with the exception of the 20 °C/45% RH condition, which was tested out to 28 days. The attenuation data for 20 °C/45% RH condition at 28 days are also included in Table 4-3 for comparison purposes. While Table 4-3 provides a quick snapshot of results and illustrates some of the confounding effects of test parameters, as mentioned above, Section 4.6 provides a detailed discussion of the statistical assessment of significant effects of test variables.

Table 4-3. Average Percent Attenuation Obtained for Each Environmental Condition at 14 and 28 days

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%

* Average of all six materials

4.5 Attenuation Results for Tests at 40-50 °C

The tests conducted at the elevated temperatures of 40 and 50 °C are representative of environmental conditions that could be achieved in a structure with additional, ancillary heating equipment. Percent reduction results for mild steel only, for each test (15-18), are shown in Figures 4-5 through 4-8. Steel was selected for these tests based on its lower variability in attenuation results (e.g., as exhibited in Figures 4-2 to 4-4). The pure ricin shows steady degradation over time, while the attenuation of the crude ricin over time is more subdued. Over 90% reduction of the pure ricin occurs within two days at both 40 and 50 °C, with 99% reduction obtained in six days at 50 °C (Figure 4-7). For the crude ricin on mild steel, 98% attenuation occurred after one week at 50 °C. In test 18, where we focused only on crude ricin on mild steel to allow for multiple test durations for up to two weeks, the maximum attenuation obtained was only 79% at 40 °C (Figure 4-8). Detailed values for the attenuation results are provided in Appendix A.

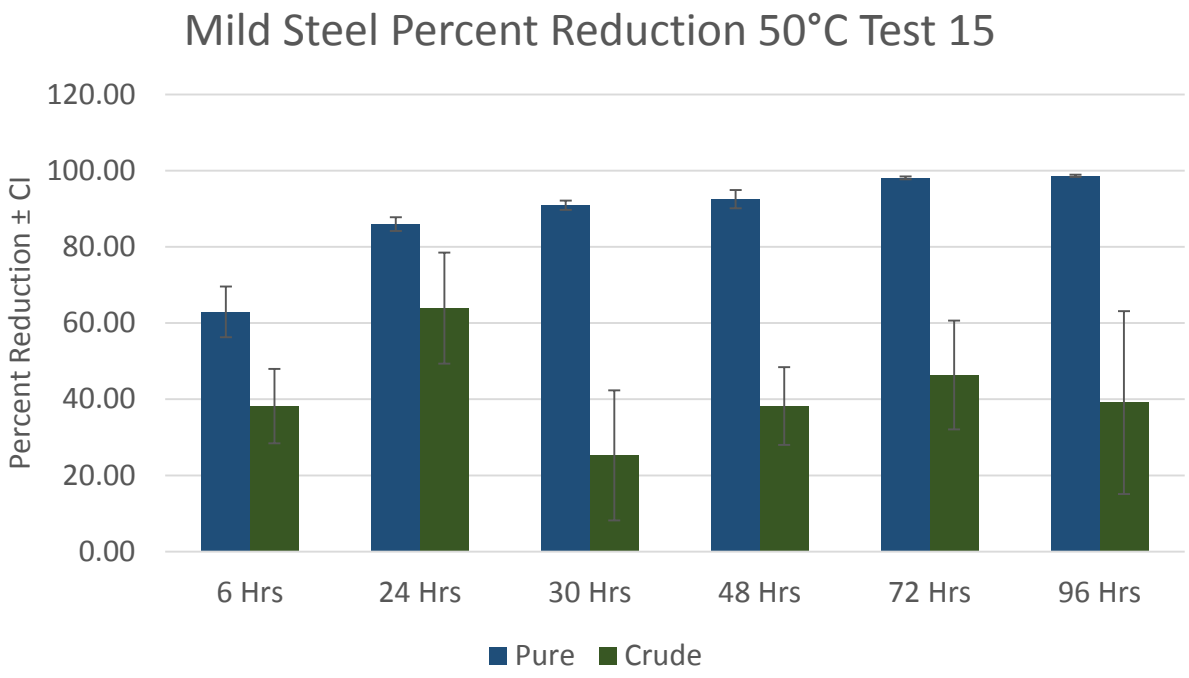

Figure 4-5. Summary of Percent Reduction (Test 15) Results at 50 °C, by Time, Comparing Pure and Crude Ricin ± 95% Confidence Interval; RH uncontrolled

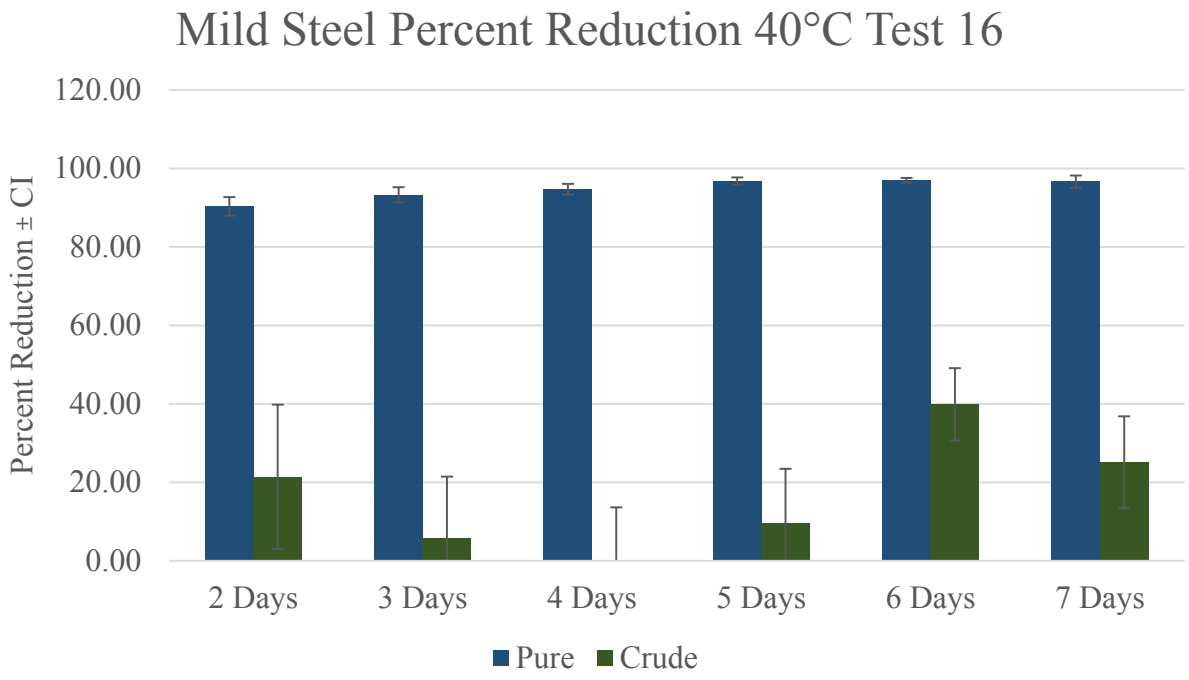

Figure 4-6. Summary of Percent Reduction (Test 16) Results at 40 °C, by Day, Comparing Pure and Crude Ricin ± 95% Confidence Interval; RH uncontrolled

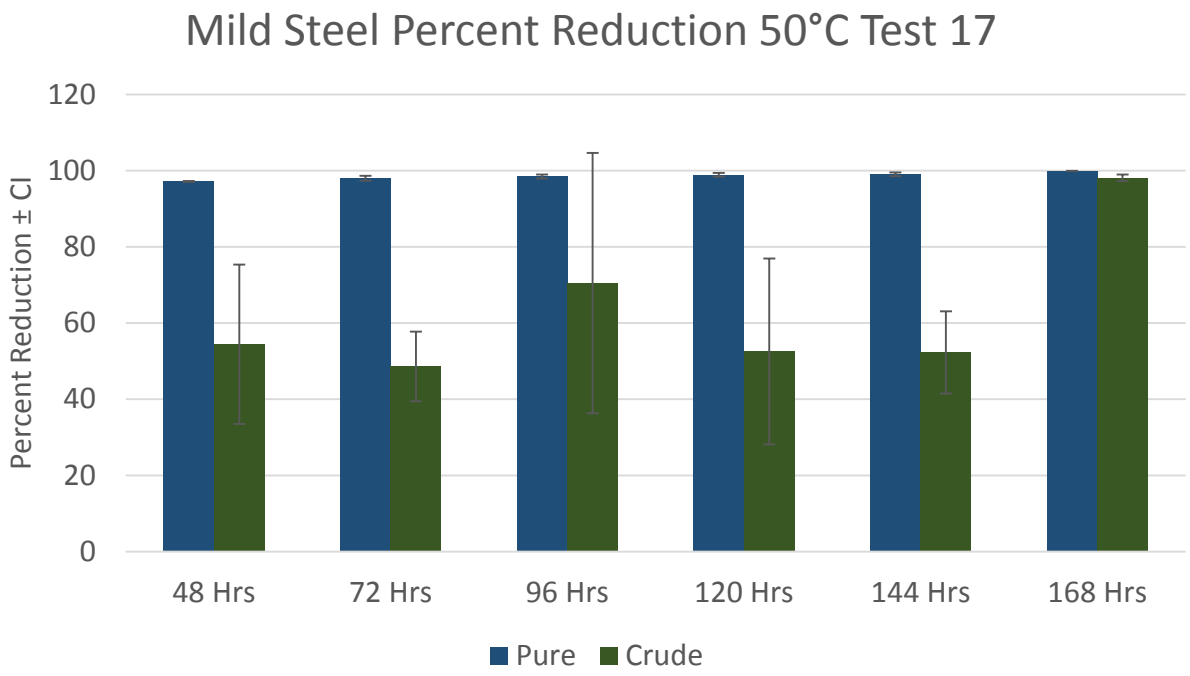

Figure 4-7. Summary of Percent Reduction (Test 17) Results, by Time, Comparing Pure and Crude Ricin ± 95% Confidence Interval; RH uncontrolled

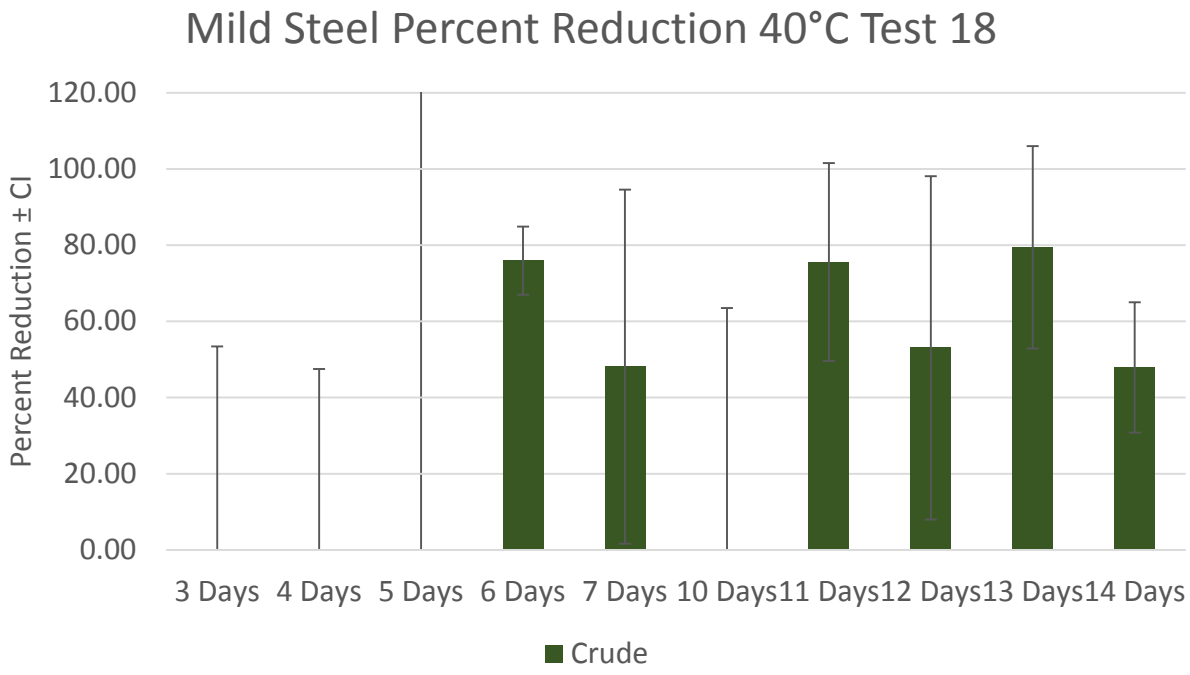

Figure 4-8. Summary of Percent Reduction (Tests 18) Results at 40 °C, by Day, for Crude Ricin ± 95% Confidence Interval; RH uncontrolled

4.6 Statistical Analyses to Assess Effect of Test Variables on Attenuation

Effect of elapsed time

The results of statistical analyses indicating whether the ricin recoveries over time were significantly different from the ricin recovered at T0 (time zero, positive control recoveries) are shown in Table 1 of Appendix B. The results for additional pair-wise comparisons for all other time points for a given experimental condition and material are shown in Table 3 of Appendix B. Ricin was generally more stable at the lower temperatures, and increasing elapsed times at the lower temperatures in many cases did not improve attenuation. This effect was more pronounced with the crude ricin. For example, there was generally no significant attenuation of crude ricin up to 14 days at 20 °C/45% RH (Table 1A of Appendix B). At this same environmental condition, minor but statistically significant attenuation occurred for the pure ricin at 7 days on most of the materials. Tables 3a, 3b, and 3c of Appendix B also illustrate this effect. Even at the higher temperature of 40 °C, there was no significant attenuation of the crude ricin at five days (Table 1H of Appendix B). Alternatively, there was also the case in which no significant additional attenuation occurred with each successive time point if the attenuation was already relatively high. See, for example, the pure ricin attenuation over time at 50 °C shown in Figure 4-7.

Effect of temperature

The statistical results of the pair-wise comparisons to assess the effect of temperature on the attenuation of ricin are shown in Appendix B, Tables 6 and 7 (refer to Tukey-adjusted P-values). In the majority of cases, there was either no significant change in attenuation or there was an increase in attenuation associated with increasing temperature. Very few comparisons showed that the attenuation decreased with increasing temperature. The effect of increasing temperature on attenuation was more pronounced when comparing results with a 10 °C temperature difference (e.g., attenuation at 20 versus 30 °C).

Effect of material

The results of the nearly 500 pair-wise comparisons between materials are shown in Table 2 of Appendix B. (Refer to Tukey-adjusted P-values; each material was compared a total of 140 times.) In the majority of the comparisons, there was no significant difference in attenuation between materials. However, the mild steel material did have the largest number of comparisons in which ricin was attenuated to a significantly greater extent than the other material being compared. The ranking of materials by the number of comparisons where ricin was attenuated to a significantly greater extent than the material it was compared with (shown in parentheses) is as follows: steel (58), paper (30), carpet (24), plastic (12), rubber (7) and wood (2). According to this ranking, ricin was least attenuated on the wood material.

Effect of RH

With all other factors being equivalent, increasing the RH from 45 to 75% does not appear to affect attenuation. As Table 5 of Appendix B shows, in over half the pair-wise comparisons, there was no significant effect on attenuation when increasing the RH (refer to Tukey-adjusted P-values). Further, in the 33 cases where there was a significant effect of increasing the RH from 45 to 75%, 16 of the cases showed an increase in attenuation while 17 of the cases showed a decrease in attenuation. The effect of RH (or lack thereof) was similar for both ricin types. However, the effect of RH appears to be somewhat dependent on the material. For example, for both mild steel and wood materials, there

was either no effect of RH, or an increase in RH significantly increased the ricin attenuation in nearly all of the comparisons. The opposite effect occurred with the plastic and rubber materials.

Effect of Ricin Preparation

A summary of the attenuation results, comparing the average percent reduction ± SD for the pure and crude ricin, by material, is shown in Figure 4-9. These results are averages for Tests 1-14, in which all materials were subject to the same environmental conditions. Overall, the average percent reductions by material ranged from 46.4 to 66.8% for crude ricin and 38.4 to 93.5% for pure ricin. The pure ricin on mild steel, neoprene rubber, optical plastic, and wood exhibited a higher percent reduction as compared to the crude material. The average attenuation of both ricin types was highest on the steel material, although this effect was significant only for the pure form.

Similar to the positive control recovery results, these attenuation results also highlight the higher variability of the crude ricin as compared to the pure ricin. This variability is most likely due to the potential for interactions of other proteins in solution that were not removed as they would be in the commercially available pure material. Additionally, while the crude ricin suspension constituents likely increased variability, they may also have shielded or protected the ricin proteins from the environmental conditions, as evidenced by the increased stability.

Tables 4a-4h of Appendix B show the p-values associated with each pair-wise comparison to assess the effect of the ricin preparation on attenuation and indicate the comparisons where there was a significant difference (refer to Tukey-adjusted P-values). The comparison of percent reduction by ricin type is presented for each material, environmental condition, and test duration. The majority of comparisons show that there was either no significant effect of ricin type, or that the crude preparation was attenuated significantly less. There were only a few cases in which the crude ricin preparation was attenuated significantly more than the pure. Interestingly, the tests at the elevated temperatures of 40 and 50 °C and with mild steel allowed for significant differences in the two types of ricin to become more readily apparent. With these higher temperatures, the crude form was significantly less attenuated than the pure form at all but two time points (Tables 4g and 4h of Appendix B).

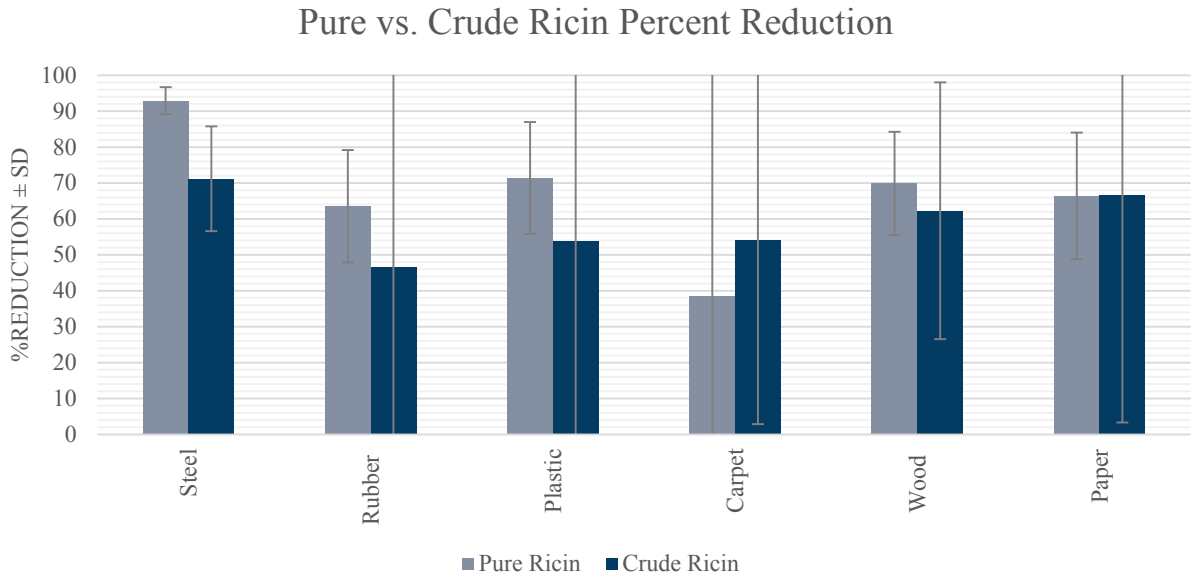

Figure 4-9. Comparison of Average Percent Reduction for Pure Ricin and Crude Ricin by Material Type ± Standard Deviation, for Tests 1-14 (20-30 °C)

4.7 Summary

Over the entire study, there were only seven cases (out of over 200 test combinations of ricin type, temperature, RH, material, and contact time) in which we observed greater than 99% attenuation of ricin. In general, elevated temperature or RH was required to achieve greater than 99% reduction of pure or crude ricin toxin. More specifically, there were no cases where any form of ricin was attenuated to a greater extent than 99% at 20 °C or at 25 °C/45% RH. There was only one case where the crude ricin preparation was attenuated more than 99%. Attenuation of more than 99% occurred most often on the mild steel and paper materials.

Under the environmental conditions most resembling the indoor environment, significant attenuation of the crude ricin was not observed until at least 21 days, with the exception of mild steel. At 30 °C, the average ricin attenuation across all materials and ricin forms ranged from 39-81%. Per the statistical analyses, there was either no significant change in attenuation, or there was an increase in attenuation associated with increasing temperature. The effect of increasing temperature on attenuation was more pronounced when comparing results with a 10 °C temperature difference.

For the pure ricin, heat treatments at the elevated temperatures of 40 °C for 5 days and 50 °C for 2-3 days achieved greater than 96% attenuation. For the crude ricin preparation, appreciable recovery of the ricin still occurred at 40 °C after two weeks. A seven-day heat treatment at 50 °C was required to achieve greater than 98% attenuation of the crude ricin on mild steel.

In general, increasing the RH level from 45 to 75% did not significantly affect attenuation of ricin. The effect of RH (or lack thereof) was similar for both ricin types, while the effect of RH appeared to be somewhat dependent on the material. For example, for both the mild steel and wood materials, there was either no effect of RH, or an increase in RH significantly increased the ricin attenuation in nearly all of the comparisons. The opposite effect of RH occurred with the plastic and rubber materials.

In the majority of the statistical comparisons, there was no significant difference in attenuation between the materials. However, the mild steel material did have the largest number of comparisons in which ricin was attenuated significantly more than the other material being compared. The ranking of materials by the number of comparisons in which ricin was attenuated significantly more than the material it was compared with (shown in parentheses) is as follows: steel (58), paper (30), carpet (24), plastic (12), rubber (7) and wood (2).

In the majority of tests, the crude ricin was more stable, i.e., more difficult to attenuate, than the pure ricin. The attenuation results for the crude ricin also exhibited more variability than the pure ricin. The use of a biological system, i.e., a cell-based assay, to quantitate ricin toxicity, regardless of ricin type, may also have contributed to variability in results. The sometimes high variability in results may mask the effects of test variables, and so a robust statistical analysis was conducted. From this statistical analysis, we found that the crude ricin was attenuated significantly more than the pure form in only a few cases. In other words, in the overwhelming majority of the statistical comparisons made, there was either no significant effect of ricin preparation on attenuation, or the pure ricin was attenuated significantly more than the crude form. The tests on mild steel at the elevated temperatures of 40 and 50 °C allow for significant differences between the two ricin forms to become more readily apparent, i.e., the pure ricin form is more easily attenuated.

5.0 References

1. US Centers for Disease Control and Prevention. 2009. Biosafety in Microbiological and Biomedical Laboratories. 5th Edition, HHS Publication No. (CDC) 21-1112.

2. US EPA, 2006. Impact of Temperature and Humidity on the Persistence of Vaccinia Virus and Ricin Toxin on Indoor Surfaces. US EPA Report 600/R-08/002.

3. US Army Medical Research Institute of infectious Diseases. 2005. Medical Management of Biological Casualties Handbook, Sixth Edition.

4. Barnes DJ, Baldwin BS, Braasch DA. 2009. Degradation of Ricin in Castor Seed Meal by Temperature and Chemical Treatment. Industrial Crops and Products (29): 509-515

5. Anandan S, Anil Kumar GK, Ghosh J, Ramachandra KS. 2005. Effects of Different Physical and Chemical Treatments on Detoxification of Ricin in Castor Cake. Animal Feed Science and Technology (120): 159-168

6. US EPA, 2005. Workshop on Decontamination, Cleanup, and Associated Issues for Sites Contaminated with Chemical, Biological, or Radiological Materials. US EPA Report EPA/600/R-05/083.

7. US EPA. 2014. Quality Assurance Project Plan for the Neutralization of Ricin Toxicity Using Elevated Temperature and Humidity, Version 1. June. (Available upon request by contacting EPA).

8. US EPA, 2015. Evaluation of the Inactivation of Ricin Toxin on Surfaces Using Vapor Phase Hydrogen Peroxide. US EPA Report 600/R-15/131.

9. Lin T T-S and Li S S-L. 1980. Purification and Physicochemical Properties of Ricins and Agglutinins from Ricinus communis. European Journal of Biochemistry (105):453-459.

10. US EPA. 2013. Evaluation of Ethylene Oxide for the Inactivation of *Bacillus anthracis.* US EPA Technology Evaluation Report. EPA/13/R-13-220.

11. Mosmann, T. 1983. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays." Journal of Immunological Methods (65): p. 55-63.

Appendix A
Detailed Test Results

Attenuation Results

The detailed attenuation results for varied environmental conditions against pure and crude ricin toxin on six material types (mild steel, rubber, optical plastic, carpet, bare pine wood and paper) are shown in Tables A-1 through A-3. Data highlighted green indicate ≥99 % reduction.

Table A-1. Attenuation of Pure Ricin Toxin[a]

Test Number	Test Parameters			Material	Inoculum (µg/coupon)	Mean Recovered Ricin ± SD (µg/coupon)		%Reduction ± CI
	Temp °C±SD	%RH ±SD	Time			Positive Control	Test Coupon	
1	30.09 ± 0.30	73.60 ± 2.49	7 Days	Mild Steel	250	120.170 ± 77.058	8.140 ± 4.781	93.23 ± 5.16
				Neoprene Rubber		41.843 ± 65.194	10.764 ± 3.524	74.27 ± 35.90
				Optical Plastic		87.882 ± 79.708	3.753 ± 0.580	95.73 ± 3.44
				Bare Pine Wood		9.536 ± 1.760	3.050 ± 1.148	68.02 ± 11.75
				Industrial Carpet		241.153 ± 22.577	65.399 ±49.019	72.88 ± 17.96
				Paper		95.309 ± 34.061	5.052 ± 1.162	94.70 ± 1.97
2	25.01 ± 0.09	47.03 ± 0.30	7 Days	Mild Steel	250	180.510 ± 38.728	20.703 ± 3.678	88.53 ± 2.80
				Neoprene Rubber		56.103 ± 34.557	51.101 ± 24.325	8.92 ± 62.15
				Optical Plastic		198.226 ± 80.640	34.049 ± 3.400	82.82 ± 6.31
				Bare Pine Wood		5.377 ± 2.234	7.655 ± 2.751	[f]0.00 ± 68.55
				Industrial Carpet		154.571 ± 91.412	87.719 ± 14.440	43.25 ± 30.54
				Paper		142.943 ± 59.814	65.853 ± 39.590	53.93 ± 29.58
3	24.99 ± 0.22	46.39 ± 1.06	14 Days	Mild Steel	250	180.510 ± 38.728	6.380 ± 2.652	96.47 ± 1.45
				Neoprene Rubber		56.103 ± 34.557	10.384 ± 4.298	81.49 ± 12.04
				Optical Plastic		198.226 ± 80.640	8.358 ± 2.051	95.78 ± 1.76
				Bare Pine Wood		5.377 ± 2.234	2.081 ± 1.011	61.30 ± 21.68
				Industrial Carpet		154.571 ± 91.412	7.678 ± 3.542	95.03 ± 3.27
				Paper		142.943 ± 59.814	8.147 ± 5.807	94.30 ± 4.13
4	25.95 ± 1.35	72.43 ± 7.20	7 Days	Mild Steel	250	68.378 ± 49.618	0.089 ± 0.051	99.87 ± 0.11
				Neoprene Rubber		149.260 ± 24.963	34.536 ± 11.134	76.86 ± 7.37
				Optical Plastic		50.373 ± 60.376	38.419 ± 7.183	23.73 ± 81.10
				Bare Pine Wood		12.774 ± 9.959	4.171 ± 1.793	67.35 ± 25.48
				Industrial Carpet		206.655 ± 38.789	83.698 ± 58.006	59.50 ± 25.49
				Paper		60.340 ± 39.655	20.231 ± 4.503	66.47 ± 20.39
5	25.58 ± 1.07	73.93 ± 5.50	14 Days	Mild Steel	250	68.378 ± 49.618	0.033 ± 0.011	99.95 ± 0.03
				Neoprene Rubber		149.260 ± 24.963	11.959 ± 6.604	91.99 ± 4.05
				Optical Plastic		50.373 ± 60.376	20.927 ± 1.561	58.46 ± 43.73
				Bare Pine Wood		12.774 ± 9.959	1.759 ± 1.309	86.23 ± 13.01
				Industrial Carpet		206.655 ± 38.789	15.097 ± 10.671	92.69 ± 4.68
				Paper		60.340 ± 39.655	3.403 ± 1.829	94.36 ± 4.20

[a] Data are expressed as the mean (± SD) of the mass of toxin recovered on five replicate individual samples, and attenuation (percent reduction ± CI).
[b] Positive Controls = samples inoculated, not attenuated (recovered after 1 hour).
[c] Test Coupons = samples inoculated, attenuated.
[d] CI = confidence interval (± 1.96 × standard error [SE]).
[e] % Reduction calculated as (mean ricin recovered positive controls – mean ricin recovered test coupons)/ mean ricin recovered positive controls
[f] As a result of high variability, negative value reported as "0".

Table A-1. Attenuation of Pure Ricin Toxin[a] (Continued)

Test Number	Test Parameters			Material	Inoculum (µg/coupon)	Mean Recovered Ricin ± SD (µg/coupon)		%Reduction ± CI
	Temp °C	%RH	Time			Positive Control	Test Coupon	
6	29.70 ± 0.16	48.09 ± 1.73	7 Days	Mild Steel	250	159.998 ± 26.952	25.250 ± 4.137	84.22 ± 3.25
				Neoprene Rubber		167.446 ± 40.218	20.872 ± 19.731	87.54 ± 10.66
				Optical Plastic		232.860 ± 27.712	49.522 ± 4.462	78.73 ± 2.78
				Bare Pine Wood		5.396 ± 1.198	0.694 ± 0.411	87.14 ± 7.13
				Industrial Carpet		204.026 ± 98.436	1.262 ± 0.914	99.38 ± 0.47
				Paper		56.780 ± 57.697	0.094 ± 0.121	99.83 ± 0.24
7	30.03 ± 0.42	45.61 ± 3.52	14 Days	Mild Steel	250	159.998 ± 26.952	13.685 ± 5.835	91.45 ± 3.44
				Neoprene Rubber		167.446 ± 40.218	31.055 ± 6.671	81.46 ± 5.24
				Optical Plastic		232.860 ± 27.712	21.643 ± 9.633	90.71 ± 3.75
				Bare Pine Wood		5.396 ± 1.198	3.808 ± 0.775	29.44 ± 18.63
				Industrial Carpet		204.026 ± 98.436	80.311 ± 18.555	60.64 ± 18.46
				Paper		56.780 ± 57.697	4.630 ± 3.097	91.85 ± 8.69
8	30.31 ± 0.21	72.96 ± 1.16	14 Days	Mild Steel	250	90.506 ± 41.338	4.557 ± 10.096	94.97 ± 9.98
				Neoprene Rubber		56.642 ± 18.047	22.361 ± 2.860	60.52 ± 11.88
				Optical Plastic		49.017 ± 16.041	14.399 ± 3.614	70.62 ± 10.62
				Bare Pine Wood		1.938 ± 1.531	6.836 ± 2.793	[f]0.00 ± 274.96
				Industrial Carpet		147.407 ± 89.460	44.416 ± 20.943	69.87 ± 20.30
				Paper		59.617 ± 60.120	14.708 ± 2.498	75.33 ± 22.11
9	20.41 ± 0.23	45.22 ± 1.47	7 Days	Mild Steel	250	192.891 ± 37.467	48.573 ± 15.609	74.82 ± 8.29
				Neoprene Rubber		114.993 ± 36.407	101.073 ± 9.998	12.11 ± 25.55
				Optical Plastic		199.895 ± 48.331	24.371 ± 31.664	87.81 ± 14.12
				Bare Pine Wood		13.015 ± 3.962	19.645 ± 15.955	0.00 ± 114.75
				Industrial Carpet		206.481 ± 35.515	126.246 ± 69.684	38.86 ± 30.98
				Paper		138.476 ± 13.787	80.794 ± 36.946	41.65 ± 23.93
10	20.59 ± 0.29	45.26 ± 1.40	14 Days	Mild Steel	250	192.891 ± 37.467	15.611 ± 22.539	91.91 ± 10.33
				Neoprene Rubber		114.993 ± 36.407	44.091 ± 11.210	61.66 ± 13.65
				Optical Plastic		199.895 ± 48.331	43.656 ± 10.448	78.16 ± 6.51
				Bare Pine Wood		13.015 ± 3.962	31.785 ± 24.921	0.00 ± 180.04
				Industrial Carpet		206.481 ± 35.515	14.047 ± 7.134	93.20 ± 3.20
				Paper		138.476 ± 13.787	79.301 ± 24.979	42.73 ± 16.58
11	20.80 ± 0.55	75.28 ± 1.05	7 Days	Mild Steel	250	224.689 ± 96.111	5.056 ± 0.931	97.75 ± 0.92
				Neoprene Rubber		98.829 ± 19.853	86.337 ± 7.451	12.64 ± 16.74
				Optical Plastic		78.392 ± 8.647	89.316 ± 15.869	0.00 ± 20.89
				Bare Pine Wood		2.569 ± 0.855	11.335 ± 5.337	0.00 ± 222.93
				Industrial Carpet		291.579 ± 49.341	208.112 ± 41.463	28.63 ± 16.35
				Paper		90.726 ± 57.043	86.250 ± 9.367	4.93 ± 53.17
12	20.84 ± 0.80	72.43 ± 5.14	14 Days	Mild Steel	250	224.689 ± 96.111	5.313 ± 5.312	97.64 ± 2.25
				Neoprene Rubber		98.829 ± 19.853	34.733 ± 6.186	64.86 ± 8.27
				Optical Plastic		78.392 ± 8.647	32.356 ± 15.284	58.73 ± 17.55
				Bare Pine Wood		2.569 ± 0.855	3.913 ± 0.580	0.00 ± 48.62
				Industrial Carpet		291.579 ± 49.341	107.434 ± 30.672	63.15 ± 10.72
				Paper		90.726 ± 57.043	40.351 ± 14.248	55.52 ± 28.11
13	19.80 ± 0.53	44.81 ± 4.03	21 Days	Mild Steel	250	192.38 ± 40.482	16.393 ± 7.916	91.48 ± 3.93
				Neoprene Rubber		203.877 ± 26.250	28.306 ± 5.162	86.12 ± 2.72
				Optical Plastic		214.895 ± 33.296	27.605 ± 2.390	87.15 ± 2.00
				Bare Pine Wood		4.706 ± 1.723	1.803 ± 1.039	61.68 ± 22.93
				Industrial Carpet		349.205 ± 43.469	91.676 ± 27.681	73.75 ± 7.52
				Paper		82.853 ± 23.078	54.022 ± 20.863	47.48 ± 20.56

[a] Data are expressed as the mean (± SD) of the mass of toxin recovered on five replicate individual samples, and attenuation (percent reduction ± CI).
[b] Positive Controls = samples inoculated, not attenuated.
[c] Test Coupons = samples inoculated, attenuated.
[d] CI = confidence interval (± 1.96 × standard error [SE]).
[f] As a result of high variability, negative value reported as "0".

Table A-1. Attenuation of Pure Ricin Toxin[a] (Continued)

Test Number	Test Parameters			Material	Inoculum (µg/coupon)	Mean Recovered Ricin ± SD (µg/coupon)		%Reduction ± CI
	Temp °C	%RH	Time			Positive Control	Test Coupon	
14	19.82 ± 0.47	45.06 ± 4.18	28 Days	Mild Steel	250	192.38 ± 40.482	2.265 ± 1.447	98.82 ± 0.69
				Neoprene Rubber		203.877 ± 26.250	22.566 ± 6.892	88.93 ± 3.22
				Optical Plastic		214.895 ± 33.296	18.761 ± 7.392	91.27 ± 3.24
				Bare Pine Wood		4.706 ± 1.723	5.428 ± 4.933	0.00 ± 99.06
				Industrial Carpet		349.205 ± 43.469	44.936 ± 47.379	87.13 ± 11.98
				Paper		102.853 ± 23.078	34.173 ± 12.943	66.77 ± 12.82
15	50.26 ± 0.24	21.05 ± 2.67	Multiple	Mild Steel TO	250	263.835 ± 27.709	NA	NA
				Mild Steel +6 h		NA[g]	97.816 ± 17.229	62.93 ± 6.66
				Mild Steel +24 h			37.059 ± 3.819	85.95 ± 1.81
				Mild Steel +30 h			23.953 ± 2.656	90.92 ± 1.22
				Mild Steel +48 h			19.739 ± 6.912	92.52 ± 2.40
				Mild Steel +72 h			5.002 ± 0.972	98.10 ± 0.37
				Mild Steel +96 h			3.546 ± 0.834	98.66 ± 0.30
16	39.95 ± 0.43	26.62 ±3.31	Multiple	Mild Steel TO	250	182.500 ± 32.073	NA	NA
				Mild Steel +48 h		NA	17.684 ± 3.793	90.31 ± 2.36
				Mild Steel +72 h			12.289 ± 3.437	93.27 ± 1.95
				Mild Steel +96 h			9.697 ± 2.267	94.69 ± 1.36
				Mild Steel +120 h			5.913 ± 1.685	96.76 ± 0.95
				Mild Steel +144 h			5.516 ± 0.753	96.98 ± 0.59
				Mild Steel +168 h			6.180 ± 3.056	96.61 ± 1.56
17	50.41 ± 0.72	19.79 ± 2.20	Multiple	Mild Steel TO	250	200.912 ± 42.567	NA	NA
				Mild Steel +48 h		NA	5.713 ± 1.310	97.16 ± .078
				Mild Steel +72 h			4.023 ± 1.167	98.00 ± 0.63
				Mild Steel +96 h			3.102 ± 0.974	98.46 ± 0.51
				Mild Steel +120 h			2.147 ± 1.028	98.93 ± 0.49
				Mild Steel +144 h			1.902 ± 1.018	99.05 ± 0.48
				Mild Steel +168 h			0.153 ± 0.042	99.92 ± 0.02

[a] Data are expressed as the mean (± SD) of the mass of toxin recovered on five replicate individual samples, and attenuation (percent reduction ± CI).
[b] Positive Controls = samples inoculated, not attenuated.
[c] Test Coupons = samples inoculated, attenuated.
[d] CI = confidence interval (± 1.96 × standard error [SE]).
[f] As a result of high variability, negative value reported as "0".
[g] NA = Not applicable.

Table A-2. Attenuation of Crude Ricin Toxin[a]

Test Number	Test Parameters			Material	Inoculum (µg/coupon)	Mean Recovered Ricin ± SD (µg/coupon)		%Reduction ± CI
	Temp °C	%RH	Time			Positive Control	Test Coupon	
1	30.09 ± 0.30	73.60 ± 2.49	7 Days	Mild Steel	320	215.692 ± 83.217	3.748 ± 1.744	98.26 ± 0.92
				Neoprene Rubber		303.449 ± 135.904	213.755 ± 48.948	29.56 ± 31.06
				Optical Plastic		297.116 ± 75.901	147.589 ± 78.831	50.33 ±25.78
				Bare Pine Wood		50.730 ± 30.423	25.121 ±10.499	50.48 ± 31.73
				Industrial Carpet		331.333 ± 107.507	323.101 ± 57.961	2.48 ± 31.69
				Paper		225.850 ± 334.821	12.990 ± 2.421	94.25 ± 7.53
2	25.01 ± 0.09	47.03 ± 0.30	7 Days	Mild Steel	320	174.907 ± 29.476	104.933 ± 10.086	40.01 ± 10.20
				Neoprene Rubber		383.491 ± 25.588	221.305 ± 116.120	42.29 ± 26.76
				Optical Plastic		378.131 ± 232.700	182.085 ± 50.571	51.85 ± 28.50
				Bare Pine Wood		52.002 ± 26.692	29.364 ± 13.249	43.53 ± 33.83
				Industrial Carpet		278.805 ± 234.893	361.882 ± 65.973	[f]0.00 ± 98.07
				Paper		467.700 ± 99.403	64.740 ± 87.050	86.16 ± 16.52
3	24.99 ± 0.22	46.39 ± 1.06	14 Days	Mild Steel	320	174.907 ± 29.476	114.113 ± 42.250	34.76 ± 24.64
				Neoprene Rubber		383.491 ± 25.588	44.519 ± 8.830	88.39 ± 2.13
				Optical Plastic		378.131 ± 232.700	150.286 ± 99.070	60.26 ± 31.42
				Bare Pine Wood		52.002 ± 26.692	24.654 ± 16.321	52.59 ± 34.81
				Industrial Carpet		278.805 ± 234.893	73.737 ± 57.494	73.55 ± 26.61
				Paper		467.700 ± 99.403	57.613 ± 68.103	87.68 ± 12.97
4	25.95 ± 1.35	72.43 ± 7.20	7 Days	Mild Steel	320	13.244 ± 13.139	2.330 ± 0.890	82.41 ± 16.39
				Neoprene Rubber		217.184 ± 250.392	226.775 ± 139.189	[f]0.00 ± 119.54
				Optical Plastic		18.129 ± 11.914	5.423 ± 7.397	70.08 ± 39.70
				Bare Pine Wood		10.353 ± 4.519	12.848 ± 15.967	[f]0.00 ±143.28
				Industrial Carpet		97.020 ± 178.789	45.245 ± 95.995	53.37 ± 114.87
				Paper		335.223 ± 94.148	1.557 ± 0.068	99.54 ± 0.12
5	25.58 ± 1.07	73.93 ± 5.50	14 Days	Mild Steel	320	13.244 ± 13.139	0.511 ± 0.089	96.14 ± 3.41
				Neoprene Rubber		217.184 ± 250.392	209.061 ± 94.556	3.74 ± 104.49
				Optical Plastic		18.129 ± 11.914	66.174 ± 25.146	[f]0.00 ± 242.88
				Bare Pine Wood		10.353 ± 4.519	16.577 ± 12.620	[f]0.00 ± 123.17
				Industrial Carpet		97.020 ± 178.789	2.274 ± 0.577	97.66 ± 3.82
				Paper		335.223 ± 94.148	12.283 ± 4.807	96.34 ± 1.55

[a] Data are expressed as the mean (± SD) of the mass of toxin recovered on five replicate individual samples, and attenuation (percent reduction ± CI).
[b] Positive Controls = samples inoculated, not attenuated.
[c] Test Coupons = samples inoculated, attenuated.
[d] CI = confidence interval (± 1.96 × standard error [SE]).
[f] As a result of high variability, negative value reported as "0".

Table A-2. Attenuation of Crude Ricin Toxin[a] (Continued)

Test Number	Test Parameters			Material	Inoculum (µg/coupon)	Mean Recovered Ricin ± SD (µg/coupon)		%Reduction ± CI
	Temp °C	%RH	Time			Positive Control	Test Coupon	
6	29.70 ± 0.16	48.09 ± 1.73	7 Days	Mild Steel	320	258.204 ± 50.424	124.811 ± 83.041	51.66 ± 29.38
				Neoprene Rubber		510.325 ± 107.271	59.947 ± 62.351	88.25 ± 10.93
				Optical Plastic		320.984 ± 94.865	12.334 ± 14.107	96.16 ± 3.98
				Bare Pine Wood		49.605 ± 17.156	44.749 ± 31.096	9.79 ± 61.38
				Industrial Carpet		625.155 ± 137.038	172.604 ± 222.875	72.39 ± 31.70
				Paper		33.410 ± 23.788	207.509 ± 181.400	[f]0.00 ± 613.79
7	30.03 ± 0.42	45.61 ± 3.52	14 Days	Mild Steel	320	258.204 ± 50.424	43.730 ± 41.418	83.06 ± 14.36
				Neoprene Rubber		510.325 ± 107.271	188.890 ± 202.132	62.99 ± 35.38
				Optical Plastic		320.984 ± 94.865	70.771 ± 39.473	77.95 ± 12.20
				Bare Pine Wood		49.605 ± 17.156	16.510 ± 7.833	66.72 ± 17.13
				Industrial Carpet		625.155 ± 137.038	39.462 ± 19.850	93.69 ± 3.04
				Paper		33.410 ± 23.788	25.413 ± 28.179	23.94 ± 87.86
8	30.31 ± 0.21	72.96 ± 1.16	14 Days	Mild Steel	320	99.014 ± 58.918	6.000 ± 2.856	93.94 ± 4.05
				Neoprene Rubber		126.407 ± 198.446	300.259 ± 82.964	[f]0.00 ± 331.89
				Optical Plastic		193.05 ± 133.936	201.572 ± 148.937	[f]0.00 ± 92.76
				Bare Pine Wood		13.848 ± 4.799	18.291 ± 8.236	[f]0.00 ± 65.79
				Industrial Carpet		85.453 ± 35.247	49.905 ± 34.604	41.60 ± 41.30
				Paper		31.675 ± 12.263	7.440 ± 0.312	76.51 ± 8.02
9	20.41 ± 0.23	45.22 ± 1.47	7 Days	Mild Steel	320	151.614 ± 36.500	241.433 ± 47.482	[f]0.00 ± 43.39
				Neoprene Rubber		448.312 ± 162.928	481.273 ± 106.865	[f]0.00 ± 40.08
				Optical Plastic		311.765 ± 249.232	359.029 ± 23.014	[f]0.00 ± 80.95
				Bare Pine Wood		31.458 ± 8.052	88.154 ± 27.781	[f]0.00 ± 99.72
				Industrial Carpet		343.024 ± 109.283	392.103 ± 147.069	[f]0.00 ± 49.31
				Paper		337.619 ± 162.887	376.756 ± 65.259	[f]0.00 ± 50.14
10	20.59 ± 0.29	45.26 ± 1.40	14 Days	Mild Steel	320	151.614 ± 36.500	92.125 ± 29.819	39.24 ± 21.48
				Neoprene Rubber		448.312 ± 162.928	497.513 ± 147.321	[f]0.00 ± 45.60
				Optical Plastic		311.765 ± 249.232	474.946 ± 96.155	[f]0.00 ± 110.12
				Bare Pine Wood		31.458 ± 8.052	52.176 ± 18.768	[f]0.00 ± 64.18
				Industrial Carpet		343.024 ± 109.283	407.41 ± 51.057	[f]0.00 ± 35.64
				Paper		337.619 ± 162.887	333.034 ± 34.917	1.36 ± 42.69
11	20.80 ± 0.55	75.28 ± 1.05	7 Days	Mild Steel	320	507.441 ± 61.225	58.110 ± 15.498	88.55 ± 2.94
				Neoprene Rubber		651.201 ± 123.619	461.810 ± 206.330	29.08 ± 30.18
				Optical Plastic		488.828 ± 280.165	352.736 ± 53.685	27.84 ± 37.51
				Bare Pine Wood		124.416 ± 28.624	46.347 ± 10.805	62.75 ± 10.69
				Industrial Carpet		843.428 ± 221.927	585.063 ± 306.596	30.63 ± 35.65
				Paper		533.712 ± 34.554	280.187 ± 97.739	47.50 ± 16.33
12	20.84 ± 0.80	72.43 ± 5.14	14 Days	Mild Steel	320	507.441 ± 61.225	27.470 ± 8.556	94.59 ± 1.58
				Neoprene Rubber		651.201 ± 123.619	364.574 ± 79.840	44.02 ± 14.22
				Optical Plastic		488.828 ± 280.165	429.044 ± 17.369	12.23 ± 44.20
				Bare Pine Wood		124.416 ± 28.624	37.655 ± 12.467	69.73 ± 10.70
				Industrial Carpet		843.428 ± 221.927	428.759 ± 43.394	49.16 ± 12.56
				Paper		533.712 ± 34.554	327.182 ± 68.640	38.70 ± 11.80
13	19.80 ± 0.53	44.81 ± 4.03	21 Days	Mild Steel	320	327.676 ± 81.598	127.425 ± 60.295	61.11 ± 18.23
				Neoprene Rubber		453.217 ± 55.596	417.753 ± 93.573	7.82 ± 20.63
				Optical Plastic		427.380 ± 21.900	423.727 ± 46.716	0.85 ± 10.57
				Bare Pine Wood		95.760 ± 13.843	37.268 ± 11.525	61.08 ± 11.65
				Industrial Carpet		655.811 ± 167.921	36.078 ± 20.466	94.50 ± 3.00
				Paper		421.922 ± 51.930	139.322 ± 50.402	66.98 ± 11.06

[a] Data are expressed as the mean (± SD) of the mass of toxin recovered on five replicate individual samples, and attenuation (percent reduction ± CI).

[b] Positive Controls = samples inoculated, not attenuated.

[c] Test Coupons = samples inoculated, attenuated.

[d] CI = confidence interval (± 1.96 × standard error [SE]).

[f] As a result of high variability, negative value reported as "0".

Table A-2. Attenuation of Crude Ricin Toxin[a] (Continued)

Test Number	Test Parameters			Material	Inoculum (µg/coupon)	Mean Recovered Ricin ± SD (µg/coupon)		%Reduction ± CI
	Temp °C	%RH	Time			Positive Control	Test Coupon	
14	19.82 ± 0.47	45.06 ± 4.18	28 Days	Mild Steel	320	327.676 ± 81.598	124.88 ± 39.143	61.89 ± 13.37
				Neoprene Rubber		453.217 ± 55.596	141.939 ± 92.140	68.68 ± 18.14
				Optical Plastic		427.380 ± 21.900	36.307 ± 29.923	91.50 ± 6.15
				Bare Pine Wood		95.760 ± 13.843	27.814 ± 10.679	70.95 ± 10.45
				Industrial Carpet		655.811 ± 167.921	157.749 ± 88.907	75.95 ± 13.05
				Paper		421.922 ± 51.930	75.495 ± 35.585	82.11 ± 7.64
15	50.26 ± 0.24	21.05 ± 2.67	Multiple	Mild Steel TO	320	345.189 ± 30.327	NA	NA
				Mild Steel +6 h		NA[g]	213.405 ± 33.609	38.18 ± 9.77
				Mild Steel +24 h			124.504 ± 56.319	63.93 ± 14.57
				Mild Steel +30 h			258.024 ± 63.255	25.25 ± 17.06
				Mild Steel +48 h			213.280 ± 35.543	38.21 ± 10.20
				Mild Steel +72 h			185.231 ± 53.836	46.34 ± 14.28
				Mild Steel +96 h			210.139 ± 92.689	39.12 ± 24.00
16	39.95 ± 0.43	26.62 ±3.31	Multiple	Mild Steel TO	320	326.962 ± 35.215	NA	NA
				Mild Steel +48 h		NA	256.930 ± 62.722	21.42 ± 18.38
				Mild Steel +72 h			308.604 ± 48.759	5.61 ± 15.82
				Mild Steel +96 h			331.161 ± 36.136	0.00 ± 13.61
				Mild Steel +120 h			295.797 ± 40.980	9.53 ± 13.92
				Mild Steel +144 h			196.584 ± 27.157	39.88 ± 9.23
				Mild Steel +168 h			244.837 ± 34.795	25.12 ± 11.70
17	50.41 ± 0.72	19.79 ± 2.20	Multiple	Mild Steel TO	320	358.674 ± 48.186	NA	NA
				Mild Steel +48 h		NA	163.327 ± 82.796	54.42 ± 20.93
				Mild Steel +72 h			184.327 ± 27.940	48.61 ± 9.12
				Mild Steel +96 h			145.681 ± 149.787	59.38 ± 36.92
				Mild Steel +120 h			170.249 ± 97.208	52.53 ± 24.40
				Mild Steel +144 h			171.211 ± 37.702	52.27 ± 10.79
				Mild Steel +168 h			6.661 ± 3.393	98.14 ± 0.86
18	40.37 ± 0.49	21.56 ± 2.48	Multiple	Mild Steel T0	320	200.575 ± 44.939	NA	NA
				Mild Steel +3 Days		NA	279.570 ± 105.000	0.00 ± 53.43
				Mild Steel +4 Days			216.049 ± 97.292	0.00 ± 47.49
				Mild Steel +5 Days			321.147 ± 381.795	0.00 ± 169.79
				Mild Steel +6 Days			48.251 ± 17.368	75.94 ± 8.94
				Mild Steel +7 Days			104.119 ± 103.739	48.09 ± 46.47
				Mild Steel +10 Days			229.036 ± 135.947	0.00 ± 63.50
				Mild Steel +11 Days			49.024 ± 58.435	75.56 ± 25.98
				Mild Steel +12 Days			94.181 ± 100.927	53.04 ± 45.06
				Mild Steel +13 Days			41.227 ± 60.082	79.45 ± 26.57
				Mild Steel +14 Days			104.536 ± 31.373	47.88 ± 17.11

[a] Data are expressed as the mean (± SD) of the mass of toxin observed on five individual samples, and attenuation (percent reduction ± CI).

[b] Positive Controls = samples inoculated, not attenuated.

[c] Test Coupons = samples inoculated, attenuated.

[d] CI = confidence interval (± 1.96 × standard error [SE]).

[f] As a result of high variability, negative value reported as "0".

[g] NA = Not applicable.

Appendix B
Detailed Statistical Analysis

Introduction

This report contains the statistical analysis of ricin percent reduction data over time generated from pure and crude ricin preparations on mild steel, rubber, plastic, wood, carpet, and paper at various temperatures and percent humidity.

Results

The ANOVA models fitted with effects for material, ricin type and time were fitted to each combination of temperature and relative humidity. These models were used to generate estimates and 95 percent confidence intervals for the percent reduction at each combination of material, ricin type and time. Tables B-1a through B-1i present these results and a p-value testing whether the percent reduction was significantly different from zero. Figures B-1a through B-1i plot the descriptive statistics and the individual percent reductions by material, ricin type and time point for each combination of temperature and relative humidity. Figures B-2a through B-2f plot the descriptive statistics and the individual percent reductions by material, ricin type and relative humidity for each combination of temperature and time.

The same ANOVA models were used to test for significant differences between different combinations of material, ricin type, and time. The tables of results comparing the percent reductions for varying conditions show unadjusted and Tukey's adjusted p-values for each comparison of interest. An up or down arrow is included indicating whether the specific level of the factor had a percent reduction greater or less than that for the level being compared. Given the large number of comparisons, it is recommended that the Tukey's adjusted p-values be used for interpreting the results. If the unadjusted p-values are used, it is likely that there would be a large number of significant comparisons from random variability when no true difference between the conditions exists.

Tables B-2a through B-2g present results from the models testing for significant differences among the materials for each combination of temperature, humidity, time, and ricin type. Tables B-3a through B-3g present results from the models testing for significant differences among the time points for each combination of temperature, humidity, material, and ricin type. Tables B-4a through B-4h present results from the models testing for significant differences among the ricin types for each combination of temperature, humidity, material, and time point.

For each ricin type and material, separate ANOVA models were fitted with effects for temperature, relative humidity, and time. Tables B-5a and B-5b present results from this second set of models testing for significant differences among the different levels of humidity for each combination of temperature and time. For each ricin type and material, Tables B-6a through B-

6g present results from this second set of models testing for significant differences among the different temperatures for each combination of relative humidity and time.

Table B-1a. Mean Percent Reduction, 95% Confidence Interval, and P-value for Crude Ricin, by Material and Time at 20 °C and 45% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
20	45	Crude	Carpet	7 Days	-8.12 (-82.82, 36.06)	0.7697
				14 Days	-18.07 (-99.65, 30.17)	0.5335
				21 Days	95.39 (92.20, 97.27)	<0.0001*
				28 Days	79.22 (64.87, 87.71)	<0.0001*
			Paper	7 Days	-10.37 (-86.62, 34.73)	0.7115
				14 Days	1.78 (-66.08, 41.92)	0.9462
				21 Days	68.77 (47.19, 81.53)	<0.0001*
				28 Days	83.57 (72.22, 90.29)	<0.0001*
			Plastic	7 Days	-10.47 (-86.80, 34.67)	0.7089
				14 Days	-50.24 (-154.05, 11.15)	0.1280
				21 Days	8.11 (-55.38, 45.66)	0.7512
				28 Days	89.81 (82.77, 93.97)	<0.0001*
			Rubber	7 Days	-5.33 (-78.11, 37.71)	0.8455
				14 Days	-7.26 (-81.37, 36.57)	0.7926
				21 Days	10.08 (-52.04, 46.82)	0.6902
				28 Days	73.59 (55.34, 84.38)	<0.0001*
			Steel	7 Days	-56.38 (-164.42, 7.52)	0.0948
				14 Days	41.62 (1.29, 65.48)	0.0447*
				21 Days	64.78 (40.45, 79.17)	0.0001*
				28 Days	63.59 (38.44, 78.47)	0.0002*
			Wood	7 Days	-169.02 (-354.90, -59.10)	0.0003*
				14 Days	-56.17 (-164.07, 7.64)	0.0958
				21 Days	62.28 (36.22, 77.69)	0.0003*
				28 Days	72.50 (53.50, 83.74)	<0.0001*

*****Significant at the 0.05 level.

Table B-1b. Mean Percent Reduction, 95% Confidence Interval, and P-value for Pure Ricin, by Material and Time at 20 °C and 45% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
20	45	Pure	Carpet	7 Days	47.37 (11.00, 68.87)	0.0169*
				14 Days	93.84 (89.59, 96.36)	<0.0001*
				21 Days	74.67 (57.17, 85.02)	<0.0001*
				28 Days	90.74 (84.35, 94.52)	<0.0001*
			Paper	7 Days	46.82 (10.08, 68.55)	0.0187*
				14 Days	44.91 (6.84, 67.42)	0.0263*
				21 Days	50.57 (16.42, 70.77)	0.0088*
				28 Days	68.28 (46.36, 81.24)	<0.0001*
			Plastic	7 Days	92.48 (87.29, 95.55)	<0.0001*
				14 Days	78.63 (63.86, 87.36)	<0.0001*
				21 Days	87.19 (78.34, 92.42)	<0.0001*
				28 Days	91.83 (86.18, 95.17)	<0.0001*
			Rubber	7 Days	12.46 (-48.02, 48.23)	0.6178
				14 Days	62.54 (36.66, 77.85)	0.0003*
				21 Days	86.33 (76.89, 91.92)	<0.0001*
				28 Days	89.28 (81.88, 93.66)	<0.0001*
			Steel	7 Days	75.77 (59.03, 85.67)	<0.0001*
				14 Days	97.08 (95.07, 98.28)	<0.0001*
				21 Days	92.75 (87.74, 95.71)	<0.0001*
				28 Days	99.00 (98.30, 99.41)	<0.0001*
			Wood	7 Days	-25.36 (-111.98, 25.86)	0.3971
				14 Days	-44.21 (-143.85, 14.71)	0.1708
				21 Days	68.12 (46.09, 81.15)	<0.0001*
				28 Days	42.91 (3.46, 66.24)	0.0366*

*****Significant at the 0.05 level.

Table B-1c. Mean Percent Reduction, 95% Confidence Interval, and P-value by Ricin Type, Material, and Time at 20 °C and 75% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
20	75	Crude	Carpet	7 Days	37.61 (12.43, 55.55)	0.0069*
				14 Days	49.38 (28.94, 63.94)	0.0001*
			Paper	7 Days	50.04 (29.87, 64.41)	<0.0001*
				14 Days	39.77 (15.45, 57.09)	0.0038*
			Plastic	7 Days	48.16 (27.24, 63.07)	0.0002*
				14 Days	34.11 (7.51, 53.06)	0.0164*
			Rubber	7 Days	33.51 (6.67, 52.63)	0.0188*
				14 Days	45.01 (22.81, 60.82)	0.0007*
			Steel	7 Days	88.87 (84.37, 92.07)	<0.0001*
				14 Days	94.76 (92.64, 96.27)	<0.0001*
			Wood	7 Days	63.46 (48.71, 73.97)	<0.0001*
				14 Days	71.09 (59.42, 79.41)	<0.0001*
		Pure	Carpet	7 Days	29.85 (1.53, 50.02)	0.0407*
				14 Days	64.17 (49.71, 74.47)	<0.0001*
			Paper	7 Days	5.38 (-32.81, 32.59)	0.7466
				14 Days	57.31 (40.07, 69.58)	<0.0001*
			Plastic	7 Days	-12.54 (-57.97, 19.82)	0.4907
				14 Days	65.45 (51.50, 75.38)	<0.0001*
			Rubber	7 Days	12.91 (-22.25, 37.95)	0.4205
				14 Days	65.26 (51.23, 75.25)	<0.0001*
			Steel	7 Days	97.78 (96.89, 98.42)	<0.0001*
				14 Days	98.58 (98.01, 98.99)	<0.0001*
			Wood	7 Days	-305.49 (-469.17, -188.88)	<0.0001*
				14 Days	-50.87 (-111.77, -7.48)	0.0180*

***Significant at the 0.05 level.

Table B-1d. Mean Percent Reduction, 95% Confidence Interval, and P-value by Ricin Type, Material, and Time at 25 °C and 45% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
25	45	Crude	Carpet	7 Days	-28.16 (-131.11, 28.93)	0.4056
				14 Days	81.05 (65.82, 89.49)	<0.0001*
			Paper	7 Days	93.84 (88.88, 96.58)	<0.0001*
				14 Days	95.15 (91.25, 97.31)	<0.0001*
			Plastic	7 Days	52.01 (13.46, 73.39)	0.0152*
				14 Days	72.19 (49.85, 84.58)	<0.0001*
			Rubber	7 Days	54.53 (18.00, 74.78)	0.0093*
				14 Days	88.61 (79.46, 93.68)	<0.0001*
			Steel	7 Days	40.22 (-7.81, 66.85)	0.0865
				14 Days	38.66 (-10.61, 65.99)	0.1031
			Wood	7 Days	47.72 (5.72, 71.01)	0.0315*
				14 Days	60.54 (28.84, 78.12)	0.0023*
		Pure	Carpet	7 Days	43.85 (-1.26, 68.86)	0.0550
				14 Days	95.43 (91.75, 97.46)	<0.0001*
			Paper	7 Days	61.19 (30.02, 78.48)	0.0019*
				14 Days	95.40 (91.71, 97.45)	<0.0001*
			Plastic	7 Days	82.89 (69.15, 90.51)	<0.0001*
				14 Days	95.87 (92.56, 97.71)	<0.0001*
			Rubber	7 Days	17.61 (-48.58, 54.31)	0.5159
				14 Days	82.86 (69.09, 90.49)	<0.0001*
			Steel	7 Days	88.68 (79.58, 93.72)	<0.0001*
				14 Days	96.74 (94.12, 98.19)	<0.0001*
			Wood	7 Days	-35.80 (-144.90, 24.69)	0.3055
				14 Days	64.19 (35.43, 80.14)	0.0008*

***Significant at the 0.05 level.

Table B-1e. Mean Percent Reduction, 95% Confidence Interval, and P-value by Ricin Type, Material, and Time at 25 °C and 75% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
25	75	Crude	Carpet	7 Days	94.16 (88.38, 97.06)	<0.0001*
				14 Days	97.71 (95.44, 98.85)	<0.0001*
			Paper	7 Days	99.54 (99.08, 99.77)	<0.0001*
				14 Days	96.59 (93.22, 98.29)	<0.0001*
			Plastic	7 Days	84.44 (69.05, 92.18)	<0.0001*
				14 Days	-243.63 (-583.68, -72.71)	0.0006*
			Rubber	7 Days	12.65 (-73.79, 56.10)	0.6972
				14 Days	12.89 (-73.31, 56.22)	0.6914
			Steel	7 Days	83.52 (67.21, 91.72)	<0.0001*
				14 Days	96.19 (92.43, 98.09)	<0.0001*
			Wood	7 Days	49.83 (0.18, 74.78)	0.0494*
				14 Days	-25.49 (-149.68, 36.92)	0.5139
		Pure	Carpet	7 Days	73.49 (47.26, 86.68)	0.0002*
				14 Days	94.06 (88.17, 97.01)	<0.0001*
			Paper	7 Days	67.31 (34.97, 83.57)	0.0017*
				14 Days	95.03 (90.12, 97.50)	<0.0001*
			Plastic	7 Days	24.98 (-49.25, 62.30)	0.4089
				14 Days	58.55 (17.53, 79.17)	0.0127*
			Rubber	7 Days	77.75 (55.74, 88.82)	<0.0001*
				14 Days	93.35 (86.76, 96.66)	<0.0001*
			Steel	7 Days	99.88 (99.77, 99.94)	<0.0001*
				14 Days	99.95 (99.91, 99.98)	<0.0001*
			Wood	7 Days	70.33 (40.97, 85.09)	0.0007*
				14 Days	89.41 (78.94, 94.68)	<0.0001*

*Significant at the 0.05 level.

Table B-1f. Mean Percent Reduction, 95% Confidence Interval, and P-value by Ricin Type, Material, and Time at 30 °C and 45% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
30	45	Crude	Carpet	7 Days	92.93 (82.05, 97.21)	<0.0001*
				14 Days	94.34 (85.63, 97.77)	<0.0001*
			Paper	7 Days	-134.36 (-494.83, 7.67)	0.0726
				14 Days	64.05 (8.76, 85.84)	0.0317*
			Plastic	7 Days	95.90 (89.60, 98.39)	<0.0001*
				14 Days	88.92 (71.88, 95.63)	<0.0001*
			Rubber	7 Days	92.13 (80.02, 96.90)	<0.0001*
				14 Days	82.57 (55.77, 93.13)	0.0003*
			Steel	7 Days	59.75 (-2.17, 84.14)	0.0554
				14 Days	87.64 (68.63, 95.13)	<0.0001*
			Wood	7 Days	36.58 (-60.98, 75.01)	0.3343
				14 Days	69.55 (22.72, 88.00)	0.0129*
		Pure	Carpet	7 Days	99.50 (98.73, 99.80)	<0.0001*
				14 Days	61.38 (1.99, 84.79)	0.0454*
			Paper	7 Days	99.93 (99.82, 99.97)	<0.0001*
				14 Days	93.29 (82.96, 97.36)	<0.0001*
			Plastic	7 Days	78.80 (46.19, 91.65)	0.0013*
				14 Days	92.02 (79.74, 96.86)	<0.0001*
			Rubber	7 Days	92.00 (79.68, 96.85)	<0.0001*
				14 Days	81.79 (53.79, 92.83)	0.0005*
			Steel	7 Days	84.37 (60.34, 93.84)	0.0001*
				14 Days	92.10 (79.96, 96.89)	<0.0001*
			Wood	7 Days	89.08 (72.29, 95.70)	<0.0001*
				14 Days	30.54 (-76.29, 72.64)	0.4392

*****Significant at the 0.05 level.

Table B-1g. Mean Percent Reduction, 95% Confidence Interval, and P-value by Ricin Type, Material, and Time at 30 °C and 75% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
30	75	Crude	Carpet	7 Days	3.71 (-103.85, 54.52)	0.9205
				14 Days	54.46 (3.59, 78.49)	0.0400*
			Paper	7 Days	94.33 (88.00, 97.32)	<0.0001*
				14 Days	76.53 (50.31, 88.91)	0.0002*
			Plastic	7 Days	56.45 (7.80, 79.43)	0.0302*
				14 Days	8.61 (-93.49, 56.83)	0.8122
			Rubber	7 Days	31.40 (-45.23, 67.60)	0.3211
				14 Days	-131.30 (-389.70, -9.25)	0.0288*
			Steel	7 Days	98.42 (96.66, 99.26)	<0.0001*
				14 Days	94.59 (88.54, 97.44)	<0.0001*
			Wood	7 Days	54.57 (3.83, 78.54)	0.0394*
				14 Days	-21.79 (-157.84, 42.47)	0.6031
		Pure	Carpet	7 Days	82.29 (62.50, 91.63)	<0.0001*
				14 Days	72.57 (41.93, 87.04)	0.0009*
			Paper	7 Days	94.81 (89.01, 97.55)	<0.0001*
				14 Days	75.62 (48.38, 88.48)	0.0003*
			Plastic	7 Days	95.77 (91.05, 98.00)	<0.0001*
				14 Days	71.48 (39.62, 86.53)	0.0013*
			Rubber	7 Days	75.66 (48.46, 88.50)	0.0003*
				14 Days	60.78 (16.97, 81.48)	0.0150*
			Steel	7 Days	94.78 (88.96, 97.54)	<0.0001*
				14 Days	99.90 (99.80, 99.95)	<0.0001*
			Wood	7 Days	70.08 (36.67, 85.87)	0.0019*
				14 Days	-232.49 (-603.92, -57.05)	0.0020*

***Significant at the 0.05 level.

Table B-1h. Mean Percent Reduction, 95% Confidence Interval, and P-value for Steel, by Ricin Type and Time at 40 °C and 20% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
40	20	Crude	Steel	48 Hours	23.16 (-74.05, 66.08)	0.5239
				72 Hours	-10.32 (-96.67, 38.12)	0.7366
				96 Hours	0.90 (-76.68, 44.41)	0.9754
				120 Hours	14.51 (-52.41, 52.04)	0.5916
				144 Hours	63.66 (35.22, 79.62)	0.0008*
				7 Days	55.37 (20.44, 74.97)	0.0067*
				10 Days	41.54 (-32.42, 74.19)	0.1955
				11 Days	83.82 (63.36, 92.86)	<0.0001*
				12 Days	79.21 (52.92, 90.82)	0.0002*
				13 Days	91.70 (81.21, 96.34)	<0.0001*
				14 Days	49.96 (-13.35, 77.91)	0.0960
		Pure	Steel	48 Hours	90.51 (78.51, 95.81)	<0.0001*
				72 Hours	93.51 (85.30, 97.14)	<0.0001*
				96 Hours	94.80 (88.22, 97.70)	<0.0001*
				120 Hours	96.86 (92.89, 98.61)	<0.0001*
				144 Hours	97.00 (93.20, 98.68)	<0.0001*
				7 Days	96.87 (92.90, 98.62)	<0.0001*

*****Significant at the 0.05 level.

Table B-1i. Mean Percent Reduction, 95% Confidence Interval, and P-value for Steel, by Ricin Type and Time at 50 °C and 20% Humidity.

Temperature (°C)	Humidity (%)	Ricin Type	Material	Time	Percent Reduction Estimate and 95% Confidence Interval	P-Value
50	20	Crude	Steel	6 Hours	38.74 (3.43, 61.14)	0.0351*
				24 Hours	67.94 (49.47, 79.66)	<0.0001*
				30 Hours	31.53 (-7.93, 56.57)	0.1018
				48 Hours	50.99 (32.38, 64.48)	<0.0001*
				72 Hours	48.63 (29.13, 62.77)	<0.0001*
				96 Hours	64.69 (51.28, 74.40)	<0.0001*
				120 Hours	60.45 (37.66, 74.91)	0.0001*
				144 Hours	53.24 (26.29, 70.34)	0.0013*
				7 Days	98.29 (97.30, 98.91)	<0.0001*
		Pure	Steel	6 Hours	63.36 (42.24, 76.75)	<0.0001*
				24 Hours	86.01 (77.95, 91.13)	<0.0001*
				30 Hours	90.96 (85.76, 94.27)	<0.0001*
				48 Hours	95.55 (93.85, 96.77)	<0.0001*
				72 Hours	98.10 (97.38, 98.62)	<0.0001*
				96 Hours	98.60 (98.07, 98.99)	<0.0001*
				120 Hours	99.12 (98.62, 99.44)	<0.0001*
				144 Hours	99.17 (98.69, 99.47)	<0.0001*
				7 Days	99.93 (99.88, 99.95)	<0.0001*

*****Significant at the 0.05 level.

Table B-2a. Unadjusted and Tukey Adjusted P-values Comparing Materials for 20 °C and 45% Humidity, Crude Ricin by Time.

					Unadjusted P-Value					Tukey Adjusted P-Value				
Ricin Type	**Temperature (°C)**	**Humidity (%)**	**Time**	**Material**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**
Crude	20	45	7 Days	Paper	0.9565					1.0000				
				Plastic	0.9545	0.9980				1.0000	1.0000			
				Rubber	0.9448	0.9015	0.8995			1.0000	1.0000	1.0000		
				Steel	0.3284	0.3560	0.3573	0.2954		1.0000	1.0000	1.0000	1.0000	
				Wood	0.0164* ↓	0.0190* ↓	0.0191* ↓	0.0136* ↓	0.1514	0.9525	0.9654	0.9659	0.9316	1.0000
			14 Days	Paper	0.6255					1.0000				
				Plastic	0.5231	0.2605				1.0000	1.0000			
				Rubber	0.7991	0.8153	0.3721			1.0000	1.0000	1.0000		
				Steel	0.0630	0.1688	0.0129* ↑	0.1079		0.9995	1.0000	0.9243	1.0000	
				Wood	0.4587	0.2197	0.9183	0.3198	0.0097* ↓	1.0000	1.0000	1.0000	1.0000	0.8800
			21 Days	Paper	<0.0001* ↓					0.0009* ↓				
				Plastic	<0.0001* ↓	0.0046* ↓				<0.0001* ↓	0.7179			
				Rubber	<0.0001* ↓	0.0055* ↓	0.9540			<0.0001* ↓	0.7607	1.0000		
				Steel	<0.0001* ↓	0.7502	0.0117* ↑	0.0137* ↑		0.0002* ↓	1.0000	0.9100	0.9318	
				Wood	<0.0001* ↓	0.6169	0.0191* ↑	0.0221* ↑	0.8556	<0.0001* ↓	1.0000	0.9657	0.9762	1.0000
			28 Days	Paper	0.5335					1.0000				
				Plastic	0.0600	0.2062				0.9994	1.0000			
				Rubber	0.5249	0.2089	0.0122* ↓			1.0000	1.0000	0.9171		
				Steel	0.1380	0.0359* ↓	0.0009* ↓	0.3950		1.0000	0.9945	0.3104	1.0000	
				Wood	0.4575	0.1728	0.0091* ↓	0.9145	0.4572	1.0000	1.0000	0.8679	1.0000	1.0000

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates the mean percent reduction of the row material is significantly greater than the column material**,** while "↓" indicates the mean percent reduction of the row material is significantly less than the column material.

Table B-2b. Unadjusted and Tukey Adjusted P-values Comparing Materials for 20 °C and 45% Humidity, Pure Ricin by Time.

					Unadjusted P-Value					Tukey Adjusted P-Value				
Ricin Type	**Temperature (°C)**	**Humidity (%)**	**Time**	**Material**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**
Pure	20	45	7 Days	Paper	0.9782					1.0000				
				Plastic	<0.0001* ↑	<0.0001* ↑				0.0006* ↑	0.0005* ↑			
				Rubber	0.1784	0.1873	<0.0001* ↓			1.0000	1.0000	<0.0001* ↓		
				Steel	0.0408* ↑	0.0382* ↑	0.0022* ↓	0.0008* ↑		0.9966	0.9956	0.5201	0.2914	
				Wood	0.0223* ↓	0.0239* ↓	<0.0001* ↓	0.3415	<0.0001* ↓	0.9765	0.9805	<0.0001* ↓	1.0000	0.0161* ↓
			14 Days	Paper	<0.0001* ↓					<0.0001* ↓				
				Plastic	0.0011* ↓	0.0127* ↑				0.3649	0.9227			
				Rubber	<0.0001* ↓	0.3070	0.1378			0.0029* ↓	1.0000	1.0000		
				Steel	0.0486* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		0.9984	<0.0001* ↑	0.0003* ↑	<0.0001* ↑	
				Wood	<0.0001* ↓	0.0114* ↓	<0.0001* ↓	0.0004* ↓	<0.0001* ↓	<0.0001* ↓	0.9065	0.0009* ↓	0.1945	<0.0001* ↓
			21 Days	Paper	0.0774					0.9999				
				Plastic	0.0718	0.0004* ↑				0.9998	0.1915			
				Rubber	0.1030	0.0008* ↑	0.8637			1.0000	0.2893	1.0000		
				Steel	0.0011* ↑	<0.0001* ↑	0.1325	0.0941		0.3528	0.0008* ↑	1.0000	1.0000	
				Wood	0.5420	0.2457	0.0164* ↓	0.0256* ↓	0.0001* ↓	1.0000	1.0000	0.9523	0.9839	0.0712
			28 Days	Paper	0.0013* ↓					0.3906				
				Plastic	0.7409	0.0004* ↑				1.0000	0.1840			
				Rubber	0.6984	0.0044* ↑	0.4729			1.0000	0.7054	1.0000		
				Steel	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	
				Wood	<0.0001* ↓	0.1203	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	0.0025* ↓	1.0000	0.0006* ↓	0.0120* ↓	<0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates the mean percent reduction of the row material is significantly greater than the column material**,** while "↓" indicates the mean percent reduction of the row material is significantly less than the column material.

Table B-2c. Unadjusted and Tukey Adjusted P-values Comparing Materials for 20 °C and 75% Humidity, Crude and Pure Ricin by Time.

					Unadjusted P-Value					Tukey Adjusted P-Value				
Ricin Type	**Temperature (°C)**	**Humidity (%)**	**Time**	**Material**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**
Crude	20	75	7 Days	Paper	0.3602					1.0000				
				Plastic	0.4452	0.8789				1.0000	1.0000			
				Rubber	0.7926	0.2397	0.3055			1.0000	0.9999	1.0000		
				Steel	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	
				Wood	0.0292* ↑	0.1986	0.1510	0.0150* ↑	<0.0001* ↓	0.8530	0.9998	0.9988	0.6890	0.0008* ↓
			14 Days	Paper	0.4735					1.0000				
				Plastic	0.2780	0.7111				1.0000	1.0000			
				Rubber	0.7325	0.7071	0.4561			1.0000	1.0000	1.0000		
				Steel	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	
				Wood	0.0225* ↑	0.0031* ↑	0.0010* ↑	0.0091* ↑	<0.0001* ↓	0.7943	0.2928	0.1256	0.5526	<0.0001* ↓
Pure	20	75	7 Days	Paper	0.2187					0.9999				
				Plastic	0.0533	0.4743				0.9501	1.0000			
				Rubber	0.3729	0.7324	0.2912			1.0000	1.0000	1.0000		
				Steel	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	
				Wood	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	0.0002* ↓	<0.0001* ↓	<0.0001* ↓
			14 Days	Paper	0.4699					1.0000				
				Plastic	0.8810	0.3834				1.0000	1.0000			
				Rubber	0.8989	0.3958	0.9820			1.0000	1.0000	1.0000		
				Steel	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	
				Wood	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	0.0002* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates the mean percent reduction of the row material is significantly greater than the column material**,** while "↓" indicates the mean percent reduction of the row material is significantly less than the column material.

Table B-2d. Unadjusted and Tukey Adjusted P-values Comparing Materials for 25 °C and45% Humidity, Crude and Pure Ricin by Time.

					Unadjusted P-Value					Tukey Adjusted P-Value				
Ricin Type	**Temperature (°C)**	**Humidity (%)**	**Time**	**Material**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**
Crude	25	45	7 Days	Paper	<0.0001* ↑					<0.0001* ↑				
				Plastic	0.0215* ↑	<0.0001* ↓				0.7826	0.0010* ↓			
				Rubber	0.0154* ↑	<0.0001* ↓	0.8982			0.6969	0.0016* ↓	1.0000		
				Steel	0.0726	<0.0001* ↓	0.6022	0.5164		0.9772	0.0001* ↓	1.0000	1.0000	
				Wood	0.0354* ↑	<0.0001* ↓	0.8389	0.7405	0.7503	0.8903	0.0004* ↓	1.0000	1.0000	1.0000
			14 Days	Paper	0.0016* ↑					0.1880				
				Plastic	0.3638	<0.0001* ↓				1.0000	0.0136* ↓			
				Rubber	0.2283	0.0449* ↓	0.0362* ↑			0.9999	0.9286	0.8943		
				Steel	0.0063* ↓	<0.0001* ↓	0.0627	0.0001* ↓		0.4535	<0.0001* ↓	0.9662	0.0222* ↓	
				Wood	0.0841	<0.0001* ↓	0.4068	0.0039* ↓	0.2964	0.9854	0.0006* ↓	1.0000	0.3422	1.0000
Pure	25	45	7 Days	Paper	0.3813					1.0000				
				Plastic	0.0057* ↑	0.0541				0.4294	0.9518			
				Rubber	0.3637	0.0762	0.0003* ↓			1.0000	0.9802	0.0505		
				Steel	0.0002* ↑	0.0042* ↑	0.3282	<0.0001* ↑		0.0410* ↑	0.3589	1.0000	0.0018* ↑	
				Wood	0.0381* ↓	0.0036* ↓	<0.0001* ↓	0.2371	<0.0001* ↓	0.9034	0.3272	0.0008* ↓	0.9999	<0.0001* ↓
			14 Days	Paper	0.9901					1.0000				
				Plastic	0.8074	0.7978				1.0000	1.0000			
				Rubber	0.0022* ↓	0.0023* ↓	0.0010* ↓			0.2348	0.2412	0.1325		
				Steel	0.4230	0.4159	0.5767	0.0001* ↑		1.0000	1.0000	1.0000	0.0268* ↑	
				Wood	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	0.0827	<0.0001* ↓	0.0009* ↓	0.0010* ↓	0.0003* ↓	0.9846	<0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates the mean percent reduction of the row material is significantly greater than the column material**,** while "↓" indicates the mean percent reduction of the row material is significantly less than the column material.

Table B-2e. Unadjusted and Tukey Adjusted P-values Comparing Materials for 25 °C and 75% Humidity, Crude and Pure Ricin by Time.

					Unadjusted P-Value					Tukey Adjusted P-Value				
Ricin Type	**Temperature (°C)**	**Humidity (%)**	**Time**	**Material**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**
Crude	25	75	7 Days	Paper	<0.0001* ↑					0.0003* ↑				
				Plastic	0.0485* ↓	<0.0001* ↓				0.9389	<0.0001* ↓			
				Rubber	<0.0001* ↓	<0.0001* ↓	0.0007* ↓			<0.0001* ↓	<0.0001* ↓	0.0943		
				Steel	0.0369* ↓	<0.0001* ↓	0.9066	0.0010* ↑		0.8980	<0.0001* ↓	1.0000	0.1281	
				Wood	<0.0001* ↓	<0.0001* ↓	0.0188* ↓	0.2608	0.0254* ↓	0.0062* ↓	<0.0001* ↓	0.7500	1.0000	0.8223
			14 Days	Paper	0.4216					1.0000				
				Plastic	<0.0001* ↓	<0.0001* ↓				<0.0001* ↓	<0.0001* ↓			
				Rubber	<0.0001* ↓	<0.0001* ↓	0.0062* ↑			<0.0001* ↓	<0.0001* ↓	0.4502		
				Steel	0.3038	0.8211	<0.0001* ↑	<0.0001* ↑		1.0000	1.0000	<0.0001* ↑	<0.0001* ↑	
				Wood	<0.0001* ↓	<0.0001* ↓	0.0426* ↑	0.4582	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	0.9208	1.0000	<0.0001* ↓
Pure	25	75	7 Days	Paper	0.6700					1.0000				
				Plastic	0.0364* ↓	0.0933				0.8953	0.9897			
				Rubber	0.7215	0.4344	0.0149* ↑			1.0000	1.0000	0.6873		
				Steel	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	
				Wood	0.8187	0.8437	0.0614	0.5583	<0.0001* ↓	1.0000	1.0000	0.9643	1.0000	<0.0001* ↓
			14 Days	Paper	0.7152					1.0000				
				Plastic	0.0001* ↓	<0.0001* ↓				0.0257* ↓	0.0076* ↓			
				Rubber	0.8187	0.5526	0.0003* ↑			1.0000	1.0000	0.0519		
				Steel	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	
				Wood	0.2418	0.1259	0.0065* ↑	0.3455	<0.0001* ↓	1.0000	0.9969	0.4612	1.0000	<0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates the mean percent reduction of the row material is significantly greater than the column material**,** while "↓" indicates the mean percent reduction of the row material is significantly less than the column material.

Table B-2f. Unadjusted and Tukey Adjusted P-values Comparing Materials for 30 °C and 45% Humidity, Crude and Pure Ricin by Time.

					Unadjusted P-Value					Tukey Adjusted P-Value				
Ricin Type	**Temperature (°C)**	**Humidity (%)**	**Time**	**Material**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**
Crude	30	45	7 Days	Paper	<0.0001* ↓					0.0002* ↓				
				Plastic	0.4130	<0.0001* ↑				1.0000	<0.0001* ↑			
				Rubber	0.8722	<0.0001* ↑	0.3278			1.0000	0.0004* ↑	1.0000		
				Steel	0.0102* ↓	0.0093* ↑	0.0009* ↓	0.0157* ↓		0.5839	0.5581	0.1157	0.7022	
				Wood	0.0013* ↓	0.0518	<0.0001* ↓	0.0022* ↓	0.4949	0.1627	0.9468	0.0150* ↓	0.2351	1.0000
			14 Days	Paper	0.0064* ↓					0.4607				
				Plastic	0.3140	0.0793				1.0000	0.9825			
				Rubber	0.0934	0.2780	0.4965			0.9898	1.0000	1.0000		
				Steel	0.2421	0.1110	0.8694	0.6059		1.0000	0.9947	1.0000	1.0000	
				Wood	0.0129* ↓	0.8029	0.1310	0.4026	0.1775	0.6473	1.0000	0.9974	1.0000	0.9995
Pure	30	45	7 Days	Paper	0.0045* ↑					0.3741				
				Plastic	<0.0001* ↓	<0.0001* ↓				<0.0001* ↓	<0.0001* ↓			
				Rubber	<0.0001* ↓	<0.0001* ↓	0.1455			0.0125* ↓	<0.0001* ↓	0.9985		
				Steel	<0.0001* ↓	<0.0001* ↓	0.6468	0.3160		0.0003* ↓	<0.0001* ↓	1.0000	1.0000	
				Wood	<0.0001* ↓	<0.0001* ↓	0.3199	0.6410	0.5903	0.0024* ↓	<0.0001* ↓	1.0000	1.0000	1.0000
			14 Days	Paper	0.0098* ↑					0.5716				
				Plastic	0.0195* ↑	0.7949				0.7586	1.0000			
				Rubber	0.2600	0.1360	0.2170			1.0000	0.9979	0.9999		
				Steel	0.0187* ↑	0.8072	0.9872	0.2111		0.7481	1.0000	1.0000	0.9999	
				Wood	0.3786	0.0007* ↓	0.0015* ↓	0.0464* ↓	0.0015* ↓	1.0000	0.0940	0.1810	0.9330	0.1743

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates the mean percent reduction of the row material is significantly greater than the column material**,** while "↓" indicates the mean percent reduction of the row material is significantly less than the column material.

Table B-2g. Unadjusted and Tukey Adjusted P-values Comparing Materials for 30 °C and 70% Humidity, Crude and Pure Ricin by Time.

					Unadjusted P-Value					Tukey Adjusted P-Value				
Ricin Type	**Temperature (°C)**	**Humidity (%)**	**Time**	**Material**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**	**Carpet**	**Paper**	**Plastic**	**Rubber**	**Steel**
Crude	30	75	7 Days	Paper	<0.0001* ↑					0.0002* ↑				
				Plastic	0.1409	0.0002* ↓				0.9982	0.0405* ↓			
				Rubber	0.5273	<0.0001* ↓	0.3972			1.0000	0.0022* ↓	1.0000		
				Steel	<0.0001* ↑	0.0186* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	0.7463	<0.0001* ↑	<0.0001* ↑	
			14 Days	Wood	0.1630	0.0002* ↓	0.9372	0.4424	<0.0001* ↓	0.9992	0.0318* ↓	1.0000	1.0000	<0.0001* ↓
				Paper	0.2179					0.9999				
				Plastic	0.1955	0.0126* ↓				0.9997	0.6410			
				Rubber	0.0030* ↓	<0.0001* ↓	0.0855			0.2913	0.0089* ↓	0.9862		
				Steel	0.0001* ↑	0.0072* ↑	<0.0001* ↑	<0.0001* ↑		0.0240* ↑	0.4908	0.0002* ↑	<0.0001* ↑	
				Wood	0.0687	0.0027* ↓	0.5923	0.2330	<0.0001* ↓	0.9734	0.2686	1.0000	0.9999	<0.0001* ↓
Pure	30	75	7 Days	Paper	0.0238* ↑					0.8074				
				Plastic	0.0086* ↑	0.7013				0.5375	1.0000			
				Rubber	0.5534	0.0047* ↓	0.0015* ↓			1.0000	0.3858	0.1742		
				Steel	0.0243* ↑	0.9927	0.6945	0.0049* ↑		0.8127	1.0000	1.0000	0.3920	
				Wood	0.3293	0.0015* ↓	0.0004* ↓	0.7005	0.0015* ↓	1.0000	0.1738	0.0636	1.0000	0.1776
			14 Days	Paper	0.8261					1.0000				
				Plastic	0.9419	0.7699				1.0000	1.0000			
				Rubber	0.5051	0.3761	0.5526			1.0000	1.0000	1.0000		
				Steel	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑		<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	
				Wood	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	0.0001* ↓	<0.0001* ↓	0.0022* ↓	0.0009* ↓	0.0029* ↓	0.0228* ↓	<0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates the mean percent reduction of the row material is significantly greater than the column material**,** while "↓" indicates the mean percent reduction of the row material is significantly less than the column material.

Table B-3a. Unadjusted and Tukey Adjusted P-values Comparing Times for Crude Ricin, by Temperature, Humidity, and Material.

Ricin Type				Unadjusted P-Value	Tukey Adjusted P-Value
	Temperature (°C)	Humidity (%)	Material	Day 7 vs. 14	Day 7 vs. 14
Crude	20	75	Carpet	0.3892	1.0000
			Paper	0.4408	1.0000
			Plastic	0.3233	1.0000
			Rubber	0.4339	1.0000
			Steel	0.0024* ↓	0.2481
			Wood	0.3344	1.0000
	25	45	Carpet	<0.0001* ↓	0.0034* ↓
			Paper	0.5696	1.0000
			Plastic	0.1970	0.9998
			Rubber	0.0014* ↓	0.1666
			Steel	0.9514	1.0000
			Wood	0.5047	1.0000
		75	Carpet	0.0593	0.9612
			Paper	<0.0001* ↑	0.0184* ↑
			Plastic	<0.0001* ↑	<0.0001* ↑
			Rubber	0.9956	1.0000
			Steel	0.0035* ↓	0.3218
			Wood	0.0644	0.9684
	30	45	Carpet	0.7378	1.0000
			Paper	0.0057* ↓	0.4323
			Plastic	0.1373	0.9980
			Rubber	0.2340	0.9999
			Steel	0.0784	0.9818
			Wood	0.2715	1.0000
		75	Carpet	0.1644	0.9992
			Paper	0.0092* ↑	0.5547
			Plastic	0.1686	0.9993
			Rubber	0.0252* ↑	0.8205
			Steel	0.0231* ↑	0.8003
			Wood	0.0681	0.9727
	40	20	Steel	0.8210	1.0000

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first time point is significantly greater than the second time point**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first time point is significantly less than the second time point. For example, the p-value and arrow <0.0001* ↑ for Paper in the Day 7 vs. 14 column would indicate that the mean percent reduction for Paper on Day 7 was significantly greater than the mean percent reduction of Day 14.

Table B-3b. Unadjusted and Tukey Adjusted P-values Comparing Times for Pure Ricin, by Temperature, Humidity, and Material.

Ricin Type				Unadjusted P-Value	Tukey Adjusted P-Value
	Temperature (°C)	Humidity (%)	Material	Day 7 vs. 14	Day 7 vs. 14
Pure	20	75	Carpet	0.0065* ↓	0.4639
			Paper	0.0014* ↓	0.1673
			Plastic	<0.0001* ↓	0.0010* ↓
			Rubber	0.0003* ↓	0.0420* ↓
			Steel	0.0665	0.9709
			Wood	<0.0001* ↓	0.0169* ↓
	25	45	Carpet	<0.0001* ↓	<0.0001* ↓
			Paper	<0.0001* ↓	0.0005* ↓
			Plastic	0.0010* ↓	0.1340
			Rubber	0.0003* ↓	0.0512
			Steel	0.0038* ↓	0.3395
			Wood	0.0020* ↓	0.2206
		75	Carpet	0.0030* ↓	0.2859
			Paper	0.0002* ↓	0.0372* ↓
			Plastic	0.2292	0.9999
			Rubber	0.0156* ↓	0.6993
			Steel	0.0647	0.9687
			Wood	0.0381* ↓	0.9033
	30	45	Carpet	<0.0001* ↑	<0.0001* ↑
			Paper	<0.0001* ↑	<0.0001* ↑
			Plastic	0.1442	0.9984
			Rubber	0.2187	0.9999
			Steel	0.3062	1.0000
			Wood	0.0064* ↑	0.4589
		70	Carpet	0.4153	1.0000
			Paper	0.0047* ↑	0.3836
			Plastic	0.0006* ↑	0.0816
			Rubber	0.3744	1.0000
			Steel	<0.0001* ↓	<0.0001* ↓
			Wood	<0.0001* ↑	0.0040* ↑

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first time point is significantly greater than the second time point**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first time point is significantly less than the second time point. For example, the p-value and arrow <0.0001* ↑ for Paper in the Day 7 vs. 14 column would indicate that the mean percent reduction for Paper on Day 7 was significantly greater than the mean percent reduction of Day 14.

Table B-3c. Unadjusted and Tukey Adjusted P-values Comparing Times for 20°C and 45% Humidity, by Ricin Type and Material.

				Unadjusted P-Value					
Temperature (°C)	**Humidity (%)**	**Ricin Type**	**Material**	**Day 7 vs. 14**	**Day 7 vs. 21**	**Day 7 vs. 28**	**Day 14 vs. 21**	**Day 14 vs. 28**	**Day 21 vs. 28**
20	45	Crude	Carpet	0.8154	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↓	<0.0001* ↑
			Paper	0.7571	0.0010* ↓	<0.0001* ↓	0.0027* ↓	<0.0001* ↓	0.0896
			Plastic	0.4152	0.6255	<0.0001* ↓	0.1933	<0.0001* ↓	<0.0001* ↓
			Rubber	0.9616	0.6748	0.0003* ↓	0.6400	0.0003* ↓	0.0013* ↓
			Steel	0.0096* ↓	0.0001* ↓	0.0001* ↓	0.1812	0.2115	0.9296
			Wood	0.1504	<0.0001* ↓	<0.0001* ↓	0.0002* ↓	<0.0001* ↓	0.4027
		Pure	Carpet	<0.0001* ↓	0.0536	<0.0001* ↓	0.0002* ↑	0.2801	0.0082* ↓
			Paper	0.9252	0.8463	0.1718	0.7736	0.1444	0.2404
			Plastic	0.0061* ↑	0.1586	0.8244	0.1757	0.0115* ↓	0.2343
			Rubber	0.0253* ↓	<0.0001* ↓	<0.0001* ↓	0.0081* ↓	0.0011* ↓	0.5192
			Steel	<0.0001* ↓	0.0016* ↓	<0.0001* ↓	0.0165* ↑	0.0051* ↓	<0.0001* ↓
			Wood	0.7103	0.0004* ↓	0.0381* ↓	<0.0001* ↓	0.0148* ↓	0.1235
				Tukey Adjusted P-Value					
Temperature (°C)	**Humidity (%)**	**Ricin Type**	**Material**	**Day 7 vs. 14**	**Day 7 vs. 21**	**Day 7 vs. 28**	**Day 14 vs. 21**	**Day 14 vs. 28**	**Day 21 vs. 28**
20	45	Crude	Carpet	1.0000	<0.0001* ↓	0.0152* ↓	<0.0001* ↓	0.0062* ↓	0.0580
			Paper	1.0000	0.3307	0.0009* ↓	0.5749	0.0036* ↓	0.9999
			Plastic	1.0000	1.0000	<0.0001* ↓	1.0000	<0.0001* ↓	<0.0001* ↓
			Rubber	1.0000	1.0000	0.1519	1.0000	0.1330	0.4035
			Steel	0.8783	0.0655	0.0862	1.0000	1.0000	1.0000
			Wood	1.0000	0.0005* ↓	<0.0001* ↓	0.1150	0.0062* ↓	1.0000
		Pure	Carpet	<0.0001* ↓	0.9990	0.0061* ↓	0.1208	1.0000	0.8483
			Paper	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
			Plastic	0.7840	1.0000	1.0000	1.0000	0.9077	1.0000
			Rubber	0.9834	0.0016* ↓	<0.0001* ↓	0.8453	0.3511	1.0000
			Steel	<0.0001* ↓	0.4424	<0.0001* ↓	0.9528	0.7426	0.0004* ↓
			Wood	1.0000	0.1681	0.9956	0.0559	0.9411	1.0000

* Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first time point is significantly greater than the second time point, while "↓" indicates, for the specific column, that the mean percent reduction of the first time point is significantly less than the second time point. For example, the p-value and arrow <0.0001* ↑ for Paper in the Day 7 vs. 14 column would indicate that the mean percent reduction for Paper on Day 7 was significantly greater than the mean percent reduction of Day 14.

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Table B-3d. Unadjusted P-values Comparing Times for 40 °C and 20% Humidity, for Steel by Ricin Type.

				Unadjusted P-Value									
Temperature (°C)	**Humidity (%)**	**Ricin Type**	**Time**	**48 Hours**	**72 Hours**	**96 Hours**	**120 Hours**	**144 Hours**	**7 Days**	**10 Days**	**11 Days**	**12 Days**	**13 Days**
40	20	Crude	72 Hours	0.4751									
			96 Hours	0.6150	0.7952								
			120 Hours	0.8329	0.5373	0.7206							
			144 Hours	0.1409	0.0083* ↑	0.0167* ↑	0.0405* ↑						
			7 Days	0.2840	0.0304* ↑	0.0557	0.1178	0.6188					
			10 Days	0.6399	0.2111	0.2980	0.4529	0.3481	0.5936				
			11 Days	0.0088* ↑	0.0003* ↑	0.0005* ↑	0.0014* ↑	0.1120	0.0471* ↑	0.0298* ↑			
			12 Days	0.0271* ↑	0.0013* ↑	0.0026* ↑	0.0061* ↑	0.2709	0.1331	0.0790	0.6678		
			13 Days	0.0002* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.0043* ↑	0.0012* ↑	0.0012* ↑	0.2544	0.1181	
			14 Days	0.4632	0.1203	0.1787	0.2909	0.5273		0.7899	0.0555	0.1348	0.0027* ↓
		Pure	72 Hours	0.5159									
			96 Hours	0.3048	0.7050								
			120 Hours	0.0606	0.2156	0.3881							
			144 Hours	0.0510	0.1885	0.3471	0.9382						
			7 Days	0.0603	0.2147	0.3868	0.9981	0.9400					

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row time point is significantly greater than the column time point**,** while " ↓ " indicates that the mean percent reduction of the row time point is significantly less than the column time point.

Table B-3e. Tukey Adjusted P-values Comparing Times for 40 °C and 20% Humidity, for Steel by Ricin Type.

				Tukey Adjusted P-Value									
Temperature (°C)	**Humidity (%)**	**Ricin Type**	**Time**	**48 Hours**	**72 Hours**	**96 Hours**	**120 Hours**	**144 Hours**	**7 Days**	**10 Days**	**11 Days**	**12 Days**	**13 Days**
40	20	Crude	72 Hours	1.0000									
			96 Hours	1.0000	1.0000								
			120 Hours	1.0000	1.0000	1.0000							
			144 Hours	0.9863	0.3731	0.5550	0.7989						
			7 Days	0.9996	0.7241	0.8718	0.9754	1.0000					
			10 Days	1.0000	0.9976	0.9997	1.0000	0.9999	1.0000				
			11 Days	0.3871	0.0236* ↑	0.0448* ↑	0.1002	0.9715	0.8352	0.7185			
			12 Days	0.6921	0.0981	0.1653	0.3071	0.9995	0.9833	0.9327	1.0000		
			13 Days	0.0225* ↑	0.0002* ↑	0.0005* ↑	0.0014* ↑	0.2395	0.0915	0.0875	0.9992	0.9756	
			14 Days	1.0000	0.9770	0.9946	0.9997	1.0000		1.0000	0.8710	0.9840	0.1696
		Pure	72 Hours	1.0000									
			96 Hours	0.9998	1.0000								
			120 Hours	0.8886	0.9979	1.0000							
			144 Hours	0.8532	0.9958	0.9999	1.0000						
			7 Days	0.8876	0.9978	1.0000	1.0000	1.0000					

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row time point is significantly greater than the column time point**,** while "↓" indicates that the mean percent reduction of the row time point is significantly less than the column time point.

Table B-3f. Unadjusted P-values Comparing Times for 50 °C and 20% Humidity, for Steel by Ricin Type.

				Unadjusted P-Value							
Temperature (°C)	**Humidity (%)**	**Ricin Type**	**Time**	**6 Hours**	**24 Hours**	**30 Hours**	**48 Hours**	**72 Hours**	**96 Hours**	**120 Hours**	**144 Hours**
50	20	Crude	24 Hours	0.0486* ↑							
			30 Hours	0.7326	0.0213* ↓						
			48 Hours	0.4290	0.1339	0.2369					
			72 Hours	0.5320	0.0964	0.3089	0.8383				
			96 Hours	0.0526	0.7314	0.0204* ↑	0.1561	0.1055			
			120 Hours	0.1803	0.5191	0.0938	0.4469	0.3542	0.6878		
			144 Hours	0.4071	0.2473	0.2427	0.8675	0.7389	0.3200	0.6066	
			7 Days	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑
		Pure	24 Hours	0.0037* ↑							
			30 Hours	<0.0001* ↑	0.1812						
			48 Hours	<0.0001* ↑	<0.0001* ↑	0.0134* ↑					
			72 Hours	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.0003* ↑				
			96 Hours	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.1825			
			120 Hours	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.0072* ↑	0.1022		
			144 Hours	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.0042* ↑	0.0692	0.8713	
			7 Days	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row time point is significantly greater than the column time point**,** while "↓" indicates that the mean percent reduction of the row time point is significantly less than the column time point.

Table B-3g. Tukey Adjusted P-values Comparing Times for 50 °C and 20% Humidity, for Steel by Ricin Type.

				Tukey Adjusted P-Value							
Temperature (°C)	**Humidity (%)**	**Ricin Type**	**Time**	**6 Hours**	**24 Hours**	**30 Hours**	**48 Hours**	**72 Hours**	**96 Hours**	**120 Hours**	**144 Hours**
50	20	Crude	24 Hours	0.8641							
			30 Hours	1.0000	0.6539						
			48 Hours	1.0000	0.9880	0.9992					
			72 Hours	1.0000	0.9665	0.9999	1.0000				
			96 Hours	0.8803	1.0000	0.6412	0.9933	0.9739			
			120 Hours	0.9965	1.0000	0.9640	1.0000	1.0000	1.0000		
			144 Hours	1.0000	0.9994	0.9993	1.0000	1.0000	0.9999	1.0000	
			7 Days	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑
		Pure	24 Hours	0.2357							
			30 Hours	0.0045* ↑	0.9966						
			48 Hours	<0.0001* ↑	0.0106* ↑	0.5238					
			72 Hours	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.0334* ↑				
			96 Hours	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.0003* ↑	0.9967			
			120 Hours	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.3643	0.9715		
			144 Hours	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.2539	0.9272	1.0000	
			7 Days	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates the mean percent reduction of the row time point is significantly greater than the column time point**,** while "↓" indicates the mean percent reduction of the row time point is significantly less than the column time point.

Table B-4a. Unadjusted and Tukey Adjusted P-values Comparing Ricin Types, for 20 °C and 45% Humidity, by Time and Material.

				Unadjusted P-Value	Tukey Adjusted P-Value
Temperature (°C)	**Humidity (%)**	**Time**	**Material**	**Crude vs. Pure**	**Crude vs. Pure**
20	45	7 Days	Carpet	0.0574	0.9993
			Paper	0.0540	0.9990
			Plastic	<0.0001* ↓	<0.0001* ↓
			Rubber	0.6237	1.0000
			Steel	<0.0001* ↓	0.0015* ↓
			Wood	0.0440* ↓	0.9975
		14 Days	Carpet	<0.0001* ↓	<0.0001* ↓
			Paper	0.1264	1.0000
			Plastic	<0.0001* ↓	0.0006* ↓
			Rubber	0.0057* ↓	0.7710
			Steel	<0.0001* ↓	<0.0001* ↓
			Wood	0.8327	1.0000
		21 Days	Carpet	<0.0001* ↑	0.0088* ↑
			Paper	0.2243	1.0000
			Plastic	<0.0001* ↓	0.0004* ↓
			Rubber	<0.0001* ↓	0.0012* ↓
			Steel	<0.0001* ↓	0.0296* ↓
			Wood	0.6558	1.0000
		28 Days	Carpet	0.0331* ↓	0.9927
			Paper	0.0822	0.9999
			Plastic	0.5590	1.0000
			Rubber	0.0176* ↓	0.9589
			Steel	<0.0001* ↓	<0.0001* ↓
			Wood	0.0539	0.9990

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly greater than the mean percent reduction of the second ricin type**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly less than the mean percent reduction of the second ricin type. For example, the p-value and arrow <0.0001* ↑ for Paper in the Crude vs. Pure column would indicate that the mean percent reduction for Crude Paper was significantly greater than the mean percent reduction of the Pure.

Table B-4b. Unadjusted and Tukey Adjusted P-values Comparing Ricin Types, for 20 °C and 75% Humidity, by Time and Material.

				Unadjusted P-Value	Tukey Adjusted P-Value
Temperature (°C)	**Humidity (%)**	**Time**	**Material**	**Crude vs. Pure**	**Crude vs. Pure**
20	75	7 Days	Carpet	0.6282	1.0000
			Paper	0.0096* ↑	0.5665
			Plastic	0.0018* ↑	0.2037
			Rubber	0.2666	1.0000
			Steel	<0.0001* ↓	<0.0001* ↓
			Wood	<0.0001* ↑	<0.0001* ↑
		14 Days	Carpet	0.1558	0.9989
			Paper	0.1575	0.9990
			Plastic	0.0089* ↓	0.5453
			Rubber	0.0603	0.9627
			Steel	<0.0001* ↓	0.0001* ↓
			Wood	<0.0001* ↑	<0.0001* ↑

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly greater than the mean percent reduction of the second ricin type**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly less than the mean percent reduction of the second ricin type. For example, the p-value and arrow <0.0001* ↑ for Paper in the Crude vs. Pure column would indicate that the mean percent reduction for Crude Paper was significantly greater than the mean percent reduction of the Pure.

Table B-4c. Unadjusted and Tukey Adjusted P-values Comparing Ricin Types, for 25 °C and 45% Humidity, by Time and Material.

				Unadjusted P-Value	Tukey Adjusted P-Value
Temperature (°C)	**Humidity (%)**	**Time**	**Material**	**Crude vs. Pure**	**Crude vs. Pure**
25	45	7 Days	Carpet	0.0524	0.9481
			Paper	<0.0001* ↑	0.0063* ↑
			Plastic	0.0159* ↓	0.7048
			Rubber	0.1604	0.9991
			Steel	0.0001* ↓	0.0258* ↓
			Wood	0.0253* ↑	0.8218
		14 Days	Carpet	0.0010* ↓	0.1342
			Paper	0.8988	1.0000
			Plastic	<0.0001* ↓	0.0035* ↓
			Rubber	0.3330	1.0000
			Steel	<0.0001* ↓	<0.0001* ↓
			Wood	0.8176	1.0000

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly greater than the mean percent reduction of the second ricin type**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly less than the mean percent reduction of the second ricin type. For example, the p-value and arrow <0.0001* ↑ for Paper in the Crude vs. Pure column would indicate that the mean percent reduction for Crude Paper was significantly greater than the mean percent reduction of the Pure.

Table B-4d. Unadjusted and Tukey Adjusted P-values Comparing Ricin Types, for 25 °C and 75% Humidity, by Time and Material.

				Unadjusted P-Value	Tukey Adjusted P-Value
Temperature (°C)	**Humidity (%)**	**Time**	**Material**	**Crude vs. Pure**	**Crude vs. Pure**
25	75	7 Days	Carpet	0.0027* ↑	0.2662
			Paper	<0.0001* ↑	<0.0001* ↑
			Plastic	0.0018* ↑	0.2034
			Rubber	0.0063* ↓	0.4571
			Steel	<0.0001* ↓	<0.0001* ↓
		14 Days	Wood	0.2865	1.0000
			Carpet	0.0549	0.9533
			Paper	0.4430	1.0000
			Plastic	<0.0001* ↓	0.0079* ↓
			Rubber	<0.0001* ↓	0.0002* ↓
			Steel	<0.0001* ↓	<0.0001* ↓
			Wood	<0.0001* ↓	0.0005* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly greater than the mean percent reduction of the second ricin type**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly less than the mean percent reduction of the second ricin type. For example, the p-value and arrow <0.0001* ↑ for Paper in the Crude vs. Pure column would indicate that the mean percent reduction for Crude Paper was significantly greater than the mean percent reduction of Pure.

Table B-4e. Unadjusted and Tukey Adjusted P-values Comparing Ricin Types, for 30 °C and 45% Humidity, by Time and Material.

				Unadjusted P-Value	Tukey Adjusted P-Value
Temperature (°C)	**Humidity (%)**	**Time**	**Material**	**Crude vs. Pure**	**Crude vs. Pure**
30	45	7 Days	Carpet	0.0001* ↓	0.0231* ↓
			Paper	<0.0001* ↓	<0.0001* ↓
			Plastic	0.0150* ↑	0.6900
			Rubber	0.9800	1.0000
			Steel	0.1572	0.9990
			Wood	0.0094* ↓	0.5606
			Carpet	0.0047* ↑	0.3848
		14 Days	Paper	0.0131* ↓	0.6521
			Plastic	0.6221	1.0000
			Rubber	0.9477	1.0000
			Steel	0.5011	1.0000
			Wood	0.2170	0.9999

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly greater than the mean percent reduction of the second ricin type**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly less than the mean percent reduction of the second ricin type. For example, the p-value and arrow <0.0001* ↑ for Paper in the Crude vs. Pure column would indicate that the mean percent reduction for Crude Paper was significantly greater than the mean percent reduction of Pure.

Table B-4f. Unadjusted and Tukey Adjusted P-values Comparing Ricin Types, for 30 °C and 70% Humidity, by Time and Material.

				Unadjusted P-Value	Tukey Adjusted P-Value
Temperature (°C)	**Humidity (%)**	**Time**	**Material**	**Crude vs. Pure**	**Crude vs. Pure**
30	70	7 Days	Carpet	0.0021* ↓	0.2232
			Paper	0.8694	1.0000
			Plastic	<0.0001* ↓	0.0067* ↓
			Rubber	0.0555	0.9544
			Steel	0.0275* ↑	0.8400
			Wood	0.4363	1.0000
		14 Days	Carpet	0.3452	1.0000
			Paper	0.9433	1.0000
			Plastic	0.0318* ↓	0.8701
			Rubber	0.0013* ↓	0.1568
			Steel	<0.0001* ↓	<0.0001* ↓
			Wood	0.0632	0.9668

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, the mean percent reduction of the first ricin type is significantly greater than the mean percent reduction of the second ricin type**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly less than the mean percent reduction of the second ricin type. For example, the p-value and arrow <0.0001* ↑ for Paper in the Crude vs. Pure column would indicate that the mean percent reduction for Crude Paper was significantly greater than the mean percent reduction of Pure.

Table B-4g. Unadjusted and Tukey Adjusted P-values Comparing Ricin Types, for Steel at 40 °C and 20% Humidity, by Time.

				Unadjusted P-Value	Tukey Adjusted P-Value
Temperature (°C)	**Humidity (%)**	**Time**	**Material**	**Crude vs. Pure**	**Crude vs. Pure**
40	20	48 Hours	Steel	0.0005* ↓	0.0450* ↓
		72 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		96 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		120 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		144 Hours	Steel	<0.0001* ↓	0.0004* ↓
		7 Days	Steel	<0.0001* ↓	0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly greater than the mean percent reduction of the second ricin type**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly less than the mean percent reduction of the second ricin type. For example, the p-value and arrow <0.0001* ↑ for Paper in the Crude vs. Pure column would indicate that the mean percent reduction for Crude Paper was significantly greater than the mean percent reduction of Pure.

Table B-4h. Unadjusted and Tukey Adjusted P-values Comparing Ricin Types, for Steel at 50 °C and 20% Humidity, by Time.

				Unadjusted P-Value	Tukey Adjusted P-Value
Temperature (°C)	**Humidity (%)**	**Time**	**Material**	**Crude vs. Pure**	**Crude vs. Pure**
50	20	6 Hours	Steel	0.1163	0.9806
		24 Hours	Steel	0.0121* ↓	0.4950
		30 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		48 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		72 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		96 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		120 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		144 Hours	Steel	<0.0001* ↓	<0.0001* ↓
		7 Days	Steel	<0.0001* ↓	<0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly greater than the mean percent reduction of the second ricin type**,** while "↓" indicates, for the specific column, that the mean percent reduction of the first ricin type is significantly less than the mean percent reduction of the second ricin type. For example, the p-value and arrow <0.0001* ↑ for Paper in the Crude vs. Pure column would indicate that the mean percent reduction for Crude Paper was significantly greater than the mean percent reduction of Pure.

Table B-5a. Unadjusted and Tukey Adjusted P-values Comparing Humidity, for Crude Ricin, by Material, Time and Temperature.

Ricin Type				Unadjusted P-Value	Tukey Adjusted P-Value
	Material	Time	Temperature (°C)	45% vs. 75%	45% vs. 75%
Crude	Carpet	7 Days	20	0.3652	0.9986
			25	<0.0001* ↓	0.0003* ↓
			30	<0.0001* ↑	0.0038* ↑
		14 Days	20	0.1656	0.9562
			25	0.0010* ↓	0.0409* ↓
			30	0.0011* ↑	0.0460* ↑
	Paper	7 Days	20	0.2180	0.9817
			25	0.0002* ↓	0.0085* ↓
			30	<0.0001* ↓	<0.0001* ↓
		14 Days	20	0.4451	0.9997
			25	0.5801	1.0000
			30	0.5053	0.9999
	Plastic	7 Days	20	0.1092	0.8882
			25	0.0189* ↓	0.4068
			30	<0.0001* ↑	0.0003* ↑
		14 Days	20	0.0817	0.8213
			25	<0.0001* ↑	0.0001* ↑
			30	<0.0001* ↑	0.0020* ↑
	Rubber	7 Days	20	0.2906	0.9947
			25	0.1361	0.9287
			30	<0.0001* ↑	0.0004* ↑
		14 Days	20	0.1273	0.9176
			25	<0.0001* ↑	0.0011* ↑
			30	<0.0001* ↑	<0.0001* ↑
	Steel	7 Days	20	<0.0001* ↓	<0.0001* ↓
			25	0.0012* ↓	0.0697
			30	<0.0001* ↓	<0.0001* ↓
		14 Days	20	<0.0001* ↓	<0.0001* ↓
			25	<0.0001* ↓	<0.0001* ↓
			30	0.0330* ↓	0.6786
	Wood	7 Days	20	<0.0001* ↓	0.0031* ↓
			25	0.9279	1.0000
			30	0.4648	0.9998
		14 Days	20	0.0005* ↓	0.0231* ↓
			25	0.0139* ↑	0.3333
			30	0.0036* ↑	0.1226

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction for the first humidity is significantly greater than the mean percent reduction for the second humidity**,** while "↓" indicates, for the specific column, the mean percent reduction for the first humidity is significantly less than the mean percent reduction for the second humidity. For example, the p-value and arrow <0.0001* ↑ for Paper in the 45% vs. 75% column would indicate that the mean percent reduction for Paper at 45% humidity was significantly greater than the mean percent reduction for 75% humidity.

Table B-5b. Unadjusted and Tukey Adjusted P-values Comparing Humidity, for Pure Ricin, by Material, Time and Temperature.

Ricin Type				Unadjusted P-Value	Tukey Adjusted P-Value
	Material	Time	Temperature (°C)	45% vs. 75%	45% vs. 75%
Pure	Carpet	7 Days	20	0.5020	0.9999
			25	0.0836	0.8272
			30	<0.0001* ↑	<0.0001* ↑
		14 Days	20	0.0001* ↑	0.0068* ↑
			25	0.5405	1.0000
			30	0.4248	0.9996
	Paper	7 Days	20	0.1482	0.9417
			25	0.6636	1.0000
			30	<0.0001* ↑	<0.0001* ↑
		14 Days	20	0.5186	0.9999
			25	0.8445	1.0000
			30	0.0019* ↑	0.0716
	Plastic	7 Days	20	<0.0001* ↑	<0.0001* ↑
			25	<0.0001* ↑	0.0002* ↑
			30	<0.0001* ↓	<0.0001* ↓
		14 Days	20	0.0974	0.8636
			25	<0.0001* ↑	<0.0001* ↑
			30	<0.0001* ↑	0.0024* ↑
	Rubber	7 Days	20	0.9864	1.0000
			25	<0.0001* ↓	0.0030* ↓
			30	0.0005* ↑	0.0212* ↑
		14 Days	20	0.8006	1.0000
			25	0.0025* ↓	0.0902
			30	0.0127* ↑	0.3135
	Steel	7 Days	20	0.0012* ↓	0.0613
			25	<0.0001* ↓	<0.0001* ↓
			30	0.1215	0.9460
		14 Days	20	0.3052	0.9987
			25	<0.0001* ↓	<0.0001* ↓
			30	<0.0001* ↓	<0.0001* ↓
	Wood	7 Days	20	0.0042* ↑	0.1386
			25	0.0003* ↓	0.0143* ↓
			30	0.0130* ↑	0.3188
		14 Days	20	0.9085	1.0000
			25	0.0031* ↓	0.1073
			30	0.0002* ↑	0.0102* ↑

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates, for the specific column, that the mean percent reduction for the first humidity is significantly greater than the mean percent reduction for the second humidity**,** while "↓" indicates, for the specific column, that the mean percent reduction for the first humidity is significantly less than the mean percent reduction for the second humidity. For example, the p-value and arrow <0.0001* ↑ for Paper in the 45% vs. 75% column would indicate that the mean percent reduction for Paper at 45% humidity was significantly greater than the mean percent reduction at 75% humidity.

Table B-6a. Unadjusted and Tukey Adjusted P-values Comparing Temperatures, for Carpet by Ricin Type, Humidity and Time.

					Unadjusted P-Value		Tukey Adjusted P-Value	
Ricin Type	**Material**	**Humidity (%)**	**Time**	**Temperature (°C)**	**20 °C**	**25 °C**	**20 °C**	**25 °C**
Crude	Carpet	45	7 Days	25	0.7786		1.0000	
				30	<0.0001* ↑	<0.0001* ↑	0.0021* ↑	0.0008* ↑
			14 Days	25	0.0038* ↑		0.1281	
				30	<0.0001* ↑	0.0502	0.0004* ↑	0.6851
		75	7 Days	25	0.0003* ↑		0.0126* ↑	
				30	0.4741	<0.0001* ↓	0.9999	0.0014* ↓
			14 Days	25	<0.0001* ↑		0.0003* ↑	
				30	0.8611	<0.0001* ↓	1.0000	0.0005* ↓
Pure	Carpet	45	7 Days	25	0.8795		1.0000	
				30	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑
			14 Days	25	0.4876		0.9999	
				30	<0.0001* ↓	<0.0001* ↓	0.0040* ↓	0.0004* ↓
		75	7 Days	25	0.0264* ↑		0.4967	
				30	0.0022* ↑	0.3476	0.0810	0.9981
			14 Days	25	0.0001* ↑		0.0053* ↑	
				30	0.5325	0.0008* ↓	1.0000	0.0324* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

Table B-6b. Unadjusted and Tukey Adjusted P-values Comparing Temperatures, for Paper by Ricin Type, Humidity and Time.

					Unadjusted P-Value		Tukey Adjusted P-Value	
Ricin Type	**Material**	**Humidity (%)**	**Time**	**Temperature (°C)**	**20 °C**	**25 °C**	**20 °C**	**25 °C**
Crude	Paper	45	7 Days	25	<0.0001* ↑		0.0020* ↑	
				30	0.2415	<0.0001* ↓	0.9877	<0.0001* ↓
			14 Days	25	<0.0001* ↑		0.0011* ↑	
				30	0.1200	0.0028* ↓	0.9069	0.0991
		75	7 Days	25	<0.0001* ↑		<0.0001* ↑	
				30	0.0013* ↑	0.0003* ↓	0.0508	0.0125* ↓
			14 Days	25	<0.0001* ↑		0.0021* ↑	
				30	0.1444	0.0038* ↓	0.9378	0.1285
Pure	Paper	45	7 Days	25	0.4256		0.9996	
				30	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑
			14 Days	25	<0.0001* ↑		<0.0001* ↑	
				30	<0.0001* ↑	0.3394	0.0001* ↑	0.9978
		75	7 Days	25	0.0093* ↑		0.2523	
				30	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.0012* ↑
			14 Days	25	<0.0001* ↑		<0.0001* ↑	
				30	0.1596	0.0002* ↓	0.9517	0.0089* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates that the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

Table B-6c. Unadjusted and Tukey Adjusted P-values Comparing Temperatures, for Plastic by Ricin Type, Humidity and Time.

					Unadjusted P-Value		Tukey Adjusted P-Value	
Ricin Type	**Material**	**Humidity (%)**	**Time**	**Temperature (°C)**	**20 °C**	**25 °C**	**20 °C**	**25 °C**
Crude	Plastic	45	7 Days	25	0.0784		0.8107	
				30	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	0.0002* ↑
			14 Days	25	0.0007* ↑		0.0291* ↑	
				30	<0.0001* ↑	0.0529	<0.0001* ↑	0.7006
		75	7 Days	25	0.0125* ↑		0.3101	
				30	0.7086	0.0311* ↓	1.0000	0.5437
			14 Days	25	0.0008* ↓		0.0357* ↓	
				30	0.4837	0.0063* ↑	0.9999	0.1896
Pure	Plastic	45	7 Days	25	0.0057* ↓		0.1758	
				30	0.0006* ↓	0.4543	0.0284* ↓	0.9998
			14 Days	25	<0.0001* ↑		<0.0001* ↑	
				30	0.0011* ↑	0.0247* ↓	0.0460* ↑	0.4775
		75	7 Days	25	0.1600		0.9520	
				30	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑
			14 Days	25	0.5249		1.0000	
				30	0.5028	0.1946	0.9999	0.9729

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates that the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

Table B-6d. Unadjusted and Tukey Adjusted P-values Comparing Temperatures, for Rubber by Ricin Type, Humidity and Time.

					Unadjusted P-Value		Tukey Adjusted P-Value	
Ricin Type	**Material**	**Humidity (%)**	**Time**	**Temperature (°C)**	**20 °C**	**25 °C**	**20 °C**	**25 °C**
Crude	Rubber	45	7 Days	25	0.0569		0.7223	
				30	<0.0001* ↑	0.0002* ↑	<0.0001* ↑	0.0085* ↑
			14 Days	25	<0.0001* ↑		0.0002* ↑	
				30	0.0001* ↑	0.3282	0.0055* ↑	0.9973
		75	7 Days	25	0.5293		1.0000	
				30	0.9424	0.5773	1.0000	1.0000
			14 Days	25	0.2907		0.9947	
				30	0.0016* ↓	0.0279* ↓	0.0639	0.5119
Pure	Rubber	45	7 Days	25	0.8389		1.0000	
				30	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑
			14 Days	25	0.0112* ↑		0.2879	
				30	0.0186* ↑	0.8397	0.4035	1.0000
		75	7 Days	25	<0.0001* ↑		0.0016* ↑	
				30	<0.0001* ↑	0.7626	0.0043* ↑	1.0000
			14 Days	25	<0.0001* ↑		<0.0001* ↑	
				30	0.6845	<0.0001* ↓	1.0000	<0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates that the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

Table B-6e. Unadjusted and Tukey Adjusted P-values Comparing Temperatures, for Steel by Ricin Type, Humidity and Time.

					Unadjusted P-Value		Tukey Adjusted P-Value	
Ricin Type	**Material**	**Humidity (%)**	**Time**	**Temperature (°C)**	**20 °C**	**25 °C**	**20 °C**	**25 °C**
Crude	Steel	45	7 Days	25	0.0136* ↑		0.4299	
				30	0.0007* ↑	0.3004	0.0429* ↑	0.9992
			14 Days	25	0.8965		1.0000	
				30	0.0001* ↑	<0.0001* ↑	0.0092* ↑	0.0061* ↑
		75	7 Days	25	0.3044		0.9992	
				30	<0.0001* ↑	<0.0001* ↑	0.0002* ↑	<0.0001* ↑
			14 Days	25	0.4017		0.9999	
				30	0.9315	0.3556	1.0000	0.9998
Pure	Steel	45	7 Days	25	0.2802		0.9977	
				30	0.5321	0.6460	1.0000	1.0000
			14 Days	25	0.8727		1.0000	
				30	0.1589	0.2105	0.9743	0.9907
		75	7 Days	25	<0.0001* ↑		0.0059* ↑	
				30	0.2254	<0.0001* ↓	0.9931	<0.0001* ↓
			14 Days	25	<0.0001* ↑		0.0006* ↑	
				30	0.0003* ↑	0.2929	0.0193* ↑	0.9983

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates that the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

Table B-6f. Unadjusted and Tukey Adjusted P-values Comparing Temperatures, for Wood by Ricin Type, Humidity and Time.

					Unadjusted P-Value		Tukey Adjusted P-Value	
Ricin Type	**Material**	**Humidity (%)**	**Time**	**Temperature (°C)**	**20 °C**	**25 °C**	**20 °C**	**25 °C**
Crude	Wood	45	7 Days	25	0.0007* ↑		0.0309* ↑	
				30	0.0025* ↑	0.6716	0.0911	1.0000
			14 Days	25	0.0039* ↑		0.1293	
				30	0.0007* ↑	0.5695	0.0315* ↑	1.0000
		75	7 Days	25	0.4874		0.9999	
				30	0.6330	0.8273	1.0000	1.0000
			14 Days	25	0.0022* ↓		0.0806	
				30	0.0026* ↓	0.9475	0.0943	1.0000
Pure	Wood	45	7 Days	25	0.8386		1.0000	
				30	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑
			14 Days	25	0.0008* ↑		0.0354* ↑	
				30	0.0675	0.0963	0.7707	0.8611
		75	7 Days	25	<0.0001* ↑		<0.0001* ↑	
				30	<0.0001* ↑	0.9832	<0.0001* ↑	1.0000
			14 Days	25	<0.0001* ↑		<0.0001* ↑	
				30	0.0487* ↓	<0.0001* ↓	0.6761	<0.0001* ↓

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

Table B-6g. Unadjusted and Tukey Adjusted P-values Comparing 40°C and 50°C, for Steel by Ricin Type, Humidity and Time.

					Unadjusted P-Value	Tukey Adjusted P-Value
Ricin Type	**Material**	**Humidity (%)**	**Time**	**Temperature (°C)**	**40 °C**	**40 °C**
Crude	Steel	20	48 Hours	50	0.2126	0.9985
			72 Hours	50	0.0103* ↑	0.4554
			96 Hours	50	0.0006* ↑	0.0575
			120 Hours	50	0.0338* ↑	0.7811
			144 Hours	50	0.4834	1.0000
			7 Days	50	<0.0001* ↑	<0.0001* ↑
Pure	Steel	20	48 Hours	50	0.0019* ↑	0.1419
			72 Hours	50	<0.0001* ↑	0.0002* ↑
			96 Hours	50	<0.0001* ↑	<0.0001* ↑
			120 Hours	50	<0.0001* ↑	0.0014* ↑
			144 Hours	50	<0.0001* ↑	0.0013* ↑
			7 Days	50	<0.0001* ↑	<0.0001* ↑

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

Table B-7a. Unadjusted and Tukey Adjusted P-values Comparing Temperatures, for Steel by Ricin Type at Day 7 with Relative Humidity of 20% or 45%.

			Unadjusted P-Value			Tukey Adjusted P-Value		
Ricin Type	**Time**	**Temperature (°C)**	**20 °C**	**25 °C**	**40 °C**	**20 °C**	**25 °C**	**40 °C**
Crude	7 Days	25	0.0337* ↑			0.4261		
		30	0.0035* ↑	0.3713		0.0753	0.9915	
		40	0.0020* ↑	0.4448		0.0457* ↑	0.9971	
		50	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑
Pure	7 Days	25	0.0813			0.6247		
		30	0.3072	0.4516		0.9647	0.9940	
		40	<0.0001* ↑	0.0047* ↑		0.0008* ↑	0.0789	
		50	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑	<0.0001* ↑

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates that the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

Table B-7b. Unadjusted and Tukey Adjusted P-values Comparing Temperatures, for Steel by Ricin Type at Day 14 with Relative Humidity of 20% or 45%.

			Unadjusted P-Value		Tukey Adjusted P-Value	
Ricin Type	**Time**	**Temperature (°C)**	**20 °C**	**25 °C**	**20 °C**	**25 °C**
Crude	14 Days	25	0.9105		1.0000	
		30	0.0010* ↑	0.0007* ↑	0.0248* ↑	0.0184* ↑
		40	0.7265	0.6442	1.0000	0.9999
Pure	14 Days	25	0.7922		1.0000	
		30	0.0247* ↓	0.0446* ↓	0.2961	0.4417

***** Significant at the 0.05 level.

↑, ↓ "↑" indicates that the mean percent reduction of the row temperature is significantly greater than the mean percent reduction of the column temperature**,** while "↓" indicates that the mean percent reduction of the row temperature is significantly less than the mean percent reduction of the column temperature.

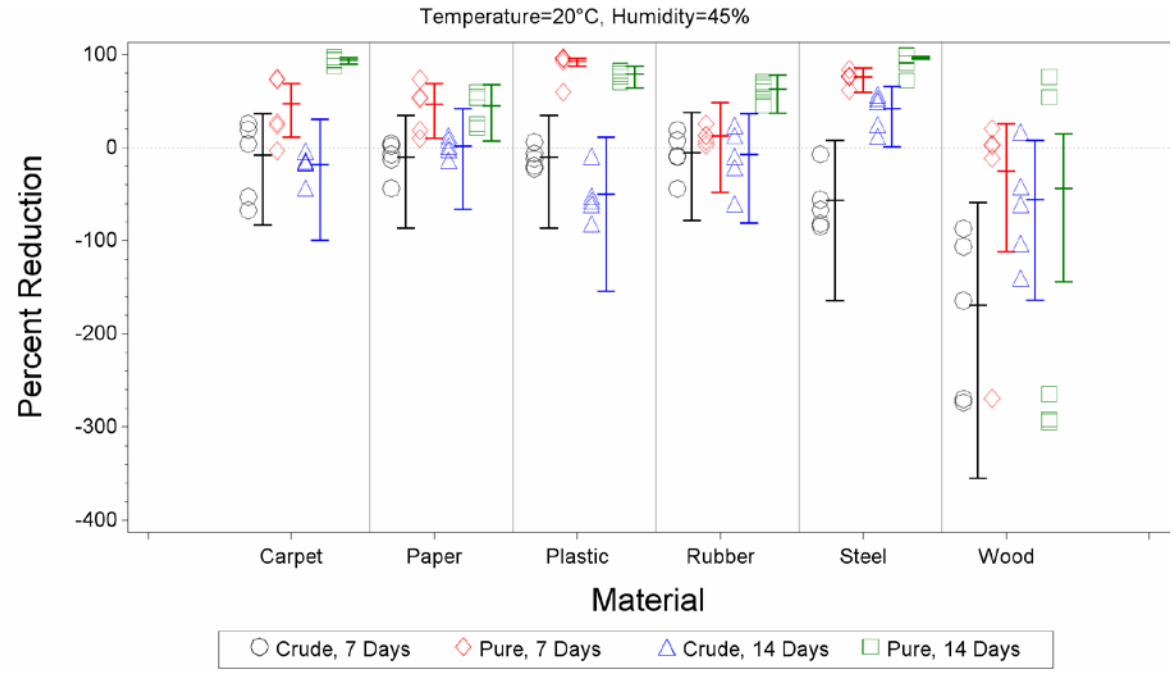

Figure B-1a. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Days 7 and 14, 20 °C and 45% Humidity.

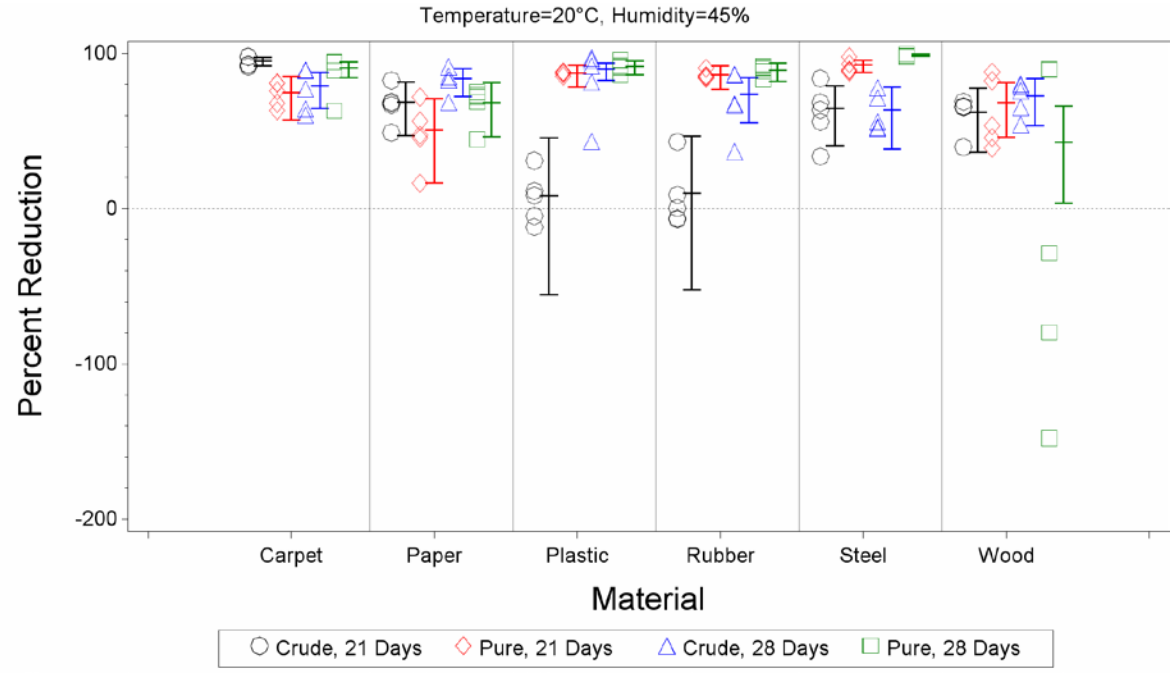

Figure B-1b. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Days 21 and 28, 20 °C and 45% Humidity.

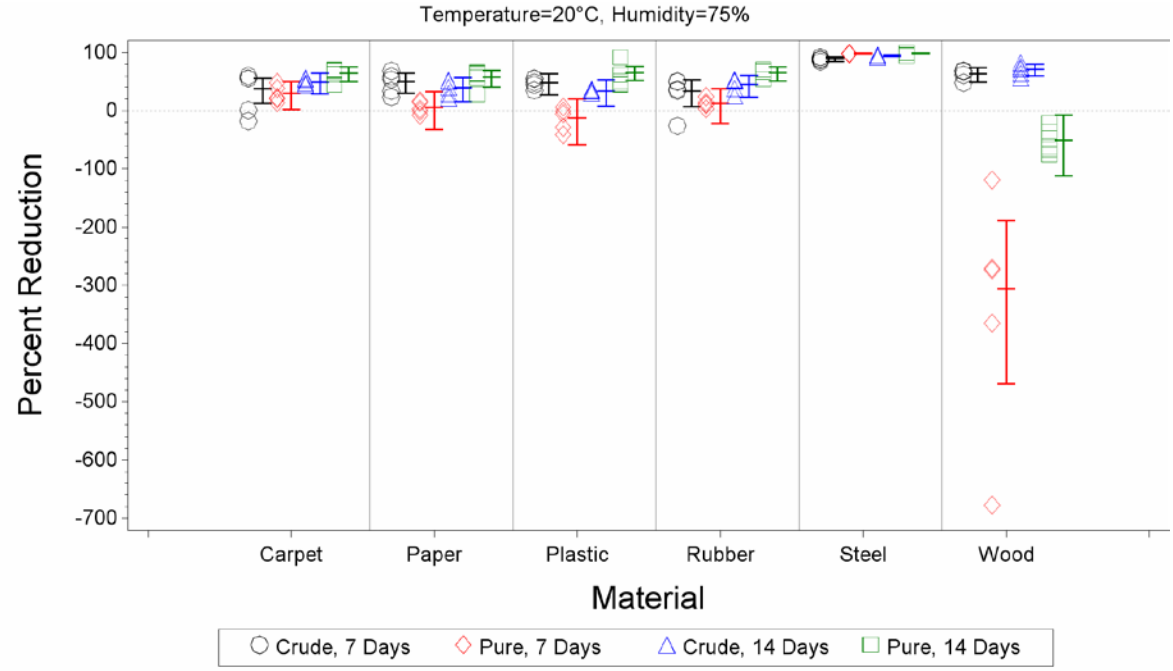

Figure B-1c. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Days 7 and 14, 20 °C and 75% Humidity.

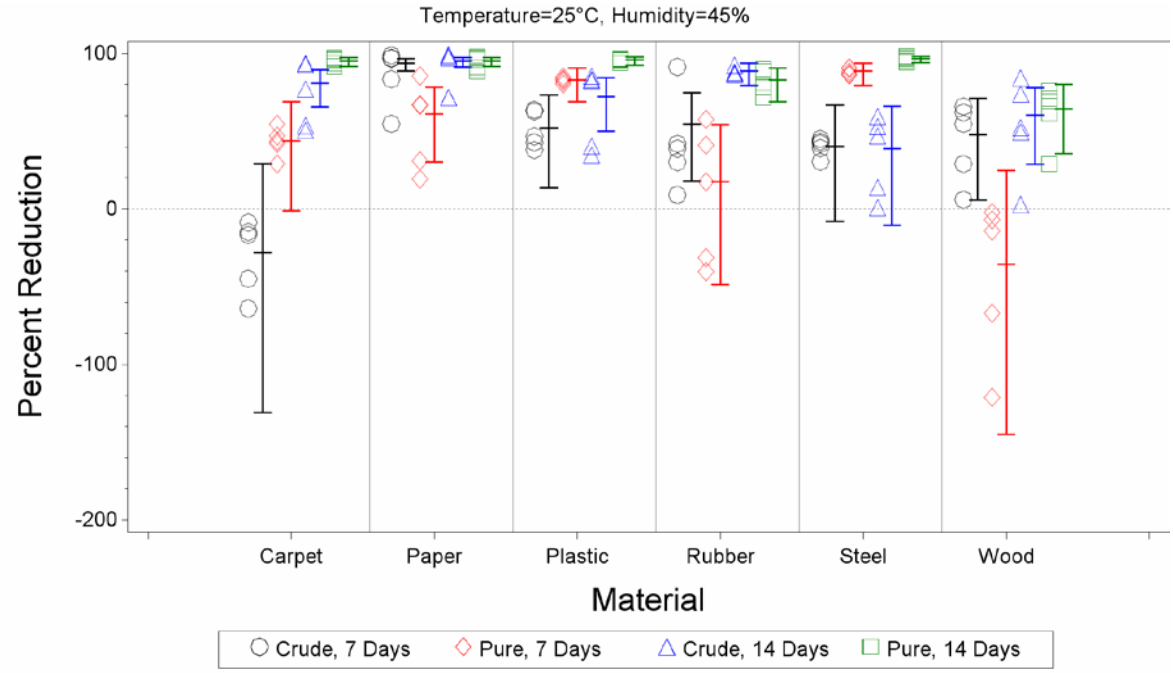

Figure B-1d. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Days 7 and 14, 25 °C and 45% Humidity.

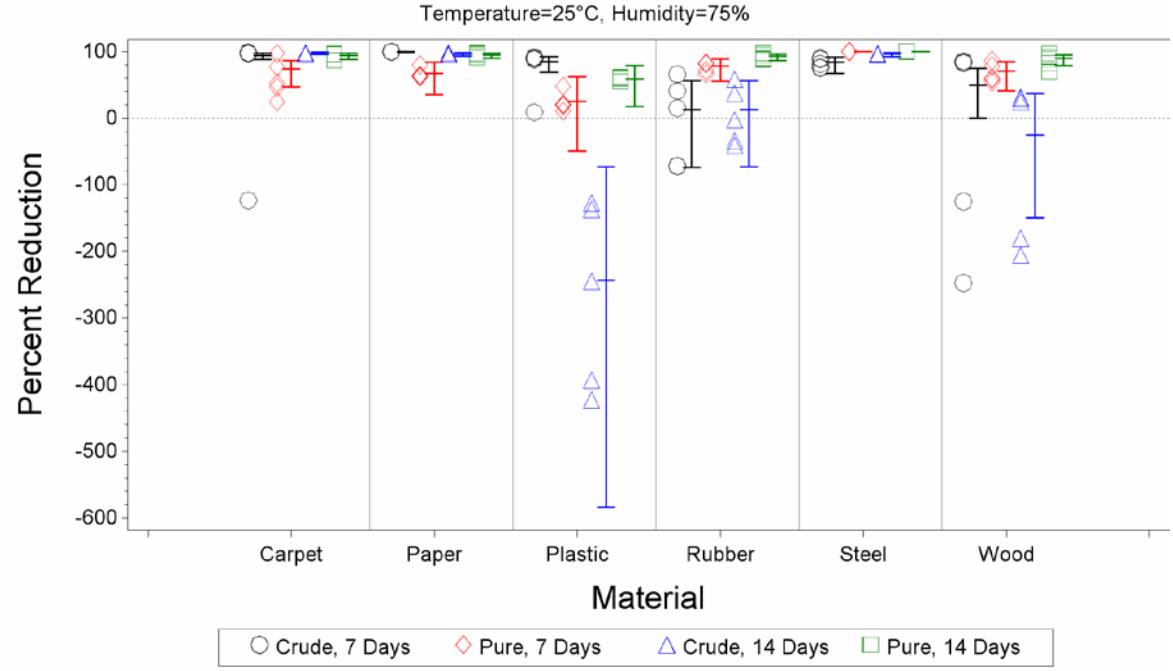

Figure B-1e. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Days 7 and 14, 25 °C and 75% Humidity.

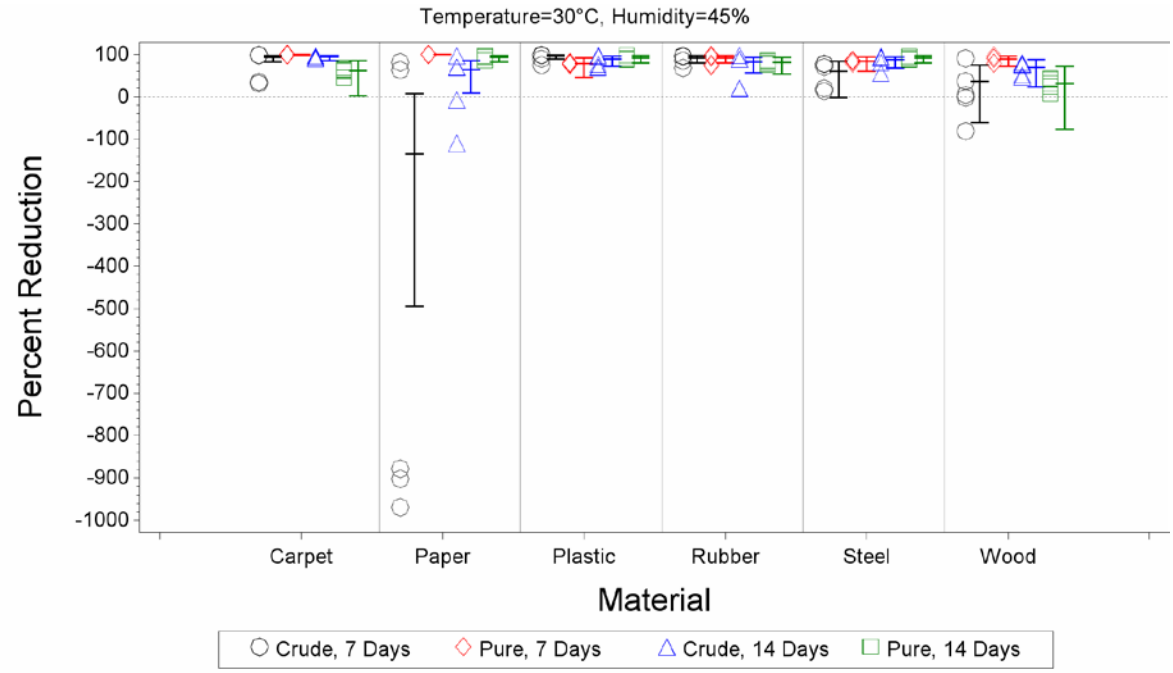

Figure B-1f. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Days 7 and 14, 30 °C and 45% Humidity.

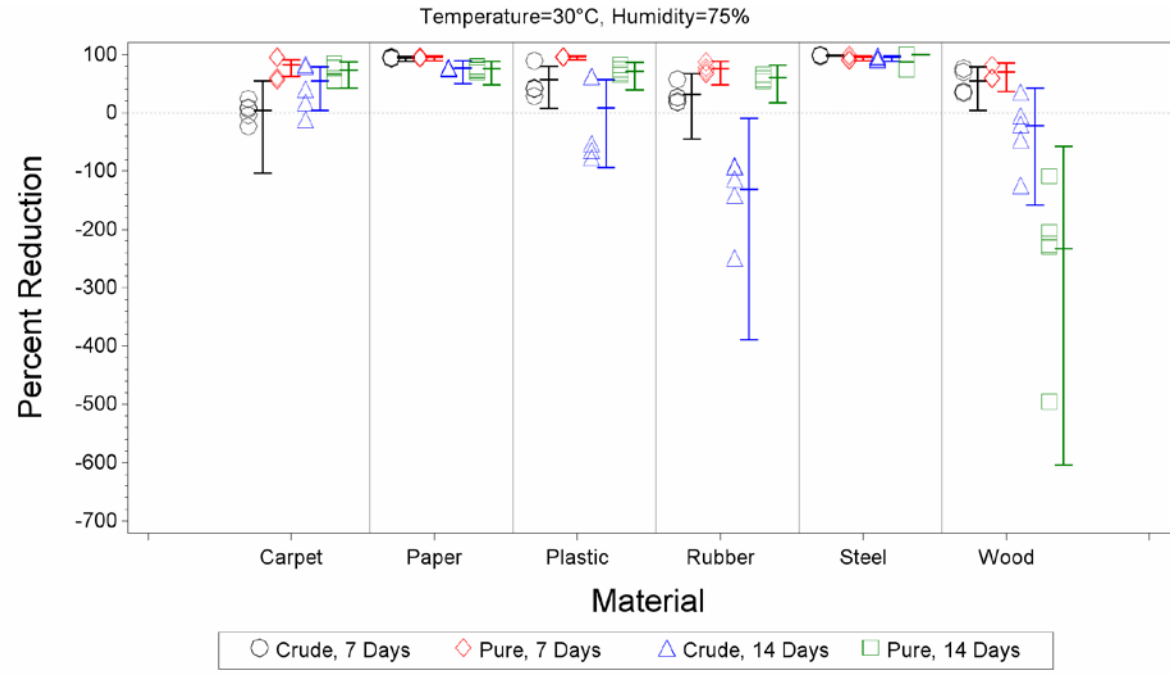

Figure B-1g. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Days 7 and 14, 30 °C and 75% Humidity.

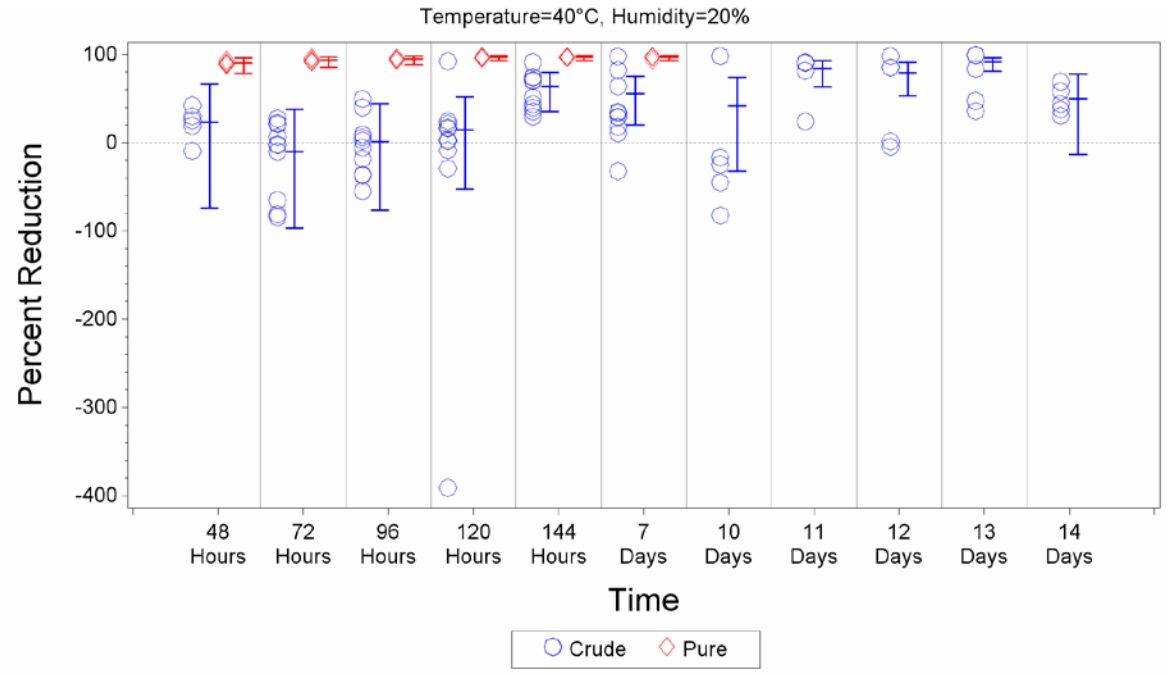

Figure B-1h. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Ricin Type and Time for Steel, 40 °C and 20% Humidity.

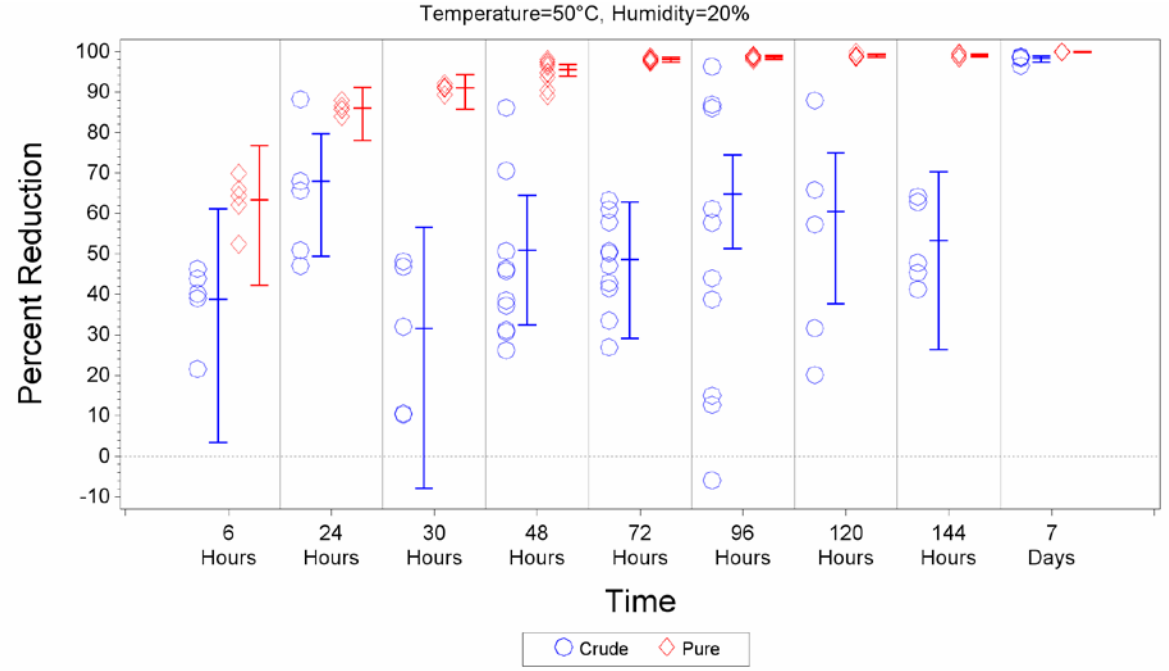

Figure B-1i. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Ricin Type and Time for Steel, 50 °C and 20% Humidity.

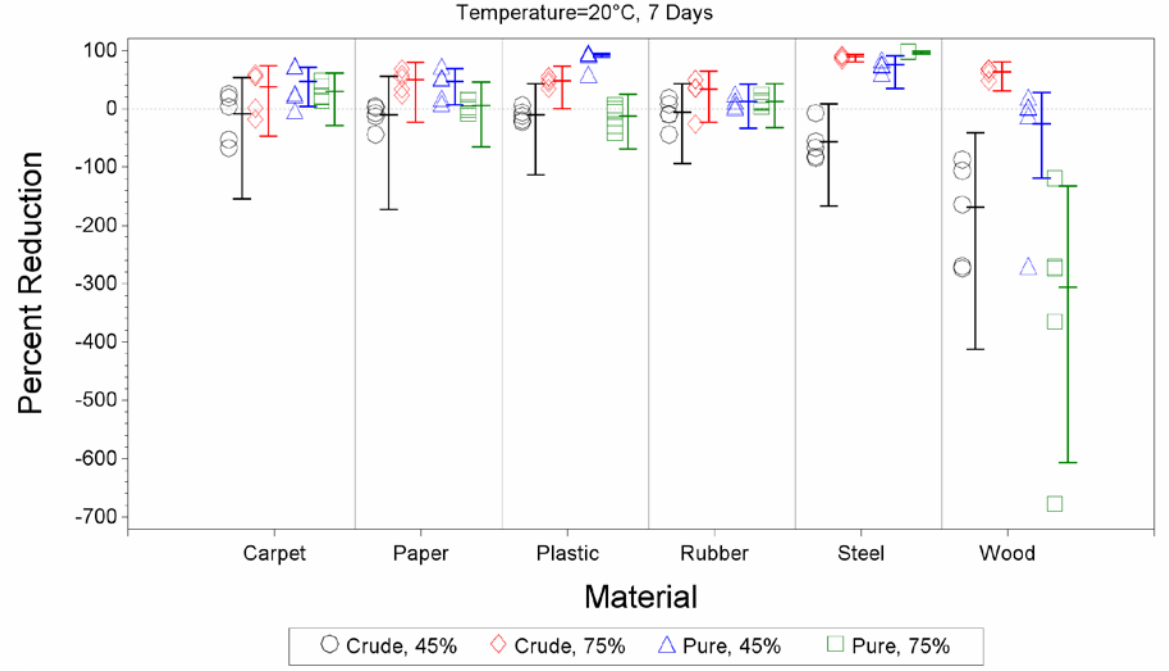

Figure B-2a. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Day 7 and 20 °C, 45% and 75% Humidity.

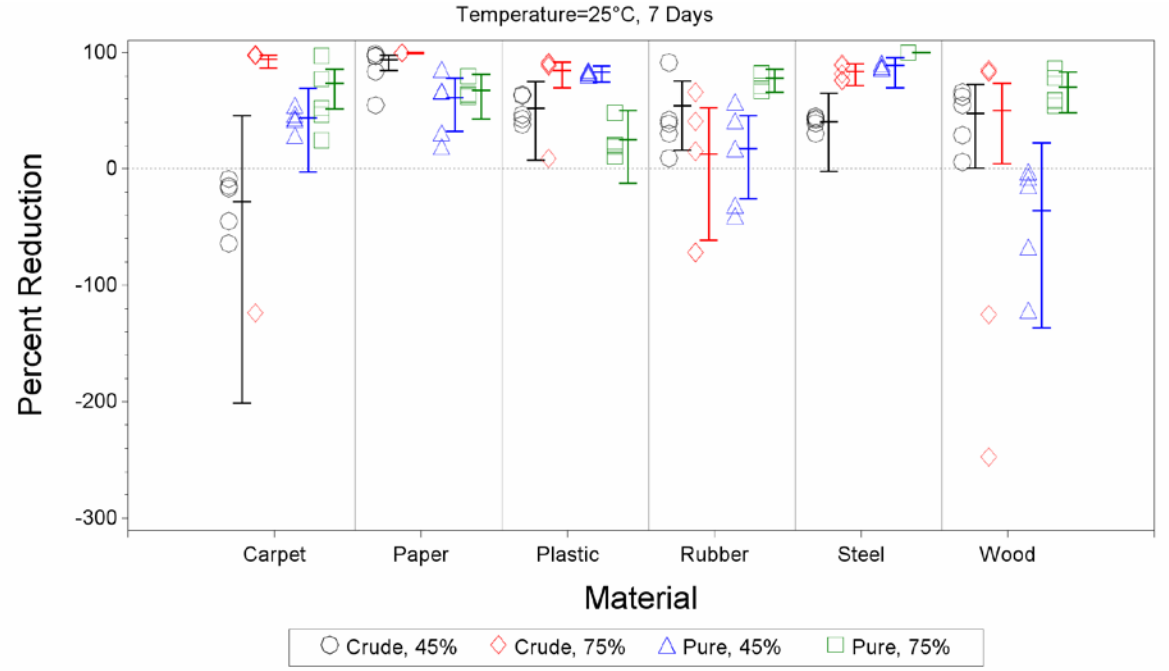

Figure B-2b. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Day 7 and 25 °C, 45% and 75% Humidity.

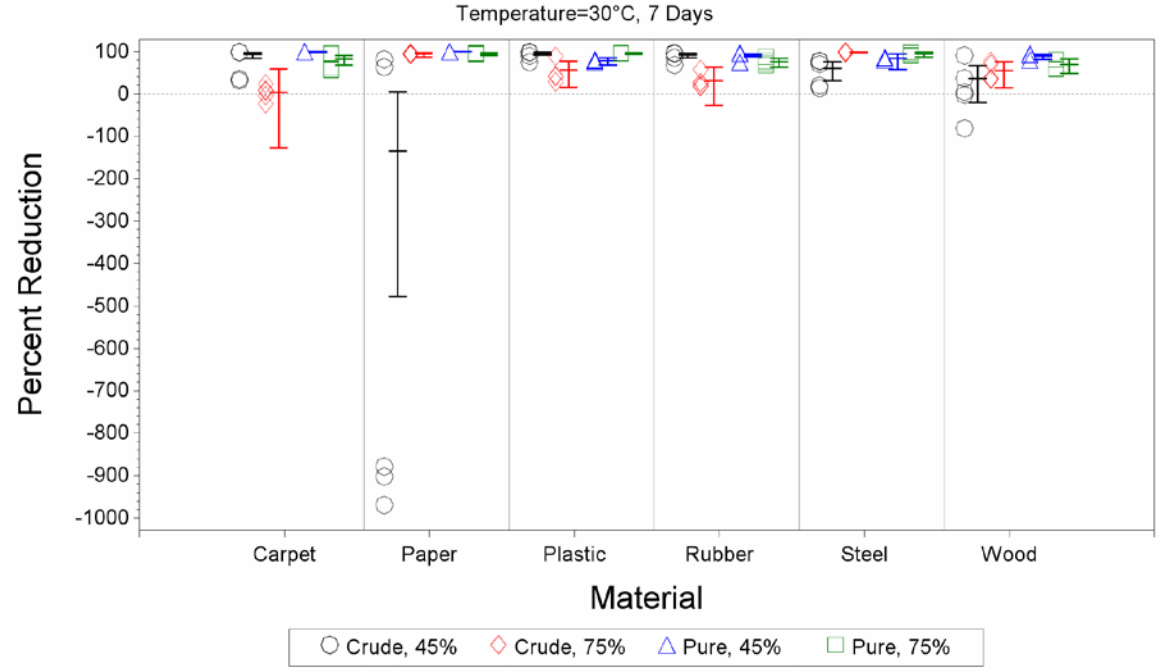

Figure B-2c. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Day 7 and 30 °C, 45% and 75% Humidity.

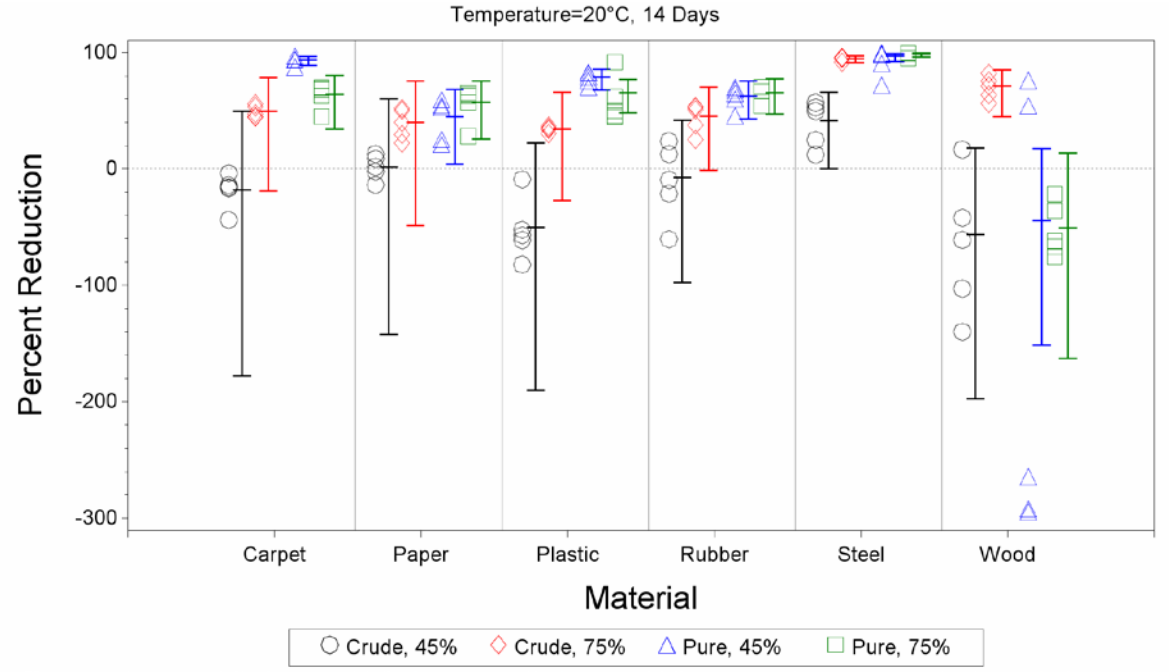

Figure B-2d. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Day 14 and 20 °C, 45% and 75% Humidity.

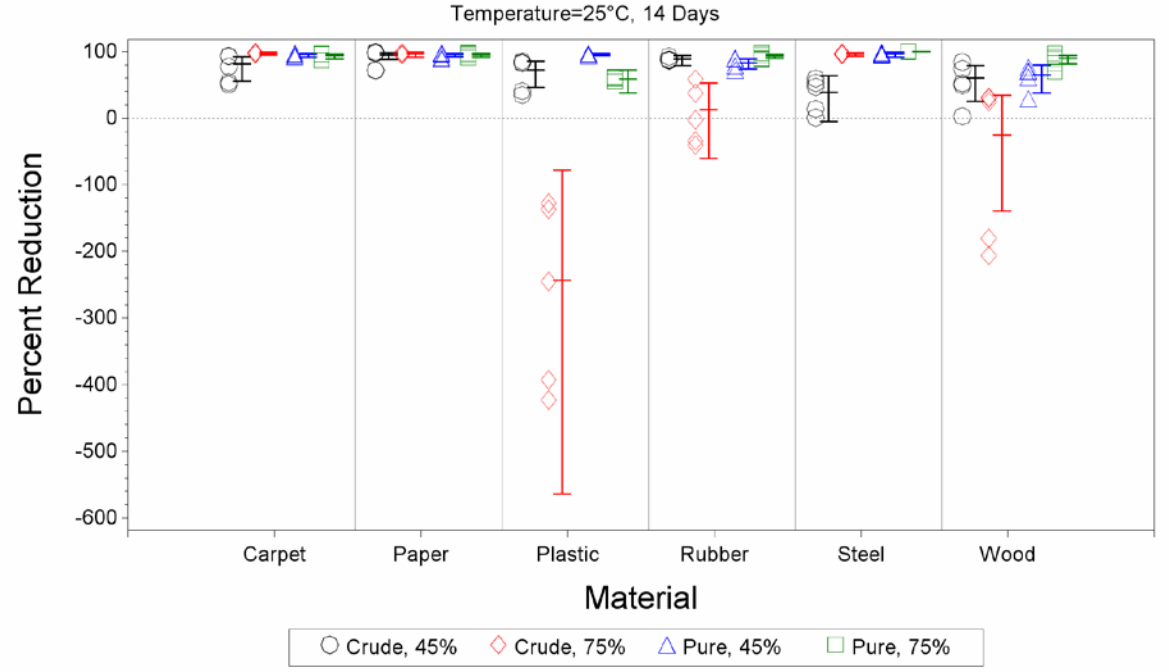

Figure B-2e. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Day 14 and 25 °C, 45% and 75% Humidity.

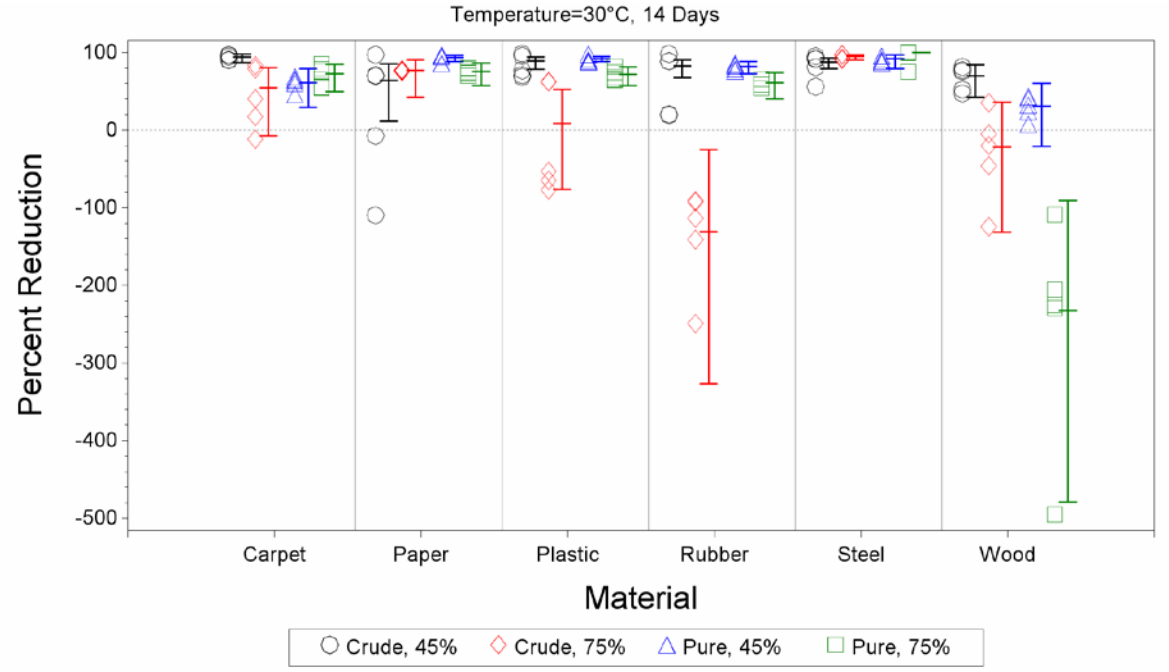

Figure B-2f. Geometric Mean Percent Reduction, 95% Confidence Intervals, and Observed Percent Reduction Plotted by Material and Ricin Type for Day 14 and 30 °C, 45% and 75% Humidity.

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