ANALYSIS OF KEY EXPERIMENTS OBTAINED IN DISCOVERY IN A *QUI TAM* CASE

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Numerical and radiobiological data subpoenaed during Discovery in a *qui tam* lawsuit were analyzed. Coulter ZM cell counts and survival colony counts that comprised data in 8 publications, a successful R01 grant proposal and its renewal were reviewed. Chi-squared analysis was performed on the terminal digits of the recorded numbers, which were expected to be approximately uniformly distributed. Counts produced by one researcher in the laboratory diverged significantly from this expectation and raised questions about the data. The p-value for the goodness of fit to a uniform distribution applied to the terminal digits of 5,155 Coulter ZM particle counter values from 171 of this individual's experiments was less than 2.2×10^{-16}. The chi-squared p-value for the terminal digits of 3,501 of colony counts in 114 experiments was also less than 2.2×10^{-16}. In data produced by several other members of the same laboratory, the terminal digits in 2,759 cell counts in 99 experiments from the same Coulter ZM particle counter and 1,556 colony counts in 59 experiments were consistent with uniformity: the p-values similarly obtained were 0.12 and 0.57, respectively. In the analyzed data of the questioned researcher, the average or near-average value of triplicate colony counts appears as one of the triples at a frequency that also greatly exceeds expectations. Additionally, results of two key experiments could not be replicated in 22 attempts. Tritiated thymidine survival kinetics in the 22 experiments conform to radiobiological predictions but differ by orders of magnitude from the questioned individual's exponential survivals. This analysis underscores the importance of access to raw data that form the bases of publications, reports and grant applications in order to evaluate the correctness of the conclusions. Methods employed in this study may prove to be useful to others in screening numerical data for anomalous results.

Introduction

Various scientists have expressed concern regarding the lack of transparency of the research data that form the bases of publications, reports and grant applications (Baggerly and Coombes 2011; Tenopir, Allard et al. 2011). Tenopir *et al.* recently stated that "Data [sharing] helps verify results data, which is a key part of the scientific process…data availability provides safeguards against misconduct related to data fabrication and falsification (Tenopir, Allard et al. 2011)." The need for open access to data became eminently apparent recently, when Baggerly and Coombes re-evaluated studies that purported to provide oncologists with information regarding patient tumor responses derived from microarray analysis (Baggerly and Coombes 2009). As a result of their investigations, at least three clinical trials were suspended, several lawsuits have been filed, at least ten articles have been retracted and the principal investigator, Anil Potti, has resigned. Even more recently, the data in more than 150 papers co-authored by the eminent Dutch social psychologist Diederik Stapel have come under fire after the revelation of statistical irregularities. The theses of 14 of Stapel's 21 graduate students are believed to contain spurious results, and Stapel himself has admitted to wrong-doing (Vogel 2011).

The raw data we analyzed in this study were made available to us through subpoena during Discovery in a *qui tam* lawsuit[1] in which Roger W. Howell (principal investigator), Anupam Bishayee (post-doctoral fellow) and the University of Medicine and Dentistry of New Jersey were co-defendants. One of us, the relator (HZH), examined approximately 30,000 PDF copies of notebook pages and other documents from the university and the laboratory that Howell supervised from 1992 to 2004. Each document presented as evidence in the *qui tam* case bears a unique Bates stamped number. Bishayee was employed by UMDNJ as a post-doctoral fellow in the laboratory from October 1997 through July 2001. The examination of the raw data was critical for the detection of the statistical and radiobiological irregularities reported herein. Bishayee's results were presented in 8 peer-reviewed publications co-authored with Howell (Howell, Goddu et al. 1998; Bishayee, Rao et al. 1999; Bishayee, Rao et al. 2000; Bishayee, Rao et al. 2000; Bishayee, Rao et al. 2000; Goddu, Bishayee et al. 2000; Bishayee, Hill et al. 2001; Howell and Bishayee 2002) as well as in the original grant application (Howell 2000-2006) and its renewal (Howell 2006-2011). Howell and colleagues continued to cite these publications as recently as June 2011(Rajon, Bolch et al. 2011).

A second post-doctoral fellow, Marek Lenarczyk, found in his preliminary experiments that he could not reproduce Bishayee's results that pertained to the bystander effect for tritiated thymidine and that had been reported in two publications (Bishayee, Rao et al. 1999; Bishayee, Hill et al. 2001). Subsequently, Howell himself failed to confirm Bishayee's results in both the 100% experiments and the 50% (bystander) experiments (Bishayee, Rao et al. 1999; Bishayee, Hill et al. 2001). Both Howell and Lenarczyk followed the same two protocols that Bishayee followed, and 16 of the attempts utilized the same cell line, Chinese hamster lung V79. Lenarczyk also studied a closely related cell line, Chinese hamster ovary CHO-K1, in six experiments. Scientists generally hold that the "reproducibility of scientific analyses and processes" is a "requirement …at the core of the scientific method." (Gil, Deelman et al. 2007)

[1]United States District Court District of New Jersey case no. 03-4837 (DMC). *Qui tam* is a suit filed by a private individual – relator – on behalf of the federal government charging violation of the False Claims Act. The Court ruled in favor of the defendants. The ruling was sustained on appeal.

Armed with the foregoing information, we undertook in-depth statistical analyses of the available data to evaluate the possibility that Bishayee's anomalous results could have occurred by chance or were the result of honest error. Our analyses led us to explore new approaches beyond the standard chi-squared test to probe more deeply.

Our results are not based on statistics alone; they are bolstered by the reported radiobiological results, which in our opinion are highly unlikely because of the experimental conditions that prevailed, i.e., no deoxycytidine, which would have antagonized the tritiated thymidine block of the cell cycle, was present during the exposure of the cells to the tritiated thymidine. Bishayee's results fail to demonstrate this block, whereas Howell, Lenarczyk and two other post-doctoral fellows in the laboratory did observe the block, which was also apparent in similar studies of the bystander effect for tritiated thymidine in Chinese hamster cells by Persaud, *et al.* (Persaud, Zhou et al. 2005).

The Discovery documents made the in-depth statistical and radiobiological analyses we have performed possible. This observation leads us to argue strongly for open access to the raw data that support scientific publications.

Methods

Statistics

The raw data were processed using the statistical program R. R is a widely used, free, open-source statistical package that is available in source form and in versions for Windows, Mac and Linux through www.r- project.org. The primary data sets that we analyzed were triplicate Coulter and colony counts that were independently drawn from suspensions of mammalian cells. The Coulter ZM is a particle counter that counts single cells as they pass randomly through a narrow orifice. Colonies arise from single cells that are distributed randomly onto tissue culture dishes. Throughout this report, the accumulated data from Bishayee's experiments are independently paralleled to the accumulated data of other investigators.

Terminal Digit Analysis: The Mosimann method (Mosimann, Wiseman et al. 1995; Mosimann, Dahlberg et al. 2002) (see below) was applied to test data sets of Coulter and colony counts produced by Bishayee and other investigators (as controls) by forming new data sets of the extracted right-most digits from the data values in the test data sets. The counts of individual digits were tabulated, and the native R chi-squared test function (chisq.test ()) was used to test the null hypothesis that the digits were drawn uniformly. The other investigators included eight members of the laboratory other than Bishayee who utilized the same Coulter counter and/or counted colonies in the same manner and two professors from out-of-state universities who contributed data from their Coulter ZM counters. Significantly low p-values in the absence of any other explanation of non-uniformity can indicate data manipulation in certain cases. The Mossimann *et al.* statistical method is recommended as a forensic tool on the website of the Office of Research Integrity of the Unites States Public Health Service (http://ori.hhs.gov/misconduct/Tips_StatisticalForensics2.shtml). Tables 1 and 2 present two examples of this analysis. Table 1 shows the actual counts in an experiment conducted by Bishayee and an experiment conducted by another investigator in the laboratory. Table 2 tabulates the occurrence as terminal digits for each of the 10 digits (0 through 9) in the two data

sets, along with the chi-squared distribution results. Note that both Coulter and colony counts are, by definition, integers.

Equal Digit Analysis: An R function was written to extract pairs of terminal digits while counting the total number of pairs (*p*) and the total number of equal pairs (*e*). The value of the R pbinom(e,p,0.10) function was subtracted from 1 to obtain the probability that the number of equal pairs exceeded the actual under the null hypothesis that the probability of equal terminal digits is 0.10. Table 1 shows double equal terminal digits highlighted in red.

Calculation of mid-ratios: A function created in R was used to calculate the mid-ratios. For the triplicate colony counts a, b, and c, in ascending order, the mid-ratio is the ratio of the difference between the middle number and the lowest number to the difference between the highest number and the lowest number (i.e.(b-a)/(c-a)). When one of the counts in a triple is close to the average of the triple, the mid-ratio of that triple will be close to 0.5. (cf. Pitt, J Expert Report, in the supporting material).

Table 3 shows the triplicate colony counts in an experiment by Bishayee and an experiment by another investigator in the laboratory, along with the derived mid-ratios. The occurrence of the rounded average in a number of the triples is shown in bold. Figure 1 is a PDF copy of the notebook page of the same experiment by Bishayee with the rounded averages highlighted in blue. Note that 9 of 10 of Bishayee's triples contain the rounded triple mean, while none of the 10 triples of the other investigator contain the rounded mean.

Analysis of the number of triples that contain their rounded mean: We developed a method to estimate the likelihood that a given number of triples in a collection contained their own rounded mean. To do so, we needed to evaluate the probability that a randomly chosen triple contains its own mean. It seems reasonable that this probability varies with the size of the gap, i.e., the difference between the largest and smallest colony counts of the triple (See Table 4 for gaps of the triples in Table 3). There is, however, an important difference that affects the estimates of the probability that a triple with an even gap contains its rounded mean and the probability that a triple with an odd gap contains its mean. This difference arises from the simple algebraic fact that when the gap between the highest and lowest value of a triple is even, there will only be one middle value that could complete it as a triple containing its own mean, whereas when the gap is odd there will be two middle values. For example, for colony counts with a =10 and c =20, the gap is 10 (even), and the only possible middle value that would create a triple that contains its mean is 15. The rounded mean of the triples (10, 14, 20; mean=14.7), (10, 15, 20; mean=15) and (10, 16, 20; mean=15.3) is 15 for all three triples. However, only (10, 15, 20) actually contains the rounded mean. For colony counts with a = 10 and c = 21, the gap is 11 (odd), and there are 2 middle values: 15 and 16. The mean of the triple (10, 15, 21) is 15.33, which rounds (down) to 15, whereas the mean of (10, 16, 21) is 15.67, which rounds (up) to 16. The weighted triple counts in Table 4 serve to compensate for the impact of odd versus even gaps in these and other experiments.

Simulations led to the hypothesis that the probability that a given, randomly selected triple contains its rounded mean should not exceed 1.3/(gap +1) if the gap is even and 2.6/(gap +1) if the gap is odd. We refer to this value as the max prob case and use it to obtain an upper bound estimate of the probability that, in a given collection of triples with known gaps, the actual number of triples that contain their mean equals or exceeds an observed value. Indeed, as the parameter of the Poisson distribution from which the triples are drawn becomes larger, the

probability that the mean is one of the counts of a triple with gap *d* appears to approach 1/(*d*+1) when *d* is even and 2/(*d*+1) when *d* is odd.

To count the number of triples that contain their rounded mean, we assign a value of 1 to the triples that contain their rounded mean and a value of 0 to the triples that do not contain their mean; we then sum these ones and zeros. Hence, under the max prob case, we view the number of triples that contain their mean as the value of the sum of variables, each of which takes the value 1 with probability 1.3/(*d* +1) if the gap is even and 2.6/(*d*+1) if the gap is odd. We can obtain the expected value of this sum by summing the various individual Bernoulli probabilities, i.e., by writing the probability of a value of 1 for a given gap as p(gap). Thus, for a collection of triples with gaps gap_1, gap_2, gap_3, ..., gap_n, the expectation is $p(gap_1) + p(gap_2) + p(gap_3) +...+ p(gap_n)$. The 6th column in Table 4 shows these expectations for the various triples. Similarly, we find the variance of the sum by adding the individual Bernoulli variances. For a given gap, the variance will be p(gap)*(1-p(gap)) (cf column 7 in Table 4). After adding these individual variances, we find the standard deviation by taking the square root (http://en.wikipedia.org/wiki/Standard_score) (column 8 in Table 4).

The Lyapunov version of the Central Limit Theorem (Fisz 1961) applies, which indicates that the sum of these Bernoulli random variables will have a near-normal distribution. Accordingly, we use tables for the normal distribution to estimate the tail probabilities. To double-check the applicability of our use of the normal approximation, we performed bootstrap calculations of the probabilities, using R to perform sets of 200,000 trials. These results did not deviate significantly from our estimates using the normal distribution.

Because the probability distribution of the number of triples in a given set that actually contain their mean would be approximately normal in the max prob case, the mean and standard deviation for that normal distribution can be readily computed from the sizes of the gaps in the collection. We can use our knowledge of the distribution in the max prob case to construct a simple test of the weaker hypothesis that the probability that the mean of a triple with gap *d* is one of the counts is less than 1.3/(*d*+1) when *d* is even or 2.6/(*d*+1) when d is odd. We construct this test by observing that, under this hypothesis, the probability that the actual number of triples that contain their mean meets or exceeds a given value is certainly less than the probability of meeting or exceeding that same value in the max prob case. Thus, once we know the mean and standard deviation for the max prob case, we can obtain an upper bound for the p-value of the actual number of triples that contained their mean by calculating the z-score (the difference between the actual and expected values divided by the standard deviation) for that number and determining the probability that a standard normal variable exceeds that value. Table 4 shows the z-scores for the two data sets, along with the corresponding probabilities.

Results and Discussion

Terminal Digit Analysis of Cell and Colony Counts

Mosimann *et al.* (Mosimann, Wiseman et al. 1995; Mosimann, Dahlberg et al. 2002) observed that when "digits are ... recorded well beyond the repeatability of an experimental procedure," the insignificant and particularly the right-most/terminal digits tend to be uniformly distributed. They added that "most people are unable to choose digits randomly." In many experiments performed in the laboratory, the concentrations of cells in single-cell suspensions were evaluated in triplicate using a Coulter ZM particle counter. The numbers of colonies distributed on the plastic surfaces of tissue-culture dishes were also evaluated in triplicate by

counting and marking each (stained) colony on the underside of its dish. We expected that because the rightmost digits of these counts were determined to be "well beyond the repeatability of the experimental procedure," they would be randomly or uniformly distributed, as had been demonstrated in a similar situation by Mosimann *et al.* for radioactivity counts recorded on hard copies from a scintillation counter.

The histograms in Figure 2 show the frequencies of the terminal digits of the Coulter counts from other members of the laboratory (Figure 2A) and from Bishayee (Figure 2B). The terminal digit counts of the other researchers exhibit reasonable uniformity, in that no single digit appears in fewer than 8% or more than 12% of the data values, whereas the digits 2, 5 and 9 appear as the right-most digit in more than 14% of Bishayee's data values, and the digit 4 appears in fewer than 6% of Bishayee's data values. Similarly, the histogram for the terminal digits drawn from the colony counts of other researchers in the laboratory is consistent with the expectation that these digits should be uniform **(**Figure 2C); again, Bishayee's values are not uniform (Figure 2D). As in Bishayee's Coulter counts, the frequencies of 2, 5 and 9 are high, whereas the frequencies of 1, 3, 4 and 7 are low.

The results of applying chi-squared tests for goodness of fit to a uniform distribution to the sets of the terminal digits of the data collected by various investigators are shown in Table 5**.** The results of other investigators are fully consistent with the expectation that the least significant digits of these types of data values are ordinarily uniform and stand in sharp contrast to the significant rejection of the uniformity hypothesis that results from applying the same test to Bishayee's data. The p-values for the chi-squared test applied to Bishayee's data sets are not only less than 0.05, which is conventionally the α level used to determine whether the results of a statistical test are "significant" enough to permit the rejection of the null hypothesis (in this case, the hypothesis that the digits are a random sample from a uniform distribution), but they are also far less than the 0.01 level at which results are often termed "very significant" and even far less than the "extremely significant" 0.001 level.

Figure 3 depicts, on a semi-log scale, the separate chi-squared p-values (for goodness of fit to a uniform distribution) for the terminal digits of the Coulter counts from 288 individual experiments conducted in the laboratory between February 1995 and October 2001. For 45 of these experiments, the hypothesis that the terminal digits of the data values were drawn uniformly would be rejected at the 0.01 level (a stringent testing condition); all 45 of these experiments were conducted by Bishayee. The many experiments in which his chi-squared values are consistent with uniformity (>0.01) indicate that Bishayee's results are not likely the result of instrument malfunction.

If the two right-most digits in the Coulter data are uniformly or randomly distributed, then the probability that they will be equal is 0.1. Whereas 280, or 10.1%, of the right-most digit pairs of 2,759 Coulter values from the other investigators were equal, 636, or 12.3%, of the pairs from 5,155 of Bishayee's Coulter values were equal. The proportion of equal right-most digit pairs from the other investigators falls into the 95% confidence interval (0.092, 0.111), whereas the 95% confidence interval for Bishayee's equal-pair frequency is (0.116, 0.130). Assuming that these right-most pairs were generated uniformly, the probability of 636 or more equal pairs in 5,155 Coulter values is less than 2.8×10^{-8}, which significantly contraindicates their expected randomness. In contrast, the probability of 280 or more equal pairs among 2,759 Coulter values for the other researchers is 0.38, which is consistent with our randomness hypothesis. (cf. Pitt, J Expert Report, in the supporting material). Obviously, the terminal duplicates would be

expected to be randomly or uniformly distributed by digit, as are the terminal digits. This property holds for the other members in the laboratory: the chi-squared p-value is 0.30. However, the p-value for Bishayee's data is, again, considerably less than 2.2×10^{-16}. The terminal doublet distributions are shown in Figure 2A and 2B.

Frequency that the Average Value of Three Colony Counts Appears as One of the Triples

The appearance of the average or near-average (average ± 1) value of the colony triples appeared frequently as one of the three values in Bishayee's colony counts. To evaluate this observation in greater depth, we sorted each colony count triple in ascending order and calculated its mid-ratio (cf. Methods). The results of these calculations are shown in Figure 4. The graph in 4A shows the mid-ratio distribution of 486 triples in 58 experiments that were performed by investigators other than Bishayee in the laboratory, whereas graphs 4B – 4I show the mid-ratio distributions from Bishayee's experiments with various radioactive isotopes. (The graph in 4C represents 190 triples in 13 100% tritiated-thymidine experiments and will be discussed below.) The contrast between 4A and the other graphs is immediately apparent. The distribution of the mid-ratios of the triples reported by the investigators other than Bishayee is reasonably uniform across the set of all of the potential values, which range from 0 to 1 in increments of 0.1. In the graphs of Bishayee's mid-ratios, the bars between 0.4 and 0.6 protrude distinctly above the others.

Upon closer examination, we noticed that the relative frequency with which the average or near-average appeared as one of the values of a triple was not exceptionally high in the data values for the control (usually not radioactively exposed) colonies in Bishayee's experiments, although it seemed unusually high in the test sample (usually radioactively exposed) triples. To provide a better quantification of our observation, we identified the triples that contained at least one colony count that was exactly equal to the rounded value of the mean of the triple and performed separate counts of these triples for Bishayee's control triples and test triples as well as the control triples and test triples of the other investigators. We calculated the relative frequency with which such triples occurred in each group. When the difference between the lowest and highest values of a triple is less than two, the rounded average of the counts in the triple will always be a count in the triple. Therefore, we considered only those triples in which the difference was greater than or equal to 2. The results of this analysis are shown in Table 6. Sixty-four percent of Bishayee's test triples contain the rounded mean as one of the counts, whereas the frequency of this effect in the other three data sets is less than 18%.

When the counts of triples are selected by the same random mechanism, the likelihood that one of the counts is equal to the mean value of the triple varies with the gap size of the triple (i.e., the difference between the largest and smallest counts in the triple). The variation is complicated by the fact that when the gap is odd, there are two possible mid-values that can complete a triple that includes its own mean, whereas when the gap is even, there is only one such value (cf. Methods).

It is, of course, possible that the unusually high frequency of triples containing their mean in Bishayee's test data might be the result of a preponderance of triples in which the gap is small. To compensate for this possibility, we performed a more elaborate calculation (described in detail in the next paragraph) in which we effectively weighted the triples in which the mean appeared, based on the foregoing observations regarding gap sizes.

Rather than counting the number of triples that contained the mean, we summed the gaps for all of the triples that contained their mean and divided that sum by the sum of all of the gaps. The results are shown in Table 7. Although this approach is not standard, it is more conservative because it takes the size of the gaps into account, and once again, the result (60.3%) that we obtained for Bishayee's test triples is markedly larger than the results for the other three data sets, which were all less than 15%.

We pursued the investigation one step further to estimate the probability that the discordance between the rate at which the average value appeared in Bishayee's test triples and the rate at which it appeared in the other data sets might have been the result of chance. We developed the concept of the max prob case, as explained in the Methods section.

We applied this test to the triples that Bishayee recorded for colony count controls and colony count test runs, as well as to those recorded by the other investigators. The entries in the first column of Table 8 identify the specific sets of triples that we considered. Each entry in the second column is the calculated mean in the max prob case; the third column displays the standard deviation for the max prob case; the fourth column displays the actual number of triples in the set that contained their mean; the fifth column displays the corresponding z-value; and the sixth column displays the probability of meeting or exceeding that z-value in a standard normal distribution.

Bishayee's control and test counts are both significantly above the expected levels. The z-score for the test values indicates there is a very small probability that so many triples could have contained their means by pure chance.

The results for the triples produced by other investigators are entirely consistent with our null hypothesis. Indeed, the actual number of triples that contain their means is less in both cases than the expected value under the null hypothesis, which is not entirely surprising given that the expectation is computed based on the max prob case, which is hypothesized to overestimate the likelihood that a triple contains its mean.

In Table 9, we display the results of applying the same analysis to the distinct sets of triples that correspond to the different isotopes for which Bishayee recorded colony counts. All of the results are highly significant.

Although we formulated our hypothesis after observing the triples of the colony counts, the same analysis applies to the triples of the Coulter counts. Consequently, we applied the foregoing hypothesis test to the Bishayee Coulter count triples, the Coulter count triples for the other investigators in the same laboratory and the Coulter count triples that we had obtained from investigators in other laboratories. The results are shown in Table 10. Again, the number of triples that contain their mean in Bishayee's data sets is significantly higher than would be expected under the null hypothesis, whereas the value for the triples reported by the other investigators are entirely consistent with the null hypothesis.

Tritiated Thymidine Experiments and the Role of Deoxycytidine

Thymidine concentrations of approximately 1 mM or greater in the tissue culture medium block the cell cycle at the G1/S interface and during S phase (Tobey, Anderson et al. 1967; Hall and Giaccia 2005). Radiation blocks the cell cycle primarily in G2 (Hall and Giaccia 2005). High-specific-activity tritiated thymidine blocks the cell cycle and kills cells at nanomolar concentrations (Ehmann, Williams et al. 1975; Pollack, Bagwell et al. 1979; Hoy, Lewis et al.

1990; Hu, Black et al. 2002). The cell-cycle-blocking effect of thymidine is reversed by deoxycytidine (Morris and Fischer 1960; Bjursell and Reichard 1973; Fox, Tripp et al. 1980; Wheater and Roberts 1987; Hiramoto, Narahara et al. 1990).

Numerous reports have described the killing effect of tritiated thymidine on cultured mammalian cells. The cells are generally incubated with the isotope up to or beyond the cell-cycle time before plating for colony formation. Storage under non-growing conditions allows more tritium decay before plating. The survival curves take 3 forms: (1) monotonic exponential; (2) initial shoulder, then exponential; and (3) biphasic, i.e., a rapid decline in survival followed by a slower decline or plateau. When the exponential killing occurred without (Burki and Okada 1970; Chan, Lisco et al. 1976) or with (Marin and Bender 1963; Burki, Roots et al. 1973; Bedford, Mitchell et al. 1975; Burki, Koch et al. 1978) a shoulder, deoxycytidine was also present in the medium, or its presence was inferred (Elkind and Sutton 1960; Sinclair 1964; Burki, Roots et al. 1973). In its absence, the survival curve was biphasic (Drew and Painter 1959; Drew and Painter 1962; Burki and Okada 1970; Keprtova and Minarova 1985; Hu, Black et al. 2002; Persaud, Zhou et al. 2005), with only one exception (Panter 1981).

In six experiments from the laboratory obtained during Discovery, the V79 cells were incubated with increasing concentrations of high-specific-activity tritiated thymidine with and without deoxycytidine for approximately one cell cycle before plating for colony formation (Figure 5). Both survival curves are biphasic, but the final plateau is approximately 15-fold lower with deoxycytidine than in its absence, which confirms the reversal of the cell cycle-blocking effect of tritiated thymidine by deoxycytidine.

Howell and Lenarczyk performed a total of ten 100% experiments (all cells exposed to tritiated thymidine): 7 experiments involving V79 cells and 3 experiments with CHO-K1 cells. No deoxycytidine was present. The V79 survival curves were biphasic and reached a plateau of approximately 0.5, except for one curve that reached a plateau at approximately 0.3 (Figure 6A). These survival results contrast with Bishayee's exponential decline, which was reported in two publications (Bishayee, Rao et al. 1999; Bishayee, Hill et al. 2001) (dashed line). The CHO-K1 survival curves are similar, but they reach a plateau at approximately 0.3 (data not shown). Howell's and Lenarczyk's V79 results are consistent with the V79 biphasic survival curves reported by Keprtova and Minarova (Keprtova and Minarova 1985) under similar conditions. Howell's ultimate survival at 5 mBq is approximately 160 times greater than Bishayee's following the same protocol. (See supporting material, Expert Report of Michael Robbins).

The defense's expert witness in the *qui tam* case argued that the exponential survival slope in Bishayee's 100% experiments was predicted from radiobiological principles (cf. Table 1 in both references (Bishayee, Rao et al. 1999; Bishayee, Hill et al. 2001)) (Figure 6A). The 100% slope, i.e., A_1 in reference 2, was computed from the survival curves of eight experiments to be the predicted 0.8 mBq per labeled cell. Thirteen 100% experiments were found in the PDF files examined during Discovery; the triple colony analysis is shown in Figure 4C. The p-values for the uniformity of the chi-squared tests for the terminal digits of the Coulter and colony data for these experiments are 1.26×10^{-7} and 2.5×10^{-4}, respectively. These values are significant indicators that the terminal digits were not drawn uniformly. The distribution of the triples in Figure 4C does not conform to that of the controls in Figure 4A. (See supporting material, Expert Report of Ludwig Feinendegen.)

The defense expert also posited that exponential survival curves would occur in the absence of deoxycytidine, as seen in Bishayee's 100% experiments, because the thymidine concentration in the high-specific-activity nuclide is too low to affect the cell cycle. "Under these conditions [high specific activity] ^{3}H-TdR becomes a true tracer of physiological metabolism and does not perturb the … nucleotide pool" (from Expert Report, Ludwig E. Feinendegen, MD., June 2009). However, both Keprtova and Minarova (Keprtova and Minarova 1985) and Hu *et al.* (Hu, Black et al. 2002) reported biphasic survivals when using similar high-specific-activity tritiated thymidine. Furthermore, Cleaver and Holford found that a 10-minute pre-incubation with 10^{-9} M thymidine decreased the incorporation of 2.5×10^{-6} M tritiated thymidine into the DNA by 10%, which indicates that minute amounts of thymidine perturb the intracellular thymidine pool (Cleaver and Holford 1965). The results in Figure 5 also support the conclusion that deoxycytidine is needed to abrogate the blocking of the cell cycle by tritiated thymidine. (See supporting material, Expert Report of Ludwig Feinendegen.)

Fifty Percent Experiments and the Bystander Effect

In 1992, Nagasawa and Little showed that 30% of an irradiated population of CHO cells had increased sister chromatid exchanges, which were indicative of DNA damage (Nagasawa and Little 1992), even though only 1% of the cells were traversed by an alpha particle. This phenomenon is known as the bystander effect and also occurs following exposure to other types of radiation. Bishayee and Howell claim to be the first to show a bystander effect using tritium (Bishayee, Rao et al. 1999; Bishayee, Hill et al. 2001); they claim that the bystander effect is abrogated by DMSO, which is a scavenger of reactive oxygen species (ROS), and by lindane, which is an inhibitor of gap junction intracellular communication (GJIC), thereby implying that the bystander effect is transmitted by cell-to-cell communication involving the transfer of ROS. However, others have shown that bystander effects in CHO cells are transmitted through the medium (Vines, Lyng et al. 2009). Bystander effects in radiation impact both radiation therapy and nuclear medicine (Mothersill, Moriarty et al. 2004; Banaz-Yasar, Lennartz et al. 2008). Inaccurate information could lead to errors in treatment planning, allowable doses in diagnostic radiation and permissible workplace exposures.

"Clusters," or mixtures of cells, of which half were exposed overnight to tritiated thymidine and half were unexposed (bystanders), were incubated for 72 h in the cold. Data in Bishayee's experiments purports to demonstrate that both exposed cells and bystanders are killed during this union. Lenarczyk performed five such "50%" experiments with V79 cells and three with CHO-K1 cells. Howell also performed four 50% experiments with V79 cells. The V79 experiments are summarized in Figure 6B and are compared to the 50% experiments in the two published reports (Bishayee, Rao et al. 1999; Bishayee, Hill et al. 2001) (dashed line). There was a 70-fold difference in the survival at the highest radiation levels, although the experiments followed the same protocol. Lenarczyk's and Howell's experiments reach a survival plateau at approximately 0.70, whereas their 100% survival (cf. **Figure 6A**) leveled at approximately 0.5. Because the exposed cells comprise half the mix, the overall expected survival would be 0.75, assuming that no bystanders are killed.

The Helena Tubes Are Hypoxic

Hypoxic conditions decrease the lethal effects of low linear energy transfer (LET) radiation (tritium, gamma and X-rays) by up to approximately 3-fold; this is the so-called "oxygen effect." (Hall and Giaccia 2005)

Howell designed an experiment to test the clusters for hypoxia (see details in the supporting material). The cells in half the tubes were undisturbed and potentially hypoxic, and the cells in the other half were re-aerated (resuspended) after 72 h of incubation in 0.4 ml of medium in capped Helena tubes (0.4 ml capacity). The survival and mutation (Hsie, McElheny et al. 1979) were measured in ten samples (5 re-aerated, 5 potentially hypoxic). In Bishayee's experiment, the mutation testing required 120 Coulter data counts; the terminal digit analysis for uniformity determined a chi-squared p-value of 7.4×10^{-13}. The average of the three survival colony counts for each sample appeared as one of the counts of the triple in the 6 irradiated samples and 1 control (Z-score 5.86, $p(z) = 2.31 \times 10^{-9}$). The relator examined some of the dishes that were believed to contain mutant colonies and reported to Howell that she found no mutant colonies on them, even though Bishayee reported mutant colonies for all the data points in this experiment. Howell made no comment. Bishayee's results showed somewhat less killing and fewer mutants in the undisturbed samples, "clusters". These results were displayed in Figure 7 of the grant application and allowed the referees to conclude that severe hypoxia was not present in Howell's clusters, which were designed to model the human isotope distribution in nuclear medicine.

Two weeks earlier, the relator had followed the same mutation protocol with V79 cells. The re-aerated cultures showed a marked increase in mutants, but there was no increase in mutants in the undisturbed cultures. The relator concluded that severe hypoxia was present in the Helena tubes; these results conflicted with Bishayee's results for the same protocol. Bishayee's experiments and the relator's experiments are included in the supporting material (B007891-B007900; B013918-B013926; B020145-B020151).

No repetitions of this experiment were found in the Discovery documents. The gamma ray survival studies in Helena tubes performed by Lenarczyk and a later post-doctoral fellow similarly indicated severe hypoxia (cf. Robbins, MR Expert Report in the Supplemental Material).

Investigators at Columbia University using CHO cells in microfuge tubes that allowed 100 ml of air above the medium demonstrated a bystander effect for tritiated thymidine (Persaud, Zhou et al. 2005). Their 100% survival tends towards a plateau of approximately 30%, which supports the results of Howell and Lenarczyk but not Bishayee.

Summary

We have recorded the following observations:

1. Coulter count terminal digits: Bishayee's digit distributions differ significantly from the expected (near) uniform frequencies; those of the other investigators do not.

2. Colony count terminal digits: Bishayee's digit distributions differ significantly from the expected (near) uniform frequencies; those of the other investigators do not.

3. Right-most and next to right-most Coulter digits: Bishayee's doublets are equal at a frequency that deviates from the expectation of randomness; those of the other investigators do not deviate significantly from the expectation of randomness. Furthermore, the distribution of the doublet values of the other investigators is also not significantly different from the expectation of randomness, whereas the distribution of Bishayee's doublet values is considerably different from the random expectation.

4. Values that are very near the average appear as one of the counts in Bishayee's triple colony counts at significantly higher frequencies than expected. The rate at which this phenomenon occurs in the triple data from others is consistent with expectations of random.

5. One hundred percent experiments: In the absence of deoxycytidine, Bishayee's exponential survival curves differ from the results in the literature; additionally, the results of Howell and Lenarczyk indicate biphasic survival curves that are similar to the majority of the reports that were found in the literature.

6. Fifty percent experiments: Bishayee's results differ markedly from the results of Howell and Lenarczyk, who followed the same protocols.

7. Twenty-two attempts by Howell and Lenarczyk failed to replicate or confirm Bishayee's 100% and 50% tritiated thymidine results.

8. It is likely that the "clusters" in the Helena tubes are hypoxic.

9. There is an eye-witness report by the relator that there were no mutant colonies in Bishayee's dishes that she examined in an external beam gamma ray experiment, and his purported report of mutant induction in the absence of re-aeration conflicts markedly with the relator's results following the same protocol. Additionally, the chi-squared probability that the terminal digits in Bishayee's 120 Coulter counts in this experiment are uniformly distributed is less than 8×10^{-13}.

As of December 2011, there were 267 citations of the papers of Bishayee and Howell, of which the most recent occurred in September 2011. Howell has cited the papers in his own publications as recently as June 2011.

This analysis raises serious questions about the results that provided the experimental background for several published papers as well as a grant application and its renewal. Had this information been known to the study section and the referees, would it have altered their decisions? With the vast capabilities of the worldwide web to store information, should not the raw data on which such decisions are based be made publicly available? A prototype for the web storage of experimental data has already been established by the physics community (Ginsparg 2011), and electronic notebooks are encouraged to facilitate data sharing and analysis (Butler 2005) (see also (Tenopir, Allard et al. 2011)). These practices could help to avoid the publication of material that does not have strong experimental support or that is otherwise unreliable.

The statistical analysis of numerical data can be used to identify aberrant results (Tomkins, Penrose et al. 2010; Postma 2011; Tomkins, Penrose et al. 2011). Terminal digit analysis, as described by Mossimann *et al.* (Mosimann, Wiseman et al. 1995; Mosimann, Dahlberg et al. 2002), provides a simple method for detecting anomalous data, and we show in this study that the careful observation and analysis of recorded values can lead to additional understanding of deviations from expectations of randomness. Recently, a rigorous statistical analysis of data that purported to predict the responses to chemotherapeutic agents of human lung, breast and ovarian cancers demonstrated the erroneous nature of the results (Baggerly and Coombes 2009; Baggerly and Coombes 2011) and led to several retractions (Baggerly and Coombes 2010; Goldberg 2010; 2011; 2011) and a resignation (2010). In this case, patients were potentially directly affected by the use of the wrong drug and/or the withholding of the right drug. The experiments reported in the Howell studies were designed to "correlate biological response with mean absorbed dose … in diagnostic and therapeutic nuclear medicine…. In….diagnosis, the risk of radiation insult can …be drastically underestimated and … lead to increased risk of inducing cancer. In contrast,

patients can be over or under treated in radionuclide therapy of cancer."(Howell 2000-2006) Howell's research was designed to prevent these untoward outcomes in nuclear medicine in the use of radionuclides for the diagnosis and treatment of cancer. The implementation of Howell's published results could lead to the underestimation of doses in diagnosis and to over- or under-treatment in radionuclide cancer therapy. It is disturbing that his results are potentially misleading in this way.

Limitations

Our studies are limited to the analysis of data that may not always have been in forms that we would have chosen. For example, the control experiments by other investigators that were available to us were fewer in number than the experiments performed by Bishayee. Because smaller sample sizes have less power, we randomly selected 314 terminal digits from Bishayee's Coulter results and ran chi-squared analyses 100,000 times to test for uniformity. All of the runs would have rejected the null hypothesis for uniformity at the 0.00001 level; one run rejected the hypothesis at the 0.000000001 level. The value of 314 was selected because it is the total number of digits supplied by one of the two outside contributors and is the smallest Coulter sample set (cf Table 5).

During the time that Bishayee was working in the laboratory, few experiments were being performed simultaneously by others, which resulted in some temporal disparity. However, the protocols that we analyzed were followed almost identically by all of the members of the laboratory. There is no *a priori* evidence that the cells, instrumentation, equipment and consumable supplies used by the other researchers were any different from those utilized by Bishayee. There is also no evidence that different operators could influence the terminal digits seen on the display of the Coulter counter. All of the investigators used similar techniques to stain and count the colonies. Nevertheless, automatic colony counters are commercially available, and their use should be encouraged. Similarly, a simple oxygen electrode could have expeditiously determined whether the Helena tubes were hypoxic, and the counts from the Coulter ZM could have been recorded on a printer.

Conclusion

Bishayee's results challenge radiobiological expectations, and statistical analyses indicate that his numerical results diverge significantly from expectations of uniformity or randomness and are extremely unlikely to have resulted from chance alone. Because the experiments that he performed form the background of as many as 8 publications, we suggest that it would be appropriate for Howell, the Principal Investigator, and Bishayee to retract these papers.

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Appendix I

Brief summary of protocols for the experiments in the laboratory (cf. also the PDF files in the supporting material):

Cells are plated into 150-cm^2 flasks and incubated for 1 to 2 days. The cells are trypsinized, harvested and transferred to Falcon tubes (17x100 mm) containing 2 to 4 million cells in 1 ml of medium (MEM with 10% FBS), which are placed on rollers in a 37°C incubator. Radioisotopes are added as appropriate after 3 hours. The tubes are rolled overnight, following which the cells are harvested and washed several times to remove any unincorporated isotope.

In the 100% experiments, each Falcon tube contains 4 million cells.

In the 50% experiments, each Falcon tube contains 2 million cells. Half of the tubes are incubated with the radioisotope.

In the external beam ^{137}Cs experiments, each Falcon tube contains 4 million cells and no radioisotope.

After the overnight rolling, washing and pairing of labeled and unlabeled cells for the 50% experiments as appropriate, 4 million cells from each tube are transferred to 400-µl Helena tubes with attached caps, with or without additives such as DMSO and/or lindane, in a total volume of 400 µl. The tubes are closed.

The Helena tubes are lightly centrifuged (1,000 rpm, 5 min, 4°C) to allow the cells to form "clusters" and are transferred to a 10°C incubator for a 72-hour incubation.

In the external beam ^{137}Cs experiments, after the 72-hour incubation, the cells in half of the Helena tubes are resuspended for aeration, and the other half remain as clusters. The tubes are irradiated as appropriate.

The cells are then harvested from the Helena tubes, washed several times, diluted and plated in triplicate for colonies. Three aliquots of the original suspension are counted in the Coulter counter to assess the cell number and in the scintillation counter to assess the radioactivity.

The dishes for the colonies are incubated at 37°C for approximately one week; the colonies are then fixed, stained and counted.

Supporting material:

1. The three expert reports
2. Lenarczyk's experiments
3. The PDF files obtained through Discovery, purged of extraneous and irrelevant files
4. A locator for the raw data in the PDF files (Colony and Coulter lists by Bates and Date)
5. Tritiated thymidine survivals with and without deoxycytidine
6. The R routines stated in the methods

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Table 1: Examples of Triplicate Coulter Counts

Sample #	AB Triplicate Counts			OI Triplicate Counts		
1	577	592	563	89	97	86
2	611	607	653	331	316	329
3	581	593	617	378	330	375
4	633	645	619	333	404	367
5	511	537	549	396	382	408
6	544	562	573	342	331	344
7	666	672	693	340	349	344
8	601	572	633	325	347	304
9	511	529	541	315	291	283
10	532	555	562	307	339	323
11	513	549	562	285	314	323
12	562	539	547	260	262	284
13	560	542	522	361	315	298
14	680	669	671	355	324	356

These were reported in an experiment by Bishayee (AB) performed on 10/9/2000 (Bates # B001357, cf. supporting material) and from an experiment by another laboratory investigator (OI) performed on 8/11/2000 (Bates # B007230). The terminal digits are shown in bold. The terminal duplicates are shown in red. There are 10 doubles in Bishayee's samples (23.8%) and 4 in the other investigator's samples (9.5%).

Table 2: The Terminal Digit Counts from Table 1 and The Chi-Squared Probability of Uniform Distribution.

Digit	0	1	2	3	4	5	6	7	8	9	Total	Chi Sq	Chi sq p for uniform
AB Freq	2	7	9	8	1	2	2	5	0	6	**42**	**21.8**	**9.5×10^{-3}**
OI Freq	3	4	3	4	7	6	4	4	3	4	**42**	**3.7**	**9.3×10^{-1}**
Ctrl Freq	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	**42**		

The chi-squared goodness of fit was determined in Microsoft Excel (9 degrees of freedom) for the digit frequencies of AB and OI compared with the control uniform distribution.

Table 3: Examples of Triplicate Colony Counts and Mid-Ratios

Sample #	AB Triplicate Counts			Average	Mid-ratio (b-a)/(c-a)	OI Triplicate Counts			Average	Mid-ratio (b-a)/(c-a)
1	130	149	142	140.33	0.63	92	111	119	107.33	0.70
2	131	**137**	143	137.00	0.50	78	85	74	79.00	0.36
3	123	**131**	138	130.66	0.53	142	126	120	129.33	0.27
4	128	**134**	140	134.00	0.50	120	129	121	123.33	0.11
5	125	**130**	136	130.33	0.45	64	68	79	70.33	0.27
6	115	**126**	137	126.00	0.50	92	101	78	90.33	0.61
7	17	**20**	24	20.33	0.43	74	62	94	76.67	0.38
8	29	**35**	41	35.00	0.50	89	69	67	75.00	0.091
9	**62**	70	54	62.00	0.50	85	87	97	89.67	0.17
10	**70**	79	62	70.33	0.47	71	58	55	61.33	0.19

These are from an experiment by Bishayee (AB) performed on 2/22/1999 (Bates # B008127, cf. supporting material) and from an experiment by another laboratory investigator (OI) performed on 4/19/2001 (Bates # B007385). Triple values that are equal to the rounded average of the triple are shown in bold.

Table 4: Demonstration of Results of Various Calculations on the Data in Table 3

	Sample #	Gap	Weighted Triple Counts	Weighted Triple Counts with Rounded Means	Max Prob Expected Rounded Means of Triples	Max Prob Variances	Standard Deviation	Actual Number of Triples with Rounded Means	Z-Score for Actual Number	Probability of Z-Score
AB	**1**	19	38		0.130	0.113				
	2	12	12	12	0.100	0.090				
	3	15	30	30	0.163	0.136				
	4	12	12	12	0.100	0.090				
	5	11	22	22	0.217	0.170				
	6	22	22	22	0.057	0.053				
	7	7	14	14	0.325	0.219				
	8	12	12	12	0.100	0.090				
	9	16	16	16	0.076	0.071				
	10	17	34	34	0.144	0.124				
	Sum		**212**	**174**	**1.412**	**1.156**	**1.075**	**9**	**7.06**	**$<9.9x10^{-10}$**
	%			**82.08**						
OI	**1**	27	54	0	0.047	0.045				
	2	11	22	0	0.113	0.100				
	3	22	22	0	0.057	0.053				
	4	18	18	0	0.137	0.118				
	5	30	30	0	0.084	0.077				
	6	46	46	0	0.055	0.052				
	7	20	20	0	0.062	0.058				
	8	22	22	0	0.057	0.053				
	9	12	12	0	0.100	0.090				
	10	16	16	0	0.077	0.071				
	Sum		**262**	**0**	**0.788**	**0.718**	**0.847**	**0**	**-.930**	**0.176**
	%			**0**						

The gap values from Table 3 are shown along with the weighted triple counts (1∗even gap; 2∗odd gap); weighted triple counts with rounded means; max prob expected rounded means of triples (1.3/(even gap + 1); 2.6/(odd gap +1)); variances of same (max prob ∗(1-max prob expected)); standard deviation; actual number of triples with rounded means; z-score for same ((actual – expected)/standard deviation) and probability of z-score

Table 5: Terminal Digit Analysis of Coulter and Colony Counts

		Digit												
Type	**Investigator**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**	**Chi-sq**	**P-value**
Coulter	**Bishayee 171 experiments**	472	612	730	416	335	725	362	422	370	711	5155	456.4	$< 2.2x10^{-16}$
Coulter	**Others 99 experiments**	249	294	276	244	296	270	284	258	306	282	2759	13.9	0.13
Coulter	**Outside lab 11 experiments**	28	34	29	24	27	36	44	33	26	33	314	9.9	0.36
Coulter	**Outside lab 17 experiments**	34	38	45	35	32	42	31	35	35	33	360	4.9	0.84
Colonies	**Bishayee 114 experiments**	514	267	395	265	262	418	306	261	342	471	3501	228.4	$< 2.2x10^{-16}$
Colonies	**Others 59 experiments**	173	154	166	140	163	137	147	156	163	157	1556	7.6	0.57

The terminal digits 0 through 9 were quantified as noted. A chi-squared test for goodness of fit was calculated by comparing each distribution with a uniform distribution with the same total digit count. See **Tables 1** and **2** for examples. "Others" refers to other investigators in the laboratory. Outside labs contributed two sets of Coulter data.

Table 6: Count and Relative Frequency of Triples and Triples Containing the Rounded Mean

Set of Triples	Number of Experiments	Number of Complete Triples With Gap (High-Low) $\geq$ 2	Number of Such Triples Where the Rounded Mean of the Triple is One of Its 3 Counts	Percentage of Triples Containing Their Rounded Mean
Bishayee-Control	128	349	61	17.5%
Bishayee-Test	128	985	625	63.5%
Bishayee-All	128	1334	686	51.4%
Others-Control	50	140	19	13.6%
Others-Test	51	337	57	16.9%
Others-All	51	478	76	15.9%

Counts and frequencies in the Bishayee Control, the Bishayee Test, the other investigators in the laboratory Control and the other investigators in the laboratory Test Sets. Triples were only counted if the difference between the high value and the low value was at least 2. See **Figure 1** for examples of triples containing their rounded means.

Table 7: **Weighted Counts of Triples and Triples Containing the Rounded Mean.**

Set of Triples	Number of Experiments	Sum of the Weighted Counts of Triples	Sum of the Weighted Count of Triples that Contain their Rounded Mean	Weighted Count of Triples Containing their Rounded Mean as a Percentage of the Weighted Count of Triples
Bishayee-Control	128	5886	828	14.1%
Bishayee-Test	128	12878	7767	60.3%
Other-Control	50	2403	222	9.2%
Other-Test	51	4879	526	10.8%

Triples are weighted by the difference between the high and low count of the triple if the difference is even and twice the difference if the difference is odd. See Table 4 for examples.

Table 8: **Max Prob Case Analysis of Colony Triples**

Set of Triples	Number of Experiments	Max Prob Case: Expected Number of Triples Containing Rounded Mean	Max Prob Case: Std Deviation of Number of Triples Containing Rounded Mean	Actual Number of Triples Containing Rounded Mean	z-Score for Actual Number	Probability of z-Score or Higher
Bishayee-Control	128	49.0	6.2	61	1.92	0.027
Bishayee-Test	128	183.8	11.5	625	38.39	$<<<9.9x10^{-10}$
Other-Control	50	21.7	4.0	19	-0.67	0.748
Other-Test	51	66.0	6.8	57	-1.32	0.907

Shown are the max prob expectation and standard deviation of the number of colony triples containing the rounded mean, actual value, corresponding z-score and probability values $\geq$ Actual for various colony data sets.

Table 9: **Max Prob Case Analysis of Colony Triples for Various Isotopes**

Set of Bishayee Triples Grouped By Isotope	**Max Prob Case: Expected Number of Triples Containing Rounded Mean**	**Max Prob Case: Std Deviation of Expected Number of Triples Containing Rounded Mean**	**Actual Number of Triples Containing Rounded Mean**	**z-Score for Actual Number**	**Probability of z-Score or Higher**
^{125}I	66.1	6.9	172	15.35	$<9.9\text{x}10^{-10}$
^{131}I	39.0	5.1	59	3.92	0.00004
^{137}Cs	6.2	2.1	14	3.71	0.00010
^{210}Po	25.6	4.3	68	9.86	$<9.9\text{x}10^{-10}$
^{117m}Sn	8.0	2.5	29	8.47	$<9.9\text{x}10^{-10}$
$^{3}H_2O$	15.6	3.4	49	9.82	$<9.9\text{x}10^{-10}$
^{3}HdThd	73.7	7.6	296	29.25	$<<9.9\text{x}10^{-10}$

Shown are max prob expectation and standard deviation of the number of triples containing the rounded mean, actual value, corresponding z-score and probability of values $\geq$ actual for Bishayee colony triples by isotope.

Table 10: Max Prob Analysis of Coulter Triples

Set of Triples	Max Prob Case: Expected Number of Triples Containing Rounded Mean	Max Prob Case: Std Deviation of Number of Triples Containing Rounded Mean	Actual Number of Triples Containing Rounded Mean	z-Score for Actual Number	Probability of z-Score or Higher
Bishayee-Control	30.9	5.2	45	2.69	0.004
Bishayee-Test	106.2	9.6	127	2.17	0.015
Others in Lab	37.8	5.7	36	-0.67	0.75
Outside Lab	1.5	1.1	0	-1.27	0.90

Shown are the max prob case expectation and standard deviation of number of triples containing the rounded mean, actual value, corresponding z-score and probability of values ≥ actual for various Coulter count data sets.

Figure Legends:

Fig. 1: Colony counts from a 50% (bystander) experiment performed by Bishayee. (Bates # B008127, 2/22/1999). The rounded average appears as one of the triplicate counts in 9 of the 10 samples; it is highlighted in blue in all conditions except for the double negative control (sample 1.2: no radioactivity, no lindane). Samples 2.2-5.2 were exposed to increasing concentrations of lindane during the 72-hour incubation in the cold, as were samples 7.2-10.2. Samples 6.3-10.2 were exposed to radioactivity (4 μCi/ml tritiated thymidine overnight) and increasing concentrations of lindane (the last column on the right outside the Table) during the cold incubation. The results in Column SF are the surviving fractions.

Fig. 2: Terminal digit analysis. The solid black bars represent the terminal digits (0-9) quantified for the Coulter and colony count data values, excluding the triples containing one or more values of less than 10. **A.** Data from researchers other than Bishayee using the Coulter ZM in the laboratory, consisting of 99 experiments and 2,759 data values. **B.** Bishayee's Coulter ZM counts, consisting of 171 experiments and 5,155 data values. **C.** The colony counts of other researchers, consisting of 59 experiments and 1,556 data values. **D.** Bishayee's colony counts, consisting of 114 experiments and 3,501 data values. Note the similarity of the patterns in **B** and **D.** The narrow grey bars represent the terminal doublet distributions in Coulter count data **Fig. 3: Coulter chi-squared p-values over time.** Filled circles: Bishayee; open circles: other laboratory investigators. Only Bishayee's experimental results fall below the 0.01 line. The lowest p-value is 1.41×10^{-8} for 30 data values from a 50% experiment on 3/12/99 (B008219).

Fig. 4: Distributions of the ratios (middle – low)/(high – low) for colony triples **A.** The ratio distribution of 486 triples from 58 experiments of researchers other than Bishayee **B – I.** Bishayee's data. **B.** 478 triples, 46 ^{3}H experiments; **C**. 190 triples, 13 100% ^{3}H experiments, 8 used to calculate A_1 (Bishayee, Rao et al. 1999; Bishayee, Hill et al. 2001); **D.** 36 triples, 4 external beam ^{137}Cs experiments; **E.** 87 triples, 9 $^{3}H_2O$ experiments; **F.** 188 triples, 19 ^{131}I experiments; **G.** 366 triples, 33 ^{125}I experiments; **H.** 50 triples, 5 ^{117m}Sn experiments; **I.** 140 triples, 14 ^{210} Po experiments. Some of the ^{131}I experiments were performed earliest and these distributions are the least anomalous.

Fig. 5: Tritiated thymidine survival curves with and without deoxycytidine. Three experiments in each group were pooled. The regressions were calculated using the surviving fraction $(S/S_0) = y = a*e^{-bx} + (1-a)*e^{-cx}$ with weight = $1/y^2$. No deoxycytidine: circles; with deoxycytidine: squares. The ultimate survival plateau is decreased by approximately 15-fold in the presence of deoxycytidine, which indicates a partial reversal of the tritiated thymidine block. (Cf supporting material folder "tritiated thymidine survivals wwout deoxycytidine".)

Fig. 6: Tritiated thymidine survival curves. A. The 100% experiments. Symbols: experiments of Lenarczyk and Howell. Dashed line: regression based on survival data (symbol = letter X) estimated from the 100% survival in Figure 1 of reference 2. Dotted red line: survival calculated for $A_1 = 0.8$ mBq per labeled cell. The Lenarczyk and Howell survival curves are biphasic, whereas Bishayee's curve is exponential (note that Bishayee's regression line coincides exactly with the calculated survival for $A_1 = 0.8$ mBq); the curves differ at 5 mBq by more than two orders of magnitude. **B. The 50% experiments.** Symbols: experiments of Lenarczyk and Howell. Solid line: survival fraction of 0.70. Dashed line: survival estimated from the 50% survival of Figure 3 of reference 2. Bishayee's survival curve is exponential; the survival curves differ at 20 mBq by approximately 70-fold.

Figure 1

Expt. #: 2 Date: 02/22/99

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony for x.2	SF	
1·2	130	149	142	140·33	—	
2·2	131	137	143	137·0	0·9762	
3·2	123	131	138	130·66	0·9311	
4·2	128	134	140	134	0·9548	
5·2	125	130	136	130·33	0·9287	uM
6·3	115	126	137	12·6	0·089	C
7·2	17	20	24	20·33	0·1484	20
8·2	29	35	41	35	0·2678	40
9·2	62	70	54	62	0·4626	80
10·2	70	79	62	70·33	0·5396	100

Figure 2

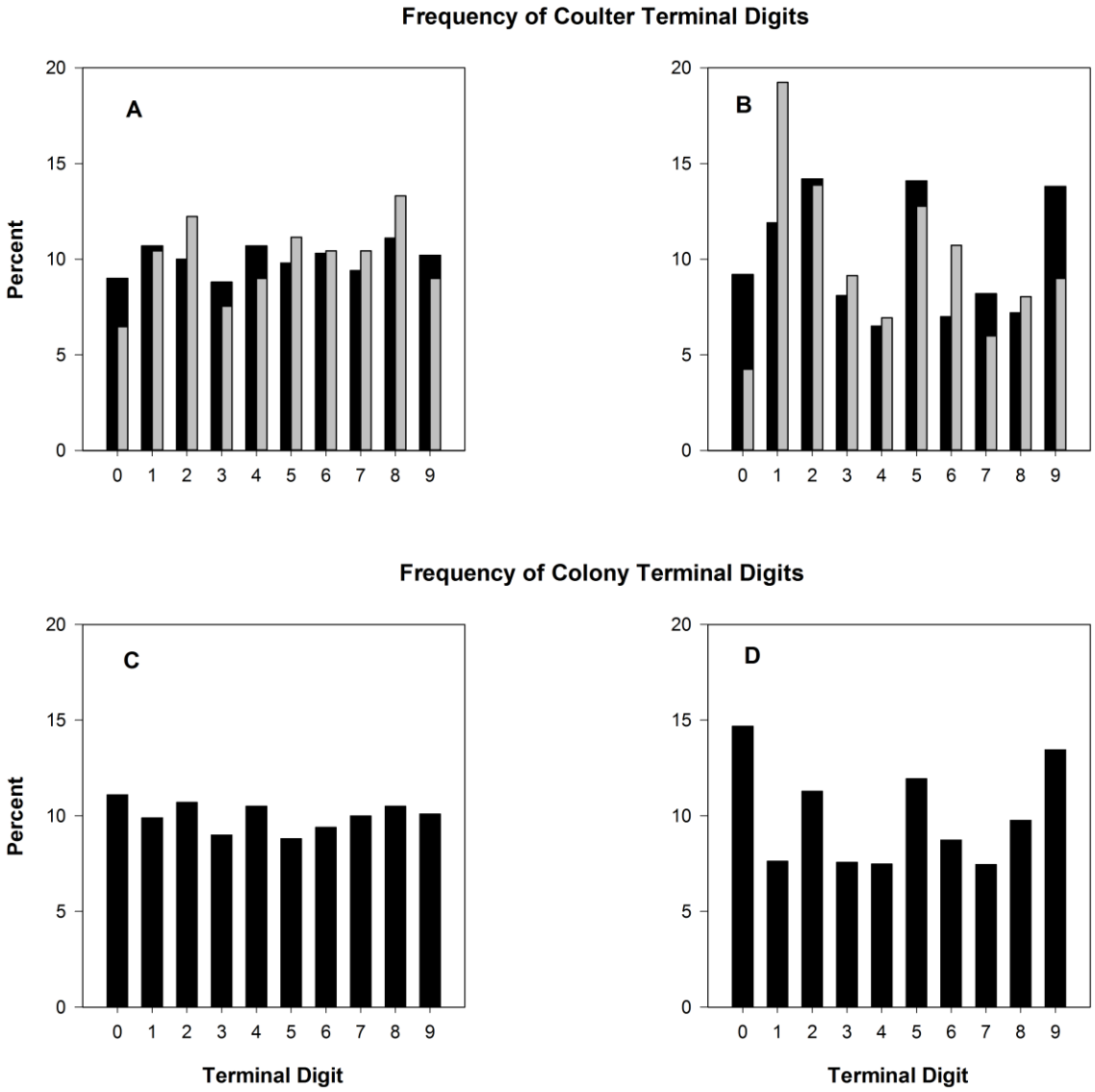

Frequency of Coulter Terminal Digits
A
B
Percent
Frequency of Colony Terminal Digits
C
D
Percent
Terminal Digit
Terminal Digit

Figure 3

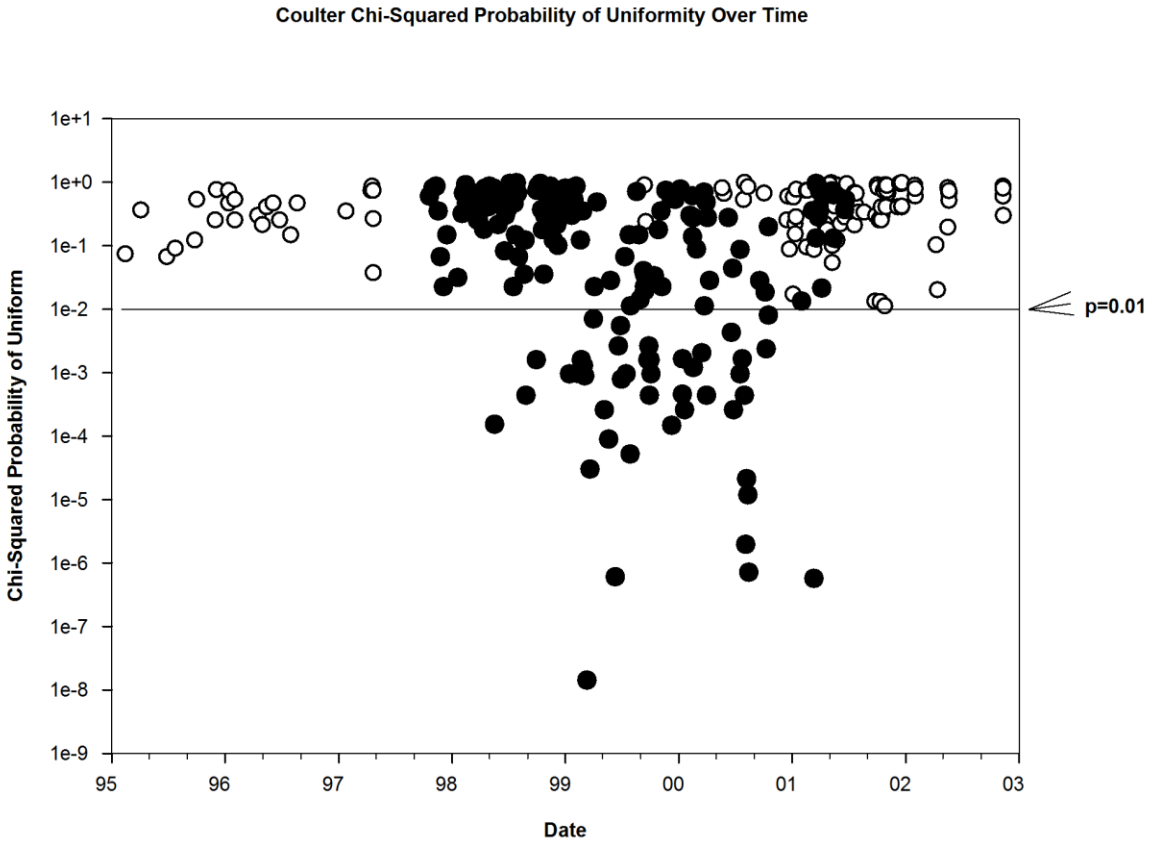

Coulter Chi-Squared Probability of Uniformity Over Time
Chi-Squared Probability of Uniform
1e+1
1e+0
1e-1
1e-2
1e-3
1e-4
1e-5
1e-6
1e-7
1e-8
1e-9
p=0.01
95
96
97
98
99
00
01
02
03
Date

Figure 4

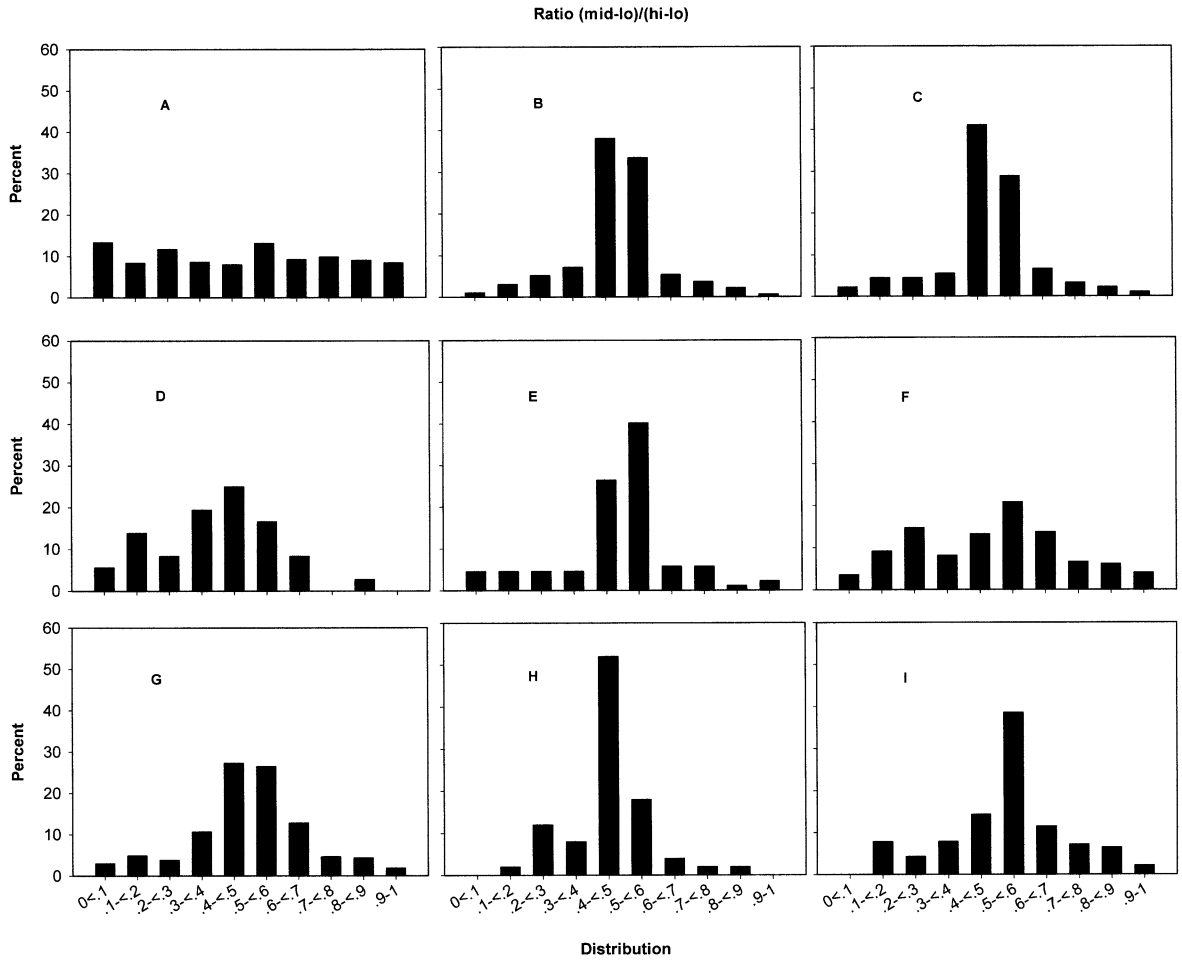

Ratio (mid-lo)/(hi-lo)
A
B
C
D
E
F
G
H
I
Percent
0
10
20
30
40
50
60
0<.1
.1-<.2
.2-<.3
.3-<.4
.4-<.5
.5-<.6
.6-<.7
.7-<.8
.8-<.9
.9-1
Distribution

Figure 5

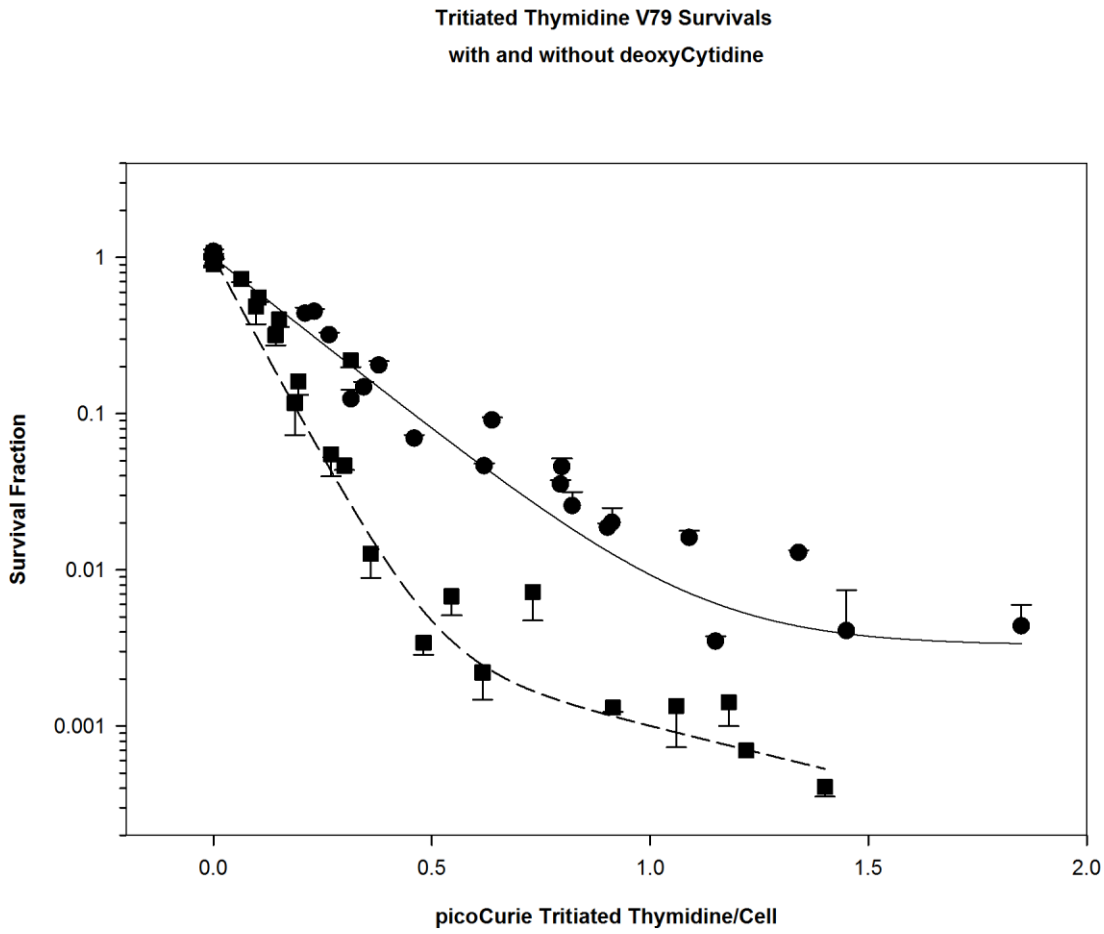
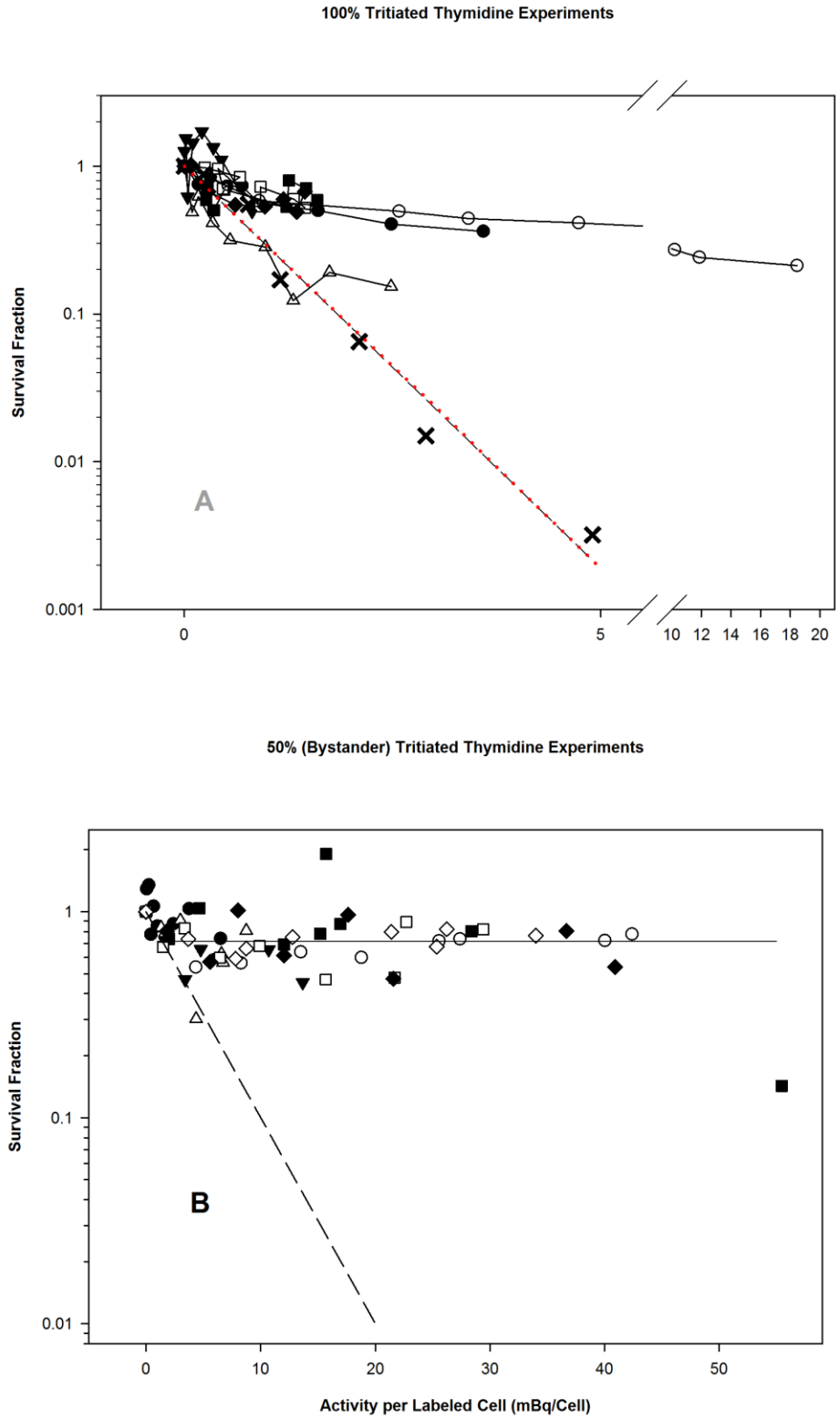

Tritiated Thymidine V79 Survivals
with and without deoxyCytidine
Survival Fraction
1
0.1
0.01
0.001
0.0
0.5
1.0
1.5
2.0
picoCurie Tritiated Thymidine/Cell

Figure 6


100% Tritiated Thymidine Experiments
Survival Fraction
1
0.1
0.01
0.001
A
0
5
10
12
14
16
18
20
50% (Bystander) Tritiated Thymidine Experiments
Survival Fraction
1
0.1
0.01
B
0
10
20
30
40
50
Activity per Labeled Cell (mBq/Cell)