SILS 2016 Undergraduate Student Workshop on Responsible Conduct of Research

Acquisition, Management, and Interpretation of Data: Best Practices in Life Science Research

CASE 1 *(Extensively modified from "Teaching the Responsible Conduct of Research Through a Case Study Approach", a handbook prepared by the Association of American Medical Colleges (Korenman SG and Shipp AC, 1994). The original version of this case was contributed by Allan Shipp (acshipp@aamc.org) of the Association of American Medical Colleges. ©1994 For further information about credit and copyright, see: rcr.ucsd.edu/copyright.htm)*

Jerry Miller has completed a series of experiments characterizing the receptor for a new class of hormones. During the course of his work, he has studied binding characteristics and hormonal responses in tissue culture and in vitro, utilizing gel electrophoresis to characterize the molecular weights of receptor variants, and fluorescence microscopy of live cells to follow the location of the receptor in cells, using anti-receptor antibodies and fusions of the receptor protein with Green Fluorescent Protein (GFP). This has been exciting work for a second-year graduate student doing his first project. One day, Jerry's laboratory chief asks him to prepare an abstract and poster for an upcoming meeting, based on the work Jerry has been doing. The poster has to be completed by the end of the following week. As Jerry examines his accumulated data, he notes that a number of cell culture plates failed to respond to the hormonal stimulus in the same way as the others, and that there was considerable variability in the dose-response relationship. Furthermore, on re-examination, he notes that a number of his gels are not very esthetic in appearance, with some of the lanes showing smearing or uneven migration of proteins. Even so, taken together, he is sure that they demonstrate the correct molecular weight, agonist binding, and subunit characteristics of the receptor. Finally, his fluorescence pictures of individual cells are variable, with most cells showing predominantly plasma membrane fluorescence as expected, but some cells showing a lot of fluorescence inside the cells.

Jerry talks with a post-doc in the lab, Karen Fallow, asking for advice on how to best use the short one-week time until the poster is due. Her response is, "*Why don't you clean up your data? You'll never get the poster in good shape in time unless you do. And if you later want to publish the results, it will improve the chances of the manuscript being accepted if the data are cleaner*." She then suggests that the four culture points failing to show a response be dropped, after he tells her that the cells may have looked sick. She also points out that he could eliminate the top data point at the 45 min interval as a statistical outlier. She examines the pictures of the SDS gels and suggests combining the "best looking" gel lanes from two different experiments, into a single panel for presentation. Finally for the fluorescence micrographs, she advises that he show only a blow- up of a cell with clear plasma membrane fluorescence, since this is the majority phenotype and also is expected from general knowledge of receptors. *"These changes will greatly improve the quality of your poster,*" she advises. Jerry agrees that the poster would be more effective with Karen's suggested changes, but is uncertain whether the changes are proper.

Questions:
1. What do you think about Karen's several suggestions for "cleaning up" the data? Under what circumstances is it acceptable, and under what circumstances unacceptable, to drop "outliers", to disregard "failed" or "imperfect" experiments, to combine data from different experiments, in this case lanes from SDS PAGE, or to show only one picture of a cell that is "typical" of the majority?

2. Given Jerry's uncertainty on how to evaluate Karen's suggestions, what course(s) of action would you recommend for him to take?

3. Would your reaction to this hypothetical case be different if Jerry were preparing a manuscript for publication rather than an abstract and poster for a conference? If so, what is the fundamental difference between these two modes of presenting results and conclusions to an audience?

4. In the modern age, what do journals (i.e. editors and reviewers) do to try to ensure that data are not misleading because they are "cleaned up"? How would you find out what is accepted and what is not accepted for publication?

CASE 2 *(Extensively modified from "Moral Reasoning in Scientific Research", developed by Muriel Bebeau, University of Minnesota, for a project entitled "Teaching Research Ethics: A Workshop at Indiana University". © 1995 by Indiana University).*

Reena Samson is a graduate student at a major research university. She is investigating the potential utility of transgenic tobacco plants as "factories" for the production of foreign proteins. The potential benefit of this research to human medicine is clear. The non-plant gene that Samson is working with is human Factor VIII, a protein essential for blood clotting and the protein that most people with hemophilia lack. In her current experiments, Samson has introduced a gene encoding Factor VIII into tobacco, and she has 50 transgenic plants that she is studying in a developmental time course. She is following both Factor VIII production and the plants' growth to assess the effect of the foreign gene on the plant's development, and vice versa. Samson is excited about the success of her experiment thus far, and she feels that the potential uses for the findings make it imperative to publish as soon as possible.

One day in January, Samson checks on the 50 transgenic tobacco plants that have now been in the greenhouse for about a month. She discovers that 6 of them (plants 4, 9, 18, 21, 35, and 42) are beginning to look somewhat sickly, with their leaves drooping a bit and turning yellow on the edges. She records this in her notebook, noting the number of each plant. Later, she analyzes Factor VIII production in one leaf from each of the 50 plants, which have been stored frozen. She makes a crude extract from each leaf, dividing the resulting extract into four equal parts to "generate statistics", and then carries out a "radioimmune assay" (RIA) on each aliquot, using commercially available antibodies to Factor VIII. RIAs are known to be very sensitive, but also to have high backgrounds, i.e. in this case give a significant signal in the absence of any Factor VIII.

Reena finds that for each plant, the four samples usually show levels of Factor VIII that all are within 10% of each other. For the 6 plants that looked sickly in the greenhouse,

the average levels of Factor VIII are much lower than the levels in the other plants, some as low as 50% of the average of the others. Only one other plant has an average Factor VIII level in same range as the 6, although quite a few come close. Feeling pressed for time, Samson decides not to investigate the cause of the poorer growth of the 6 plants any further. She recalls that at least some of the 6 plants were near the greenhouse door, but does not remember if all of them were. She also remembers that because of the sickly appearance, for the biochemical analyses she may have picked a leaf that was younger and farther up on the plant than for the other plants. Reena hypothesizes that repeated exposure to lower temperatures when the door was opened might explain why the plants were sickly, and that choosing a younger leaf also might have contributed to the lower Factor VIII levels.

Early the following week, Samson is working on integrating her most recent transgenic plant data into the first draft of the manuscript on which she is working. It is entitled it "*Human Factor VIII from Transgenic Tobacco: High Levels of Production and No Deleterious Effect on Plant Growth*." When Samson comes to the data on the 6 sickly plants, she considers whether she should exclude these plants from the analysis. She thinks that doing so would be justified because of the plants' proximity to the greenhouse door, and/or because the analyzed leaves might have been of different age, although her notebook does not have definitive data for either of these possibilities. The paper clearly would be more impressive without the uncertainty associated with the data from these plants. She weighs the relevance of the data from those 6 plants against the principle that there is nothing wrong with excluding "outliers" and irrelevant data.

In looking at the Factor VIII values from the 44 plants that appeared healthy, she sees that these values appear to fall into two classes, with one class of 20 of the plants showing almost twice the values as the 24 other plants. She remembers that part way through the RIA analysis, the antibody stock ran out and she used a new vial of the commercial antibody. She reflects on the possibility that the new batch of antibody might have given a higher background signal or been different in some other way, compared with the earlier batch of antibody.

Since the journal to which the paper is to be submitted requires some statistical analysis of data, she puzzles what and how to present this. She has a total of 200 measurements (4 x 50). One way would be to consider all 200 as the data set, and then calculate a standard deviation (for example). Or she could use only 176 data points, not including any data from the 6 sickly plants. Or she could use the average values for each plant, either all 50, or 44 if the 6 plants are excluded. Or she could present the data for the 24 high producers and the 20 lower producers separately, under the assumption that the change in RIA reagents is the cause for the two classes of plants. Reena is uncertain how present the results in the best way.

Questions:
1.Should Samson leave out the data from the 6 sickly plants plants? Why or why not?

2.How should she present the statistics generated by this experiment? Using the concepts of "biological replicates" and "technical replicates" may help to clarify your reasoning.

3.If it were repeated in the future, what could she do differently to firm up the conclusions from this experiment?