Arch. Immunol. Ther. Exp. (2014) 62:81–84
DOI 10.1007/s00005-014-0272-3

LETTER TO THE EDITOR

Research Integrity: the Experience of a Doubting Thomas

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Received: 18 November 2013 / Accepted: 10 January 2014 / Published online: 9 February 2014

Abstract The sensational "reactome array" paper published in Science in 2009 was investigated in Spain by the Ethics Committee of Consejo Superior de Investigaciones Cientificas (CSIC) after Science issued an editorial expression of concern. The paper was retracted in 2010 because of "skepticism" due to "errors" in chemistry. The "errors" were so profound that many readers expressed doubt that they were really errors, but part of an elaborate hoax. I conducted a forensic analysis of mass spectrometry data in the paper's Supporting Online Material (SOM) and was able to prove that thousands of data values were in fact fabricated. The SOM contains signatures of improper extensive spreadsheet manipulations of incorrect atomic and molecular mass values as well as impossibly repetitive deviations of found molecular mass values from their expected values. No evidence of real mass spectrometry data was detected. Both CSIC and Science have been content to retract the paper without acknowledging the fabrications or assigning responsibility for them. Neither CSIC nor Science has expressed interest in having an independent investigation determining how the paper came to be written, reviewed and published. Their weak response to this episode is a daunting signal that there is an impending crisis in research integrity and science journalism.

Keywords Ethics · Forensic analysis · Fraud · Reactome array · Research misconduct · Science journalism

Introduction

Dr. Puigdomènech identified several cases of research integrity investigations carried out in Spain by the Ethics Committee of Consejo Superior de Investigaciones Cientificas (CSIC) (Puigdomènech 2014). Research integrity is a concern not just for Spain and CSIC, but for all scientists and lay people alike. We expect researchers in science to be truthful and to make noble attempts to search for the truth. Research misconduct in the form of fabrication or falsification of data is destructive to science culture because its occurrence means we are not able to distinguish truth from fiction. Fraudulent research diminishes honest research and undermines public trust in science. Most countries accept that intentional fabrication, falsification or plagiarism in the conduct of research constitutes misconduct. When misconduct is alleged to occur, it is important that a thorough and unbiased investigation take place, so that legitimate findings are acknowledged and false claims are discredited. Furthermore, parties responsible for misconduct should be identified and innocent parties should be exonerated. To do less would leave everyone under a cloud of suspicion.

The "Reactome Array" Paper

One investigation by the Ethics Committee of CSIC involved the notorious "reactome array" paper that was published in Science in 2009 (Beloqui et al. 2009) and

Electronic supplementary material The online version of this article (doi:10.1007/s00005-014-0272-3) contains supplementary material, which is available to authorized users.

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subsequently retracted in 2010 (Beloqui et al. 2010) after serious questions were raised about the scientific validity of the paper. The retraction notice restated the paper's main conclusions as if they were fact. The wording of the retraction notice did not include renunciation of the imaginary array chemistry, the fictitious data in the Supporting Online Material (SOM) or the unjustified conclusions of the paper, but implied that the retraction was forced upon the authors because of "skepticism" due to "errors" in chemistry. The wording is a disservice to researchers who raised substantial and legitimate concerns, and is tantamount to blaming the victims. In a letter to Science in 2010 (no longer available online), Dr. Puigdomènech and the Ethics Committee of CSIC recommended that the paper be retracted in part because the "content of the publication does not have all the necessary experimental support for the conclusions reached" (Travis 2010). This opaque statement fails to explain how the 6 pages of the main text and 553 pages of SOM (http://www.sciencemag.org/content/suppl/2009/10/08/326.5950.252.DC1/Beloqui_SOM.pdf) (accessed October 25, 2013) were insufficient to support the conclusions. Despite the editorial expression of concern and subsequent retraction and suspicions of fraud, there is no evidence that a formal charge of research misconduct was ever made or that any investigation resulted in a finding of research misconduct.

Errors in Chemistry

The concept behind the "reactome array" is the creation of a generic process for using an array of enzyme substrates tethered to a solid support to detect, trap and release the cognate enzymes from biological samples for analysis. This ambitious goal is difficult to achieve because of the idiosyncratic nature of chemical reactions. The basic scheme for the functioning of the array as reported in the "reactome array" paper has several serious errors in chemistry, including an error in the structure of the fluorescent cyanine dye, an implausible anhydride linkage involving histidine, an unstable quaternary aminomethanol derivative, impractical chemical methods for linking the substrate to the dye and questionable anchoring to a cobalt complex in the solid support. These errors were so egregious that organic chemists immediately recognized that the entire scheme was unworkable and probably a hoax. The authors attempted to correct some of these errors in a 672-page revision of the SOM. All of the major errors still remained in the revised SOM, and new errors were introduced, including a generic mysterious procedure, not mentioned in the original SOM, for preparing di-iodo derivatives of all of the substrates. This post hoc chemical synthesis scheme in the revised SOM seems to have been generated by the need for two substrate attachment sites, yet only one site was shown for each substrate in the original SOM Table S2. The word "iodide" does not even occur in the original SOM even though the revised SOM indicates that synthesis of iodide-metabolite derivatives of the substrates is a key step in the production of the "reactome array". The authors should reinstate the link to the revised SOM so that readers can judge its scientific merit.

Forensic Analysis of Mass Spectrometry Data

The SOM provides no information about how the 1,676 array elements mentioned in the abstract were actually synthesized. Data presented in SOM Table S2 claim to show the molecular attachment points in the substrates, but these structures are wholly hypothetical because there are no established chemical methods to form universal derivatives of diverse chemical compounds. The SOM Table S1 lists the calculated and found M + H peaks of the mass spectra of the supposed derivatives. The mass spectrometry data are enlightening because they contain consistent errors showing that the "found" values were not measured at all, but only calculated.

I provide in Electronic Supplementary Material, a forensic analysis of the mass spectrometry data for the first 150 substrates that were listed in Beloqui et al. (2009) SOM Table S1. They are representative of the 2,483 substrates listed in both the original and revised SOM. The data from both SOM versions were imported into Microsoft Excel and analyzed by spreadsheet formulas available in that program.

Most of the "found" molecular mass values in Table S1 for the M + H peaks are larger than the "calculated" values by the same factor of 1.00021. Furthermore, the found values are given with an accuracy to 7 decimal places that is exactly the same for all substrates with the same chemical composition. For example, in the SOM Table S1 page 46, for 1,3,4-oxadiazine and methyl-1,3,4-oxadiazole, which have the same empirical formula ($C_3H_4N_2O$), their derivatives have the same calculated mass (879.82148) and the same found mass (880.0062425), exactly equal to the calculated mass multiplied by 1.00021. Such precision can be made only by rote calculation and not by physical measurement. The excessive precision and lack of variation of the found values prove that they have been fabricated.

The calculation of the expected molecular masses in the mass spectrometry data shown in Table S1 is conceptually incorrect because it does not make the important distinction between molecular weight and molecular mass. The molecular weight of any pure chemical substance is determined by adding the atomic weights of all of the

component elements. The atomic weights, in turn, depend on the isotopic compositions of the elements. In mass spectrometry, each isotope produces a separate peak such that the molecular mass of a compound is usually described by the combined atomic masses of the major isotope of each element rather than the average isotopic composition. The SOM data show that, by subtraction of what are claimed to be the calculated M + H peaks of various substrates, forensic analysis proves that the theoretical atomic masses are those of natural isotopic compositions and not the dominant isotopes as required for analysis by mass spectrometry. For example, in the SOM Table S1 page 46, by subtracting the mass of derivatized triazole $C_2H_3N_3$ (864.80966) from the mass of derivatized triazine $C_3H_3N_3$ (876.82081), a value of 12.01115 is obtained that should be the atomic mass of carbon, but is actually the atomic weight of carbon (Coplen and Peiser 1997). The value of 12.01115 was the accepted value for the atomic weight of carbon from 1961 to 1968—the current value of 12.0107 has been in use since 1995—and includes contributions from carbon isotopes 12 (dominant) and 13 (minor). In actual mass spectrometry, carbon-12 is used as the standard mass with a defined value of exactly 12, and molecules differing by one carbon atom will have a theoretical mass difference of exactly 12. The same subtraction procedure with other elements shows inappropriate theoretical values for the masses of the dominant isotopes. Notwithstanding this crucial error, the so-called "found" masses of the M + H peaks closely matched the incorrect calculated values. Thus, it is clear that no mass spectra data were actually collected and that thousands of data values were fabricated.

Similar evidence for data fabrication is evident in the revised SOM, though even more confusion was added because different atomic masses for the elements were used for the substrates and their derivatives. The atomic mass of carbon in the substrates was forensically calculated to have the nonsense value of 12.1064 and that of the derivatives was 12.011, when both should have been equal to exactly 12. As a consequence, with the subtractive method used above, the theoretical atomic mass of iodine, which should have a constant value of 126.90447, varied in a revealing and consistent manner between 122 and 127, depending on the molecular mass of the substrate. The "found" values for the presumed di-iodo derivatives of the substrates closely paralleled the calculated values—that included the variable mass of iodine—within a common factor of 1.00001, proving that the entire mass spectrometry dataset was fabricated. In some cases, the formation of a di-iodo derivative by substitution of 2 hydrogen atoms by 2 iodine atoms according to the synthetic scheme is categorically impossible. For example, one of the substrates listed in the revised SOM Table S1, oxatriazole CHN_3O—itself a non-existent compound—has single hydrogen that can be replaced by iodine. Nevertheless, the mass of the presumed di-iodo derivative is given in the table with the "found" value corresponding to that expected for incorporation of 2 iodine atoms. In order for this to happen, the derivative would need to have the patently absurd formula $CH_{-1}N_3OI_2$. This again is an evidence of fabrication by simple spreadsheet math.

Discussion

The two versions of SOM of the "reactome array" paper provide a trove of evidence of intentional data fabrication that is apparent even without having to inspect data books or interview authors of the paper. Signatures of spreadsheet manipulations of incorrect and highly repetitive mass spectrometry data values offer a much simpler explanation for data generation than the monumental effort that would have been needed to carry out thousands of unlikely chemical reactions and analyses. The only real question is who is responsible for the fraudulent data, which can only be answered by an onsite investigation at the institutions involved. From what Dr. Puigdomènech has described (Puigdomènech 2014) as the "Spanish Experience", the main purpose of any investigation carried out by the Ethics Committee of CSIC is to report in secret its findings to the president of CSIC. Even when findings would suggest or prove misconduct, the only further action would be to submit retraction(s) of the offending paper(s) without providing clear reasons for the retraction(s). Following the publication of the retraction notice of the "reactome array" paper, I contacted Science and suggested that they commission an independent body to investigate how the paper came to be written, reviewed and published. They declined my suggestion, saying only that retraction of the paper was their main concern and that it would be up to the research institutions involved to investigate and to determine if any misconduct had occurred. Considering the ambiguous response of CSIC to the editorial expression of concern, I doubted that CSIC would give a full account of the reason(s) for the retraction.

If the article by Dr. Puigdomènech is all that the Ethics Committee of CSIC has to say publicly about the "reactome array" paper, it is a sad day for researchers interested in openness and clarity. Both Dr. Puigdomènech and the journal Science have obvious conflicts of interest in the matter, Dr. Puigdomènech because he belongs to the same institution as some of the authors, and Science because it published the bogus sensational paper without adequate review. It is noteworthy that the authors, using much of the same material, applied for a patent (EP2230312A1) based on the "reactome array" at about the same time that the

paper was published in Science. Financial interests could explain why the authors published the paper in the first place and why they were reluctant to give clear reasons for the retraction. Many scientists have wasted a lot of time trying to make sense of the paper and to determine what value it may have. To a certain extent it is possible to do a critical forensic analysis of the paper from a distance, but CSIC has a proximity advantage as well as the responsibility to uncover and report the facts. CSIC and Science should be more forthcoming and clearly explain to readers how the fiasco occurred. It is also disconcerting that none of the 18 authors has come forward to explain what happened. Stonewalling will delay uncovering the truth and will increase the chances of further damage to people and institutions.

There are probably many lessons to be learned from the "reactome array" episode, though we do not yet know what they are. Shoddy research can often hide behind elaborate methodologies and extensive informatics databases, but forensic analysis can use knowledge of the same methods and data resources to uncover erroneous and fraudulent research. It would be a mistake to fault the enterprise of science itself for the errors of researchers. Science may be self-correcting, but it still needs help. We could divide responsibility for research integrity between authors, research institutions, reviewers and journals, but that may be too simple a concept because they interact in diverse and peculiar ways. I doubt that reviewers should share much of the blame for aberrant publications, because they have limited resources to evaluate the complex interdisciplinary research that is currently emphasized, particularly in high-profile journals. A single reviewer having expertise in a specialized area may have only a day or two to review voluminous material prepared over a period of months or years by an array of authors who are experts in numerous fields. Reviewers generally do not assume that a manuscript is a product of deception, and will make every effort to be fair and give the authors the benefit of the doubt. It is mainly the authors, institutions and journals who should accept responsibility when science goes wrong. When we find out how it happened, we can work toward a solution.

Conclusion

Perhaps what Dr. Puigdomènech was saying all along—albeit in a very obscure way—is that the "reactome array" paper "does not have all the necessary experimental support for the conclusions reached" because the paper contains no real experimental data at all—just fabricated data. The retraction notice in Science would also have made more sense if instead of "errors" they used the word "fraud", and if the authors totally renounced the chemistry, SOM and conclusions. The "reactome array" paper is bad science, but the institutional responses to it are not much better. One might think that this case is an isolated event, but there have been numerous other cryptic retractions of high-profile papers riddled with fictitious data. The "reactome array" episode has exposed pervasive research misconduct and a developing credibility crisis in science journalism.

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