RE: Query publication

From: Sibbett, Ben <bsibbett@wiley.com>

To: helmy.m@protonmail.com <helmy.m@protonmail.com>

CC: Rieseberg, Loren <lriesebe@mail.ubc.ca>

Date: Friday, April 2nd, 2021 at 4:48 PM

Dear Dr. Helmy,

Thank you again for your patience.

I have been back in contact with the corresponding author of these two manuscripts. I must inform you that as a journal, we have reported this issue to the Research Integrity Officer at Nanyang Technological University. I have asked for the matter to be investigated and to be kept informed of the decision. I therefore cannot make any further comment at this time.

Kind Regards

Benjamin Sibbett

Managing Editor

From: Sibbett, Ben
Sent: 29 March 2021 10:37
To: Helmy, M. <helmy.m@protonmail.com>
Cc: Rieseberg, Loren <lriesebe@mail.ubc.ca>; Manager.Molecol <manager.molecol@wiley.com>
Subject: RE: Query publication

Dear Dr. Helmy,

Thank you for following up with me.

I must apologise that your previous email was missed in my inbox. Your responses have been passed onto the corresponding author and I will keep you informed once a reply has been received, hopefully in the next week.

In the meantime, I appreciate your discretion whilst the conversation is ongoing.

Kind Regards

Benjamin Sibbett

Managing Editor

From: Helmy, M. <helmy.m@protonmail.com>
Sent: 29 March 2021 06:51
To: Sibbett, Ben <bsibbett@wiley.com>
Cc: Rieseberg, Loren <lriesebe@mail.ubc.ca>; Manager.Molecol <manager.molecol@wiley.com>
Subject: RE: Query publication

⛔ This is an external email.

Dear Professor Rieseberg and Dr. Sibbett,

Regarding articles published by Vyas and others in *Molecular Ecology*, please see correspondence below.

I believe it is unethical to publicly comment on an ongoing-investigation. However, if the investigation is closed or there is no response by the authors during a reasonable period of time and no expression of concern was made on the article, then it is my duty to the academic community to raise the issue in the public domain. Please let me know the status of the current investigation.

I look forward to your reply.

Kind regards,

Mohamed Helmy

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------- Original Message -------

On Sunday, February 14, 2021 11:32 PM, Helmy, M. <helmy.m@protonmail.com> wrote:

Dear Dr. Sibbett,

Thank you kindly for your message and my sincere apologies for the delay in reply.

Regarding the response from the authors on Hari Dass and Vyas (2014) in the document they kindly sent, *Ajai Mol Ecol.docx* and in reference to the points mentioned in my previous message:

1. I must apologize for writing 'µM' as 'mM' it was an honest mistake! I thank the authors for the comprehensive recipe on how to dissolve RG108. The *in vivo* grade of RG108 used by the authors from Sigma is sold in a 10 mg pack and I tried to dissolve the lot in 600 µl of 100% DMSO *per* the authors' recipe - it did not dissolve so there was no point in trying to make up the volume to 10 ml using buffer. I will attempt the feat once more with *in vitro* grade of RG108 from MedChemExpress. Am I correct to say the final concentration of RG108 in 6% DMSO was 2.9911 mM?

2. I respect the "...Freedom to disagree..." mentioned by the authors. However, scientific literature indicates that 2.9911 mM into brain is a lot. Something in the region of 100 µM may have been appropriate or excessive (for comparison, see for example Barbier et al., 2017; Figge, Jaunarajs & Standaert, 2016). At 2.9911 mM it will diffuse down a concentration gradient into brain and body, and one may as well administer it intraperitoneally because RG108 crosses the blood-brain barrier (see for example Todorov, Mayilvahanan, Ashurov & Cunha, 2019). May the authors justify why such a mammoth dose was used?

Regarding the response from the authors on Lim, Kumar, Hari Dass and Vyas (2013) in the document they kindly sent, *Ajai Mol Ecol.docx* and in reference to the points mentioned in my previous message:

1. I used positive controls and samples with Enzo Testosterone ELISA kit Catalog #: ADI-900-065. When manufacturer instructions are followed as recommended by the authors, in other words, when the sample dried under nitrogen is dissolved in Assay Buffer 3, 27 mL, Catalog No. 80-0145, a positive result *is* obtained. I believe the problem could be attempting to dissolve putative testosterone in the sample in aqueous buffer and not under nitrogen which is what is reported by the authors. Please consider publishing a correigendum to the article to rectify the method reported by the authors dissolving testosterone in aqueous buffer after drying in room air.

2. I thank the authors for clarifying that Stanford datasets were used in the article. Please consider publishing an addendum to the article to correctly attribute and acknowledge work contribution in the article, and to comply with animal experiment reporting requirements in place at Stanford and Nanyang Technological University at the time of publication.

Once more, thank you for your time and concern and I look forward to your reply.

Kind regards,

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------- Original Message -------

On Wednesday, January 20, 2021 7:34 PM, Sibbett, Ben <bsibbett@wiley.com> wrote:

Dear Dr. Helmy,

I would firstly like to apologise for the delay in getting back to you.

I have received the attached response from the corresponding author of these papers. I hope this additional information is useful. Please let me know if your require anything further.

Kind Regards

Benjamin Sibbett

Managing Editor

From: "Helmy, M." <helmy.m@protonmail.com>

Subject: Query publication

Date: December 15, 2020 at 8:44:13 PM PST

To: "lriesebe@interchange.ubc.ca" <lriesebe@interchange.ubc.ca>, "manager.molecol@wiley.com" <manager.molecol@wiley.com>

Reply-To: "Helmy, M." <helmy.m@protonmail.com>

[**CAUTION:** Non-UBC Email]

Dear Professor Rieseberg and Dr. Sibbett,

Regarding the article published in *Molecular Ecology*, Hari Dass and Vyas (2014):

1. The dilution reported for RG108 is not possible. If 1 mg of RG108 was dissolved in 1 ml 6% DMSO in physiological saline or 'ACSF', then its concentration was 2.9911 mM. Sigma-Aldrich states that RG108 is soluble in 100% DMSO to >10 mg/ml. Other suppliers and information sources have values around 50 mg/ml. The highest solubility I found was 100 mg/ml or 299.1 mM (MedChemExpress, 2020). It's for *in vitro* work and it's ten times higher than Sigma's recommendation, but let's use the highest one for the sake of argument. If the concentration of RG108 infusate in Hari Dass and Vyas (2014) was 1 mg/ml or 2.9911 mM, then a 10 ml aliquot of 299.1 mM RG108 in 100% DMSO needs to be diluted with ACSF to a final volume of 1 ml: that is a dilution factor of 100. In other words, DMSO in the infusate was 1%. If the RG108 did not come out of solution, it would be an amazing and hitherto unreported feat (done frequently because RG108 should be prepared fresh), and also misrepresented in the article as in 6% DMSO. If the final concentration of DMSO was indeed 6%, then an aliquot of 60 ml of RG108 in 100% DMSO would have been diluted to 1 ml of ACSF. That is a dilution factor of 16.67. But then the final concentration of RG108 in the infusate was 17.95 mM (6 mg/ml). That's 1.2 mg of RG108 in 200 ml of infusate into brain. Because it was infused bilaterally, the total is 2.2 mg RG108 into brain. Either accomplishment, 200 ml of 1 mg/ml RG108 in 1% DMSO or 6 mg/ml RG108 in 6% DMSO, are noteworthy, probably impossible, and perhaps lethal. Is there a typographical error perhaps?

2. 2.9911 mM into brain sounds like a lot. Something in the region of 100 mM may have been appropriate or excessive, time of infusion notwithstanding, though it was reported to have been infused over a longer period (for comparison, see for example Barbier et al., 2017; Figge, Jaunarajs & Standaert, 2016). At 2.9911 mM it will diffuse down a concentration gradient into brain and body, and one may as well administer it intraperitoneally because RG108 crosses the blood-brain barrier (see for example Todorov, Mayilvahanan, Ashurov & Cunha, 2019).

3. This high or mammoth concentration and impossible dilution is reproduced in doctoral theses supervised by Ajai Vyas (Hari Dass, S.A., 2014 and Singh, D.K., 2017, page 78 and 110 respectively).

Regarding the article published in *Molecular Ecology,* Lim, Kumar, Hari Dass and Vyas (2013):

1. I am unable to replicate the testosterone extraction process, which uses diethyl ether and finally dissolves testosterone thus extracted from testes in phosphate-buffered saline or PBS. May we have clarification on the procedure please or how testosterone is otherwise rendered water-soluble?

2. It is stated that pilot data for testes testosterone is from Long-Evans rats. However, I could not find a record for Long-Evans rats at Nanyang Technological University in this context. Was the pilot data used from Vyas' Stanford dataset (for example see House, Vyas & Sapolsky, 2011)? May the Animal Use Protocol for the experiments be reported and source of pilot data be clarified?

Regarding both articles discussed above published in *Molecular Ecology,* (Hari Dass & Vyas, 2014; Lim et al., 2013), why are Author Contributions under References and not for example under Acknowledgements like other articles published in *Molecular Ecology* in the same issues?

Unfortunately the corresponding author did not respond to my queries. I may submit a Comment if necessary.

Kind regards,

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Barbier, E., Johnstone, A.L., Khomtchouk, B.B., Tapocik, J.D., Pitcairn, C., Rehman, F., Augier, E., Borich, A., Schank, J.R., Rienas, C.A., Van Booven, D. J., Sun, H., Nätt, D. Wahlestedt, C., & Heilig, M. (2017). Dependence-induced increase of alcohol self-administration and compulsive drinking mediated by the histone methyltransferase PRDM2. *Molecular Psychiatry*, *22*(12), 1746-1758. doi: 10.1038/mp.2016.131

Figge, D.A., Jaunarajs, K.L.E., & Standaert, D.G. (2016). Dynamic DNA methylation regulates levodopa-induced dyskinesia. *Journal of Neuroscience*, *36*(24), 6514-6524.

Hari Dass, S.A. (2014). Role of arginine vasopressin in modulating plasticity in defensive behavior. Doctoral thesis, Nanyang Technological University, Singapore. doi:10.32657/10356/61632 https://hdl.handle.net/10356/61632

Hari Dass, S.A., & Vyas, A. (2014). Toxoplasma gondii infection reduces predator aversion in rats through epigenetic modulation in the host medial amygdala. *Molecular Ecology*, *23*(24), 6114-6122. doi: 10.1111/mec.12888

House, P.K., Vyas, A., & Sapolsky, R. (2011). Predator Cat Odors Activate Sexual Arousal Pathways in Brains of Toxoplasma gondii Infected Rats. *PLOS ONE*, *6*(8), e23277. doi: 10.1371/journal.pone.0023277

Lim, A., Kumar, V., Hari Dass, S.A., & Vyas, A. (2013). Toxoplasma gondii infection enhances testicular steroidogenesis in rats. *Molecular Ecology*, *22*(1), 102-110. doi: 10.1111/mec.12042

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Singh, D.K. (2017). Role of testosterone in parasitic host behavioral change in Rattus norvegicus: Toxoplasma gondii association. Doctoral thesis, Nanyang Technological University, Singapore. http://hdl.handle.net/10356/69806

Todorov, G., Mayilvahanan, K., Ashurov, D., & Cunha, C. (2019). Amelioration of obsessive-compulsive disorder in three mouse models treated with one epigenetic drug: unraveling the underlying mechanism. *Scientific Reports*, *9*(1), 8741. doi: 10.1038/s41598-019-45325-6

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