Dear Editor,

We would like to thank you and the reviewers for their constructive remarks of our manuscript entitled ‘***Assessment of Cognitive Deficits Associated with Paint Thinner Inhalation Using Morris Water Maze***’. By addressing the editorial’s comments and those raised by the reviewers, we provide an improved version of the draft and emphasize more explicitly the main steps of our protocol.

**NB: all changes in the draft are highlighted in ‘Track changes’ mode for recognition.**

**Responses to Editorial comments:**

•The manuscript would benefit from proofreading.

**We read the final draft at multiple occasions to correct any remaining typos and errors.**

•The authors do not have a materials table, which should include paint thinner, filter paper, the pool, any software used for data analysis, and any other purchased equipment.

**We added a material table listing all used equipment in this protocol.**

•There are some formatting issues which need to be corrected:

-Line 78: Avoid use of personal pronoun “we”

**We have removed the personal pronoun and replaced the whole sentence by the following one: ‘The purpose of this protocol is to model solvent abuse in humans using mice. …’.**

-Why is 2.9 in bold?

**This is not anymore in bold characters.**

•Additional detail is required in a number of areas:

-2.8: How is this recorded? By hand or by video recording? How is the time spent in the quadrant determined?

**We added the following paragraph in page 5 in order to stress the possibility of extracting data either directly while conducting the behavioural tests or from videotapes:**

**‘All these parameters could be monitored and obtained manually while conducting the essays. If however, video recording is used to film the behavioural tests, then the parameters could be extracted from the videotapes’.**

**As stated in step 2.8, the time spent by an animal swimming in any quadrant of the maze is simply the relative amount of seconds the animal is swimming inside that quadrant compared to the full 60s dedicated to the test.**

-2.9.1: How are latency and velocity measured?

**We added the following sentences to explain the meaning of each term (page 5, step 2.9):**

**Latency: time separating the start of the trial and the finding of the platform.**

**Velocity: Distance travelled while swimming per time unit.**

-The timeline of training, probing, exposure, and testing is a little unclear. A diagram of the timeline would be helpful, for both acute and chronic exposure.

**The overall timeline of the entire experimental protocol is now shown in a separate figure (Figure 1).**

-Figure 1: Please include number of mice tested.

**We added in both the text and the figure legend the number of animals used for each group.**

•Discussion: What are the limitations of the protocol?

**In the discussion, limitations of the protocol (such as potential CO2 intoxication) as well as ways to overcome these limitations are discussed. We also included a discussion of the possible adjustments of the protocol depending of the scientific question addressed.**

• Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.

**Indeed, all previous comments from the editor have been addressed in this new version as well.**

• Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

**We read the final draft at multiple occasions to correct any remaining typos and errors.**

• If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

**We included a phrase “Re-print with permission from (reference#)” in the legend of figure1. We also contacted the editor of Metab Brain Dis journal for a permission to re-publish our figure. As soon as we get the permission, we will forward it to you.**

\* JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

**When available, we added the DOIs of the references mentioned in the paper.**

**Response to Reviewers' comments:**

**Reviewer #1:**

The presented protocol is relatively straightforward, based on methods generally used in the field and I have no concerns over the accuracy of the authors' observations. However, I have to make a number of important points:

1. The presented results have been already published in the cited Metab Brain Dis 2014 paper. Panels A and B from the manuscript were published as Figure 1 graphs in the 2014 paper by Fifel, Bennis and Ba-M'hamed, while panel C graphically represents the data contained in text of results in the 2014 paper. Is there a re-publishing issue? In addition, having in mind the authorships and acknowledgments in these two manuscripts, it would be of interest to check and make clear in which of two mentioned laboratories the presented experiments have been performed.

**Indeed the figures are reprinted from my previous study upon which this protocol paper is based. For this, we have asked for a re-print permission from the Metab. Brain Dis. Journal.**

**The work described in the current protocol paper was conducted in Caddi Ayyad. We have made this more explicit in the disclosure section of the draft in page 2.**

2. There is no expanded description of the protocol used in the Metab Brain Dis 2014 paper. For example, what was the color of the pool and platform? What about details of lighting conditions? The details of housing conditions throughout the experiment are also necessary for any replication efforts.

**We added as much details as possible regarding the color of the pool and lighting conditions to make replication of the protocol straightforward.**

3. It is stated: "Give the mouse 60 s to find the hidden platform." Panel B demonstrates means of Day 1 escape latencies of some 65 s. This is a striking and serious discordance between the method description and (already published) results.

**Indeed, the timing allowed for the animals to find the hidden platform is 60s per trial. However, as stated in the protocol, if the animals do not find the platform within those 60s, they are gently guided towards it. The few seconds that this additional manoeuvre takes were behind the latencies of ≈ 65s that we recorded in Day1 of our test. There is therefore no discordance between the description of our protocol and the results we obtained (and published).**

4. As soon as on Day 3, control escape latencies reached 20 s or so, and on Day 4, no more than 10 s. The authors stated that they used a "large circular pool of about 90 cm in diameter and 35 cm high". The results suggest quite oppositely: the pool was not large enough. The acquisition task encountered a clear-cut ceiling effect. Most probably, the diameter of the pool adequate for the given setup would be 120 cm for mice (and 200 cm for rats).

**We do not think that the diameter of the pool used in our study (90cm) is necessary associated with a ceiling effect. The latencies of <10s observed in Day4&5 are mainly due to training. Similar latencies have been observed in several comparable paradigms even with pools larger than 1m in diameter (Morris RG et al., *Nature*, 1982). We do however agree with the reviewer that pools with a diameter of 120cm can also be used and lead to optimal outcomes. We added a sentence and a reference discussing the impact of pool’s diameter of the performances of mice in the MWM in page 4 of our draft.**

**Reviewer #2:**

Manuscript Summary:

The title and abstract correspond to the purpose and the contents of this manuscript.

The test MWM more than 30 years used in many different neuroscience applications. Since MWM has many modified versions. Authors propose modified step for animals inhalation. This is 5 min interval between 15 min inhalation. The main argument for adequate performance of MWM is possible accumulation of carbon dioxide (CO2) over time in the inhalation chamber.

But it could be Plexiglas box more than (L:30cm, W:17,5cm, H:15cm) volume.

**We do agree with the reviewer that Boxes larger than the one we used could be used to expose the animals to the paint thinner. However, unless continuous ventilation is allowed through the boxes, we do not think that larger boxes will avoid potential accumulation of respiratory CO2. For this reason, we highly recommend the 5min interval between the two 15min inhalation periods if one is using the protocol of inhalation we describe in this paper.**

Any way proposed protocol is correct as well observed results.

Every sreps of protocol clearly explained.

Some questions are rising on become familiar with manuscript.

Authors should note

- the conditions for control animals group. Control mice should keep into chamber the same time without thinner inhalation;

**We would like to thank the reviewer for this reminder. Indeed, control mice are exposed to the same protocol except that thinner is not injected into the inhalation boxe. We added this just after step 1.4 of the protocol (page 3).**

- the chemicals contain of "paint thinner" that used in this issue; it could be different chemicals with distinct effects.

**Although Toluene is by far the main constituent of paint thinners, we agree with the reviewer that the exact composition of the thinner may differ depending on the manufacturer. Consequently the behavioural and pathophysiological effects following its inhalation may differ.**

- argue that 5 days treatment enough for the evidence of cognitive impairment.

**As we made clear in many occasions throughout the draft, 5 days of training in the MWM are largely enough to sort out any potential cognitive impairment following intoxication by inhalants.**

Sincerely,

The authors