Author's response to reviews

Title: Physiological and pharmacokinetic effects of oral 1,3-dimethylamylamine administration in men

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Author's response to reviews: see over
DMAA response to Reviewers

Please provide the full name of the ethics committee that approved the manuscript in the 'Methods' section of your manuscript.

Changed as suggested.

Reviewer: Ajit S. Narang

* Title should not include abbreviation, or it should also include the full, expanded name of the compound.

Changed as suggested.

* Line 27, the meaning of the word 'combined' is confusing. Should be rephrased.

Changed as suggested.

* Line 34, what does lag time refer to?

Changed as suggested.

* Line 67, what is the proposed combined effect?

Information added.

* Line 76, 'Jack3d' should be clarified.

Information added.

* Line 80, contents of OxyELITE should be clarified.

The contents of the supplements are proprietary, and the exact quantities of each ingredient are not available. This fact has been clarified in the manuscript.

* Line 86, AUC should be expanded.

Changed as suggested.

* Line 90, which trends?

Changed to remove reference to trends.

* Line 110, what was the implication of measuring body fat? How did this factor into the PK calculations?

There are no implications for body fat, this is only a descriptive variable. This fact has been added to the manuscript.

* Line 119, why specifically cellulose capsule? Was in vitro drug release rate measured?

This is how the product is usually presented for oral consumption. In vitro rate was not measured.

* Formatting of some references should be improved such as #1, 10, 12.

We have attempted to update using the formatting for the journal in Refworks. Copy editors may suggest improvements.

* Line 161, typo, period after 0.05.

Changed.

* Statistical significance or lack thereof, is not reported in any figure legends.
Statistical significance is shown in the legends that are in the paper. We have corrected the discrepancy between the paper and figures.

Which data were subject to statistical analyses?

This is reported in the statistical analysis section.
Reviewer: Paulo Paixão

1 - In the background section, it is important to contextualize the current legal status of DMAA that is on ban in various countries, namely Canada, New Zealand, Sweden, Australia, Brazil, UK to name a few. FDA itself had challenged the marketing of DMAA products for lack of safety evidence.

We have added a comment on the current state of DMAA on the US market.

2 - Still in the background section, authors have commented that “several prospective investigations to date using recommended label dose have not shown any side effect”. It is important to further characterize the “recommended” label doses. Looking at one of the products that the authors describe on their paper, Oxyelite pro, there is no indication on the amount of DMAA present on the capsules and the suggested use propose 1-2 capsules on an empty stomach and an additional capsule six to eight hours later. It would be important to compare the expected daily amount of DMAA on these “suggested uses” against the dose presented in the current paper.

The exact doses of DMAA in Jack3d and Oxyelite Pro are not available, as the supplements have a proprietary blend.

This comment has been added.

3 - The plasma analysis section should be further explained. No references are made to any published study and, as such, indications on the plasma precipitation procedure, columns, eluents, etc. should be presented. The lower limit of quantification of 1-2 ppb is outside the linearity spiked samples (5-50 ppb), and this should be explained. A chromatogram should also be presented.

This section has been added to in order to provide more information and references. Chromatogram also added.

4 - On the Results section, it is indicated that extremely high blood levels of DMAA were observed in a subject, including a high baseline value. These unexplained results should be presented. What was the baseline value? What was the Cmax on this subject? Was the terminal half-life comparable to the remaining subjects? What was the change on the pharmacodynamic variables on this subject?

This has been added, however individual 1 does not have a terminal half-life as the individual doesn’t have a terminal phase (ie the individual’s DMAA level is at steady state). The tmax and Cmax skewed mean values by 70 and 30% respectively. The last value, tlag that was calculated for the subject didn’t skew mean values only due to the fact that at steady state blood concentrations of DMAA, there would be no tlag. We have added to the manuscript in this section.

5 - On the discussion section, the authors should comment on an interesting paper of Venhuis and Dries (Venhuis, Bastiaan J.; De Kaste, Dries "Scientific opinion on the regulatory status of 1,3-Dimethylamylamine (DMAA)" European Journal for Food Research and Review 2012 2 (4): 93–100 (http://www.sciencedomain.org/issue.php?id=155&id=1/)). In that paper, the authors concluded that oral DMAA acts as a bronchodilator (above 4 mg), increases heart rate (above 50–75 mg), and increases blood pressure (above 100 mg). They also conclude that serious adverse effects are expected for oral dosages above 200 mg. The studied dose of 25 mg is below these values, but on a multiple dose administration, DMAA plasma concentrations may increase to higher and more relevant levels, and this should be discussed.

We have added this paper to the references and address it in the discussion.

6 - Also on the discussion section, the authors reported that the peak plasma DMAA concentrations are 15-30 times lower than those reported on the intoxication case studies and hypothesized that these patients likely ingested dosages of DMAA of 375-750 mg. It is important to mention that these conclusions are based on the assumption of linearity of DMAA PK and on the assumption that no previous DMAA was ingested by the patients. It is also important to mention that no conclusions on linearity can be drawn from the present study, as only one dose strength was tested. It is also important to mention that, assuming this linearity would allow us to simulate plasma profiles on multiple dose administrations and that in this case, a regime of two 50 mg capsules and a 25 mg capsule 6 hours later, a day, would result in plasma DMAA concentrations as high as 230 ng/ml. This value is only 10 times less than the ones reported on the intoxication cases.

We have added content to address these points.

- Minor Essential Revisions
7 - There are many bibliography references that are wrongly numbered throughout the document. This should be corrected.

We have attempted to correct the numbering.

8 - Figure 4 and 5 should be merged, as they contain the same type of information.

These figures have been combined.
Reviewer: evelyn lobo

1. The question the authors are addressing is too narrow in scope and therefore limited in its value. The limitations of the current study design are that it does not address the question, what is the pharmacokinetics of oral DMAA on multiple dosing and in the dose range it is marketed as a supplement. An important question to address is, what is the maximum tolerated dose of DMAA administration?

We have added information on the LD$_{50}$ for this compound in order to give more background information and an estimation of the maximum tolerated dose.

2. Background: Lacks sufficient information to understand the relevance and impact of purpose of the study. What is DMAA used for and by who? What is the dose/s it is sold as? An example says 278 mg per capsule. It is unclear what is it used for at high doses or in combination with caffeine/alcohol. What dose range was studied (line 68 to 70)? Lines 92 to 98, didn’t understand how lack of systematic study on safety drives the need to conduct a PK study. Its not clear why heart rate, blood pressure and temperature are being evaluated.

Information about this has been added.

3. Methods: It is unclear what the inclusion and exclusion criteria were for enrolment in the study. Why was DMAA given under fasted condition? Is it how it is directed for use? What is the source of DMAA, marketed source, USP labs?

Enrollment criteria have been added, along with source.

4. Results: The PK profile of one of the subjects excluded should be showed and concentration values reported. Report the variability in pharmacokinetics as %.

% CV added to Table 2.

5. Discussion.

Limitations of the work are not clearly stated (see comment 1). Why enroll men only?

Discussion added to address this.

This has been added.

Comparison of concentration data in this study to that in the literature needs more discussion. Are they only 2 references that reported concentration values?

Only the studies of Bloomer listed the actual dosage of DMAA. The other studies used proprietary blends.

Difficult to follow the comparison with ref 2,3 (lines 202-208) with the concentration reported are not listed. While concentrations are listed for ref 2 in the background, couldn’t find for ref 1. Consider comparing the range of the concentrations in this study to those reported in handful of individuals. Expecting individuals to match with the mean of the population is misleading. Authors haven’t discussed the scientific reason for why DMAA would be expected to have an effect on women taking contraceptives or caffeine. There is a pharmacokinetic reason for the drug interaction between caffeine and oral contraceptives.

Reference to women taking oral contraceptives has been deleted. Only Bloomer et al. in JCR and P&SM used non-proprietary doses.

It is not clear what dose range is safe. What kind of studies have been conducting to investigate the safety or physiologic effects?

All of the studies below have information that could be used to predict safety and physiologic effect. All are discussed in the paper.
Consider stating findings as preliminary, to confirm lack of an effect, one needs a placebo-controlled randomized study.

Several of the above studies are randomized and placebo-controlled. All are discussed and referenced in the paper.

Conclusion: Lines 270, Higher doses is one potential reason for higher concentrations of DMAA, but there could be others, drug interaction, errors in bioanalytical methods, bioavailability from different sources could vary, variability in exposure, nonlinear pharmacokinetics etc. Last 2 sentences in the conclusions are irrelevant.

Changes made to reflect other variables, and last sentences deleted.

Minor comments:

The order of the title, pharmacokinetic and physiologic effects is not reflected in the order of the results reported.

Changed as suggested.

Line 130: What is biochemistry? Was any lab work done on the subjects? No results reported.

“biochemistry” changed to “processing”

Title should mention that the investigation is in men

Changed.

Line 76, there appears to be some error, “to consume their condition”? What is Jack3d?

Description of Jack3D is added, and we have attempted to clarify the condition information.

Line 142, consider using ng/mL instead of ppb. Why is lower limit stated as 1 to 2, instead of 1.

Changed.

Line 190: Use same units minutes vs hr

Changed.

Line 228: Clarify significance related to statistical vs clinical

Information added.
Why are the lines not connected between 6 to 24 h for figures 1-3

We decided to make the 12-hour time delay on the 24-hour sample clear by avoiding connecting the samples.

Can figures 4 and 5 be combined. Showing mean (no SD) and individual profiles.

Done.