Author’s response to reviews

Title: Ancestral DDT Exposures Promote Epigenetic Transgenerational Inheritance of Obesity

Authors:

Michael K Skinner (skinner@wsu.edu)
Mohan Manikkam (mohan.manikkam@email.wsu.edu)
Rebecca Tracey (rebecca.h.tracey@gmail.com)
Carlos Guerrero-Bosagna (catelo@wsu.edu)
Md. M Haque (mhaque@eecs.wsu.edu)
Eric E Nilsson (nilsson@wsu.edu)

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Author’s response to reviews: see over
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Dr. Sabina Alam, PhD
Editor
BMC Medicine

Dear Dr. Alam:

The enclosed revised manuscript MS#: 3183211929952274 entitled “Ancestral DDT Exposures Promote Epigenetic Transgenerational Inheritance of Obesity” is a submission for consideration for publication as an Article in BMC Medicine.

All the comments of the reviewers were addressed with revisions and outlined in the Discussion of Revisions enclosed. We feel the manuscript has been improved by responding to the reviewers comments.

The manuscript was submitted electronically as requested by BMC Medicine. Supplementary information is also submitted and required for review. A list of potential reviewers is provided. We look forward to your comments and hope the manuscript is now suitable for publication in BMC Medicine. Thank you for considering the manuscript and please let me know if any additional information would be useful.

Sincerely

Michael K. Skinner
Professor
Referee Suggestions. None of the individuals listed have read the manuscript. Due to the topic and area of science we feel investigators in industry and government will have potential conflicts of interest, so request only university based investigators be considered as reviewers.

Dr. Lou Guilette  
Medical University of South Carolina  
Dept. of Obstetrics & Gynecology  
221 Ft Johnson Rd  
Charleston, SC 29412  
Telephone: 843-876-2051  
Email: guillett@musc.edu  
(Expert in DDT and Endocrine Disruptors)

Dr. Shanna Swan  
Professor  
Mt. Sinai School of Medicine  
17 East 102 Street Floor 3 West Room D3-135  
New York, NY 10029  
Tel: 212-824-7025  
Email: shanna.swan@mssm.edu  
(Expert in Endocrine Disruptors and Disease)

Dr. Bruce Blumberg, Ph.D.  
Professor  
4351 Natural Science II  
University of California Irvine  
Irvine, CA 92697  
Telephone: (949) 824-8573  
Email: blumberg@uci.edu  
(Expert in Obesity and Toxicology)

Dr. Fred vom Saal, Ph.D.  
Curators' Professor  
University of Missouri-Columbia  
Division of Biological Sciences  
105 Lefevre Hall  
Columbia, MO 65211  
Email: vomsaalF@missouri.edu  
(Expert in Endocrine Disruptors and Disease)

Dr. Robert Waterland, Ph.D.  
Associate Professor of Pediatrics  
Baylor College of Medicine  
One Baylor Plaza  
Houston, Texas 77030  
Email: waterland@bcm.edu  
(Expert in Epigenetics and Obesity)

Dr. Nanette Santoro, MD  
Professor and E Stewart Taylor Chair of OB/Gyn  
University of Colorado  
School of Medicine  
Anschutz Medical Campus  
Aurora, CO 80045  
Email: Nanette.Santoro@ucdenver.edu  
(Expert in Obesity and Reproduction)
Epigenetic Transgenerational Inheritance of Obesity

Transgenerational Obesity

Transgenerational Sperm Epimutations
Discussion of Revisions

MS#: 3183211929952274

Reviewer 1 –

1) Question on DDT source information.

As requested, we have provided in the revised Methods the source, isomer and general information on the DDT used in the study.

2) Clarification on Low Dose and dose used in the study.

As requested, all reference to “Low Dose” was deleted from the revised manuscript and only reference to lower dose for the two doses used. In addition, as requested, in the revised Discussion section we have added the information and references suggested by the reviewer in the discussion of dose used, environmental doses known and IP vs. oral dose. We appreciate the comment and valuable information provided by the reviewer.

3) Clarification of environmental DDT dose.

As requested, the environmental dose reference made is clarified and this is now put in the context of the environmental dose and references the reviewer provided. We appreciate the information and agree this clarifies the environmental dose issue.

4) Clarification of sacrifice range (2 months) and age match date.

As requested, the sacrifice range of 2 months used did not show the weight gain change observed and the individual data was not biased to one treatment or another, and this is now clarified in the revised Methods. We ran statistics on this as a potential confounder and did not find any difference, which is also discussed.

5) Concern on obesity associated disease references and discussion.

As requested, the obesity related disease, previously established in the literature is now clarified in regards to its link and reference with obesity. The revised Introduction, Results and Discussion now clarify the previous literature establishment of these related diseases in the animals in the study. The continued reference to “association” has been maintained throughout the text, but correlation was deleted. Therefore, previously a number of diseases have been linked with obesity in regards to increased incidence in the literature, and that many of the same diseases are also observed in the current study.

6) Clarification of the prostate disease phenotype.

As requested, the prostate abnormalities are now clarified in the revised Methods and the association with prostate disease discussed with the addition of references in the revised Results section. In addition, the Figure S1 panels and histology is now clarified in the revised figure legends as requested.

7) Question on future experiments.
As requested, the future experiments and limitations to the current study are now clarified in the revised Discussion section. The generational DMR for F1, F2 and F3 comparison study suggested is more thoroughly discussed as a critical future experiment. The current study was done to see if a transgenerational phenotype was observed and obesity to our surprise was a major unexpected phenotype. The observations can now be expanded to address a number of the critical tissues such as the generational DMR issue raised. Although we feel this is beyond the scope of the current initial study, the issue is now more thoroughly discussed in the revised Discussion.

Reviewer 2 –

1) Question on definition of epigenetic transgenerational inheritance.

As requested, the definition is now provided in the revised Discussion and clearly the transgenerational F3 generation sperm have an altered epigenome that was not corrected, so previously programmed, and there is transgenerational disease, such that there is epigenetic transgenerational inheritance of diseases induced by DDT. The causal link between the sperm epigenome alterations and the dose needs to be further investigated, and this is now stated in the revised Discussion.

2) Question on F1 generation expected disease.

As requested, the previous literature DDT induced F1 generation disease is now clarified in the revised Introduction and Discussion, and its correlation to the F1 generation disease observed in the current study.

3) Question on outcross observation mechanisms.

As requested, the revised Discussion now has an expanded discussion of how a parental organ imprinted like site can in a sex specific manner generationally transmit the phenotype.

4) Question on metabolic syndrome focus.

As requested, the revised Discussion now clarifies the study was done to examine the potential transgenerational actions of DDT and not to study obesity, however one of the most dramatic phenotypes was obesity. Therefore, future studies to examine the more classic metabolic syndrome phenotypes are now needed, but are beyond the scope of the current study. As requested, this issue and limitations of the current study are now clarified.

5) Question on rodent obesity.

As requested, the revised Introduction and Discussion now clarifies that rodent obesity and its relationship with human obesity is well established with the appropriate references provided. Those phenotypes that are investigated are clarified and those that need to be investigated clarified in the revised text.

6) Question of IP pharmacological dose.

As requested, this issue is addressed as outlined in Reviewer 1, #2 & #3 and the expanded discussion in the revised Discussion. The initial study needed to establish if there is a phenomenon that is worth following up with a risk assessment study. Since this is the first DDT
transgenerational study the protocol of IP pharmacological dose was used to see if a transgenerational phenotype exists. Now that this is shown, future studies can now be designed for risk assessment and know what to look for. This issue is now clarified in the revised text. Although a large number of grant requests for this type of analysis have been proposed, no funding has yet materialized.

7) Question on Methods details on the animal experiments.

As requested, expanded details are now presented in the revised Methods for housing, diet, water, litters, and breeding strategy. Additional references to previous studies and details are also provided. Since a vehicle exposed control generation lineage of animals were generated along side the DDT lineages and housed and managed in the same manner, the EDC confounder issue questioned does not appear to be a confounder. This issue is also discussed in the revised text.

8) Question on abdominal adiposity.

As requested, the revised Results now clarifies the reproducibility of the abdominal adiposity obtained. Since this was an unexpected observation further studies of adipose cell size, and specific tissue adipose analysis are now needed. The limitations and the future experiments are now clarified in the revised Discussion.

9) Question on Figure 2 and liver disease.

As requested, Figure 2 has been revised to include liver disease.

10) Question on litter and analysis.

As requested, the revised Methods discussion of litter and analyses is expanded to clarify individual animals for a litter were used for the analyses for both the epimutations and pathology. The statistics also determined any confounders for litter and did not detect any. This issue is now clarified in the revised text.

11) Question on obesity and associated disease.

As requested, the term associated was detected in reference to obesity and other diseases. The revised Introduction, Results and Discussion now clarify that the previous literature has established the associations that are observed in the current study, but the formal functional links need to be established.

12) Question on outcross data trends.

As requested, the outcross data and statistical trends are indicated with the appropriate p-value, and the text revised to clarify the lack of statistical significance with p<0.05 but trends and the p-value. As now stated, further studies with high n-value are needed to investigate the phenomenon.

13) Question on Discussion exposure.

As requested, the Discussion section is now expanded to indicate the issues raised on parental transmission, epimutations and mechanisms.