Reviewer's report

Title: Ancestral DDT Exposures Promote Epigenetic Transgenerational Inheritance of Obesity

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Reviewer: Frederick vomsaal

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This is a continuation of a series of important studies by the senior author showing transgenerational effects of exposure during development to different chemicals. These findings were initially controversial but are now accepted based on numerous replications of the phenomenon. The issue of DDT being implicated in effects across generations is still relevant, since as noted by the authors, DDT is being widely used in developing countries for malaria control.

Specific Comments

Methods. DDT was administered. There is no indication what isomer or mixture was used, its purity or where it was purchased – all critical information. Sigma-Aldrich sells o,p'DDT, p,p'DDT and a mixture of both. Also identify the volume injected, including of DMSO.

The authors state the following –
the females were administered daily intraperitoneal injections of DDT (either 50 or 25 mg/kg BW/day) or dimethyl sulfoxide (vehicle). Treatment lineages are designated ‘control’, ‘DDT’ or lower dose DDT lineages.

However, the oral LOAEL (IRIS) is 0.25 mg/kg/day, which is 100 x lower than the “lower dose” referred to here – and in the text this is the low dose “LD” group. There is a “low dose” definition used by the NTP in 2001 - low-dose effects refer to biological changes that occur in the range of human exposures or at doses that are lower than those typically used in the U.S. EPA's standard testing paradigm for evaluating reproductive and developmental toxicity.

The rationale for using these doses and the choice of ip route of administration should be included. Regarding the route by ip, I suggest adding that a study was just published that reports that restraint plus gavage to pregnant rats receiving vehicle alters gene expression relative to restraint only, indicating that this procedure would be problematic for the current study and relative to gavage, ip injection is likely to be a less stressful procedure (Cao, J. et al (2013) Prenatal Bisphenol A (BPA) Exposure Alters Sex Specific Estrogen Receptor Expression in the Neonatal Rat Hypothalamus and Amygdala. Toxicol Sci, Online March 3).

At the beginning of the results the authors state the following –
The doses of DDT used are anticipated environmental exposures [22, 52]. However, the ATSDR reference identifies that typical exposure was a few
micrograms/kg/day in the USA prior to the ban on DDT, so the basis for identifying the doses as being anticipated environmental exposures decades ago is unclear. Instead of addressing this in the results, this information should be presented where the doses are first presented. I have no problem with a dose above those predicted for the general public, since subpopulations are exposed to higher amounts, but this issue needs to be accurately described.

Since animals were sacrificed over 2 months, it is important to indicate that this was done to match ages of animals from different groups, since body weight could change as much as the non-obese compared to obese animals in 2 months if one group was killed earlier than another.

Results

I found the continued reference to obesity related disease throughout the manuscript difficult to understand in terms of the presentation of results; for example, the author’s state: Therefore, DDT was found to promote obesity associated transgenerational testis disease.

A proportion of treated F1 – F4 animals showed obesity and showed disease. The way the authors draw the conclusion that there is a link between DDT and obesity and then obesity and the other diseases makes it seem as if the disease data are only being presented for the subset of animals that also showed obesity or that the disease data are statistically related to obesity status, but this did not seem to be the case. Thus, referring in the results to these diseases as being “obesity related” does not seem appropriate unless the diseases did not occur without obesity as a comorbidity. Also, there was no statistical analysis to justify presenting the disease findings as “obesity related”. The study sought to examine the relationship of these diseases to F0 exposure to DDT, not their relationship to obesity – correlations could be conducted to determine those relationships, however.

The author’s state: Prostate disease was characterized by atrophic prostate duct epithelium and hyperplasia (Supplementary Figure S1A).

It appears that atrophic and hyperplastic prostates were found but combined together as prostate disease, although these are clearly opposite and should not be treated the same. Also was there evidence for PIN lesions instead of hyperplasia? For the prostate there is a difference between Fig S1-Panel A and Panel C. There is no explanation of the figures in these panels in the figure legend.

There is a constant phenotype, but the DMR is found in the F3 and not checked in the F2, which is an important issue if your hypothesis is that this mediates the phenotype. It will also be interesting if the same DMR are found at lower doses.

Quality of written English: Acceptable
**Statistical review**: No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests**:  
I have no conflict of interest to disclose.