**Reviewer's report**

**Title:** Perinatal exposure to a chemical mixture based on Arctic maternal body burden results in a long-term elevation of hypothalamic cytokines.

**Version:** 1  **Date:** 5 January 2011

**Reviewer:** Larissa Takser

**Reviewer's report:**

**General comments:**

This paper addresses an original research question and reports interesting results. Pro-inflammatory mechanisms of toxicity of xenobiotics are rarely explored in environmental toxicology. Moreover, the dosing, the mixture, the route of exposure, and LPS charge protocol are relevant for situations found in human populations from Canadian North. There are some concerns about the methodology of the “second study” with LPS stimulation and its results which were never shown, and general presentation of results. Results section needs extensive revision. All the raw data of experiments reported as “data not shown” should be presented. I have impression that the second experiment was not designed to answer the research question, but available female animals from another study were used. In addition, no rationale is given for the dose of LPS. As only females were used and cytokines were analyzed in adult age, the question about hormonal cycle should be addressed: were they all ovariectomized? Does hormonal cycle change cytokine levels in hypothalamus? How this parameter was controlled? Also, authors should show target blood levels of contaminants and those found in exposed animals. In Discussion section, the affirmation that “these findings are consistent with the substantial evidence indicating that exposure...can influence central nervous system functioning” is speculative without any experimental evidence showing how IL-6 and IL-10 can be involved in development of neurocognitive deficits similar to those observed in exposed subjects. The Discussion should be focused on disturbed cytokines, the pathway they are involved, and the link between them and brain development and functioning. Also, the delayed effect should be addressed – how may it be explained? By persistence of exposure? By abnormal glia development? How can we conclude on persistent changes by using the only one time point, the 120th postnatal day? Some published data exists on cytokines and pituitary D1 activity – this subject should also be addressed considering thyroid disruption related to organochlorine contaminants.

**Major remarks:**

Abstract: the first sentence of Methods is the objective of the study, not methods. All sentences of the Abstract should be more concise (i.e. no statistical details, no rationale for cytokines etc).

Background:
the last sentence of the first paragraph – please, give more details about pesticides (type), are these citations all relevant for the present research question?

Page 6, 1st paragraph, last sentence – what is the relevance of the information about multiple sclerosis?

Methods:
Page 9, last paragraph – it would be interesting to know the nature of “plastic cages”, knowing the ability of plastics to leach some potentially toxic additives, i.e. BPA, even at room temperature.

The rationale on the choice of hypothalamus should be moved to the Background.

Page 10 – “As described…”, this and the following sentence should be removed from this section entitled “Chemical administration procedures” or another section should be created to describe the overall experimentation protocol.

“In separate groups of aged female rats…” – it is not clear if there was animals from the same experiment, which was described before or a different experimentation, it would be useful also to indicate the age of animals.

Table 1 should be moved to Tables section.

Statistical analyses: why Tukey’s test was selected for multiple comparisons? If all exposed groups are compared to the control, Dunnet’s test would be more appropriate. Two separate studies are described – what is the difference between? Why do the authors describe the 5 groups of exposure for this “second study” in this section? I suggest to add more details about this experimentation in previous sections.

Results:
The results about LPS charge should be shown to readers.

The presentation of results should be more concise, no need to comment ANOVA and post hoc tests separately. No need to use terms like “omnibus ANOVAs”, “just missed significance” etc.

As Results are presented now, they are too descriptive for a scientific paper, no one citation of a Figure or a Table is done!

Page 17, “Perinatal exposure…” – it would be more informative to give the % of increase of IL-6.

Discussion:
The second affirmation should be followed by a reference to a published paper or to results of this study.

Page 23, second paragraph – this paragraph refers to not significant results, even not cited in Results section.

Conclusion section should be more concise.
References – please check the format for references – they are in Author-Year format in the text and in Numbered format in the list.

Figures – five figures should be grouped in one or in one Table, the number of animals per group should be indicated. Asterisk should be explained – ANOVA? p<0.05?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.