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Letter to the Editor

Letter to the Editor Regarding Banu *et al.* (2018). Chromium Accumulation on Human Placental Oxidative Stress and Apoptosis

Hexavalent chromium (Cr(VI)) is detected in U.S. public drinking water at an average concentration of 0.002 ppm (U.S. EPA, 2017). Studies in rodents indicate that exposure to 50 ppm Cr(VI) (It is unclear whether this is 50 ppm Cr(VI) ion or 50 ppm potassium dichromate.) increases markers of oxidative stress in placental tissue (Banu *et al.*, 2017). It has been demonstrated that exposure to such high levels of Cr(VI) overwhelm protective reductive processes that otherwise limit Cr(VI) bioavailability (Kirman *et al.*, 2017). We therefore read with great interest a new report in *Toxicological Sciences* that chromium levels in human placentas obtained from a Michigan hospital were associated with increased markers of oxidative stress and apoptosis (Banu *et al.*, 2018). According to data collected by the U.S. EPA, the average Cr(VI) concentration in 1371 water samples in Michigan is 0.00014 ppm, with a maximum reported detection of 0.0015 ppm (U.S. EPA, 2017). Thus, the highest detected levels of Cr(VI) in Michigan are $\geq$30 000 lower than those that caused placental effects in rodents. Human pharmacokinetic data indicate that Cr(VI) levels present in U.S. drinking water are well within the capacity of gastric fluid to reduce Cr(VI) to Cr(III) (Kirman *et al.*, 2017). Therefore, we believe that the attribution of the oxidative stress in human placentas to environmental exposure to Cr(VI) is not biologically plausible, and that methodological issues in Banu *et al.* (2018) further weaken any such association.

The 50 placentas collected by Banu *et al.* (2018) were de-identified and thus relatively little is known about the mothers or birth outcomes. As such, sources of chromium exposure such as occupation, prenatal vitamin use, and treatment received while in the hospital are unknown. Furthermore, it is common to use Cr-free instruments to collect tissues for inductively coupled plasma mass spectrometry; however, Banu *et al.* (2018) do not indicate whether such instruments were used. It is therefore conceivable that some placental samples were contaminated with small pieces of stainless steel from medical/cutting instruments. Banu *et al.* also provide no details on the time from delivery to sample stabilization, which likely influences the variability in oxidative stress across the 50 samples.

Critically, the potential for confirmation bias is high in this study. First, Banu *et al.* state that many metals "accumulate" in the placenta, yet accumulation can only be determined by sampling over time. Second, Banu *et al.* omitted 30 of the 50 placental samples from biochemical analyses. For each sex, the 5 placentas with the lowest Cr and the 5 placentas with the highest Cr concentration comprised the experimental groups. Visual inspection of Figure 1 in their paper seems to indicate 3 clusters, with the majority of samples forming a low Cr cluster. The Banu *et al.* findings might be more informative if all samples had been examined in order to test for correlations between placenta Cr concentration and oxidative stress. Third, and most importantly, Banu *et al.* only measured Cr and therefore attribute their findings of oxidative stress to Cr. Only in the last sentence of the paper do they acknowledge that "metals such as Cd, Ni, As, and Mn and other endocrine disruptors in the placenta may have also played a role in the development of such adverse effects." The human placenta weighs approximately 600 g (ICRP, 2002), so there was likely sufficient tissue to measure these other metals.

The presentation of results for mRNA and protein expression is confusing. The y-axis in Figure 3, "mRNA(fold change)", is unclear as there is no reference group from which to calculate fold change. The Western analyses presented in Figure 4 are far from transparent, as small snippets of bands are shown (some vertical, some horizontal) rather than intact Western blots with samples loaded into adjacent lanes. The Methods indicate that samples were run on 7.5%, 10%, or 12% SDS-PAGE, but it is not specified which samples were run on each gel or how exactly the plots were normalized and quantified. The Western data should be presented in a clear and convincing manner in order to support the potentially important findings of oxidative stress in human placentas; however, this is not the case.

In the Discussion, Banu *et al.* compare placental Cr concentrations (0.02–1.25 ppm) with drinking water standards (0.05–0.1 ppm) and comment that the placental levels are similar to "levels of Cr in the worst contaminated places in the country". Notwithstanding the aforementioned concerns about placental Cr measurements and the low Cr(VI) levels measured in Michigan, this comparison demonstrates a gross misunderstanding of toxicity criteria, which is unfortunate for a publication in *Toxicological Sciences*. Other misleading statements include "environmental exposure to Cr(VI) is increasing and is a growing concern", for which no data are cited to demonstrate that exposure is increasing. Banu *et al.* state that "significant contamination with CrVI has been found in approximately 30% of the drinking water sources in California"; however, it is unclear what is meant by "significant" in this context. The aforementioned environmental monitoring data indicate that among 10 008 samples taken in California, the average concentration was 0.002 ppm with a 95th percentile value of 0.0093 ppm and maximum detected value of 0.047 ppm (U.S. EPA, 2017).

The findings in Banu *et al.* (2018) are undermined by methodological issues. Environmental monitoring and pharmacokinetic data also limit biological plausibility.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

CONFLICT OF INTEREST

ToxStrategies is a private consulting firm providing services to private and public organizations on toxicology and risk assessment issues. The work was supported by the Cr(VI) Panel of the American Chemistry Council (ACC). ACC was given the opportunity to review the draft. The purpose of this review was for the authors to receive input on the clarity of the science presented but not on the interpretation of research results. The authors' scientific conclusions and professional judgments were not

subject to the funders' control. The contents of this letter reflect solely the view of the authors.

FUNDING

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REFERENCES

Available as a Supplementary File.

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Response Letter to the Editor

Chromium Burden in Human Placenta: Author(s) Responses to Thompson *et al.*

A large body of data from the epidemiologic studies conducted in the U.S, Finland, China and Russia [Hemminki *et al.*, 1980; Hemminki *et al.*, 1983; Remy *et al.*, 2017; Shmitova, 1980; Yang *et al.*, 2013], have established concerns regarding adverse reproductive outcomes in women and their children who were exposed to hexavalent chromium (Cr(VI)) through the environmental and occupational sources. These women suffer from various gynecological illnesses, including preterm labor or premature abortion [Hemminki *et al.*, 1980; Yang *et al.*, 2013]. Cr(VI) toxicity in female [Banu *et al.*, 2008; Mishra *et al.*, 2008; Murthy *et al.*, 1996; Rao *et al.*, 2009; Sivakumar *et al.*, 2014; Stanley *et al.*, 2013] and male [Aruldhas *et al.*, 2004; Kumar *et al.*, 2017; Marouani *et al.*, 2017; Wise *et al.*, 2008] animals is evident from wide range of investigations. Our previous study identified that regulatory dose of Cr (100 ppb or 0.1 ppm) causes germ cell apoptosis in an *ex vivo* fetal ovarian culture model [Stanley *et al.*, 2015]. Although it is clear that Cr(VI) is a *reproductive toxicant* in females and males, information regarding the pharmacokinetics and mechanisms of Cr toxicity are unknown, in particular in humans. Our preliminary study published in *Toxicological Sciences* identified a "*positive association*" between Cr accumulation, oxidative stress and apoptosis in human placenta collected from de-identified human placental samples from Michigan [Banu *et al.*, 2018]. Although we continue to further explore the mechanisms (by incorporating more samples) and possibilities to identify biomarkers for Cr(VI) and other heavy metal(s) exposure, this article is a response to Thompson *et al* regarding critiques and comments about our and other published data and its interpretation. Some of the questions, critiques and comments by Thompson *et al.*, are valuable and reasonable, and these points are addressed here, and will be carefully considered in future reports.

Comment 1: "The findings in Banu *et al.* (2018) are undermined by methodological issues. Environmental monitoring and pharmacokinetic data also limit biological plausibility".
Response: It is conceivable that the environmental monitoring and pharmacokinetic data could limit biological plausibility. This is because we used tissue samples from the repository. However, the methodologies used were validated and accurate, peer reviewed and published [Banu *et al.*, 2016; Sivakumar *et al.*, 2014; Stanley *et al.*, 2013; Wang *et al.*, 2005; Zadorozhnaja *et al.*, 2000].

Comment 2: "Cr(VI) in Michigan (0.0015 ppm) are 30 000 times lower than those that caused placental effects in rodents (50 ppm)".
Response: As Thompson *et al* indicates, the maximum reported detection of Cr(VI) in Michigan water is 0.0015 ppm (less than the approved safety limits). However, there are multiple other sources besides water such as, from the food, lifestyle factors (such as smoking), vitamin supplements, and exposure from the air. The placenta is one of the organs with highest vasculature [Pretorius *et al.*, 1998]. It is not known how much Cr could possibly be *periodically accumulated* in the placental tissue or Cr-DNA-adducts during the course of pregnancy. The erythrocyte has a high capacity for Cr(VI) uptake and binding. Cr(VI) enters the erythrocyte through a sulfate ion channel; once inside the cell, it is rapidly reduced to reactive intermediates Cr(V) and Cr(IV) and binds to the beta chain of human hemoglobin [Kerger *et al.*, 1997] and Cr-hemoglobin complex is stable and remains sequestered within the cell over the lifespan of the erythrocyte [Paustenbach *et al.*, 2003]. A recent study indicate that estimation of Cr in erythrocyte is a direct indicator of Cr(VI)