

LETTER TO EDITOR

Duodenal GSH/GSSG Ratios in Mice Following Oral Exposure to Cr(VI)

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Mice chronically exposed to high concentrations of hexavalent chromium (Cr(VI)), as sodium dichromate dihydrate (SDD), in drinking water develop duodenal tumors (NTP, 2008). In our recent publication in *Toxicological Sciences*, we reported that mice exposed to Cr(VI) for 90 days exhibited significant decreases in the reduced-to-oxidized glutathione (GSH/GSSG) ratio (Thompson *et al.*, 2011). The GSH/GSSG ratio is well recognized as an indicator of cellular redox status (Schafer and Buettner, 2001). GSH is present in cells at mM concentrations and is likely a key reductant of Cr(VI) (De Flora and Wetterhahn, 1989). At study termination, the GSH/GSSG ratio in the duodenum was significantly decreased at ≥ 14 mg/l SDD. Stern asserts that “at face value, one might conclude from this report that 14 mg/l is a threshold for saturation of the reductive capacity of the mouse stomach.” In our view, the lack of statistical significance at < 14 mg/l does not demonstrate that ingested Cr(VI) was entirely reduced in the stomach but simply that the effect on the GSH/GSSG ratio in the duodenum was not statistically significant. Like any study, a larger sample size would have greater power to detect differences among treatment groups. We certainly did not state or imply that, based on the GSH/GSSG ratio data, there is a threshold for Cr(VI) reduction in the mouse stomach at 14 mg/l SDD.

Stern further states that benchmark dose (BMD) analysis is “the more appropriate approach to the analysis of these data.” We agree that BMD modeling of biologically meaningful data is appropriate for risk assessment and in no way implied that the statistical analyses described or any no-observable-effect level that could be derived from data in Thompson *et al.* (2011) preclude BMD analysis. However, the small sample size (five per group) will result in wide confidence intervals on the BMD. Thus, Stern’s warning in the final sentence that “readers should interpret the statistical analysis ... presented by Thompson *et al.* (2011) with great care” could be applied to most of the data in the published scientific literature. BMD analysis is not routinely conducted in toxicological studies but rather is used

to obtain point-of-departure values for quantitative risk assessment. As indicated by the title, Thompson *et al.* (2011) was meant to inform the mode of action (MOA) of Cr(VI) in the intestine, and analysis of these data for risk assessment was beyond the scope of the paper.

Finally, it appears that Stern wishes to draw attention to his 2010 article where he posits that redox processes in the stomach compete with the kinetics of gastric emptying and absorption within the stomach even at low Cr(VI) concentrations, and thus, low levels of Cr(VI) can pose a cancer risk to the small intestine (Stern, 2010). Stern’s approach for cancer risk assessment of Cr(VI) appears to focus on the “determination of a threshold (or nonthreshold) for the passage of Cr⁺⁶ from the stomach to the duodenum.” In our view, to estimate the duodenal (target) tissue dose at which key events in the MOA for intestinal carcinogenesis occur in mice, it is necessary to develop species-specific pharmacokinetic models that account for dynamics of competing rates of reduction, absorption, gastric emptying, and transit through the small intestine. Attempts to estimate a reductive threshold in the mouse stomach based on the GSH/GSSG ratio or other such measures are an overly simplistic approach. The findings of Thompson *et al.* (2011) inform key events in the MOA for intestinal carcinogenesis in mice and with the other biochemical, toxicogenomic, and toxicokinetic data from complimentary investigations will contribute to understanding the potential human relevance of rodent tumors and the cancer risk assessment for ingested Cr(VI) at environmentally relevant exposures.

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