## Developmental Perchlorate Exposure and Synaptic Transmission in Hippocampus

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In "Developmental Exposure to Perchlorate Alters Synaptic Transmission in Hippocampus of the Adult Rat," Gilbert and Sui (2008) reported results of exposure of pregnant rat dams to perchlorate in drinking water, with the purpose of evaluating neurologic development in rat pups after *in utero* exposure to perchlorate.

We cannot agree with the authors' conclusion that their findings

> indicate that neurologic impairment is associated with modest degrees of thyroid hormone insufficiency and support previous animal studies of neurodevelopmental sequelae associated with low levels of perchlorate exposure.

Also, we do not agree that their data (Gilbert and Sui 2008) "provide evidence in a rodent model that modest degrees of thyroid hormone reduction induced by perchlorate result in persistent decrements in brain function."

The "association" of the electrophysiologic anomalies measured in the hippocampus [interpreted by Gilbert and Sui (2008) as "neurologic impairment" or "persistent decrements in brain function"] with "modest degrees of thyroid hormone insufficiency" in the pups is not, in our opinion, a credible interpretation of the authors' results, because the evidence for "thyroid hormone insufficiency" is questionable, the electrophysiologic anomalies did not demonstrate a consistent dose–response relationship, and behavioral tests, chosen for their sensitivity to hippocampal deficiencies, did not show any behavioral changes associated with the measured electrophysiologic anomalies.

Pregnant rat dams were exposed to perchlorate in drinking water in four experimental dose groups (0, 30, 300, and 1,000 ppm) from gestational day 6 until pups were weaned on postnatal day (PND) 30. The active thyroid hormone triiodothyronine ($T_3$), its inactive precursor thyroxine ($T_4$), and thyroid-stimulating hormone (TSH) were measured in the serum of rat pups on PNDs 4, 14, and 21. Hormonal levels were not affected on PNDs 4 or 14, with the exception of a marginal but statistically significant increase in serum TSH on PND14 in the two lower dose groups (30 and 300 ppm perchlorate). The statistical significance of these results is questionable because changes of similar or greater magnitude in serum TSH on PND14 in the highest dose group (1,000 ppm) were not statistically significant.

On PND21, serum $T_3$ was reduced approximately 10–14% in the two higher dose groups (Gilbert and Sui 2008). These changes are similar to the intra- and interassay variations in these measurements, stated in the "Methods" to be 9–12%. Serum $T_4$ at PND21 was reduced by approximately 11% in the 300-ppm dose group and 27% in the 1,000-ppm dose group. The authors observed no statistically significant change in TSH in any of the dose groups on PND21.

Thus, in three dose groups at three time points for three serum thyroid hormones—a total of 27 data points—Gilbert and Sui (2008) found no changes in the active serum thyroid hormone $T_3$ that were greater than the intra- and interassay variations. Only 3 of 27 possible data points showed changes in any of the serum thyroid hormones: TSH at PND14 increased marginally in the two lower dose groups (but not in the highest dose group), and $T_4$ (the precursor to the active hormone $T_3$) decreased by 27% on PND21 in the highest dose group.

Because Gilbert and Sui (2008) found no changes in the active thyroid hormone $T_3$ and questionable changes in other serum thyroid hormones measured in rat pups, we cannot agree with their interpretation that these data denote "thyroid hormone insufficiency" or "thyroid hormone reduction."

The implication by Gilbert and Sui (2008) that the development of the hippocampus in rat pups is impaired as a result of perchlorate exposure of pregnant dams is weakened by the lack of consistent dose responses in the electrophysiologic parameters measured in the hippocampus of the rat pups. For example, the essentially identical curves for the 30- and 300-ppm dose groups shown in their Figure 4B, and the statistical equivalence of these curves with the control curve, leads us to question the relationship of these changes to perchlorate dosage. In Figure 5, the lack of dose dependence is evident as the changes in 300-ppm dose values often exceed or equal the changes in the 1,000-ppm dose values. The apparently random nature of the results of the various electrophysiologic tests used undermines any claim of reproducibility of their findings.

Interpretation of the reported electrophysiologic anomalies in the hippocampus as "neurologic impairment" or "decrements in brain function" by Gilbert and Sui (2008) was not supported by the results of behavioral testing. The authors chose four different behavioral tests for motor activity, spatial learning, and fear conditioning because of their sensitivity to hippocampal deficiencies. Rat pups in the three experimental dose groups performed equivalently to those in the control (unexposed) group on all behavioral tests. Thus, none of these tests demonstrated any "neurologic impairment" or "decrements in brain function."

We also wish to comment on the relevance of this study (Gilbert and Sui 2008) to human health risk assessment. The perchlorate concentrations in drinking water the authors used (30–1,000 ppm) are 2–3 orders of magnitude higher than environmental concentrations, which range up to a maximum of 200 ppb in the United States (National Research Council 2005). The lack of biologically functional effects observed at the 30-ppm drinking water dose (Gilbert and Sui 2008) indicate that the environmental concentrations found in the United States have a 150-fold margin (30 ppm ÷ 200 ppb) of safety against any effects suggested by these authors, based on water concentrations alone. Considering that rats consume approximately 5 times more water per kilogram body weight than humans would increase the margin of safety to 600-fold (5 × 150).

In addition to the shortcomings of the study noted above, the rat is questionable as a model for the sensitivity of the human thyroid system to perchlorate because of differences in thyroid hormone storage. Humans are less sensitive than rats to inhibition of thyroid hormone synthesis by perchlorate because humans are capable of storing several months supply of sequestered $T_4$ and $T_3$, whereas rats are capable of storing only a few days supply of these hormones. Given the inadequacies of the experimental model, the absence of a dose response in the findings, and lack of corroboration of alleged hippocampal deficiency by behavioral tests, we believe that Gilbert and Sui's (2008) conclusions are not substantiated.

*The authors are employed by Noblis, a nonprofit science and technology company that has worked under contract for several branches of the federal government. J.M.D. has been deposed about his opinion on several chemicals, including perchlorate. These depositions and all Noblis work involved no financial incentive given (or expected) for the authors personally or for Noblis, other than the normal cost of doing contractual business in the public interest.* *Evaluation of the subject article (Gilbert and Sui 2008) was conducted under Air Force contract FA8903-04-D-8715.0076.*

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## Developmental Perchlorate Exposure: Gilbert Responds

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I welcome the opportunity to respond to the comments of Mavis and DeSesso regarding our article on the developmental effects of perchlorate exposure on brain function (Gilbert and Sui 2008). Although couched in strong terms, their criticisms are not pertinent to the underlying physiologic processes that we investigated, and many of the points they raised were addressed in our original article. The comments of Mavis and De Sesso alter neither the significance of our study nor the integrity of our conclusions.

Mavis and DeSesso opine that the "evidence for 'thyroid hormone insufficiency' is questionable." They base this opinion on two points. First, they argue that there is no "dose–response" effect of perchlorate, and second, they focus on serum triiodothyronine ($T_3$) as the biologically active hormone. They completely ignore the effect of perchlorate in the dams, and they do not acknowledge that circulating levels of $T_3$ do not reflect thyroid function. More than 80% of serum $T_3$ is derived from peripheral deiodination of thyroxine ($T_4$) and is not tightly linked to thyroid hormone action in the developing brain. Tissue (not serum) concentration of $T_3$, as well as the critical window over which the $T_3$ is required, dictates the nuclear action of thyroid hormone. Different tissues, including brain, can be deficient in $T_3$ while serum levels of this hormone are unchanged. In addition $T_4$ and other iodothyronines interact with membrane-bound hormone receptors and can directly affect, through nonnuclear actions, the biological activity in the cells of many tissues. Mavis and DeSesso also inaccurately limit the discussion of thyroid hormones and tissue function to circulating serum levels of $T_3$ in developing pups.

Mavis and DeSesso confuse analytical variability of the hormone measures with biological effect size: One characterizes the precision and accuracy of the specific assay (inter- and intra-assay variation); the other characterizes the variation among animals attributable to treatment. The performance of the $T_3$ and $T_4$ assays we reported (Gilbert and Sui 2008) fall well within the manufacturer's recommended limits, and variation between replicate samples was typically < 3%. Although thyroid-stimulating hormone (TSH) assays are inherently more variable, performance also fell within acceptable limits (9–12%). These sources of variance, as Mavis and DeSesso portend, do not underlie the reported effects on serum hormones in pups.

The consequences of small reductions in serum $T_4$ on brain development in pups and dams are not trivial and should not be dismissed (for review, see Zoeller and Rovet 2004). Unless perchlorate is acting through a nonthyroidal mechanism, our study fully supports recent data indicating that small changes in maternal and/or neonatal serum thyroid hormone can impact brain development.

We are perplexed by Mavis and DeSesso's erroneous characterization of *in vivo* field potentials as "electrophysiologic anomalies." This seems to reflect a misconception of the value of these measures as functional indices of integrated physiologic responses in an intact neural circuit. These end points are widely acknowledged to reflect the functional integrity of the hippocampus. Our data (Gilbert and Sui 2008) go beyond the more commonly reported acute response in an isolated hippocampal brain slice to reveal impairments in the fundamentals of neuronal communication in living animals, and the changes were demonstrated months after exposure to perchlorate had ceased. Certainly a permanent impairment in the synaptic function of any brain structure must be considered an adverse neurotoxicologic insult. Input/output (I/O) functions [Figure 4 (Gilbert and Sui 2008)] reflect the ability of a population of neurons to transmit signals across a monosynaptic connection. Synaptic plasticity, the ability to adapt to stimuli, tested in the form of paired pulse (PP) depression and facilitation measure the influence of local circuit neurons to modulate that synaptic output [Figure 5 (Gilbert and Sui 2008)]. The clear relationship between increasing stimulus strength and increases in the amplitude of the physiologic response, evident in both the I/O and PP data, validates the high degree of experimental control maintained over these biological responses. Contrary to the allegations of Mavis and DeSesso, both sets of measures demonstrate dose-dependent perturbations as a function of perinatal perchlorate exposure and represent important contributions to a literature largely lacking examinations of dose–response relationships. Furthermore, these findings are in complete agreement with observations using graded levels of the known goitrogen propylthiouracil (PTU) over a similar dosing regimen.

Mavis and DeSesso state that electrophysiologic anomalies are not evidence of neurologic impairment as they occurred in the absence of behavioral changes (Gilbert and Sui 2008). As discussed in our article, the neuroscience literature holds many instances where molecular, neurochemical, anatomical, and electrophysiologic indices do not correlate with apical behavioral measures. This does not negate the significance of these downstream observations, but rather reveals the relative bluntness of some of the behavioral tools available to assess cognitive function in rodent models. Attempts to evaluate subtle perturbations of the thyroid axis will require further refinement of existing paradigms to increase sensitivity or the utilization of more sophisticated evaluations of behavioral dysfunction. This paradigm shift is not dissimilar from what was necessary in the behavioral evaluation of developmental lead exposure two decades ago.

Mavis and DeSesso state that the concentrations used in our study (Gilbert and Sui 2008) bear no relevance to the human health risk assessment for perchlorate. However, the purpose of our study was not to emulate human exposures to perchlorate. Rather, percholorate was used to disrupt the thyroid axis via a mechanism distinct from standard model compounds (i.e., PTU and methimazole) to examine the impact of mild perturbations of the thyroid axis on neurodevelopment. Nonetheless, the results revealed a significant reduction in synaptic function at a dose (30 ppm = 4.5 mg/kg body weight per day) consistent with the lowest observable adverse effect levels identified from the review of all available animal data and summarized in the U.S. Environmental Protection Agency's perchlorate risk assessment document (U.S. EPA 2002). As such, these findings corroborate previous findings and add additional weight to the existing evidence from animal studies on the negative impact of perchlorate on brain development.

Finally, according to Mavis and DeSesso, the rat model is questionable for sensitivity of the human thyroid system to perchlorate because of differences in thyroid hormone storage. The hypothalamic–pituitary–thyroid axis is very similar in its chemistry and its function in rodents and humans, and rodent models have provided important information on the fundamental biology of endocrine systems informing medical practice and public health protection. Although differences in thyroid hormone economy of adult rats make rodents less than ideal for the assessment of thyroid tumors, the dependence of the fetus on

the maternal supply for thyroid hormone makes the rat a suitable model for neurodevelopment. In humans, differences in the capacities and the relative immaturity of compensatory mechanisms of the fetus and neonate increase the vulnerability of these life stages and have significant implications for tolerance to perturbations of the thyroid axis. Rather than detracting from the utility of the model, the limited storage capacities of the rodent offer a reasonable parallel to the immature human.

In conclusion, the comments of Mavis and DeSesso are not consistent with current thinking in the fields of thyroid endocrinology or neuroscience. Their critique does not effectively challenge the veracity of our observations or the soundness of our conclusions.

*The content of this letter does not reflect U.S. EPA policy.*

*The authors declare they have no competing financial interests.*

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## Erratum

In "Cumulative Exposure to Lead in Relation to Cognitive Function in Older Women" [Environ Health Perspect 117:574–580 (2009)], Mark Weisskopf's middle initial is incorrect. Instead of "Marc A. Weisskopf," it should be "Marc G. Weisskopf."

The authors apologize for the error.