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Letter to the editor

Critical effect of perchlorate on neonates is iodide uptake inhibition

Strawson et al. (2004) calculate a reference dose for perchlorate based on thyroid hormone (TH) change in pregnant women as the critical effect. There are two issues that are not well developed, which renders the overall analysis misleading.

1. *Critical Effect* Because normal adult humans have a large storage capacity of hormone in the thyroid gland, the 14-day Greer study (Greer et al., 2002), even with high perchlorate exposures, does not inform us about the relationship between perchlorate, iodide inhibition, TH synthesis, and TH levels. Applied to a 3 kg newborn, the Greer findings indicate that ~18–20 μg perchlorate per day will begin to inhibit iodine uptake. Empirical measurements show that neonates do not have TH stored in the thyroid gland (Savin et al., 2003; van den Hove et al., 1999); they must synthesize new hormone daily to meet known requirements. Therefore, any decrease in TH synthesis in a neonate will result in a reduction in serum T_4. Even a short duration (14 days) of TH insufficiency can result in measurable neurological or cognitive deficits in neonates (van Vliet, 1999). But, newborn thyroxine levels do not provide a measure of neonatal thyroid function. A significant proportion of T_4 at birth is derived transplacentally, and the half-life of serum T4 in neonates is approximately 3.5 days (Vulsma et al., 1989). Therefore, data derived from the neonatal screening programs do not measure the impact of perchlorate exposure to neonates and infants directly exposed to perchlorate. These facts are important to incorporate into a risk analysis for perchlorate.
2. *Compensatory or adverse effects.* Capen clearly articulates that direct measures of cell proliferation in the thyroid gland (i.e., hyperplasia versus hypertrophy) are required to determine whether the responsive increase in serum TSH following TH insufficiency is adverse or compensatory within the context of increased risk of thyroid cancer (Capen, 1994, 1997). Similarly, overt measures of neurodevelopment are required to determine whether changes in the HPT axis are adverse or adaptive within the context of neurodevelopment. The unpublished Argus (2001) study found statistically significant changes in measures of neurodevelopment, and these changes were upheld by an independent analysis (TERA, 2001). Although unpublished and controversial, Strawson et al. had no obvious reason to exclude it from their discussion since other unpublished and controversial studies were cited.

The uncertainties surrounding the application of the no observable effect level (NOEL) of Greer et al. to a human neonate seems greater than that described by Strawson et al. Specifically, the establishment of the NOEL was based on seven adults; while useful information, it may not provide a good estimate of the variance in the population for this important "threshold." Moreover, we do not know whether neonates are more or less sensitive than adults to perchlorate. We do not know the degree of iodine uptake inhibition required to inhibit thyroid hormone synthesis. And we do not know specifically the degree, and duration, of thyroid hormone insufficiency in neonates required to produce adverse effects. Finally, there are no clinical data on the effect of perchlorate on neonates that would provide even estimates of these uncertainties.

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Toxicology for Excellence in Risk Assessment (TERA) 2001 Report on five expert peer reviews of the Primedica 2001 Study report (hormone, thyroid and neurohistochemical effects of oral (drinking water) exposure to ammonium perchlorate in pregnant and lactating rats and in fetuses and nursing pups exposed to ammonium perchlorate during gestation or via maternal milk) TERA, Cincinnati, OH

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Response to letter to the editor

Response to "Critical effect of perchlorate on neonates is iodide uptake inhibition" by Zoeller

We thank Drs. Zoeller and Rice for their comments. While we agree with their points concerning uncertainties in the response of neonates to perchlorate, we disagree that these uncertainties prevent the development of a reference dose (RfD) based on human data. Perchlorate has been detected in public water supplies. To regulate perchlorate, an RfD *must* be developed. Given the available perchlorate database, the RfD must be derived from either rat or human studies—and we believe that the rat studies introduce an even greater degree of uncertainty into the risk assessment.

Because our focus was the human studies, we limited our manuscript to primarily discussing these studies. The Argus 2001 developmental toxicity study did find some statistically significant changes in the thickness of some regions of pup brains using a pair-wise comparison. In 2001, TERA asked experts on neurodevelopment to review this study. Far from confirming these findings, this analysis concluded that the statistical methods were inadequate to assess whether any treatment-related effects were observed. In addition, all reviewers concluded that design flaws prevented drawing any conclusions about the effects of perchlorate on neurodevelopment. In 2002, one reviewer conducted a further re-analysis of the Argus 2001 data (Wahlsten, 2002). He did find treatment-related, *very small* increases in the thickness of three brain regions. But the effect was so small that Dr. Wahlsten concluded it was smaller than normal variation in controls and had no biological significance. Because we concluded that the Argus study did not demonstrate neurodevelopmental effects, we did not include it in our paper.

Our RfD is not based on the clinical study by Greer et al. (2002) as our colleagues seem to suggest. We used Crump et al. (2000), which studied thyroid function in 9784 newborns and 162 school-age children in three cities in Northern Chile with perchlorate in public water. We selected this study because it included a large population of neonates—one of the sensitive populations for perchlorate, and it included 127 children approximately age 7 who were likely exposed both in utero and for their entire lifespan. Therefore, use of Crump et al., 2000 as the critical study reduces some of the uncertainties associated with the short-term clinical studies.

Pregnant women are also a sensitive population for perchlorate because metabolic changes that occur during pregnancy require an increased hormonal output by the maternal thyroid (Glinoer, 2001). Therefore, they are sensitive to situations that deplete the availability of iodine. Ongoing studies (Téllez et al., 2004) are examining whether perchlorate affects pregnant women in Chile. Maternal T4, TSH, urinary iodine, and breast milk iodine are comparable among the three cities. Perchlorate was detected in maternal serum, cord serum, and breast milk in women exposed to 114 μg/L perchlorate in water. Therefore, a perchlorate concentration of 114 μg/L appears to be a NOAEL; it is not affecting the ability of pregnant women to maintain an increased output of thyroid hormones.

Next, we address several other uncertainties mentioned in the letter, including (1) relative sensitivity of neonates to adults, (2) degree of iodine uptake inhibition required to inhibit thyroid hormone synthesis, (3) the degree and duration of thyroid hormone insufficiency that produces adverse effects in neonates.

Issue 1. The Chilean studies (Crump et al., 2000; Téllez et al., 2004) provide reasonable data on the response of neonates at doses equivalent to the threshold of iodine uptake inhibition observed in Greer et al. (2002). If neonates were significantly more sensitive than adults to perchlorate, they would respond at lower doses than adults. They do not. Physiologically based pharmacokinetic models demonstrate that the predicted threshold for iodine uptake inhibition in fetuses is approximately 2-fold lower than the predicted threshold in adults (Mattie et al., 2004).

Issue 2. In healthy adults, both a short- and long-term exposure at the highest perchlorate doses resulted in serum perchlorate concentrations that inhibited iodine uptake by 70% without affecting thyroid hormone synthesis. We do not know if this relationship holds true for pregnant women and neonates. However, by basing an RfD on actual measured water concentrations that do not result in the inhibition of thyroid hormones in pregnant women or neonates, we are

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confident that we are protecting these populations. We do not know what perchlorate dose would be required to inhibit hormone synthesis in these populations, but we are confident that it is higher— not lower—than our RfD

Issue 3. No studies in humans have quantified the degree of T4 suppression that can be tolerated before neurodevelopmental effects are observed. Some data in rats suggest that a >50% decrease of maternal serum T4 would be required before any effect on thyroid hormone levels in pup brains would be observed (Calvo et al., 1990; Pleus personal communication), but the relevance of this to humans is unclear. Nonetheless, no studies of perchlorate in healthy humans have involved doses high enough to result in *any* suppression of T4, much less result in adverse effects from T4 suppression.

In closing, we emphasize that the purpose of developing an RfD is to provide an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily perchlorate exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD we propose for perchlorate is based on a NOAEL in neonates and young children, is supported by new data in pregnant women, and includes an uncertainty factor to account for the remaining lack of data regarding pregnant women and their fetuses. We may never be able to exactly quantify what perchlorate dose may result in adverse effects in pregnant women and neonates, but we are confident that our RfD is lower than this dose—perhaps by an order of magnitude.

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Letter to the editor

Interspecies differences in susceptibility to perturbation of thyroid hormone homeostasis requires a definition of "sensitivity" that is informative for risk analysis

Lewandowski et al. (2004) develop a case for comparing the sensitivity of various mammalian species to thyroid toxicants on the basis of the lowest dose of perchlorate required to alter circulating levels of thyroid hormones. Two important issues are not addressed in this analysis, which weakens the authors' conclusion that the rat is "more sensitive than humans" to perchlorate.

The authors review the ability of perchlorate to inhibit iodide uptake into the thyroid gland of humans and rats (their Figs. 1 and 2) and its ability to reduce circulating levels of thyroid hormones (their Figs. 3–8). These data indicate that rats and humans are similar in their sensitivity to perchlorate's ability to inhibit iodide uptake into the thyroid gland, but that rats are far "more sensitive" to the ability of perchlorate to decrease serum thyroid hormone levels. In principle, blood levels of a hormone represent a balance between the rates of hormone secretion and clearance. Likewise, the amount of hormone stored in an endocrine gland represents a balance between hormone synthesis and release. Thus, the ability of perchlorate to reduce thyroid hormones in any animal will be determined by its ability to: (1) inhibit thyroidal iodide uptake, (2) inhibit thyroid hormone synthesis, (3) exhaust intrathyroidal stores of hormone, and (4) reduce thyroid hormone secretion.

It is obvious from this sequence that the duration of perchlorate exposure required to cause a reduction in circulating thyroid hormone level will depend on the size of the intrathyroidal store and the serum half-life of thyroid hormones. Because adult euthyroid humans have a serum half-life of T_4 of around 7 days, and intrathyroidal stores of T_4 are estimated to be several month's worth (Greer et al., 2002), it is clear why perchlorate caused a reduction in serum thyroid hormones in rats but not in humans. However, rats and humans may be similarly sensitive to perchlorate's ability to reduce thyroid hormone *synthesis*—a seemingly important issue. Likewise, considering that a human neonate has a serum half-life of T_4 of around 3 days (Vulsma et al., 1989) and intrathyroidal stores of T_4 estimated to be less than one day's worth (van den Hove et al., 1999), it is easily predictable that human neonates will exhibit a decrease in serum thyroid hormone levels within 14 days of exposure to doses of perchlorate that would clearly not affect serum T_4 in normal adults. Thus, if we assume that a human neonate is no more *sensitive* to perchlorate's ability to inhibit thyroid hormone synthesis than are adults, we can still predict that they will be more *vulnerable* to the adverse effects of perchlorate.

The definition of "sensitivity" to thyroid disruption by exogenous chemicals in general should be debated, especially within the context of neurodevelopment. The lowest dose of toxicant that causes a reduction in serum hormone levels is one possible definition, but it does not take into account that animals may differ in their sensitivity to thyroid hormone insufficiency per se, which is likely to be a more significant issue than simply the reduction in hormone levels.

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Response to letter to the editor

Response to: Interspecies differences in susceptibility to perturbation of thyroid hormone homeostasis requires a definition of "sensitivity" that is informative for risk analysis

In his comments on our article, Dr. Zoeller raises a number of interesting points regarding "sensitive populations" and use of toxicological data to characterize such populations. However, as discussed below, these points do not support the use of data collected in rats for quantitative assessment of the potential effects of perchlorate in humans.

Zoeller's comment that differences in half-life and intrathyroidal stores of T_4 make it "clear why perchlorate caused a reduction in serum thyroid hormones in rats but not in humans" is not quite germane to the appropriateness of the use of the rat for quantifying effects of perchlorate exposure in humans. We presented data for species other than the rat, including pregnant rabbits and occupationally exposed humans. In none of these species were effects of perchlorate on thyroid hormone levels or developmental effects seen, even in pregnant rabbits exposed to doses many orders of magnitude higher than those given to rats (York et al., 2003). We also noted a lack of effects in the human chronic exposure studies by Gibbs et al. (1998) and Lamm et al. (1999). While these studies involved populations with intermittent exposure patterns, such studies nonetheless demonstrated no effect whatsoever on serum thyroid hormones at exposure levels that clearly affected rats. As a whole, these data indicate that, in terms of the thyroidal response to perchlorate, rats differ not only from humans but also from mice and rabbits.

Zoeller also implies that because of issues of thyroid hormone economy that the same effects seen in the rat will eventually be seen in the human once thyroid hormone stores are depleted. U.S. EPA has previously made this point in their "parallelogram" approach for extrapolating between the rat data and humans (U.S. EPA, 2002). We note, however, that this notion does not consider the potential for differences in the *magnitude* of the effect; the greater resilience of the thyroid hormone pool in humans and species other than the rat allows for adaptation or compensation. This is consistent with studies of chronic perchlorate exposures in human occupational (Gibbs et al., 1998; Lamm et al., 1999) and residential (Crump et al., 2000; Gibbs et al., 2004) populations (including children and adults) that have not reported adverse effects even at exposure levels well above those causing such effects in rats.

Zoeller cites studies indicating the child is more vulnerable to disruption of thyroid homeostasis than the adult. Our work examined inter-species differences in thyroid responsivity to perchlorate and did not address the issue of children's increased vulnerability except for a brief summary of some of the epidemiology studies. In the absence of a child-specific model, U.S. EPA's RfD methodology (currently being used to develop regulatory levels for perchlorate) relies upon uncertainty factors to address issues such as children being a particularly sensitive subpopulation (i.e., via the intra-species uncertainty factor). In contrast, the aim of our article was to evaluate the relevance of a particular animal model for predicting human risks, i.e., for purposes of developing an RfD. We do not see that an increased sensitivity of the fetus or neonate relative to adults provides a basis for selecting the rat as an appropriate model for the human. The choice of the animal model should be based on the overall appropriateness of the model and intra-species differences can be addressed subsequently via careful selection of uncertainty factors. Thus the rat model may be appropriate for hazard evaluation or mechanistic studies, but it may not be appropriate to use data collected in rats for direct quantitative dose-response assessment in humans potentially exposed to perchlorate.

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