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From: Andrew M. Geller, Neurotoxicology Division, NHEERL, ORD, USEPA
Date: 1 November 2001
Subject: Profile Analysis of Brain Morphometry Data from Argus/Primedica "Effects" Protocol 1416-003

This memo is to transmit results of EPA statistical analyses on the brain morphometry effects in the study entitled *Hormone, Thyroid, and Neurohistological 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* (Argus, 2001). Brain weights and morphometric measurements were made on preserved and sectioned brains from male and female rats at the 0, 0.01, 0.1, 1.0, and 30 mg/kg ammonium perchlorate in drinking water dose levels. Linear morphometric measurements on 10 different brain regions were made. Data from postpartum days 10 and 22 were included (These ages were calculated using the day of birth as Day 1; standard USEPA practice is to use day of birth as day 0. These ages will be referred to by EPA convention as postnatal day (PND) 9 and 21). At each of these time points, one male and one female from each litter was examined. Different litters were used at the different PD timepoints.

As discussed in the Argus report, analyses consisted of Students t-test comparisons between the control and the corresponding group of each sex at each separate dose level. For example, PND 9 male control striatum measurements were compared to PND 9 male 30 mg/kg dose group, then day 10 male control striatum measurements were compared to PND 9 1 mg/kg dose group. These analyses were run separately for both sexes and ages and all brain areas, right and left sides. These analyses found a large number of significant effects on brain morphometry at doses of 0.1 and 0.01 mg/kg/day ammonium perchlorate in drinking water.

Guidelines on the assessment of neurotoxicity (USEPA, 1998a) specify that alterations in brain structure should be considered adverse and relevant to human health risk assessment. Alterations in brain structure are consistent with the mode-of-action for perchlorate, i.e., transient decrements in thyroxine (T4) and tri-iodothyronine (T3) during development can result in neurodevelopmental effects. The significant findings reported in the Argus report strongly argue,

therefore, that adverse effects of ammonium perchlorate are present at the lowest dose tested, and that this data set contains only LOAELs, no NOAELs.

While the analysis in the Argus report was provocative, the number of t-tests run increases the risk of introducing Type I error into this analysis. To address this, a more conservative multivariate analysis, profile analysis (Johnson and Wichern, 1988; Tabachnick and Fidell, 2001), was run by the USEPA. Profile analysis is more conservative than the analysis described above because a multivariate analysis of variance takes into account any correlations between the independent variables, where the multiple t-tests assumes complete independence. This analysis also reduced the number of main effects tests by nesting gender within litter and by constructing a vector composed of all of the morphometric data from each animal, then comparing these vectors. This is explained in more detail below.

Profile analysis: When a series of measurements are made from a single animal, i.e. within-subjects measurements, they can be used to build a profile or vector of scores across the measurement variables. Profile analysis makes between-groups comparisons using a vector composed of all of the (within-subject) measurements taken from each animal. Its primary test, for parallelism of the vectors, establishes whether the pattern of results between treatment groups is the same or different. This simple determination allowed us to examine the entire set of data without an *a priori* expectation of effect in one brain region or another, or indeed of the direction of the effect. While there is indication that certain areas of the brain are likely susceptible to the effects on thyroid hormones of perchlorate (e.g., Madeira, et al, 1991, 1992, 1993) and the previous study performed by Argus indicated that the corpus callosum was affected (USEPA, 1998b, Crofton, 1998), definitive gestational windows for specific brain areas are unknown. Profile analysis determines whether there were exposure-related changes in the pattern of brain growth, i.e., brain growth in one region relative to another, while precluding prior expectations about specific areas of the brain or the degree of these changes.

Profile analysis can be broken down into three questions:

1) Are the profiles from the different treatment groups parallel? This tests whether the levels of the various measurements are similar for all treatment groups, i.e. between-groups, and is essentially a test of the interaction between the treatment factor and the within-subjects scores. If the profiles are not parallel, an interaction exists, and univariate analyses of each measurement for the interaction factors may be made to elucidate the effects.

2) Assuming the profiles are parallel, are they equal? This tests for the main effects of treatment, testing for differences among the groups by collapsing data across all of the within-subject measurements. In other words, if the profiles are parallel, one group may be above the other, or the profiles may be coincident. This tests for coincidence.

3) Assuming coincidence, are the profiles of a constant value across measurements? This question is irrelevant to the current analysis given that the different brain areas vary greatly in size, resulting in profiles that are clearly not flat, i.e. of constant value. One may consider, however, the effect of normalizing the data for each measurement to put them all on the same scale. To foreshadow the result of this analysis slightly, this was not necessary, since profiles were not parallel.

The profile analysis was run on the data from the PND 9 and PND 21 animals separately, with gender nested within litter (PROC GLM, SAS Institute, Inc, Cary,NC). Profile analysis requires data from each endpoint for each animal. Data from individual brain regions, both right and left sides, were missing from 8 animals in the PND 9 cohort and 3 animals in the PND 21 cohort, eliminating these animals from the analysis (Table 1). If a sex by treatment interaction was found, separate analyses were run on males and females. Treatment effects within a brain region were examined with univariate analyses of variance with gender nested within litter. Dunnett's two-tailed t-test was used to compare each dose group to controls at $\alpha = 0.05$ for step-down tests of treatment effects within a brain region as guided by the overall (univariate) treatment or sex by treatment effects.

Right and left side measures of the same brain structures were examined with profile analyses (whole set of data) and repeated measures analyses of variance (univariate analysis on each brain region). While there was no *a priori* reason to expect other than a bilateral effect, the presence of this kind of bias could reflect either anisometries in brain regions, i.e., lateralization, or sectioning that was not perfectly perpendicular to the anterior-posterior axis of the brain, resulting in sampling brain regions at different depths on right and left side. These analyses, together with examination of the images of the brain sections (Jean Harry, 2001) demonstrated some systematic variability in the sectioning resulting in differences in right vs left measurements in different brain regions. The magnitude of the variability was small, and it was not always in the same direction, even within a brain region (varying with the dose group sampled). The small magnitude of difference relative to the dose-related changes found in this study, the fact that different brain regions varied in their laterality bias in different directions, and that different dose groups varied in different directions all argue for simply averaging the right and left brain region measurements for each animal rather than tailoring different analyses for different brain regions. In addition, averaging could help to reduce variability in the data due to sampling only one histological section/brain region/animal. Therefore, data from right and left sides of the brain were averaged before the analysis of dose effects. Where data were missing from only one side of the brain, the remaining measurement was used for the analysis.

Two additional analyses were run with adjustments to the raw morphometry data designed to subtract variability due to variation in brain size and focus on changes in the sizes of brain areas relative to one another. One analysis was run dividing all of the linear dimensions through by the post-fixation brain weight from each brain. We note, however, that there is little historical data for normalizing data with post-fixation brain weight (Harry, 2001), and that

fixation results in the loss of any evidence of hydration-related changes such as edema or other swelling.

The second additional analysis also adjusted for brain size, using the anterior-posterior (a-p) measurements of cerebrum and cerebellum and the full width measure of hippocampus to adjust the linear dimensions. In this analysis, frontal, parietal, and corpus collosum dimensions were divided by a-p cerebrum size; dentate, CA1, and CA3 were divided by hippocampal width; and the cerebellar linear measurement was divided by the a-p cerebellum measurement. Hippocampus, a-p cerebrum, and a-p cerebellum were not included in the analysis as separate measures. The striatum and external germinal layer measurements were not adjusted by these other linear dimensions.

An additional two analyses were run on the PND 22 data. These analyses omitted 1) the posterior corpus callosum measurement or 2) the posterior corpus collosum and all hippocampal measures, i.e., all measures that came from the level II section, since there was some indication that there may have been a systematic difference in the plane of sectioning with dose (Harry, 2001).

Results:

Profile Analysis

PND 9: The brain morphometry profiles were not parallel across treatment groups (Table 2). The absence of parallel profiles obviates further analysis for equal profiles. This means that the effects of developmental dosing with ammonium perchlorate were different on different brain regions. Planned contrasts show that the 0.01 and 1.0 mg/kg/day doses were significantly different than controls (Table 2A). Adjusting for brain weight had little effect on these results (Table 2B), though the adjustment for the linear size of the different brain regions made the effect at the highest dose (30 mg/kg) also significantly different from control (Table 2C).

The profile analysis was done using the raw (right-left averaged) data values. Because the brain structures measured yield a range of measurements varying 10-fold, it is difficult to plot the raw data vector in a meaningful way to see the differences driving the findings of significant differences between dose groups. I have attempted to do this, however, in Figure 1. Figure 1 plots the (unadjusted) region by region size of each brain structure normalized by the mean size of that brain structure in the controls, male and female combined. The control group is therefore represented by a horizontal line at 1.0 with associated variability. The other dose groups differ from this horizontal line to different extents and the parallel profiles analysis tests, in essence, whether these departures make the other dose groups significantly "non-horizontal". Note that the analysis was not done on the normalized data; dividing through by the control values was done simply to aid in visualizing the data vectors used in this analysis. The 99% confidence intervals around the control means represent an envelope inside of which comparable values ± standard error of the mean (SEM) are not significantly different from controls.

PND 21: The brain morphometry profiles were not parallel across treatment groups (Table 2A). Contrasts between each of the dose groups and controls showed that the controls differed from all other dose groups at better than $p < 0.0001$, including at the lowest dose used, 0.01 mg/kg/day ammonium perchlorate in drinking water. The absence of parallel profiles obviates further analysis for equal profiles. The analysis adjusting for brain weight or regional size yielded similar, highly significant effects (Tables 2B, 2C). Sex X Dose interactions were significant in the parallel profiles analysis of the raw data and with the data adjusted by brain region size. The parallel profile manova remained significant at $p < 0.0001$ in the overall and contrast tests with the posterior corpus callosum or posterior corpus callosum and all hippocampal measurements, i.e. measurements taken on section 2, removed from the analysis.

Figure 2 is similar to Figure 1, described above. The differences between the vectors of the groups treated with ammonium perchlorate and the control group should be readily apparent.

Univariate analyses

Gross measurements: There were no significant effects of treatment or sex on brain weight, anterior-posterior cerebrum length, or anterior-posterior cerebellar size at either age tested.

PND 9: Univariate tests yield significant effects of treatment with ammonium perchlorate in the frontal and parietal regions of the cerebral cortex, the striatum, region CA1 of the hippocampus, the corpus callosum, and the external germinal layer (Table 3A). There is an increase in size at the 1.0 mg/kg/day dose in the frontal, parietal, and striatum measurements, and decreases in size in CA1 and the external germinal layer. There were also treatment by sex interactions in the corpus callosum and CA1 (Table 3). Both of these brain regions showed a treatment-related decrease in linear extent in females while showing an increase in size in males. While most of the changes in linear extent measured in the sampled brain regions were ± 5 - 11%, the male corpus callosum was increased 23% at both the 0.1 and 1.0 mg/kg doses.

The adjustment for brain size reduced the significance of treatment effects in the striatum, CA1, and external germinal layer (Table 3A, center). The analysis using adjustment for regional size (Table 3A, right) was nearly identical to the raw data analysis, with the addition of significant effects being noted on cerebellum.

A comparison of the profile analysis and the analysis presented in Argus 1416-003 shows similar results were obtained on the PND 9 brain morphometry, with one exception. Both analyses found an increase in linear extent of frontal, parietal, and striatum at 1.0 mg/kg ammonium perchlorate and corpus callosum at the 0.1 and 1.0 mg/kg dose, with the corpus callosum increase limited to males. There was a decrease in the linear extent of the striatum at 0.1 mg/kg, and decreases in the size of region CA1 of females at doses 0.01, 0.1, and 1.0 mg/kg. The Argus 1416-003 analysis did not detect a significant difference in female CA1 at 0.01 mg/kg.

A post-hoc analysis of the plane of cut of the PND 9 brain sections suggests that the 0.1 and 1.0 mg/kg dose groups were sectioned at a different depth than were the other dose groups (Jean Harry, 2001). This likely contributed to the small but significant increase in size of the frontal, parietal, and striatum sections in the 1.0 mg/kg dose groups and the and may have contributed to the large increase in size of the anterior corpus callosum seen in the PND 9 males.

A post-hoc analysis of the PND 21 brain sections showed none of the sectioning-depth bias seen in the PND 9 sample.

PND 21: The striatum, cerebellum, and corpus callosum II (posterior sample) all showed significant changes with the lowest administered dose of ammonium perchlorate, 0.01 mg/kg/day (Table 3B, left). The striatum was significantly reduced in size at all but the highest dose. Region CA3 of the hippocampus similarly showed a u-shaped dose response. The cerebellum and the posterior corpus callosum increased in size with dose in an inverted u-shape. There were sex by treatment interactions in striatum and frontal cortex such that the female rats showed a stronger dose-related decrease in linear measurement than males. Both males and females show a complex dose response in the anterior corpus callosum measurement. As in the PND 9 animals, the changes in linear extent were generally in the ± 5- 11% range with the exception of the posterior portion of the corpus callosum, which showed an increase in size of 24% in the 0.1 and 1.0 mg/kg dose groups, and a 39% increase in the 0.1 mg/kg dose group.

The adjustments for brain size had little effect on the region by region results at PND 21 (Table 3B, center, right). Dividing through by the a-p or hippocampal measurements resulted in additional significant dose effects noted on CA1 and a sex by dose effect on cerebellum.

The Argus 1416-003 and current analyses agreed. Both analyses found a significant decrease in size of the striatum at 0.01, 0.1, and 1.0 mg/kg doses, and increases in size of the corpus callosum II (posterior) and cerebellum at the same doses. Both analyses noted the decrease in size of CA3 at the 0.1 mg/kg dose, the decreased anterior corpus callosum in females at 0.01 mg/kg, and the increased size of the frontal region in males at 0.1 and 30 mg/kg.

A post-hoc analysis of the PND 21 brain sections showed none of the sectioning-depth bias seen in the PND 9 sample (Jean Harry, personal communication).

Discussion:

There were significant differences in brain morphometry due to treatment with ammonium perchlorate at both PND 9 and 21 in this study. Tables 2 and 3 enumerate strong effects of developmental exposure to ammonium perchlorate on brain morphometry considered either across all regions tested and in the analysis of individual brain regions. These effects were present at PND 9 and PND 21, with the latter age group showing stronger effects. Many of these effects represent a increase or decrease of ± 10% in the size of a brain region, similar to the range of morphometric alteration noted in a recent study of fetal alcohol syndrome (Bookstein, et. al.,

2001). The corpus callosum showed a notable increase in linear extent of 24% or more at PND 21 in the 0.01, 0.1, and 1.0 mg/kg ammonium perchlorate dosing groups. Adjusting the raw morphometric determinations by either brain weight or measurements of larger brain areas, i.e. cerebrum, cerebellum, and hippocampus, had no strong effect on the results of the analysis.

The significant differences on the parallel profiles test demonstrate exposure-related changes in relative growth of different brain areas, even at the lowest administered dose (Table 2). Univariate analyses to further investigate these effects showed effects on a number of different brain regions at both ages tested. The most sensitive endpoints were the linear dimensions of the striatum, corpus callosum, and cerebellum at the 0.01 mg/kg/day dose when males and females were considered together at PND 21. Thus these analyses ultimately agree with those submitted in Argus, 2001: exposure to 0.01 mg/kg/day ammonium perchlorate during gestational and post-partum (weanling) development resulted in measurable changes in brain structures.

While no attempt is made in this memo to relate changes in brain morphometry to changes in the thyroid hormone economy, it should be noted that changes in thyroid hormone levels effect different brain regions differently during development. For example, developmental hypothyroidism has been linked to increases in the size of the cerebellum (Figure 3) (Lauder, et. al., 1994). Different brain regions show an inverted U or U-shape dose response; this is not uncommon in biological systems as compensatory or other mechanisms may be triggered at high doses. There is some concern over sectioning artifacts, since the brains from the different dose groups were sectioned at different intervals after sacrifice (Argus, 2001), and indeed post-hoc analysis of the brain sections did reveal some systematic differences in the PND 9 animals and in a limited sample of sections examined from the PND 21 animals. There is less concern over fixation artifacts, since all brains were fixed and embedded at the same time. In addition, dose-related effects were seen as both increases and decreases in brain region size. One might conclude from this that whatever artifacts may be present were not large enough to obviate alterations of the magnitude observed.

The increase in the size of the corpus callosum in this study replicates that seen in the previous morphometric analysis of rats developmentally exposed to ammonium perchlorate (USEPA, 1998b, Crofton, 1998). This is notable given the differences between the two studies. The previous data were obtained from tissues from rats aged PND 11 rather than PND 9 and 21 as in this study, and dose spacing included high doses of 3 and 10 mg/kg rather than 1 and 30 mg/kg as in this study. Fewer animals were used in the previous study (6/dose/sex) than in the current study (approximately 15/dose/sex), and litter identity was considered in the current analysis.

In summary, two different analyses of the brain morphometry data from the Argus/Primedica "Effects" Study yielded significant effects, i.e., alteration of brain structures, of developmental exposure to ammonium perchlorate in drinking water at doses of 0.01 mg/kg/day and higher in a mammalian (rat) model of neurodevelopment. These alterations included a 23 -

39% increase in the size of the corpus callosum over controls in the progeny of dams dosed with 0.01 to 1.0 mg/kg of ammonium perchlorate in drinking water. Alteration of brain structures in a laboratory animal model is considered to be an adverse neurotoxic effect (USEPA, 1998a). One of the analyses used a series of t-tests, the other a more conservative multivariate analysis employing a nested model profile analysis followed by univariate analysis of specific brain regions. The latter method is more likely to be considered a valid analytic method, since it better incorporates the design elements of the study and reduces the likelihood of Type I statistical error.

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Figure Captions

Figure 1. PND 9 pup brain region linear thickness measurements normalized by the control mean of each region, male and female data combined. The control data are represented by a horizontal line at 1.0. Profile analysis determines whether the vectors of measurements from each treatment group differ from each other and control. The heavy lines represent ± 99% confidence intervals around the mean control values. Note that while this plot uses the normalized data to more easily illustrate the data vectors, the actual analysis was performed using raw data values.

Figure 2. PND 21 pup brain region linear thickness measurements normalized by the control mean of each region, male and female data combined. Other details as in Figure 1.

Table 1: Animals with missing data for profile analysis.

Post-Natal Day 9	**Sex**	**Treatment Group**	**Brain Regions Missing**
16618	F	1; control	Dentate, CA1, CA3
16656	F	2; 0.01 mg/kg/day	Cerebellum
16654	M	2; 0.01 mg/kg/day	Corpus callosum I
16676	F	3; 0.1 mg/kg/day	Cerebellum
16681	F	3; 0.1 mg/kg/day	Hippocampus, Dentate, CA1, CA3
16667	M	3; 0.1 mg/kg/day	Corpus callosum I
16670	M	3; 0.1 mg/kg/day	Cerebellum
16725	M	5; 30 mg/kg/day	Cerebellum
Post-Natal Day 21			
16826	F	5; 30 mg/kg/day	Corpus callosum I
16831	F	5; 30 mg/kg/day	Corpus callosum I
16833	F	5; 30 mg/kg/day	Corpus callosum I

Table 2A: Effects of ammonium perchlorate on sizes of brain regions estimated by histological morphometry: Parallel Profiles. Contrasts show p-value for treatment effects.

	Day 9	**Day 21**
Overall treatment / sex * dose	$F_{36,245} = 2.47$, $p < 0.0001$ / NS	$F_{36,249} = 4.77$, $p < 0.0001$ / $F_{36,245} = 1.54$, 0.03
Control vs 0.01 mg/kg/day	$F_{9,65} = 2.80$, $p < 0.008$	$F_{9,66} = 14.14$, $p < 0.0001$
Control vs 0.1 mg/kg/day	NS	$F_{9,66} = 9.99$, $p < 0.0001$
Control vs 1 mg/kg/day	$F_{9,65} = 4.45$, $p < 0.0001$	$F_{9,66} = 10.50$, $p < 0.0001$
Control vs 30 mg/kg/day	NS	$F_{9,66} = 11.92$, $p < 0.0001$

Table 2B: Adjustment for Brain Weight: Effects of ammonium perchlorate on sizes of brain regions estimated by histological morphometry, with linear dimensions divided by total brain weight: Parallel Profiles. Contrasts show p-value for treatment effects.

	Day 9	**Day 21**
Overall treatment / sex * dose	$F_{36,245.} = 1.99$, $p < 0.0013$ / NS	$F_{36,249} = 4.98$, $p < 0.0001$ / NS
Control vs 0.01 mg/kg/day	$F_{9,65} = 2.57$, $p < 0.0135$	$F_{9,66} = 15.22$, $p < 0.0001$
Control vs 0.1 mg/kg/day	NS	$F_{9,66} = 10.43$, $p < 0.0001$
Control vs 1 mg/kg/day	$F_{9,65} = 4.15$, $p < 0.0003$	$F_{9,66} = 10.87$, $p < 0.0001$
Control vs 30 mg/kg/day	NS	$F_{9,66} = 11.89$, $p < 0.0001$

Table 2C: Adjustment for regional dimensions: Effects of ammonium perchlorate on sizes of brain regions estimated by histological morphometry, with linear dimensions divided by anterior-posterior length estimates of cerebrum, cerebellum, and total width of hippocampus, as appropriate: Parallel Profiles. Contrasts show p-value for treatment effects.

	Day 9	**Day 21**
Overall treatment / sex * dose	$F_{32,245} = 2.32$, $p < 0.0002$ / NS	$F_{32,249} = 4.005$, $p < 0.0001$ / $F_{32,249} = 1.84$, $p < 0.0054$
Control vs 0.01 mg/kg/day	$F_{8,66} = 2.83$, $p < 0.009$	$F_{8,67} = 9.62$, $p < 0.0001$
Control vs 0.1 mg/kg/day	NS	$F_{8,67} = 7.28$, $p < 0.0001$
Control vs 1 mg/kg/day	$F_{8,66} = 2.73$, $p < 0.012$	$F_{8,67} = 6.66$, $p < 0.0001$
Control vs 30 mg/kg/day	$F_{8,66} = 3.93$, $p < 0.0008$	$F_{8,67} = 8.82$, $p < 0.0001$

Table 3A: Effects of ammonium perchlorate on sizes of brain regions estimated by histological morphometry, individual brain areas: Postnatal Day 9

	Postnatal Day 9: Raw Data **dose** **sex * dose**	**Postnatal Day 9: Brain Weight Adjusted** **dose** **sex * dose**	**Postnatal Day 9: Regional Size Adjusted** **dose** **sex * dose**
Frontal	$F_{4,73} = 4.11$, $p < 0.005$ NS	$F_{4,73} = 6.55$, $p < 0.00015$ NS	$F_{4,73} = 5.09$, $p < 0.00113$ NS
Parietal	$F_{4,73} = 2.82$, $p < 0.03$ NS	$F_{4,73} = 2.81$, $p < 0.032$ NS	$F_{4,73} = 2.80$, $p < 0.032$ NS
Striatum	$F_{4,73} = 5.74$, $p < 0.00045$ NS	NS NS	$F_{4,73} = 5.74$, $p < 0.00045$ NS
Hippocampus	NS NS	NS NS	----------
Dentate	NS NS	NS NS	NS / NS
CA1	$F_{4,73} = 2.81$, $p < 0.03$ $F_{4,56} = 4.24$, $p < 0.0046$	NS $F_{4,56} = 3.46$, $p < 0.01$	NS / NS
CA3	NS NS	NS NS	NS / NS
Cerebellum	NS NS	NS NS	$F_{4,73} = 5.24$, $p < 0.036$ $F_{4,56} = 2.81$, $p < 0.034$
External Germinal Layer	$F_{4,73} = 3.31$, $p < 0.015$ NS	NS NS	$F_{4,73} = 3.31$, $p < 0.015$ NS
Corpus Callosum - anterior	$F_{4,73} = 3.02$, $p < 0.02$ $F_{4,56} = 5.83$, $p < 0.0005$	NS $F_{4,56} = 5.79$, $p < 0.0006$	NS $F_{4,56} = 6.02$, $p < 0.0004$

Table 3B: Effects of ammonium perchlorate on sizes of brain regions estimated by histological morphometry, individual brain areas: Postnatal Day 22

	Postnatal Day 21: Raw Data **dose** **sex * dose**	**Postnatal Day 21: Brain Weight Adjusted** **dose** **sex * dose**	**Postnatal Day 21: Regional Size Adjusted** **dose** **sex * dose**
Frontal	NS $F_{4,71} = 2.84, p < 0.03$	NS NS	NS / 0.01
Parietal	NS NS	NS NS	NS / NS
Striatum	$F_{4,74} = 19.02, p < 0.000001$ $F_{4,71} = 5.87, p < 0.0004$	$F_{4,74} = 13.9, p < 0.000001$ $F_{4,71} = 2.75, p < 0.035$	$F_{4,74} = 19.02, p < 0.000001$ / $F_{4,71} = 5.87, p < 0.0004$
Hippocampus	NS NS	NS NS	----------
Dentate	NS NS	NS NS	NS NS
CA1	NS NS	NS NS	$F_{4,74} = 3.26, p < 0.016$ NS
CA3	$F_{4,74} = 5.47, p < 0.00065$ NS	$F_{4,74} = 9.38, p < 0.000001$ NS	$F_{4,74} = 5.20, p < 0.00095$ NS
Cerebellum	$F_{4,74} = 16.40, p < 0.000001$ NS	$F_{4,74} = 7.45, p < 0.00004$ NS	$F_{4,74} = 5.24, p < 0.0009$ $F_{4,71} = 2.64, p < 0.04$
Corpus Callosum - anterior	$F_{4,74} = 4.57, p < 0.002$ $F_{4,71} = 2.64, p < 0.04$	$F_{4,74} = 4.57, p < 0.0024$ NS	$F_{4,74} = 4.46, p < 0.0028$ $F_{4,71} = 2.66, p < 0.039$
Corpus Callosum - posterior	$F_{4,74} = 5.91, p < 0.00035$ NS	$F_{4,74} = 5.25, p < 0.0009$ NS	$F_{4,74} = 5.38, p < 0.0007$ NS

PND 9: Normalized Brain Structure Thickness measured on coronal sections from rats treated during development with ammonium perchlorate

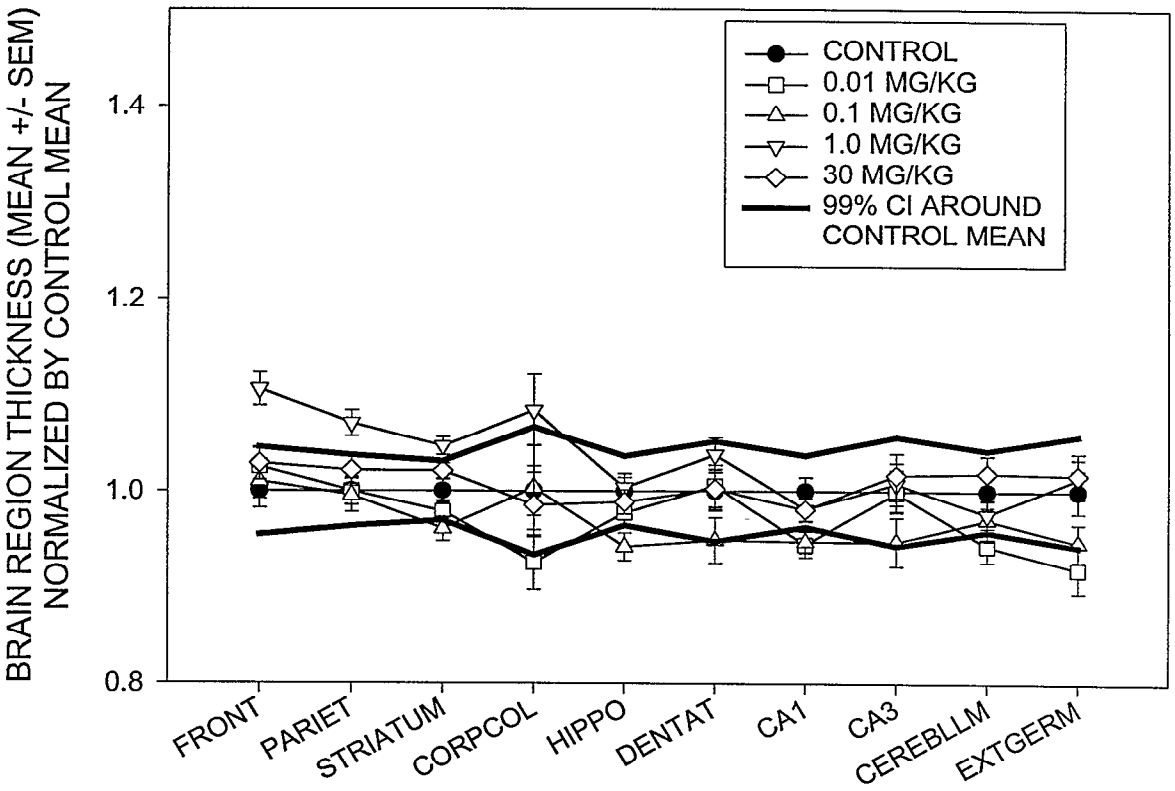

Figure 1

PND 21: Normalized Brain Structure Thickness measured on coronal sections from rats treated during development with ammonium perchlorate

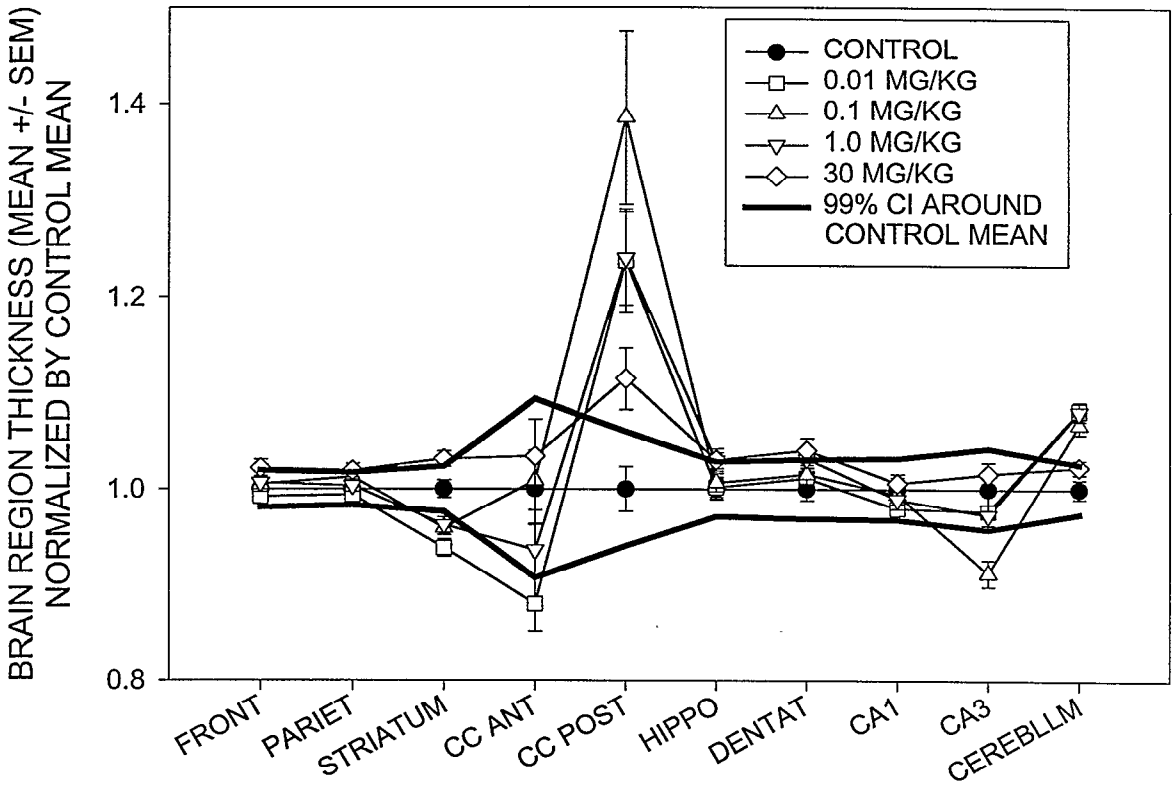

Figure 2