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DEPARTMENT OF HEALTH AND HUMAN SERVICES

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Date: October 11, 2001

To: Annie M. Jarabek, National Center for Environmental Assessment, US EPA

From: Jean Harry, Ph.D., Acting Chief, Laboratory of Toxicology
Neurotoxicology Group Leader, NIEHS

Re:Comments on Original Experimental Design, Study Performance, and Brain Morphometry Results of Argus Research Laboratories, Inc. 14 March 2001 Study (Protocol Number 1416-003) and Supplemental Materials Provided by Dr. Robert Garman, Consultants in Veterinary Pathology, Inc.

Basis for examining structural formation of the brain as a measure of developmental neurotoxicity:

The formation and maturation of the nervous system is critically dependent upon both a temporal and spatial organization pattern. Within this framework, there has been demonstrated an interdependency between the various cells types in the brain and precise spatial relationship to one another. During this time the developing system is learning how to organize and regulate itself. Thus, the disruption of the developmental profile of one cell type may significantly influence critical events in later development resulting in an alteration of the normal formation of the brain and it's functional connections. Many toxic agents have been demonstrated to interfere with one or more of the developmental processes of the brain, such as, cell division of neuronal and glia precursor cells, cell interaction with the immediate environment through surface receptors or cell adhesion molecules, regulation of cytoskeletal processes that control proliferation and migration, cell-cell interactions that underlie synaptogenesis, development of the cerebral circulation and the blood-brain barrier, meylination, and programmed cell death. Such perturbations may not be evident by standard histological assessments as often there can be little, if any, evidence of cell death. Rather what is seen is a delay or disruption in the normal development and maturation of specific neural regions. Given the critical interactions and signalling processes that occur between various cell types, a delay in the normal progression of any cell population or specific developmental event may have significant influence on the formation of the final neural network critical for the normal functioning of the nervous system.

Purpose of Memo:

This memo addresses the involvement and response of NIEHS in 1) the evaluation of data from the 1998 Argus Research Labs study on developmental perchlorate exposure and altered brain morphometry, 2) recommendations with regard to the experimental design for the replication study (Argus Research Laboratories, Inc., 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", Protocol Number: 1416-003 (Final Report dated 14 March 2001), 3) an evaluation of the quality of the sections and brain morphometry data from the Argus, 2001 study and supplemental materials provided separately by Dr. Robert Garman on July 17, 2001, and 4) where appropriate, a response to comments submitted to US EPA from expert reviewers regarding the brain morphometric data from the Argus, 2001 study.

Review of brain sections from PND 11 (Argus PND 12) rat pups (Argus Research Labs, 1998)

During peer review of the developmental neurotoxicity study entitled, "A Neurobehavioral Developmental Study of Ammonium Perchlorate Administered Orally in Drinking Water to Rats" (Argus, 1998; Protocol Number 1613-002) and the associated EPPA 1998 assessment, it was recommended that the blocks of brain sections be evaluated for the possibility of additional use (Research Triangle Institute, 1999). Brain sections from the Postnatal day 11 male and female rats were examined by Dr. Harry at NIEHS as follows: Using an atlas of the rat brain (Sherwood and Timiras, 1970), the plane of cut for each section was estimated according to stereotaxic coordinates. A large variability in the plane of cut was detected that could significantly affect measurements of brain regions. This level of variability was most likely due to the small size of the brain at PND 11 and the technical difficulties in precisely blocking the brain at this age.

However, the examination continued in spite of this high level of variability. For evaluation of the corpus callosum, the section containing the corpus callosum was examined for each animal using a computer video imaging program (NIH image). This was conducted both in consultation with two developmental neurobiologists/neuropathologists and with the assistance of NIEHS's Pathology Laboratory. As the site of measurement for the corpus callosum as conducted in the Argus, 1998 study was not known, three areas were identified for measurement in the coronal sections. Two measurements at distinct locations in each hemisphere and while possibly compromised in coronal sections by artifacts due to close proximity to the ventricle, a measurement at the midline between the two hemispheres. There were substantial differences in the measurements both across hemispheres in individual animals and between animals dependent upon the plane of cut that could confound the evaluation of a treatment-related effect. The lack of uniform sections of the brain in addition to the small sample size prevented any conclusion to be made with regard to the possible effects of perchlorate on brain development. It was determined that the inconsistency in the plane of cut did not allow for additional evaluations to be conducted using the existing blocks or for re-analysis of the original sections from the Argus 1998 study.

Issues raised with regard to a replication study according to a mutually agreed upon protocol (Argus Laboratories, Inc., protocol #1416-003, 2001)

One major objective of the Argus, 2001 study, was replication of brain morphometric measurements in order to address concerns raised by the US EPA, NIEHS, and the external peer review panel regarding results observed in the 1998 developmental neurotoxicity study (Argus, Protocol Number 1613-002, 1998; EPA, 1998). The purpose was to evaluate, under more rigorous experimental conditions and according to the US EPA developmental neurotoxicity guidelines, whether the proported effect in the corpus callosum as "called out" by the USEPA (1998) would be replicated. In addition, to identify effects that may occur in other brain regions.

The following suggestions were made by NIEHS, with regard to conducting a replication of the earlier study and toward developing a mutually agreed upon protocol. Some recommendations were incorporated into the study design while others were not due to either the cost and time demands or in the requirement to conduct a study that would adhere to the US EPA Developmental Neurotoxicity guidelines.

1. While US EPA developmental neurotoxicity guidelines recommend 6 animals per group, data from the Argus, 1998 study suggested that this was an insufficient number of animals to evaluate linear measurements of brain regions. It was recommended that the number of animals within each group be increased to a minimum of 10. The final study design resulted in 15-16 animals per group.

2. In discussions on protocol design, comments were raised with regard to the need or value of collecting tissue at an earlier age. In the proposed design, PND 5 animals would be available for such collection. While it was felt that little would be gained with regard to morphometric analysis, if excess animals were available, they could be used to establish methods to handle and prepare such immature brains. However, it was viewed to be unlikely that the tissue would be examined morphometrically for determining perchlorate effects.

3. While the tissue fixation method of choice in adult rodents is via cardiac perfusion, even this procedure is not without problems that can compromise tissue integrity. It has been documented that immersion fixation artifacts can influence histological and morphometric evaluations of adult brains however, a less than optimal cardiac perfusion can also result in morphological artifacts. In the younger animal there is less of a consensus on the proper manner of fixation. With the decreasing size and blood volume of the younger animal (PND10-11), the difficulty of ensuring a good fixation via cardiac perfusion is significantly increased over that in the adult. Since comparisons were to be made between the 1998 and the 2001 study, consistency in method of fixation was considered to be important to maintain across studies. Thus, it was decided that with all of its limitations, immersion fixation was the tissue processing method of choice and recommended for the Argus 2001 study.

4. It was recommended that the analysis be conducted progressively with the PND 22 animals being evaluated first. Following this analysis and the evidence of an alteration in the morphometric analysis of the brain structures, the PND 11 brains would be examined for comparison to the Argus 1998 study. This recommendation was based on two features. One was the difficulty that became evident in the 1998 study in obtaining consistent plane of cut for comparison in the immature PND 10 brains. The second was based on the timing of the developmental process in the formation of the corpus callosum as it is associated with the initiation and accumulation of myelination.

5. Given the number of samples that were to be processed, it was recommended that a step procedure routinely used in pathology studies be used for analysis. All tissue was to be processed to the stage of sectioning at one time to minimize fixation artifacts The control and the high dose groups would be sectioned and analyzed first followed by additional dose groups. Based on the availability of animals, it was recommended that additional control samples be included for processing with each dose group. These additional control animals could be used to evaluate various components of fixation and processing that may vary with the dose groups. An alternative approach was discussed in which a representative sampling across all groups would be processed and examined at any one time. It was strongly recommended that all efforts be made to ensure that samples were handled and processed in such a way to minimize artifacts.

6. It was considered to be critical that all efforts would be made to ensure sectioning of the brain and sites of measurement be in a consistent plane of the brain. All were aware that this would become more difficult with decreasing age of animals. Documentation of plane of cut for each section should be provided by a full image of the brain, location of each measurement site, and anterior-posterior plane of axis as identified by stereotaxic atlas of the immature brain (e.g., "A Stereotaxic Atlas of the Developing Rat Brain" by Nancy M. Sherwood and Paola S. Timiras, University of California Press, 1970).

7. Following the review of the Argus, 1998 study, and in considering design considerations for the subsequent study, the plane of cut for the brain was discussed. While sagittal sections for analysis were recommended for some aspects of morphometric analysis, coronal sections were ultimately adopted, since comparisons were to be made between the 1998 and the 2001 study. This final design of the study also adhered to the US EPA developmental neurotoxicity guidelines which calls for coronal sections

8. It was recommended that measurements of the corpus callosum in coronal sections should not be conducted at the midline due to possible edema artifacts that can occur due to the close proximity of the ventricle. Three sites were recommended for measurement that would have been consistent with the evaluation conducted by NIEHS on the sections from the Argus, 1998 study. It was agreed upon in the final design meeting that, given the time constraints and need for comparison to the Argus, 1998 study, one measurement per hemisphere would be recorded at the same site as used for the Argus, 1998 study. This was a site just off of the midline of the two hemispheres.

9. Given the critical nature of plane of cut and the high degree of variability seen in the Argus, 1998 study, it was recommended that measurements of each brain region would be recorded for each hemisphere. The availability of such data for individual animals would allow for an assessment of the consistency in the plane of cut and bilateral nature of any effect identified. For each brain

section from each animal, images would be archived, including measurement site. If images confirmed a consistent plane of cut across hemispheres, analysis could be conducted on the averaged measurement across both hemispheres. If, however, the plane of cut differed an analysis might be possible with anterior-posterior plane of cut as a factor. This design is not intended to measure exposure-related effects on a hemisphere comparison but rather to offer data to support a consistency of plane of cut in each section thus, increase confidence in the data set.

10. Given that the various neuronal populations of the hippocampus develop and mature at different times during development, it was suggested that measurements of the hippocampus should not be limited to the full width of the hippocampal formation but rather should include width measurements of the various neuronal bands e.g., the dentate granule cell layer and the pyramidal neurons of the CA1 and CA3 cell layers. This would allow for changes in any specific region of the hippocampus to be examined relative to the overall size of the hippocampus. This approach may serve to minimize the influence that plane of cut may have on the analysis of hippocampal morphometric measurements.

11. It was initially recommended that morphometric determinations be conducted on digital images using imaging quantitation software e.g., NIH image. In the final design meeting, it was agreed that due to time constraints of the study altering the established method of analysis of the contractor was not feasible. However, digital images were to be captured for documentation.

Comments on Experimental Design and Study Performance. (Argus Laboratories, Inc., protocol #1416-003, 2001)

A review of the Argus 2001 brain morphology study was conducted by a number of expert reviewers (Toxicology Excellence for Risk Assessment, 2001). These comments were provided to both the US EPA and to NIEHS. This section is to provide a review of specific aspects of the study design and data obtained and serve to address a number of issues raised by the expert panel.

1. As mentioned in a previous section (item #3), immersion fixation was the method of choice for a number of reasons including consistency with the Argus, 1998 study and the difficulty in obtaining a good cardiac perfusion in young animals.

2. The postnatal days for tissue collection and brain evaluation were based on the developmental neurotoxicity guidelines (EPA, 1998) which calls for processing of tissues on PND11, with the day of birth designated as PND 0. An additional time point of PND 21 was included in the Argus, 2001 study as animals were available under the experimental study design and the additional evaluation would occur during a time of active myelination. Argus Laboratories identified the day of birth as PND1, therefore the age nomenclature of PND 10 and PND 22 is off by one day as referenced to the EPA definition and would be PND 9 and PND21, respectively. The Argus 1998 study, also performed by Argus on PND12, was actually performed per EPA guideline nomenclature on PND 11. While the actual ages were slightly different between the two studies, the concept of capturing an active process of development remains in effect.

3. In the review by TERA, questions were raised with regard to the age of sampling as it related to myelin formation. The process of myelination is a "developmental landmark" for the maturation of the brain, is initiated upon the presence of the axon, and continues over an extended period of time. It is a structure that matures over time with the accumulation of protein and structural lamella. One major period of myelin protein and lipid synthesis occurs approximately between PND19 and PND35. Thus, while examination at PND21 would not capture the final accumulation of myelin it would capture events occurring at a time in which myelin processing and lamella wrapping of the axon is actively occurring and therefore may represent a period of critical development of the myelin sheath. Examination of animals with a mature myelin sheath e.g. ages greater than PND 40, may offer information regarding whether any of the changes seen at earlier time points would represent a permanent structural alteration. The majority of studies that have examined myelin development and/or alterations in this developmental process have employed biochemical, molecular, as well as, morphological evaluations to make such determinations as delay or hypomyelination. From such studies, the timing for examination has been demonstrated to be

most appropriate between the ages of PND15 and PND35. Thus, examination of the corpus callosum at PND 9 is probably at the limit of early development where the data would represent an evaluation of the myelin sheath. In addition, the development of the axonal pathways connecting the two hemispheres via the corpus callosum also continues to develop over this time period. While the study design allowed for the collection of tissue at PND5 it is felt that any measurements recorded at such age would be very limited in their contribution to the interpretation of the currently available data set. In addition, given the variability of the plane of cut and the difficulty in examining brains of young animals, examination of the corpus callosum in younger animals would present an even greater problem.

4. Two pathology laboratories, Consultants in Veterinary Pathology, Inc. (CVP) and Experimental Pathology Laboratories (EPL) were involved in the processing of tissue blocks. Since CVP did the blocking and sectioning for both the PND9 and PND21 brains, it was concluded that EPL could excise the brains without introducing additional variability. Blocking of brain into discrete sections for paraffin embedding can be influenced by a number of factors. As the expert reviewers noted a number of the histology sections gave indication of a preference for one side in the plane of cut. Significance of shifting of the plane of cut that may have occurred due to "handedness" of the technician can be determined by an analysis of the two hemispheres.

5. There can often be a concern for evaluator knowledge of the identity of the samples at time of measurement (blind reading). However, in reality, the number of slides read often obviates the practicality of such a concern. In this case and the data collected it was not felt to be a major issue of concern.

6. In the review by TERA it was suggested that brain measurements be normalized to a more general feature such as brain weight. While brain wet weight is often used there is little historical data for using fixed brain weight as a normalization factor. In the Argus, 2001 study, the brain weights did not significantly differ between the groups as demonstrated by the statistical analysis of Argus and an additional analysis conducted by Dr. Geller, US EPA. Additional analysis were conducted on individual brain measurements and fixed brain weight. As an alternative measurement, the various brain regions were evaluated relative to a measurement of a more general structure. Measurement of cortical structures were examined relative to the linear anterior - posterior measurement of the brain. In addition, the individual cell layers in the hippocampus were examined relative to the overall measurement of the hippocampus. The data and results have been provided by Dr. Geller, US EPA (2001).

7. TERA reviewers questioned if the appropriate endpoints were examined based upon an hypothesis of alterations in thyroid hormone functioning. Additional measurements are available that would offer a more detailed evaluation of the effects on brain development such as, morphological evaluation at the electron microscope level, individual cellular measures, or other biological/biochemical markers of axonal and myelin development. However, these measures are not conducive to a screening/testing approach. They are more appropriate for understanding the process by which the delay or alteration is occurring rather than hazard identification.

8. TERA reviewers raised the issue of conducting studies in non-human primates to determine human health effects. While the ability to conduct developmental and developmental neurotoxicity studies in non-human primates would provide additional information regarding adverse effects in humans, that is the case for all compounds both pharmaceutical and environmental. However, the difficulty and ethical issues of conducting screening studies in non-human primates is one of the driving forces over the last few decades in establishing rodent models for brain development. Not only has a pattern of the critical developmental features been established but these patterns have been compared and contrasted with what is known in the developing human. There is a wealth of information in the scientific literature both on the process of normal development and alterations in such normal processes. These data support the use of rodent models in determining potential adverse effects on the developing brain.

9. Dr. Douglas Wahlston suggested in his review (Toxicology Excellence for Risk Assessment, 2001), that the external granular layer (EGL) may be a particularly good indicator of developmental rate in certain age ranges. He asserts that PND 21 would likely be near the age when the EGL layer disappears and would therefore reveal major gross abnormal retardation. Upon visual examination

of the cerebelllum brain images, there was no obvious disruption with neuronal migration from the external to the internal layers. Measurement of the cerebellar cortical region would not provide additional information with regard to this developmental endpoint. It is doubtful whether or not examination of the cerebellum at PND 9 would offer information on this process due to the active nature of the process during the first two weeks of life. The visual examination of the PND 9 cerebellum in the Argus 2001 study supports this conclusion. Thus, it is not recommended that this endpoint be examined for exposure related effects at ages earlier than the currently available PND 21 time point.

NIEHS Review of the Sections and measurements (Argus, 2001 study)

1. Overall the images of the brain sections from the PND 9 and PND 21 time points demonstrated that the processing of the brain was adequate for conducting limited morphometric measurements as outlined in the study design and in the US EPA Developmental Neurotoxicity Guidelines. However, concern is raised over the consistency of the plane of cut of these sections and the lack of comparability across dose groups. As mentioned by the TERA reviewers and stated in the study report provided by Consultants in Veterinary Pathology, there was a greater degree of variation in the PND 9 brain sections than in the PND 21 brain sections. It was speculated that this would be associated with the greater variability seen in both the size and degree of development of brains from PND 9 rat. Again as also noted in the contract report, many sections in the PND 9 brains showed signs of disruption or damage that may have compromised morphometric measurement. Details of the evaluation of brain sections by NIEHS are discussed below.

2. Review of the brain images at PND 9 indicated a concern for the variance of the plane of cut in all three sections. This was also a concern raised by the expert reviewers. Dr. Harry at NIEHS viewed all of the sections and determined the plane of cut as identified by a rat brain atlas at PND 9. All sections, #1-3, showed differences in the plane of cut within all treatment groups. Following the statistical analysis conducted by US EPA (Geller, 2001), section 1 was examined more detailed for plane of cut and possible influence on the structural measurements (striatum and the corpus callosum). Each section 1 was viewed for plane of cut, consistency of plane across hemispheres, visual indication of section blocking side bias, and noted for anterior-posterior (AP) coordinates using stereotaxic atlas of the developing rat brain (Sherwood and Timiras, 1970). A/P coordinates are set as millimeter units. As was noted in the report provided by Consultants in Veterinary Pathology, there are slight variations between this atlas that is based on Long –Evans hooded rats and the actual images generated from the Sprague-Dawley rats used in this study. Thus, the atlas was used as a reference point from which to examine the images generated in this study and any evaluation was based upon both the atlas and actual images and structural landmarks. In the normal/control brain, sections located at A/P coordinates between 2.1 and 3.1 appeared to be relatively similar for many of the structures measured. However, as the plane of cut progressed in an anterior direction changes occurred in the structures measured. For example, using measurements provided from control animals in the Argus study and measurements of width from the atlas images, the the width of the corpus callosum was increased approximately 20% from A/P coordinates of 2.8 to 4.1. A variability in plane of cut was evident both across animals and across hemispheres within the same animal (see representative image in appendix). While there was variability in the plane of cut within each exposure group it was not uniformly distributed across groups. In examination of the images, normal widening of the corpus callosum was evident by A/P coordinates of 3.5. In the control group and in exposure group V, only 10 and 18% of the sections, respectively were above AP 3.5. In exposure group III, a number of sections were missing or not adequate for measurement. Of those that were 23% in A/P planes greater than 3.5. In group IV, 56% of the sections were in an A/P plane of cut greater than 3.5. While the emphasis for examination was the corpus callosum, it should be noted that these differences in plane of cut could also significantly influence the measurements of the striatum and the frontal cortex.

3. The variability and integrity of the cerebellum sections at PND9 were viewed as being inadequate for morphometric measurement due to the significant variability in the plane of sectioning and often missing tissue in the line of measurement. This was considered to be an inherent problem with the examination of such small brains and fragile tissue rather than any question of the performance of the contractors conducting these studies.

4. Visual examination of brain section from PND 21 animals showed less variability in the plane of cut than that seen at PND 9. Brief reviewing of brain sections showed no prominent differences in plane of cut however, a detailed evaluation of the plane of cut was conducted only on section 2. Section 2 showed a variability in the anterior/posterior plane of cut for section 2 was seen that could influence any morphometric measurements recorded.

5. In section 2, the plane of cut, as determined primarily by hippocampal landmarks, demonstrated an anterior/posterior gradient in the width of the corpus callosum with a greater width occurring as the sections progressed through the brain. It was noted in the report provided by Consultants in Veterinary Pathology, that the "posterior measurement of the corpus callosum within the PND 22 rat brains was taken quite close to or within the region of the splenium, a location in which the corpus calllosum normally becomes thicker." As the corpus callosum was identified as a structure showing the greatest percentage increase these sections were evaluated to determine the impact that plane of section would have of the measurements. All A/P coordinated are in millimeter units. Images were examined from males at PND 21 for dose groups I, II, III, IV, and V. Groups I and V were sectioned and evaluated concurrently in the Argus, 2001 study followed by the additional treatment groups. In group I (controls) 100% of the sections were in an anterior plane of cut. In group II (0.01mg/kg/day), out of 16 sections 4 were in an anterior plane, 1 anterior/mid, 3 in the mid plane, 1 in mid/posterior plane, and 7 in the posterior plane. In group III (0.1mg/kg/day), out of 15 sections, 2 were anterior, 2 were anterior/mid level, 1 at mid level, 2 were mid/posterior level, and 8 were at a posterior level of sectioning, In dose group IV (1mg.kg.day), out of 16 sections, 6 were anterior, 2 mid level, 2 mid/posterior, and 6 posterior. In dose group V (30 mg/kg/day) out of 16 sections, 11 were in the anterior plane of cut, 2 were in the anterior/mid level, 2 in the mid/posterior level, 1 posterior level of sectioning. The anterior level of sectioning (A/P 3.5) resulted in measurements of the corpus callosum (approx. 192-210) that were less than measurements taken in either the mid level (A/P 2.9, approx. 220-240) or the posterior level (A/P 2.6-2.0, approx. 260-420). The variability in the plane of cut for section 2 resulted in non-comparable data sets across dose groups. The evaluation of the plane of cut was conducted independent of the measurements of the corpus callosum. Whether or not this occurred in the other sections was not determined by a detailed re-examination of the sections nor was this level of examination conducted for the females.

Using the images and morphometric measurements provided by Argus the plane of cut for section 2 and measurements of the corpus callosum fall in the following groupings:

	I	II	III	IV	V
Anterior	16	4	2	6	11
Ant/Mid		1	2	0	2
Mid		3	1	2	0
Post/Mid		1	2	2	2
Posterior		7	8	6	1

Summary

In the review of the data from the Argus, 2001 study it became apparent that the quality of the sections for use in morphometric analysis was a major limitation. The criteria for obtaining uniform sections at the same level of the brain was not met at either age. For PND 9 no conclusions can be made about the possible effects of perchlorate on brain development either as an overall treatment effect or dose response effect. For PND 21, matching of samples by plane of cut is not possible for section 2 as only the control group and high dose groups contained a preponderance of sections in the anterior plane of cut as determined by distinct hippocampal landmarks. It may be possible to obtain adequate data if additional brain sections are provided to produce comparable data sets with regard to plane of cut, e.g., additional samples of section 2 in the posterior plane be provided for analysis of the corpus callosum. In addition, detailed examination of the other two may be necessary to determine if these data sets are comparable across dose groups.

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G. Jean Harry

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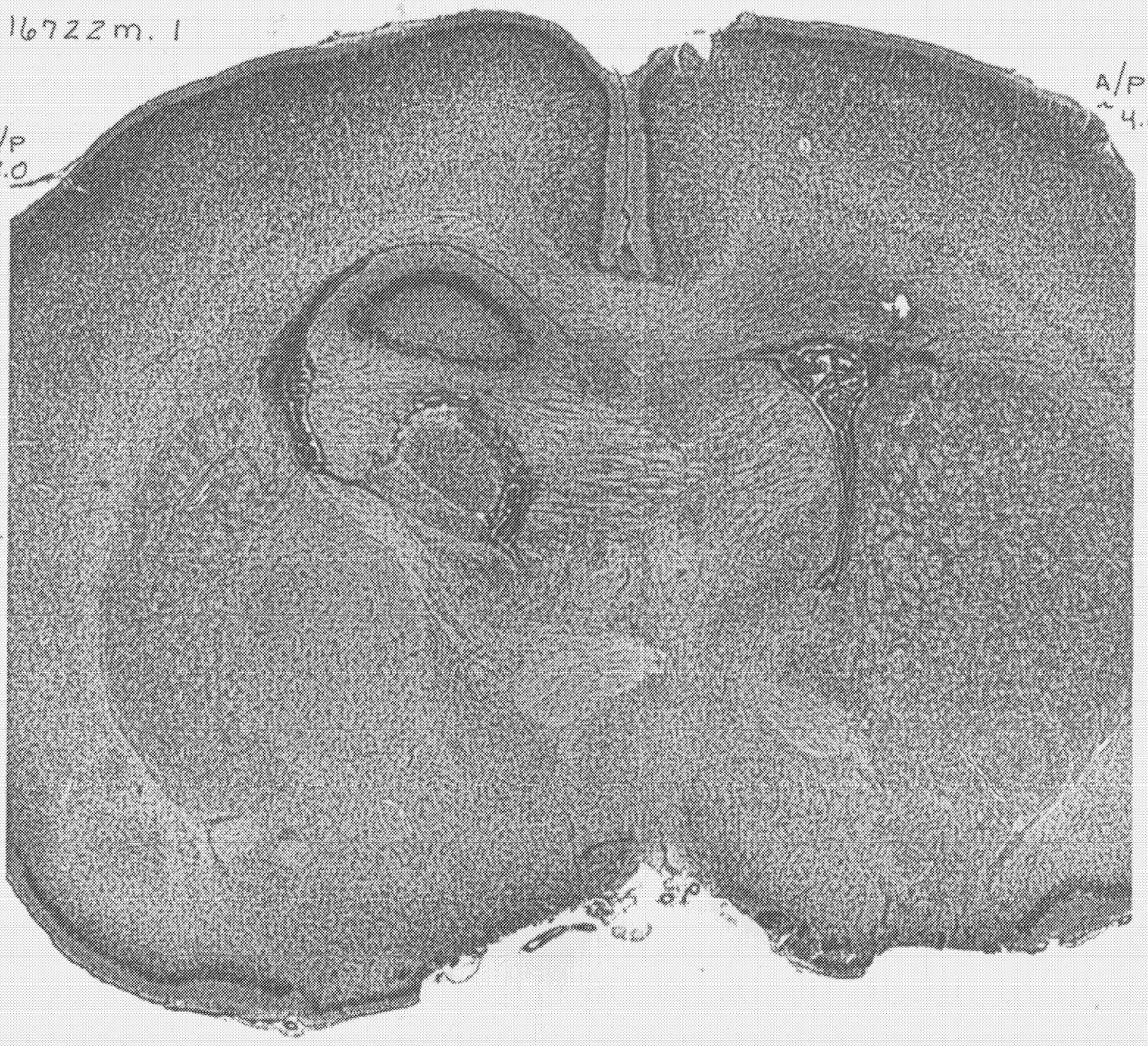
Appendix B:

:T	SEX	LITTER	Posterior Corpus Callosum, µM	Qualitative depth
1	M	16731	192	ant
1	M	16732	192	ant
1	M	16734	192	ant
1	M	16735	202	ant
1	M	16736	182	ant
1	M	16737	235	ant
1	M	16738	134.5	ant
1	M	16740	187	ant
1	M	16741	158.5	ant
1	M	16742	173	ant
1	M	16745	173	ant
1	M	16746	211	ant
1	M	16747	192	ant
1	M	16749	216	ant
1	M	16750	192	ant
1	M	16753	192	ant
2	M	16761	211.5	ant
2	M	16762	197	ant
2	M	16765	177.5	ant
2	M	16769	153.5	ant
2	M	16774	225.5	ant/mid
2	M	16758	201.5	mid
2	M	16771	235.5	mid
2	M	16773	250	mid
2	M	16755	278	post
2	M	16760	297.5	post
2	M	16763	273.5	post
2	M	16764	321.5	post
2	M	16767	302.5	post
2	M	16770	326	post
2	M	16776	370	post
2	M	16768	283	post/mid
3	M	16783	187	ant
3	M	16798	149	ant
3	M	16782	221	ant/mid
3	M	16786	173	ant/mid
3	M	16797	168	mid
3	M	16781	202	post
3	M	16787	245	post
3	M	16791	230	post
3	M	16792	460.5	post
3	M	16794	321.5	post
3	M	16795	273.5	post
3	M	16796	341	post
3	M	16799	408	post
3	M	16784	192	post/mid
3	M	16793	235	post/mid
4	M	16800	192	ant
4	M	16805	211	ant

4	M	16807	225.5	ant
4	M	16808	177.5	ant
4	M	16817	177.5	ant
4	M	16818	158.5	ant
4	M	16806	197	mid
4	M	16810	206.5	mid
4	M	16801	317	post
4	M	16802	302	post
4	M	16803	264	post
4	M	16811	369.5	post
4	M	16814	345.5	post
4	M	16815	321.5	post
4	M	16820	254.5	post/mid
4	M	16822	240	post/mid
5	M	16823	206.5	ant
5	M	16825	197	ant
5	M	16826	206.5	ant
5	M	16827	201.5	ant
5	M	16828	134	ant
5	M	16830	187.5	ant
5	M	16833	216	ant
5	M	16834	177.5	ant
5	M	16839	206.5	ant
5	M	16840	201.5	ant
5	M	16843	206.5	ant
5	M	16837	230	ant/mid
5	M	16842	235	ant/mid
5	M	16831	336	post
5	M	16836	278.5	post/mid
5	M	16844	230	post/mid

Appendix A:

16722m. 1



MALES

GROUP I

#16616	(No section #1)	
#16617	3.0	3.0
#16618	2.9 (tear in corpus callosum, no measurement)	2.9
#16619	3.0	3.2
#16620	2.6	3.0
#16621	3.0	2.6
#16622	2.5	2.5
#16625	2.6	2.5 (torn no measurement)
#16626	4.1	4.1 (torn, may measure)
#16629		
#16630	3.0	3.1 (torn no measurement)
#16634	2.6	2.9
#16636	3.1	3.0
#16637	2.6 (torn on left side)	(not appropriate for measurement)

GROUP II

#16640	5.0	5.0
#16641	5.3	5.3
#16643	3.2	4.1
#16644	5.3	5.3
#16646	2.6	2.5
#16647	3.0	4.7 (torn corpus callosum)
#16649	2.6	2.8
#16650	3.0	5.0
#16651	5.3	3.0
#16654	2.3	2.5
#16655	3.0	5.3
#16656	5.0	4.8
#16657	4.8	5.0
#16659	3.0	3.0

GROUP III

#16664	4.1	4.1
#16667	(No measurement)	
#16668	(Ventricle large – disrupt corpus callosum)	3.0
#16669	2.8	(No corpus callosum)
#16670	2.5	2.8
#16673	3.0	3.0
#16675	3.3 (no corpus callosum)	4.4
#16676	3.0	3.2 (torn)
#16678	3.1	3.1
#16679	4.6	4.6
#16683	4.4	4.4

GROUP IV

#16685	4.7	4.7
#16686	4.6	4.6
#16687	4.4	4.4
#16688	4.4	4.4
#16689	4.7	4.7
#16690	3.0	4.4
#16692	3.1	4.7
#16693	3.1	4.7
#16695	2.1	2.8
#16696	5.0	5.0
#16697	4.7	4.7
#16698	3.0	3.1
#16699	3.2	3.2
#16701	4.7	3.2
#16706	2.9	3.0
#16707	2.9	2.9

GROUP V

#16708	3.0	(torn)
#16709	3.0	3.0
#16711	3.0	3.0
#16712	2.9	3.0
#16713	3.0	3.0
#16715	3.0	3.1
#16717	4.7	4.7
#16718	2.6	2.5
#16719	2.9 (poor fix)	2.9
#16722	3.0	4.1
#16723	3.0	3.2
#16724	3.0	4.1
#16725	2.6 (torn corpus callosum)	2.6 (torn corpus callosum)
#16728	4.6	4.6
#16730	2.9 (poor fix)	2.9