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# RADIATION INDUCED MICRENCEPHALY IN GUINEA PIGS[1]

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## INTRODUCTION

Morphologic effects of ionizing radiation at doses of less than 100 mGy when delivered in utero have been reported infrequently and are difficult to verify. Roux et al (2) have observed radiation-induced resorption of conceptuses of rats at doses of about 50 mGy when delivered prior to implantation. Brent (3) has reported a similar observation at doses of about 100 mGy in mice. The organ system shown to be most affected by low doses of ionizing radiation is the central nervous system. Hicks and D'Amato (4) regularly observed alterations of the dendrites of cortical neurons of newborn rats irradiated in utero at doses of 100 mGy. The morphologic effects most frequently observed among those exposed in utero to atomic-bomb radiation at Hiroshima and Nagasaki were small head size and mental retardation (5-8). At conceptus doses less than 500 mGy effects were observed for those individuals who were at least two weeks and up to 15 weeks post-conception at the time of the detonations. The data are consistent with a linear no-threshold relationship between frequency of occurrence and dose but a threshold for this relationship at a conceptus dose in excess of 100 mGy is also consistent with the data. Cell depletion is commonly invoked as a mechanism for the effects of small head size and mental retardation (9) and is likely a predominant mechanism at high doses (e.g. >0.2 Gy). However, at low doses other mechanisms may be at work (10,11). In order to explain the correlation of radiation-induced mental retardation in those exposed to atomic-bomb radiation at Hiroshima and Nagasaki and the gestation at which the effect is induced, Otake and Schull (7,8) and Rakic (10) have suggested that the migration of neurons from their

production sites in the ventricles of the developing brain may be interrupted by nonspecific damage to the migrating pathways, preventing the neurons from localizing and connecting in their proper biological configuration. Congenital migration anomalies of neurons have been observed on magnetic resonance scans of individuals who are mentally retarded and who were exposed in utero to atomic-bomb radiation at doses in excess of 500 mGy (12).

One interesting finding in the data of Otake and Schull (7,8) is that there was no radiation-induced mental retardation in individuals exposed in utero prior to the 8th week post conception. This is different from temporal patterns observed in the data of Miller and Mulvihill (6), who demonstrated microcephaly in individuals exposed in utero at Hiroshima and Nagasaki prior to the 8th week post-conception as well as after. Therefore, if the finding of radiation-induced microcephaly in individuals exposed prior to 8 weeks post-conception is a valid finding, then it is likely that the mechanism for microcephaly alone in this group is different from the mechanism for radiation-induced mental retardation or microcephaly associated with mental retardation.

Wanner and Edwards (13) demonstrated a linear regression technique that is sensitive to detect radiation-induced micrencephaly in guinea pigs. Their data showed a consistently decreasing effect with decreasing radiation dose when radiation was delivered on the 21st day after conception in guinea pigs. This corresponds with a developmental stage in the human between the 5th and 6th weeks post-conception (14), when microcephaly is induced but not mental retardation. The data of Wanner and Edwards (13) also suggested an effect at doses less than 100 mGy was possible and that a threshold in the vicinity of 50 mGy might exist. An effect at less than 100 mGy during this

stage would suggest a mechanism different from those previously proposed. The purposes of the experiments in our study were to use the technique proposed by Wanner and Edwards (13) to a) provide independent information at the 100 mGy level, b) to determine whether micrencephaly might be induced at a dose on the order of 75 mGy, and c) to study potentially confounding factors such as sex, litter size, dam weight, and others. The data reported here was acquired in a blind study in which one investigator was responsible for irradiation of pregnant guinea pigs while three other investigators were responsible for data on the brain weight of guinea pig pups without prior knowledge of the irradiation history. A fourth investigator examined 31 brains or 5% of the total and was involved in the irradiation process. However, the conclusions of the study are the same when this data is excluded from the analysis. Our data demonstrate that irradiation at 75 mGy and 100 mGy has a statistically significant effect on brain weight independent of body weight, litter size, or sex. Additionally, brain weight relative to body weight and litter size is different for males and females and the housing conditions of dams had a small effect. Litter size may have a small influence, also. No dependence on dam weight or on the person performing the excisions was observed.

## MATERIALS AND METHODS

### *Data Acquisition*

Timed-pregnant Hartley albino guinea pigs were supplied by a vendor (Hilltop Lab Animals, Inc., Scottdale, PA) in twenty-five shipments of 8 to 12 guinea pigs per shipment. The dams were from the breeding stock of the vendor and were mated during postpartum estrus. Mating took place on a Wednesday and 13 days later the dams were shipped by same-day express to the

housing facility at our institution. To ensure that no irradiation of the dams took place during shipment, thermoluminescent dosimeters were included in the shipping crates with the dams. The TLD indicated an exposure of approximately 0.02 mGy per crate, consistent with the doses expected from air travel.

Upon arrival at our institution, animals were weighed to the nearest gram on a triple beam balance and assigned to cages. Guinea pigs from the first nine shipments were assigned cages with wire bottoms, and the remaining 16 shipments were assigned cages with solid bottoms and hardwood chips as bedding. Animals were maintained *ad libitum* on a diet of water and guinea pig chow (Purina Lab Chow, Purina Mills, Inc., Richmond, Indiana). Animals were observed daily and any unusual conditions or behavior were brought to the attention of a staff veterinarian. On the 21st day following conception, the animals were transferred in individual cages to a fluoroscopic x-ray room where they were divided into three groups: a control group, a 75-mGy irradiation group, and a 100-mGy irradiation group. Typically, four animals were assigned to the control group, four to the 75-mGy group, and two to the 100-mGy group.

Individually, each animal was constrained in a plexiglas cylinder with outside and inside diameters of 100 mm and 88 mm. One end of the cylinder was sealed with a conically shaped attachment that had a 25 mm opening at the end. The animal's head fit snugly into the cone with the snout positioned in the opening. To ensure uniform exposure across the abdomen of the animal, it was necessary to seal the other end with a device that could compress the hind end of the animal into the cylinder. This forced the abdomen to fully occupy the spaces of the cylinder.

A General Electric LU fluoroscopic system with MPX generator (General Electric Co., Medical Systems Division, Milwaukee, Wisconsin) was used to irradiate the dams. The x rays were generated at 110 kVp, the half-value layer was 3.85 mm of 1100-type aluminum and the tube current was about 4 mA. The image intensifier was positioned at 110 cm from the focal spot and a 1-mm thick lead plate was placed in front of the image intensifier to reduce the x-ray intensity at the input phosphor of the image intensifier. This ensured that the fluoroscopic image brightness at the output of the image intensifier was within the range of the pick up tube for the TV system. The animal was positioned laterally in the beam with the center of the abdomen approximately 75 cm from the focal spot. An ionization chamber was placed at the output of the x-ray tube to monitor the amount of radiation delivered in each case. The system was set up in an identical manner each time and was calibrated for radiation output prior to irradiations.

Irradiation of the full abdomen was verified on the fluoroscopic image. Calibration employed the use of the same constraint cylinder as was used for the animals, but an insert of water was used in the cylinder to simulate the abdomen of the guinea pig. The insert cylinder was constructed of polystyrene in such a way that an 0.6-$cm^3$ Farmer ionization chamber (Model 2505/3B, Nuclear Enterprises, Ltd., Brookshire, England) could be inserted into the cylinder to measure the internally administered dose. The Farmer chamber and electrometer (Model 602 dosimeter, Keithley Instruments, Inc., Cleveland, Ohio) interfaced to a digital multimeter were calibrated by the M.D. Anderson Accredited Dosimetry Calibration Laboratory, Houston, Texas and at beam qualities similar to those used in this test. Doses at the center of the abdomen and around the periphery of the abdomen were examined. The calibration involved normalizing the dose delivered

internally in the water to the monitor chamber reading. In order to achieve a relatively uniform dose distribution across the abdomen of the animal, the radiation was delivered in a parallel-opposed manner in which the x-ray tube was first positioned laterally on the left side of the animal with half the radiation dose delivered. The tube was then positioned on the other side of the animal and the remaining dose delivered. In this manner, the uniformity of the dose across the entire volume was measured and found to spatially vary by no more than ±6%. To verify the accuracy of the dosimetry, TLD were placed in the water insert and other TLD were placed in the cylinder along side one of the animals that was irradiated. The dose was determined at the Radiation Physics Dosimetry Laboratory of the M.D. Anderson Cancer Center, Houston, Texas. The doses as determined from the ionization chamber measurements and the TLD measurements agreed to within 10% of each other.

The total constraint time to irradiate animals in the 100-mGy group was approximately four minutes. In order to assure that the stress imposed by this procedure was the same for all animals, all animals were constrained in the device for four minutes and placed on the irradiation table simulating the same procedures in all cases. The x rays were not engaged for the control group and were engaged for the appropriate duration for animals in the 75-mGy and 100-mGy groups.

Following this procedure, the animals were weighed on a triple beam balance to the nearest gram, and marked with a color in order to identify them according to their dose group. The animals were then returned to the animal care facility until parturition.

Approximately 1 week prior to parturition, the animals were transferred to large solid bottom cages with a bedding of hardwood chips. The cages were checked daily for pups.

Within 24-hours after delivery, information was recorded regarding the total litter size and the number of live pups. The live pups were then transferred to the laboratory where they were weighed to the nearest gram on a triple beam balance (Ohaus, Florham Park, NJ) and sacrificed with an overdose of sodium pentabarbitol (approximately 0.3 ml). The brains were excised with the spinal cord severed at the atlantoepistrophic articulation. They were immediately weighed to within the nearest milligram on an electronic balance (Sartorius Instruments, Ltd., Surrey, England). The accuracy of the scales was checked with a set of calibration weights (Ohaus, Florham Park, NJ) and periodically verified for consistency over the course of the experiment. The electronic scales did not vary in reading by more than 1 milligram and the balance was consistent to within 1 gram. The person performing the cerebral excision and the sex of each animal were recorded in most cases. The excisions were performed by four individuals identified as A, B, C, and D.

## RESULTS

Characteristics of caging, dam weight and weight change, gestation time, litter size, pup weight, sex distribution of the pups, and examiners processing the pup brains were studied to determine their possible effects on the results. A summary of characteristics is given in Table 1. The data from pups of one animal were discarded because the animal developed an ear infection which was treated with antibiotics. Data from pups of another dam were discarded because the dam was too large (>1 kg) to fit into the constraint device.

*Caging and Weight Change*

The data demonstrate systematically different changes in dam weight during the 8-day interval between arrival at our facility and the time of irradiation as a function of shipment number (Table 2). This suggests that there was a systematic improvement in our ability to accommodate the adaptation of the guinea pigs to their new environment. This was directly correlated with the type of caging used for the animals upon arrival at our facility. Therefore, the analysis of the brain weight data for the pups was separated into two groups, those pups from the first nine shipments and those pups from the following 16 shipments, to determine if there was some systematic effect as a result of the accommodation to caging of the dams. This grouping was selected because animals in groups 10-25 were housed in identical solid bottom caging conditions and received similar upkeep. In groups 1-9 the caging was wire bottom and conditions varied. In addition, the data from the pups of another animal were discarded because of the dam's excessive weight loss (25%) in the first week.

*Gestation Time and Litter Size*

The data were also analyzed according to gestation time with an average gestation time (defined as the time from the day of mating to the day that live pups were observed in the cage) of 69.7 days with a standard deviation of 1.9 days. In one case, the gestation lasted for 83 days which fell well outside the norm and the data from pups of this animal were discarded.

There were no significant trends in gestation time with shipment number or dam weight (Table 2 and 3). No significant differences in average gestation time were observed for the dams in individual dose groups (Table 4). There was a significant correlation between gestation time and litter

size with smaller litters having an average gestation slightly longer than those of larger litters (Table 5), consistent with trends previously observed (15). The potential effects of this observation on brain weight were accounted for by using litter size as an independent variable in the regression analysis. The litter size distributions among dose groups was similar (Table 6) and the distribution of numbers of dams producing live pups in each dose group was similar to the distribution of numbers of dams assigned to each dose group (Table 7). These latter two observations do not suggest any differences in early mortality among the different dose groups. Dam weight at shipment was correlated with litter size (Table 3), consistent with previous findings (16). To examine the potential impact of dam weight on brain weight of the pups, each of the two shipment groups of dams was sub-divided into two groups of dams, those greater than 800 grams and those less than or equal to 800 grams at shipment.

*Pup Weight and Litter Size*

The account for the effect of body weights of pups on their brain weights and the potential effect of litter size on brain weights, multiple linear regression with the brain weight of the pups as the dependent variable and the pup weight and litter size as independent variables was performed using the SPSS/PC+ statistical package (SPSS Inc., Chicago, Illinois). Each of the three dose categories for each of the four combinations of shipment and dam weight were analyzed for a total of 12 regression analyses (Table 8). The dependence of brain weight on pup weight is obvious from the positive slope, b, in the analysis, but the dependence on litter size, if any, is subtle. For groups of less than 50 pups, the slope, c, associated with the litter size is sometimes positive, sometimes

negative, and frequently the standard deviation is larger than the slope, suggesting a true slope of nearly zero. For those study groups of more than 50 pups, there is a consistent negative correlation with litter size and the standard errors are smaller than the values, suggesting a slightly negative slope with litter size. Using the data of Table 8, the brain weight of each pup was adjusted to the equivalent value for a pup of weight of 92 grams and a litter size of four pups, which were chosen as representative norms for the entire study group. The formula used was:

$$B' = B + b(92-P) + c(4-L)$$

Where B' is the adjusted brain weight, B is the measured brain weight, b is the slope for pup weight, P is the measured pup weight, c is the slope for litter size, and L is the actual litter size. The data are summarized in Tables 8, 9, and 10.

The data of Table 8 demonstrate a significant difference ($p<0.001$) in mean adjusted brain weights as a function of dose. The adjusted brain weights of the controls is on the average 77 mg greater than those of the 75-mGy group with a 95% confidence interval of 57 to 99 mg. The adjusted brain weights of the controls were on the average 69 mg greater than those of the 100-mGy group with a 95% confidence interval of 39 to 99 mg. The 75-mGy and 100-mGy adjusted brain weights are not significantly different from each other. We attribute this lack of an observed dose dependence due to the small dif-ference in the doses applied in the two groups and due to the large variance in brain weight and the limited number of animals in the dose groups.

The data, when adjusted for pup weight and litter size, did not indicate any effect of dam weight on pup brain weight (Table 9). We conclude therefore that dam weight is not a significant confounding effect on brain

weight of pups at birth.

Also observed in the data of Table 8 is a trend in adjusted pup brain weight with shipment, summarized in Table 10. This effect was significant at the $p < 0.05$ level. The adjusted pup brain weight for dams received in the first nine shipments was on the average about 20 mg less than those of later shipments (Table 10). This effect was independent of dose category.

*Examiner and Sex of Pups*

To determine the effects of the sex of the pups and the effects of the person performing the excisions on the measured brain weights, the data from the different dam weights were pooled, since dam weight does not appear to be a significant factor influencing brain weight. The data for the adjusted brain weights for each shipment group, those less than 9-weeks and those greater than 9-weeks, were broken down by sex, by person performing the excision, and by dose group. In this analysis data of examiners C and D were excluded, even though they were consistent with results of A and B, because of previously discussed problems associated with small numbers of animals examined by these individuals and the potential for bias on the part of examiner D. The data are summarized in Tables 12 and 13. The sex of the animals had a significant effect on the relationship of brain weight with body weight and litter size, independent of examiner, dose group, or shipment group. Females, for a given body weight and litter size, demonstrate a brain weight approximately 44 mg less than those of males. This result was significant at the $p < 0.001$ level, suggesting that it is important to correct for sex distributions amongst examiners when trying to ascertain the effect of radiation dose on brain weight.

There was no statistically significant difference between the brain weights excised by examiner A and those excised by examiner B, suggesting that both examiners severed the spinal cord at roughly the same positions.

Again, the data of Table 13 demonstrate a very strong dependence of mean adjusted brain weight on radiation dose and a weaker effect with shipment category, suggesting that the previously discussed results are valid, even when data from examiners C and D are excluded from the pool.

*Inter- and Intralitter Variance*

Jensh and Brent (17) demonstrated that variance of fetal weight, placental weight and placental/fetal weight ratio among litters of rats is an important factor to consider in the design of teratological investigations in polytocous animals. The principal potential confounding effect of interlitter variance is bias if the numbers of litters are small. Tables 4 and 5 show the numbers of litters in each dose group and Table 6 shows that the distributions of litter sizes representing dose groups are the same. A concerted effort to avoid litter bias was made in the assignments of dams to each dose category by assuring that weight categories of dams were equally distributed among the dose groups. We have also separately analyzed the inter- and the intralitter variances for brain weight in our control populations, after correcting for body weight, litter size, sex and shipment category, to determine the magnitude of the independent variances and how they might affect the interpretation of the results of this study.

The variance in adjusted brain weight due to intralitter variations was substantially greater than variance due to interlitter variations, but interlitter variance was significant, confirming the applicability of the

findings of Jensh and Brent (17) to guinea pigs. The consistency of the results regarding the effects of dose for both sexes, for both shipment categories, for both categories of dam weight, and for each examiner do not suggest any bias due to interlitter variance that might invalidate the conclusions.

## CONCLUSIONS

A brain weight deficit of about 70 mg was induced at doses of approximately 75-mGy and a deficit of 60 mg was induced at 100 mGy. This confirms the effects projected and observed by Wanner and Edwards (13). Although the data do not demonstrate a clear dose-response relationship between the 75-mGy and 100-mGy groups, the data are statistically consistent with a dose-response effect because of the overlapping confidence intervals. The lack of a statistically significant observation is most likely related to the small difference in doses and the limited numbers of animals examined.

There are several factors that can influence the brain weight of guinea pig pups. Our data have demonstrated that caging and housing conditions can influence brain weight, and they must be taken into account in such an experiment. Uniform caging conditions are preferred. The sex of the animals is an important factor in determining brain weight and females have approximately 44 mg less brain weight per average body weight and letter size than males. Litter size may have a slight effect on the pup brain weight and should be taken into account for accurate analysis. Dam weight did not appear to have a significant effect.

The confirmation of a micrencephalic effect induced by x rays at doses of 75-mGy during this late embryonic stage of development is consistent with the findings of small head size induced in those exposed prior to the eighth

week of conception at Hiroshima. This implies a mechanism for micrencephaly different from those previously suggested and lends credence to a causal relation between radiation and small head size in humans at low doses as reported by Miller and Mulvihill (6).

(GUINEA_PIG)

# TABLE 1
## Characteristics of Study

| | |
|---|---|
| Total number of dams shipped by vendor: | 262 |
| Total number of dams delivering live full-term pups: | 174 |
| Number of litters excluded from the study as non-characteristic of study: **(one due to weight loss of dam, 1 due to infection, one due to extended gestation, and one because dam was too large to fit into constraint device)** | 4 |
| Average gestation ± standard deviation = | 69.7 ± 1.9 days |
| Average litter size per dam delivering live pups ± standard deviation = | 4.2 ± 1.8 |
| Average number of live pups per dam delivering live pups: | 3.5 |
| Number of live pups in study: **(290 males, 304 females, 9 with sex not recorded)** | 603 |
| Number of individuals performing excisions: | 4 |

# TABLE 2

## Average percent change in dam weight after first eight days at facility and gestation time versus shipment number

| | Shipment No. | | | | |
|---|---|---|---|---|---|
| | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 |
| Mean % weight change (standard deviation) | -1.3 (3.8) | -1.6[a] (4.7) | 0.2 (5.2) | 0.8 (5.3) | 1.8[a] (5.1) |
| Mean gestation time[b] in days (standard deviation) | 69.5 (2.1) | 69.8 (1.5) | 69.6[c] (3.5) | 70.1 (1.7) | 70.0 (1.6) |
| No. of dams in group | 28 | 38 | 31 | 33 | 41 |

a Difference in these two groups is significant ($p<0.05$).

b No two groups are significantly different ($p>0.05$).

c Standard deviation high due to one dam with gestation of 83 days. Excluding this value the mean gestation time is $69.1 \pm 2.5$ and footnote b still holds.

# TABLE 3
## Gestation time and litter size by dam weight

| | Dam weight at shipment | |
|---|---|---|
| | ≤800 g | >800 g |
| Litter size[a] (standard deviation) | 4.0[a] ± 1.7 | 4.7[a] ± 1.8 |
| Gestation time in days (standard deviation) | 69.8 ± 1.9 | 69.5 ± 1.9 |
| Number of dams | 120 | 50 |

a Difference in litter size between groups is significant ($p<0.05$)

# TABLE 4
## Gestation time by dose group

| | DOSE GROUP | | |
|---|---|---|---|
| | Controls | 75-mGy | 100-mGy |
| Gestation[a] in days (standard deviation) | 69.8 (1.9) | 69.5 (.9) | 70.0 (1.7) |
| Number of dams | 78 | 68 | 24 |

[a] No two groups are significantly different ($p>0.05$).

# TABLE 5
# Characteristics of Litters in Study

| | Number of pups in litter | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | TOTALS |
| No. of dams with live pups | 10 | 24 | 24 | 39 | 33 | 22 | 14 | 3 | 1 | 170 |
| Mean Gestation time[a] in days | 71.4 | 70.8 | 70.7 | 69.9 | 69.0 | 68.0 | 69.3 | 69.3[b] | 67.0 | 69.7 |
| (standard deviation) | (2.5) | (1.5) | (2.2) | (0.9) | (1.1) | (2.1) | (1.7) | (0.6) | (-) | (1.9) |
| No. of live pups | 10 | 46 | 67 | 145 | 142 | 106 | 70 | 13 | 4 | 603[c] |
| % Survival at birth | 100 | 96 | 93 | 93 | 86 | 80 | 71 | 54 | 44 | 84 |

[a] Groups 1, 2, and 3 are significantly different from groups 5 and 6; group 4 is significantly different from 6; and group 1 is significantly different from 7($p<0.05$).

[b] Dam with 83-day gestation excluded from this data.

[c] One pup in this group was excluded from brain weight analysis because brain weight was not recorded.

# TABLE 6
## Distribution of litter sizes among dose groups[a]

| | Dose Group | | |
|---|---|---|---|
| **Litter size** | **Controls** | **75-mGy** | **100-mGy** |
| Small (1 to 3 pups) | 23 (26.6) | 24 (23.2) | 11 (8.2) |
| Medium (4 to 6 pups) | 44 (43.1) | 40 (37.6) | 10 (13.3) |
| Large (7 to 9 pups) | 11 (8.3) | 4 (7.2) | 3 (2.5) |

[a] Values in parenthesis are expected values. There are no significant differences in the distributions of litter sizes with dose ($p>0.1$) based on a chi-squared contingency table analysis.

# TABLE 7

## Comparison of distributions of dams assigned to dose groups versus dams delivering live pups

| | Dose Group | | |
|---|---|---|---|
| | **Controls** | **75-mGy** | **100-mGy** |
| Assigned | 113 (43.3) | 103 (39.5) | 45 (17.2) |
| Dams delivering live pups | 78 (45.9) | 68 (40.0) | 24 (14.1) |

[a] Values in parenthesis are percents of total.

# TABLE 8

## Multiple linear regression of brain weight versus pup weight and litter size by dose group, shipment number, and dam weight at shipment

| Dose Category (mGy) | Shipment number Ship weight (g) Category | a±SE (g) | b±SE x10$^3$ | c±SE x10$^3$ | Mean[a] Litter Size ±SD | Mean Pupweight ±SD (g) | Mean brain weight ±SD (g) | Mean Adjusted[b] brain weight ±SD (g) | Number of Pups |
|---|---|---|---|---|---|---|---|---|---|
| 0 | ≤9/≤800 | 2.3031 ± 0.1208 | 5.851 ± 0.923 | -17.4 ± 9.9 | 4.9 ± 1.7 | 92.7 ± 18.6 | 2.7607 ± 0.1715 | 2.772 ± 0.113 | 68 |
| 0 | ≤9/>800 | 2.3219 ± 0.1980 | 5.502 ± 1.482 | -25.5 ± 15.9 | 5.6 ± 1.5 | 91.9 ± 15.9 | 2.6840 ± 0.1690 | 2.726 ± 0.127 | 42 |
| 0 | >9/≤800 | 2.3806 ± 0.1146 | 5.022 ± 0.887 | -21.7 ± 10.8 | 4.4 ± 1.4 | 91.7 ± 17.4 | 2.7443 ± 0.1749 | 2.756 ± 0.139 | 113 |
| 0 | >9/>800 | 2.1995 ± 0.1089 | 7.499 ± 0.871 | -23.2 ± 10.5 | 5.5 ± 1.6 | 85.5 ± 19.7 | 2.7132 ± 0.2037 | 2.797 ± 0.121 | 58 |
| 75 | ≤9/≤800 | 2.0683 ± 0.1870 | 6.168 ± 1.527 | +11.2 ± 15.6 | 5.0 ± 1.6 | 90.5 ± 16.1 | 2.6825 ± 0.1764 | 2.681 ± 0.150 | 48 |
| 75 | ≤9/>800 | 1.9546 ± 0.1553 | 6.930 ± 1.201 | +25.0 ± 14.2 | 4.6 ± 1.8 | 92.3 ± 20.8 | 2.6725 ± 0.1582 | 2.655 ± 0.103 | 25 |
| 75 | >9/≤800 | 2.0235 ± 0.1008 | 7.947 ± 0.726 | -14.3 ± 10.0 | 4.4 ± 1.4 | 92.9 ± 18.2 | 2.6978 ± 0.2100 | 2.697 ± 0.130 | 132 |
| 75 | >9/>800 | 2.2503 ± 0.1629 | 4.996 ± 1.006 | -6.4 ± 18.6 | 4.6 ± 1.1 | 89.9 ± 20.2 | 2.6697 ± 0.1404 | 2.684 ± 0.092 | 41 |
| 100 | ≤9/≤800 | 2.1192 ± 0.1970 | 6.676 ± 1.574 | -13.3 ± 14.7 | 5.2 ± 1.5 | 93.6 ± 14.1 | 2.6757 ± 0.1317 | 2.680 ± 0.080 | 20 |
| 100 | ≤9/>800 | 2.4329 ± 0.3054 | 1.407 ± 3.698 | +11.8 ± 38.1 | 5.7 ± 0.8 | 84.9 ± 7.8 | 2.6200 ± 0.0557 | 2.610 ± 0.053 | 7 |
| 100 | >9/≤800 | 2.3062 ± 0.1912 | 5.298 ± 1.426 | -24.7 ± 16.9 | 3.6 ± 1.4 | 106.5 ± 16.1 | 2.7801 ± 0.1499 | 2.695 ± 0.106 | 31 |
| 100 | >9/>800 | 2.6912 ± 0.3438 | 1.384 ± 2.762 | -17.5 ± 20.4 | 4.6 ± 2.7 | 95.2 ± 19.8 | 2.7428 ± 0.1427 | 2.749 ± 0.124 | 17 |

[a] The mean litter size here is generally larger than that of Table 1 because this table gives the mean litter size per pup and Table 1 is the mean litter size per dam.

[b] The mean adjusted brain weights of the control group are significantly different ($p<0.001$) from those of the 75-mGy and 100-mGy groups.

# TABLE 9

## Mean adjusted brain weights in grams by dam weight at shipment and dose groups

| Dam Weight (g) | Dose Group[a] | | |
|---|---|---|---|
| | Controls (n) | 75-mGy (n) | 100-mGy (n) |
| ≤800[b] | 2.762 (181) | 2.693 (180) | 2.689 (51) |
| >800[b] | 2.767 (100) | 2.673 (66) | 2.708 (24) |

[a] n = the number of pups in each category and the standard deviation is about 0.12 g.

[b] The two weight groups are not significantly different ($p>0.05$)

# TABLE 10

## Mean adjusted brain weights in grams by shipment number and dose group

| Shipment | Dose Group | | |
|---|---|---|---|
| | Controls ± SD (n) | 75-mGy ± SD (n) | 100 mGy ± SD (n) |
| ≤9[a] | 2.754 ± 0.120 (110) | 2.672 ± 0.1325 (73) | 2.662 ± 0.079 (27) |
| >9[a] | 2.770 ± 0.134 (171) | 2.694 ± 0.122 (173) | 2.714 ± 0.114 (48) |

[a] Values in two groups significantly differ ($p<0.05$) and mean difference is 22 mg with a 95% confidence range of about 2 mg to 42 mg.

# TABLE 11
# Distribution by Sex and Dose Groups for Examiners Performing Excisions

| Examiner | Total no. of pups processed | | | Males | | | Females | | |
|---|---|---|---|---|---|---|---|---|---|
| | Males | Females | Totals | Controls | 75-mGy Group | 100-mGy Group | Controls | 75-mGy Group | 100 mGy Group |
| A | 144 | 164 | 308 | 64 | 61 | 19 | 70 | 72 | 22 |
| B | 115 | 104 | 219 | 49 | 55 | 11 | 51 | 36 | 17 |
| C | 9 | 15 | 24 | 5 | 4 | 0 | 12 | 3 | 0 |
| D | 16 | 15 | 31 | 10 | 5 | 1 | 8 | 5 | 2 |
| Total | 284 | 298 | 582* | 128 | 125 | 31 | 141 | 116 | 41 |

*Twenty pups were excluded from this catagorization because either sex or examiner was not recorded.

# TABLE 12

## Mean adjusted brain weight in grams by dose, sex, and examiner

| Dose | Sex[a] | Examiner A[b] | Examiner B[b] |
|---|---|---|---|
| Control | M | 2.769 ± 0.107 (64) | 2.807 ± 0.105 (49) |
| Control | F | 2.717 ± 0.138 (70) | 2.743 ± 0.120 (51) |
| 75-mGy | M | 2.710 ± 0.109 (61) | 2.707 ± 0.142 (55) |
| 75-mGy | F | 2.663 ± 0.133 (72) | 2.673 ± 0.112 (36) |
| 100-mGy | M | 2.724 ± 0.112 (19) | 2.644 ± 0.084 (11) |
| 100-mGy | F | 2.691 ± 0.120 (22) | 2.716 ± 0.079 (17) |

a Difference between sexes are significant ($p<0.001$) with mean difference of 44 milligrams and a 95% confidence range of 24 to 64 mg.

# TABLE 13

Results of analysis of variance for examiners A and B taking into account the effects of pup weight, litter size, sex of the pups, shipment category of the dams, and dose. Provided are the mean adjusted brain weights in grams for each item and the number of pups involved. The standard deviation in all cases is about 0.12 grams.

| | Controls | 75-mGy | 100-mGy |
|---|---|---|---|
| Dose effect[a] | 2.757 (234) | 2.685 (224) | 2.703 (69) |
| | **Males** | **Females** | |
| Sex differences[a] | 2.742 (259) | 2.698 (268) | |
| | ≤9 | >9 | |
| Shipment differences[b] | 2.698 (181) | 2.731 (346) | |
| | **A** | **B** | |
| Examiner differences[c] | 2.714 (308) | 2.728 (219) | |

a) $p<0.001$ for difference between controls and each dose group

b) $p<0.01$

c) $p>0.19$

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