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PCB Effects on Development of Behavior in Rats

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HONORS PROJECT

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Abstract

PCBs were originally used as industrial chemicals in many devices and applications, including electrical capacitors and transformers, lubricants, and sealants in buildings. PCBs have been investigated and their use has been slowly phased out starting in the U.S. in the 1970s. A worldwide ban was put on the use and manufacturing of PCB in 2001, however they remain in the food chain because of their lipophilic properties and their long half-life. Major sources of PCB include wild-life, fish, and other seafood. Individuals exposed to PCB during development can experience negative effects on neurobiological, cognitive, and behavior functioning in both humans and non-humans. Additional studies have shown that PCB can lead to changes in the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4). The goal of this research project was to determine if any one week of PCB exposure during the three-week rat pregnancy has a more detrimental impact on development than any other. Sprague-Dawley rats were exposed to PCB in their diet during gestation and lactation at different one week intervals. Multiple tests were done to investigate behavioral changes as well as thyroid hormone concentrations in the blood. After analyzing the data it appears that exposure to PCB may have a more negative effect on development during earlier stages of gestation.

Introduction

After years of experimenting and exposure it is now understood that polychlorinated biphenyl (PCB) has negative effects on the development and growth of many organisms. PCBs are organic compounds that can bioaccumulate because of their chemical stability (Johansen et al., 2011). PCBs were originally used as industrial chemicals in many devices and applications, including electrical capacitors and transformers, lubricants, and sealants in buildings (Selvakumar et al., 2013; Lilienthal et al., 2014). The dangers of PCBs have been investigated and their use has been slowly phased out starting in U.S. in the 1970s. A worldwide ban was put

on the use and manufacturing of PCB in 2001, however they remain in the food chain because of their lipophilic properties and their long half-life (Lilienthal et al., 2014). According to Johansen et al., (2011) PCBs are composed of two phenyl rings. Chlorine atoms can substitute at the ortho, meta, and para positions on the phenyl rings giving a possibility of 209 different PCB congeners. Based on where the chlorine substituents bind to the PCB structure and the number of chlorines, determines the chemical and toxic properties of a PCB congener. Some of the congeners that humans are most exposed to are the ortho-substituted non-planar PCBs 118, 138, 153, and 180, and also the less chlorinated di-ortho-substituted non-planar congener PCB 52. Unfortunately humans are exposed to PCB through trans-placental transfer, breast milk after birth, and during adolescence and adulthood because of a diet that frequently includes organisms that contain PCB (Johansenet al., 2011). Major sources of PCB include wild-life, fish, and other seafood (Lilienthal et al., 2014).

According to the research, individuals exposed to PCB during development can experience negative effects on neurobiological, cognitive, and behavior functioning in both humans and non-humans (Johansen et al., 2011). Hyperactivity is one element that an organism exposed to PBC usually experiences, (Cauli et al., 2013; Eubig et al., 2010). The studies that have been done on animals indicate that there are many factors that may alter the effects of PCB exposure which include; PCB dose, age when exposed, age when effects are investigated, sex, and species. It has also been noted through research that the tests done on animals generally show the same results on humans indicating that PCB exposure affects learning and memory, activity level, and cognitive functions (Johansen et al., 2011).

Additional studies have shown that PCB can lead to changes in the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4). The thyroid gland secrets T_4 in greater concentration,

but T_3 is more biologically activate. T_3 concentrations in circulation are not as affected by PCB as are those concentrations of circulation of T_4, but PCB lowers the amount of T_4 in the body, a condition known as hypothyroidism (Fritsche et al., 2005). These thyroid hormones are transported in the blood (circulation) and are involved in development, growth, body temperature, metabolic rate (Abdelouahab et al., 2013; Ren et al., 2013). Research has demonstrated that during pregnancy, if a mother has the condition known as hypothyroidism then it is possible for there to be negative effects on the cognitive development of the child. (Roman et al., 2013). These authors explained that short lasting and even slight deficiencies in T_4 hormone during the early stages of pregnancy can result in lifelong abnormalities in cortical development of the progeny. These abnormalities could be a cause of faulty neuronal migration in hippocampus and somatosensory cortex (Roman et al., 2013). The authors also noted that thyroid deficiency in rats for as few as three days resulted in similar findings. A lack of thyroid hormone also causes problems in human development. Through experimental results Roman (2007) believes that maternal (free T_4) deficiency in the early stages of pregnancy could be influential to autistic analysis because hypothyroxinemia may disrupt critical stages of neuronal migration in the brain and cerebellum. Recently it has been discovered that infants born with abnormally low fT_4 had an increased risk of autism (Roman et al., 2013).

It is believed that deficiencies in thyroid hormone can cause negative effects on development. Current research shows that there is a possible connection between PCB and thyroid hormone concentrations. Thyroid hormones play a critical role in the growth of many organs, especially the brain. PCBs interact with the thyroid pathway and may disturb neurodevelopment (Abdelouahab et al., 2013). During some of the first few weeks of pregnancy the embryo in the mother is nearly completely dependent on maternal thyroid hormone supply.

PCBs are endocrine disruptors that interact with the thyroid pathway (Abdelouahab et al., 2013). The effect of PCB on thyroid hormone could be a cause of developmental problem.

The goal of this research project was to determine if any one week of PCB exposure during the three-week rat pregnancy has a more detrimental impact on development than any other. Even though the research today shows a great deal of the negative impacts of PCB on development fewer studies have been done to show when PCB effects development the most. The end result of this research would be to focus one narrow window either during pregnancy or after birth in rats to determine when PCB exposure may have a more profound negative effect on the offspring. If any particular week of exposure to PCB results in a much greater effect on development than other weeks, then the information could be crucial to human medicine. We could learn when in the early life of organisms they are the most vulnerable to PCB exposure. Also, and most importantly, if a particular week of rat development is more harmed by PCB exposure we should certainly have human mothers avoid eating food likely to contain PCB, or being in areas with high concentrations of PCB during the time of pregnancy that correlates to when rats are the most effected. Developing humans exposed to PCB have been known to have increased chances of suffering from autism along with other developmental disorders (Kimura-Kuroda et al., 2007).

Materials and Methods

The entire experiment for the effect of PCB exposure is part of a much larger research project in Dr. Meserve's lab. For this university and departmental honors project I have selected a portion of the project for own report and presentation. Other aspects of the research have been completed by other undergraduate and graduate students in Dr. Meserve's lab. All procedures used in the present study were approved by the BGSU Institutional Animal Care and Use Committee (IACUC protocol #13-3003).We have all put in the individual time and effort to

complete the background research, animal care, data collection, and analysis for our own projects that will contribute to the overall research. For this project I will test the effect week of PCB exposure on the thyroid hormone concentration (physiology) and on open field activity (behavior). The procedures for these tests will be described along with basic care of the animals.

Animals

The rats used in the experiment are from the species Rattus norvegicus and the strain Sprague-Dawley. The adult rats weighed between 200 and 250g and were obtained from Harlan-Sprague Dawley, Indianapolis, IN. Upon arrival at BGSU the females were housed in pairs for one week to help them acclimate to the new surroundings. In the experiment we used eighteen female rats and four male rats. Litter size varied for each mother and will be noted later.

Animal Care and Housing

The housing and experimental procedures were performed in the animal research facilities in the life sciences building on Bowling Green State University's main campus. All rats were on a 12 hr. light/ 12 hr. dark cycle and the room was kept at a constant temperature of 23°C. After the week of acclimation two females were housed with one male to increase the chances of mating. Mating was determined by a sperm-positive vaginal washing. After collecting the sample from the vagina it was examined microscopically to detect the presence of sperm. If sperm was present the female was determined to be pregnant (gestational day 0 or GD0) and randomly assigned to a PCB exposure group. All rats were supplied with unlimited food and water throughout the experiment. After successful mating each female was housed individually for the remainder of the pregnancy and experimental procedures. Upon being housed individually all female rats were given daily 100 g of powdered diet. For the assigned week of exposure the powdered diet contained PCB and the other weeks were the plain powdered diet.

The normal food contained powered Lab Chow and the PCB diet contained powdered Lab Chow mixed with a total of 25 mg of equal parts of PCB 47 and 77 per kg of diet.

Each morning after mating the rats were weighed and returned to their individual cages, and the food weight was recorded. Food weight and amount of food eaten were recorded in grams and then the food was refilled to 100 g of the appropriate diet for each rat. These procedures were repeated each day until the birth of the pups. On the morning of the pup's birth the mother and pups were left alone and the same food measurements were taken. On each of the succeeding days until the pups were twenty-two days old similar measurements were recorded. Each morning the mother was weighed, pups were weighed as a group and individually. The food was also weighed; the amount eaten was calculated and replenished to 100 g of the appropriate diet. After the twenty-two days the experimental procedures were completed and the mother rats are housed individually and returned to the regular Lab Chow diet (non-powdered).

Experimental Groups

The research on the control groups for the large scale PCB exposure experiment were completed previously and the data results were recorded. These data were used in this as a matter of comparison to the effects of PCB exposure. All control data for thyroid hormone are presented as an average from the previous analyses. For the experimental groups eighteen female rats were mated and randomly assigned to one of the four PCB exposure weeks. These weeks included the first week of gestation, the second week of gestation, the third week of gestation, and the first week after birth (post-natal week one) beginning on postnatal day 0 (PND0). For the entire experiment each female rat lasted for six weeks. All groups received the PCB diet a total of seven days. Gestation group 1 had four females, gestation week 2 had four females, gestation week 3 had four females and lactation week 1 had five females. Within these groups some of the females were sperm positive, gained weight initially, and then had no pups. If these females

ingested PCB it was noted. It is important to note that rat J was excluded from the experiment. After being pregnant and briefly gaining weight it became apart that rat J lost weight and then maintained a steady healthy weight. Rat J was never exposed to any PCB and as a result the PCB could not be to blame for the termination of the pregnancy and therefore rat J was excluded from the results.

Testing Procedures

Tests of thyroid status and behavior were completed on PND 3, 7, 14, 21, and 22. On the initial day of testing, PND 3, the rat litter was culled to eight individual pups, 4 males and 4 females when possible. The pups not included in the eight were tested on PND 3 and blood samples were collected from all of these pups. On each proceeding landmark day a male and a female from the litter were tested. After each test pups were euthanized and a blood sample was collected for hormone concentration experiments. *Figure 1* below shows more detail about what tests were performed on each particular postnatal testing day. The tests being presented for this research are a test for thyroid hormone concentration in the blood and also a behavioral test using an open field experiment (PND21).

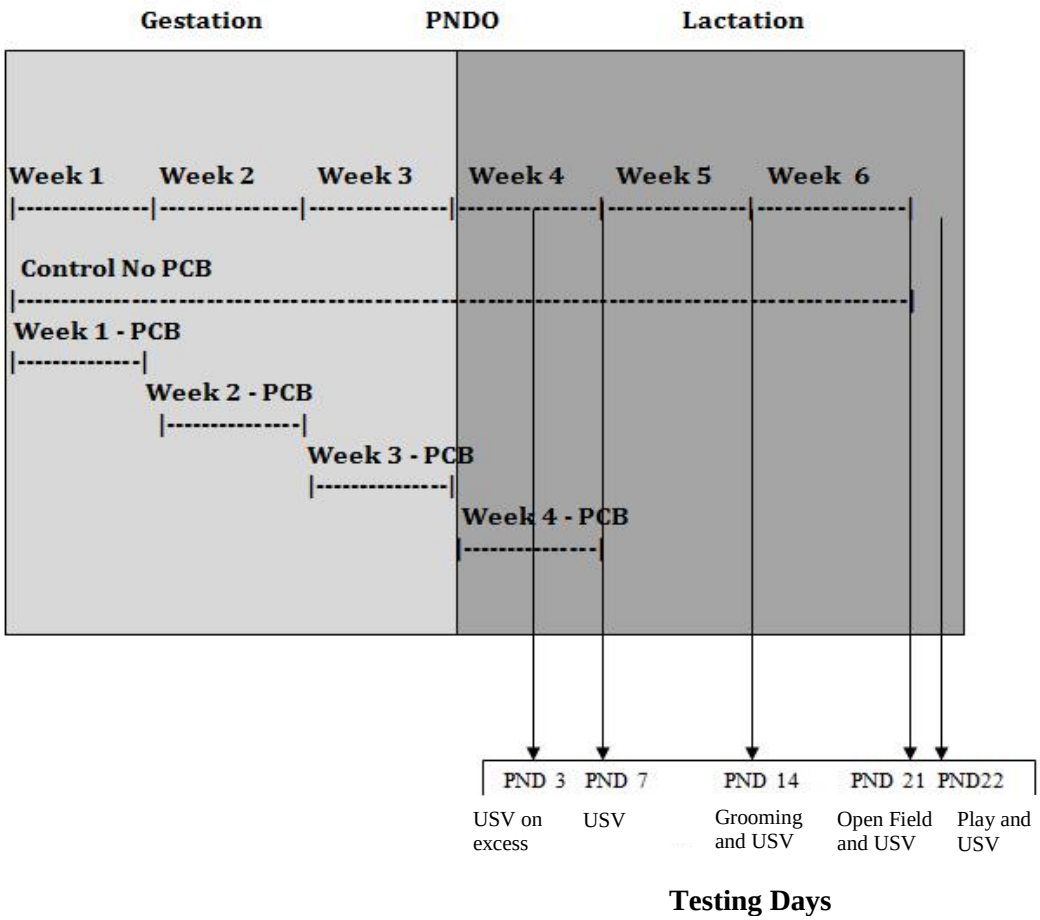


Figure 1. Experimental Overview. Detailed overview of the different PCB exposure groups along with the corresponding week of gestation or lactation. The figure also shows the different testing days as they fit into the duration of the experiment.

The open field test was done on postnatal day 21. As noted two rats from each litter were used for this test, typically one male and one female. One rat pup was tested at a time in the open field. The pup was placed in the center of a 40 x 50 x 20 cm open field with nine squares of equal dimensions drawn on the bottom. The squares measured 13.3 x 16.6 cm and were clearly marked so that they were easily viewable. Pups were placed in the middle square and remained in the field for ten minutes. Activity was recorded with a video camera with the investigator out of the room. Three different movements were recorded during each test. Horizontal moments were counted as the number of total lines the pup crossed throughout the ten minutes. Vertical

movements were counted as number of rears, or when the rat stood on its hind legs either with or without the assistance of the sides of the container. The number of times the pup traveled through the center square was also noted.

Euthanasia, Blood Collection, and TH Determination

After each test the pups were euthanized by intraperitoneal (IP) injection using a pentobarbital based solution (100 mg/kg). A deep stage of anesthesia was determined by the rat not responding to a toe pinch. Once in deep anesthesia the rats were decapitated and the blood was collected. The blood sample was centrifuged for twenty minutes and then the serum was collected and frozen for thyroid hormone determination. After serum samples were collected from all experimental pups an enzyme immunoassay test kit (MP Biomedicals, Solon, OH) was used to quantitatively determine the concentration of total triiodothyronine (T_3) and thyroxine (T_4) in the serum for each pup. The blood test done that show the lowest amount of T4 could determine which week of exposure to PCB had the most negative effect on thyroid status and potentially development, when compared to the control group because it has been shown in research that low thyroid hormone can negatively alter development of an organism.

Statistical Analysis

An ANOVA was performed on the results obtained for the open field behavioral test as well as the T_4 and T_3 concentration results. The results were analyzed using an ANOVA: single factor and anything that was less that 5% difference was determined to be statistically significant.

Results

Litter Sizes

Below are the average litter sizes for each PCB test group.

Gestation Week 1	3.75	Gestation Week 3	11.5
Gestation Week 2	11	Lactation Week 1	14

*Average litter size for rats in general is around 12

**Litter A was born with 13 pups, but the mother began eating her babies around PND 7 and as a result some testing data for rat A was unavailable because there were not enough pups.

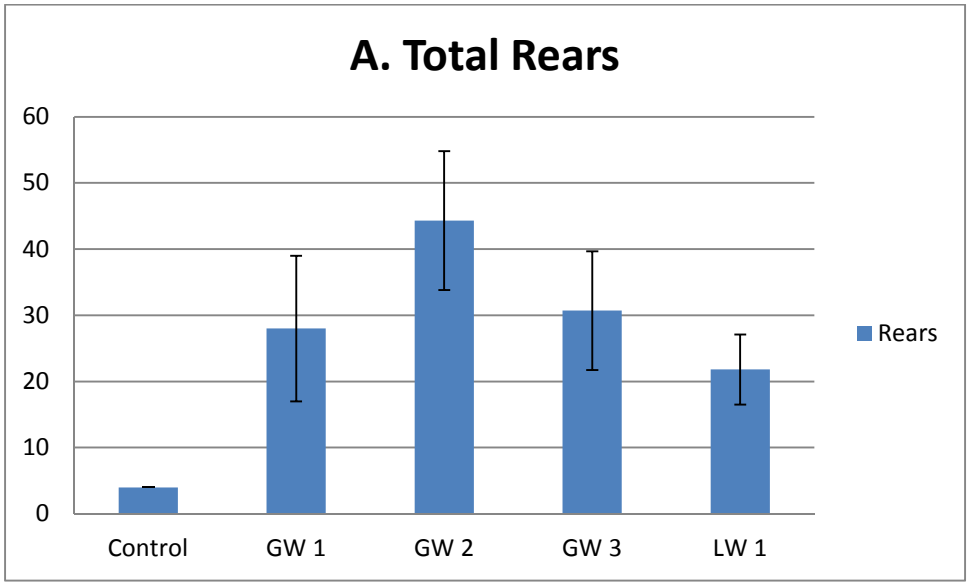
Given that the typical rat litter averages 12 pups, PCB exposure during gestation week 1 resulted in a much smaller average than all other PCB exposure weeks. Gestation week 2, 3 and lactation week 1 seem to be right around the average. In addition to gestation week 1 having the lowest average number of pups born, this PCB exposure group also had three litters that resulted in zero pups. The moms were pregnant at first, gained weight, and produced no offspring. Gestation week 2 had two litters that had these same results and gestation week 3 had one mother that produced no offspring. Lactation week 1 pregnant rats produced offspring in all litters. The mean for gestation week 1 may be slightly misleading as a result of the three litters not being produced. The one litter that did produce offspring had a normal amount (15 pups), but the average was severely hindered by the other three litters.

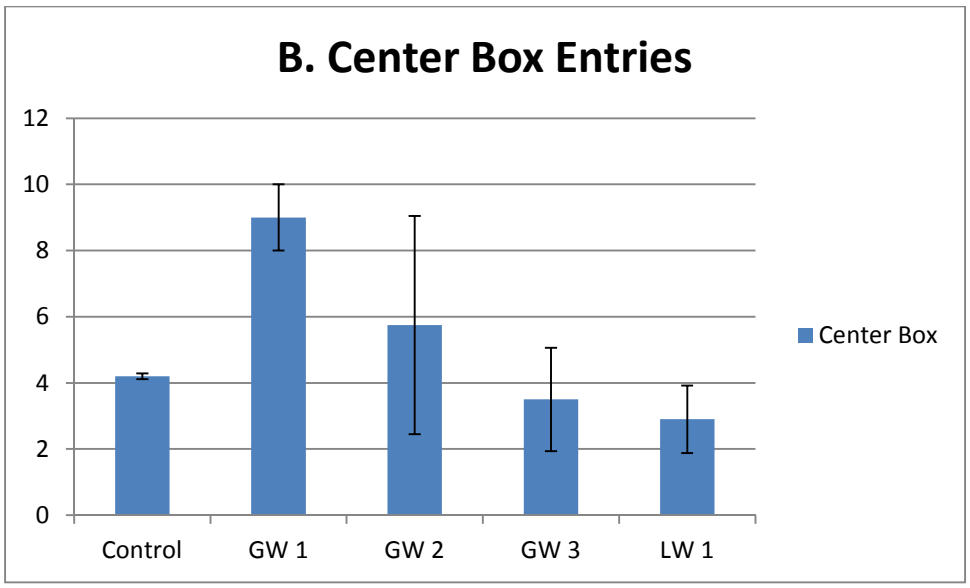
Open Field Behavioral Test

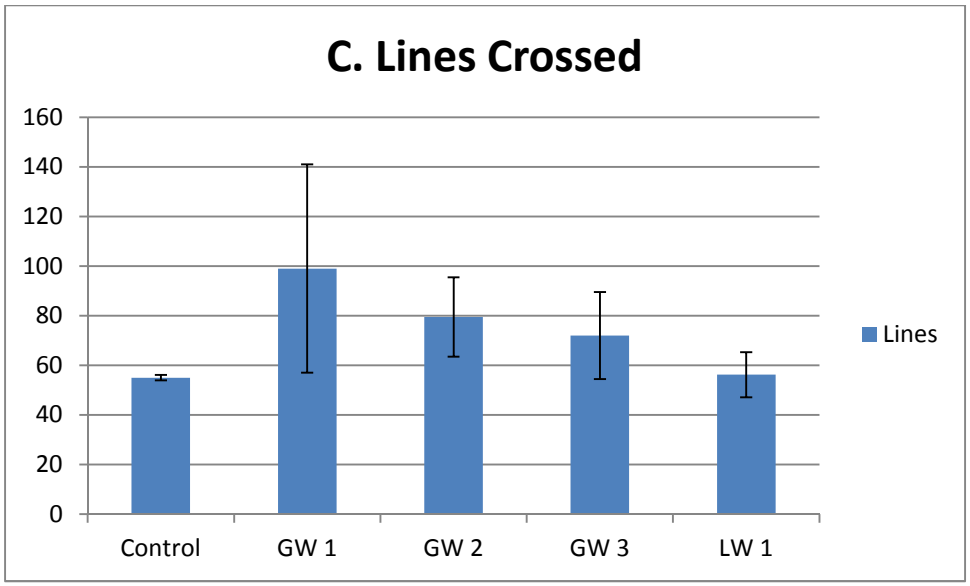
Table 1. Open Field Test.

		Open Field Test Data				
PCB EW	Animal ID	Sex	Rears	Center Box	Lines	Total
No PCB	Control	Average	**4±0 (40)**	**4.2±.60 (40)**	**55±7.07 (40)**	**63.2±7.67**
GW 1	N	M	-	-	-	-
		F	-	-	-	-
	O	M	39	10	120	169
		F	17	8	78	103
	P	M	-	-	-	-
		F	-	-	-	-
	Q	M	-	-	-	-
		F	-	-	-	-
		Average	**28.0±15.6**	**9.00±1.41**	**99.0±29.7**	**136.±46.7**
GW 2	A	M	-	-	-	-
		F	-	-	-	-
	C	M	73	15	111	199
		F	34	2	51	87
	D	M	27	0	53	80
		F	43	6	103	152
	G	M	-	-	-	-
		F	-	-	-	-
		Average	**44.3±20.3**	**5.75±6.65**	**79.5±31.9**	**130.±56.5**
GW 3	B	M	19	0	46	65
		F	11	0	15	26
	E	M	63	7	134	204
		F	54	9	105	168
	F	M	-	-	-	-
		F	-	-	-	-
	L	M	17	1	52	70
		F	20	4	80	104
		Average	**30.7±22.0**	**3.50±3.83**	**72±43.2**	**106±67.6**
LW 1	H	M	14	1	54	69
		F	32	7	75	114
	I	M	18	3	45	66
		F	11	0	34	45
	K	M	32	2	60	94
		F	16	2	75	93
	M	M	63	10	122	195
		F	9	1	42	52
	R	M	12	3	30	45
		F	11	0	25	36
		Average	**21.8±16.7**	**2.9±3.21**	**56.2±28.9**	**80.9±47.4**

All numbers in *Table 1* represent a tally mark when a particular action was observed as indicated by the testing categories. Each individual result for each litter was used to determine a mean for each PCB exposure week per category. A "-" mark represents no data collected. GW stands for gestation week and LW stands for lactation week. The ± values in the table are a calculation of standard deviation.







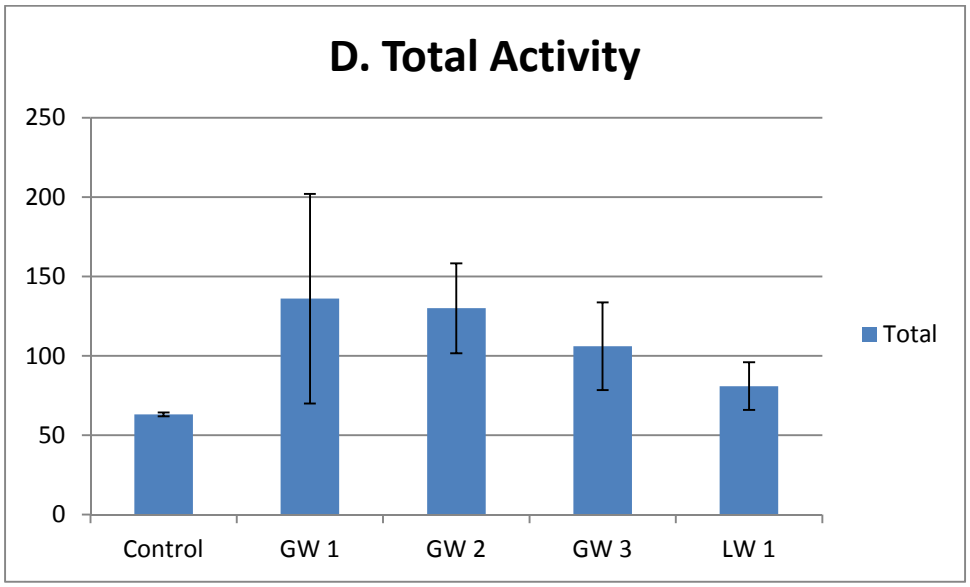


Figure 3. Open Field Results. Total Rears *(3A)* refers to the number of times an animal stood on its hind legs per 10 minutes. Center box entries *(3B)* refers to the number of times an animal walked through the center square on the grid per 10 minutes. Total lines crossed *(3C)* refers to the number of times an animal crossed any line inside the grid per 10 minutes. The total activity *(3D)* refers to the sum of the three previous behavioral categories. The vertical axis in each of the

graphs depicts the numerical value that each behavior was observed in the 10 minute testing sessions. Bars represent the means of values in Table 1, ± standard error of the mean (SEM).

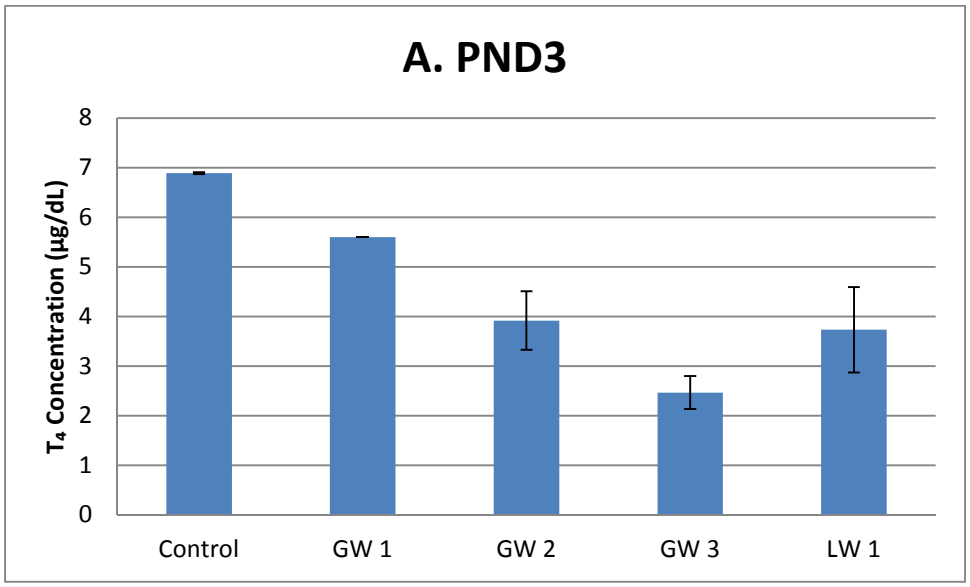
The relatively small number of animals examined did not allow ascription of statistical significant by ANOVA ($P<0.05$). However, observation of mean differences revealed a trend. Taken together these results depict the amount of activity performed by the pups at different PCB exposure weeks *(Figure 3*). There is a general trend toward hyperactivity in pups exposed to PCB, with those earlier in gestation being more hyperactive than those exposed later. The trend seems to be that the earlier in gestation that the pups were exposed to PCB the more hyperactive they seem to be. The most activity was revealed by the number of lines crossed followed by number of rears, then center box entries. Total activity *(Figure 3D)* also reflects this trend. After doing an analysis of variance on all of the results, all of the p values were greater than 5%. This indicates that there was no significance between the data points. It is believed that the results obtained begin to show a trend of what most research is currently showing in regards to PCB exposure. A possible reason as to why the analysis of variance did not show significant differences may have been because the sample sizes were too small. The trend of the graphs depicts that all pups exposed to PCB have a much greater recorded activity level in all of the categories tested. It also appears that the earlier in gestation that a pup is exposed to PCB the greater the amount of activity in all categories.

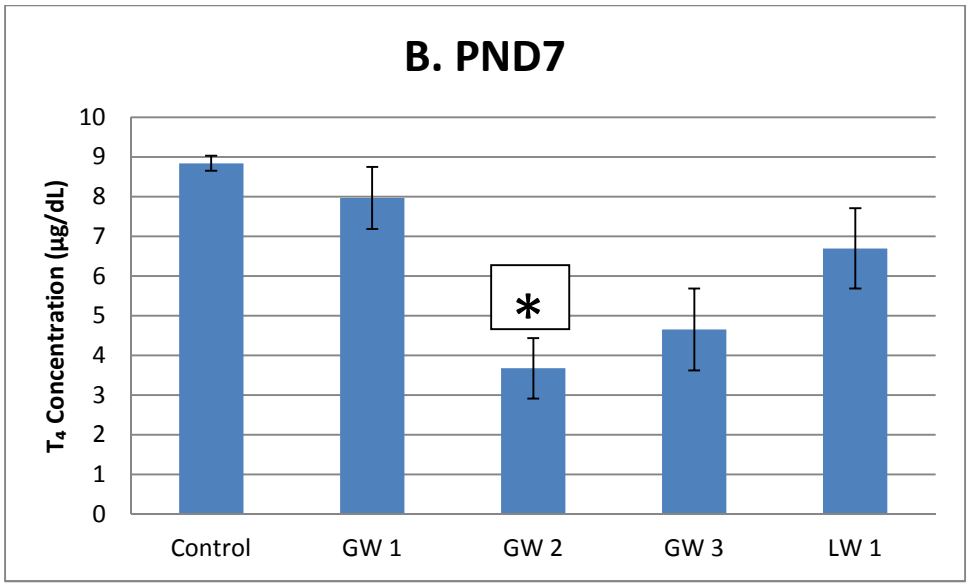
Thyroid Concentrations

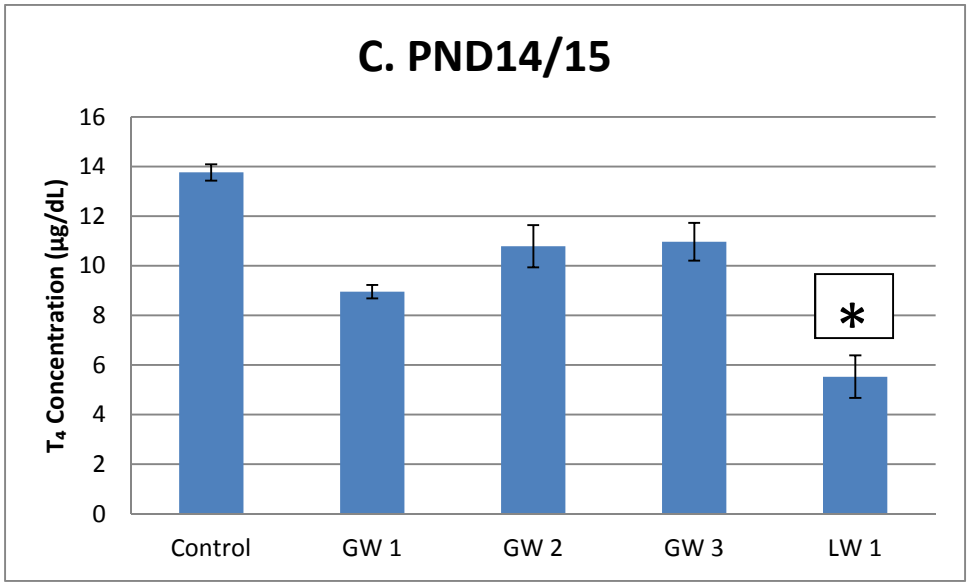
Table 2. Total Thyroxine (T_4) Concentration

		Total Thyroxine (T_4) Concentration (µg/dL)						
	Animal ID		PND 3 (17)	PND7 (6)	PND15 (6)	PND21 (6)		
No PCB	Control	Average	**6.89±0.07**	**8.84±0.47**	**13.76±0.82**	**9.01±0.77**		
	Animal ID	Sex	PND3	PND7	PND14	PND21	PND22	PND21/22 AV
GW 1	N	M	-	-	-	-	-	
		F	-	-	-	-	-	
	O	M	5.598	7.181	8.682	5.515	4.645	
		F	^	8.750	9.219	3.629	4.935	
	P	M	-	-	-	-	-	
		F	-	-	-	-	-	
	Q	M	-	-	-	-	-	
		F	-	-	-	-	-	
		Average	**5.598**	**7.966±1.11**	**8.951±0.38**	**4.572±1.33**	**4.79±0.21**	**4.681±0.79**
GW 2	A	M	3.308	2.330	10.522	-	-	
		F	^	2.494	^	-	-	
	C	M	2.298	6.533	11.253	13.063	9.043	
		F	^	^	11.898	8.382	11.658	
	D	M	3.471	3.392	7.689	-	9.913	
		F	^	3.619	12.571	-	5.739	
	G	M	4.994	-	-	-	-	
		F	5.518	-	-	-	-	
		Average	**3.918±1.31**	**3.674±1.69**	**10.787±1.89**	**10.723±3.31**	**9.088±2.48**	**9.633±2.57**
GW 3	B	M	1.871	2.767	8.642	9.875	7.996	
		F	^	3.343	12.064	10.888	9.166	
	E	M	3.378	5.189	9.634	9.905	7.239	
		F	^	^	^	14.544	10.217	
	F	M	2.113	-	-	-	-	
		F	^	-	-	-	-	
	L	M	2.504	7.312	12.401	13.440	8.002	
		F	^	^	12.091	11.175	7.514	
		Average	**2.467±0.66**	**4.653±2.05**	**10.966±1.71**	**11.638±1.93**	**8.356±1.13**	**9.997±2.28**
LW 1	H	M	5.200	6.477	6.457	5.684	10.707	
		F	^	^	6.048	3.845	10.99	
	I	M	7.133	5.327	5.339	8.531	7.315	
		F	^	^	4.357	9.043	7.792	
	K	M	2.045	10.628	10.431	9.671	7.315	
		F	^	^	9.561	8.204	7.822	
	M	M	3.873	5.703	4.701	2.218	2.661	
		F	^	5.338	3.038	4.621	2.656	
	R	M	1.975	-	3.250	4.383	2.945	
		F	2.174	-	2.076	2.971	2.369	
		Average	**3.733±2.10**	**6.695±2.25**	**5.526±2.73**	**5.917±2.72**	**6.257±3.35**	**6.087±2.98**

The numbers *(Table 2)* in () next to the control's data points signify the number of pups tested. A "-" mark signifies that no data was collected for that particular set of pups. A ^ mark is used when the male and female serum was pooled together for a particular litter. Serum was pooled on earlier testing days when sample size per pup was insignificant for the thyroid hormone concentration test. GW, gestation week LW, lactation week. The ± values in the table are a calculation of standard deviation. PND 21 and 22 were averaged together so that they could be compared to the controls which were tested on PND 21.




B. PND7
T4 Concentration (µg/dL)
10
9
8
7
6
5
4
3
2
1
0
*
Control
GW 1
GW 2
GW 3
LW 1


C. PND14/15
T4 Concentration (µg/dL)
16
14
12
10
8
6
4
2
0
*
Control
GW 1
GW 2
GW 3
LW 1

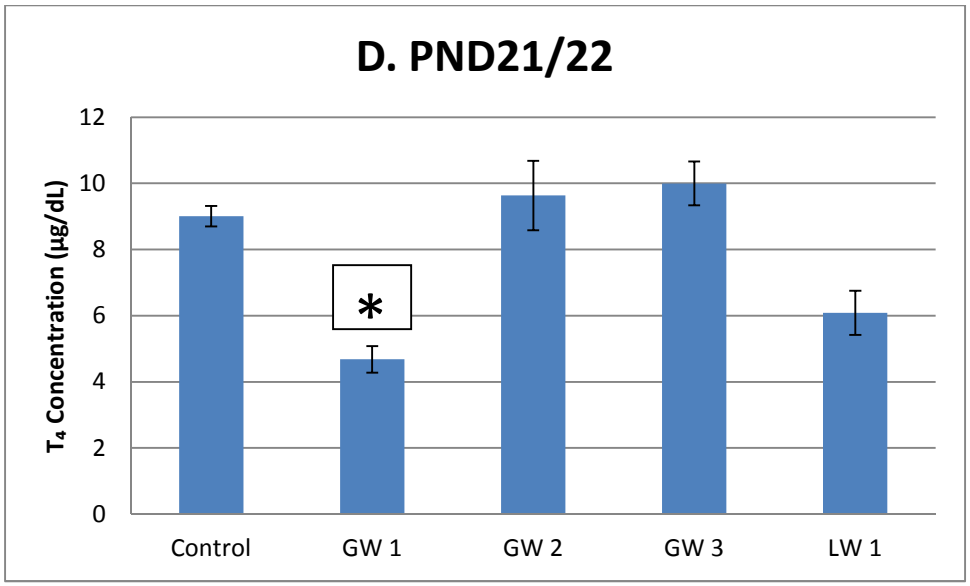


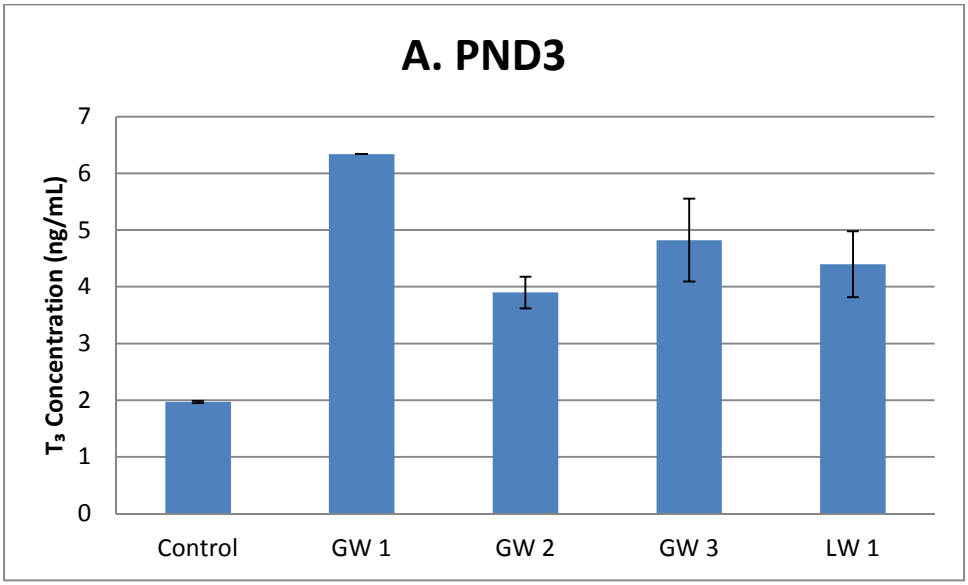
Figure 4. T_4 concentration at PND 3*(4A)*, 7*(4B)*, 14/15*(4C)*, and 21/22*(4D)*. Values used for these graphs were obtained from the means displayed in *Table 2*. GW, gestation week LW, lactation week. The "*" marks on the graphs represent the PCB exposure groups that were significantly different than the control groups. Bars represent the concentration of T_4 in circulation per PCB exposure group. The error bars are a calculation of standard error.

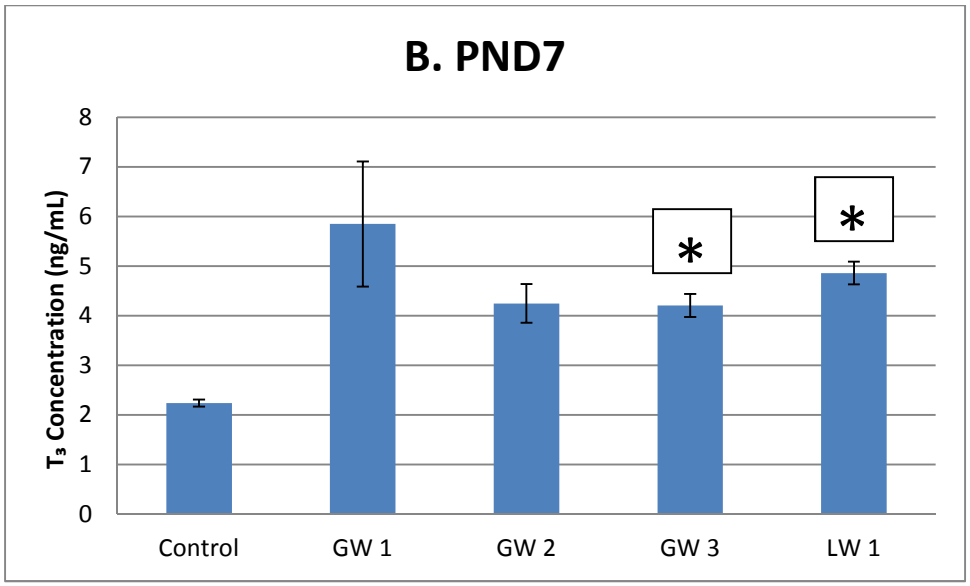
Thus T_4 concentrations did not reveal as clear a trend as did open field behavior. For the most part, the T_4 concentration in the serum of the pups was depressed by PCB when compared to that of the controls. One of the more noticeable trends is that as the testing days increase from PND 3 to PND 21/22, the T_4 hormone concentration in PCB treated rats raised to a level of that in the controls. The ANOVA performed on T_4 concentrations revealed a main effect of PCB treatment on PND 7 *(Figure 4B)*, 14/15 *(Figure 4C)*, and 21/22 *(Figure 4D)*. ($p<0.05$). The influence of PCB exposure week varied with pup age with GW2 having the greatest depressive effect on T_4 at PND 7 *(Figure 4B)*, LW1 at PND 14/15 *(Figure 4C)*, and GW1 at PND 21/22 *(Figure 4D)*.

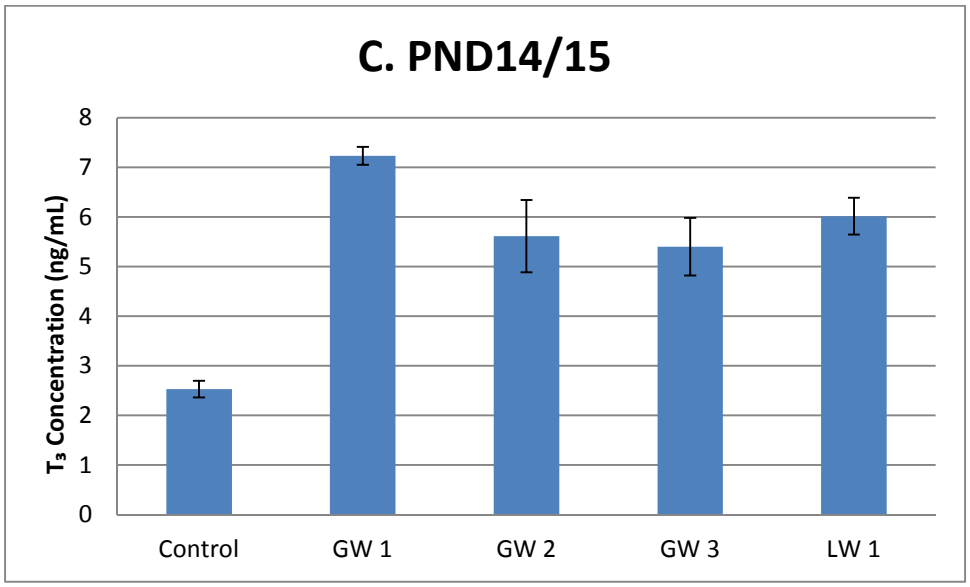
Table 3. Total Triiodothyronine (T_3) Concentration

		Total Triiodothyronine (T_3) Concentration (ng/mL)						
	Animal ID		PND3 (17)	PND7 (6)	PND15 (6)	PND21 (6)		
No PCB	Control	Average	**1.97±0.08**	**2.24±0.17**	**2.53±0.42**	**3.99±0.33**		
	Animal ID	Sex	PND3	PND7	PND14	PND21	PND22	PND21/22 AV
GW 1	N	M	-	-	-	-	-	
		F	-	-	-	-	-	
	O	M	6.339	7.111	7.053	8.355	5.260	
		F	^	4.587	7.406	6.557	6.781	
	P	M	-	-	-	-	-	
		F	-	-	-	-	-	
	Q	M	-	-	-	-	-	
		F	-	-	-	-	-	
		Average	**6.339**	**5.849±1.78**	**7.23±0.25**	**7.456±1.27**	**6.021±1.08**	**6.738±1.27**
GW 2	A	M	3.372	3.372	4.199	-	-	
		F	^	3.394	^	-	-	
	C	M	3.364	5.426	6.420	8.981	7.817	
		F	^	^	8.064	6.587	7.944	
	D	M	4.906	4.612	4.230	-	8.359	
		F	^	4.431	5.151	-	8.259	
	G	M	3.798	-	-	-	-	
		F	4.056	-	-	-	-	
		Average	**3.899±0.63**	**4.247±0.87**	**5.613±1.64**	**7.784±1.69**	**8.095±0.26**	**7.991±0.80**
GW 3	B	M	3.621	3.757	5.266	6.313	5.172	
		F	^	3.895	4.069	9.306	10.078	
	E	M	4.179	4.652	4.838	6.091	6.715	
		F	^	^	^	9.064	8.945	
	F	M	6.921	-	-	-	-	
		F	^	-	-	-	-	
	L	M	4.566	4.523	5.281	8.665	5.681	
		F	^	^	7.538	8.804	7.722	
		Average	**4.822±1.45**	**4.207±0.45**	**5.398±1.29**	**8.041±1.44**	**7.386±1.90**	**7.713±1.64**
LW1	H	M	5.592	5.051	3.950	5.259	6.252	
		F	^	^	6.385	10.212	8.921	
	I	M	3.274	5.186	6.225	8.437	5.178	
		F	^	^	6.621	6.948	8.352	
	K	M	3.489	3.950	4.704	7.497	9.471	
		F	^	^	4.925	9.768	10.157	
	M	M	2.667	5.129	6.678	8.099	6.339	
		F	^	4.981	5.716	5.886	7.699	
	R	M	5.393	-	7.46	8.718	8.232	
		F	5.959	-	7.472	9.241	7.573	
		Average	**4.396±1.41**	**4.859±0.51**	**6.014±1.18**	**8.007±1.62**	**7.817±1.55**	**7.912±1.55**

Averages are taken within a group for each postnatal testing day and compared across PCB exposure groups. The numbers *(Table 3)* in () next to the control's data points signify the number of pups tested. A "-" mark signifies that no data was collected for that particular set of pups. A ^ mark is used when the male and female serum was pooled together for a particular litter. Serum was pooled on earlier testing days when sample size per pup was insignificant for the thyroid hormone concentration test. GW, gestation week LW, lactation week. The ± values in the table are a calculation of standard deviation. PND 21 and 22 were averaged together so that they could be compared to the controls which were tested on PND 21.




B. PND7
T₃ Concentration (ng/mL)
0
1
2
3
4
5
6
7
8
*
*
Control
GW 1
GW 2
GW 3
LW 1


C. PND14/15
T₃ Concentration (ng/mL)
0
1
2
3
4
5
6
7
8
Control
GW 1
GW 2
GW 3
LW 1

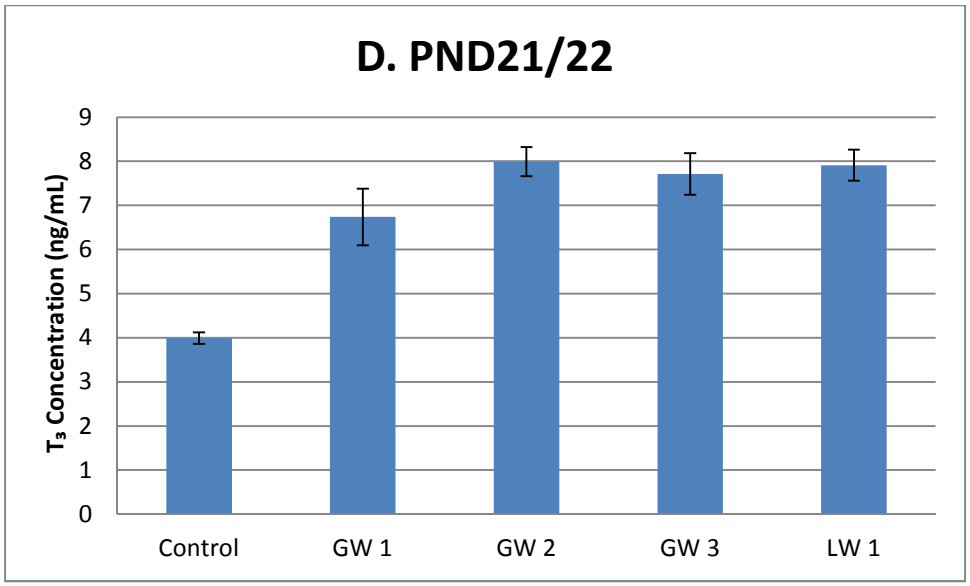


Figure 5. T_3 concentration at PND 3*(5A)*, 7*(5B)*, 14/15*(5C)*, and 21/22*(5D)*. Values used for these graphs were obtained from the averages displayed in *Table 3*. GW, gestation week LW, lactation week. The "*" marks on the graphs represent the PCB exposure groups that were significantly different than the control groups. Bars represent the concentration of T_3 in circulation per PCB exposure group. The error bars are a calculation of standard error.

The common effect of PCB exposure on T_3 concentrations appears to be an elevation of the concentration of the hormone when compared to the controls *(Figure 5 A-D)*. This result occurs at each of the four days tested (PND 3- PND 21/22), and is not strongly influenced by the time of PCB exposure (GW1-LW1). However, exposure during GW1 results in the greatest elevation of T_3 on PND3, with pups from other exposure times reaching that level by PND 21/22.

Discussion

The results for the litter size seem to depict that there is an effect of the PCB exposure on gestation week 1. Again it should be mentioned that the mean value for GW1 was severely lowered by the three litters not producing offspring. It may be possible that the small one week

PCB exposure window at the beginning of pregnancy followed by no PCB altered the pregnancy. In other research done in the present study for a two week PCB exposure window there were no results observed where the mothers seemed to produce no offspring in so many cases.

Observation of PCB effects on open field behavior revealed a trend for groups being hyperactive when exposure occurred during the early gestation weeks. Hyperactivity was determined by a larger number of lines crossed, center box entries, and rears. In an experiment that also used an open field design to test activity (Selvakumar et al., 2013) they found that in the PCB exposed rats the frequency of exploratory behavior was increased with regards to number of lines crossed, rears, and center squares. Another variety of behavior that has been reported to be altered by PCB (Deleon et al., 2013) is bird's song production in areas of high PCB contamination. The results were that PCB exposure effected the song production and communications for the birds from two different regions that differed in the kind of PCB present in the area. These results support the idea that all PCB in general is behaviorally disruptive, but also has varying effects. The authors also noted that ramifications for song quality may extend further than the toxic effects of environmental PCB exposure. From these two studies and the present results, it is important to note that analysis of the results of behavioral tests can be very complex. PCB seems to have an effect, but there may be many other uncontrollable variables involved. Some of these variables may include variability within litters, and other hormones acting on behavioral activity.

When comparing the T_4 and T_3 hormone concentration level results to one another in the present study some interesting trends were revealed. The trends that were observed in T_4, which were that the concentration of hormone became more similar to the controls as the testing days advanced, are a point of interest. One possible explanation may be that because the PCB

congeners that we used were less highly chlorinated, they did not remain in the rat as long and the thyroid hormone was able to rebound (Ren et al., 2013). The T_4 seemed to rebound much more easily than the T_3. As a result of T_3 being in less concentration in the blood it may not be able to return to normal levels as quickly as T_4 which may be why T_3 remains elevated above the control at PND 21/22. Results from Ren et al. (2013) help explain why the less chlorinated PCB may have an effect on the concentration of T_4 on the final testing days in the current study. They used PBDE which is similar to PCB, but with bromine substituents instead of chlorine. The results indicated that the more highly brominated the PBDE the greater the effect on the thyroid hormone concentrations. These results would support the conclusion that as the number of days since PCB exposure increase the T_4 levels seem to increase to a more normal level (Ren et al., 2013).

The T_4 concentration results were much more varied, but as expected T_4 was decreased when compared to the controls. As noted before, T_4 is secreted from the thyroid gland in greater quantity than is T_3, but T_3 is more biologically active. Also, PCB has more of an effect on T_4 directly by decreasing its circulating concentration. The results obtained by Ren et al. (2013) indicate that PCB may bind to thyroid hormone receptors. In a study by Cattani et al. (2013) they found that depressed T_4 resulted in a greater concentration of thyroid stimulating hormone. As a result of T_3 being more biologically active than T_4 it may act as somewhat of a safety net with other thyroid hormones to respond to lower level of T_4. In yet another study Fritshe et al. (2005) found that PCB-118 acted by interfering with the thyroid receptor complex and ultimately interfered with thyroid hormone signaling. These results help sum up some conclusions from the present results.

The present study illustrated that PCB exposure for as little as one week during rat development depresses T_4. As a result of thyroid receptors being blocked and thyroid stimulating hormones being elevated T_3 concentrations may increase. With T_3 usually being in a less concentration of circulation in the body and also being more biologically active, the concentration of T_3 hormone may possibly be a better indicator of when PCB has a greater effect on development. As a result of T_4 being blocked by PCB the T_3 may try to compensate. The present results reveal that exposure during gestational week 1 results in a much greater concentration of T_3 when compared to the other PCB exposure weeks. When compared to the behavioral tests, this may indicate that the earlier in gestation there is exposure to PCB a more negative effect may result.

In summary, as a result of looking over the open field behavioral test results, the thyroid hormone results for concentration of T_4 and T_3, and also the average litter sizes produced there may be an interesting trend developing. It appears across these different tests that the earlier in gestation that the rats were exposed to PCB the more negative the effects on the offspring were. Further studies should increase the numbers of animals per PCB exposure group to result in greater statistical strength of the observations. These conclusions were drawn from comparing all of the tests done in this study to try and focus on a window of PCB exposure during gestation and lactation resulting in the most negative effects on the offspring. The remaining results obtained from the tests done by other members of the group will likely continue to help identify the most dangerous time during development to be exposed to PCB.

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