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Dietary Exposure to Aroclor 1254 Impairs Radial Arm Maze Acquisition and Performance in Rats

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DIETARY EXPOSURE TO AROCLOR 1254 IMPAIRS RADIAL ARM MAZE ACQUISITION AND PERFORMANCE IN RATS

by

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Western Michigan University, 2004

Exposure to polychlorinated biphenyls (PCBs) is suspected to produce long lasting cognitive deficits in children and adults. This study assessed the effects of dietary exposure to a commercial mixture of PCBs on spatial learning and memory in Fisher 344 rats. Aroclor 1254 (0, 10, 50 ppm) was administered for 28 consecutive days in the daily diet. Seven days after the last dietary exposure, acquisition training began in an eight-arm radial maze. Following 28 days of acquisition training, "working" memory was assessed using a delayed win/shift procedure. Each delay (20 minutes, 2 hours, and 6 hours) was examined on three consecutive days. The results demonstrated that developmental dietary exposure to Aroclor 1254 impaired RAM acquisition, with little impact on performance during the delayed win/shift procedure. Specifically, animals treated with the 50 ppm dose exhibited significantly more errors during the acquisition phase than those treated with the 10 ppm dose or those in the control group. Suggestions for extensions to this research are discussed.

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INTRODUCTION

Polychlorinated Biphenyls (PCBs) are chemical contaminants used in a variety of forms and, in the past, randomly discarded without concern. PCBs were originally used as dielectric fluids for capacitors and transformers. They were also manufactured for a variety of uses, such as sealants, flame-retardants, pesticide extenders, and in carbonless copy paper (Roegge, et al., 2000: Safe, 1994). PCBs were produced from the 1930s to the late 1970s, when they were banned from production due to extreme health concerns. PCBs are resistant to biodegradation and are extremely environmentally persistent. Due to their lipophilic structure, they accumulate in tissues of many animals, specifically adipose tissues in mammals (Roegge et. al., 2000). Environmental toxicant exposure to fetuses and newborns often manifests itself as learning and memory impairments. The Toxic Substances Control Act of 1976 mandated testing of industrial chemicals including environmental toxicants. Due to the known hazards of toxicant exposure, behavioral testing was mandated to examine the specific effects of toxicant exposure.

PCB Chemical and Structural Background

There are 209 possible PCBs. This is possibly due to the many combinations in both the number and position of chlorines on the biphenyl moiety. The types and degree of toxic response are also dependent on the placement and number of chlorines present (Mullin et al, 1984; Parkinson and Safe, 1987; Safe, 1994; Seegal et al, 1997). There are three classes of PCB congeners; coplanar dioxin-like congeners, di-ortho-subtituted congeners, and mono-ortho-substituted congeners. Coplanar dioxin-like congeners interact at the aromatic hydrocarbon (Ah) receptor and induce

associated hepatic enzymes. Coplanar dioxin-like congeners share many toxicological properties with dibenzofurans and dioxins. Di-ortho-substituted congeners do not interact with the Ah receptor, nor do they induce hepatic enzymes. The third class, mono-ortho-substituted congeners are intermediary congeners where the toxicity of this PCB ranges from the effects of each of the other two congeners (Safe, 1990). In the past, the concern of PCB toxicity was based on their dioxin-like actions, mediated via activation of aromatic hydrocarbon receptors and induction of cytochrome P450 oxidases (Niemi, 1998).

Human Exposure to PCBs

Research has shown a positive correlation between PCB exposure and neurological dysfunction in adults and cognitive deficits in children (Choksi, et al., 1997). Two incidents of accidental exposure to PCBs alerted the scientific community to the dangers and long-term effects of accidental exposure. In Taiwan and Japan, people consumed contaminated rice oil. This oil contained PCBs and other polychlorinated thermal conversion products. The result was a strange disease termed "Yuko", also known as oil disease. The population most drastically affected were the children born to mothers who had consumed the contaminated oil. Even children born many years after the exposure were affected. The primary effects found were on birth weight, physical growth and development, calcium metabolism, intelligence, epithelial tissue, and behavior (Niemi et al., 1998; Miller R.W., 1985; Yamashita F. and Hayashi M., 1985; Yu et al., 1994). These children showed deficits in formal developmental testing, were shorter and weighed less than age-matched controls, and exhibited significant behavioral delays and abnormalities on behavioral assessment

(Niemi et al, 1998; Harada, M., 1976; Hsu et al., 1985; Rogan, W.J. and Gladen, B.C., 1992; Rogan et al., 1988; Yu et al., 1994). Studies in Taiwan showed that children who were exposed to PCBs scored lower on standardized tests (Chen et al, 1992; Roegge et al, 2000).

Jacobson and Jacobson studied children exposed prenatally due to maternal consumption of PCB-contaminated Lake Michigan fish. These children showed deficits in short-term memory and visual recognition memory. The extent of impairment was found to be dose dependent. These effects were found to persist many years after exposure and infancy (Niemi, 1998; Jacobson and Jacobson, 1985, 1990, 1996; Jacobson et al., 1990).

Human populations exposed to PCBs show a decrease in birth weight and increased exposure leads to a decrease in gestational age (www.EPA.gov). Moreover, cognitive impairments have been shown in children exposed perinatally to PCBs. Mothers who consumed Lake Michigan or Ontario fish, which contained PCBs, bore children with significant memory deficits at 7 months of age, decreased IQ and physical growth at 4 years-old, and long-term IQ deficits at 11 years of age (Gellar, et. al., 2000: Jacobson et al, 1985: Jacobson and Jacobson, 1993, 1996). Correlations were found between PCB pre-natal exposure and impaired short-term memory, reduction in sustained attention, and reduced cognitive function (Gellar et al, 2000; Jacobson et al, 1992; Patandin et al, 1999).

Routes of Human Exposure to PCBs

The vast majority of human exposure comes from diet. PCBs bioaccumulate in fatty tissue and can reach extremely high levels. Due to PCB bioaccumulation,

PCBs are found in the fatty tissue of most animals including, fish, meat, meat products, and milk. PCBs are also found in human breast milk (CDC, 2003). PCBs move into the human brain by a saturatable transport system (Seegal et al., 1996). PCB exposure can occur through inhalation, skin contact, or from eating contaminated fish, meat, or dairy products. A fetus may be exposed to PCBs through the mother's consumption of contaminated food sources. PCBs pass from the mother's blood to the fetal blood through the placenta. Infants may also receive significant exposure through breastfeeding (Niemi, 1997). The EPA currently has guidelines for safe consumption of PCB contaminated food. The limit is 20 ppm.

Non-Human Studies of PCB Exposure

In utero and lactational exposure to PCBs is associated with behavioral and neurochemical alterations in rats and primates (Pantaleoni et al., 1988; Schantz et al., 1991; Seegal et al., 1991, Seegal et al., 1997). EPA animal studies showed a decrease in birth weight, conception and live birth rate, and reduced sperm counts. Coplanar and ortho-substituted PCBs may have different mechanisms of action on the CNS (Schantz et al., 1996). Studies in primates have shown PCB-induced persistent and significant reductions in visual recognition, short-term memory, learning (www.EPA.gov), and adverse behavioral effects (CDC, 2003).

Neurochemical Effects of PCB Exposure

Many studies have shown learning deficits as well as decreased neurotransmitter concentrations in rats (Seegal et al., 2002; Choksi et at., 1997; Mariussen et al., 1999; Roegge et al., 2000). Seegal et al. (2002) reported that after three days of dietary exposure, Aroclor 1254 (25 mg/kg/day: oral delivery through

food) produced an initial increase in dopamine. However, further exposure caused a prolonged decrease significantly below baseline levels. This suggests that minor exposure may have neurotoxic effects. The specific mechanisms underlying the dopaminergic alterations produced by dietary Aroclor 1254 exposure are poorly understood at the present time. Ortho-chlorinated biphenyls are competitive inhibitors of dopamine transport into the synaptic vesicles in rat brain (Mariussen et al., 1999). Inhibition of dopamine uptake in both vesicles and synaptosomes may contribute to the decrease of dopamine levels in nervous tissue after exposure to PCBs (Mariussen et al., 2001). Seegal et al. (1991) and Shain et al. (1991) have shown that some PCB congeners cause reduction in the amount of the neurotransmitter dopamine, present in cultured neurons and adult animal brains, presumably via inhibition of the enzyme tyrosine hydroxylase (Niemi, 1997). Choksi et al (1997) found that ortho-substituted PCBs inhibit the activity of the rate limiting enzyme in the biosynthesis of dopamine, tyrosine hydroxylase, and therefore decrease dopamine synthesis in the human brain. Seegal et al (1997) also found that ortho-substituted PCB (2,4,2',4'-TCB: 1, 10, 20 mg/kg/day) dietary (oral delivery through food) exposure during critical periods of development (GD 6- PND 21) reduced brain dopamine concentrations due to inhibition of the synthesis of dopamine in combination with changes in cholinergic receptor function.

Effects of PCB Exposure on Learning and Synaptic Plasticity

PCB exposure produces inhibition of nitric oxide synthase (NOS), which may lead to deficits in learning and memory. Research has shown that NOS is selectively sensitive to dichloro-ortho-substituted PCBs. NOS is critically important to many

processes in the brain, including long term potentiation (LTP), learning, and memory processes involving the hippocampus (Sharma et al, 2001).

Long term potentiation represents a measure of long-term changes in synaptic plasticity, which appears to be related to learning and memory. LTP is long-lasting and synapse specific. Patterned input or brief titanic stimulation can initiate a prolonged alteration in synaptic efficiency or LTP. Higher nervous system functions involved in learning are thought to be associated with LTP (Niemi et al, 1998; Baskys et al., 1990; Gomaz et al., 1990; Silva et al., 1992; Teyler et al., 1989).

Developmental exposure to PCBs has been shown to produce impairments in learning and memory processes as well as inhibition of LTP (Sharma et al., 2001). Neimi et al. (1998) examined two commercial preparations of Aroclor on hippocampal tissue. Both mixtures were found to selectively impair LTP in the CA1 neurons of the hippocampus, which is the primary brain structure associated with learning and memory (Niemi et al., 1998). Concentrations of Aroclor 1016 (1-100ppm) that had little effect on synaptic transmission were found to reduce LTP in a dose-dependent manner. These authors also reported that Aroclor 1254 (1-100 ppm) was found to inhibit LTP at low concentrations, and higher concentrations produced a decrease in synaptic transmission.

PCB Effects on Learning and Memory

The Radial Arm Maze (RAM) is a behavioral assay frequently used to study spatial learning and memory in rodents. The radial arm maze offers a unique way to test the behavioral characteristics of memory and learning (Walsh and Chrobak, 1987).

The apparatus contains circular center with eight arms radiating out. At the distal end of each arm is a dish that is baited with food (e.g., cereal). A food-deprived rat is placed in the center of the maze and is allowed to enter each arm and eat a piece of food. Many versions of the RAM are used. To assess "working memory" and "reference memory", some arms may be left un-baited. Working memory errors are defined as repeated entries into arms from which food was already retrieved (Packard and White, 1989). The process of working memory depends on the cholinergic innervation to the hippocampus (Walsh and Chrobak, 1987). Reference memory errors are defined as entries into arms that were never baited. The radial arm maze was originally used to assess animal's memory capabilities as it pertains to spatial locations. Rats have been shown to use extramaze special cues to determine which arms have food. This shows that rats use memory in the RAM task (Olton, 1987).

Using a 12-arm radial arm maze procedures, Roegge et al. (2000) reported that gestational and lactational exposure to PCBs (Aroclor 1254) decreased the reference and working memory performance in male rats. Roegge et al. (2000) found that rats exposed to Aroclor 1254 made significantly more reference memory and working memory errors in the radial arm maze when compared to control animals. In-utero and lactational exposure to ortho-substituted PCB congeners resulted in a learning deficit on a delayed spatial alternation task in female rats (Schantz et al, 1996).

The present study implemented a delay win/shift RAM procedure, which is an established hippocampal- sensitive paradigm (Packard and White, 1989). The win-shift procedure is similar to natural foraging behavior of rats. It is comparable to an

animal's ability to memorize where food was buried at some time in the past without having to return to the same location. Animals have shown a decreased ability to remember where food has been placed in the middle of a series of locations. Food which was buried first and last are best remembered (Olton, 1987). The win-shift procedure is comparable to a delayed-match-to-non-sample task used to test learning in primates and humans.

The purpose of this study was to examine the effects of dietary Aroclor 1254 exposure on radial arm maze performance. Aroclor 1254 is a commercial mixture of coplanar and ortho-substituted PCBs. The PCB composition of Aroclor 1254 closely resembles the PCB mixture found in the environment. At this time, there is only one published study examining the effects of Aroclor 1254 on RAM performance (Roegge et al, 2000). This study examined gestational and lactational exposure, while the present study examined developmental exposure.

METHOD

Subjects

Eighteen male Fischer 344 rats (Charles River, Portage MI) were obtained at 21days old and delivered to Haenicke Hall animal colony, Western Michigan University. Western Michigan University animal colony is maintained at constant 20-22˚C and 20-24% humidity under a 12 hour light/dark cycle. The animals were singly housed in standard plastic hanging cages with free access to water. They were fed PCB contaminated food, except control, for 28 days. One week followed of free feeding to increase body weights. Two days before habituation began, the animals were food deprived and restricted to 85% of free feeding weight. All subjects were weighed prior to each session.

Materials

Apparatus

A private room within the animal colony was used to conduct the RAM experiments. A standard eight-arm radial arm maze was used to train and test working and reference memory. Kellogg's Fruitloops® were placed at the end of each arm. Between every session, each arm was wiped down with 70% isopropyl alcohol to diminish scent traces left from the previous rat. Pictures of different colored shapes were placed on the wall for spatial orientation. Data were collected by visual observation and recording. Each session was videotaped for further analysis. Data were graphed and analyzed using Prism Graph Pad version 3.0 software.

Drug

The polychlorinated biphenyl mixture, Aroclor 1254, was prepared in two different doses, 10 ppm and 50 ppm. 500 ml ethanol was mixed with 5 or 10 ml Aroclor 1254. This solution was then poured over 2.0 kg rat chow (Purina). The rat chow/ Aroclor mixture was placed on a rotary shaker for four hours and manually shaken every 15 minutes for the first hour and every half hour thereafter. The food was then placed in a single layer in aluminum pans to dry for three days. This allows the ethanol to evaporate while the Aroclor remains in the food and is spread throughout evenly. Each dose was made in individual batches of food. The control food was also soaked in ethanol and dried to control for any unknown differences in smell or texture. The animals were given free access to the treated food for 28 days. On the 28th day all food was replaced with standard Purina rat chow and free access to food was continued for one week then food was restricted to achieve 85% of free feeding body weight.

Habituation

A small handful of Fruitloops® were placed at thee end of each arm of the radial arm maze. The animals were placed in pairs in the center of the maze and allowed to roam throughout the maze, with free access to the Fruitloops®. Each pair was left in the maze for ten minutes. This procedure was repeated for three consecutive days.

Acquisition Phase

Training began on the day following the last habituation session. One Fruitloop® was placed at the end of each arm of the radial arm maze. Each animal was placed in the maze individually and allowed to explore the maze. Each animal was taken out of the maze after all eight cereal pieces were eaten or 16 arms were entered. Training sessions occurred once a day per animal for 28 days or until each group achieved a mean of 80% accuracy for two consecutive days. An error was considered an entry into a previously entered arm. Six measures were recorded or calculated during acquisition; total time to complete the maze, latency to first bite of food, total number of arm entries, repeat arm entries, number of entries made until first error, and number of correct entries out of the first eight entries.

Delay Win-Shift Phase

In the second phase a delay win-shift procedure was utilized. A Fruitloop® was placed at the end of six randomly chosen arms, while the other two arms were blocked from entry by an adhesive index card. The blocked arms were randomly chosen prior to each session for every rat. Each animal was removed from the maze when all six Fruitloops® were consumed or 16 arms were entered. Three delays, 20 minutes, 2 hours, and 6 hours were established. After the given delay, each animal was placed back in the maze and the previously blocked arms were now baited while the other six arms were not baited. Each delay was tested for three consecutive days (sessions). Each animal was removed from the maze when either both Fruitloops® were eaten or 16 arms were entered. In this task, 100% accuracy was defined as one entry into each of the two previously unbaited arms.

All data were recorded by two human observers and each session was videotaped for further data collection. All entries were recorded in order of entry and each entry that resulted in food consumption was circled. The total number of arms entered, repeated arm entries, number of entries until an error occurred, and the number of correct entries out of the first eight were all recorded during or calculated after each session. A stopwatch was used to record the latency, time until the first Fruitloop® was eaten, and the total session time.

Analysis

Days 6-10 of the 28 day exposure period, food consumption was calculated by subtracting the amount of food not eaten from the amount of food given. The amount consumed in grams was calculated and multiplied by the microgram per gram dose (0, 10, 50). Daily consumption was estimated from the 5-day calculated average. An estimate of the total amount of PCB exposure over the 28 day period was 108 μg/g for the 50 ppm group and 22 μg/g for the 10 ppm group. The total daily consumption was estimated at: 3.88 μg/g for the 50 ppm group with a range from 3.63- 4.1 μg/g and 0.79 μg/g for the 10 ppm group with a range of 0.74- 0.86 μg/g.

Graph Pad version 3.0 (Prism) software was used to graph and statistically analyze the data. Questionable entries were reviewed on videotape to ensure validity. Graphs were constructed to visually examine the effects of Aroclor 1254 on the dependant variables described above. Accuracy was determined by the number of errors, which were defined as repeated arm entries. Three primary dependent measures were analyzed using a repeated measures two-way (treatment x session)

analysis of variance (ANOVA): repeat entries, entries to error, and the number of correct entries out of the first eight entries.

RESULTS

Phase one (acquisition) was conducted to train spatial location in the radial arm maze. The average number of errors (repeat arm entries) in each session was calculated for each treatment group. Although the prearranged criterion of 80% accuracy on two consecutive sessions was met by several individual animals in less than 14 days, none of the group averages were at least 80% on two consecutive sessions within this time frame. Therefore, the acquisition phase was extended to 28 days. All six of the control animals met the above-stated criterion for acquisition. In both the 10 ppm and 50 ppm treatment groups, five of the six animals met this criterion.

A two-way (treatment x session) repeated measures ANOVA showed a statistically significant treatment effect on the number of repeated arm entries ($F_{2, 420}=15.25$, $p< 0.0001$), entries to error ($F_{2, 420}=3.76$, $p< 0.05$), and the correct number of entries out of the first eight ($F_{2, 420}=7.47$, $p< 0.001$) during the acquisition phase. There was not a statistically significant difference within groups across sessions. Since visual illustrations of these three measures of accuracy were very similar, only the number of repeated arm entries are graphically displayed below. The graph depicted in figure 1 best exemplifies the differences observed among treatment groups during the acquisition phase. The 50 ppm group as a whole, made significantly more errors compared to the other treatment groups.

Figure 1: Mean number of errors per dose group during each session of acquisition.

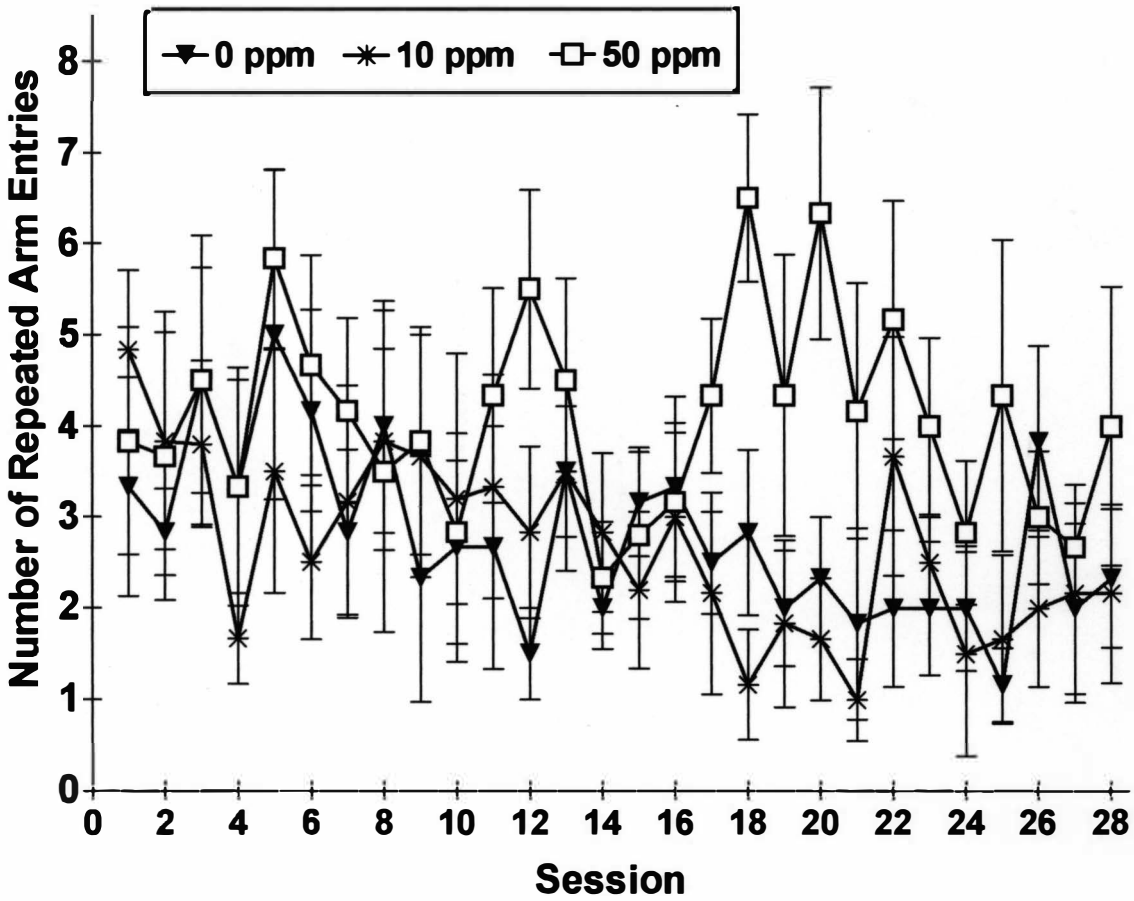


Figure 2 depicts the results from the delayed win/shift phase. The average number of repeated entries for each treatment group was plotted for each of three trials conducted following each delay. These results were also analyzed using a two way (treatment group x session) repeated measures ANOVA. Separate ANOVAs were conducted for each delay. None of these analyses showed statistically significant

differences among the treatment groups or across the three sessions within each delay.

Figure 2: Treatment group mean errors for each delay session.

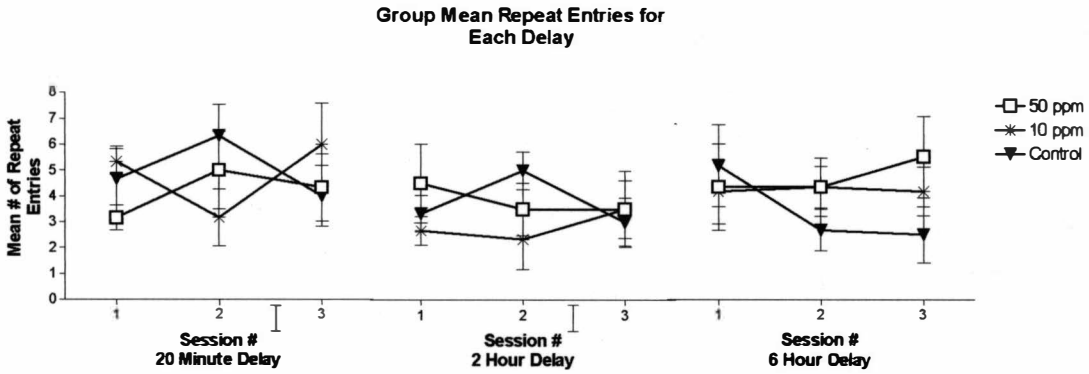
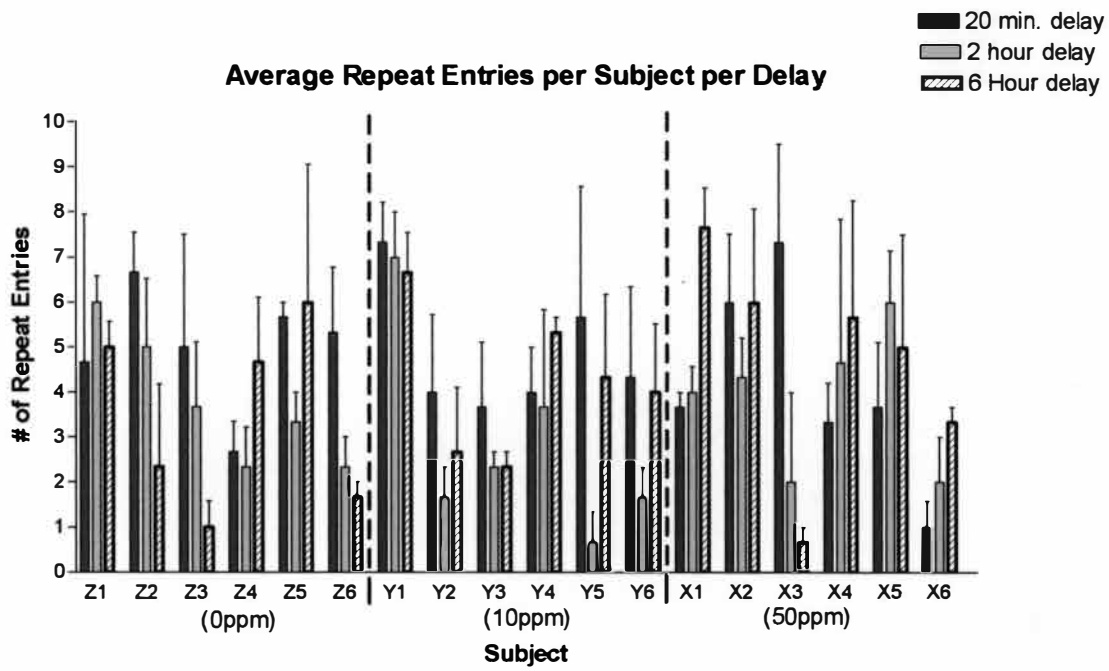


Figure 3 illustrates the number of repeated arm entries made by individual subjects following each of the three delays. For this graph, individual subject data were averaged across the three trials from each delay. This graph clearly illustrates the variability among subjects as well as within subjects. A few animals exhibited a systematic decrease in the number of errors as they progressed through the win/shift procedure. Specifically, three of the control animals exhibited this pattern, whereas none of the 10 ppm treated animals and one of the 50 ppm treated animals exhibited this pattern.

Figure 3: Average repeat entries per subject during delayed win/shift phase.



DISCUSSION

The results of this study provide evidence that post-weaning dietary exposure to Aroclor 1254 (50 ppm for 28 days) impairs spatial working memory in male Fischer 344 rats. Rats in the 50 ppm treatment group made significantly more errors during RAM acquisition trials than those in the 10 ppm group or the control group. The greatest difference was particularly evident in later trials. There was substantial variability among animals in all three groups.

In the win-shift procedure, each delay (20 min., 2 hr., 6 hr.) was assessed on three consecutive days, with two sessions per day (before delay, after delay). There were no significant differences among the three treatment groups in the number of repeat entries following any of the delays. This may be in part due to the extended time since exposure. The acquisition phase started one week after exposure was stopped, which may account for the increased errors, however this does not explain the increase in group differences closer to the end of the acquisition phase. It is possible that PCB induced neurological changes within the brain take time. This would account for the increase in errors in the later half of the acquisition phase. The lack of significant findings in the delay phase may also be due to the small number of sessions per delay. Only three sessions were completed for each delay. Following the 6 hr delay, there was an evident dose-dependent trend, with increased errors by the 50 ppm treatment group. It is possible that greater group differences may have been observed if additional win-shift trials were conducted.

Learning from one delay session to another can be seen in a number of subjects mainly from the control group in Figure 3. Learning is defined as a smaller mean number of errors or repeat entries from one delay to another. This also suggests a better reference memory. The 50 ppm group, notably X1, X4, and X6 exhibited more errors as the delay increased.

The present results are consistent with those of a recent report by Roegge et al, (2000) that Aroclor 1254 impaired radial arm maze performance in rats. Roegge et al. examined gestational and lactational PCB exposure on memory of male Long Evans rats in a 12-arm radial maze. There are several procedural differences between that study and the present study. For example, Roegge et al. administered Aroclor 1254 by oral gavage to dams from gestational day (GD) 6 to post-natal day (PND) 21, whereas the present study administered Aroclor in the daily diet from PND 21 to 49. Additionally, Roegge et al. employed a shaping procedure for 5 days, but subjects did not begin testing until they were 120-150 days old. Furthermore, testing procedures were conducted for 12 weeks, drug challenges were introduced, and gender differences were examined. However, the dependent measures were defined and recorded similar to those in the present study. Roegge et al., (2000) obtained similar results to the present study with respect to the number of errors within treatment groups in the male animals. Errors were not recorded during the equivalent acquisition or shaping phase, however impairments were found during the testing phase. The testing phase lasted eight weeks, at which point drug challenges were introduced.

Berger et al. (2001) examined the effects of 30-day dietary exposure (oral delivery through food) to either 0.5μg/g Aroclor 1248 or 1.15g mashed contaminated fish during adolescence in male Sprague-Dawley rats. An increase in hyperactivity in the animals exposed to PCBs was found. This is relevant because the animals were exposed at essentially the same age as were animals in the present study. Animals exposed to Aroclor 1248 or contaminated fish did not perform as efficiently. This suggests that the increased errors may be the result of decreased attention rather than memory. Further testing should examine the differences between the effects of PCBs on memory verses attention span.

Schantz et al. (1997) dosed by oral gavage, Sprague-Dawley rats GD10-16 with PCB 95 (8 or 32mg/kg/day). Spatial learning and memory were assessed using an eight-arm radial arm maze at 60 days. The animals were "shaped" for 5 sessions prior to testing. Testing was conducted 5 days a week for 4 weeks for a total of 20 test sessions. PCB 95-exposed animals actually made fewer errors, suggesting faster acquisition. This result can also be interpreted as animals exposed to PCB 95 adopted a response strategy of choosing the arm next to the one just entered, leading to faster acquisition and fewer errors. The use of this type of response strategy has been suggested to be the result of brain damage. One significant finding showed that animals exposed to ortho-substituted PCBs do not show the same decrease in errors compared to the control group. Also, ortho-substituted PCBs lead to hyperactivity, whereas PCB 95 produces hypoactivity in adult animals.

Other studies involving non-human-primates and rats also show poorer performance when exposed to PCBs during prenatal and early post-natal

development. Deficits were found in spatial discrimination as well as differential reinforcement of low-rate operant schedules (Bowman et al., 1978; Levin et al., 1988; Rice, 1997, 1998; Rice and Hayward, 1997; Schantz et al., 1991).

Not all PCB exposure studies have demonstrated impaired performance. Some studies have shown that learning deficits are not caused by individual coplanar PCBs. Studies using Long-Evans rats have failed to find any deficits in a number of learning and memory measures including delayed spatial attention, visuospatial attention, and sustained attention when exposed to coplanar PCB 126 throughout gestation and lactation (Roegge et al, 2000; Bushnell and Rice, 1999; Rice, 1999; Rice and Hayward, 1998, 1999). Schantz et al. (1996) exposed Sprague Dawley rats to two PCB coplanar congeners, PCB 77 or 126, from GD 10 to GD 16 and found no impairments on the 8-arm radial arm maze. The same group in 1995 found no impairments on male Sprague Dawley rats when exposed at the same gestational period with individual ortho-substituted PCBs.

Walsh and Chrobak, (1987) reviewed the use of the radial arm maze in the study of neurotoxic substances and found that the RAM is sensitive to a number of compounds including some heavy metals and their alkyl derivatives. However, other compounds tested that were known to have neurotoxic effects produced no significant differences between dose groups in the RAM. They concluded that the RAM is not well suited for primary neurotoxicity screening. However, they reported that damage to the cholinergic efferent or afferent innervations produces long-lasting and damaging effects to the hippocampus and long-lasting impairment of performance in the radial arm maze.

Gilbert and colleagues exposed rodents to Aroclor 1254 (6 mg/kg/day: oral delivery through food) gestationnally and lactationally (GD 6- PND 21) and found no deficits in the male rats at 5-7 months in the Morris water maze. Both the Morris water maze and the radial arm maze are considered assessments of hippocampal integrity. However, these two tasks may be examining completely different processes, despite their similarities. This might explain the lack of deficits found by Gilbert et al. (2000).

Bushnell et al.(2002), found that perinatal exposure to Aroclor 1254 (1, 6 mg/kg/day: oral gavage) does not affect behavior during adulthood in Long Evans hooded rats. The animals were exposed gestationally and lactationally from GD6 through PND 21. Behavioral tests were conducted throughout adulthood. Sustained attention tasks tested on PND 300-360. No significant differences found for behavioral measures. Sex-dependent effects were examined and the male rats made more errors than the female rats.

Freeman et al., (2000) found no significant neurotoxicity after 52 weeks of dietary exposure (mixed in food) to one of the following Aroclors; 1016, 1242, 1254, and 1260 (25-200 ppm) in male and female Sprague-Dawley rats. Some general toxicity observed for Aroclor 1254 treatment group. Dosing began between GD49 and GD56, considered adult exposure.

When comparing the present results with previous findings, a few points must be made. Roegge et al. (2000) used a 12-arm radial arm maze, which is reportedly more sensitive to subtle impairments than the 8-arm maze. The time and duration of both exposure and testing appear to be critical to the specific types of deficits.

Gestational and lactational exposure may lead to different memory impairments than adolescent or adult exposure. The present study examined post-weaning exposure during adolescence/puberty. Research has shown a distinct difference between the effects of mono-substituted PCBs, the more heavily chlorinated PCBs, and the different commercial mixtures on learning and memory effects in rats. The present study examined a commercial mixture, Aroclor 1254, because that is more likely to be encountered in the environment and the complex actions of a commercial mixture might have a more pronounced effect than individual congeners.

In conclusion, the present study demonstrated that dietary exposure to Aroclor 1254, a commercial PCB mixture, impairs RAM acquisition, with little impact on performance during the win-shift procedure. Although deficits were observed in maze acquisition, this study could be expanded in further research. For example, blood levels of PCB could be assessed to more accurately determine the level of exposure. It is also recommended that the effects of longer periods of exposure be examined, and that additional measures of learning be assessed.

Appendix A:
Institutional Animal Care and Use Committee

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

ANNUAL REVIEW OF VERTEBRATE ANIMAL USE

PROJECT OR COURSE TITLE: Investigation of Markers of Cell Death and Immune Function in Rats Exposed to Selected Chemicals

RECEIVED
MAY 19 2004
I.A.C.U.C.

IACUC Protocol Number: 03-04-02
Date of Review Request: 06/10/03 Date of Last Approval: 6/10/03
Purpose of project (select one): ☐Teaching ☒Research ☐Other (specify):

PRINCIPAL INVESTIGATOR OR ADVISOR
Name: JayC. Means Title: Professor
Department: CHEM Electronic Mail Address: means@wmich.edu

CO-PRINCIPAL OR STUDENT INVESTIGATOR
Name: Lisa Baker Title: Assoc./Assist. Professor
Department: PSHC Electronic Mail Address: lisa.baker@wmich.edu

1. The research, as approved by the IACUC, is completed:
   ☐Yes (Continue with items 4-5 below.) ☒No (Continue with items 2-5 below.)

If the answer to any of the following questions (items 2-4) is "Yes," please provide a detailed explanation on an attached sheet of paper. Include details of any modifications made to the protocol based on new findings or publications, adverse events or mortalities.

2. Have there been any changes in Principal or Co-Principal Investigators? ☒Yes ☐No
3. Have there been any new findings or publications relative to this research? ☒Yes ☐No
   Describe the sources used to determine the availability of new findings or publications:
   - ☐No search conducted (Please provide a justification on an attached sheet.)
   - ☐Animal Welfare Information Center (AWIC)
   - ☒Search of literature databases (select all applicable)
     - ☐AGRICOLA ☐Current Research Information Service (CRIS)
     - ☒Biological Abstracts ☒Medline
     - ☒Other (please specify): CAS

   Date of search: 05/10/04 Years covered by the search: 1980-present
   Key words: gene expression, benzo[a]pyrene, PCBs, lead, immune system effects, DNA adducts, rat (Fisher 344), tumors, cancer, behavior, learning, maze
   - ☐Additional search strategy narrative:
4. Are there any adverse events, in terms of animal well being, or mortalities to report as a result of this research? ☐Yes ☒No
   Cumulative number of mortalities: 0 prior to euthanasia
5. Animal usage: Number of animals used during this quarter (3 months): 4 Cumulative number of animals used to date: 88

✏ Jay C. Means 5/17/04
Principal Investigator/Faculty Advisor Signature Date

✏ Lisa E. Baker 5/18/04
Co-Principal or Student Investigator Signature Date

IACUC REVIEW AND APPROVAL
Upon review of the relevant information regarding this protocol, the IACUC approval for this project has been extended for one year from the date of this signature.

✏ 05/26/04
IACUC Chair Signature Date

Revised 10/01 WMU IACUC
All other copies obsolete.

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