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# Exposure to Polychlorinated Biphenyls (PCBs) Produces Hyperactivity Differentially in Male and Female Rats

## Introduction

This paper is based on an examination of some of the behavioral and physiological effects of exposure to polychlorinated biphenyls (PCBs). The examination started in response to a request from colleagues at the NY State Department of Health who were involved in a USA Environmental Protection Agency Superfund grant studying the effects of contamination at the St. Regis Reservation on the St. Lawrence River near Massena (see Figure 1). We wondered whether exposure to that PCB-contaminated environment could produce the symptoms of attention deficit/hyperactivity disorder (AD/HD) in the Mohawk children, and. decided to use Terje Sagvolden's procedures to measure the effects of exposure to environmental levels of PCBs on the behavior of normal (non-hyperactive) Sprague-Dawley (SD) rats. The majority of this presentation was focused on the recent results of our studies, much of which has not yet been published.

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**Figure 1.** The Great Lakes – St. Lawrence River System.

## Polychlorinated biphenyls

PCBs are a family of 209 manufactured compounds (congeners) that was once produced in large quantities in the USA for use as

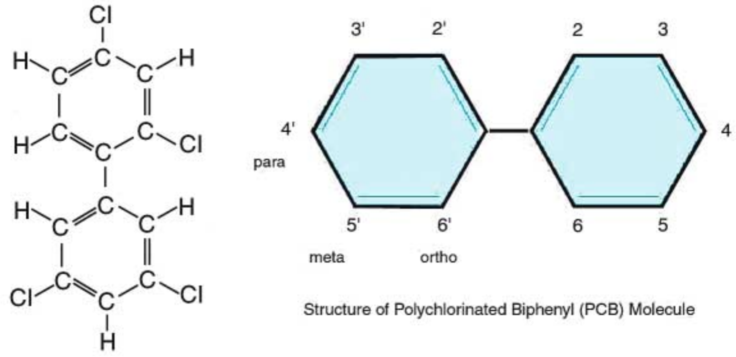
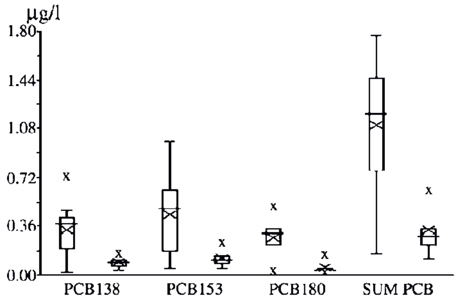



**Figure 2.** Chemical Identity.

heat resistant electrical insulators, brake liners, etc. An Aroclor is a commercial mixture of PCB congeners. Large amounts have leached or been dumped into bodies of water, including the Great Lakes, and the St. Lawrence and Hudson Rivers, where they continue to be found today. PCBs are highly stable, bioaccumulate, and because of their stability, have entered the food chain, even north of the Arctic Circle. Diagrams of their chemical identity are shown in Figure 2. A specific example of a congener is shown on the left, and the general structure of the molecules on the right. Congeners are formed by the addition of Chlorine ($Cl_2$) to Biphenyl ($C_{12}H_{10}$), which is a dual-ring structure comprising two 6-carbon Benzene rings linked by a single carbon-carbon bond. The nature of an "aromatic" (Benzene) ring allows a single attachment to each carbon. This means that there are 10 possible positions for chlorine substitution (replacing the hydrogens in the original Biphenyl).

The positions of the chlorine substituents on the rings are denoted by numbers assigned to each of the carbon atoms, with the carbons supporting the bond between the rings being designated 1 and 1'. Manufacture of PCBs was banned in 1977 because of evidence that they were persistent in the environment and because of wide spread concern that they were associated with health risks. By the end of 1980 worldwide production of PCBs totaled 1,054,800 tons. For many purposes they are divided into classes based on whether and how many chlorines are found on ortho positions or are not present in any ortho position, where the compound is called a coplanar PCB. Without ortho-substituted chlorines the molecule can assume a coplanar configuration and exert some actions similar to 3,4,7,8-tetrachloro-dibenzo-dioxin (TCDD), the most toxic of dioxin congeners. Unlike the coplanar, ortho-substituted PCBs have the




Box and Whisker-diagram of serum PCB concentrations (PCB congeners 138, 153, 180 and ΣPCB) in breast-fed (left column and bottle-fed (right column) infants at the age of six weeks.

**Figure 3.** Organochlorine Compounds in Breast-Fed vs. Bottle-Fed Infants: Preliminary Results at Six Weeks of Age. From: Lachmann et al. (2004). *Science of the Total Environment*, 329, 289–293.

ability to reduce levels of the neurotransmitter dopamine, both in vivo and in vitro. Unfortunately, when ingested, PCBs are readily absorbed, and because they are lipophilic and resistant to metabolism they can persist in fat tissue and human breast milk. Lachmann et al. (2004) demonstrated this recently by comparing concentrations of specific congeners in the sera of breast- versus bottle-fed infants (see Figure 3).

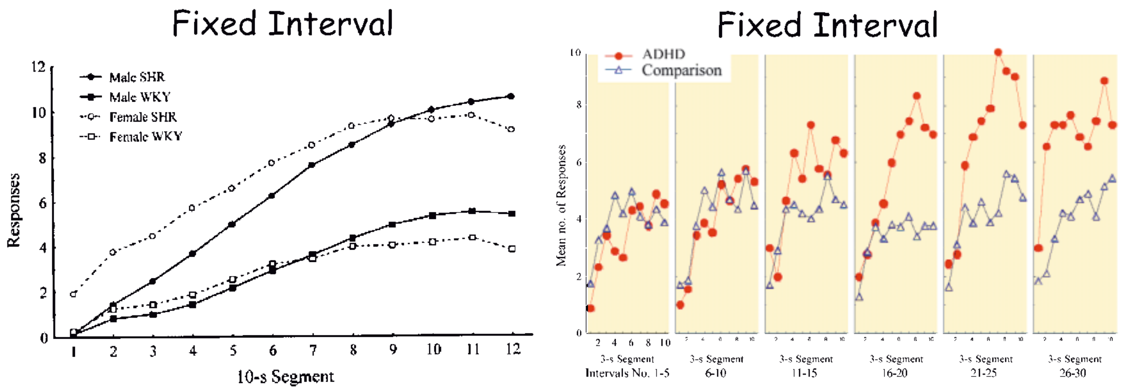
## Attention Deficit Hyperactivity Disorder

AD/HD is the most commonly diagnosed disorder in children (Barkley, 1989). It is estimated to affect between 3–7% of children (Barkley, 1997). However some (e.g., Gillis, et al., 1992) suggested that the percentage of affected children may be as high as 20%. The American Academy of Pediatrics (2000) indicated that between 2% and 12% of grade school children are affected. Cantwell (1996) suggested that between 50% and 70% of children diagnosed with this disorder will have problems related to social adjustment and functioning, and/or psychiatric problems as adolescents and young adults. For example, Barkley et al. (2003) and Murphy et al. (2002) pointed out that affected adolescents and adults are at increased risk of developing conduct disorder, dysthymia, alcohol-cannabis abuse/dependence to receive psychiatric medication and other mental health services. In these individuals there is also an increased risk of suicide (Murphy et al., 2002). One peculiar aspect of the disorder is that it affects more boys than girls. The behavioral problems of ADHD boys seem to differ from those of ADHD girls. The primary deficit in girls may be associated with attention problems, without hyperactivity (Predominantly Inattentive Type) whereas the primary deficit in the boys may be associated with overactivity, impulsiveness (Predominantly Hyperactive-Impulsive Type).

## Research Précis

Validating the SHR as an animal model. The behavioral apparatus for the operant conditioning procedures used by Sagvolden and associates to establish the viability of the SHR as an animal model of AD/HD are shown in Figure 4. The animals are trained to press the lever for drops of water (left panel), and the children press the nose of the clown (right panel) for plastic toys and Norwegian Crowns. The operant training is with a multiple fixed interval (FI), extinction (EXT) schedule of reinforcement. Hyperactivity is indicated by the tendency to press the lever more often, and to produce more response bursts – responses with short (≤ 1.0 s) interresponse times (IRTs) – than the comparison groups. For our purposes impulsiveness is responding prematurely in anticipation of the end of the fixed time interval prior to the next reinforcer. Berger and Sagvolden (1998) reported this behavior in male and female SHRs (Figure 5, left panel), and Sagvolden et al. (1998) with children diagnosed with AD/HD (Figure 5, right panel), compared to their respective comparison

**Figure 4.** The Research Apparatus for Rats and Children.




Berger & Sagvolden (1998). *Behavioural Brain Research*, 94, 73–82.

Sagvolden et al. (1998). *Behavioural Brain Research*, 94, 61–71.

**Figure 5.** The Operant Behavior of the SHR (Animal Model) and Children Diagnosed With AD/HD

groups. The obvious similarity between the children's behavior during the final sessions, to that of the animals, helps establish the external validity of the animal model. A compilation of the FI timing behavior of various strains of rats by Sagvolden (2000) is shown in Figure 6. Data from Holene et al. (1998) are included (marked in the figure as PCB 153 hnss) showing hyperactive/impulsive behavior, similar to that of SHRs, but produced in male rats by exposure to PCB congener 153 in their mother's milk.

Findings from our laboratory. Instead of using individual congeners, we exposed male and female SD rats to environmentally relevant concentrations of the PCB mixtures found in the St. Lawrence River (Aroclor1248) or Lake Ontario (Aroclors 1254 & 1260) via ingestion or inhalation around puberty, or during the perinatal period (in *utero* and during lactation). The experimental protocol for the ingestion and inhalation studies is presented in Figure 7. Berger et al. (2001)

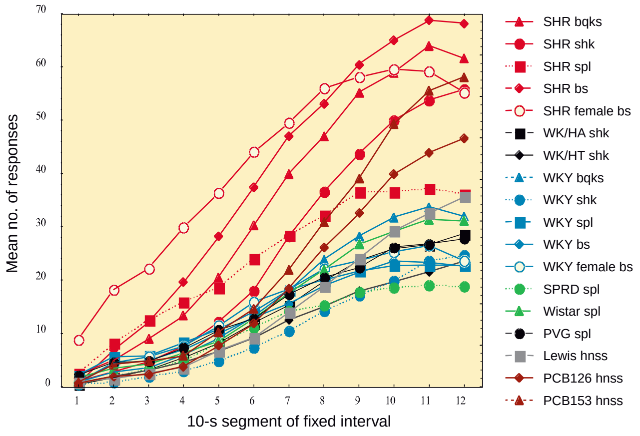



**Figure 6.** Comparison of the Operant Behavior of the Spontaneously Hypertensive Rat With Other Animals. From: Sagvolden, T. (2000). *Neuroscience and Biobehavioral Reviews*, 24, 31–39.

| Procedure | Duration | Comments |
|---|---|---|
| Exposure Phase | 30 Days – 1 hr per day | Age of rats – 35–64 Days |
| Habituation to apparatus | 1 Day – 30-min session | House-Lights off |
| Dipper Training | 4 Days – 30-min sessions | Begin 22-hrs Water deprivation House-Lights on |
| Lever Press Training | 5 Days | Shaping Procedure – House-Lights on |
| Continuous Reinforcement | 5 Days – 40-min sessions | House-Lights on |
| FI 30 s-EXT 5 min | 2 Days – 40-min sessions | FI-lights on, EXT – House-lights off |
| FI 60 s-EXT 5 min | 2 Days – 40-min sessions | FI-lights on, EXT – House-lights off |
| FI 120 s-EXT 5 min | Until behavior stabilized – 40-min sessions | FI-lights on, EXT – House-lights off |

**Figure 7.** Experimental Protocol.

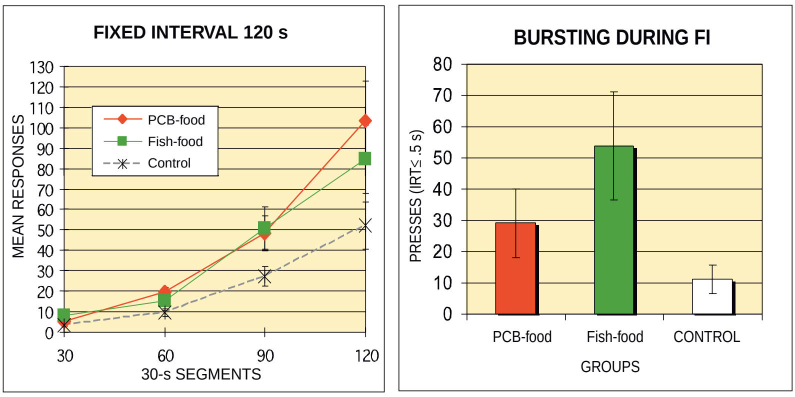



**Figure 8.** Ingestion Experiment – Males.
Berger, Lombardo, Jeffers, Hunt, Bush, Casey & Quimby (2001)

reported the behavioral effects, measured with FI-EXT procedure (e.g. Berger & Sagvolden, 1998), of ingestion by males of a diet augmented with either corn oil containing 0.5 µg/g Aroclor 1248 (PCB-Food), contaminated St. Lawrence River carp (Fish-Food, total PCBs ~0.72 µg/g), or corn oil alone (Control). The left panel of Figure 8 shows that both exposed groups lever pressed more often (hyperactivity) and earlier in the FI (impulsiveness) than controls, and the exposed groups also produced relatively more response bursts (right panel). Thus, the PCB-exposed males behaved like genetically hyperactive SHRs. However, hyperactive/impulsive behavior was not produced in females that ingested the same PCB-Food diet and were tested under conditions identical to those used with the males. The sex difference displayed in Figure 9 indicates that the exposed females tended to be hypoactive compared to controls. These differential sex effects replicate, with a different exposure method, those of Holene et al. (1999).

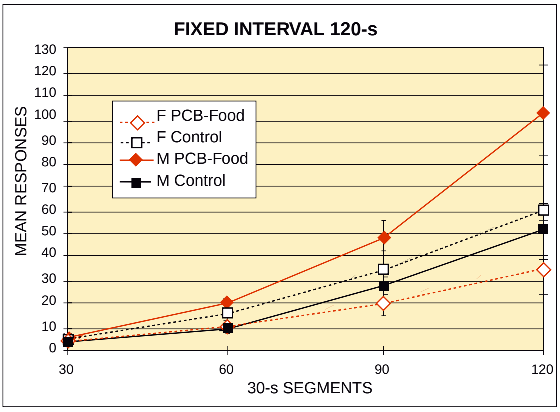



**Figure 9.** Ingestion of Aroclor 1248 (0.5 µg/g). Sex Differences.

Berger, Lombardo and Hunt (2004) exposed male and female SD rats, via inhalation, to either vapor from Aroclor 1248, or vapor generated from PCB-contaminated St. Lawrence River sediment. All were exposed for 23 hours per day in a sealed environment. The unexposed control groups lived in a similarly sealed environment containing essentially uncontaminated air. All animals were tested with FI-EXT procedure, as above. The differential sex effects are depicted in Figure 10. The sediment vapor (SED) produced hyperactivity in both sexes. In contrast, the Aroclor (PCB) produced relatively more responding in males than females, and compared to their respective unexposed controls; again in line with Holene et al. (1999).

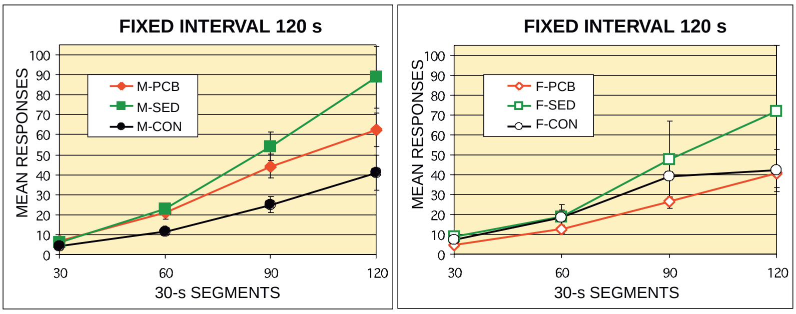



**Figure 10.** Inhalation Experiment. Sex Differences.

More recently, Lombardo, Berger, and Jeffers (2005) we examined the effects of perinatal exposure to the above diet conditions. Groups of pregnant rats were fed either the PCB-Food, Fish-Food, or Control diets used by Berger et al. (2001) during 9 days of their 21-day gestation period. After their pups were weaned at 21 days of age, all offspring received uncontaminated food for the remainder of the study. They were tested as adults using the same FI-EXT procedure as above. Figure 11 represents

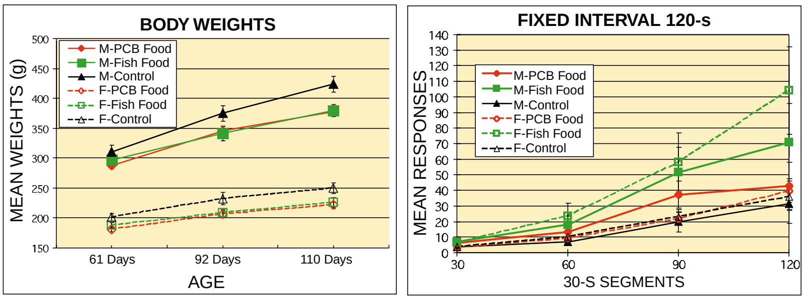



**Figure 11.** Effects of Perinatal Exposure to PCBs on the Male and Female *Offspring*.

the body weights of the male and female offspring from the three dams' diet groups. The mean body weights of the groups perinatally exposed to PCBs were lower that their unexposed same-sex counterparts. This observation serves as a manipulation check of exposure conditions. The mean number of responses by male and female offspring in the three dams'-diet conditions is plotted in Figure 12 as a function of 30-s segments of the FI. The results show that only perinatal exposure to the PCB-contaminated fish was associated with hyperactivity and impulsiveness in both male and female offspring, relative to unexposed controls. This is the second time that we were able to observe these behavioral effects in female SD rats. In the present study perinatal exposure to the PCB-Food diet did not produce hyperactivity and impulsiveness in either the males or the females. The dose may have been too low, perhaps because the dams only consumed about 67% of their daily portions of the diet. However the reproductive (estrous) cycles of the female offspring were affected (see Figure 12). The peri-

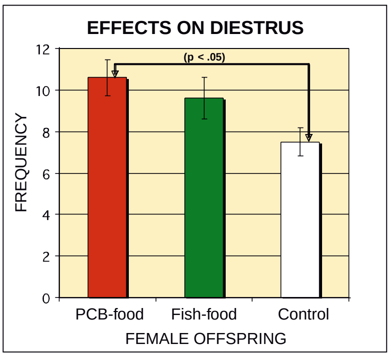



**Figure 12.** Effects of the Perinatal Exposure on Estrous Cycles.

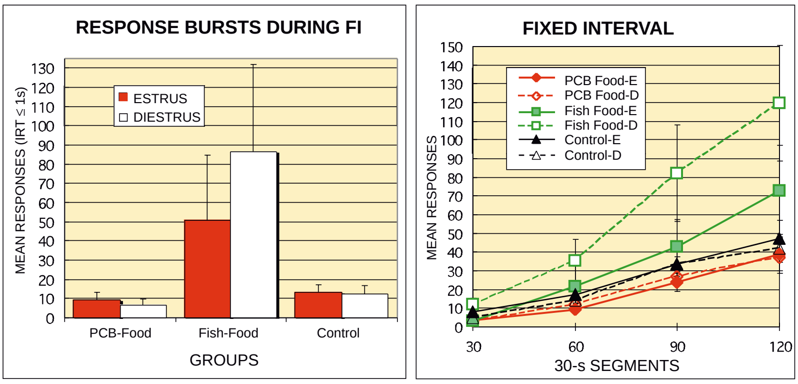



**Figure 13.** Perinatal Exposure to PCBs: Estrous-Effects on Behavior of the Female *Offspring.*

natal exposure to the PCB-Food diet resulted in this offspring group spending more time in the diestrus phase of their cycle than controls. Estrogen levels are lowest during this phase, and females will not accept a male. Interestingly, Mendola, Buck, Sever, Zielezny, and Vena (1997) had observed a reduction in the length of menstrual cycles in pre-menopausal women who had eaten PCB-contaminated fish more than once a month. The effects of perinatal exposure to the contaminated fish on the behavior of the female offspring were also more pronounced during diestrus than estrus. Figure 13 shows relatively more bursts (left panel) and hyperactivity and impulsiveness (right panel).

Most recently we replicated the sex differences in the effects of PCB exposure during adolescence, shown above, with the mixtures found in the Great Lakes and in mothers' milk, following the protocol presented in Figure 7. Groups of male and female SD rats ate cookies on to which two different doses had been placed prior to being tested with the FI-EXT procedure. Figure 14 shows the resulting dose-related hyperactive/impulsive behavior in the males (left), but the opposite in the females (right), compared to same-sex controls.

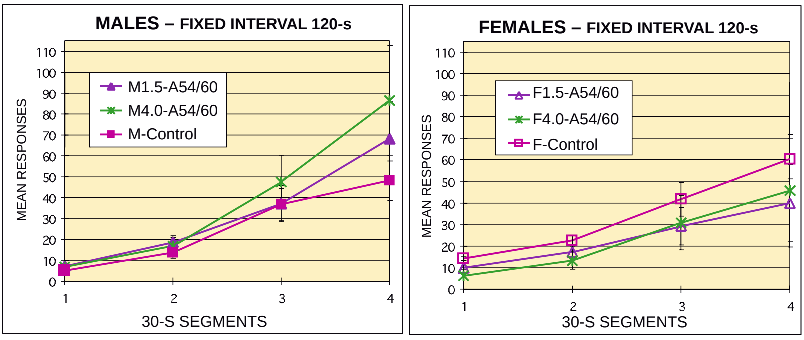



**Figure 14.** Ingestion of 1.5 v 4.0 µg/g Aroclor 1254/1260. Sex Differences.

Clearly the PCB-contaminated St. Lawrence River sediment and fish contain other toxins such as methylmercury, organochlorine pesticides, polybrominated diethyl ethers, and hydroxylated metabolites of PCBs that may also affect behavior. Some of their neurotoxic and endocrine disruptive effects appear to be sex-specific. More research is needed to separate and elucidate all these effects.

In conclusion, we have shown that exposure to environmental levels of PCBs and related toxicants, by varied means and at different times during development, affects the behavior and physiology of an animal model relevant for human beings. We believe it is important to study the effects of ingesting a contaminated food source because ingestion of PCB contaminated food, not a specific congener or Aroclor, is the main source of human exposure to PCBs. Our data and others suggest that exposure to PCBs and other environmental toxins are partly responsible for the increases in the prevalence of AD/HD in the USA and other parts of the world. A theoretical interpretation appears in a recent paper by Sagvolden, Johansen, Aase and Russell (in press), and from which Figure 15 was taken.

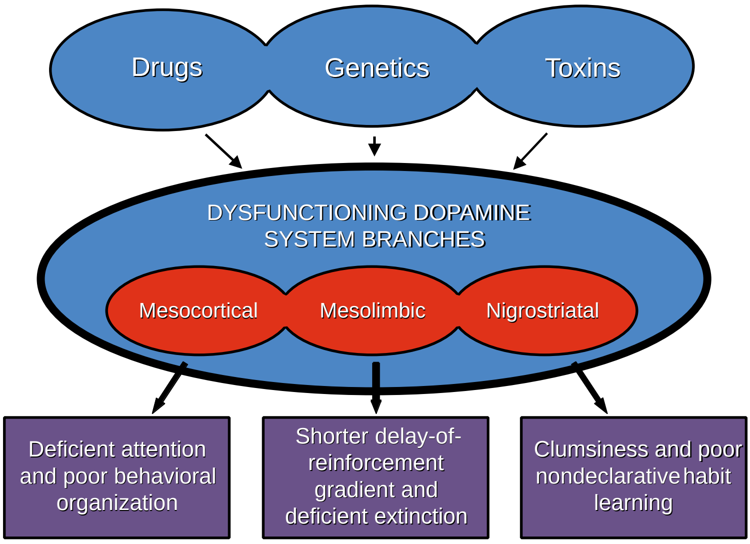



**Figure 15.** From Sagvolden, Johansen, Aase & Russell, BBS

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