Behavioral effects of PCB exposure in an animal model of Attention-Deficit/ Hyperactivity Disorder – the Spontaneously Hypertensive rat

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Summary

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Title: Behavioral effects of PCB exposure in an animal model of Attention–Deficit/ Hyperactivity Disorder- the Spontaneously Hypertensive rat

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Background: Polychlorinated biphenyls are a class of organic compounds that bioaccumulate due to their hydrophobic and lipophilic properties. The main source of exposure for humans is through food consumption as adolescents or adults and through breast milk as infants. Exposure might have a variety of adverse effects, like impairment of cognitive function as well as effects on neurological development, depending on the organisms´ age at exposure. Effects of PCBs are associated with the same impairments in neurobehavioral function as in ADHD.

Methods: A total of 212 rats were tested in this study. 104 Spontaneously Hypertensive rats (SHR) and 108 Wistar Kyoto rats (WKY) were orally given three different doses of PCB 153; 1mg/kg, 3mg/kg or 6mg/kg, between postnatal day 8 and 21. Control groups were given pure corn oil. The animals were tested on a variable interval reinforcement schedule measuring activity level, impulsivity and sustained attention.

Results: Rats exposed to PCB 153 showed changes in activity, impulsivity and sustained attention. This was true for SHR, but not WKY. There was an effect of dose in exposed animals. The SHR given 1mg/kg of PCB were different on behavioral outcome in comparison with animals given 3mg/kg of PCB, 6mg/kg of PCB or corn oil. Both male and female SHR showed behavioral effects following PCB exposure.

Conclusion: The two strains of rats responded differently to the doses of PCB 153. There was an increased sensitivity to PCB exposure in the animal model of ADHD, SHR, both males and females, but not in WKY.

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Introduction

There have been massive expansions of the chemical industry the last century, where new chemicals are developed which have been of importance and benefit to humans (Smith & Gangolli, 2002). Many of the compounds have a variety of applications, including usage as defoliants and insecticides and as coolants in electrical equipment. It took many years before the damaging consequences to the ecosystem and to man as a result of use of these compounds were discovered (Smith & Gangolli, 2002). Organohalogen compounds (OHCs) are some of these environmental pollutants with a known toxic effect (Kodavanti & Curras-Collazo, 2010). Today, the use of OHCs are severely restricted or banned in most parts of the world, but still they remains ubiquitous in the environment and persistent in soil and water (Kodavanti & Curras-Collazo, 2010; Mariussen & Fonnum, 2006). They also persist in human tissue, including breast milk. One of the OHCs that have been most popular in use is the polychlorinated biphenyls (PCBs) (Mariussen & Fonnum, 2006).

Polychlorinated biphenyls

PCBs consist of two phenyl rings (biphenyl), as shown in figure 1. The hydrogen atoms can be substituted with chlorine in 1 to10 sites giving a total of 209 possible structures (congeners) of PCBs (Faroon, Jones, & de, 2001; Smith & Gangolli, 2002).

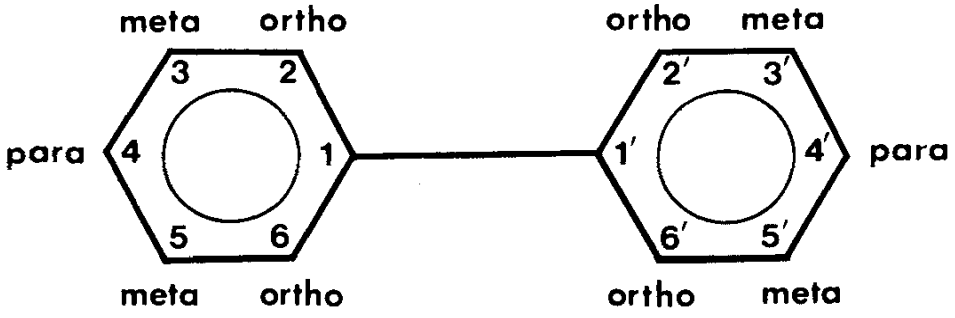

Figure 1. Biphenyl. Chlorine atoms can be bound to positions 2-6. From Safe (1984).

There are three main structural classes of PCBs, depending on the site of chlorine substitution: (**1**) coplanar, dioxin-like PCBs which have chlorine substitutions at the *para* (4, 4`) and *meta* (3, 3` or 5, 5`) positions, with no chlorine substitutions at the *ortho* (2, 2` or 6, 6`) position. (**2**) *Mono-ortho*–substituted PCBs with one chlorine substitution at the *ortho* (2, 2` or 6, 6`)

position. This class of PCBs may attain coplanarity, which is when the whole molecule is in the same plane. (**3**) Non-coplanar, with two or more *ortho*-substitutions (Fonnum & Mariussen, 2009). Molecule stability increases proportionally with the number of chlorine atoms and their placement at *ortho*-, *meta*- or *para*- position is of importance for their toxicity (Safe, 1984). The PCBs have generally been available in mixtures given a chlorine content dependent on the chlorination process. This gives them distinct properties adapted for their use. Common names for the mixtures are Aroclor 12XX. The two X´es refer to percentage of chlorine in the mixture, for example A1254, which contains 54 % chlorine by weight (Fonnum & Mariussen, 2009; Giesy & Kannan, 1998).

Distribution and properties

PCBs were first synthesized at the end of the nineteenth century, but were only introduced to the commercial market in 1929. They are nonflammable, thermo-stable, extremely persistent, lipophilic and resistant to biological decomposition (Mariussen & Fonnum, 2006). Due to their versatility, physical properties and miscibility with organic compounds, they have been extensively used in hydraulic fluids, plasticizers, as additives in different types of glue, cement and paint, in cooling and insulating fluids for transformers and capacitors as well as insulating materials for windows (Fonnum & Mariussen, 2009; Safe, 1984). Because of their versatile use, worldwide production increased annually until it was banned in Western Europe and North America in the 1970s. Today, there is no known production in the industrialized world (Giesy & Kannan, 1998). Despite this, PCBs are still detected all over the world because of atmospheric deposition, careless dumping of electrical equipment containing PCBs or leakage from dump sites (Seegal, 1996). This is of great environmental concern because PCBs are resistant to both chemical and biological degradation and in addition they accumulate in the food chain (Rice, 1995). Due to their properties, PCBs are therefore persistent in the environment and easily transported to remote areas from the localized or regional sites of contamination. This makes them present in almost every part of the environment (Giesy & Kannan, 1998).

PCBs are lipid soluble and have hydrophobic properties. An implication of this, is that under normal circumstances one will find the highest concentration of PCBs in lipid rich tissue, not water (Fonnum & Mariussen, 2009). Consequently, there will be more PCBs in sediments and sediment dwelling organisms than in the water (Voie, Johnsen, & Rossland, 2002). There is an accumulation in the marine food chain from edible shellfish to fish and sea mammals.

Some of these species are serving as food for humans. Surveys have shown that the main source of daily exposure to PCBs for humans is through the diet (La & Mantovani, 2006; Smith & Gangolli, 2002). Fish found in exposed waters, such as the Great Lake and Hudson River, are part of the human food chain. For the general population, contaminated fish are seen as the most significant source of the chemical, exposing those consuming them to greater background levels of PCBs (Rice, 1995; Smith & Gangolli, 2002). Since PCBs are lipophilic they accumulate in fatty materials, like adipose tissue and breast milk, and because they are resistant to metabolism they can persist for years (Berger et al., 2001). In humans, it is the *ortho*-substituted PCBs that dominate in adipose tissue (Mariussen, Andersson, Tysklind, & Fonnum, 2001) and PCB congener 153 is one of these. The half life of this congener is estimated to be 27.5 years, mainly due to its structure and level of chlorination (Duarte-Davidson, Wilson, & Jones, 1994). This is also one of the congeners with the highest level in breast milk (Kostyniak et al., 1999). Breast milk is an efficient medium for transference of toxic chemicals from mothers to infants (Weiss, 2000). The fact that PCBs are transferred from mother`s milk to infants is alarming, because early development seems to be a critical period of exposure (Fonnum, Mariussen, & Reistad, 2006; Mariussen & Fonnum, 2006; Weiss, 2000). Studies suggest that the human nervous system is particularly sensitive during development (Kodavanti, 2005).

Effects of exposure

PCB exposure can have a variety of adverse effects, for adults and children alike. One believes that different PCB congeners, depending on structure and number of chlorine atoms, give different toxicological responses after exposure. The effects seen after exposure also depend on the individual`s developmental stage (Seegal, 1996).

There are several epidemiological studies on humans following exposure to PCBs, both after accidental and environmental exposure (Fonnum & Mariussen, 2009; Fonnum et al., 2006). These show that PCBs might impair cognitive function as well as affect neurological development (Fonnum et al., 2006; Schantz, Widholm, & Rice, 2003; Seegal, 1996).

Accidental exposure

There were two cases of accidental exposure to PCBs and other related chemicals in Japan and Taiwan in 1968 and 1979, respectively. 4000 people were exposed to rice oil which was contaminated during the manufacturing process (Chen, Yu, Rogan, Gladen, & Hsu, 1994;

Faroon et al., 2001). The adults exposed showed severe chloracne, thickening of the nail bed, increased skin pigmentation and peripheral nervous system symptoms, such as numbness and weakness in the limbs and decreased nerve conduction velocities. Central nervous system symptoms, such as tiredness and respiratory disturbances, were also observed (Fonnum & Mariussen, 2009; Seegal, 1996). The children in these cases were either exposed in utero or during lactation. The children born after the incident in Japan displayed dark pigmentation of the skin, early eruption of teeth and low birth weight. Some had hypotonic reflexes and were dull and apathetic (Rice, 1995; Yamashita & Hayashi, 1985). Physical deficits resembling the ones seen in the children born in Japan were also found in children born after the incident in Taiwan (Rogan et al., 1988). The Taiwan children also showed developmental delay, behavioral problems and higher activity levels (Chen et al., 1994). Both cohorts had cognitive defects such as problems with short term memory and lower scores on IQ tests (Seegal, 1996). Due to the fact that the contaminated oil also contained other chemicals, one cannot be sure that PCBs were the causative agent for the all symptoms observed (Fonnum et al., 2006; Seegal, 1996).

Environmental exposure

There are also other epidemiological studies on the effects of exposure to PCBs. In the Unites States there are studies on children whose mothers had consumed fish presumed to be contaminated with PCBs during pregnancies. This has given the opportunity to examine transplacental and lactational exposure of children (Faroon et al., 2001; Fonnum & Mariussen, 2009; Seegal, 1996). The conclusion drawn from these studies points to a correlation between levels of PCB exposure during development and delays is psychomotor development and defects in cognitive development (Seegal, 1996). This may lead to increased impulsivity, lower IQ scores, deficit in short term and long term memory, and deficit in focused and sustained attention (Jacobson & Jacobson, 2003). In addition, there are other epidemiological studies from different parts of the world, such as Holland, Germany and the Faroe Islands, where all but one found a negative association between prenatal PCB exposure and cognitive function in infancy and childhood (Schantz et al., 2003). These epidemiological studies of the neurobehavioral effects of PCBs are valuable because they provide evidence of subtle, but measureable, deficits in prenatally exposed children (Stewart et al., 2005).

Many of these studies have been criticized because there are several unmeasurable factors involved, like effects of other environmental toxins present, making it difficult to correlate the

findings to one contaminant, such as PCBs (Mariussen & Fonnum, 2006). In addition, most of the studies done regarding PCBs and health address total PCB exposure. This means that much knowledge accumulated on the topic is not congener specific (Longnecker, Rogan, & Lucier, 1997).

Animal studies

Studies examining the neurobehavioral and neurochemical effects of PCBs in animals strengthen the assumption that PCBs are a major cause of symptoms seen in humans. Still, there are several factors influencing the effects seen in animals following PCB exposure. Several species, like rats, mice and monkeys, have been exposed for various durations to different types of PCB formulations in a range of designs (Faroon et al., 2001). Route of exposure (in utero, through gavage, through mother`s milk), dose (low, moderate, high), sex and animals age at exposure are all some of the variables involved (Kodavanti, 2005), making it hard to summarize and draw conclusions in this research field.

Neurobehavioral effects

The most common findings in animals exposed to PCBs during development are effects on motor activity and cognitive functioning, memory and learning. Adult exposure is correlated with decreased motor activity (Faroon et al., 2001; Fonnum & Mariussen, 2009; Fonnum et al., 2006). The following review will discuss neurobehavioral effects according to type of PCBs, mixtures and single congeners.

There are several studies exposing animals to a commercial mixture of PCB, Aroclor 1254 (A1254). This contains a high proportion of *ortho*-substituted, highly chlorinated biphenyls (Bushnell et al., 2002). Branchi et al. (2005) exposed mice to A1254 during gestation and lactation. The results of the study showed increased activity following exposure. This was mainly seen in adulthood and may point to a long-lasting alteration throughout the life span. The mixture is also shown to have effects on spatial learning and memory in adult male, but not female rats (Roegge, Seo, Crofton, & Schantz, 2000). However, Bushnell et al. (2002) exposed pregnant rats to A1254 during gestation and nursing. A variety of neurobehavioral screening was done. It was found that the mixture had little effect on loco motor activity and visual attention during development as well as in adulthood.

There are also studies using a mixture of PCB congeners which resemble the congeners found in mother`s milk. Rice (1999;2000) exposed monkeys to the mixture from birth until they

were 20 weeks of age. It was found that they showed problems with temporal organization of behavior, perseverative behavior, problems with learning from previous consequences and inability to inhibit inappropriate responding.

Most of the studies investigating behavioral effects of PCBs use commercial mixtures, but some have used single congeners. Holene, Nafstad, Skaare, Krogh, and Sagvolden (1999) and Holene, Nafstad, Skaare, and Sagvolden (1998) did two studies where rats were exposed to either di-*ortho* substituted PCB 153 or non-*ortho* PCB 126 during lactation. These types of PCBs represent two different classes when it comes to toxicity and chemical properties. It has been generally accepted that the *ortho*-substituted PCBs (like PCB 153) have a more toxic effect than the non-ortho PCBs (like PCB 126) (Giesy & Kannan, 1998; Seegal, 1996). It was found that rats given PCB 153 and rats given PCB 126 in the studies showed a higher activity level and attention deficit on a reinforcement task. This was true for males, but not female rats (Holene et al., 1999; Holene et al., 1998). Another study exposed rats during pregnancy and lactation to PCB 153 and 126 and found impaired learning ability. This was true for young, but not adult rats (Piedrafita, Erceg, Cauli, Monfort, & Felipo, 2008). However, Bushnell and Rice (1999) exposed rats prenatally to PCB 126, and found no evidence of effects on learning or attention

Neurochemical effects

Linking the behavioral effects seen after exposure to a toxicant, such as PCB, to neurochemical changes, is a challenge since the effects probably is a result of influence on several neurochemical targets. Still, PCBs are environmental toxicants that are extensively studied, and it is found that PCBs act on both neurochemical targets, such as neurotransmitter systems, and neuroendocrine targets, such as the thyroid hormone system (Fonnum & Mariussen, 2009).

Dopamine. The most consistent finding when it comes to neurochemical targets of PCBs is the decrease in dopamine concentration in different areas of the brain. This is found in rats and non human primates (Faroon et al., 2001; Fonnum et al., 2006). The effect is primarily seen with *ortho*-substituted PCBs. Evidence suggests that PCBs are an inhibitor of dopamine uptake in synaptosomes and the synaptic vesicles (Fonnum & Mariussen, 2009; Mariussen et al., 2001; Seegal, 1996). Dreiem, Okoniewski, Brosch, Miller and Seegal (2010) examined the effects of a PBC mix called Fox River Mix (FRM) in striatal synaptosomes derived from rats at three different times, postnatally. They found that in synaptosomes from animals of all

ages, FRM significantly reduced dopamine concentrations. It is noteworthy that the effect was age dependent with an increased reduction in dopamine synaptosomal levels from 7 days old rats to 14 days old rats and 21 days old rats. This is in line with previous research done by Mariussen and Fonnum (2001). They investigated the effect of Aroclor 1242 and 1254 in addition to specific congeners with varying degrees of chlorination in rat brain synaptosomes. The overall results pointed to an inhibition of neurotransmitter uptake into synaptosomes with *ortho*-chlorinated biphenyls and a decrease in dopamine concentration. The same was found regarding uptake of dopamine in synaptic vesicles. In a study which included PCBs with large structural variations, the results showed that the *ortho*-substituted PCBs inhibited dopamine uptake (Mariussen et al., 2001).

Other neurotransmitter systems. Serotonin is another neurotransmitter believed to be affected by PCB exposure. In the same way as dopamine, a decrease in serotonin levels is seen in the brain after PCB exposure (Fonnum & Mariussen, 2009). Rosin and Martin (1981) did a study on isolated mice brain synaptosomes and found inhibited serotonin uptake following exposure to various concentrations of PCB (as cited in Seegal, 1996, p 726). Khan and Thomas (2004) studied the effect of PCB exposure on serotonin in the rat brain. Rats were given a single dose of A1254 and the results showed an inhibition of tryptophan hydroxylase (TPH) activity and reduced serotonin (5-HT) concentration in selected brain areas. However, in a study done by Kodavanti et al.(1998), no change in TPH were found in the rat brain after postnatal exposure to Aroclor 1254.

In addition to dopamine and serotonin, it is believed that other neurotransmitters might be involved in the effects seen after PCB exposure, such as glutamate and GABA (Mariussen & Fonnum, 2001). Mariussen and Fonnum (2001) found that PCB mixtures, A 1242 and A 1254, inhibited glutamate and GABA uptake in rat brain synaptosomes. Further testing with specific congeners showed that *ortho*-PCBs with five, or less than five chlorines inhibit uptake, while hexa- or hepta- chlorinated PCBs were found to be partial or poor inhibitors. The effects found on uptake of glutamate and GABA may play a part in PCB mediated influence of cognitive functions (Mariussen & Fonnum, 2001).

When researching the effects of PCBs on neurotransmitters, one should be aware of the fact that there is an extensive interaction between all the systems. This makes it hard to draw any conclusions regarding the links between behavioral effects seen and neurotransmitter systems involved (Myhrer, 2003).

PCB and related disorders

Some of the behavioral effects and the consistent findings of decreased dopamine levels seen in PCB exposed animals resemble the aeitology of other disorders. Attention deficit hyperactivity disorder is one disorder found to have a dopamine deficiency.

Attention deficit hyperactivity disorder

Attention deficit hyperactivity disorder (ADHD) is a heterogeneous neurobehavioral disorder with onset in childhood. It affects 8-12% of children worldwide and is characterized by inattention, impulsivity and hyperactivity (Faraone, Sergeant, Gillberg, & Biederman, 2003). The attention problems in ADHD children typically includes difficulty in sustaining attention with lack of persistence and distractibility, and the hyperactivity-impulsivity dimension includes impulsive responding and excessive motor activity (Lahey et al., 1998). The Diagnostic and Statistical Manual of the American Psychiatric Association 4th edition (American Psychiatric Association, 1994) defines ADHD based on the two behavioral dimensions and these allows for three subtypes of ADHD (1) predominantly inattentive subtype, (2) predominantly hyperactive / impulsive subtype and (3) the combined subtype. The symptoms are associated with functional impairments such as problems at school and conflicts with peers and family (Biederman & Faraone, 2005). The problems may persist into adulthood, but there is an age dependent decline in symptoms. In adults, there is also an absence of the hyperactivity seen in childhood which makes it more difficult to diagnose adults than children (Knutson & O'Malley, 2010).

The aeitology of ADHD is not fully understood, but it is a heritable disease, and studies suggest a heritability of about 80% (Smith, Mick, & Faraone, 2009). Molecular genetic studies together with pharmacological studies of stimulant drugs and neuroimaging give support to a dopamine deficiency in ADHD (Biederman & Faraone, 2005; Varrone & Halldin, 2010). It has been suggested that a hypofunctioning dopamine system is central in the disorder and may result in a changed basic learning process (Sagvolden, Johansen, Aase, & Russell, 2005). Still, evidence points to an interaction between genetic, environmental and social factors (Pennington et al., 2009).

Exposure to different environmental toxins like lead, mercury or PCBs during early human development is identified as factors that may contribute to ADHD (Williams & Ross, 2007). Research on both ADHD and PCBs has shown that prenatal exposure to PCBs results in behavioral impairments that share significant similarities with ADHD (Eubig, Aguiar, &

Schantz, 2010). Although the underlying mechanisms are not clear, it is believed that PCBs acts on different neurotransmitter systems assumed to be involved in the development of ADHD.

Animal models of neurobehavioral disorders

Most of the studies regarding the relation between PCBs and the effects observed are done using animal models. Animal models help to simplify and promote an understanding of the topic of interest (Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). Van der Staay, Arndt and Nordquist (2009) define animal models in behavioral neurosciences as follows:

> *An animal model with biological and/or clinical relevance in the behavioral neurosciences is a living organism used to study brain-behavior relation under controlled conditions, with the final goal to gain insight into, and to enable predictions about, these relations in humans and/or a species other than the one studied, or in the same species under conditions different from those under which the study was performed.* (p. 133-134)

A model of a neurobehavioral disorder needs to be broken down to phenotypes that are observable, measureable and testable. This implies elements that can be observed and measured directly, elements assigned qualitative or quantitative attributes and, finally, measures that can be submitted to statistical analysis, in order to confirm or falsify a hypothesis (Smoller & Tsuang, 1998).

Animal models of ADHD

There are several animal models of ADHD (Sagvolden, 2000). Rodent models have proven to have several advantages for the purpose. Rats are genetically homogenous, inexpensive to maintain, and easily available. Moreover, the researcher has control over certain important variables, like diet, environment and learning history (Russell, Sagvolden, & Johansen, 2005).

A valid model of ADHD needs to meet certain criteria. An ADHD model must mimic the behavioral characteristics of ADHD, it must conform to a theoretical rationale for the disorder and it must predict previously unknown aspects of behavior, genetics and neurobiology. It is shown that Spontaneously Hypertensive rats (SHR) fulfill many of the criteria in addition to compare well with clinical cases (Sagvolden et al., 2005). SHR shows all the behavioral characteristics of ADHD with hyperactivity, impulsiveness and impaired sustained attention

(Russell et al., 2005; Sagvolden, 2000). The SHR are bred from progenitor Wistar Kyoto rats (WKY). WKY often serve as valid control for SHR due to the fact that their behavioral characteristics are similar to that of other rat strains when tested in operant tasks (Sagvolden, 2000).

Aims of the study

The present study investigated behavioral effects in rats postnatally exposed to different doses of PCB 153; an *ortho*-substituted PCB, as compared to a non exposed control group. This specific congener was chosen because it is widespread in the environment, bioaccumulates in humans and wildlife and is found in human breast milk (Mariussen et al., 2001). Two strains of rats, SHR and WKY, were orally given a mixture of corn oil and PCB three times between post natal day 8 and 20 at a dose of 1, 3 or 6 mg/kg body weight or only corn oil. The behavioral effects were measured as changes in activity, impulsivity and attention using an operant test shown reliable for the purpose (Sagvolden, 2000; Sagvolden & Xu, 2008).

The aim of the present study was to investigate whether SHR rats, an animal model of ADHD, are more vulnerable than controls to exposure of PCB 153 and if exposure to PCB might give ADHD-like symptoms in WKY rats. The study also explored whether there will be a dose-response effect in the exposed animals and if there is a sex difference in behavioral response following exposure.

Method

This method has previously been described (Sagvolden & Xu, 2008).

Subjects

In this study a total of 212 rats were behaviorally tested. There were 104 Spontaneously Hypertensive rats (SHR/ NCrl) from Charles River, Germany, and 108 Wistar Kyoto rats (WKY/ NHsd) from Harlan, England.

During the first three weeks, the rats were under the care of a veterinarian at the Norwegian Defense Research Establishment, Kjeller. At this time the PCB was administered. At postnatal day (PND) 24 the rats were sent to the University of Oslo for behavioral testing. The rats were experimentally naive on arrival. Young rats were required since ADHD primarily is a child and adolescent disorder (Sagvolden & Xu, 2008).

During habituation and shaping the rats were housed together in twos in transparent cages of 41 x 25 x 25 (height) cm. Following acquisition of lever pressing and throughout the rest of the study, they were housed in individual cages of the same size.

The rats had access to food (RM3 (E) from Special Diet Services, Witham, Essex CM8 3AD, UK) in their home cages at all times, and free access to water at all times prior to the experimental procedure. When the experimental procedure started and through the rest of the study, they were deprived of water for 21.5 hours pr. day to ensure sufficient motivation. During the experimental sessions they were given water as reinforcers and they had free access to water for 50 minutes in their home cages immediately following each day`s sessions.

The cages were kept in a housing area with a stable temperature of about 22 °C. The light was on from 0700 to 1900 hours every day for a period of 40 days. The experimental sessions took place between 1000 and 1500 hours seven days a week.

This study was approved by the Norwegian Animal Research Authority (NARA) and was conducted in accordance with laws and regulations controlling experiments and procedures with live animals in Norway.

Apparatus

Sixteen Campden Instruments operant chambers were used in the experiment. They were located in two different rooms with eight chambers in each that were run by two different computers. Each chamber was enclosed in a light and sound resistant outer housing, and was ventilated and equipped with a grid floor. The animals´ working space in eight of the chambers were 25 x 25 x 30 (height) and 25 x 25 x 20 (height) in the other eight chambers.

A house light of 2.8 W, located in the ceiling, and a fan ensuring air circulation and producing low masking noises were on during the entire experimental session.

Each chamber was equipped with two retractable levers. Above both of these a 2.8 W cue light was located. The levers required a dead weight of at least 3 g to activate a micro switch. A liquid dipper delivered a drop of water (0.05 ml) as a reinforcer when activated. The dipper was located in a small cubicle separated from the rats´ working space by a transparent plastic flap of 7 x 5 cm. A 2.8 W cue light lit up in the cubicle when a reinforcer was presented and the rat could easily open the flap with a light push with the nose or paw to collect the reinforcer. The reinforcer was available for 3 s after opening of the flap. If it was not collected within 5 s after activation, the light would be turned off and the drop of water would no longer be available.

Before the experiment started, the rats were assigned to the different cages and given a time for testing. This was done in a randomized and balanced fashion. Each rat was tested in the same chamber throughout the experiment and was returned to its living cage after each session.

The animals´ behavior was recorded by a computer program, Lab VIEW 7.1 (National Instruments, 2004). This program also scheduled lights and reinforcers.

Procedure

PCB exposure

The animals were randomized and assigned either to an experimental group or to a control group. Because the experiment was done over a period of time (from spring 2008 to spring 2009), with different doses of PCB 153 tested at different times, the animals were not randomized and assigned to their group all at once. Still, the procedure was the same each time with the different groups. The animals in the experimental group were orally given PCB

dissolved in corn oil, while the animals in the control group were exposed to corn oil only. The animals in the experimental group were exposed to one of three doses of PCB 153 (2, 2`, 4, 4`, 5, 5`- Hexachlorobiphenyl). Group 1 received 1 mg/kg, group 2 was fed 3 mg/kg and group 3 was given 6 mg/kg purchased from Patrick Anderson, Department of Chemistry, University of Umeå, Sweden. Group size after randomization is shown below in table 1.

Table 1
Group size after randomization

	Group 1 PCB 1 mg/kg		Group 2 PCB 3 mg/kg		Group 3 PCB 6 mg/kg		Control Corn oil	
	Male	Female	Male	Female	Male	Female	Male	Female
SHR	12	13	12	13	16	12	14	12
WKY	12	12	14	16	14	14	14	12

The animals were exposed to PCB at PND 8, PND 14, and PND 20 at a dose of 1, 3 or 6 mg/ kg each time. Time and route of exposure were chosen to reflect the transferal through mother`s milk in humans. The administration was done by a veterinarian at the Norwegian Defense Research Establishment, Kjeller.

Habituation, dipper training and shaping

Before the experimental sessions started, the animals went through a period of training. Habituation training started the day following arrival at the lab, at PND 25, and lasted 30 min. During this session, the flap between the working space and the cubicle was taped open. The house light was on, but no levers were present, no cue lights were on above the levers and no reinforcers were delivered. After this training session and throughout the rest of the training and the experimental sessions, the rats were deprived of water for 21.5 hours a day.

The following day the rats went through two 30 min dipper training sessions. The flap was taped open, both levers were retracted and the cue lights were off. A variable time schedule was used, so independent of the rats' behavior the computer delivered a drop of water on average every 10 s. The cue light lit up in the cubicle with each water delivery.

In the following two sessions, the tape was removed from the flap and the rats were trained to open the flap to gain access to the drop of water. The levers were retracted and the cue lights above the levers were off. Each flap opening turned on the cue light in the cubicle and

produced a presentation of a single drop of water. Irrespective of the animals' behavior, the water dipper was lowered after 5 s. The rats were left in the cages until they had fully learned the procedure of the flap opening.

During the subsequent two sessions lever pressing was shaped according to the method of successive approximation (Catania, 1998). In the first session, the left lever was present while the right was still retracted. The cue light above the left lever was lit while it was turned off above the right lever. Every time the left lever was pressed, the rat could collect a reinforcer in the cubicle immediately following each press. During the presentation of the reinforcer, the light in the water cubicle was turned on while the cue light was turned off. On the second session the right lever was present while the left lever was retracted. During this session the cue light above the right lever was lit while it was turned off above the left lever except during the presentation of the reinforcer when the light in the water cubicle was lit. After both sessions of response shaping, the rat was monitored to make sure that the response was learned. It was then left in the chamber for another 15 min to strengthen the newly learned behavior.

Variable interval 3 s schedule

The shaping procedure was followed by five 30 min long training sessions (sessions 8-12) using a variable interval (VI) 3 s reinforcement schedule. During these VI 3 s sessions and throughout the rest of the study both levers were present at all times. The computer program semi-randomly selected which lever would produce the reinforcer. Lever selection was limited to a maximum of 4 consecutive reinforcers on the same lever. This was to avoid the development of lever preference. The lever associated with reinforcers was signaled by the lit cue light above the lever (discriminative stimulus). The cue light stayed lit for as long as the lever was associated with reinforcers, but was turned off during presentation of the reinforcer when the light in the cubicle was on. When the dipper was presented, the timer for the next interval started. Scheduled reinforcers and produced reinforcers which were not collected were accumulated and scheduled for the first correct response. Reinforcers were available for 3 s after the flap into the cubicle was opened, except during habituation and dipper training sessions. After 3 s, the dipper was lowered and the cubicle light was turned off. If the flap into the cubicle was not opened within 5 s after a reinforcer presentation, the dipper was lowered and the light in the cubicle was turned off.

The VI 3 s schedule combined with the interval timer starting when the dipper was presented, accumulation of scheduled reinforcers, and the 3 s access to the reinforcers had the effect that practically every response produced a reinforcer. A concurrent extinction schedule was in effect for the lever which would not produce a reinforcer. The light above the extinction lever was turned off at all times. Due to this, the task can be described as a simultaneous visual discrimination task.

Variable interval 180 s schedule

The initial five training sessions were followed by 28 sessions using a variable interval (VI) 180 s schedule (session 13 - 40) which lasted for 90 min. This program was run for 28 days. This VI 180 s schedule scheduled reinforcers on the average every 3 minutes. A concurrent extinction schedule was in effect for the lever which would not produce a reinforcer. The inter-reinforcer intervals during the VI 180 s ranged from 6 s to 719 s in a semi-randomized fashion and with a constant probability distribution after the Catania-Reynolds distribution (Catania & Reynolds, 1968). The animals were given no indications as to when a reinforcer was programmed, or how much time had passed since last response.

Table 2 shows the behavioral procedure and reinforcement schedules used.

Table 2
Summary of the experimental procedure.

Behavioral procedure	Session number	Reinforcement schedule
Habituation	1	
Dipper training	2-3	VT 10s
Flap training	4-5	CRF
Lever pressing	6-7	Response shaping
Training sessions	8-12	VI 3 s
Main schedule	13-40	VI 180 s

Note. VT = variable time schedule. CRF = continuous reinforcement schedule. VI = variable interval schedule.

Behavioral measures

Each 90 min session with the VI 180 s schedule was divided into 5 segments, each with a time duration of 18 min. This was done to monitor intra-session changes in the rats´ behavior. For each segment, the computer recorded total number of presses on the lever producing

reinforcers and on the lever not producing reinforcers, number of flap openings to the cubicle, numbers of reinforcers produced and collected and the time of the events. Hyperactivity, impulsivity and sustained attention were then calculated based on the recordings in the following way:

Hyperactivity: Activity level was measured by the total numbers of lever presses on the two levers combined.
Impulsivity: The time interval between two consecutive lever presses, inter-response times (IRTs), were recorded and divided into short IRTs (< 0.67 s) and long IRTs (>0. 67 s). Responses closer than 0.67 s in time will almost never produce a reinforcer and can be used as a measure of impulsivity ("inability to wait" or "inability to withhold inadequate behavior")
Sustained attention: To produce a reinforcer the animal had to pay attention to the cue light above the lever in order to press the lever producing the reinforcers. The percentage of presses on the lever associated with reinforcers was then used as a measure of sustained attention. If the animal paid attention to the cue light and consequently pressed the lever due to this signal, percentage correct would be high. If the animal did not pay attention to the cue light and pressed both levers equally often, the percentage would be at chance level (about 50%). Stimulus control is the behavioral term for percent correct.

Data analysis

Data management was done in Excel (Microsoft, 2003) and SPSS 14.0 (SPSS, 2005) but all statistical analyses were done in Statistica 7.0 (StatSoft, 2005). Data were evaluated by univariate analyses of variance (ANOVAs) and multivariate analysis (MANOVAs) using Wilks lambda when the degrees of freedom relative to the number of levels of the repeated factor permitted this approach. Post hoc tests on main effects were performed using Unequal N HSD, which is a test for differences in group means where the N in each group is unequal (StatSoft, 2011). Sessions were used as the within-subject factor, while treatment was used as the between-subject factor. Segment 5 in the 28 sessions (13-40) on the VI 180 s schedule was used in the analysis.

Missing data were substituted by calculating the means from the previous and the following session. Grubbs´ test was used to identify outliers. The test assumes that the data set comes from a normally distributed population. To check for this, normal probability plot was used. Grubbs´ test is based on the difference of the mean of the sample and the most extreme data considering the standard deviation. Grubbs have tabulated critical values for Z which

increases with sample size. These values were used for determining outliers in each group according to group size (Grubbs, 1969).

Results

General

In general, the rats showed different behavior during variable interval (VI) 3 s, sessions 8-12, compared to the variable interval (VI) 180 s, sessions 13-40.

During the VI 3 s there were no differences between the SHR and WKY. All the animals showed a low activity level, no impulsivity and no problems with sustained attention. This was probably due to shorter session length (30 min) and a relatively larger proportion of the time spent drinking water because the rats produced more reinforcers on VI 3s than on VI 180 s.

During the VI 180 s the overall activity level increased, as did impulsivity. The SHR had a higher number of total lever presses and more short IRTs (<0.67s) than WKY. This is shown in figure 2 and figure 3 below.

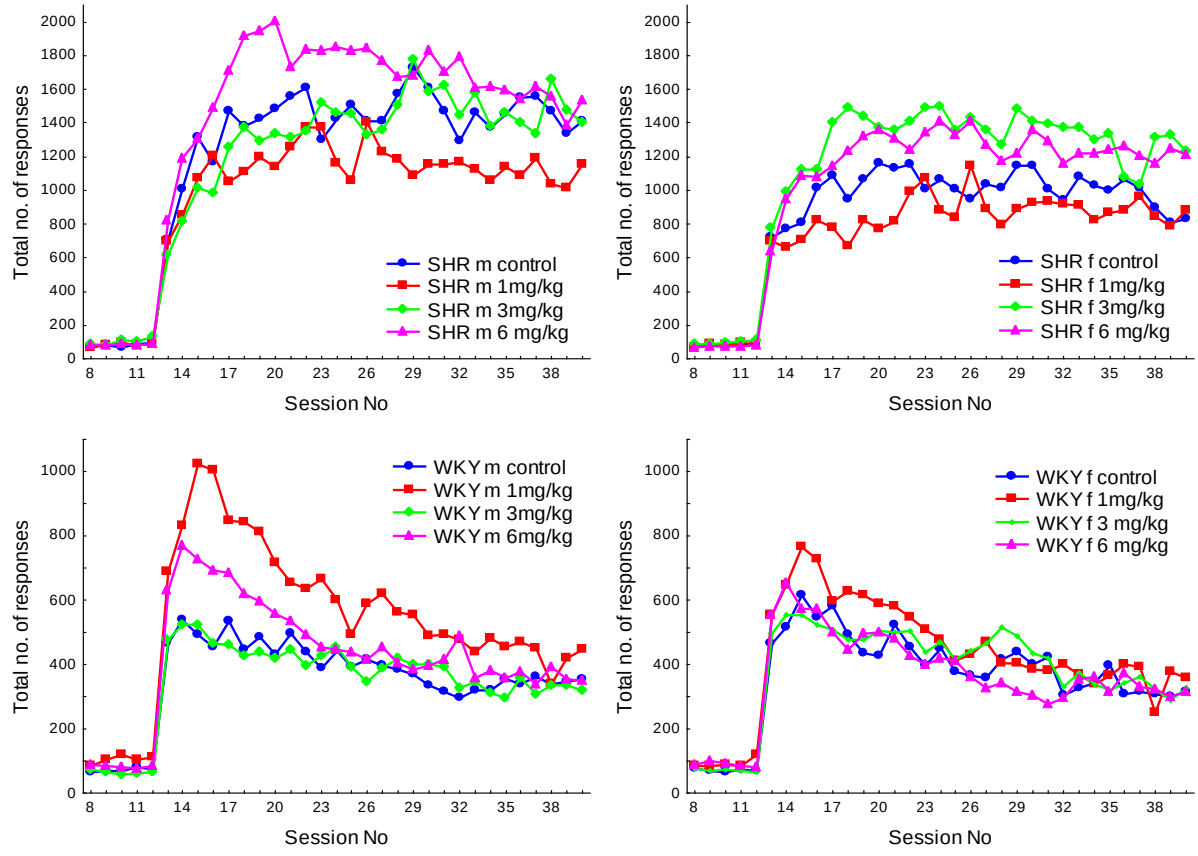

Figure 2. Activity level across sessions.

Note. Different scaling on the y-axis. Top: 0 - 2000. Bottom: 0 - 1100. m= male. f= female.

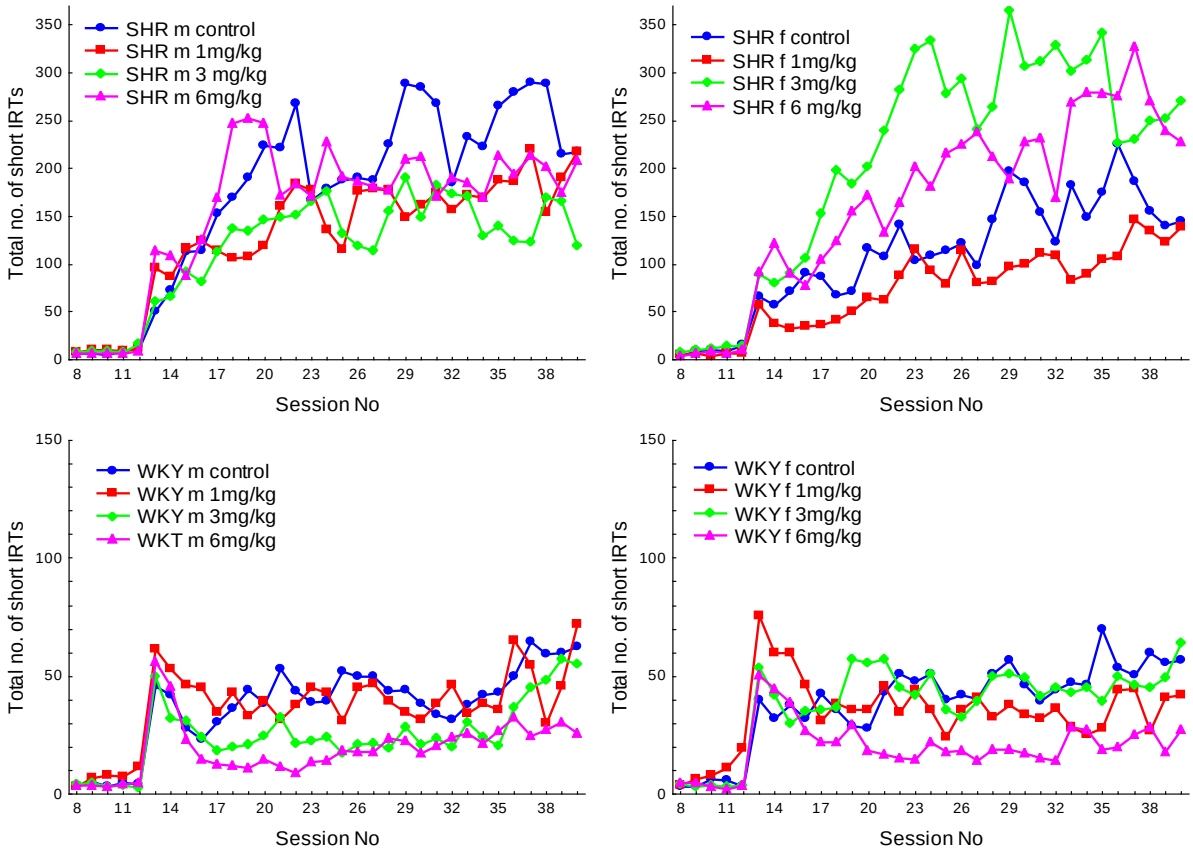

Figure 3. Total number of short ITRs.

Note. Different scaling on the y-axis. Top: 0 - 350. Bottom: 0 - 150. m= male. f= female.

Sustained attention, proportion of percent correct lever presses, decreased on VI 180 s. Both WKY and SHR dropped from about 80% to 85% correct lever presses on the VI 3 s to between 55% and 65% on the VI 180 s as shown in figure 4.

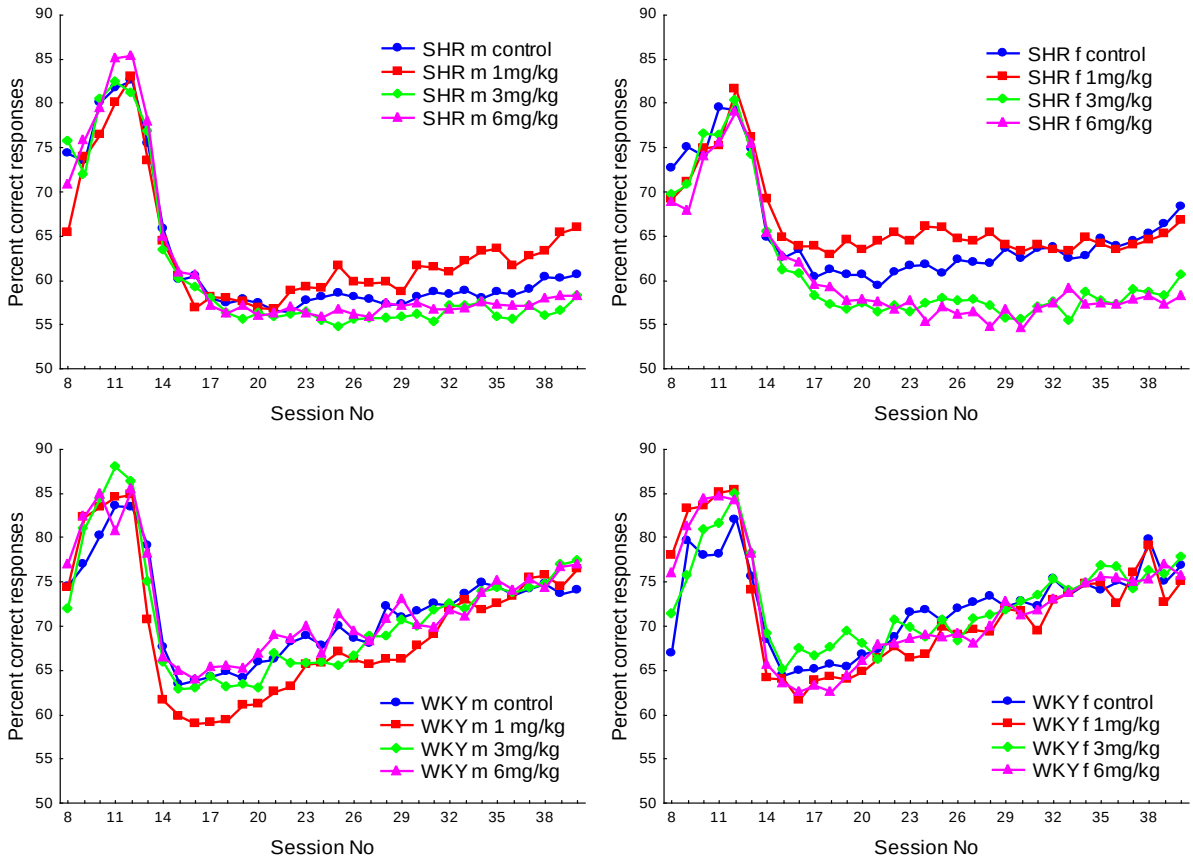

Figure 4. Percent correct responses.

Note. m= male. f= female.

Variable Interval 180 s schedule

Activity

The results from the analyses showed that the SHR were significantly more active than the WKY and produced more lever presses. The males were more active than females and the activity level was affected by the dose the animal was given. The animals exposed to 1mg/kg of PCB were less active than controls, while the animals exposed to 3mg/kg and 6mg/kg of PCB were more active than controls. The activity level decreased from session 0 to session 28. It was also shown that the activity level seen in the SHR and WKY depended on the animals' sex as well as the dose they were given. The SHRs were less active than controls given 1mg/kg of PCB and more active than controls given 3mg/kg and 6mg/kg of PCB. The WKY showed the same activity level as controls regardless of the dose they were given. Males were most active given 6mg/kg and females most active given 3mg/kg of PCB. Both SHR and WKY showed a different activity level across sessions, as did males and females.

All the significant results from the analysis of activity are displayed in table 3 below. No other significant effects were found.

Table 3
Results from univariate analysis of variance, ANOVA, and multivariate analysis, MANOVA.

Measure	Variable	ANOVA		Multivariate	
		Df	F	Df	F
Activity	Strain	1.172	664.973***		
	Sex	1.172	30.408***		
	Dose	3.172	6.388***		
	Session	27.464	26.086***	27.146	14.985***
	Strain*Sex	1.172	13.505***		
	Strain*Dose	3.172	15.171***		
	Sex*Dose	3.172	2.911*		
	Session*Strain	27.464	52.098***	27.146	21.157***
	Session*Sex	27.464	1.635*	27.146	1.771*
	Session*Dose	81.464	4.539***	81.437	4.927***
	Session*Strain*Sex	27.464	2.147***	27.146	2.197**
	Session*Strain*Dose	81.464	3.223***	81.437	3.639***
	Session*Sex*Dose	81.464	1.966***		

Note. *:p< .05. **:p< .01. ***:p< .001

Post hoc analyses of sex, strain and dose using Unequal N HSD showed that there were statistically significant differences within the groups of the SHR. The male SHR rats that were given 1mg/kg and 6 mg/kg of PCB were significantly different from each other (p<.001). The female SHRs were also different from each other. There was a significant difference between the controls and the group that received 3mg/kg of PCB (p<.05). There were also differences between the group that received 1 mg/kg of PCB and the ones that received 3mg/kg (p<.001) or 6mg/kg of PCB (p<.001). It was seen that the ones that got 3mg/kg of PCB were different from both controls (p<.05) and the ones that got 1mg/kg of PCB (p<.001). Finally the ones that got 6 mg/kg of PCB were different from the ones that got 1mg/kg of PCB (p<.001). There were no significant differences within the WKY group regarding activity. Group differences on activity level are shown in figure 5.

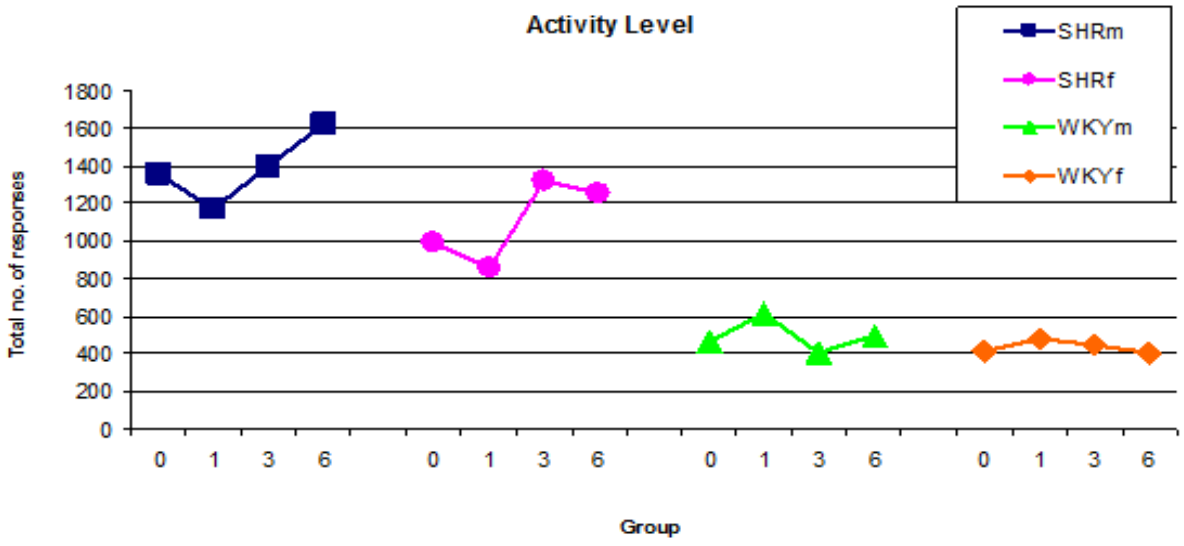

Figure 5. Group differences on activity level.

Note. m= male. f= female. 0=control group. 1= 1mg/kg. 3= 3mg/kg. 6= 6mg/kg.

Impulsivity

There results from the analysis showed that SHR had more short IRTs than the WKY and that short IRTs increased from session 0 to session 28. It was observed that the SHR showed a reduction in impulsivity when given 1mg/kg of PCB in comparison with the control group and there was an increase in impulsivity when they were given 3mg/kg and 6mg/kg of PCB compared to the control group. The WKYs showed no significant effect of the different doses on measures of impulsivity. The males showed most short IRTs when given 6mg/kg of PCB, while the females had most short IRTs when given 3mg/kg. The SHR and WKY showed a different level of impulsivity across sessions and males and females were different across sessions depending on the strain. All the significant effects of the analysis regarding impulsivity are displayed in table 4 below. No other significant effects were found.

Table 4

Results from univariate analysis of variance, ANOVA, and multivariate analysis, MANOVA.

Measure	Variable	ANOVA		Multivariate	
		Df	F	Df	F
Impulsivity	Strain	1.154	122.869***		
	Session	27.415	16.201***	27.128	5.054***
	Strain*Dose	3.154	3.146*		
	Sex*Dose	3.154	4.397**		
	Session*Strain	27.415	15.660***	27.128	4.821***
	Session*Dose	81.415	1.930***	81.383	2.650***
	Session*Strain*Sex	27.415	2.148***	27.128	1.807*
	Session*Strain*Dose	81.415	1.587***	81.383	2.014***
	Session*Sex*Dose	81.415	1.415**	81.383	1.444*

Note. *:p< .05. **:p< .01. ***:p< .001

Unequal N HSD follow-up analysis of strain, sex and dose showed a significant difference between the SHR females that received 1 mg/kg of PCB and 3 mg/kg of PCB (p<.001).There were no significant differences between the WKY groups. Group differences are shown in figure 6.

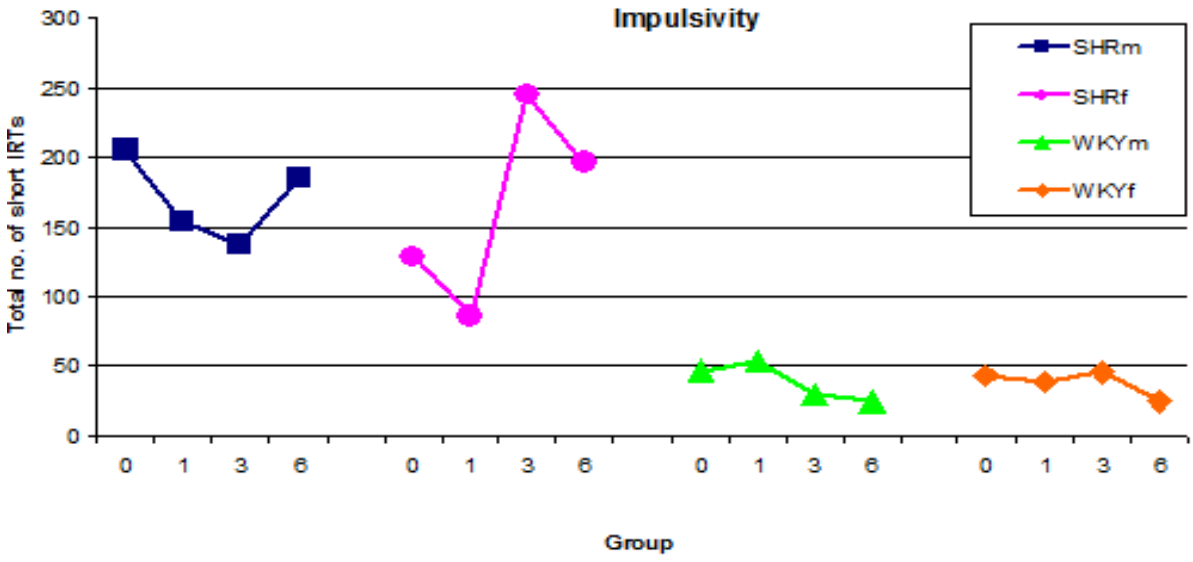

Figure 6. Group differences on impulsivity.

Note. m= male. f= female. 0= control group. 1= 1mg/kg. 3= 3mg/kg. 6= 6mg/kg.

Sustained attention

The results from the analysis showed that the SHR had fewer percent correct responses than the WKY and the females had more correct responses than males. There was also observed

that sustained attention was improved from session 0 to session 28. The analyses showed that the SHR had better sustained attention when given 1mg/kg of PCB and poorer sustained attention when given 3mg/kg and 6mg/kg of PCB, while the WKY did not show any differences in attention in relation to dose given. Both SHR and WKY showed different levels of attention across sessions. All the significant effects of the analysis regarding sustained attention are displayed below in table 5. No other significant effects were found.

Table 5

Results from univariate analysis of variance, ANOVA, and multivariate analysis, MANOVA.

Measure	Variable	ANOVA		Multivariate	
		Df	F	Df	F
Attention	Strain	1.178	202.730***		
	Sex	1.178	7.690**		
	Session	27.480	105.800***	27.152	65.143***
	Strain*Dose	3.178	5.050**		
	Session*Strain	27.480	56.880***	27.152	7.470***
	Session*Dose	81.480	2.370***	81.455	1.470**

Note. *:p< .05. **:p< .01. ***:p< .001

Follow- up analysis with Unequal N HSD showed no significant effects. Group differences are shown in figure 7.

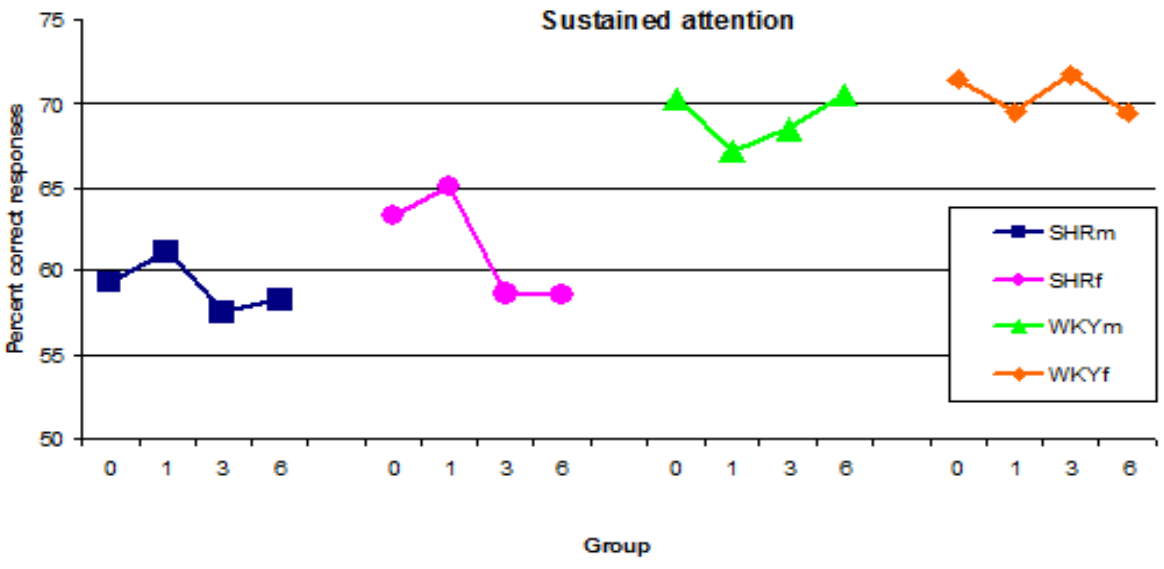

Figure 7. Group differences on sustained attention.

Note. m= male. f= female. 0= control group. 1= 1mg/kg. 3= 3mg/kg. 6= 6mg/kg.

Reinforcers produced and reinforcers collected

The ANOVA showed that there were no group differences on reinforcers produced, but there was a statistically significant difference on reinforcers collected between the groups, $F(15,195) = 3$; $p = 0.002$. The group that collected the most reinforcers collected on average 28.536 reinforcers, and the group that collected the least reinforcers collected on average 27.995 reinforcers. The mean difference between the two groups was .54 reinforcers. Due to the fact that the variance was small, even very small effects were statistically significant.

Discussion

The present study investigated behavioral effects in rats postnatally exposed to three different doses of the di-*ortho*-substituted PCB 153 as compared to a non-exposed control group. The PCB was administered orally three times between postnatal day 8 and 20 at a dose of 1mg/kg, 3mg/kg or 6mg/kg. The effects of exposure were examined in an animal model of ADHD and in controls using a procedure developed for studying behavioral changes on measures of hyperactivity, impulsivity and sustained attention.

The aim was to investigate whether the Spontaneously Hypertensive rat (SHR) was more vulnerable than controls to exposure to PCB 153 and if the Wistar Kyoto rat (WKY) would show ADHD-like behavior following exposure. The study also explored the possibility of a dose-response effect and whether there was a sex difference in behavioral response following exposure.

The procedure used can be described as an operant visual discrimination task measuring activity as total number of lever presses, impulsivity as responses with inter-response times shorter than 0.67 s, and sustained attention as percentage responding on the lever producing reinforcers.

The results showed a statistically significant difference between SHR and WKY after exposure to different doses of PCB 153. SHRs were more active, more impulsive and had poorer sustained attention in comparison with the WKYs. The WKYs did not show any behavioral effects following exposure. There was an effect of dose in the exposed SHR. The SHR given 1mg/kg of PCB were different from SHR given 3mg/kg and 6mg/kg of PCB on all behavioral measures. Animals exposed to 1mg/kg of PCB showed a reduction in activity, were less impulsive and had better sustained attention in comparison with the animals exposed to 3mg/kg of PCB, 6mg/kg of PCB and controls. In general, the animals given both 3mg/kg and 6mg/kg of PCB showed a higher level of activity, were more impulsive and had poorer sustained attention than controls. No effects were seen in the WKYs. Both male and female SHR showed effects of exposure to PCB and there were sex differences within the SHRs regarding effect of dose. The males were more active and showed increased impulsivity given 6mg/kg of PCB, the same was true for females given 3mg/kg of PCB. No differences were observed on sustained attention. No sex differences were found within the WKY groups.

Vulnerability to PCB exposure in SHR and WKY

Results from previous studies on animals exposed to PCB 153 have come to different conclusions regarding behavioral effects on activity, impulsivity and sustained attention using different strains of animals (Berger et al., 2001; Holene et al., 1999; Holene et al., 1998). Holene et al. (1999) and Holene et al. (1998) used an experimental design similar to the one used in the present study. Male and female rats, from a strain that resembles the WKY, were postnatally exposed to PCB 153, 5mg/kg. It was reported behavioral changes in males, but not female rats, showing the behavioral pattern of hyperactivity, impulsivity and impaired sustained attention seen in the SHR model. This is opposite of what was found in the present study where the WKYs, both males and females, were unaffected by exposure. Similar results as Holene et al. (1999) and Holene et al. (1998) reported, were found by Berger et al. (2001). They studied male Sprague Dawley rats exposed to low doses of PCB contaminated fish or to a commercial mixture of PCB, Aroclor 1248, containing PCB 153. Other studies have found impaired learning ability in young, but not adult, Wistar rats exposed to PCB 153 and PCB 126. The impairment was seen in both males and females when tested in a Y-shaped maze (Piedrafita et al., 2008). Others have not found any changes on behavioral measures, such as activity, learning and sustained attention in Long-Evans rats exposed to the PCB mixture Aroclor 1254. This contains a high proportion of *ortho*-substituted biphenyls, like PCB 153 (Bushnell et al., 2002). It has also been reported that adult Long-Evans rats have become hypoactive after acute and repeated exposure to Aroclor 1254 (Nishida, Farmer, Kodavanti, Tilson, & MacPhail, 1997). The different results reported in studies using different strains of animals, supports a notion that effect of PCB exposure is dependent on the strain used in a study together with variables like different experimental design, different doses used and route of exposure.

Due to the fact that behavioral changes were only found in SHRs in the present study, it seems that SHRs are more vulnerable to PCB exposure than WKYs. The underlying mechanism for differences in vulnerability might be related to genetics. Previous research has shown that expression of many genes is different in different strains of rats. It is also observed an alteration in gene expression after exposure to PCB (DasBanerjee et al., 2008). Genes and environment might interact, resulting in specific neurochemical changes that give similar symptoms, but one might hypothesize that they also might cause different symptoms and that there are different effect of toxins.

Effects of different doses

In toxicology the term hormesis is used to describe a dose-response relationship where there is a stimulatory response to a low dose and an inhibitory response to a high dose of a chemical, resulting in an inverted U-shaped dose-response. The effects of a low dose are often thought of as a beneficial response to a stressor agent, but the opposite is true for effects of a high dose (Calabrese, 2010; Calabrese & Baldwin, 2001; Stebbing, 2003). Calabrese and Baldwin (2001) concluded that this phenomenon is applicable in biological, pharmacological and other bio-medical disciplines. Hormesis might be implicated in, and describe the non-linear dose-response relationship observed in SHR behavior following exposure to different doses of PCB.

Activity level

Rats given 3mg/kg and 6mg/kg of PCB became more active than rats in the control group in the present study, and this is in line with other research where it is reported a higher activity level after exposure to moderate doses of PCB (Berger et al., 2001; Holene et al., 1999; Holene et al., 1998). However, our observation of decreased activity in the group exposed to 1mg/kg of PCB in comparison with a control group, is contradictory to these results. Hypoactivity has, however, been found in rats given high doses of A 1254. The larger dose the rats were exposed to, the more hypoactive they became (Nishida et al., 1997). This is a linear dose-response as opposed to the curvilinear relationship observed in the present study. Hypoactivity was also found in a study using the same experimental design as in this study, where rats were exposed to PCB 153, 10mg/kg (Johansen et al., 2011).

The findings from previous studies regarding hypoactivity in rats exposed to high doses of PCBs may be linked to the effects *ortho*-substituted PCBs have on the dopamine system (Fonnum & Mariussen, 2009). Previous studies have shown a dose-dependent reduction in dopamine transporter levels (DAT) in mice exposed to A1254 and A 1260, 7.5 mg/kg and 15mg/kg (Caudle et al., 2006), resulting in a lower activity level. Possible mechanisms for these effects could be that low doses give specific effects, while higher doses influence on a more systemic level.

Taken together, there are different findings regarding effects of different doses of PCB, where both hyperactivity and hypoactivity is reported. This might suggest a non-linear dose-response relationship between dose and behavioral outcome.

Impulsivity

Increased impulsivity was seen in SHRs exposed to 3mg/kg and 6mg/kg of PCB in the present study. Previous studies have also shown increased impulsivity in rats exposed to PCB 153 (Holene et al., 1999; Holene et al., 1998). Another study exposed rats to 0, 1, 3 or 6 mg/kg of a PCB mixture and tested them on different reinforcement schedules. The results showed that developmental exposure to the highest dose of PCB resulted in more responses with short inter-response times (IRTs). (Sable, Powers, Wang, Widholm, & Schantz, 2006). One study has, however, reported a decrease in impulsivity after exposure to a high dose of PCB 153 (Johansen et al., 2011). In our study, there was seen a decrease in impulsivity after exposure to a low dose of PCB, 1mg/kg, in comparison with controls.

A hypothesized mechanism underlying the increased impulsivity seen after exposure to 3mg/kg and 6mg/kg of PCB might be related to dopamine. Developmental exposure to PCB has been shown to reduce dopamine concentration in some areas of the rat brain (Mariussen & Fonnum, 2001). These alterations seem to be related to inhibition of the dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT) (Sable et al., 2006; Jones & Miller, 2008).

As described above, there was a decrease in impulsivity, with fewer short IRTs, after exposure to 1mg/kg of PCB in the present study. An alternative explanation for the observed effect, could be that activity level is linked to other responses, such as impulsivity. A response pattern with fewer short IRTS could be secondary to a decreased activity level as a result of exposure to 1mg/kg of PCB.

Attention

The results from this study showed that PCB has an effect on sustained attention. The animals given 1mg/kg of PCB displayed better sustained attention, while the animals given 3mg/kg and 6mg/kg of PCB showed poorer attention in comparison with the control group. The effect was smaller than what was observed regarding activity and impulsivity. Poorer sustained attention following PCB exposure is in line with other research findings (Holene et al., 1999; Holene et al., 1998). However, Bushnell et al. (2002) found no effects on attention after exposure to A 1254.

Hormesis. Together with previous findings and the results from the present study, it seems possible that an inverted U-curve can be used to describe the effects of different doses following PCB exposure. In this study it was found that a low dose resulted in a decrease in both activity and impulsivity and an increase in sustained attention in comparison with a control group. The doses of 3mg/kg and 6mg/kg of PCB resulted in increased activity and impulsivity as well as poorer sustained attention in comparison with a control group. Other studies have shown that high doses of PCB gave an inhibitory response, like hypoactivity (Johansen et al., 2011; Nishida et al., 1997).

Sex differences

Several studies have shown that the developing male and female respond differently to chemical agents, with different behavioral responses as a result (Weiss, 2002). Overall, both male and female SHR showed effects on all the behavioral measures when exposed to different doses of PCB 153 in this study. This is opposite of what Holene et al. (1999) and Holene et al. (1998) found in their studies, where effects were only seen in male rats after exposure to PCB 153. Roegge et al. (2000) also found a sex difference following PCB exposure. They studied effects of A1254 on spatial learning and memory in rats and found effects in male, but not female, adult rats. On the contrary, McConnell (1985) claimed that females are more sensitive than males when exposed to PCB. Others have not found any sex differences (Piedrafita et al., 2008). In our study, some sex differences were observed in SHR, but not WKY, on the various measures depending on the given dose. The conflicting findings reported in relation to sex differences may be due to variables and procedures involved in the different experiments done.

As described above, there is a range of critical factors likely to contribute to the different results, and sometimes opposing conclusions regarding effect of exposure to PCBs. There might be several different factors determining the degree of neurological, cognitive, and behavioral changes. The organisms´ age when exposed is important. When exposed at a young age, there are several characteristics involved; physiological characteristics, uptake characteristics and possible inherent susceptibilities. The developing nervous system is more susceptible to toxicants in a period due to the brain growth spurt and maturation of the brain, than when the organism is older (Kodavanti, 2005). Other factors involved could be time of exposure as well as the measures used to test the outcome. Findings show that changes following PCB exposure vary with age and measure used (Branchi et al., 2005; Piedrafita et

al., 2008). Other important factors are the experimental design (Bushnell et al., 2002; Holene et al., 1998; Sable et al., 2006), type of animal used; rats, mice, monkeys (Berger et al., 2001; Branchi et al., 2005; Holene et al., 1998; Rice, 2000), administration route; orally, through gavage, with cookies (Branchi et al., 2005; Sable et al., 2006; Taylor, Crofton, & MacPhail, 2002) and the usage of single congeners as opposed to commercial mixtures (Branchi et al., 2005; Holene et al., 1998; Piedrafita et al., 2008). These might all be implicated in the findings seen in the different studies regarding the effects following PCB exposure.

ADHD-like behavior in SHR and WKY

The aeitology of ADHD is not fully understood, but a possible explanation may be that some people have a genetic vulnerability for developing the disorder. It is estimated that environmental factors may contribute to about 20% of prevalence of ADHD (Barkley, 1998), and it is believed that environmental chemicals, like PCBs, can contribute to the development by influence of the dopaminergic system (Eubig et al., 2010; Sagvolden et al., 2005). The effects seen after exposure to PCB in this study were different in the two strains of rats tested. No ADHD-like symptoms were observed in the WKYs following PCB exposure, while the SHRs showed an increase in hyperactivity, impulsivity and poorer sustained attention when given 3mg/kg or 6 mg/kg of PCB in comparison with controls. It seems that there is an additive effect of exposure in SHR where PCB interacts with already existing behavioral symptoms when the dose exceeds a not yet known amount mg/kg in comparison with a control group.

The result from this study does not show that male rats are more vulnerable to exposure to PCB, as is previously found and described as a parallel to the findings in epidemiological studies of children with ADHD, where boys more often than girls are diagnosed with the disorder (Sagvolden, Aase, Zeiner, & Berger, 1998). Other effects, however, found in our study are parallel to some of the epidemiological findings regarding ADHD. Sagvolden et al. (1998) did a study with boys using a two-component reinforcement schedule with a 30 s fixed interval schedule and an extinction schedule. They found that boys with ADHD showed increased activity, impulsivity and problems with sustained attention in comparison with boys without the disorder. The boys with ADHD showed similar aspects of symptoms as animals exposed to PCB.

The results from the present study showed no effects following PCB exposure in WKYs. This may be due to a different vulnerability in SHR and WKY. It could be possible that the WKYs

are not vulnerable to PCB exposure. The lack of behavioral responses seen might also be a result of doses given in the present study. If a higher dose were to be used, this might have an effect on behavioral responses in WKY, as seen in the SHR.

Limitations and future challenges

Trying to draw any conclusions about the effects of PCBs requires one to choose between using mixtures or single congeners and look at them separately in an experimental design. The animals in this study were exposed to a single congener in a laboratory setting, while most natural exposure for animals and humans outside the laboratory, is to mixtures and several isomers (McConnell, 1985). The present study is limited regarding information on how PCBs may interact, or interact with other chemicals or toxins, like lead and mercury, which is often the case in the normal environment (McConnell, 1985). Another limitation is the operationalization of the behavioral measures. Hyperactivity, impulsivity and sustained attention are heterogeneous terms which might consist of different components. This implicates different possibilities on how to measure the behavior of interest.

The lack of consistency on the effects following PCB exposure makes it a complex research field consisting of several studies with different, and sometimes opposing conclusions. The fact that there are a range of different variables involved makes it difficult to draw uniform conclusions about the topic. This underlines the need for more systematic studies on the matter in the future. Moreover, to find out more about the underlying neurochemical changes one should include neurochemical measures as well as behavioral measures. Another challenge for the future would be to involve different doses of PCBs in experiments. This might shed light on the dose-response effect possibly implicated in the effects of exposure and unravel if hormesis could be used to describe the effects, or if another pattern emerges.

Conclusion

Two different strains of rats responded differently to different doses of PCB 153. In SHRs there were found additive effects on measures of hyperactivity, impulsivity and sustained attention after exposure to 3mg/kg and 6mg/kg of PCB. The opposite effect was seen after exposure to 1mg/kg of PCB. WKYs did not show changes on any behavioral measures after exposure in comparison with a control group. Both male and female SHR showed behavioral changes, but there was a difference between males and females regarding effect of dose. The effects observed in the SHR might be described as a dose-response relationship which is like an inverted U-curve. Together with different results from previous studies done, this raises the need for doing other studies with different doses of PCB investigating the relationship between dose and behavioral outcome. As more is known about the link between PCB exposure and behavioral effects, one might get a better understanding of the relationship between PCB and ADHD-like behavior and the underlying mechanisms assumed to be involved in the development of ADHD.

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