# RELATIVE LIVER WEIGHT IN RATS SUBACUTELY EXPOSED TO POLYCHLORINATED BIPHENYLS

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**Abstract**. Polychlorinated biphenyls (PCBs) are pollutants abundantly present in the environment. Before they were banned, commercial mixtures of PCBs had been produced in many countries and were used for a number of technical purposes. This is considered to be the main source of the current levels of PCBs found in the environment due to their lipophilic properties and high persistence. The aim of this study was to evaluate dose-response relationship for the effects of the commercial PCBs mixture on relative liver weight by Benchmark dose (BMD) approach, and to derive the BMD10 for the effect. In this study, commercial mixture of PCBs, Aroclor 1254 was used in a 28-day toxicity study in young adult Wistar rats. The rats were provided with food and water ad libitum and maintained in a controlled environment. Commercial mixture of PCBs dissolved in corn oil or corn oil (control) was administered by oral gavage at 1 µL/g. Rats were given doses of 0.5, 1, 2, 4, 8, 16 mg PCBs/kg b.w./day. After 28 days rats were sacrificed and their livers were weighted. Relative liver weight was calculated as ratio of liver weight and body weight. Using PROAST software a dose-response relationship has been confirmed for the influence of PCBs on relative liver weight and associated CED10 was 0.154 mg/kg bw/day, and its lower confidence limit (CEDL) was 0.1127 mg/kg bw/day proving CED10/CEDL ratio under 10. Our results showed that relatively low doses of PCBs may produce effect on liver weight causing liver hypertrophy in a dose response manner.

**Keywords**: polychlorinated biphenyls, benchmark dose, relative liver weight, rats



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## 1. Introduction

Polychlorinared biphenyls (PCBs) are ubiquitous environmental contaminants which are considered to be persistent organic pollutants that bioaccumulate in individuals and biomagnify in food chain. Being highly persistent in living organisms, together with other organochlorides, PCBs comprise the bulk of organochlorine residues in human tissues (Longnecker et al., 2003).

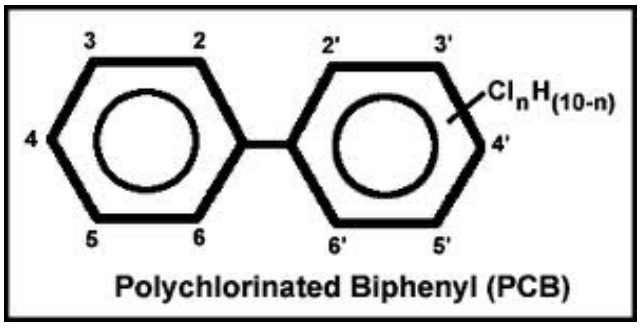


*Figure 1*. Chemical structure of polychlorinated biphenyls.

The structure of all PCBs consists of biphenyl molecule with minimum of 1, and no more than 10 chlorine atoms attached (Figure 1). Theoretically, 209 different PCB congeners are possible, depending on the place of Cl, although only 130 congeners are found in commercial PCB mixtures. These synthetic organic chemicals have no known natural sources in the environment; they were commercially manufactured as industrial mixtures known for their stability under broad range of chemical, thermal and electrical conditions. They have been used as heat transfer agents in electrical transformers, flame retardands, adhesives, carbonless copy paper, as well as pesticide extenders. After the public's outcry concerning the discovery of PCBs detrimental health effect and apparent link between PCBs and widespread environmental problems, PCBs were the first industrial compounds to experience a world-wide ban on production. The production of PCBs in USA known under commercial name Aroclor ceased in 1979 and international ban on production was enacted at the Stockholm Convention on Persistent Organic Pollutants in 2001. However, exposure to PCBs due to their resistance to biodegradation and their lipophilicity which allows them to biomagnify in the food chain is still significant (Alleva et al., 2006). General population may be exposed to PCBs by contaminated food and by contaminated air. Food consumption, especially consumption of fish, meat and poultry remains to be the major source of PCBs

intake in humans. The concentration of these chemicals in blood, fat tissue and mothers` milk have decreased over the past 30 years, but are still detectable in blood of the general population all over the globe (Jonsson et al., 2005).

Exposure to PCBs has been associated with neurobehavioral, immune, developmental, reproductive and hepatic abnormalities in both humans and experimental animals (Branchi et al., 2005; Ulbrich and Stahlmann, 2004; Whysner and Wang, 2001). International Agency for Research on Cancer (IARC) has determined that PCBs are probably carcinogenic to humans and assigned them to group 2A (IARC, 1987). The environmental Protection Agency (EPA) has determined that PCBs are probable human carcinogens and assigned them the cancer weight-of-evidence classification B2 (IRIS, 2000).

Hepatotoxicity of PCBs is well-documented in animals exposed to acute, intermediate (subacute and subchronic), or chronic poisonings with commercial PCBs mixtures or single congener by different relevant routes of exposure (Andrews, 1989; Carter, 1984; Casey et al., 1999; Roos et al., 2011; Wlostowski et al., 2008). PCB-induced liver effects in animals include microsomal enzyme induction, liver enlargement, increased serum levels of liver-related enzymes and lipids, altered porphyrin and vitamin A metabolism, and histopathological changes. Hepatotoxic effects are considered one of the most sensitive endpoints for PCBs mixtures (ATSDR, 2000), relative liver weight being one of the relevant parameters for determination of no observable adverse effect levels (NOAEL) and lowest observable adverse effect level (LOAEL).

Risk assessments for health effects that may result from exposure to environmental agents such as PCBs require an analysis of the dose-response relationship that can be defined by NOAEL/LOAEL levels. However, this approach has several disadvantages which can be overcome by benchmark dose (BMD) approach.

The aims of this study were to evaluate dose-response relationship for the effects of the commercial PCBs mixture, Aroclor 1254 on relative liver weight by using BMD approach, to derive critical dose effect ($CED_{10}$) for this effect and to compare it with NOAEL values obtained for liver in previous studies.

## 2. Materials and Methods

Male albino Wistar rats weighting 200 to 250 g free of typical rodent pathogens were used through the study. The animals were housed in cages with a 12-hour day and night cycle at the temperature of 20C to 24C and relative humidity between 40% and 60%. The rats were provided with food and tap water ad libitum and were treated in compliance with Guidelines for Animal studies no. 9667-1/2011. Rats had been accommodated to the experimental conditions for

one week before they were randomly assigned into control and treated groups, seven animals each. Six treated groups were receiving Aroclor 1254 dissolved in corn oil in the doses of 0.5, 1, 2, 4, 8 and 16 mg PCBs/kg body weight/day by oral gavage in a volume of 1 µl/g body weight for 28 days. Control animals were receiving only corn oil also by orogastric tube during 28 days. Doses were selected on the basis of literature data (ATSDR 2000; Martin and Klaassen, 2010; Wade et al., 2002). On the day following the final dose, rats were sacrificed. The liver was removed, dissected out and weighted. Relative liver weight was calculated as ratio of liver and body weight at the time of sacrifiction.

In order to establish significantly important differences between the observed groups, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) as post hoc test. The level of significance for all tests was set at $p<0.05$. For this statistical analysis SPSS software (version 11.5) was used.

Collected data on relative liver weight were then analyzed by the Benchmark dose approach using PROAST software. PROAST is a software package that has been developed by the Dutch National Institute for Public Health and the Environment for the statistical analysis of dose-response data and deriving a BMD in risk assessment. The dose-response data are statistically evaluated by fitting a dose-response model to the data followed by established low but measurable change in response-Benchmark response (BMR) and the associated dose level-BMD. The BMDs lower confidence level (BMDL) is generally used as the reference point. The terms critical effect size (CES) and critical effect dose (CED) can be used instead of BMR and BMD when obtained data are continuous (Slob, 2002). In this study, $CED_{10}$ was determined since critical effect size at 10% level appears to minimize the adversity and adaptivity, as well as animal variations (Van der Ven et al., 2008).

## 3. Results

Relative liver weight was significantly higher in all treated group when compared to control group with a maximum increase of 63.84% observed in the group receiving the highest dose of 16 mg PCBs/kg bw/day. As it can be seen in Figure 2, the increase in doses of PCBs was followed by elevated relative liver weight.

Therefore, the next step was to characterize a dose-response relationship for the effect of PCBs on relative liver weight (Figure 3). Lower confidence leveld of CED at CES of 10% was calculated from the best fitted curve. PROAST software has proved dose-response relationship and $CED_{10}$ was shown to be 0.154 mg PCBs/kg with the lower confidence level of 0.1127 mg PCBs/kg. The

obtained CED/CEDL ratio, which is used as measure of statistical uncertainty, was under 10 proving that data on relative liver weight can be considered relevant.

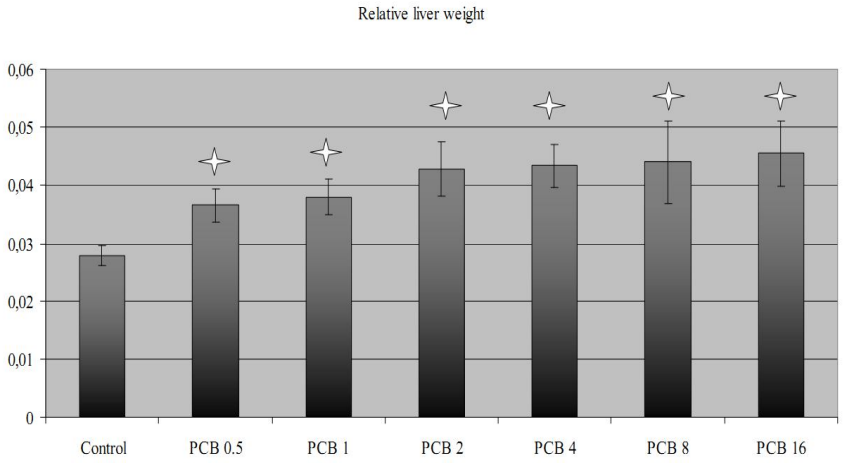


*Figure 2.* Relative liver weights in rats exposed to different doses of PCBs. The values are expressed as mean values±SD and statistical evaluation was performed by ANOVA followed by LSD test. Stars represent significant difference between the treated group and control group ($p<0.01$).

## 4. Discussion

Common way of analyzing dose-response data collected from animal studies is to statistically test each dose group against control groups for significant difference and to determine NOAEL. Due to serious objections against this approach, Crump (1984) introduced the BMD concept as an alternative to the NOAEL approach. This approach is currently gaining more and more attention and acceptance in the process of risk assessment of various chemicals. The scientific committee of European Food Safety Authority (EFSA) recommended this approach for deriving reference points for risk assessment claiming that BMD approach is scientifically more advanced approach than NOAEL/LOAEL approach (EFSA, 2009). Moreover, BMD approach is supported by the REACH

legislation guidance document (ECHA, 2008). It is scientifically accepted that BMD approach is applicable to all toxicological effects estimating the shape of the overall dose-response relationship for a particular endpoint.

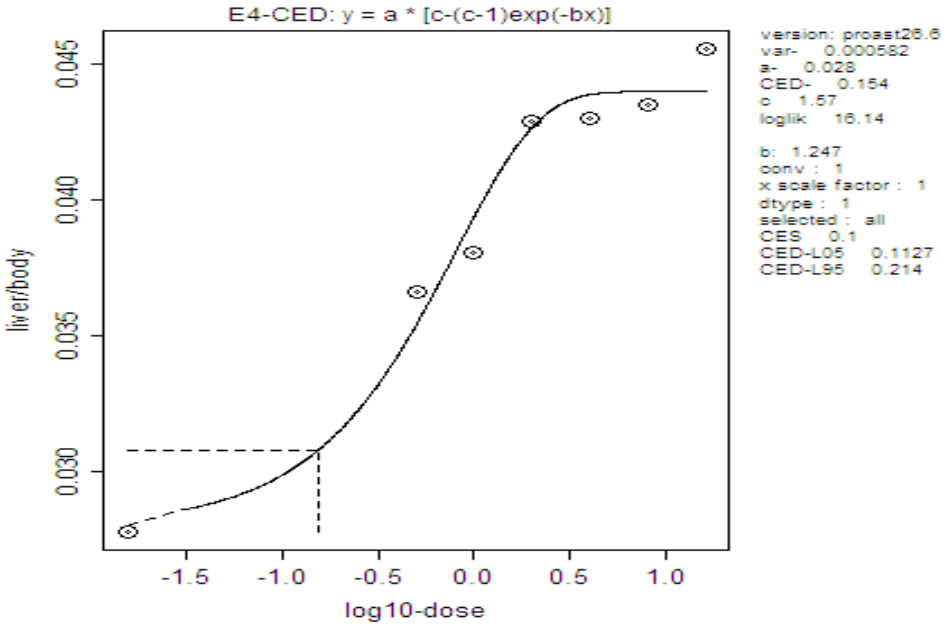


*Figure 3*. Dose-response curves of the increased relative weight of liver against PCB doses. Exposure dose (x-axis) is on a logarithmic scale. CES for this endpoint was set at 10% ((horizontal dotted line), while a corresponding CED is represented by the vertical dotted line; L05 and L95 represent the lower and upper bound of 90% confidence level.

In this study, Aroclor 1254 (known to produce the most prominent effects on liver) was applied to adult rats in 28 days gavage study. Benchmark dose approach was appropriate since all doses in a study were associated with a significant adverse response and therefore no NOAEL could be estimated.

Relative liver weight was significantly higher in all treated animals when compared to controls. Literature data confirm increased absolute and relative liver weights in rats treated either with PCBs or with single congener. Thus, in rats fed with estimated dose of 1 mg/kg/day Aroclor 1254 for 4 days relative liver weight and serum total cholesterol were increased while no changes were observed in rats fed with estimated dose of 0.5 mg/kg (Carter, 1984). The

lowest reported hepatic effect level (microsomal enzyme induction) was 0.03 mg/kg in orally treated rats with Aroclor 1242, 1248, 1254, or 1260 through food for 4 weeks, while increased relative liver weight was determined at the dose of 2.5 mg/kg (Litterst et al., 1972). Andrews (1989) found increased liver-to-body weight ratio in rats intragastrically treated with 10 or 25 mg PCBs/kg during 10 weeks. Dietary ingestion of 0.25 mg/kg Aroclor 1254 for 35 days induced hepatic microsomal enzymes in rats, while increased liver weight occurred only at a higher dose of 1.25 mg/kg (Bruckner et al., 1977). No changes in liver weight were found in rats exposed to dietary dose of 0.033 mg/kg Aroclor 1242 for 30 days (Casey et al., 1999). However, recent investigations carried out on bank voles treated orally with 10 and 50 mg/kg Aroclor 1254 for 12 weeks showed significant increase in liver weight (Wlostowski et al., 2008). Treatment with single congener also affects liver weight. Dose dependent increase in liver weight was observed in male rats receiving doses higher than 300 mg PCB 180/kg by gavage for 28 days (Roos et al., 2011). However, the results of this study indicate that even the lowest applied dose of 0.5 mg PCBs/kg can induce changes in relative liver weight. Furthermore, hepatic NOAEL values obtained in previously conducted studies, i.e. NOAEL of 1 mg/kg during 5 weeks in study on rats (Andrews, 1989) and NOAEL of 0.25 mg/kg during 35 days in study on rats (Bruckner et al., 1974) were higher than $CED_{10}$ value obtained in this study. The results of BMD analysis showed that the dose of 0.1127 mg PCBs/kg Aroclor 1254 produce the response in relative liver weight that is likely (95%-confidence) to be smaller than 10%.

## 5. Conclusion

Based on performed BMD analysis it can be concluded that relative liver weight is a sensitive parameter for PCBs exposure, since relatively low doses produced effect on liver weight causing liver hypertrophy in a dose-response manner. The $CED_{10}$ value obtained for this effect suggests that BMD approach can be used for determining critical levels of change in the effect pattern that may later serve as a basis for risk assessment. Further investigations that would determine possible histopathological changes in liver caused by these doses of PCBs are needed.

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