EFFECTS OF PURIFIED PCB180 ON LOCOMOTOR ACTIVITY IN ADULT RATS IN A SUBACUTE TOXICITY STUDY

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Introduction

Whereas Ah-receptor mediated toxicity of several coplanar and mono-ortho-substituted polychlorinated biphenyls (PCBs) is well characterized, systematic knowledge is lacking for many non-dioxin-like PCB congeners (NDL-PCBs). Most previous studies of NDL-PCBs have used compounds which were not highly purified to remove possible contamination by Ah-receptor active ligands. The amounts of these impurities may be small, but they can influence the effects reported in toxicity studies because of their high toxic potency. Also, the toxic profile of many NDL congeners is incomplete with respect to certain endpoints, including neurotoxicity. These issues are addressed within the framework of the EU-collaborative project ATHON of which the present study is a part. The purpose of this experiment was to investigate possible neurobehavioral effects of purified PCB180 in a 28-day toxicity study in rats. Because of the subacute exposure period, a test of locomotor activity was selected as the behavioral endpoint which allowed efficient testing of large numbers of rats. In addition, effects on activity levels were frequently reported in studies of PCB mixtures and single congeners (review in [1]).

Materials and methods

Eighty adult Sprague Dawley rats (6 weeks of age) were randomly assigned to 8 groups (5 males and 5 females per group). They were housed in stainless steel wire-bottom cages (45 x 38 x 19 cm) in groups of 5 in a room with an ambient temperature of 21±0.5 °C (mean±SD) and a relative humidity of 48±7 %. Rats had *ad libitum* access to water and laboratory diet (R36, Lactamin, Sweden). Groups were exposed to purified PCB180 (2,2',3,4,4',5,5'-heptaCB) dissolved in corn oil at total dose levels of 0 (vehicle), 3, 10, 30, 100, 300, 1000, or 1700 mg/kg body wt. for 28 days. In general, protocols followed OECD guideline 407. The total amount of dioxin-like impurities was 2.7 ng TEQ_{WHO}/g PCB180. In order to reach the kinetic steady-state at early stage of the study the total dose was divided into 6 daily loading doses and three weekly maintenance doses, which were calculated[2], using a half-life of 90 days[3,4]. During the last 5 days of the treatment period, rats were tested for locomotor activity in an octagonal open field (diameter 75 cm). The behavior was recorded on videotapes and automatically evaluated with a commercial program for behavioral analyses (Ethovision, Noldus, NL). Each rat was tested for 5 min on each of the 5 days to allow the examination of habituation. Sequence of the rats to be tested was varied according to a permutation scheme to exclude a systematic influence of daytime on the outcome. For analyses, the area of the open field was divided in an inner zone (diameter 50 cm) and an outer ring (width 12.5 cm). Total distance moved in the 5-min period, distance moved in the inner zone of the open field, distance moved in outer zone, time in inner zone, and time in outer zone were extracted as parameters from the recordings. Results were analyzed for significant dose-response relationships by trend analyses and for group differences with different ANOVA models. Error probabilities of < 0.05 were accepted as statistical significance.

Results and discussion

Body weight development was dose-dependently delayed at the two highest dose levels during the initial loading dose period, but recovered by the end of the study. Preliminary results indicate effects of exposure to PCB180 on open field behavior only in females. According to trend analyses, the distribution of activity between the inner and the outer zones was significantly affected by PCB180 in female rats ($p<0.05$). On day 1, there were dose-related increases in percentage of time in the inner zone ($p<0.05$; Figure 1, left) and, conversely, decreases in percentage of time in the outer zone of the open field. These differences ameliorated across the five days of testing, thus, indicating a shift of activity from the inner to the outer zone in exposed rats. Consequently, the dose-response relationships were no longer significant on day 5 of the measurement. Using a measure of

habituation by dividing the mean of time in inner zone across days 2-5 by time in inner zone on day 1, revealed a dose-dependent decrease by PCB180 exposure ($p<0.05$; Figure 1, right) and, vice versa, an increase in time in outer zone ($p<0.05$). This demonstrates that habituation accounts for the increasing similarity of behavior in exposed female rats compared to controls during the course of testing. In essential the same results were detected, when the percentage of distance moved was evaluated for inner and outer zones, with a higher percentage of distance moved in inner zone on day 1 in exposed females and a decrease due to habituation during the test days (Figure 2). Trend analysis of total distance moved revealed a quadratic relation of total distance moved to dose across all test days ($p<0.05$; Figure 3, left) and on each of the days 2-5 ($p<0.05$), with elevated activity values at intermediate dose levels compared to controls and the top dose group. There were no clear-cut differences in total distance moved between test days ($p>0.1$), irrespective of exposure, thus, showing that total activity did not habituate across the days of measurement (Figure 3, right).

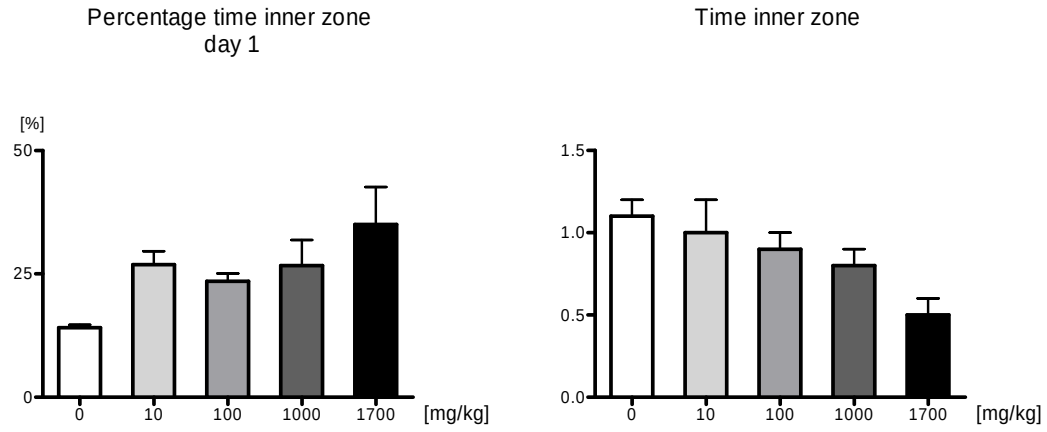

Figure 1. (left) There was a significant dose-dependent increase in percentage of time in inner zone of the open field in female rats on day 1. (right) To evaluate habituation of the time in inner zone, the mean of values for this parameter on days 2-5 was divided by time in inner zone on day 1. Results show a dose-dependent decrease, thus, demonstrating rapid habituation across test days ($p<0.005$; n = 5/group; means $\pm$ SEM).

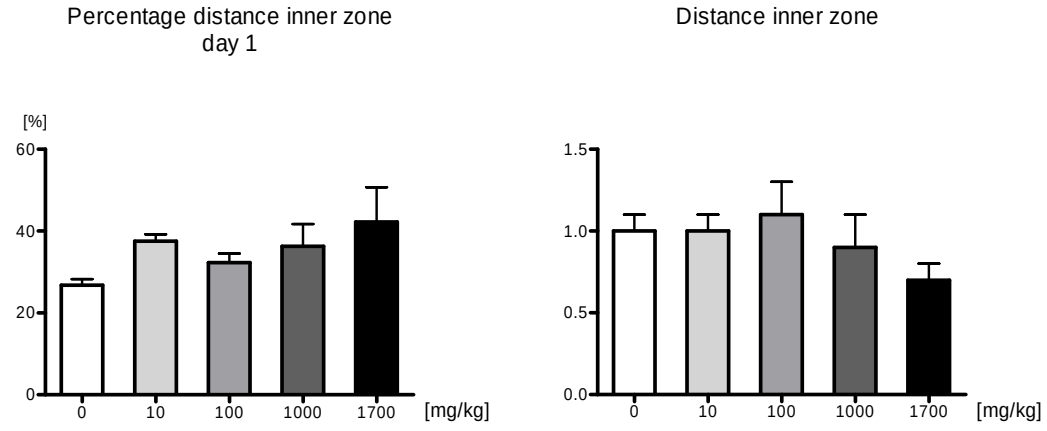

Figure 2. (left) In addition to time in inner zone, percentage of distance moved in inner zone of the open field was significantly increased in a dose-dependent manner in female rats on day 1. (right) To evaluate habituation of the distance moved in inner zone, the mean of values for this parameter on days 2-5 was divided by distance moved in inner zone on day 1. Results show a dose-dependent decrease at higher dose levels, thus, demonstrating a shift of activity to the outer zone across test days ($p<0.05$; n = 5/group; means $\pm$ SEM).

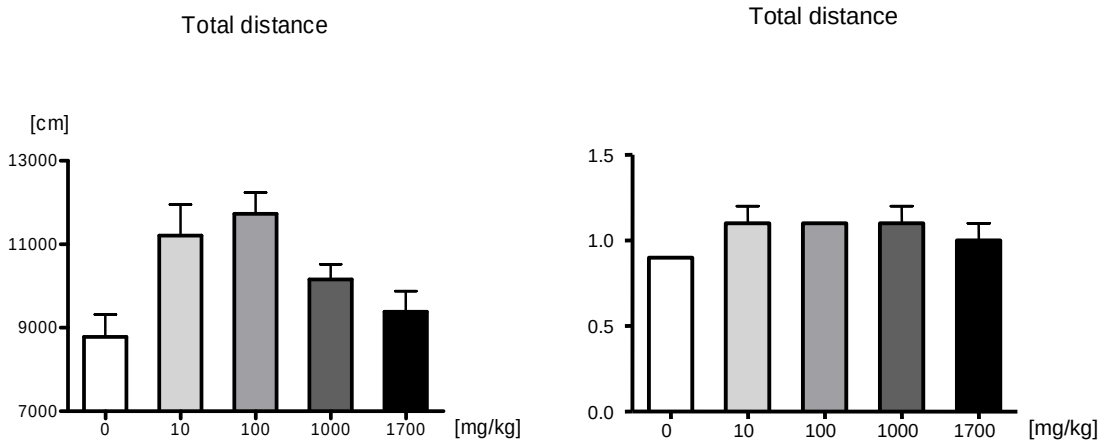

Figure 3. (left) Sum of total distance moved in both inner and outer zones of the open field on all test days showed a significant quadratic relation to dose, thus, suggesting a depressive effect of PCB180 on general motor activity at higher doses. (right) To evaluate habituation of the total activity, the mean of values for this parameter on days 2-5 was divided by total distance moved in inner zone on day 1. Results show that total activity does not change significantly across test days. SEMs are too small to be shown on columns without error bars ($p>0.1$; n = 5/group; means $\pm$ SEM).

Alterations of locomotor activity are among the most frequently reported behavioral effects after exposure to single PCB congeners and mixtures (review in[1]). Effects have been found in many species, including rats[5,6,7], mice[8,9], birds[10], and monkeys[11]. Motor impairments were also reported in humans, albeit in different test situations which examined motor coordination and cognitive components rather than spontaneous locomotor activity (reviewed by Roegge and Schantz[1]). There is evidence that effects in experimental animals can be induced by a variety of PCBs, such as coplanar[9,12,13], mono-[14] and di-ortho-chlorinated congeners[12,15,16], and even by a hydroxylated PCB metabolite[17]. Spontaneous locomotor activity is a highly integrative behavior which as such may be affected by different chemicals and via different mechanisms. In addition, the outcome largely depends on the testing conditions and the device used to study the animal's behavior, age at testing, exposure doses and periods[1]. The present finding of elevated total activity at intermediate dose levels is in general accordance with earlier reports, using developmental exposure and different congeners[8,14] or mixtures[6,18]. The depression of motor activity by PCB exposure at high levels may be due to more pronounced general toxicity. Also, a similar influence on the distribution of activity as detected here for PCB180 has been described previously[12]. Since this effect on distribution of activity was mainly expressed on day 1 of testing, an emotional response to an unfamiliar environment is likely to underlie the outcome. Control rats generally avoid crossing open areas, offering no spaces to hide, when necessary[19]. Therefore, the pattern of increased distance moved and more time resident in the inner zone of the open field could indicate a) less avoidance due to decreased fear or b) behavioral disinhibition resulting from enhanced emotional reactivity. Since exposed rats rapidly acquired a "control-like" behavior on the following 4 test days, the first alternative is less likely. Signs of PCB-induced behavioral disinhibition were previously reported in rats[20] and children[21]. Rapid habituation of the distribution of locomotor activity between zones was found in the present study, using repeated testing for short periods, in contrast to total activity which did not habituate. Using longer measurement periods, impairment of habituation of total motor activity was found in mice and rats exposed to PCBs during development[9,13,17,18]. This indicates that the observed discrepancies may be due to differences in testing duration or exposure periods. The present results further demonstrate that total activity and spatial distribution of activity are different measures. In summary, subacute exposure to PCB180 for 28 days caused elevations of total activity at intermediate doses and dose-related effects on the distribution of locomotor activity in the open field in female rats. Together, this pattern suggests an emotional effect, leading to altered responses to unfamiliar environments and involving behavioral disinhibition.

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References:
1. Roegge C.S. and Schantz S.L. *Neurotoxicol Teratol* 2006; 28: 260.
2.Gibaldi M., Perrier D. Pharmacokinetics, pp. 329. 1975. Dekker, New York.
3.Öberg M., Sjödin A., Casabona H., Nordgren I., Klasson-Wehler E., Håkansson H. *Toxicol Sci* 2002;70:171-182.
4.Tanabe S., Nakagawa Y., Tatsukawa R. *Agric Biol Chem* 1981;45:717-726.
5. Pantaleoni G., Fanini D., Sponta A.M., Palumbo G., Giorgi R. and Adams P.M. *Fund Appl Toxicol* 1988; 11: 440.
6. Lilienthal H., Neuf M., Munoz C. and Winneke G. *Fund Appl Toxicol* 1990; 15: 457.
7. Goldey E.S. and Crofton K.M. *Toxicol Sci* 1998; 45: 94.
8. Agrawal A.K., Tilson H.A. and Bondy S.C. *Toxicol Lett* 1981; 7: 417.
9. Eriksson P., Lundkvist U. and Fredriksson A. *Toxicology* 1991; 69: 27.
10. Ulfstrand, Södergren A. and Raböl J. *Nature* 1971; 231: 467.
11. Bowman R.E. and Heironimus M.P. *Neurobehav Toxicol Teratol* 1981; 3: 15.
12. Hany J., Lilienthal H., Roth-Härer A., Ostendorp G., Heinzow B. and Winneke G. 1999; 21: 147.
13. Eriksson P. and Fredriksson A. *Environ Toxicol Pharmacol* 1998; 5: 17.
14. Kuriyama S.N. and Chahoud I. *Toxicology* 2004; 202: 185.
15. Eriksson P. and Fredriksson A. *Environ Toxicol Pharmacol* 1996; 1: 155.
16. Schantz S.L., Seo B.-W., Wong P.W. and Pessah I.N. *Neurotoxicology* 1997; 18: 457.
17. Meerts I.A.T.M., Lilienthal H., Hoving S., van den Berg J.H.J., Weijers B.M., Bergman A., Koeman J.H. and Brouwer A. *Toxicol Sci* 2004; 82: 207.
18. Storm J.E., Hart J.L. and Smith R.F. *Neurobehav Toxicol Teratol* 1981; 3: 5.
19. Walsh R.N. and Cummins R.A. *PsycholBull* 1976; 83: 482.
20. Berger D.F. *Behav Brain Res* 2001; 126: 1.
21. Stewart P., Fitzgerald S., Reihman J., Gump B., Lonky E., Darvill T., Pagano J. and Hauser P. *Environ Health Persp* 2003; 111: 1670.