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Developmental Landmarks in Offspring of Rats Exposed Singly and in Combination to Aroclor 1016 and Levothyroxine

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Polychlorinated biphenyls (PCBs) have been associated with a reduction in circulating thyroid hormones (TH) (Desaulniers et al. 1997) and are speculated to interact with this system to alter normal development (reviewed in Brouwer et al. 1998; Porterfield and Hendry 1998). To date, most concern over PCB exposure has been with higher chlorinated and coplanar mixtures which have poor or minimal interaction with the thyroid hormone system when compared to the more infrequently studied mono-, di- and tri-ortho substituted CBs, which are more readily metabolized to hydroxylated metabolites (Hansen 1998). Nonplanar PCBs and hydroxylated PCB metabolites however, are prevalent as transient and pulsatile components in fish, human serum, (Gerstenberger and Hansen, 2000) and breast milk, (Kostyniak et al. 1999) and are major products of environmental and laboratory dechlorination reactions (Brown et al.1987). Hydroxylated PCB metabolites can inhibit thyroid hormone sulfation, (reviewed in Brouwer et al. 1998) and Kato et al.(1998) related reductions in serum T4 in rats to methylsulfonyl metabolites of PCBs. T4 concentrations were severely reduced in animals exposed to a tetraCB, (Brouwer and Van Den Berg 1986) and based on the similarity between TH nuclear receptors and PCB congeners with three chlorine substitutions, Porterfield and Hendry (1998) suggested these compounds may interact by competing for TH receptor sites and inhibit the hormone's gene expression.

While changes in circulating thyroid hormones can influence many physiological endpoints in adults, precise concentrations during time-limited critical periods of gestation are crucial to normal development (Haddow et al. 1999; Porterfield and Hendry 1998). Maturation of brain, lung, heart, and intestine during fetal and early postnatal life are dependent on TH. Influence on the "set points" for the human pituitary-thyroid negative feedback system (Welch et al. 1998) has been attributed to TH, as has auditory development (Goldey and Crofton 1998) in rats. The specifics of how TH effects brain function are not known, however children who have experienced deficits in TH have difficulties with attention, activity, and general cognitive functions (Matochik et al. 1996; Haddow et al. 1999; reviewed in Hauser et al. 1998). Maternal consumption of PCB contaminated fish has been associated with similar developmental and behavioral problems in animals (Beattie et al. 1996; Daly et al. 1989) and humans (Jacobson et al. 1990; Lonky et al. 1996). Children exposed to PCBs via maternal fish consumption in the Jacobsons' study, (1996) and those born to mildly hypothyroid mothers (Haddow et al.1999) had similarly reduced performance on IQ tests. The time of the appearance of

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developmental landmarks can be a good indicator of future neurological performance (Levine, Carey and Crocker 1999). Thus, the present study was designed to examine developmental landmarks following exposure to a lightly chlorinated PCB mixture (Aroclor 1016), and to determine which PCB effects may be mediated by thyroid hormone status.

MATERIALS AND METHODS

Timed pregnant Long-Evans hooded rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, Indiana) on gestational day (GD)4. Rats were housed singly in shoe box style cages in an AAALAC approved animal facility. Laboratory conditions consisted of a light-dark cycle of 12 hours, a temperature range of 21-23 degrees Celsius, and a constant 35% humidity. Throughout gestation rat dams were fed certified rat chow ad libitum and were weighed and handled daily. After a 3-day period of acclimation, 28 timed-pregnant rat dams were randomly divided into two main groups. One group was dosed with 2.5 mg/kg/day IP of Aroclor 1016 on gestational days 7-13. The other group received a similar volume of saline IP over the same time period and served as controls. Half of each main group (7 randomly chosen dams) were given levo-thyroxine sodium (Aldrich Chemical, Milwaukee, WI) supplements, creating two more groups for a total of four dose groups: A, Aroclor only; B, both Aroclor and thyroxine; T, thyroxine only; and S, saline only.

Levo-thyroxine supplements of 20μg/liter of deionized drinking water were given on gestational days 7-21. Water bottles were measured and intake in ml was calculated daily for each dam. The actual dose each animal received was calculated by multiplying the concentration of L-thyroxine by the 24 hr intake and dividing this by the animal's body weight for the same time period. At the end of the 14-day dosing period the actual dose was 2.89 μg/kg/day. A complete dosing schedule including route and type of administration and actual doses is given in Table 1.

Table 1. Dosing of timed-pregnant Long-Evans rats

Group, (No. Dams)	Aroclor 1016 or saline	L-thyroxine or Pure DI water
A-Aroclor (7)	2.5mg/kg/day IP on GD 7-13	Pure DI drinking water GD 7-21
B-Aroclor + Thyroxine (7)	2.5mg/kg/day IP on GD 7-13	2μg/kg/day in DI water GD 7-21
T- Thyroxine (7)	0.1cc saline IP on GD 7-13	2μg/kg./day in DI water GD 7-21
S -saline (7)	0.1cc saline IP on GD 7-13	Pure DI drinking water GD 7-21

Offspring were observed for selected developmental landmarks including total number of pups per dam, survival index, eye opening, incisor eruption, pinna unfolding, surface

righting, and negative geotaxis as described in Norton (1986) for all groups. On the day of birth pups were sexed, counted and the live birth index was determined for each litter. Survival index was computed at 24 hrs and at 4, 7, 14 and 21 days of age. On postnatal day (PND) 4 litters were culled to 8 pups per dam. Fostering between litters but within dose groups was done as necessary to acquire 4 females and 4 males per dam. Pinna unfolding was observed on PNDs 5-7 and recorded as the percent of pups with bilateral pinnas unfolded per liter. Pups were counted for incisor eruption and recorded as a percentage of the litter with any eruption on PNDs 9, 10 and 11. The percentage of pups per litter with both eyes opening was recorded on PNDs 15, 16 and 17.

Time in secs to right when placed supine (or surface righting) was observed and recorded for 1 pup picked randomly from each litter on PND 1 and 6, and for 1 pup of each sex per litter on PNDs 7 and 13. Negative geotaxis was determined by placing the pup head pointing down on a 45 degree sloped screen and recording the time in secs it took for the rat to orient itself head up. This was done for 1 male and 1 female randomly picked from each litter on PNDs 6-12. Reflex suspension times were determined for a pup of each sex in all litters on PNDs 7, 13 and 17 by placing the pup where it could grasp a small rod, (1.85 mm in diameter) with their forepaws and suspend themselves with no other support. The time in secs until they released their hold was recorded.

Statistical analysis was done using SPSS (Version 7.5). All developmental landmarks were analyzed statistically using a general linear model (GLM) for repeated measures to reveal group by day effects. Differences due to gender were not significant within dose groups, therefore sex was not factored in the analysis. Pups tested from the same litter were considered replicates and although this did not raise sample n, it did increase statistical power. Dunnett's or Tukey's post hoc tests were done when indicated. The saline group was used as a control for post hoc tests, when there were no significant differences between the saline and thyroid control groups.

RESULTS AND DISCUSSION

There were no significant differences between dose groups and controls in dam weight gain during gestation. After receiving Aroclor 1016, pinna unfolding and incisor eruption (Figures 1 and 2) were delayed, while the group receiving both the Aroclor and L-thyroxine (B) exhibited no significant differences in these endpoints compared to controls. No overall effect was seen in eye opening however, as seen in Figure 3. Rat pups exposed to Aroclor 1016 alone had significantly delayed surface righting times (Figure 4) and were slower at negative geotaxis, (Figure 5) but exhibited longer reflex suspension at the earliest age of testing (Figure 6). These alterations were not present in those dosed with Aroclor 1016 and supplemented with thyroxine (B).

Delayed developmental landmarks after exposure to PCBs during gestation were not unexpected, as several other studies have generated similar data, (Goldey et al. 1995; Beattie et al. 1996; Holene et al. 1998) and these behavioral observations have been standard indicators of developmental neurotoxicity (Reviewed in Weiss and O'Donoghue 1994; Jacobson et al. 1984). While thyroxine supplementation has been found to ameliorate some effects of postnatal PCB exposure in the rat, (See Cooke, Zhao and Hansen 1996; Goldey and Crofton 1998) the finding that low dose oral L-thyroxine supplementation during gestation attenuated all developmental delays experienced by

offspring of the Aroclor 1016 dosed animals is significant and unique to this study. Results support the hypothesis that TH status during discrete windows of time in utero could be a major factor in PCB-induced neurological abnormalities. In addition, the supplementation of L-thyroxine to PCB exposed rats could provide some insight into thyroid-related toxicities.

Early eye opening has been postulated to result from Ah receptor activation by dioxin-like compounds, (Schantz et al. 1997) and subsequent enhancement of epidermal growth factor (EDF) signaling pathways (Aulerich et al. 1988). Aroclor 1016 did not alter age of eye opening as did the higher chlorinated Aroclor 1254 which also has a greater proportion of Ah receptor agonists. The distinct congener content of the two Aroclors results in different, frequently opposing effects including actions on other hormone systems and on P450 oxidases (Hansen 1998; Jansen et al.1993).

Differences in L-thyroxine dose, (route and time of administration) between the two studies can also account for differing effects. The continuous oral T4 supplementation (in drinking water) appears to be more efficacious in rats than large bolus supplements. Goldey and Crofton (1998) supplemented T4 by injection and postnatally at 100μg/kg body weight. However, this method only elevated hormone concentrations for 6 hours post injection, which is evidence of the very short half life for thyroid hormones in adult rats due to a lack of thyroid binding globulin. Our intent with thyroxine supplementation was to reestablish euthyroidism in the chemically exposed rats, without causing hyperthyroidism, or severe effects in the thyroid control group. In their study of the effect of thyroid hormone on rat testis, Cooke, et al. (1996) found supplementation doses of 20-50μg/kg/day of thyroxine produced a hyperthyroid state even when given in combination with compounds known to lower circulating thyroid hormone concentrations. Figure 5 shows that pups from dams given thyroxine only (group T) tended to be slowest in eye opening, (although not significantly) the opposite of what has been seen in Goldey and Crofton's 1998 study. Since exophthalmus has long been associated with thyrotoxicosis in juvenile mammals, (Norris 1997) early eye opening may be specifically indicative of this type of severe hyperthyroidism.

The depression of thyroid hormones and the ensuing neurodevelopmental effects associated with PCB exposure are most likely due to the multiple mechanisms associated with maintaining thyroid homeostasis. A thorough examination of the interactions and feedback of circulating thyroid hormone concentrations in the bloodstream, pituitary, and hypothalamus seem warranted, especially following different doses and congener compositions. The attenuation of developmental delays by L-thyroxine has obvious implications for the understanding of developmental effects in maternally exposed populations, and certainly warrants further examination. The efficacy of Levo-thyroxine to prevent or minimize developmental and behavioral effects in maternally exposed rodent populations has provided us with an excellent foundation from which we can systematically pursue such issues.

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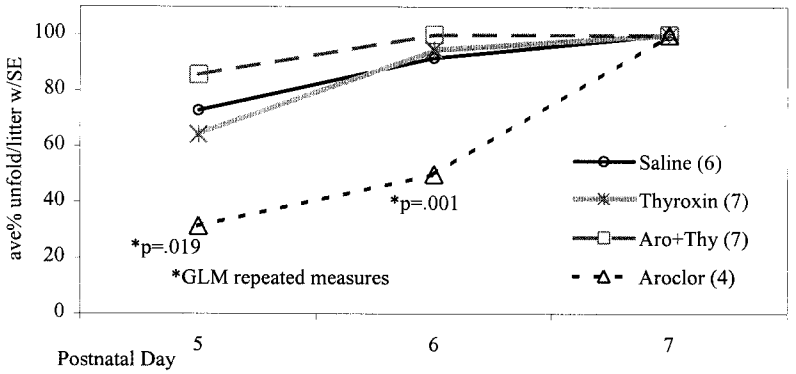

Figure 1. Pinna Unfolding in rats exposed to Aroclor 1016 singly or in combination with L-thyroxine. Doses given in Table 1.

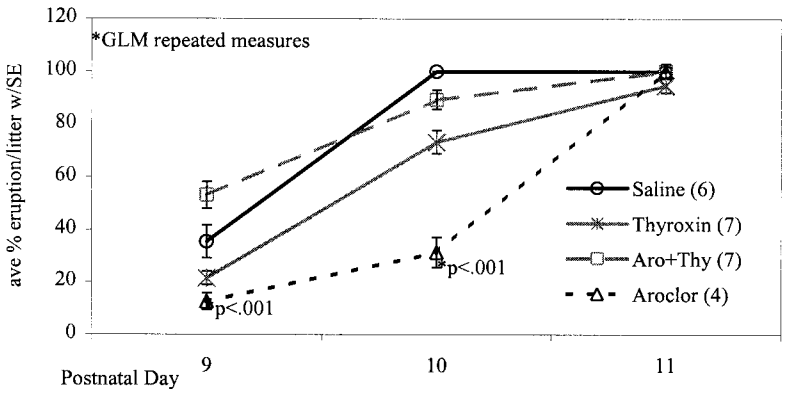

Figure 2. Incisor eruption in rat pups afer maternal exposure to Aroclor 1016 singly or in combination with L-thyroxine. Doses given in Table 1.

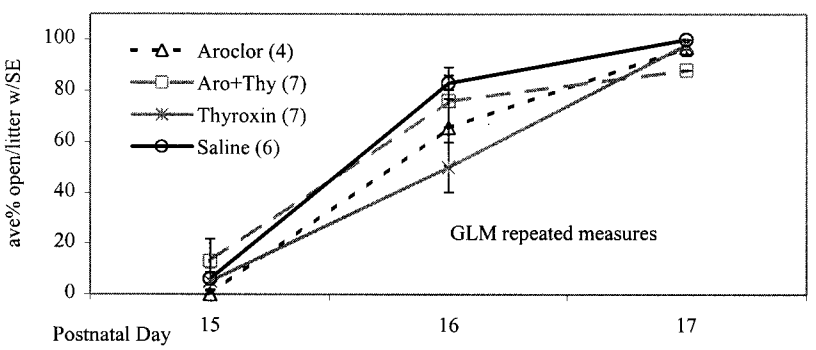

Figure 3. Eye opening in rat pups after maternal exposure to Aroclor 1016 singly or with L-thyroxine. Doses given in Table 1.

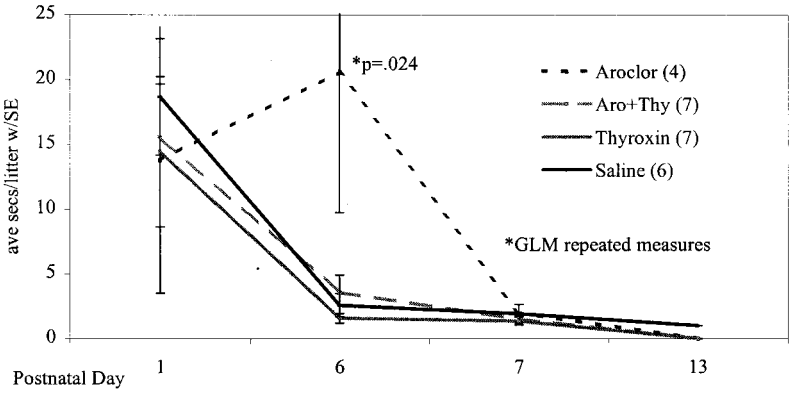

Figure 4. Surface righting in rats exposed to Aroclor 1016 singly or in combination with L-thyroxine. Doses given in Table 1.

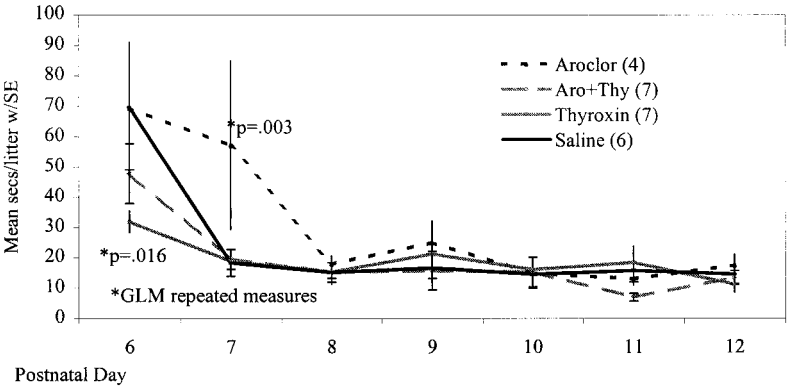

Figure 5. Negative geotaxis in rats exposed to Aroclor 1016 singly or in combination with L-thyroxine. Doses given in Table 1.

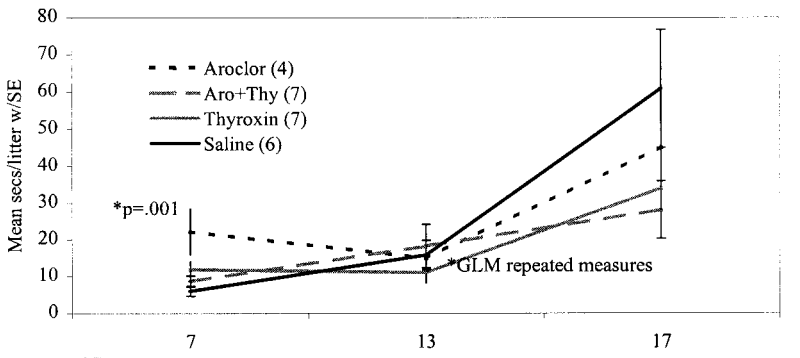

Figure 6. Reflex suspension in rats exposed to Aroclor 1016 singly or in combination with L-thyroxine. Doses given in Table 1.

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