An Assessment of Neurotoxicity of Aroclors 1016, 1242, 1254, and 1260 Administered in Diet to Sprague-Dawley Rats for One Year


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As part of a comparative chronic toxicity/oncogenicity study of different Aroclors (1016, 1242, 1254, and 1260), neurotoxicity was assessed in male and female Sprague-Dawley rats using functional observational battery (FOB) and motor activity tests, and histopathologic evaluation of selected nervous system tissues. Doses varied by Aroclor and ranged from 25 to 200 ppm in the diet. Animals were evaluated prior to initiation of dosing and at 13, 26, 39, and 52 weeks of exposure. Clinical signs, body weights, and feed consumption were evaluated weekly. Data analysis of FOB and motor activity results revealed several instances where Aroclor-treated groups were different from control. However, these were considered incidental, as they lacked any consistent dose- or time-related pattern that would suggest Aroclor-induced neurotoxicity. The nonremarkable findings during each of the four assessments were supported by the absence of any treatment-related clinical signs or mortality. Decreased body weight gain was evident in the male 100 ppm Aroclor 1254 dose group and in all female Aroclor 1254 dose groups late in the study (when a linear relationship was assumed between body weight and time), correlating with decreased feed consumption. Although a variety of incidental, spontaneous, degenerative changes were found in nervous tissue evaluated histopathologically, these changes were seen with similar incidence and severity in treated and control groups. No lesions were found that could be attributed to Aroclor-related neurotoxicity. In summary, 52 weeks of exposure to Aroclors 1016, 1242, 1254, or 1260 mixed in the diet did not yield any functional or morphologic changes indicative of PCB-induced neurotoxicity.

Key Words: Aroclor; chronic toxicity; oncogenicity; rats; PCBs; neurotoxicity.

U.S. production of polychlorinated biphenyls (PCBs) peaked in 1970 when 85 million pounds were produced, and production in the United States was halted in October, 1977, due to their potential for bioaccumulation and persistence in the environment. Although PCBs are no longer produced in the United States, many of the transformers and capacitors that were produced containing PCBs are still operational. These products are of concern as they represent potential sources for exposure to PCBs in the environment and humans (ATSDR, 1996).

Although the major target organ for PCBs is the liver, other studies have reported that they may also affect neurologic development. Prenatal exposure in mice and rats has led to altered locomotor activity, convulsions, and neuropathologic changes in the spinal cord and limbic system (Chou et al., 1979; Pantaleoni et al., 1988; Storm et al., 1981; Tilson et al., 1979). Perinatal exposure in primates has resulted in cognitive and motor dysfunction (Bowman et al., 1978, 1981; Bowman and Heironimus, 1981; Levin et al., 1988; Mele et al., 1986; Schantz et al., 1989). Behavioral and biochemical changes have also been reported for PCB-exposed rodents and nonhuman primates that have been attributed to effects on the dopamine system (Agrawal et al., 1981; Seegal et al., 1986, 1990, 1991 a,b, 1994).

Neurotoxic effects have been reported in humans as a result of PCDF-contaminated PCB exposure during the Yusho and Yu Cheng incidents (Chen and Hites, 1983; Chia and Chu, 1984; Kikuchi et al., 1979; Kuratsune et al., 1972; Murai and Kuroiwa, 1971). These effects included headaches, numbness, and altered peripheral nerve conduction velocity. Fischbein et al., 1979, reported neurologic symptoms in a cohort of PCB-exposed capacitor workers, but did not attempt to correlate either the incidence or severity of symptoms with PCB exposure. Recent epidemiologic studies have suggested that perinatal exposure to PCBs may result in delays in psychomotor development and deficits in cognitive and intellectual functioning in children (Gladen et al., 1988; Jacobson et al., 1985, 1989, 1990 a, b; Lonky et al., 1996; Patandin et al., 1999; Rogan et al., 1986, 1988; Schantz et al., 1990); an effect that other studies have reported is reversible (Koopman-Esseboom et al., 1996). However, the validity of these findings has been the subject of considerable debate (Schantz, 1996; Seegal, 1996).

The objective of this study was to assess and compare the potential chronic neurotoxicity in rats of four commercial polychlorinated biphenyl test substances (Aroclors 1016, 1242, 1254, and 1260) administered daily in the diet for up to 1 year.
The neurobehavioral and neurotoxicity evaluation methods used were those recommended in the U.S. EPA Neurotoxicity Guidelines (U.S. EPA, 1991) for assessing the neurotoxicity of environmental agents. The study was conducted in conjunction with a 2-year carcinogenicity study that has been reported elsewhere (Mayes et al., 1998).

**MATERIALS AND METHODS**

**Test System, Dosing, and In-life Parameters**

For a detailed description of the test system and a complete characterization of the test articles used in this study, refer to Mayes et al. (1998); a brief description follows. Male and female Sprague-Dawley rats (120/sex; 10/sex/dose group; 7–8 weeks of age at initiation of dosing) were obtained from Charles River Laboratories (Portage, MI). Animals were individually housed in stainless steel wire-mesh cages on a multi-tiered rack. The environmental conditions of the animal study room conformed to the standards for laboratory animal care outlined in the Guide for the Care and Use of Laboratory Animals, NIH Pub. No. 86–23, 1985. All animals were fed ad libitum Purina Certified Rodent Diet® No. 5002 (Purina Mills, Inc., Richmond, IN) in meal form to which an appropriate amount of the specific Aroclor (AccuStandard, Inc., New Haven, CT) was added. Contaminant analysis of each feed lot supplied by the vendor confirmed that the basal diet contained no more than 0.15 ppm polychlorinated biphenyls. Fresh water (municipal water supply) was provided ad libitum via an automatic watering system.

Ten rats per sex were assigned to a single control group or one of 11 treatment groups and received either blank feed or test substance mixed in feed, respectively, for approximately 52 weeks. Dose groups were as follows: Aroclor 1016 (Lot No. 129)–50, 100, and 200 ppm; Aroclor 1242 (Lot No. 01141)–50 and 100 ppm; Aroclor 1254 (Lot No. 122–078)–25, 50, and 100 ppm; Aroclor 1260 (Lot No. 021–020)–25, 50, and 100 ppm. Dosed diets were prepared by dissolving Aroclor in hexane and combining with a small amount of diet (the "premix"). The hexane was evaporated, and the premix was subsequently blended (Patterson-Kelley Cross Flow Blender) with an appropriate amount of basal diet to achieve the desired final PCB concentrations. Control feed was handled in a similar manner, without the addition of test material. Periodically during the study, samples were taken to confirm that diet preparations were achieving target PCB concentrations, were homogenous, and were not cross-contaminated. This was done by taking duplicate samples from each dose batch and extracting with hexane:acetone (1:1 v/v). The extract was diluted with hexane and analyzed by gas chromatography with electron capture (EC) detection. Dose concentrations were calculated by comparison of peak response ratios of the samples to a regression line constructed from the peak response ratios of spiked feed standards and their concentrations. Analysis results indicated that samples were homogenous and, with very few exceptions, were within 10% of the target concentrations. The postadministration analysis values generally agreed with the predadministration values. In addition, randomly selected samples of diet were periodically submitted to an independent laboratory (Northeast Analytical Laboratories, Schenectady, NY) for referee PCB concentration analysis.

The parameters used to assess toxicity during the in-life portion of the study included body weight, feed consumption, and clinical observations. These parameters were measured and recorded at weekly intervals. Prior to dosing and after 13, 26, 39, and 52 weeks of exposure, all animals were subjected to a screening battery that consisted of a functional observational battery (FOB) and a motor activity assessment as outlined in the U.S. EPA Neurotoxicity Guidelines (U.S. EPA, 1991). Postmortem studies included necropsy observations and histopathologic evaluation of selected nervous system tissues.

The study was conducted in compliance with the Environmental Protection Agency’s Good Laboratory Practice Standards (40 CFR Part 792).

**Neurotoxicity Testing**

**Functional observational battery**. The FOB included a thorough description of the animal’s appearance, behavior, and functional integrity. This was assessed through observations in the home cage, while animals were moving freely in an open field, and through manipulative tests. The various parameters were grouped into functionally related categories as shown in Table 1.

Briefly, measurements were first carried out in the home cage. The observer recorded each animal’s posture and palpebral closure. The presence or absence of convulsions was noted and, if present, described. The presence or absence of spontaneous vocalizations was also noted. The observer then removed the animal, rating the ease of removal and handling. The presence or absence of piloerection was also noted. Palpebral closure and any lacrimation or salivation were rated.

The animal was next placed in an open field arena having a piece of clean absorbent paper on the surface. A timer was started for 2 min, during which time a hand-held counter was used to count the number of rears (includes both supported and unsupported, not differentiated). At the same time, gait characteristics were noted and ranked, and the ease with which the animals ambulated was ranked (mobility score). At the end of 2 min, the number of fecal boluses and urine pools on the absorbent paper were recorded. Reflex testing began next and consisted of recording each animal’s responses to the approach of a blunt object, a touch of a blunt object to the rump, a click stimulus, tail pinch, and the constriction of the pupil to light. Degree of catalepsy and aerial righting were rated next, followed by grip strength performance. The animal was weighed, and the rectal temperature taken with the animal restrained by hand. The hind feet were then pressed onto an ink pad to facilitate measurement of landing foot splay.

**TABLE 1**

Categories for FOB Behavioral Parameters

<table>
<thead>
<tr>
<th>Autonomic</th>
<th>Muscle tone and equilibrium</th>
<th>Sensorimotor</th>
<th>Central nervous system</th>
<th>Physiological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defecation</td>
<td>Gait</td>
<td>Approach response</td>
<td>Rearing</td>
<td>Body temperature</td>
</tr>
<tr>
<td>Urination</td>
<td>Gait score</td>
<td>Click response</td>
<td>Arousal</td>
<td>Body weight</td>
</tr>
<tr>
<td>Pupil response</td>
<td>Mobility score</td>
<td>Touch response</td>
<td>Posture</td>
<td></td>
</tr>
<tr>
<td>Salivation</td>
<td>Grip strength</td>
<td>Tail pinch response</td>
<td>Ease of removal</td>
<td></td>
</tr>
<tr>
<td>Piloerection</td>
<td>Landing foot splay</td>
<td>Palpebral closure</td>
<td>Ease of handling</td>
<td></td>
</tr>
<tr>
<td>Lacrimation</td>
<td>Righting reflex</td>
<td></td>
<td>Vocalizations</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fur appearance</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clonic convulsions</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Tonic convulsions</td>
<td></td>
</tr>
</tbody>
</table>

The study was conducted in compliance with the Environmental Protection Agency’s Good Laboratory Practice Standards (40 CFR Part 792).
Motor activity. Motor activity was measured using a Figure 8 Photobeam Activity System (San Diego Instruments, San Diego, CA). This system was made of acrylic plastic and included enclosure walls and cover (one piece), an elevated enclosure top, and an enclosure floor. There were eight photobeams in the Figure 8 to detect horizontal movements, and eight additional beams to detect rearing (vertical) activity. The units used for pre study and Week 13 testing were 70 cm long by 40 cm wide. The runways were 10 cm high and 9 cm wide. The units used for the 26-, 39-, and 52-week intervals were larger to accommodate the larger size animals and were approximately 100 cm long by 62 cm wide, with runways that were both 15 cm high and wide. Motor activity was determined over six 5-min intervals for each animal. Generators of white noise at 65 db (Lafayette white noise generator, model 15800; Realistic solid state amplifier, model 32–2022; Realistic 8 ohm speakers in room and at each enclosure) were used to mask noises that could influence motor activity. Measures of total activity as well as an indication of habituation were obtained.

Neuropathologic examination methods. Rats that survived the full 12-month in-life period were submitted for neurologic tissue assessment. They were killed and perfused with Trump’s fixative (4% formalin-1% glutaraldehyde). Using the landmarks described by Boysen et al., 1993, the following areas of the central nervous system were examined: cerebral cortex (frontal, cingulate, insular, piriform, retrosplenial, occipital, temporal, rhinal), olfactory area, hippocampus, corpus callosum, internal and external capsule, caudate/putamen, globus pallidus, amygdala, hypothalamus, thalamus, ventricles, dentate, lateral/medial geniculate nucleus, cerebral peduncle, substantia nigra, red nucleus, cerebellar cortex and white matter, cranial nerve tracts/nuclei, and medulla. Spinal cord/vertebral sections were trimmed following decalcification, as was the Gasserian ganglion/cranium section. Slides were stained with hematoxylin and eosin, along with serial sections stained with luxol fast blue (a myelin stain) and cresyl violet (for nerve cell bodies). Peripheral nerves were dissected free of adjacent tissue and prepared for glycol-methacrylate embedding. Nerves were stained with toluidine blue.

Statistical Analysis
The objective of the statistical analyses was to determine when the observed difference in data between a group administered a given dose level of a given Aroclor and the control dose group was statistically significant at the 0.05 level. All statistical analyses were performed separately for each Aroclor and sex.

The neurotoxicologic data parameters measured in this study had data distributions that were either continuous or discrete in nature. Continuous data parameters included total horizontal motor activity, total vertical motor activity, body temperature, forelimb and hindlimb grip strengths, landing foot splay, number of rears, and body weight. Discrete data parameters had either ranking or categorical data values. The methods used to statistically analyze the data for a given parameter were determined according to whether the data had a discrete or continuous distribution.

Continuous Data Variables
Because continuous data variables were measured for each animal at five successive time periods in the study (prestudy, and Weeks 13, 26, 39, and 52), these endpoints were analyzed by repeated measures analysis of variance (ANOVA) using the GLM procedure in the SAS® System. Dose group effects were evaluated relative to the animal-to-animal variability within a dose group. The repeated measures ANOVA model took the following form:

$$Y_{ijk} = (\mu + \delta + p_i) + (\tau_1 + [\delta\tau]_j + \epsilon_{ik}),$$

where $Y_{ijk}$ is the observed value of a given endpoint at the $k^i$ time period for the $j^i$ animal in the $i^i$ dose group (i=1,..4; j=1,..10; k=1,..5), $\mu$ is an overall constant, $\delta$ is the effect of the $i^i$ dose group, $p_i$ represents random variability in results for the animals within a dose group, $\tau_1$ is the effect of the $k^i$ time period, $[\delta\tau]_j$ represents interaction between the dose group and time period effects, and $\epsilon_{ik}$ represents random variability in results across time periods for a study animal. It is assumed that the random errors $p_i$ are an independent sample from a normal distribution with mean zero and constant variance across dose groups, and the random errors $\epsilon_{ik}$ are a second independent sample from a normal distribution with mean zero and constant variance across time periods.

If the interaction effect ($\delta\tau$) in model (1) was not significant at the 0.05 level (via an F-test), then inferences on the dose group differences were made across the entire study by performing F-tests on the main dose group effect ($\delta$) in model (1). Otherwise, separate analyses were performed at each time period to characterize the dose group effect, using the following reduced model:

$$Y_{ijk} = \mu + \delta + p_i (\text{for fixed } k).$$

Model (2) was fitted using either parametric techniques (F-tests within a one-way ANOVA) or nonparametric techniques (the Kruskal-Wallace test, using the SAS® procedure NPAR1WAY), based on the underlying distribution of the data $Y_{ijk}$. The estimates of within-group variability from the ANOVAs were used to estimate standard errors associated with the dose group averages. If the effect of dose group in models (1) or (2) was statistically significant at the 0.05 level based on a parametric analysis, then Dunnett’s $t$-test was executed at the 0.05 level to identify those dose groups (if any) which differed significantly from the control group. Occurrence of significant differences among dose groups in the nonparametric analyses was followed by performing the Wilcoxon rank-sum test multiple times, once for each Aroclor dosing level. A particular dosing level was declared significantly different from the control group if the reported significance level of the test on data for the control group and the Aroclor group was less than 0.05/D, where D is the number of dosing levels of the given Aroclor that were considered in the study.

Body weight gain through the study (i.e., change in body weight from Day 1 to Day 372) was calculated for each study animal and averaged across animals of a given sex for each dose group. The level of significance associated with the differences across dose groups in these average gains was determined for each Aroclor and sex in an ANOVA, performed by fitting model (2) above to the differences in body weights and using Dunnett’s test to identify significant differences from the control group. The estimates of within-group variability from the ANOVAs were used to estimate standard errors associated with the dose group averages.

Discrete Data Variables
The analyses of data for parameters with discrete distributions (i.e., ranking or categorical data) evaluated the significance of the association between the data values and dose group. For a given parameter, the number of responses was determined within each combination of dose group and response category by Aroclor, sex, and study week. Response categories with extremely low numbers of responses were identified and possibly combined with neighboring categories to facilitate statistical analysis. Then, Fisher’s exact test of association was performed by applying the FREQ procedure within the SAS® System. This test was considered over the chi-square test of association due to the small numbers of data values within each category for each dose group. Data analyses were not performed on those discrete data parameters whose values were equal or nearly equal across all study animals.

RESULTS
In the presentation of results, all references to significant difference were done so at the 0.05 level, unless otherwise indicated.

Body Weight
Group mean body weight gain through the study (Day 1 to Day 372) are plotted for males and females in Figure 1. On
FIG. 1. Average body weight gain (g) over the study (day 1 to day 372) for each Aroclor dose group and sex.
average, male control animals gained 541 g (249 to 790 g), and female controls 271 g (202 to 473 g) over the study. Standard error bars associated with the mean weight gains, calculated using the estimate of within-group variability from the one-way ANOVAs [model (2)], are included in the plots within Figure 1.

For males, the only incidence of significant differences in average weight gain among dose groups occurred with Aroclor 1254 ($p < 0.001$), where average weight gain in the 100 ppm group was 74.4% of the control group average and was declared significantly different from control by Dunnett’s test. Significant differences across dose groups in average weight gain among females were observed for Aroclor 1242 ($p = 0.028$, where the 100 ppm group was declared significantly different from control by Dunnett’s test) and Aroclor 1254 ($p < 0.001$, where all groups were found to be significantly different from control by Dunnett’s test). Final mean body weights for these groups of females ranged from 71.7 to 82.9% of control.

For individual animals, body weights tended to increase over the study, with rapid growth in the early part of the study, followed by more gradual increases that became linear in nature during the later part of the study.

**Feed Consumption**

The group mean feed consumption for the control groups ranged from 25 to 30 g for males and 19 to 28 g for females. A pattern of slightly decreased food consumption was evident for males in the 100 ppm Aroclor 1254 group. Feed consumption for the remaining male groups was not different from control. For females, a dose-related pattern of decreased feed consumption was evident for all Aroclor 1254 dose groups, and in the 200 ppm Aroclor 1016 group.

The group mean dietary PCB consumption (mg/kg/day) increased in proportion to ppm administered in the diet (Table 2). This exposure index (mg/kg/day) indicated greater PCB consumption by females than males. The greater feed consumption was reflected in greater body burdens in females than males, by a factor of approximately 2-fold (Silkworth et al., 1995).

**Body Temperature**

Average body temperatures calculated for each dose group and sex at 13-week intervals are plotted in Figure 2. Standard error bars associated with each average are included, as calculated from variability estimates obtained from the ANOVAs. These plots show only slight increases in average body temperature over the study, with no dose-related pattern evident for any Aroclor. These increases do not appear to be biologically significant.

For all but Aroclor 1254 (where no significant differences were observed among dose groups), significant interaction between dose and week effects resulted in separate analyses by study week. In these analyses, the following incidences of significant dose group effects were observed, with results of Dunnett’s test for comparisons to the control group indicated when significant differences were present:

- Aroclor 1016, males, Week 13: $p = 0.001; 50$, 100 ppm significantly different from control
- Aroclor 1016, females, prestudy: $p < 0.001$
- Aroclor 1242, males, Week 13: $p = 0.028; 50$ ppm significantly different from control
- Aroclor 1242, females, prestudy: $p = 0.003$
- Aroclor 1242, females, Week 52: $p = 0.008; 50$ ppm significantly different from control
- Aroclor 1260, males, Week 13: $p = 0.032$
- Aroclor 1260, males, Week 52: $p = 0.026; 100$ ppm significantly different from control
- Aroclor 1260, females, Week 52: $p < 0.001; 50$ ppm significantly different from control.

**Landing Foot Splay**

Average values for landing foot splay are plotted in Figure 3, with standard error bars calculated from variability estimates obtained from the ANOVAs.

For males, the plots in Figure 3 indicate that average landing foot splay at Week 52 was greater in all groups, including controls, than that measured at prestudy. The increase in male controls across this period was approximately 1 cm. For Aroclor-treated males, 7 out of 11 groups showed slight increases of greater than 1 cm over this period. Only one incidence of significant difference in the average values across dose groups was observed for males: Aroclor 1254 on Week 26 ($p = 0.019$), with 100 ppm significantly different from control by Dunnett’s test (a 37% increase over the controls). This finding was not consistent across measurement intervals.

For females, average landing foot splay in control animals

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**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (ppm.)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aroclor 1016</td>
<td>50</td>
<td>2.7</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.6</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>11.3</td>
<td>14.1</td>
</tr>
<tr>
<td>Aroclor 1242</td>
<td>50</td>
<td>2.6</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Aroclor 1254</td>
<td>25</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.8</td>
<td>3.6</td>
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<tr>
<td></td>
<td>100</td>
<td>5.8</td>
<td>6.9</td>
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<tr>
<td>Aroclor 1260</td>
<td>25</td>
<td>1.3</td>
<td>1.5</td>
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<td>50</td>
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</tr>
<tr>
<td></td>
<td>100</td>
<td>5.6</td>
<td>6.7</td>
</tr>
</tbody>
</table>
FIG. 2. Average body temperature (°C) versus monitoring period for each Aroclor dose group and sex.
FIG. 3. Average landing foot splay (cm) versus monitoring period for each Aroclor dose group and sex.
generally did not change from prestudy to Week 52, and no significant differences were observed between the treatment and control groups. For the 50 ppm Aroclor 1016, 100 ppm Aroclor 1242, and 50 ppm Aroclor 1260 groups, a slight increase in landing foot splay (1–1.7 cm) was measured from prestudy to Week 52. However, this finding was not consistent by Aroclor dose group or study interval, suggesting that the statistical effect was likely not biologically relevant and possibly the result of a Type II error.

Grip Strength

Average forelimb and hindlimb grip strengths, with standard error bars calculated from variability estimates obtained from the ANOVAs, are plotted in Figures 4 and 5, respectively. Average grip strength increased considerably from prestudy to Weeks 13 and 26 for both treatment and control groups, with the rate of increase curtailing through the remainder of the study (especially in forelimb grip strength). The repeated measures ANOVAs indicate that, for both forelimb and hindlimb grip strength, this increasing pattern was statistically equivalent across the dose groups, for each Aroclor and sex.

Number of Rears

Figure 6 contains plots of average numbers of rears in the open field test, with standard error bars calculated within the ANOVAs. The incidence of rearing consistently declined for males through Week 26 and for females through Week 39, then remained steady to Week 52. Significant differences across dose groups were observed only in the following incidents, with results of Dunnett’s test for comparisons to the control group:

- Aroclor 1254, males, Week 26: $p = 0.009$; 50 ppm significantly different from control
- Aroclor 1260, males, pre-study: $p = 0.008$; 25, 50 ppm significantly different from control

These significant findings did not appear to be specifically due to dose level and were not consistent across measurement intervals.

Motor Activity

Total horizontal activity and total vertical activity, averaged across animals within each animal group, are plotted in Figures 7 and 8, respectively. Average totals are accompanied by standard error bars calculated from variability estimates obtained from the ANOVAs. Note that no vertical activity data were available for the prestudy period.

As expected with increasing age, a general decline in average total horizontal activity was observed through the study for each sex (Figure 7). The greatest decline occurred from Week 13 to Week 26. This pattern of decline was generally consistent across the dose groups. It is uncertain, however, of the extent to which enlarging the motor activity testing apparatus after Week 13 to accommodate the larger size of the animals may have had on this decline. The only incident of significant differences in average total horizontal activity across dose groups occurred at Week 13 for females treated with Aroclor 1242 ($p = 0.019$), where the 100 ppm group was significantly different from the control group according to Dunnett’s test.

A similar pattern of decline was observed for total vertical activity (Figure 8) as was seen for total horizontal activity. Due to the distributional characteristics of total vertical activity, a nonparametric analysis was occasionally necessary to determine the presence of significant differences across dose groups. The following incidents of significant differences were observed with total vertical activity:

- Aroclor 1016, females, Week 13: $p = 0.026$; 100 ppm significantly different from control
- Aroclor 1242, males, entire study: $p = 0.016$; 50, 100 ppm significantly different from control
- Aroclor 1242, females, Week 13: $p = 0.047$; 100 ppm significantly different from control
- Aroclor 1254, females, Week 26: $p = 0.004$; 25 ppm significantly different from control
- Aroclor 1254, females, Week 39: $p = 0.017$; 100 ppm significantly different from control
- Aroclor 1260, males, Week 13: $p = 0.041$; 50 ppm significantly different from control
- Aroclor 1260, males, Week 39: $p = 0.019$; 25 ppm significantly different from control

The percent habituation of motor activity was generally similar between Aroclor-treated and control animals. For each assessment time point, motor activity for Aroclor groups during the final 5-min measurement interval was approximately 70–90% lower for males and 60–80% lower for females, than during the first 5-min interval. Similar reductions in motor activity were recorded for male and female controls.

Discrete Data

Generally, frequency counts of selected responses for discrete FOB parameters within each category provided in Table 1 (autonomic, muscle tone and equilibrium, sensorimotor, and central nervous system) were similar for Aroclor-treated and control animals. There were isolated incidences when results from Aroclor dose groups and the control group differed significantly. However, these changes were not consistent among measurement intervals, demonstrated no dose-related pattern, and thus were not considered treatment related.

Neuropathology

No histomorphologic lesions were found that were attributable to Aroclor exposure. A variety of incidental, spontaneous, or degenerative changes, of minimal to mild severity, were found in the spinal cord, spinal root fibers, and cranial and peripheral nerves. These changes consisted of axonal degeneration and axon/sheath swelling, and were seen in similar inci-
FIG. 4. Average forelimb grip strength (kg) versus monitoring period for each Aroclor dose group and sex.
FIG. 5. Average hindlimb grip strength (kg) versus monitoring period for each Aroclor dose group and sex.
FIG. 6. Average number of rears versus monitoring period for each Aroclor dose group and sex.
FIG. 7. Average total horizontal activity versus monitoring period for each Aroclor dose group and sex.
FIG. 8. Average total vertical activity versus monitoring period for each Aroclor dose group and sex. No vertical activity data were available for the prestudy period.
dence and severity in treated and control animals. In the spinal cord and spinal root fibers, lesions consisted morphologically of swollen, often empty axonal sheaths, predominantly in the ventral and lateral funiculi, yielding a vacuolated appearance at low magnification. This condition, called spontaneous radiculoneuropathy, begins in the ventrolateral spinal tracts, spreads to the spinal roots, and moves distally. Older rats can be severely affected both morphologically and clinically; lesions in affected rats on this study were minimal to mild. Cranial and peripheral nerves also exhibited evidence of minimal to mild axonal degeneration and swelling.

**DISCUSSION**

Neurobehavioral testing was combined with other toxicologic end points to assess the toxicity of Aroclors 1016, 1242, 1254, and 1260 after 52 weeks of dietary exposure. The functional observational battery, motor activity, and neuropathology along with clinical observations, measurements of body weight, and feed consumption were performed at specified intervals during the 52 weeks of the study. The evaluations included distinct categories of responses that represented a wide range of behavioral and functional end points as well as anatomical correlates.

Although there were several instances where treatment with Aroclor resulted in statistically significant changes relative to control, these were considered incidental and were not associated with Aroclor exposure in any consistent way. The occurrence of statistically significant effects from the various Aroclor mixtures was sporadic, e.g., they were observed at only one test interval, and no consistent pattern for the changes was evident. For example, landing foot splay was increased at Week 26 in the 100 ppm Aroclor 1254 males, but was not different from control at other time points for this dose group. Additionally, muscle tone and equilibrium were not affected. Responses across Aroclor dose groups were generally consistent with the profiles observed for the control animals. Likewise, the effect of dosing on motor activity did not appear to be biologically significant. The lack of behavioral results was supported by the absence of clinical signs, and by the fact that upon microscopic examination, no CNS lesions were found that were attributable to Aroclor-induced neurotoxicity. A variety of minimal to mild incidental, spontaneous, or degenerative changes were found in the spinal cord, spinal root fibers, and cranial and peripheral nerves. However, these changes were seen with similar incidence and severity in treated and control groups. Such spontaneous changes are not uncommon in aging Sprague-Dawley rats and have been previously described (Burek, 1978; Jortner and Percy, 1978; King, 1994; Mitsumori and Boorman, 1990). After 12 months on test, the age of the animals in this study was approximately 14 months.

Although neurotoxicity did not develop during 52 weeks of Aroclor exposure, toxicity was evident in the Aroclor 1254 and 1016 groups based on changes in body weight and feed consumption. Growth rates late in the study were significantly decreased for Aroclor 1254 in all dose groups for females and in the 100 ppm dose group for males. In addition, the final body weight for the female 200 ppm Aroclor 1016 group was significantly less than control. The pattern of reduced feed consumption evident in these same groups correlated with the observed body weight gain decreases. The decreased feed consumption and body weight decreases are consistent with systemic toxicity rather than poor palatability of test material, which would have been characterized by a marked reduction in feed consumption at the initiation of the study. The decreased body weights and feed consumption occurred in the absence of any treatment-related clinical signs. Other than some incidental occurrences of small abrasions, alopecia, nasal/eye discharge, and abdominal swelling (tissue masses) in both control and treated animals, all rats appeared normal throughout the study.

The absence of signs of neurotoxicity in the current study is consistent with the findings of Nishida et al. (1997) for a sub-chronic study in adult rats receiving Aroclor 1254. In the current study, chronic dose levels for Aroclor 1254 ranged approximately from 1.5 to 7 mg/kg/day. Nishida et al. (1997) reported the no observed effect level (NOEL) for decreased motor activity as 10 mg/kg/day, with the effects observed at higher doses being reversible after a 3-week recovery period. Therefore, although dietary administration of Aroclor at 100 ppm is a maximum tolerated dose (MTD) for chronic administration (Mayes et al., 1998), it is below the threshold for neurotoxicity.

In summary, 52 weeks of exposure to high dose levels of several Aroclor mixtures in the diet failed to produce any functional or morphologic changes indicative of PCB-induced neurotoxicity. In contrast, body weight and feed consumption decreases suggested that some general toxicity was present for Aroclor 1254-exposed animals after 1 year of exposure.

**REFERENCES**


ATSDR. (1996). Toxicological Profile for Selected PCBs (Aroclor 1260, 1242, 1254, and 1260) at 100 ppm is a maximum tolerable dose (MTD) for chronic administration (Mayes et al., 1998), it is below the threshold for neurotoxicity. 1254-exposed animals after 1 year of exposure.


