Chronic Toxicity of Three Polychlorinated Biphenyls in Albino Rats

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Running Title: Chronic Toxicity of 3 PCB's in Albino Rats

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ABSTRACT

Chronic Toxicity of Three Polychlorinated Biphenyls in Albino Rats. Fancher, Otis E., Keplinger, M. L., Plank, J. B., Wheeler, E. P., and Calandra, J. C. (0000). Toxicol. Appl. Pharmacol. 00, 00 - 00. Two year toxicity studies were conducted in albino rats with 3 polychlorinated biphenyls (Aroclors 1242, 1254 and 1260) at dietary levels of 1, 10 and 100 ppm. Each of the three materials at the 100 ppm level caused liver changes characterized by increased liver weights, increases in liver to body weight and liver to brain weight ratios, and by slight to mild centrilobular hypertrophy with fatty vacuolization. These changes were observed only in animals sacrificed after 24 months of treatment. No other effects were observed at 100 ppm except for a weight depression for female rats with Aroclor 1254 and no effects were observed with any material at 1 or 10 ppm.

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INDEX TERMS

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Background information has been summarized in the introductory paper of this series (Fancher et al., 0000). The present study was conducted in order to determine the effect of long-term, low-level exposure of albino rats to three polychlorinated biphenyls of variable chlorine content.

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METHODS

All animals utilized in these studies were weanling albino rats of the Charles River strain obtained from Charles River Breeding Laboratories, North Wilmington, Mass. A control group and nine treatment groups, each consisting of 50 male and 50 female animals, were employed, three treatment groups for each of the 3 Aroclors studied.

All diets were prepared fresh each week by mixing the appropriate Aroclor at levels of 1, 10 and 100 ppm with Purina Rat Chow (Ralston Purina Company, St. Louis, Missouri) in a Hobart Mixer. Each individually housed rat was offered an amount of food sufficient for ad libitum feeding for one week. Unconsumed food was weighed and food consumption was recorded for five rats of each sex from each group weekly for the first three months and once monthly for the next nine months. Periodic spot checks were made thereafter. Water was freely available at all times.

Initially the body weight of each rat was determined. Individual weights were determined weekly for 13 weeks and monthly thereafter.

Blood studies, including determinations of hemoglobin concentration, hematocrit value, erythrocyte count and total and differential leukocyte counts, and urine analyses for the presence of glucose, albumin and microscopic elements and determinations of pH and specific gravity were conducted for five male and five female rats from the control groups and from each high dose level group after 3, 6, 9, 12, 18 and 24 months of treatment.

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Determinations of blood urea nitrogen concentration (BUN), serum alkaline phosphatase activity (SAP), fasting blood glucose concentration and serum glutamic-pyruvic transaminase activity (SGPT) were determined for the same animals at the same times.

A gross autopsy was performed on each animal which died during the study and when feasible tissues were preserved in formalin for histopathologic study.

After 3, 6 and 12 months of testing 5/5 animals from each group were sacrificed and subjected to complete gross pathologic examinations. Organ weights were recorded for liver, kidneys, spleen, gonads, heart and brain and organ to body weight and organ to brain weight ratios were calculated. These data were subjected to an Analysis of Variance and significant disclosures were further studied by "t"-tests.

Microscopic examinations were conducted on 33 tissues from all control and high treatment level animals sacrificed after 3, 6 and 12 months of testing following fixation in 10% formalin and staining with hematoxylin-eosin.

At termination of the study after 24 months of treatment the remaining animals were sacrificed and all were subjected to gross and microscopic examination.

A tabulation of the incidence of tumor occurrence for each group was made at the conclusion of the investigation and data relative to location, weight, size and pathologic classification for each tumor were recorded.

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RESULTS

A. Body Weights and Weight Gains:

Except for a statistically significant (99% confidence level) depression of weight gain at the 24 - month point for female rats fed 100 ppm of Aroclor 1254, no other effect was observed.

B. Food Consumption:

Food consumption measurements during the first 12 months of the study and periodic checks thereafter revealed no significant differences between the control group and any test group.

C. Mortality and Reactions:

The number of animals dying and the time frequency distribution of deaths did not differ among treatment and control groups.

D. Hematologic and Blood Chemistry Studies:

Values for all parameters were within the normal range for the albino rat. No significant differences between values for the control groups and those for any test group were observed.

E. Urine Analyses:

No differences from control data were observed for test animals from any group.

F. Pathologic Studies:

1. Three, Six, and Twelve Month Sacrifices.

Gross and microscopic findings were not different for control animals and animals from any test group. With each compound organ weights, organ to body weight ratios and organ to brain weight ratios

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disclosed several randomly occurring intergroup differences but the lack of any consistent dose related response and the absence of any deleterious histopathologic changes indicate that the differences were not directly related to the ingestion of the Aroclors.

The lesions noted in the microscopic examinations of tissues from control and test animals were those of spontaneous disease common for the albino rat; lesions of the trachea and lungs indicative of chronic murine pneumonia.

2. Final Sacrifice

a. Gross Pathologic Findings

Gross findings for animals from all treated groups were not different from those for control animals.

b. Organ Weight and Ratio Data

Each of the three Aroclors led to increased liver weights and increases in liver to body weight and liver to brain weight ratios of rats treated at the 100 ppm level, females only in the case of Aroclor 1242. The data for liver weights and ratios are summarized in Table 1.

Statistical evaluations of data for other organs disclosed additional randomly occurring intergroup differences, but the lack of any consistent dose related response and the absence of any significant histopathologic changes indicate that these differences are probably unrelated to ingestion of the Aroclors.

c. Histopathologic Changes

1. Aroclor 1242

Significant liver changes were found in animals fed 100 ppm of

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Aroclor 1242. The compound associated lesions consisted of vacuolar changes, focal hypertrophy and focal hyperplasia. In addition, there were lesions of inflammation, necrosis, fibrosis and minor degeneration in this group which, although seen in the controls and lower test groups, were more severe and frequent at the highest treatment level.

The vacuolar change which was observed occasionally in the control animals and in animals treated at levels of 1 or 10 ppm but was observed frequently in animals at the 100 ppm level. The lesion is morphologically indicative of fatty degeneration. Formalin-frozen sections of liver from representative animals which displayed vacuolar changes were stained with Oil Red O to reveal the presence of fat. The vacuolar lesion in the cytoplasm of these cells was positively identified as fat.

The hypertrophic change found in the liver was focal and often limited to the central lobular area where groups of cells were swollen to two or three times their normal size with clear pink homogenous cytoplasm. The hyperplasia was associated with the same cells and appeared to be an extension of the hypertrophic lesion. The hyperplastic cells were also usually hypertrophic. The most severe examples of hyperplasia appeared as nodular growths with limited compression of the surrounding normal hepatic tissue.

Other minor lesions of degeneration, hepatitis, ductile cell proliferation, necrosis and focal lymphoid infiltration seen in the livers of control and treated animals are lesions of spontaneous disease and not related to the Aroclor 1242. This level of liver disease is not unusual in old animals.

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Hyperplasia of the transitional epithelium was present in the urinary bladder of animals from the control group and from each of the test groups. This lesion was usually associated with cystitis.

All of the other lesions in the other tissues found in these animals are related to spontaneous disease and they are not unusual for rats of this age.

2. Aroclor 1254

The histopathologic changes observed may be described in terms identical to those given above for Aroclor 1254. The most severe hyperplastic changes in the transitional epithelium of the bladder were associated with the presence of mineralized calculi in the bladder lumen.

3. Aroclor 1260

The histopathologic changes observed were analogous to those described above for Aroclor 1242 except that hyperplasia of the urinary bladder was not observed.

The occurrence of hepatic fatty vacuolization, which is judged to be a treatment related effect, is summarized in Table 2. The bladder findings are presented in Table 3. The occurrence of epithelial hyperplasia in a control animal and the absence of a dose correlation with regard to either incidence or severity make it doubtful that the bladder lesions are related to treatment with the Aroclors.

D. Tumor Findings

Tumor data revealed no indication that any of the Aroclors are

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carcinogenic. The incidence of tumors and the tumor types for all groups, including the control group, were as would be expected for a random population of rats at the age of two years. The tumor findings are summarized in Table 4. The predominant tumors were abdominal fibroadenomas. Adenomas of the pituitary, thyroid, parathyroid, adrenals and pancreas occurred with much lower frequencies. Tumors judged to be malignant were of random occurrence and included adenocarcinoma of pancreas and abdomen, lymphosarcoma of lymph nodes, spleen or liver and lymphatic reticulum cell sarcoma.

DISCUSSION

From the results of this study it is concluded that 100 ppm is an effect level for each of the Aroclors investigated. The target organ is the liver and the effect is characterized by increased liver weights and increased liver/body weight and liver/brain weight ratios and by vacuolar lesions indicative of fatty degeneration. The effect was slow to develop, not being seen among animals sacrificed after 3, 6 or 12 months, and was not associated with functional changes in SAP or SGPT. In all cases the severity of the vacuolar changes was judged to be slight to mild (grades 1 or 2 on a 5-point grading system). Since the frequency of occurrence of fatty vacuolization was not greater for animals treated at levels of 1 and 10 ppm than for control animals, it is judged that 10 ppm is a no-effect level for each of the three Aroclors.

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TABLE 1

Liver Weight and Ratio Data for Albino Rats Treated

with Aroclors 1242, 1254 and 1260

Test Material	Dietary level (ppm)	Liver Weight (g)		Liver/Body Wgt. Ratio (g/100g)		Liver/Brain Wgt. Ratio (g/100g)	
		Males	Females	Males	Females	Males	Females
None	--	22.0	16.3	3.47	3.05	10.10	8.42
Aroclor 1242	1	21.9	15.7	2.93	3.00	9.97	8.03
	10	22.3	17.1	3.18	3.19	10.20	8.50
	100	25.6	22.4[a]	3.95	4.82[a]	11.90	11.60[a]
Aroclor 1254	1	19.5	16.1	2.99	3.10	8.76[b]	8.31
	10	23.9	19.5	3.21	3.84[b]	11.40[b]	10.30
	100	30.2[a]	32.1[a]	4.75[a]	8.62[a]	14.10[a]	17.10[a]
Aroclor 1260	1	22.0	16.6	3.40	3.35	9.93	8.62
	10	24.1	18.1	3.74[b]	3.19	11.20	9.26
	100	29.9[a]	22,7[a]	4.67[a]	4.42[a]	13.42[a]	11.60[b]

[a] Statistically significant at the 99 percent confidence level

[b] Statistically significant at the 95 percent confidence level

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TABLE 2

Occurrence of Hepatic Fatty Vacuolization in Albino Rats

Treated with Aroclors 1242, 1254 and 1260

Test Material	Dietary Level (ppm)	Incidence of Fatty Vacuolization			
		Male	Av. Grade	Female	Av. Grade
None	—	0/11	—	2/14	1
Aroclor 1242	1	0/13	—	0/14	—
	10	2/13	1	0/13	—
	100	3/ 6	1	11/14	1
Aroclor 1254	1	1/11	1	3/22	2
	10	3/12	1	1/12	1
	100	3/11	1	10/15	1
Aroclor 1260	1	0/11	—	1/14	2
	10	2/10	2	5/15	1
	100	2/10	2	5/15	1

Grading System:
1, 0 = minimal; 2 = mild; 3 = moderate; 4 = severe; 5 = extreme

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TABLE 3

Urinary Bladder Lesions of Albino Rats Treated with

Aroclors 1242, 1254 and 1260

Test Material	Dietary Level (ppm)	Animals Examined	Focal Epithelial Hyperplasia		Focal Epithelial Hyperplasia with Cystitis	
			Incidence	Av. Grade	Incidence	Av. Grade
None	—	25	1	1	—	--
Aroclor 1242	1	25	—	—	2	1.5
	10	24	—	—	2	2
	100	19	1	2	—	—
Aroclor 1254	1	29	—	—	1	3
	10	26	—	—	2	3
	100	27	1	1	1	2
Aroclor 1260	1	21	—	—	—	—
	10	21	—	—	—	—
	100	24	—	—	—	—

Grading System:
1 = minimal; 2 = mild; 3 = moderate; 4 = severe; 5 = extreme

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TABLE 4

Tumor Findings Among Albino Rats Treated with

Aroclors 1242, 1254 and 1260

Test Material	Dietary Level (ppm)	No. of Animals Examined	Tumor Incidence			No. of Animals with Tumors
			Benign	Malignant	Total	
None	–	26	9	2	13	10
Aroclor 1242	1	29	17	2	19	14
	10	27	14	1	17	13
	100	21	4	0	6	5
Aroclor 1254	1	34	15	1	16	13
	10	24	13	0	13	11
	100	27	12	3	15	15
Aroclor 1260	1	28	10	0	10	7
	10	23	12	0	12	9
	100	28	9	3	12	11

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FOOTNOTES

[1] Aroclors 1242, 1254 and 1260. Monsanto Company, St. Louis, Missouri.

[2] Present Address: 624 North Abrego Drive, Green Valley, Arizona 85614

[3] Monsanto Company, St. Louis, Missouri

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