EFFECT OF SIMULTANEOUS ADMINISTRATION OF POLYCHLORINATED BIPHENYLS AND ALKYLBENZENE SULFONIC ACID SALT IN RATS

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Administration of PCB to rats resulted in an increase in liver weight, hypertrophy with foam-like changes in cytoplasm of individual liver cells, an increase in liver cholesterol levels, an increase in liver aniline hydroxylase activity and a decrease in liver Na-K-Mg-dependent ATPase activity. When ABS was simultaneously administered with PCB, most of the above-mentioned abnormalities were observed to increase and were dependent on administration time and dosage. Testicular abnormalities were observed only in rats on long term co-administration of PCB and ABS.

In 1968, over 1,000 persons in Kita Kyushu district (Southern Japan) suffered from blindness, gastrointestinal symptoms plus abdominal pain, jaundice, edema and a dermatitis following ingestion of rice oil which contained 2,000 to 3,000 ppm of polychlorinated biphenyls (PCB) (Kuratsune *et al.* 1969). Alkylbenzene sulfonic acid salt (ABS) is an industrially used neutral cleansing agent which has universally accumulated in the waterways. The LD_{50} of ABS is in the range of 500 to 3,000 mg/kg to various mammals (Swisher 1968). Chronic toxicity was examined by Bornmann (1963). He administered ABS to the rats in the drinking water at 100 ppm for two years and no significant difference was observed between the ABS-fed animals and the controls. However, the toxicity of this substance is still highly debatable (Buehler *et al.* 1971, Yanagisawa 1963, Tokoro *et al.* 1964). As both PCB and ABS are ingested by humans, the authors attempted to discern the combined effect of these substances on rats, the results of which are outlined herein.

Materials and methods

Kanechlor 500 (a Japanese manufactured PCB containing as its main components pentachlorobiphenyl), was used.

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Reagent grade ABS (laurylbenzene sulfonic acid sodium salt, Wako Chemical Co., Japan) was employed.

Male rats of the Wistar strain were housed individually in stainless steel cages with a raised wire bottom.

Experiment No. I. Rats weighing about 60 g were divided into four groups of twelve animals each. To each group the following diets and water were given.

Group 1: normal diet and tap water.
Group 2: normal diet and water containing 1,000 ppm of ABS.
Group 3: 500 ppm of PCB-supplemented diet and tap water.
Group 4: 500 ppm of PCB-supplemented diet and tap water containing 1,000 ppm of ABS.

Experiment No. II. Rats weighing about 150 g were divided into eight groups of eight animals each. To each group the following diet and water were given.

Group 1: normal diet and tap water.
Group 2: Ten ppm of PCB-supplemented diet and tap water.
Group 3: 100 ppm of PCB-supplemented diet and tap water.
Group 4: 500 ppm of PCB-supplemented diet and tap water.
Group 5: normal diet and water containing 1,000 ppm of ABS.
Group 6: Ten ppm of PCB-supplemented diet and water containing 1,000 ppm of ABS.
Group 7: 100 ppm of PCB-supplemented diet and water containing 1,000 ppm of ABS.
Group 8: 500 ppm of PCB-supplemented diet and water containing 1,000 ppm of ABS.

Diets and water were provided *ad libitum* for the duration of the experiment. Diets and water intakes were determined every two days and no significant differences in diet (average: 17.3 g/day) and water (average: 15.2 ml/day) intakes were observed between any of the groups. The composition of normal diet (Oriental Food Co., Japan) is as follows (in 100 g diet): protein, 24.2 g; fat, 5.5 g; fiber, 4.5 g; carbohydrates, 52.6 g; salts, 6.2 g; water, 7.0 g; vitamin A, 1,000 I.U.; vitamin D, 200 I.U.; vitamin E, 1.6 mg; vitamin B_1, 0.9 mg; vitamin B_2, 0.8 mg; vitamin B_6, 0.8 mg; niacin, 4 mg; pantothenic acid, 1.9 mg; vitamin C, 50 mg; inositol, 60 mg; choline, 100 mg.

Analytical Procedure. After a given period on these dietary regimens, blood specimens were obtained from the descending aorta using a plastic syringe. Serum was centrifugally separated from blood clot, and brain, heart, liver, kidneys, spleen, and testicles were dissected and weighed. Portions of these tissues and serum were used for analysis.

Serum alkaline phosphatase activity was assayed by the method of Hansen (1966), and choline esterase activity (Weber 1966), glutamate-oxaloacetate transaminase (GOT)

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and glutamate-pyruvate transaminase (GPT) activities (Reitman and Frankel 1957) were also determined.

Cholesterol levels in serum were determined by the method of Abell *et al.* (1952), total and free cholesterol levels in liver by the method of Sperry and Webb (1950), serum and liver trigylceride levels according to Sardesai and Manning (1968), and serum and liver non-esterified fatty acids levels by the procedure of Duncombe (1964).

Aniline hydroxylase activity in liver was assayed by the method of Imai *et al.* (1966) and Na-K-Mg-dependent ATPase and Mg-dependent ATPase by the method of Nakao *et al.* (1965).

Iron levels in liver and serum were determined by the bathophenanthroline method (Beale *et al.* 1961), serum urea by the method using diacetylmonoximethiosemicarbazid (Coulombe and Favreau 1963), protein by the procedure of Lowry *et al.* (1951), and DNA and RNA levels in liver by the method of Ceriotti (1955).

For histological analysis, all tissues were fixed in 3% formaldehyde solution. Sections of 5 μ thickness were cut and stained with hematoxylin and eosin.

Results

Experiment No. I. After 1, 3 and 7 months on diets, four rats from each group were sacrificed and the body weights determined (Table I). In the control group and ABS-administered group (groups 1 and 2) good growth was observed, while growth in the PCB-administered group and the PCB-ABS co-administered group (groups 3 and 4) was depressed significantly. Actual liver weight, the weight of liver and testicles expressed as percentage of body weight for each group are shown in Table II. In PCB-administered groups 3 and 4, liver weight increased significantly; however in the ABS-administered group (group 2), liver weight was normal. Regarding actual liver weight, the weight was greater in group 4 than in group 3 at seven months. Fig. 1 shows the gross appearance of the livers.

Light-microscopic examination of livers of groups 3 and 4 revealed swelling of the individual hepatic cells, nuclei showed picnosis, cytoplasm had many vacuoles and degenerative changes were prominent in scattered areas of the liver. (Fig. 2A, normal liver; 2B, groups 3 and 4).

DNA, RNA, and protein content and concentration in livers are shown in Table III. Liver DNA concentration was decreased in the PCB-administered groups (groups 3 and 4), however the total DNA content in liver underwent no significant change as liver weight was increased in the PCB administered groups. In contrast, total RNA and protein content per liver increased significantly in proportion to the increase of liver weight in PCB-administered rats. No change was observed in RNA and protein with respect to concentrations.

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Table I. *Initial and terminal body weight of rats in experiment I*[a]

Group	Additives to		Initial[b] Weight (g)	Terminal Weight (g)[c]		
	Diet	Water		1 Month	3 Months	7 Months
1	NONE	NONE	62 ± 5[x]	216 ± 7[x]	303 ± 7[x]	371 ± 19[x]
2	NONE	ABS (1000 ppm)	62 ± 4[x]	219 ± 12[x]	263 ± 23[x]	351 ± 10[x]
3	PCB (500 ppm)	NONE	63 ± 6[x]	185 ± 10[xy]	228 ± 15[y]	250 ± 11[y]
4	PCB (500 ppm)	ABS (1000 ppm)	64 ± 8[x]	176 ± 8[y]	231 ± 13[y]	245 ± 9[y]

[a] Values represent mean ± S.E.
[b] 12 rats
[c] 4 rats average
Data were considered statistically based on the method of one factor ANOVA. Different letter superscripts (x,y) following mean and S.E. denote significant differences ($p < 0.05$) between groups in each column. (eg, entries with superscript "x" showed no significant differences between groups; those with superscript "y" showed significant differences on comparison with groups not marked "y").

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Table II. *Actual liver weight, ratio of liver and testicular weight to body weight in experiment I*

	Group	Additives to		Experimental period		
		Diet	Water	1 Month	3 Months	7 Months
				(g)	(g)	(g)
Actual Liver Weight	1	NONE	NONE	8.51 ± 0.84[a]	8.64 ± 0.51[a]	9.53 ± 0.21[a]
	2	NONE	ABS (1000 ppm)	9.11 ± 1.03[a]	8.57 ± 0.27[a]	8.78 ± 0.18[a]
	3	PCB (500 ppm)	NONE	13.73 ± 1.77[b]	13.93 ± 0.71[b]	15.60 ± 0.79[b]
	4	PCB (500 ppm)	ABS (1000 ppm)	13.83 ± 1.51[b]	16.33 ± 2.12[bc]	19.25 ± 2.15[c]
				(%)	(%)	(%)
Liver Weight/ Body Weight	1	NONE	NONE	3.94 ± 0.41[a]	2.85 ± 0.15[a]	2.57 ± 0.12[a]
	2	NONE	ABS (1000 ppm)	4.16 ± 0.32[a]	3.26 ± 0.46[a]	2.50 ± 0.30[a]
	3	PCB (500 ppm)	NONE	7.42 ± 1.11[b]	6.11 ± 0.43[b]	6.24 ± 0.83[b]
	4	PCB (500 ppm)	ABS (1000 ppm)	7.86 ± 1.23[b]	7.07 ± 1.08[b]	7.86 ± 1.50[b]
Testicles Weight/ Body Weight	1	NONE	NONE	0.56 ± 0.03[a]	0.52 ± 0.02[a]	0.44 ± 0.02[a]
	2	NONE	ABS (1000 ppm)	0.59 ± 0.02[a]	0.58 ± 0.04[a]	0.46 ± 0.01[a]
	3	PCB (500 ppm)	NONE	0.61 ± 0.04[a]	0.66 ± 0.08[a]	0.50 ± 0.02[a]
	4	PCB (500 ppm)	ABS (1000 ppm)	0.63 ± 0.06[a]	0.63 ± 0.06[a]	0.26 ± 0.03[b]

Values represent mean ± S.E. of 4 rats.
Different letter superscripts (a, b, c) denote significant difference ($p < 0.05$) in each tissue.
Statistical analysis was carried out according to the method of one factor ANOVA.

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Table III. *DNA, RNA, and protein levels in liver (7 months) in experiment I*

	Group	Additives to Diet	Additives to Water	Concentration (mg/g)	Content (mg/liver)
DNA	1	NONE	NONE	2.73 ± 0.09[a]	26.45 ± 1.31[a]
	2	NONE	ABS	2.65 ± 0.16[a]	24.72 ± 1.16[a]
	3	PCB	NONE	1.81 ± 0.11[b]	28.15 ± 0.89[a]
	4	PCB	ABS	1.69 ± 0.21[b]	28.80 ± 1.07[a]
RNA	1	NONE	NONE	8.27 ± 0.73[a]	79.9 ± 2.6[a]
	2	NONE	ABS	7.95 ± 0.90[a]	73.5 ± 4.0[a]
	3	PCB	NONE	7.45 ± 0.45[a]	117.0 ± 11.6[b]
	4	PCB	ABS	7.78 ± 0.40[a]	138.8 ± 19.2[b]
				(%)	(g/liver)
Protein	1	NONE	NONE	19.5 ± 2.0[a]	1.88 ± 0.21[a]
	2	NONE	ABS	20.4 ± 1.8[a]	1.88 ± 0.16[a]
	3	PCB	NONE	18.8 ± 2.8[a]	2.94 ± 0.26[b]
	4	PCB	ABS	18.5 ± 2.2[a]	3.35 ± 0.31[b]

Values represent mean ± S.E. of 4 rats.
Different letter superscripts denote significant difference ($p < 0.05$) in each nucleic acid or protein. Statistical analysis was carried out according to the method of one factor ANOVA.

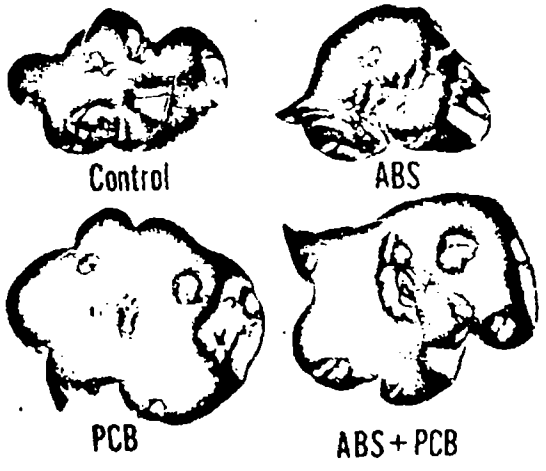
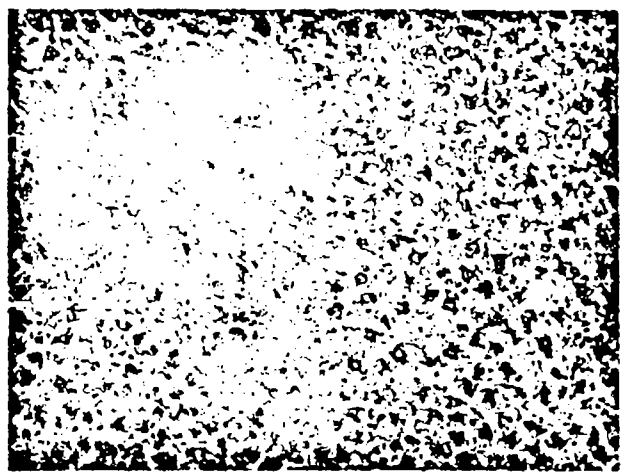


Fig. 1. Gross appearance of liver of groups 1 to 4 (Experiment I, 7 months), Blackish color change and hypertrophy seen in PCB (group 3) and ABS + PCB treated rat (group 4).

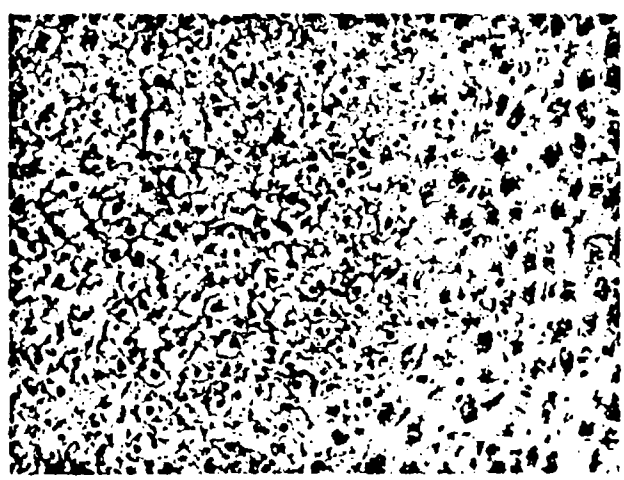
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Testicle weight decreased in the individuals in group 4 after seven months treatment (Table 2). Histological observations of the testis of these rats revealed considerable degeneration. Necrosis was observed in the seminiferous tubules (Fig. 3B), there was a disappearance of spermatogonium cells (Fig. 3B), hypertrophy of the interstitium between the tubules (Fig. 3B, 3C) and in some cases, the appearance of bizarre spermiogoniums (Fig. 3C). No significant changes in tissue weight or histological appearance were observed in other tissues.



A



B

Fig. 2. Microscopic appearance of livers. (Experiment I, 7 months) Haematoxylin-eosin, X100.
A. Normal rat (group 1). Nuclei and cytoplasma in all hepatocytes are normal.
B. PCB-ABS treated rat (group 4). Note foamy degeneration in many hepatocytes.

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Fig. 3. Microscopic appearance of testis. (Experiment I, 7 months), Haematoxylin-eosin, X100.
A. Normal rat (group 1). The seminiferous tubules and interstitial tissues are normal in appearance.
B. PCB-ABS treated rat (group 4). Note empty tubules and hyperplasia of the interstitial tissues.
C. PCB-ABS treated rat (group 4). Note appearance of bizarre spermiogoniums as well as hyperplasia of the interstitial tissue.

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GOT and GPT levels in the serum of groups 3 and 4 (seven months) increased (Table IV). There was no significant change in serum alkaline phosphatase and choline esterase activities. Cholesterol levels in the serum increased in groups 3 and 4 after 1, 3 and 7 months treatment (Table V). Total cholesterol levels in the liver increased markedly in group 3 and particularly in group 4 after 3 and 7 months. Liver total cholesterol levels increased with administration time. (Table V). Liver aniline hydroxylase activity increased significantly ($p < 0.01$) in rats on a PCB-implemented diet, the increase being augumented ($p < 0.01$) when PCB and ABS were combined. (Table VI). Liver Na-K-Mg-dependent ATPase decreased in both groups 3 and 4; however, decrease were greater in group 4. (Table VI). No significant difference was observed in Mg-dependent ATPase among the groups.

Experiment No. II. As the above experiment indicated that the effect of PCB increased when ABS was simultaneously administered, a further study was done to determine the dose response.

Growth was depressed only in groups 4 and 8 (administered 500 ppm of PCB); there was no significant change in growth among the other groups. The rats were sacrificed after one month and analysis revealed that the weights of the livers increased with increased doses of PCB. Regarding liver weight, a synergistic effect of ABS upon PCB was observed between groups 4 and 8 (500 ppm of PCB) only. (Table VII).

Serum urea levels increased in groups 4 and 8 (500 ppm of PCB). Iron levels increased in the groups 7 and 8. (Table VIII).

Serum cholesterol and liver free cholesterol levels were increased in group 8 only. Total cholesterol levels in the liver increased in the 100 and 500 ppm PCB groups 3, 4, 7 and 8. Cholesterol deposits were more marked in the PCB-ABS combined groups. (Table IX).

Anilline hydroxylase activity increased and Na-K-Mg-dependent ATPase decreased in proportion to the PCB administered. Mg-dependent ATPase was slightly decreased in the groups on 500 ppm of PCB. (Table X). There was no significant change in the triglyceride and non-esterified fatty acids levels in serum and liver.

Discussion

Monkeys which had been administered 1.4 to 16 mg/day of PCB (Kanechlor 400) for 40 to 48 days revealed a liver cell enlargement and fatty degeneration (Nishizumi 1970). A similar morphological change in rat liver was reported by Kimbrough *et al.* (1972). The present study demonstrated that the increase in size and weight of the liver in the PCB groups is attributed to the enlargement of individual liver cells. This conclusion was reached both from morphological examinations and chemical analysis of liver DNA, RNA, and protein contents (Table III). Enhancement of the liver drug metabolizing system by PCB administration has been reported by Fujita *et al.* (1971),

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Table IV. *Glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase activities (GPT) in serum in experiment I (International Units)*

Enzyme	Group	Additives to		Experimental period		
		Diet	Water	1 Month	3 Months	7 Months
GOT	1	NONE	NONE	17.9 ± 1.2[a]	20.6 ± 5.1[a]	18.7 ± 1.8[a]
	2	NONE	ABS (1000 ppm)	18.2 ± 1.7[a]	18.3 ± 5.0[a]	23.1 ± 4.2[a]
	3	PCB (500 ppm)	NONE	15.5 ± 3.6[a]	19.8 ± 3.3[a]	52.5 ± 7.5[b]
	4	PCB (500 ppm)	ABS (1000 ppm)	16.1 ± 2.7[a]	22.7 ± 4.9[a]	54.2 ± 7.4[b]
GPT	1	NONE	NONE	24.1 ± 0.7[ab]	21.8 ± 5.1[ab]	22.7 ± 2.4[ab]
	2	NONE	ABS (1000 ppm)	24.0 ± 2.7[ab]	20.9 ± 1.2[a]	24.1 ± 2.2[ab]
	3	PCB (500 ppm)	NONE	21.8 ± 3.7[ab]	31.8 ± 4.0[bc]	32.0 ± 3.9[c]
	4	PCB (500 ppm)	ABS (1000 ppm)	21.2 ± 1.2[a]	36.6 ± 6.4[bc]	44.2 ± 7.5[c]

Values represent mean ± S.E. of 4 rats.
Different letter superscripts (a,b,c) denote significant differences ($p < 0.05$) in each enzyme.
Statistical analysis was carried out according to the method of one factor ANOVA.

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Table V. *Total cholesterol levels in liver and serum in experiment I*

	Group	Additives to		Experimental period		
		Diet	Water	1 Month	3 Months	7 Months
				(mg/100 ml)		
Serum	1	NONE	NONE	106 ± 13[a]	87 ± 7[a]	94 ± 4[a]
	2	NONE	ABS (1000 ppm)	138 ± 28[ab]	124 ± 15[a]	105 ± 15[a]
	3	PCB (500 ppm)	NONE	196 ± 9[bc]	210 ± 19[bc]	211 ± 19[bc]
	4	PCB (500 ppm)	ABS (1000 ppm)	262 ± 24[c]	220 ± 24[bc]	230 ± 15[c]
				(mg/100 g wet tissue weight)		
Liver	1	NONE	NONE	241 ± 17[a]	255 ± 31[a]	257 ± 22[a]
	2	NONE	ABS (1000 ppm)	281 ± 21[a]	253 ± 25[a]	320 ± 33[a]
	3	PCB (500 ppm)	NONE	323 ± 33[b]	439 ± 32[bc]	548 ± 34[cd]
	4	PCB (500 ppm)	ABS (1000 ppm)	389 ± 74[bc]	611 ± 47[de]	768 ± 67[e]

Values represent mean ± S.E. of 4 rats.
Different letter superscripts (a,b,c,d,e) denote significant differences ($p < 0.05$) in serum or liver. Data were considered statistically based on the method of one factor ANOVA.

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Table VI. *Aniline hydroxylase, Na-K-Mg-dependent ATPase and Mg-dependent ATPase in liver in experiment I*

Enzyme	Group	Additives to		Experimental period	
		Diet	Water	3 Months	7 Months
				(mμmoles p-aminophenol/mg prot./min)	
Aniline Hydroxylase	1	NONE	NONE	1.16 ± 0.06[a]	1.48 ± 0.21[a]
	2	NONE	ABS (1000 ppm)	1.42 ± 0.12[a]	1.22 ± 0.14[a]
	3	PCB (500 ppm)	NONE	3.94 ± 0.38[b]	4.19 ± 0.46[b]
	4	PCB (500 ppm)	ABS (1000 ppm)	6.72 ± 0.57[c]	6.90 ± 0.66[c]
				(mμmoles Pi/mg prot./min)	
Na-K-Mg-ATPase	1	NONE	NONE	140 ± 7[a]	155 ± 5[a]
	2	NONE	ABS (1000 ppm)	122 ± 21[a]	130 ± 7[a]
	3	PCB (500 ppm)	NONE	78 ± 8[b]	76 ± 6[b]
	4	PCB (500 ppm)	ABS (1000 ppm)	45 ± 12[c]	44 ± 10[c]
Mg-ATPase	1	NONE	NONE	401 ± 34[a]	328 ± 47[a]
	2	NONE	ABS (1000 ppm)	424 ± 32[a]	329 ± 56[a]
	3	PCB (500 ppm)	NONE	387 ± 31[a]	342 ± 35[a]
	4	PCB (500 ppm)	ABS (1000 ppm)	343 ± 29[a]	399 ± 85[a]

Values are represent mean ± S.E. of 4 rats.
Different letter superscripts following mean and S.E. (a,b,c) denote significant differences ($p < 0.05$) in each enzyme. Data were considered statistically based on the method of one factor ANOVA.

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Table VII. *Ratio of liver weight to body weight (one month) in experiment II*

Group	Additives to		%
	Diet	Water	
1	NONE	NONE	2.94 ± 0.06^{a}
2	PCB (10 ppm)	NONE	3.01 ± 0.05^{ab}
3	PCB (100 ppm)	NONE	3.93 ± 0.14^{c}
4	PCB (500 ppm)	NONE	5.82 ± 0.50^{d}
5	NONE	ABS (1000 ppm)	2.92 ± 0.07^{a}
6	PCB (10 ppm)	ABS (1000 ppm)	3.12 ± 0.06^{b}
7	PCB (100 ppm)	ABS (1000 ppm)	4.05 ± 0.14^{c}
8	PCB (500 ppm)	ABS (1000 ppm)	6.45 ± 0.24^{e}

Values represent mean ± S.E. of 8 rats.
Different letter superscripts following mean and S.E. (a,b,c,d,e) denote significant differences ($p < 0.05$) compared by the method of one factor ANOVA.

Table VIII. *Urea and iron levels in serum (one month) in experiment II*

Group	Additives to		Urea (mg/100 ml)	Iron (μg/100 ml)
	Diet	Water		
1	NONE	NONE	20.9 ± 0.8^{a}	157 ± 18^{a}
2	PCB (10 ppm)	NONE	22.8 ± 2.1^{abc}	149 ± 16^{a}
3	PCB (100 ppm)	NONE	22.2 ± 1.5^{ab}	180 ± 17^{ab}
4	PCB (500 ppm)	NONE	27.4 ± 2.3^{bc}	176 ± 21^{ab}
5	NONE	ABS (1000 ppm)	22.8 ± 2.0^{abc}	198 ± 36^{ab}
6	PCB (10 ppm)	ABS (1000 ppm)	23.7 ± 2.5^{abc}	206 ± 43^{ab}
7	PCB (100 ppm)	ABS (1000 ppm)	23.5 ± 2.0^{abc}	216 ± 15^{ab}
8	PCB (500 ppm)	ABS (1000 ppm)	28.8 ± 2.9^{cd}	236 ± 31^{b}

Values represent mean ± S.E. of 8 rats.
Different letter superscripts following mean and S.E. (a,b,c,d) denote significant differences ($p < 0.05$) compared by the method of one factor ANOVA.

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Table IX. *Total cholesterol levels in serum and total and free cholesterol levels in liver (one month) in experiment II*

Group	Additives to		Serum Cholesterol (mg/100 ml)	Liver	
	Diet	Water		Total Cholesterol (mg/100 g wet weight)	Free Cholesterol (mg/100 g wet weight)
1	NONE	NONE	109 ± 12[a]	290 ± 14[a]	214 ± 10[a]
2	PCB (10 ppm)	NONE	104 ± 15[a]	289 ± 18[ab]	219 ± 16[a]
3	PCB (100 ppm)	NONE	113 ± 20[a]	336 ± 23[bc]	224 ± 12[a]
4	PCB (500 ppm)	NONE	141 ± 28[ab]	421 ± 25[d]	254 ± 30[ab]
5	NONE	ABS (1000 ppm)	98 ± 16[a]	268 ± 19[a]	225 ± 14[a]
6	PCB (10 ppm)	ABS (1000 ppm)	116 ± 26[a]	265 ± 16[a]	237 ± 15[a]
7	PCB (100 ppm)	ABS (1000 ppm)	123 ± 23[a]	354 ± 31[cd]	225 ± 10[a]
8	PCB (500 ppm)	ABS (1000 ppm)	180 ± 13[b]	522 ± 15[e]	313 ± 26[b]

Values represent mean ± S.E. of 8 rats.
Different letter superscripts following mean and S.E. (a, b, c, d, e) denote significant differences in each column ($p < 0.05$) compared by the method of one factor ANOVA.

Villeneuve *et al.* (1971) and Litterst *et al.* (1972). Matsuda (1959) and Tanaka *et al.* (1969) observed that total lipid, triglyceride and cholesterol contents in the liver increased when PCB was ingested. Inhibition of ATPase activity in fish has been reported by Yap *et al.* (1971). The present studies confirmed the various abnormalities attributed to PCB administration and proved that some of them are increased by simultaneous feeding of PCB and ABS. It is noteworthy that PCB inhibited the Na-K-Mg-dependent ATPase not only in a cold-blooded animal but also in mammals. In various analyses of the serum, increase of transaminase observed in the high-dosage PCB groups (seven months) could be due to degenerative changes in the liver. The hemolytic effect of ABS could explain the increase of the serum iron levels in the ABS-administered groups. It is of interest to note the morphological testicular abnormalities observed in ABS-PCB co-administered rats (seven months). With regard to the effect of PCB as a mitotic poison, Peakall *et al.* (1972) reported that frequencies of chromosome aberrations increased in PCB-treated dove embryos as compared to control embryos. The following mechanisms may be considered for the etiology of the observed testicular atrophy: (1) PCB disturbs primarily the testicular vasculature, (2) PCB affects genetic materials as a mitotic poison, (3) PCB causes a nutritional deficiency, such as Vitamin E deficiency, (4) PCB affects certain enzyme activities in the testicles, (5) PCB affects the endocrine control of testicular integrity, and (6) these effects could be caused by an enhanced absorption of PCB as a

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Table X. *Aniline hydroxylase, Na-K-Mg-dependent ATPase and Mg-dependent ATPase activities in liver (one month) in experiment II*

Group	Additives to		Aniline hydroxylase (m moles /mg prot./min)	Na-K-Mg-ATPase (m moles/mg prot./min)	Mg-ATPase (m moles/mg prot./min)
	Diet	Water			
1	NONE	NONE	1.21 ± 0.16^{a}	117 ± 16^{ab}	306 ± 59^{ab}
2	PCB (10 ppm)	NONE	1.76 ± 0.23^{a}	113 ± 20^{abc}	311 ± 37^{a}
3	PCB (100 ppm)	NONE	3.96 ± 0.48^{b}	80 ± 19^{bc}	265 ± 39^{ab}
4	PCB (500 ppm)	NONE	5.00 ± 0.62^{b}	60 ± 20^{cd}	251 ± 22^{ab}
5	NONE	ABS (1000 ppm)	1.63 ± 0.13^{a}	135 ± 21^{a}	336 ± 48^{a}
6	PCB (10 ppm)	ABS (1000 ppm)	1.81 ± 0.19^{a}	99 ± 17^{abc}	307 ± 55^{ab}
7	PCB (100 ppm)	ABS (1000 ppm)	4.15 ± 0.41^{b}	81 ± 12^{bc}	300 ± 27^{a}
8	PCB (500 ppm)	ABS (1000 ppm)	5.51 ± 0.66^{b}	42 ± 5^{d}	226 ± 20^{b}

Values represent mean ± S.E. of 8 rats.
Different letter superscripts (a,b,c,d) denote significant differences ($p < 0.05$) in each column compared by the method of one factor ANOVA.

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result of the emulsifying action of ABS. Although further experiments are necessary to elucidate the mechanism, attention is drawn to the effects of PCB (or PCB plus ABS) on the reproductive physiology of male rats.

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References

Abell, L. L., B. B. Levy, B. B. Brodie, and K. Kendall: A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem. **195**, 357 (1952).

Beale, R. N., J. O. Bostrom, and R. F. Taylor: Rapid incremental methods for the determination of serum iron and iron binding capacity. J. Clin. Pathol. **14**, 488 (1961).

Bornmann, G., and A. Loeser: The biological effects of dodecylbenzene sulfonate surfactants on rats. Fette Seifen Anstrichmittel **65**, 814 (1963).

Buehler, E. V., E. A. Newmann, and W. R. King: Two-year feeding and reproduction study in rats with linear alkylbenzene sulfonate (LAS). Toxicol. Appl. Pharmacol. **18**, 83 (1971).

Ceriotti, G.: Determination of nucleic acids in animal tissues. J. Biol. Chem. **214**, 59 (1955).

Coulombe, J. J., and L. Favreau: A new simple semimicro method for colorimetric determination of urea. Clin. Chem. **9**, 102 (1963).

Duncombe, W. G.: The colorimetric micro-determination of non-esterified fatty acids in plasma. Clin. Chim. Acta **9**, 122 (1964).

Fujita, S., H. Tsuji, K. Kato, S. Saeki, and H. Tsukamoto: Effect of biphenyl chlorides on rat liver microsomes. Fukuoka Acta Medica **62**, 30 (1971).

Hansen, P. W.: A simplification of King and King's method for determination of serum phosphatase. Scand. J. Clin. Lab. Invest. **18**, 353 (1966).

Imai, Y., A. Ito, and R. Sato: Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. J. Biochem. **60**, 417 (1966).

Kimbrough, R. D., R. E. Linder, and T. B. Gaines: Morphological changes in livers of rats fed polychlorinated biphenyls. Arch. Environ. Health **25**, 354 (1972).

Kuratsune, M., T. Yoshimura, J. Matsuzaka, and A. Yamaguchi: Yusho, a poisoning caused by rice oil contaminated with chlorobiphenyls. Fukuoka Acta Medica **60**, 513 (1969).

DSW 031479

STLCOPCB4015441

Litterst, C. J., T. M. Farber, A. M. Baker, and E. J. Van Loon: Effect of polychlorinated biphenyls on hepatic microsomal enzymes in the rat. Toxicol. Appl. Pharmacol. **23**, 112 (1972).

Lowry, O. H., N. J. Rosebrough, A. C. Farr, and R. J. Randall: Protein measurement with the folin-phenol reagent. J. Biol. Chem. **193**, 265 (1951).

Matsuda, T.: The experimental studies of effect of polychlorinated biphenyl on lipid metabolism. Kokumin Eisei 28, 672 (1959).

Nakao, T., Y. Tashima, K. Nagano, and M. Nakao: Highly specific sodium-potassium-activated adenosine triphosphatase from various tissues of rabbit. Biochem. Biophys. Res. Comm. **19**, 755 (1965).

Nishizumi, M: Light and electron microscope study of chlorobiphenyl poisoning. Arch. Environ. Health **21**, 620 (1970).

Peakall, D. B., J. L. Lincer, and S. E. Bloom: Embryonic mortality and chromosomal alterations caused by Aroclor 1254 in ring doves. Environ. Health Perspectives **1**, 103 (1972).

Reitman, S., and S. Frankel: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol. 28, 56 (1957).

Sardesai, V. M., and J. A. Manning: The determination of trigylcerides in plasma and tissues. Clin. Chem. **14**, 156 (1968).

Sperry, W. M., and M. Webb: A revision of the Schoenheimer-Sperry method for cholesterol determination. J. Biol. Chem. **187**, 97 (1950).

Swisher, R. D.: Exposure levels and oral toxicity of surfactants. Arch. Environ. Health **17**, 232 (1968).

Tanaka, K., S. Fujita, F. Komatsu, and N. Tamura: Experimental subacute poisoning of chlorobiphenyls particularly the influence on the serum lipids in rats. Fukuoka Acta Medica **60**, 544 (1969).

Tokoro, Y., M. Hirasawa, and S. Moteki: Experimental histopathologic investigation upon the biological effect of alkylbenzene sulfonate in the long term intake. Jap. J. Public Health **11**, 29 (1964).

Villeneuve, D. C., D. C. Grant, W. E. J. Phillips, M. L. Clark, and D. G. Clegg: Effects of PCB administration on microsomal enzyme activity in pregnant rabbits. Bull. Env. Contam. Toxicol. 6, 120 (1971).

Weber, H.: Rashe und einfache Ultramikromethode zur Bestimung der Serumcholin-esterase. Dtsch med. Wschr. **91**, 1927 (1966).

Yanagisawa, F.: On the neutral cleansing agent. Jap. J. Public Health **10**, 413 (1963).

Yap, H. H., D. Desaiah, L. K. Cutkomp, and R. B. Koch: Sensitivity of fish ATPase to polychlorinated biphenyls. Nature 233, 61 (1971).

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