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PUBLIC HEALTH SERVICE-CDC-ATLANTA
EPI-79-44-4 July 22, 1980

TO : Director, Center for Disease Control

FROM : Chronic Diseases Division
Bureau of Epidemiology

SUBJECT: PCB Levels in a Community with Exceptional Exposure to DDT, Triana, Alabama

SUMMARY

The geometric mean serum level of polychlorinated biphenyls (PCB) of 458 persons in a communitywide study was 17.2 parts per billion, with 80%-90% having levels within the range found in other community groups. PCB levels were found to be directly related to age--the older the age group, the higher the PCB level--even when controlled for other variables positively associated with PCB level: male sex, fish consumption, serum cholesterol level, and alcohol consumption. No major point source of PCB contamination was found, and fish taken in the dreinage of a major population center had mean PCB levels below the current enforceable Food and Drug Administration tolerance of 5 parts per million. Serum PCB levels were positively associated with measured blood pressure, with gamma-glutamyl transpeptidase level (a liver function test), and with serum cholesterol level, all independent of major confounding variables. These findings, along with the unknown remote consequences of PCB accumulation in the body, support our recommendation for Triana residents to avoid eating fish taken from contaminated areas.

INTRODUCTION

Polychlorinated biphenyls (PCB) are complex mixtures of fat-soluble aromatic hydrocarbons that were manufactured in the United States from 1929 until 1977. Their thermal and electrical insulating properties led to widespread use in transformers and capacitors. They were also used in plasticisers, inks, adhesives, pesticide extenders, and carbonless dupliceting paper. PCBs are now widely distributed in the environment. Like DDT residues, PCBs are accumulated and concentrated by fish. Nonoccupational human exposure to PCBs occurs primarily via the diet. The Food and Drug Administretion (FDA) reduced the tolerance for PCB residues in fish from 5 to 2 parts per million (ppm), but this reduction is stayed pending further hearings. Once ingested, PCB residues concentrate in adipose tissue, and human metabolites of these compounds have not been studied. Concern for human accumulation of these persistent compounds is based on their hepatic carcinogenicity in rodents and reproductive effects and immune response changes in several species. Human health effects associated with heavy PCB exposure include chloracne, liver function abnormalities, and serum lipid concentration abnormalities.

BACKGROUND

In May 1979 a communitywide study was conducted in Triana, Alabama, where the population has had exceptional exposure to DDT residues as a result of eating locally caught fish. During laboratory analyses of serum specimens for DDT residues, interference from PCBs was encountered. A pilot effort suggested that some Triana residents had PCB levels above the range found in the general population and that a strong correlation existed between serum PCB and DDT levels. The background, methods, and results of this cross-sectional study are described in EPI-79-44-3 (dated January 31, 1980). To confirm these findings and to explore the possible relation of PCB levels to health parameters, we analyzed sera frozen at -60 C in the May study for PCB levels.

In the meantime, the U.S. Environmental Protection Agency (EPA) conducted extensive environmental sampling for PCBs in sediments, water, soil, sewage, and fish. These efforts were complemented by the Department of Army and the National Aeronautics and Space Administration on Redstone Arsenal and by the Tennessee Valley Authority. Low levels of PCBs were found in a variety of samples, but no major point source of PCB contamination was discovered. Indian Creek, which receives drainage from Redstone Arsenal and from Huntsville (population 138,000), enters the Tennessee River at Triana. Fish taken from Indian Creek and Cataco Creek, the embayment on the opposite bank of the Tennessee, had PCB levels many times those found in fish taken in other embayments or in the Tennessee River (Appendix 1). A composite sample from 6 Indian Creek catfish averaged 3.64 ppm (range = 0.35 - 7.57 ppm), and from 4 Cataco Creek catfish 4.34 ppm (range = 2.04 - 6.28 ppm). Catfish from 13 other sites in the river system averaged 0.59 ppm (range = 0.05 - 4.94 ppm; N = 73). The EPA findings corroborate those reported by CDC for 12 Indian Creek fish that had PCB levels ranging from 0.36 - 3.32 ppm with a mean of 1.08 ppm.

A strong positive correlation existed between log PCB and log DDT levels in 47 individual catfish taken from the Tennessee River system (Pearson correlation coefficient 0.697; $p<10^{-5}$). This correlation resulted from correlation coefficients above 0.9 in catfish taken from Indian Creek, from Tennessee River mile 320 (the point at which Indian Creek enters the Tennessee River), and from Tennessee River mile 275 (downstream at Wheeler Dam). Catfish taken at other sites in the Tennessee River system had no significant correlation between log PCB and log DDT levels. Again, these EPA data corroborate the CDC findings of a high log PCB-log DDT correlation in 12 fish taken in Indian Creek (total DDT range = 1.2 - 498.4 ppm; r = 0.67).

METHODS

Study design, clinical chemistry, and laboratory analyses are described in EPI-79-44-3, and only supplementary information is given here. Single readings of systolic and diastolic blood pressure were taken on seated participants 12 years and older, according to procedures used by the Health and Nutrition Examination Survey (1). Normal blood pressure was defined as a systolic measurement less than 140 mm Hg and a diastolic measurement less than 90 mm Hg. Borderline hypertension was defined as a systolic measurement of 140-159 mm Hg or a diastolic measurement of 90-94 mm Hg. Definite hypertension was defined as a systolic measurement of 160 mm Hg or greater or a diastolic measurement of 95 mm Hg or greater. A body mass index for obesity was calculated from weight/height^R, where R = 2 for males, 1.5 for females. Social class was measured by Hollingshead Index (2).

Analysis of sera for PCBs as Aroclor* 1260 was performed at CDC. After protein denaturation with 1 ml of methanol, 0.5 - 2.0 ml serum was extracted with 5 ml hexane 3 times. The combined extracts were concentrated to a volume of 1 ml, and DDT residues were adsorbed onto a silver nitrate/silica column (Needham LL, Smrek AL, Head SL, Burse VW, Liddle JA. Column chromatography separation of PCBs from DDT and metabolites. Manuscript submitted for publication). After hexane elution and

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concentration, the sample was analyzed on an electron capture gas chromatograph, with a 3% SE 30 column. Injector, column, and detector temperatures were 250, 195, and 330 C, respectively. Quantification was made by adaptation of the Webb-McCall method (3) to the Sigma I data system, with Aroclor 1260 used as the calibration standard. The limit of detection was 3 parts per billion (ppb). Quality control over time was assured with 2 pools of bovine serum spiked *in vitro* with Aroclor 1260, 1 pool of unspiked bovine serum, and 1 human pool consisting of sera from Triana residents; the last pool was introduced into the daily run as a "blind" sample. Recovery was 94.5% when PCB-free bovine serum was spiked with 41 ppb Aroclor 1260.

RESULTS

PCB Levels

Of the 500 persons giving blood specimens in the study, 458 gave sufficient serum for PCB (Aroclor 1260) analysis. Those persons without sufficient serum for PCB analysis were younger (mean age 19.1 vs 32.2 yrs), were more often male (57.1 vs 42.5%) and had a lower geometric mean total DDT level (52.5 vs 79.6 ppb). For individuals with PCB measurements, the geometric mean PCB level was 17.2 ppb with a standard deviation of 20.8 (arithmetic mean 22.2 ppb; S.D. 22.3; range = 3.2 - 157.9 ppb). Ninety-eight persons (21.4%) had PCB levels greater than 30 ppb, 60 (13.1%) had levels >40 ppb, 41 (9.0%) had levels >50 ppb, and 7 (1.5%) had levels >100 ppb.

As with DDT, age was the most powerful predictor of the log PCB level (Pearson correlation coefficient = 0.56) (Table 1). Males had a higher geometric mean PCB level than females (23.5 vs. 13.7 pb; $p < .0001$), a difference present in each group (Figure 1). Race had no independent effect in multiple regression analysis.

Fish consumption before publicity about DDT contamination correlated positively with log PCB levels independent of age (Figure 2). The commercial fishermen and their adult family members had a higher geometric mean PCB level than the other study participants aged ≥19 years (32.4 vs. 20.2 ppb, $p = .0003$). Years of residence, proximity to cotton fields, farm work, and social class were not associated with PCB levels independent of age, sex, and fish consumption. In contrast to our finding with DDT, breast-fed children did not have significantly higher PCB levels than bottle-fed children.

Table 1
Relative Weight of Significant Correlates of Log PCB
in Serum, Triana, Alabama, May 1979

Variable	Standardized[β] Coefficient	F	R^2 Change
Age	.531	116.1	.310
Sex	.223	23.8	.128
Fish consumption	.210	30.0	.042
Body mass index	-.162	10.7	.015
Cholesterol	.147	11.9	.015
Alcohol consumption	.117	8.5	.012
			.523

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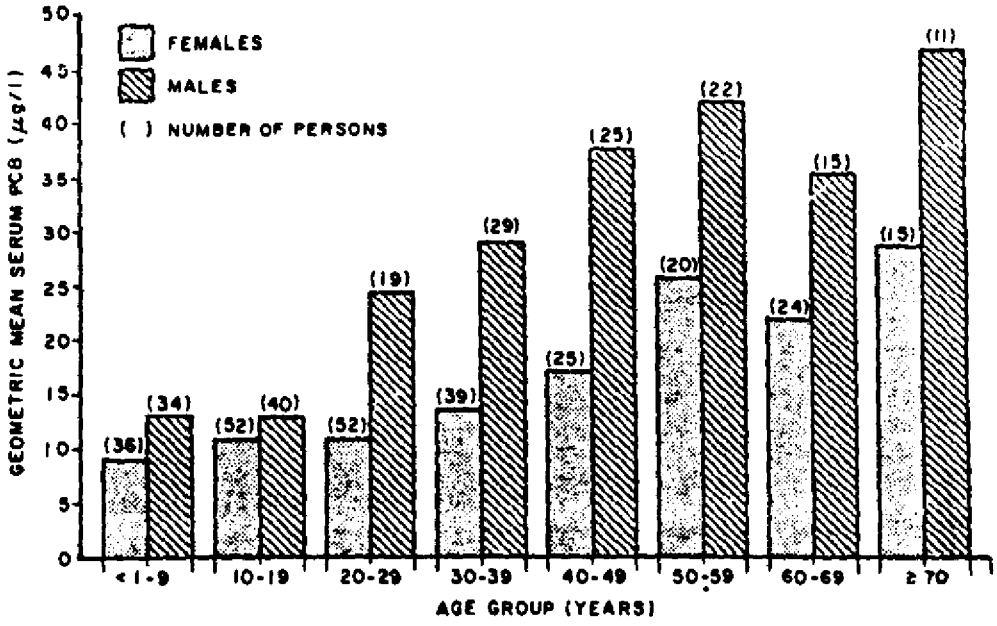
Fig. 1 PCB LEVELS IN SERUM SPECIMENS, BY AGE GROUP AND SEX, TRIANA, ALABAMA, MAY 1979



Alcohol consumption was positively associated with log PCB levels when controlled for age, sex, fish consumption, and serum cholestarol level. Cigarette smoking was not associated. The 10 adults who had taken enzyme-inducing medications in the preceding year had a lower geometric mean PCB level than the rest of the adult study population (13.7 vs. 21.7 ppb, p = .05).

Serum cholesterol was positively associated with log PCB level independent of the other variables included in Table 1. Serum triglyceride or high density lipoprotein cholesterol (HDLC) made no additional independent contribution. Obesity had a significant negative correlation with log PCB. Weight loss, gamma-glutamyl transpeptidase (GT), and creatinine were insignificant when included in multiple regression.

Health Effects

None of the following health indices were associated with log PCB independent of age group: prevalence of illness or weight loss in the preceding year, use of medication, use of medical care, history of heart disease, or percentage of pregnancies ending in miscarriage, stillbirth, or infant death.

Rates of measured borderline and definite hypertension among study participants were 30% higher than expected on the basis of national rates for a population of the same age, race, and sex composition (1). During the study, 3 persons were referred to emergency medical services for extreme hypertension, and sera for PCB levels were not obtained. Log PCB did make a significant contribution to explaining the variability of log systolic and log diastolic blood pressure in multiple regression analyses (Tables 2,3). Obesity and sex were stronger predictors of blood pressure than log PCB. Age was the strongest predictor for the systolic measurement. The PCB effect on blood pressure was independent of social class. For systolic blood pressure, addition of serum cholesterol or triglyceride to the list of independent variables rendered the PCB contribution insignificant; addition of smoking habit or race made no additional contribution. For diastolic blood pressure, however, addition of serum cholesterol, triglyceride, smoking habit, or race made no additional contribution and did not nullify the PCB effect.

Table 2
Relative Weight of Significant Correlates of Log Systolic Blood Pressure, Triana, Alabama, May 1979

Variable	Standardized$^{\beta}$ Coefficient	F	R^2 Change
Age	.427	52.9	.355
Sex	.177	13.0	.020
Body Mass Index	.158	9.0	.011
Hollingshead Index	.123	8.7	.015
Log PCB	.111	4.2	.007
			.408

Table 3
Relative Weight of Significant Correlates of Log Diastolic Blood Pressure, Triana, Alabama, May 1979

Variable	Standardized$^{\beta}$ Coefficient	F	R^2 Change
Body Mass Index	.258	18.8	.068
Sex	.198	12.8	.083
Log PCB	.176	8.2	.063
Age	.151	5.2	.015
Hollingshead Index	.109	5.3	.011
			.241

Since many factors--age, sex, obesity, and social class--influence blood pressure, simple correlation of blood pressure measurement and PCB level shows a wide scatter. Many persons with hypertension have comparatively low serum PCB levels, and conversely some persons with PCB levels of 100 ppb or greater are normotensive. The trend of persons with hypertension to have higher PCB levels is present in both young and old adult age groups (Figure 3).

The correlation between log PCB and log DDT serum levels was very high (Pearson correlation coefficient = 0.83, Figure 4). Despite this, no DDT effect on blood pressure was evident when log PCB and log DDT were both included in regressions controlled for age or obesity.

Log GT was positively correlated with log PCB (Figure 5) independent of alcohol consumption and age (Table 4). The other liver function tests (total bilirubin and serum glutamic pyruvic transaminase) were not so affected. Addition of log DDT as a third independent variable to log PCB and age made no significant contribution to log GT. The relative contributions to log GT of alcohol consumption, log PCB, and log DDT are unknown since the collinearity of the latter 2 results in no significant contribution when these 3 independent variables are used to predict log GT.

As mentioned above, serum cholesterol--but not triglyceride level--was a significant independent predictor of serum PCB level in multiple regression analysis. We then used log PCB as an independent variable to predict serum lipid levels. Although a weak correlation existed between log PCB and log triglyceride (Pearson

Fig. 2 PCB LEVELS IN SERUM SPECIMENS, BY AGE GROUP AND FISH MEALS PER MONTH, TRIANA, ALABAMA, MAY 1979

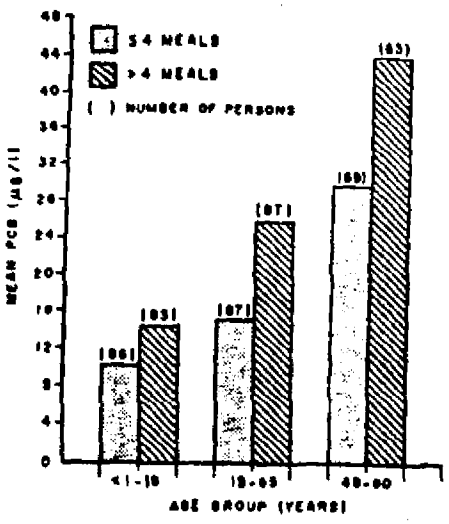


Fig. 3 SERUM PCB LEVELS FOR NORMOTENSIVE AND HYPERTENSIVE PERSONS BY AGE GROUP, TRIANA, ALABAMA, MAY 1979

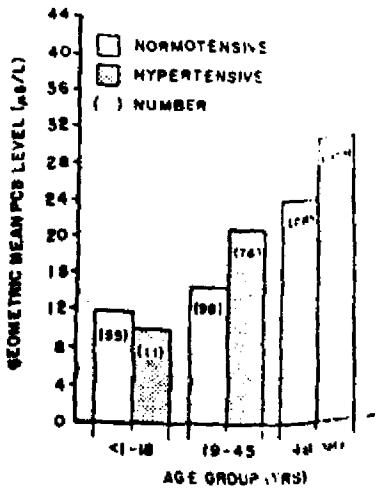


Fig. 4 CORRELATION BETWEEN LOG DDT AND LOG PCB LEVELS IN SERUM SPECIMENS, TRIANA, ALABAMA, MAY 1979

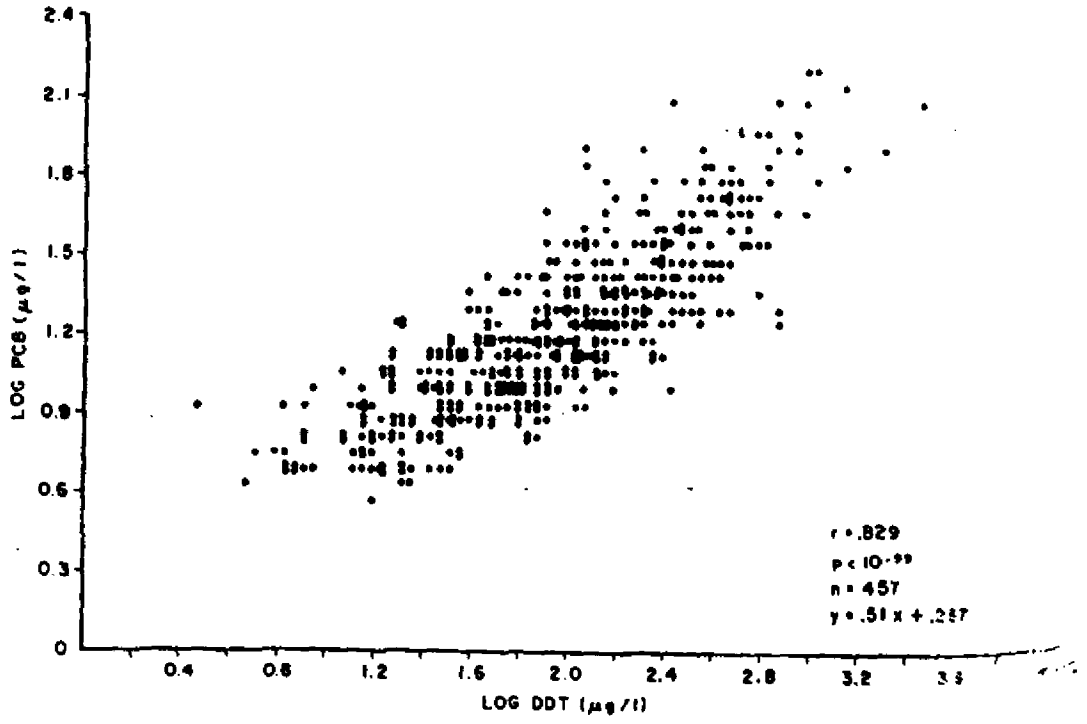


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Fig. 5 CORRELATION BETWEEN LOG PCB AND LOG GAMMA-GLUTAMYL TRANSPEPTIDASE LEVELS IN SERUM SPECIMENS, TRIANA, ALABAMA, MAY 1979

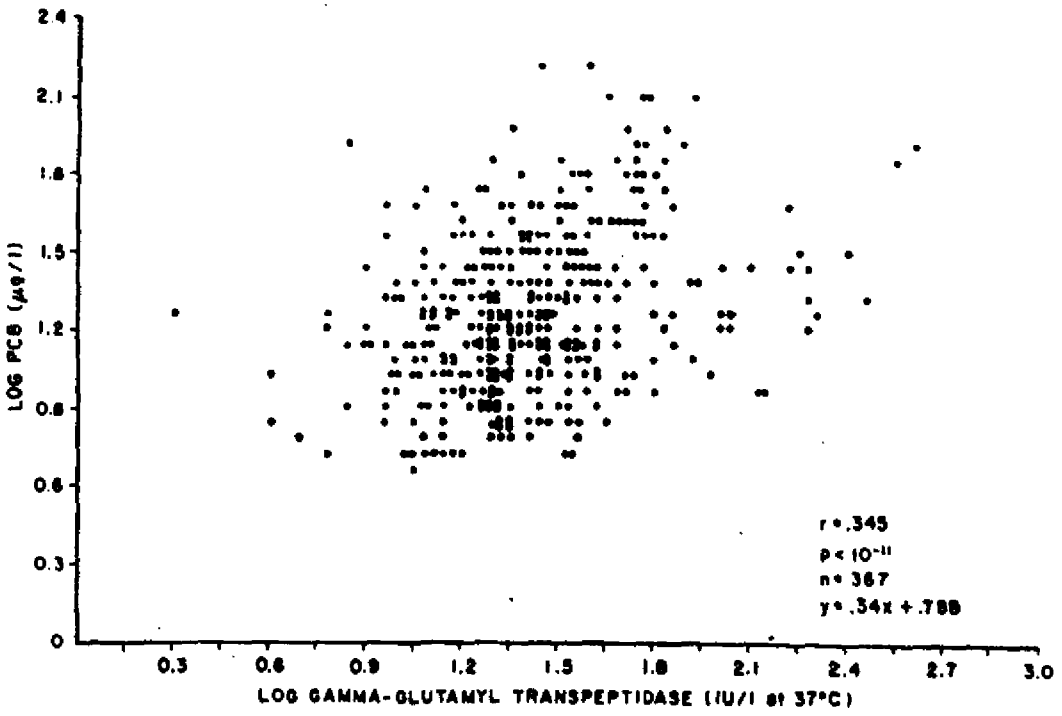


Table 4
Relative Weight of Significant Correlates of Log Gamma-Glutamyl Transpeptidase in Serum, Triana, Alabama, May 1979

Variable	Standardized[β] Coefficient	F	R^2 Change
Alcohol consumption	.242	24.8	.101
Age	.200	12.6	.082
Log PCB	.169	8.5	.019
			.201

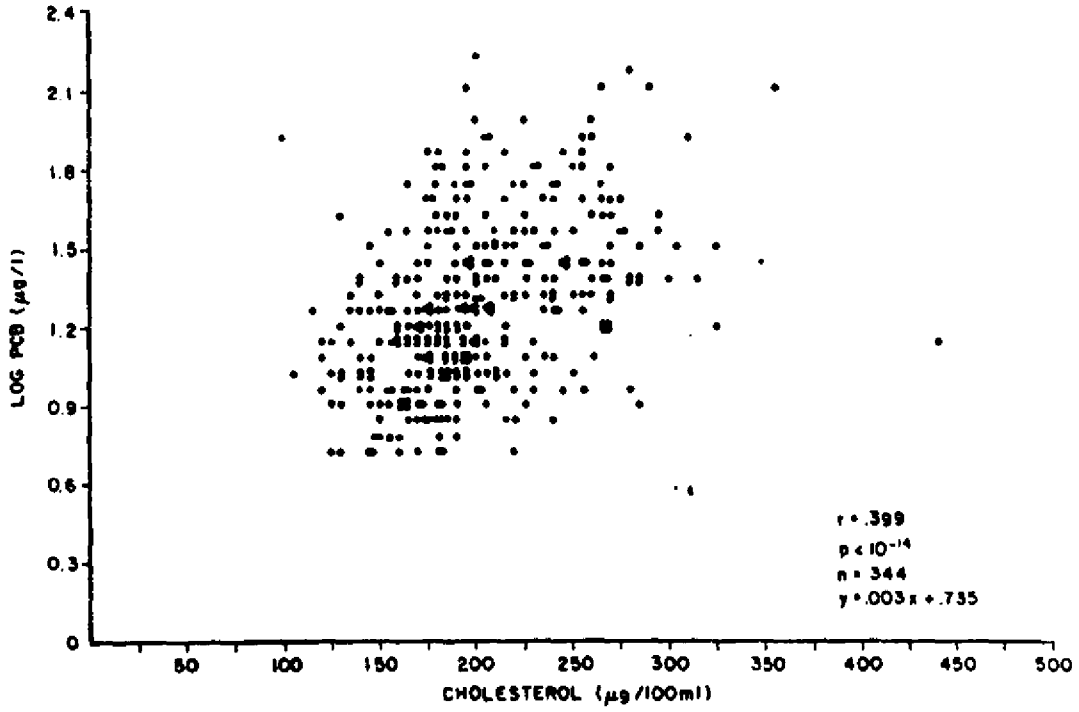
correlation coefficient = 0.298, $p = 8.5 \times 10^{-9}$), no association was found between them independent of log cholesterol and log GT, both better predictors of log triglyceride level. There was no correlation between log PCB and HDLC.

In contrast, log PCB as an independent variable was weakly associated with cholesterol (Figure 6); this association existed independent of log triglyceride and age (Table 5). When log DDT was added as a fourth independent variable in predicting log cholesterol, its contribution and that of log PCB became insignificant. Thus, the relative contribution of log PCB and log DDT to predicting serum cholesterol is unknown because of their collinearity.

Table 5
Relative Weight of Significant Correlates of Log Cholesterol in Serum, Triana, Alabama, May 1979

Variable	Standardized[β] Coefficient	F	R^2 Change
Log triglyceride	.355	60.6	.221
Age	.309	34.6	.131
Log PCB	.127	5.7	.011
			.362

Fig.6 CORRELATION BETWEEN LOG PCB AND CHOLESTEROL LEVELS IN SERUM SPECIMENS, TRIANA, ALABAMA, MAY 1979



DISCUSSION

No comparable published data exist on serum PCB levels in the general population of the United States. The only study with a large number of community volunteers (4), sampled in 1968, is difficult to interpret because no limit of detection was specified and no quality assurance in PCB measurement was described. Means for subgroups with measurable residues (43% of the study population) ranged from 1.50 - 20.6 ppb, with a maximum individual measurement of 29.0 ppb. One hundred ten persons in Bloomington, Indiana (1977), without occupational PCB exposure (sludge-users and controls), had a mean serum PCB level of 18.8 ppb (S.D. = 10.8) with a range of 6 - 79 ppb (5, plus personal communication from Matthew M. Zack). The CDC Toxicology Laboratory has unpublished data on 2 other community groups: In Billings, Montana (1979), 17 residents had a mean PCB level of 7.5 ppb with a range of 2 - 30 ppb; in Michigan (1978-79), 370 residents had a mean of 8.0 ppb (S.D. = 7.2) with a range of 1 - 61 ppb. Unpublished

data from the Michigan Department of Public Health on 16 non-fisheaters sampled in 1973-74 show a mean serum PCB level of 17 ppb with a range of 7 - 42 ppb (6). The serum PCB levels of most Triana residents fall within the range seen in other communities in the United States. Since PCB residues appear to accumulate with age and are higher in males, only age- and sex-standardized comparisons are appropriate.

The 10%-20% of Triana participants with PCB levels somewhat above those found in other investigations tended to eat fish more frequently. The only major dietary source of PCBs in the United States is fish (7). Most fish contain PCB levels well below 1 ppm in the edible portion, although freshwater fish from polluted waterways, such as the Hudson River or Lake Michigan, may have much higher levels. The Michigan Department of Public Health (6) investigated 71 sport fishermen who consumed $\geq$24 pounds per year of fish taken from Lake Michigan ($\geq$1 fish meal/wk). Their mean serum PCB level was 77 ppb with a range of 25 - 366 ppb. The mean PCB levels in whole raw fish in Lake Michigan for the 3 years 1972-1974 were 13, 19, and 23 ppm for trout and 11, 12, and 10 ppm for coho salmon. PCB levels in cooked fish collected from the Michigan fishermen were much lower: 1.03 - 4.67 ppm for cooked trout and 0.48 - 5.38 ppm for cooked salmon. Thus the level of fish contamination by PCBs in Lake Michigan is higher than that in Indian Creek; the mean and range of human PCB levels in Michigan fishermen are higher than the mean and range of PCB levels in Triana residents eating comparable amounts of fish. Nonetheless, the higher than expected number of Triana residents with high PCB serum values is probably explained by the consumption of fish that at present have a mean PCB level very close to the stayed FDA tolerance of 2 ppm.

The FDA tolerance for PCBs is based on a 200-mg total dose safety limit, the lowest PCB dose thought to have caused overt Yusho disease in the 1968 Japanese episode of rice oil contamination (7). However, Yusho disease resulted from a mixed exposure to PCBs, chlorinated quaterphenyls, and chlorinated dibenzofurans, the last of which are more toxic than PCBs. The FDA recommendation for maximum allowable ingestion of PCBs over a protracted period is 1 µg/kg/day. The only study of a population exposed to dietary PCBs of this magnitude is that in Michigan (mean daily dose = 1.7 µg/kg/day; range = 0.49 - 3.94), which identified no adverse health effects. However, blood pressure measurements, serum lipids, and gamma-glutamyl transpeptidase tests were not done.

The high correlation between total DDT and PCB residues in serum specimens is explained by their common dietary source in fish and similar accumulation in the body once ingested. We have laboratory data to demonstrate that carryover of DDT residues into the PCB measurement did not occur. Whether DDT residues affect the toxicity or metabolism of PCBs is unknown. For another pair of chlorinated hydrocarbons, kepone exposure is known to increase the excretion of lindane in rats (8). Both DDT and some PCB congeners induce cytochrome P450-type mixed function oxidases, and some PCB congeners induce cytochrome P448-type enzymes as well (9-11).

No other studies have been conducted to determine whether PCBs affect blood pressure. The contribution of PCBs to the systolic measurement was small and of borderline significance; however, the PCB contribution to the diastolic measurement was unequivocally significant. We think that the excess prevalence of measured hypertension among Triana residents is not explained by their PCB levels. The hypertension excess could be due to the environment in which they were measured, a noisy gymnasium, whereas the national prevalence data used for comparison were generated in medical vans. The absence of a control group with blood pressure measurements does not weaken our observation of the trend of persons with higher serum PCB levels to have higher blood pressure readings. We can think of no reason why participants with higher PCB levels would react to the blood pressure measurement in a different fashion, and indeed neither participants nor measurers had knowledge of PCB levels at the time of the study.

Our study confirms many others (12-13) that PCB exposure is associated with liver function test changes. In this population, GT was more sensitive than the other liver function tests. The large study population allowed demonstration of a small PCB-GT association independent of alcohol consumption, a stronger predictor of GT level.

Despite relatively low PCB levels in most Triana residents, we were able to confirm aspects of other human studies which show an association between PCB exposure

and lipid levels (5, 12 - 19). In our analyses, PCB level (as a dependent and independent variable) was positively associated with serum cholesterol level independent of other major confounding variables. Discrepancies between our results and those of others finding a PCB-triglyceride association (5, 14 - 19) may be due to this control of confounding, or to the lower PCB burdens of the Triana population, or to differing mixed exposures. We were unable to confirm the suggestion (19) of an inverse association between HDLC and PCB.

The long-term implications for human health of the associations between PCB level and blood pressure, liver function, and serum cholesterol are unknown. A recent study of the causes of death in a cohort of 2,567 PCB workers showed no excess of nervous or circulatory system causes of death (Brown DP, Jones M. Mortality and industrial hygiene study of workers exposed to polychlorinated biphenyls. Unpublished manuscript, National Institute for Occupational Safety and Health). A preliminary report (20) of an increase in malignant melanoma and pancreatic cancer among PCB-exposed workers has not been substantiated by this cohort mortality study, which showed slight excesses of mortality due to rectal and liver cancer and due to cirrhosis of the liver, none of which was statistically significant. Since 80%-90% of the Triana study population has PCB levels in the range probably found in the general population, we would not expect to be able to detect differences between causes of death in Triana and causes in the general population associated with PCB exposure.

Our preliminary finding of associations between serum PCB level and blood pressure, liver function, and cholesterol concentration merits further medical study--especially since the associations are found in a population with PCB levels overlapping those found in other communities. Triana remains unique in the degree of DDT metabolite burden of its citizens, the source of both PCB and DDT being the consumption of contaminated fish. Given the uncertain remote consequences that these 2 compounds may have on human health, especially since they both accumulate in the body, we recommend that Triana residents avoid eating fish taken from contaminated areas.

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APPENDIX 1

Means, Ranges, and Correlations of PCB and DDT Levels in Catfish, Tennessee River System, 1979*

Site	Number of Fish	Mean PCB (ppm)	PCB Range (ppm)	Mean DDT † (ppm)	DDT Range (ppm)	Log PCB-Log DDT Correlation Coefficient	P Value
TRM 400	6	0.63	0.22 - 1.15	0.1			
TRM 350	6	0.18	0.05 - 0.59	0.1			
Paint Rock River	5	0.79	0.45 - 1.04	1.03	0.2 - 2.6	-0.584	0.151
TRM 340	6	0.37	0.10 - 0.70	1.2			
Flint River	5	0.45	0.21 - 0.73	0.83	0.3 - 2.6	-0.120	0.424
Indian Creek	6	3.64	0.35 - 7.57	318.1	15.5 - 627.4	0.953	0.002
TRM 320	4	0.66	0.41 - 1.12	7.2	0.8 - 22.0	0.986	0.007
Cotaco Creek	4	4.34	2.04 - 6.28	60.0	2.3 - 139.2	0.192	0.404
Limestone Creek	6	1.10	0.13 - 3.26	0.43			
Flint Creek	6	1.19	0.09 - 4.94	6.75	0.6 - 19.1	0.653	0.183
TRM 300	6	0.84	0.08 - 1.24	9.7	1.4 - 46.3	0.593	0.107
Elk River	6	0.32	0.10 - 0.63	1.37	0.6 - 2.3	-0.049	0.463
Spring Creek	6	0.53	0.17 - 0.79	2.0			
TRM 275	5	0.51	0.38 - 0.77	4.3	1.2 - 10.1	0.902	0.018
TRM 260	6	0.09	0.03 - 0.22	0.8			

*Derived from data provided by the Environmental Protection Agency and Tennessee Valley Authority
†6-fish composite
‡Tennessee River Mile

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Mammalian Toxicology of 3,3',4,4'-Tetrachl[illegible]oazobenzene and 3,3',4,4'-Tetrachloroazoxybenzene: Implications in Environmental and Occupational Health

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3,3',4,4'-Tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) have been found in the soil as microbial degradation products from propanil, by far the most commercially important acylanilide herbicide in the United States. Recently outbreaks of chloracne, attributed to TCAB and TCAOB have occurred among workers handling contaminated 3,4-dichloroaniline or its herbicide derivatives. TCAB and TCAOB, being isosteric to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), bind with a high affinity to the TCDD receptor found in the mouse hepatic cytosol. Toxicological studies conducted in various mammalian systems by our laboratory indicate that exposure to TCAB and TCAOB should be considered as a threat to human health.

Cytotoxicity and Transformation. A cloned cell line (C3H/10T½) was used as one method of toxicity screening, since characteristics such as growth, morphology, and interaction with toxic chemicals of these mouse embryo fibroblasts have already been reported. In addition, C3H/10T½ cells are highly sensitive to post confluence inhibition of division and no spontaneous morphological transformation has been seen in any of the control cultures in the experiments reported. TCAB was found to be 2 to 5 times more toxic to C3H/10T½ cells than 2,2',5,5'-tetrachlorobiphenyl, two major components of Aroclors (PCBs), when cells were treated in suspension. A 50% growth inhibition was observed when cells were treated with 4 ug/ml of TCAB for 3 days. Numerous vacuoles were also observed throughout the cytoplasmic region in the exposed cells. C3H/10T½ cells treated with 1 ug of TCAB per ml of culture medium for 10 days showed focal morphologic alterations characteristic of transformed cells. At that time, numerous type III foci were observed, which stained darkly and were comprised of thickened spindle cells with a swirling growth pattern. Contact inhibition was lost, and cell growth continued after a monolayer was established. Cells from type III foci have been shown to produce tumors when injected into syngeneic C3H mice. The rapid appearance of these foci suggests that TCAB is oncogenic.

Unscheduled DNA Synthesis. The carcinogenic potential of TCAB was further confirmed in a subsequent study using freshly isolated rat hepatocytes. The measurement of DNA repair as unscheduled DNA (UDS) in primary cultures of rat hepatocytes was first suggested by Williams as a basis for screening chemical carcinogens. The hepatocyte not only serves as the target for DNA damage, but also has the necessary enzymes for procarcinogen activation. UDS was induced by TCAB and a dose-dependent response was demonstrated and presented in comparison to a well-known carcinogen, benzo[a]pyrene (Table I). The induction of UDS was assessed by a new procedure developed in our laboratory and based on the chloroform-isoamyl alcohol-phenol extraction of DNA from the isolated nuclei. The incorporation of ^{3}H-thymidine was determined by the scintillation counting assay. UDS as high as 333% of the controls was observed in hepatocytes treated with 5×10^{-5} M of TCAB.

Table I. Unscheduled DNA synthesis (UDS) in suspensions of freshly isolated rat hepatocytes and viability of cells at the end of a 4-hr incubation period.

Treatment	M	UDS, dpo/ug DNA	UDS, % control[a]	Viability %[b]
Benzo[a]pyrene	1×10^{-4}	610 ± 45	213	23
	5×10^{-5}	953 ± 36	332	44
	1×10^{-5}	899 ± 42	313	64
	5×10^{-6}	745 ± 45	260	62
	1×10^{-6}	398 ± 43	139	65
3,3',4,4'-TCAB	1×10^{-4}	491 ± 39	241	38
	5×10^{-5}	954 ± 59	333	47
	1×10^{-5}	834 ± 64	291	57
	5×10^{-6}	731 ± 50	255	71
	1×10^{-6}	537 ± 21	187	66

[a] The control sample (289 ± 36 dpo/ug DNA) was incubated with medium containing ^{3}H-thymidine and 1 % DMSO.
[b] Cell viability was determined by trypan blue exclusion.

Microsomal Enzyme Induction. The hepatic microsomal cytochrome P-448 level in male Sprague-Dawley rats was found to be induced by the administration of TCAB or TCAOB. Maximal stimulation of cytochrome concentration to 270% of the control level was observed with five daily consecutive i.p. injections of TCAB or TCAOB at a dose of 25 mg/kg/day. Detailed biochemical changes concerning the profiles of selected drug-metabolizing enzymes in the liver microsomes of rats exposed to TCAB and TCAOB was subsequently examined. A 10-fold increase of aryl hydrocarbon hydroxylase (AHH) level was observed in rats treated with either one of these two compounds at a dose of 10 mg/kg/day (5 daily i.p. injections). Two- and three-fold increases of UDP glucuronyl transferase activity were also observed in rats treated with TCAB and TCAOB respectively. However, other microsomal drug-metabolizing enzymes classified as phenobarbital-inducible were not affected. Further examination indicated TCAOB as being the

ost potent and persistent inducer among the three analogs
ested. In addition, stimulation of liver growth was observed in
he treated animals with a concurrent decrease in body weight
elative to control animals. This study indicates the ability of
CAB and TCADB to affect synergistically the toxicity of other
enobiotics and the pharmacological function of therapeutic drugs
y induction of hepatic microsomal enzymes.

spatocellular Pathotoxicology. The hepatotoxicity of TCAB and
CADB was examined in several short term experiments using rats
nd mice. Various histopathological alterations were observed by
ights and electron microscopic studies. Liver hypertrophy was
bserved in all animals following i.p. injection of either TCAB or
CADB. Hepatocyte profiles from treated animals were approximately
0% larger than control cells as determined by counting the number
f hepatocytes per unit area in liver sections from control and
xperimental animals. The other major histopathological
lterations were presence of cytoplasmic vacuoles, proliferation
f smooth endoplasmic reticulum, and the occurrence of numerous
esbranous arrays. The degenerative changes in the mouse liver
ections were more pronounced than that observed in rat liver.
he hepatic mitotic index for the experimental animals was
ignificantly greater than control values. TCAB appears to affect
he mitotic spindle apparatus in the mouse hepatocyte in which the
hromosomes are observed in a distinct tripolar arrangement.
imilar morphological changes were observed when hepatocyte
ultures were treated with TCAB at a dose of 5 ug/ml for 96 hr.
dditionally, the incorporation rate of selected precursors of
ellular macromolecules was affected.

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