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Correlation of PCB Body Burden with Behavioral Toxicology in Monkeys[1]

ROBERT E. BOWMAN, MARK P. HEIRONIMUS AND JAMES R. ALLEN

Wisconsin Regional Primate Research Center, Madison, WI 53706

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BOWMAN, R. E., M. P. HEIRONIMUS AND J. R. ALLEN. *Correlation of PCB body burden with behavioral toxicology in monkeys.* PHARMAC. BIOCHEM. BEHAV. 9(1) 49–56, 1978.—Eight monkeys fed 2.5 ppm PCB in their daily diet conceived, delivered and nursed five infants, three of which survived past weaning at four months of age. PCB residues in fat in the surviving infants at 8, 10½, and 23 months of age declined linearly when plotted as log concentration versus time (first order clearance), and these functions extrapolated to presumed peak PCB levels of 21, 114, and 123 μg/g fat (ppm) at 4 months of age. Behavioral tests on these three infants and four normal controls revealed hyperlocomotor activity at 6 and 12 months of age correlated with peak PCB body burdens. Higher peak PCB body burdens also were correlated with increased errors in five of nine learning tasks conducted between 8 and 24 months of age. Point estimates of zero-effect levels of PCB body burdens ranged around 21 ppm, although it was clear that even the monkey carrying only 21 ppm PCBs at four months of age exhibited some behavioral deficits persisting through the final testing at 24 months of age.

PCB Discrimination learning Hyperactivity Monkeys

POLYCHLORINATED biphenyls (PCBs) are widespread and extremely persistent environmental contaminants, present in wildlife [12, 13, 20], foods [10,15] and human tissues [4, 9, 19]. In samples of the U.S. population about one third carried measurable residues of PCBs [24]. There is also growing evidence that PCBs are extremely toxic. They have been associated with a variety of pathological symptoms in animals and in human beings accidentally exposed to large doses of the compounds [2, 7, 16], and research is needed on the toxological effects, if any, of exposure to low levels of PCBs.

The use of behavioral measures in the assessment of toxic damage, particularly to the nervous system, is a developing field and there is reason to believe that such measures can provide a highly sensitive biological endpoint. Behavioral effects have proven detectable in the absence of overt clinical pathology [5]. However, only two studies have reported on the behavioral toxicity of PCB exposure. Adult robins *(Erithacus rubecula L.)* fed 5 μg of Clophen A50 daily for three weeks exhibited heightened locomotor activity but no disturbance of migratory vectors [22]. Gravid rats fed 20 or 100 mg/kg of Kanechlor 500 (in olive oil) daily on Days 8–14 or Days 15–21 of gestation produced offspring of lower birth weight, which exhibited normal open field behavior at 12 weeks of age, and retarded learning of a water-filled multiple T-maze at 13 weeks of age [21].

The present research has examined behavioral toxicology resulting from chronic exposure to low levels of PCBs, thus modeling the most typical exposure parameters encountered by humans. The monkey was chosen as the experimental animal since it appears to metabolize PCBs similarly to the human [14], and differently from the rat [23]. On the basis of evidence that the developing nervous system is more vulnerable to toxic damage than the adult nervous system [8], it follows that any damage caused by low-level exposure would be maximized if the exposure occurred during early development. Consequently, rhesus monkeys were exposed chronically to PCBs *in utero* and in mothers' milk, following which they were tested on a variety of behavioral tasks during the first two years of life.

METHOD

Animals. Eighteen adult female rhesus monkeys *(M. mulatta)* were fed PCB (Aroclor 1248) for 16–21 months, a period which terminated at the end of 3 months of nursing for those monkeys with surviving offspring. Another 12 control mothers received no added PCB in their diet. The PCB mothers were divided into two groups of nine animals each, one of which was fed 2.5 ppm PCBs and the other 5.0 ppm in their monkey chow. One mother in each PCB group died; the remaining mothers were bred. The 5.0 ppm group showed a particularly high rate of reproductive failure with only two mothers out of the six which conceived carrying infants to term (one stillborn, one live birth) compared with five births out of eight conceptions in the 2.5 ppm group. Of the six live births of PCB-exposed infants, three 2.5 ppm offspring survived to begin testing in the present project.

Four control monkeys were selected from the group of 12 normal infants born to the above control mothers on the basis of birth dates comparable to the three surviving PCB infants. These four controls averaged 520 ± 20(SE) g in birth

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weight; the mean for the entire group of 12 normal infants was 507±17 (SE) g. The three PCB animals averaged 410±21 (SE) g which was 21% below the birth weight of the four controls and by two months of age had developed the overt physiological symptomatology characteristic of PCB poisoning: facial acneform skin lesions, loss of eyelashes, eyelid edema and focal hyperpigmentation of facial skin. The reproductive and pathological data as well as tissue content of PCBs in autopsied infants, have been described elsewhere for the above animals [1,3].

After weaning, at four months of age, which terminated the exposure of the infants to PCBs, the morphological symptomatology in the PCB infants steadily diminished. At seven months of age the body weight of the PCB and control groups averaged 1250±10 (SE) g and 1340±49 (SE) g, respectively; by 10.5 months of age the body weight of the PCB animals was only 3% below that of the normals and averaged 1523±20 (SE) g, compared to an average of 1572±33 (SE) g for the controls. The overt clinical symptoms normalized over the first year of life except for some residual hyperpigmentation lines in the facial skin. All animals showed normal appetites throughout testing.

PCB determinations. Fat biopsies were performed at 8, 9, 10.5 and 23 months of age on the PCB animals and at 10.5 and 23 months of age on the controls (subcutaneous fat was taken at 8 and 9 months of age, and mesenteric fat thereafter). The samples of fat were analyzed for PCB content as follows [6]. Each sample was homogenized in hexane, evaporated under dry nitrogen with anhydrous sodium sulfate at 40°C., passed onto a silica gel 60 (0.05–0.2) microcolumn and eluted with 1:1 benzene-hexane. Eluates were quantified by gas chromatography using a ^{63}Ni electron capture detector and a glass column packed with gas Chrom Q (80–100 mesh) coated with 2% SE–30, with argon-methane (95%–5%) as the carrier gas at a flow rate of 40 cc/min at 170°C. The lower limit of detectability of PCBs in 1.0 g of sample was less than 0.05 ppm.

Behavioral test schedule. All monkeys were tested on a sequence of 11 tasks, one task at a time, in the order and at the age reported in Table 2. All tasks are described below for the sake of completeness, although significant effects were not noted on four of them.

Locomotor activity test. (LA I and LA II). This was assessed in an activity chamber which measured 72×40×56 cm in height (about the same size as the home cage) and which was quadrasected by two photobeams 19 cm above the floor. Beam breaks were cumulated on a series of counters in 15 min blocks. All animals were given 16 consecutive daily 60 min sessions in the apparatus at 6 months of age (LA I), and 20 consecutive, daily 90 min sessions at 12 months of age (LA II).

WGTA discrimination tests and general procedure. All of the discrimination learning tasks described below were carried out in a standard Wisconsin General Test Apparatus or WGTA [11]. Briefly, the monkey was placed in a cage facing a table upon which a test tray could be rolled to within the animal's reach and out of it again. The test tray had two recessed foodwells 3 cm in dia and 1.5 cm deep centered 22 cm apart which were 8 cm from the front bars of the monkey's cage when the tray was pushed all the way forward. A typical test trial proceeded as follows: The experimenter lowered an opaque screen in front of the animal's cage and pulled the test tray back to bait the foodwell under the correct object. He then lowered an opaque screen to conceal himself, raised the animal's opaque screen and pushed the tray halfway toward the monkey, still out of reach, paused a second or two to let the animal see both stimulus objects and then rolled the tray to within the monkey's reach. The experimenter observed the animal through a small one-way window set in his opaque screen. The monkey was permitted to displace only one of the objects (noncorrection procedure) whereupon the animal's screen was lowered, the tray withdrawn and the process repeated. Intertrial intervals were around 10 sec. Each session consisted of a maximum of 50 trials, and was terminated whenever the monkey reached criterion on any given task. Rewards were either raisins, a dry breakfast cereal with the brand name Fruit Loops or small marshmallows.

Spatial reversals (SpDR). The two foodwells on the test tray were each covered with an identical, yellow, square, wooden block. After about two weeks of adaptation and object displacement training the monkeys were trained to displace the object on the nonpreferred side (as determined during adaptation) by baiting only that side. Once the animal had attained a criterion of 90% correct on two consecutive days the problem was reversed with only the opposite side being baited. The criterion of learning on all reversals was 9 correct responses in 10 trials at which time the day's testing was terminated and the opposite side baited the following day. Twenty such reversals were given.

Color reversals (CDR). Both foodwells in the test tray were covered with a square, wooden block, one red and the other green. One of the colors was baited consistently from trial to trial, and each color was presented on each side of the tray equally often. The same learning criteria were used as for Spatial Reversals above. Thirty-two color reversals were given.

Shape discrimination reversals (ShDR). Two wooden blocks, circular and cross-shaped, were placed over the foodwells on each trial and one of the shapes was rewarded consistently. One object was always red and the other always green but each color was rewarded only half the time overall; i.e., if the circle was the rewarded stimulus on a given day the red circle was used randomly on half the trials and the green circle was used on the remaining half. The same learning criteria were used as for Spatial Reversals above. Forty shape reversals were given.

Partial reinforcement (PR). The same stimulus objects as for ShDR were used, baiting the correct shape on only 60%, 50% or 33% of the trials and the incorrect shape never. Sixteen reversals were given to a 9 out of 10 criterion at 67/0, then 16 at 50/0 and finally, 16 at 33/0 reinforcement.

Probability learning (PL). The ShDR objects were again used. The series began with 16 daily sessions in which one shape was baited on 70% of the trials and the other shape baited on the remaining 30% of the trials (a ratio of 70/30). The task was then reversed for eight sessions baiting the first shape on 30% of the trials and the second shape on the remaining 70% (a ratio of 30/70). The series continued through ratios of 65/35, 60/40, 58/42, 42/58, 56/44 and 44/56 (8 sessions each).

Progressive probability shift (PPS). This was a single gradual reversal problem over 23 sessions of 50 trials each. Two new stimulus objects were used, a rectangle and a triangle, one designated the A object and the other the B object. The percentage of trials on which a reward was randomly placed under the A object vs. the B object (A/B) was as follows for the respective 23 sessions: 100/0, 90/10, 80/20, 70/30, 60/40, 58/42, 56/44, 54/46, 52/48, 50/50 (i.e., one session of an insoluble problem), 48/52, 46/54, 44/56, 42/58,

TABLE 1

PCB INTAKES IN MG AND PCB BODY BURDENS IN μG/GM OF BODY FAT

		Monkey						
		AA06	AA07	AA18	AA08	AA17	AA19	AA23
Estimated total PCB intake of mother		292	284	303	<6	<6	<6	<6
Mother's Body Burden*		224	81	—	.7	0	.09	.3
Age at Biopsy	8 months	27	21	11	—	—	—	—
	10.5 months	11	7	10	0	0	.1	.1
	23 months	0	.7	1.6	0	0	.2	.3
r†		−1.0¶	−1.0¶	−.99				
Intercept†		2.68	2.85	1.56				
Slope†		−.156	−.191	−.0584				
Estimated Peak‡		114	123	21				
Half Life§		1.93	1.58	5.16				

* PCB mothers assayed approximately 3 months prior to conception, control mothers assayed approximately 8 months postpartum.

† Correlation coefficients and linear regressions were based on log PCB concentration versus age.

‡ The regression was extrapolated back to 4 months of age to calculate the 'estimated peak' value of PCB in μg/gm fat (ppm).

§ The clearance half-life, $t_{1/2}$, in months was determined from the linear regression of log PCB on age.

¶ The regressions for AA06 and AA07 were based on only the 8 mo. and 10.5 mo. bioassays, since the assay values at 23 mo. were too low (<1 ppm) for accurate quantitation. These regressions predicted PCB values at 23 mo. (0.12 and 0.03 ppm respectively) close to those actually observed.

40/60, 38/62, 36/64, 34/66, 32/68, 30/70, 20/80, 10/90 and 0/100. To analyze the data, the variance was reduced by averaging each monkey's responses as follows. The data for each monkey was plotted as mean daily % responses to the A object (Y axis) versus the 23 successive daily reinforcement ratios to the A object (X axis) and the data points were connected by straight lines. Then horizontal lines were drawn at the arbitrarily selected response ratios to the A object of 95, 90, 80, 70, 60, 50, 40, 30, 20, 10, and 5%. Each horizontal line constituted a response criterion, and the highest and lowest reinforcement ratio at which the monkey's data curve crossed each response criterion was tabulated and averaged. This yielded for each monkey the information that each successively lower response criterion to the A object occurred at the given average reinforcement ratio to the A object. The resulting curve for each monkey of criterional response ratios to the A object versus mean reinforcement ratios to the A object was thereby constrained to be a monotonic curve without inversions. At each response criterion, the mean reinforcement ratios for the four control monkeys and the three PCB monkeys (seven data points) were paired with the peak PCB body burdens for regression analysis.

Object alternation learning (OA I and OA II). In OA I two objects which differed in both color (yellow or blue) and shape (triangular or circular) were used on each trial. The reward was alternately placed first under one shape and then under the other shape from trial to trial while the color was rewarded irrelevantly. In order to be consistently correct the monkey had to alternate between the shapes from trial to trial while ignoring the color cues. OA II was simplified by deleting the color cue and making both objects the same color throughout. Forty sessions of OA I and 24 sessions of OA II were given.

Object alternation learning set (OALS). At two years of age all animals were given 24 sessions of Object Alternation Learning Set in the WGTA, each session made up of six 6-trial problems. That is, six different pairs of differing, 3-dimensional junk objects were used for six trials each in each day's testing. The objects were baited alternately within problems so that the optimal strategy for the animal was again to alternate his choices between the objects.

RESULTS

PCB body burdens. PCB levels were measured in body fat at 8 months of age in the PCB animals and at 10.5 and 23 months of age in all animals. Addditional biopsies taken at 9 months of age were lost through contamination of the assay tubes. Table 1 gives the PCB concentrations for all animals and the least-squares linear regression of log PCB concentrations on age for the three PCB animals. The regressions using log PCB concentrations assume first order clearance kinetics, and the data for each PCB monkey were highly consistent with this assumption.

The regression equations were used to estimate the half-lives ($t^1/_2$) for the decline of PCB concentration in fat in the experimental animals and also were extrapolated backwards to four months of age to estimate the PCB body burden at the age at which PCB intake was terminated. This 4 month estimate was based on the measurements at all time points and probably approximated an important quantity, i.e., the peak PCB body burden carried by these animals at any time.

For these reasons the 4-month estimates (or peak estimates) were used as the X-variable for the PCB subjects (with performance the Y-variable) in all the regression calculations to follow. It should be noted that regressions utilizing

TABLE 2

AGE AT TIME OF TESTING, RANK ORDERS (R) AND OVERALL Z-SCORES FOR INDIVIDUAL ANIMALS ON EACH TASK, AND THE OVERALL CORRELATION COEFFICIENT, r, BETWEEN BEHAVIORAL PERFORMANCE AND PCB BODY BURDEN ON EACH TASK CALCULATED ON ALL 7 MONKEYS

Task*		LA I	SpDR	CDR	ShDR	LA II	PR	PL	PPS	OA I	OA II	OALS
Age		6 mo.	7 mo.	8 mo.	10 mo.	11 mo.	12 mo.	16 mo.	19 mo.	21 mo.	23 mo.	24 mo.
Overall r		.7596‡	.9243‡	.8162‡	.2088	.7604‡	.3107	.1466	.7925‡	.5168	.7779‡	.7228†
AA06§	R	6	6	4	4	7	5	2	7	6	7	6
	z	−.02	−1.08	−.02	−.02	−1.56	−.42	1.0	−1.50	−1.03	−1.70	−.90
AA07	R	7	7	7	5	6	4	7	6	5	6	7
	z	−2.18	−1.73	−1.66	−.25	−.83	−.41	−1.57	−1.06	−.53	−1.36	−1.31
AA18	R	2	5	5	1	2	1	1	2	4	3	4
	z	.65	.11	−.49	.50	1.08	1.07	1.24	.55	−.28	.82	−.02
AA08	R	1	1	6	2.5	1	6	4	5	7	5	5
	z	.71	1.16	−1.04	.20	1.15	−.57	.19	−.02	−1.21	−.77	−.56
AA17	R	5	3	3	2.5	5	3	3	3	1	1	1
	z	.11	.80	.18	.20	−.42	.49	.74	.23	1.84	1.80	1.83
AA19	R	4	2	1	7	3	7	5	4	3	2	2
	z	.28	.93	1.85	−.36	.43	−.73	−.59	0	.56	.98	.90
AA23	R	3	4	2	6	4	2	6	1	2	4	3
	z	.44	.02	1.50	−.26	.15	.67	−.102	1.81	.65	.23	.05

* All abbreviations identifying the tasks are defined under Methods in the text.
† $\alpha<0.10$
‡ $\alpha<0.05$
§ Animal Nos. AA06, AA07 and AA18 were the three PCB animals; AA08, AA17, AA19 and AA23 were the four control animals.

the PCB values actually measured at 8 months of age gave essentially the same findings as those reported here using the peak estimates. For the control animals, the PCB levels measured at 10.5 months (the earliest collected) were used in all the regression calculations to follow. The controls were not measurably different from zero PCB concentrations at any time actually measured, and the same was assumed to hold for their PCB body burdens at four months of age.

PCB in control diets. Samples of 1 gm each of the control diets contained undetectable levels of PCBs when subjected to assay. This indicated that the control diets contained less than 0.05 ppm PCBs, since 0.05 ppm would have been detected by the present methods.

Locomotor activity. In the 16 sessions of LA I (6 months of age) the correlation coefficients over all 7 monkeys for the successive 4-day blocks were .3075, .7116 ($p<0.10$), .7114 ($p<0.10$) and .8743 ($p<0.10$). In the 20 sessions of LA II (11 months of age) the corresponding correlation coefficients were .2144, .1715, .6169, .7881 ($p<0.05$) and .9664 ($p<0.001$). Thus, the hyperlocomotor activity shown by the PCB animals only appeared after they had been exposed to the test situation for many consecutive daily test sessions.

Discrimination learning. The linear regressions of overall errors during learning versus peak PCB body burdens yielded significant effects of PCB for five of the nine discrimination learning tasks (Table 2). The treated monkeys showed deficits on the first two discrimination reversal tasks, namely spatial (SpDR) and color (CDR), but thereafter were equivalent to controls on shape reversal (ShDR), partial reinforcement of shape reversal (PR) and probability learning of shape reversals (PL). These five tasks all required the development of object perseveration as the strategy for maximizing reinforcement. Subsequently, PCB monkeys were retarded in learning the progressive probability shift (PPS), persisting in object perseveration to the originally correct object. Finally, the PCB monkeys were retarded on the object alternation tasks (OA I, OA II, OALS), on which the correct strategy was exactly the opposite of object perseveration. All of the controls eventually learned the alternation strategy to a significant degree during the OA II and OALS tasks, revealing a significant retardation in the PCB subjects on OA II and OALS (Table 2).

Rank orders and the performance of the two high PCB monkeys. Mean performance measures for each animal over all trials on each task were ordinally ranked within each task (Table 2). The two high PCB animals began at the bottom of the rankings. They were hyperactive in the first locomotor task, and made more errors in the first two discrimination reversal tasks. As successive object perseveration tasks were presented however, they rose in rankings, becoming as expert as the controls in perseverating on object choices in order to maximize reinforcements. However, this improvement appeared to be specific to object perseveration as a maximizing strategy, as revealed by subsequent deficits. For example, the Progressive Probability task (PPS), which required a shift from one object to the other in the course of the testing, revealed that the high PCB animals had difficulty in shifting away from perseverating on the first learned object. In addition, the high PCB animals were significantly hyperactive in the second locomotor activity test. Finally, they showed deficits on the last two discrimination tasks, which required repeated object alternations to maximize reinforcement.

Performance of the intermediate-level PCB animal.

Monkey No. AA18 had an estimated 4-month, peak body burden of 21 ppm which was intermediate between that of the high-PCB animals (No. AA 06, 114 ppm and No. AA07, 123 ppm) and the control level. The extent to which that monkey was affected was therefore of interest. The ordinal rankings in Table 2 showed that when the high-PCB animals exhibited deficits, then No. AA18's performance was between their level and that of the controls. However, on the tasks on which no PCB effect was seen, i.e., tasks on which the high-PCB animals were not affected, No. AA18 was at or near the top of all of the rankings. On the object-alternation tasks No. AA18 again dropped to her intermediate rank, suggesting that the improvement shown by this animal while more dramatic than that of the two high-PCB animals, was also strategy-specific.

To further illustrate the intermediate behavioral status of No. AA18 the following procedure was used. The average z-score for tasks on which there was an overall PCB effect (significant r values) and the average z-score for tasks on which there was not were determined for each animal. The latter average was then subtracted from the former one to give the following difference scores: −.9217, −.7317, and −.4430 for the PCB monkeys and .0129, .1495, .7119 and 1.249 for the controls. The PCB animals all had negative difference scores, indicating a decline in their relative position on tasks where a PCB effect was noted. The −.4430 for No. AA18 was intermediate: negative like the scores of the high-PCB animals but less negative than either of them. The ranking of AA18 tended to improve or decline in harmony with the ranks of the two high PCB animals, although AA18's rankings averaged higher overall. Of course, since the tasks were grouped by the criterion of significant or nonsignificant regressions, and since the high PCB subjects and the controls were the extreme point in the regression analysis (thereby mainly determining the regression line), their z-difference scores were constrained to differ. However, AA18 was near the overall mean in PCB body burden, and hence was weighted as nearly zero in the regression calculations. Therefore, her z-difference behavioral score was not constrained by this procedure. It is noteworthy then that AA18's z-difference score lay near those of the other PCB monkeys, suggesting a behavioral effect on the PCB levels carried by this animal.

DISCUSSION

The significant correlation coefficients in Table 2 are evidence for a reliable dose-effect change which resulted in both hyperlocomotor activity and learning retardation as a function of PCB levels in body fat. The occurrence of these effects is consistent with the hyperactivity noted previously in adult robins fed PCBs [22] and with the learning deficits seen earlier in rats which had received PCBs *in utero* and during nursing [21], although observed at PCB doses much lower than those employed in these previous studies. It should be remarked that the hyperactivity seen here required many consecutive daily exposures to the activity cage before it emerged. This is suggestive of an early suppression of the hyperactivity through fear of the novel apparatus and the unmasking of the hyperactivity as the monkeys slowly adapted to the apparatus and lost their fear.

It is difficult to infer the mechanisms or natures of these behavioral alterations, and particularly how they might extrapolate to the human. Nevertheless, pending further research, it would be prudent to assume that these behavioral alterations in the monkey do extrapolate to the human.

Given these points, and the dose-effect functions implied by the above regression analyses, it is pertinent to consider some estimate of the lowest PCB body burden likely to produce the noted effects. Ideally, such an estimate would take the form of dose-response functions in which the proportion of population affected to some behavioral criterion is plotted against PCB body burden. Lacking the large number of subjects required for a dose-response function, it is necessary here to work with inferences drawn from the dose-effect relationships, in which the observed behavioral changes within individual subjects are plotted versus PCB body burdens. The distinction between dose-response and dose-effect relationships has been defined elsewhere [18].

One advantage of determining dose-effect functions is that one can observe where such a function for intoxicated, experimental animals intersects the mean behavioral score of nonintoxicated control monkeys. This intersection indicates an average dose level at which no behavioral effect is observed. We suggest here that this intersection point be termed the mean zero effect threshold (or mean ZET). If the theoretical ZET values for individual animals are normally distributed and have a sufficiently narrow range around the mean ZET, then the mean ZET will represent the toxin concentration at which 50% of the animals will show toxic effects and 50% will not, i.e., the ZET_{50}. Hence, dose-effect data can potentially be transformed into dose-response statements. In the absence of a real threshold for toxin effects, the estimates of the mean ZET would be near zero. Hence, the concept and estimation procedures for the ZET can handle either the presence or absence of a real threshold simply as different points on the continuum of toxin dosage.

It should also be noted that different biological endpoints could well have different mean ZET values. Hence, the ZET values derived from different measures could only be averaged if all of the measures were affected by a common mechanism of toxic action.

In attempting to estimate ZET values on the present data, one can only utilize those tasks (or blocks within tasks) on which a significant toxic effect of PCB was observed. Taking the five significant discrimination tasks observed here (Table 2), the linear regressions of behavior on peak PCB body burdens over the PCB and control animals combined yielded ZET estimates of −4, −11, +4, +6 and +3 ppm PCB in body fat. However, these values were probably constrained to lie near zero by the inclusion of the control subjects in the regression analysis.

To obviate this problem, regressions were calculated for these five tasks on only the three experimental animals. It was necessary to utilize block by block analysis to obtain significant regressions ($p<0.10$) using only the three experimental animals, and these significant regressions occurred only when the low PCB monkey (AA18) had learned the task (and was like a control) while the two high PCB monkeys had not learned. Four such blocks were found, as noted by the significant r values in Table 3. The respective regression equations for these blocks were $y=2.8+.067x$, $y=50-.26x$, $y=75-.16x$ and $y=81-.21x$, and the respective mean ZET estimates given by the points of intersection were 27, 27, 22 and 11 ppm. The control-like performance of AA18, once she learned, therefore constrained the ZET estimate to vary around her own body burden of 21 ppm. Since other data (rank order, z-score and z-score differences) had clearly indicated that AA18 was affected and was intermediate be-

TABLE 3

SIGNIFICANT CORRELATION COEFFICIENTS AND LINEAR REGRESSIONS OF BEHAVIORAL MEASURES ON PEAK PCB BODY BURDENS

Task	Block #	All Monkeys§ r	Intercept	Slope	PCB Monkeys¶ r
LA I	II	.71*	49	2.8	—
	III	.71*	50	3.8	—
	IV	.87‡	56	4.4	—
SpDR	I	.90‡	16	.11	.43
	II	.96‡	4.4	.05	.99*
	III	.72*	3.9	.02	.88
	V	.69*	1.8	.01	.82
CDR	III	.79†	8.8	.08	.90
	V	.80†	5.3	.05	.68
	VII	.98‡	4.1	.04	.97
	VIII	.96‡	3.1	.04	.99
ShDR	No significant effects in 8 blocks.				
LA II	IV	.79†	40	1.1	—
	V	.97‡	23	1.4	—
PR	No significant effects in 12 blocks.				
PL	No significant effects in 18 blocks.				
PPS	III	−.75*	50	−.11	−.82
	IV	−.77†	48	−.11	−.93
	V	−.74*	47	−.11	−.93
	VI	−.74*	46	−.11	−.92
	VII	−.87†	46	−.19	−.91
	VIII	−.86†	44	−.21	−1.00†
	IX	−.83†	41	−.26	−.90
	X	−.75*	37	−.23	−.89
	XI	−.74*	29	−.20	−.98
OA I	No significant effects in 10 blocks.				
OA II	III	−.88‡	72	−.14	−1.00*
	IV	−.84†	71	−.17	−.96
	V	−.84†	73	−.14	−.95
	VI	−.72*	70	−.11	−.97
OALS	II	−.72*	74	−.16	−.95
	III	−.77†	80	−.19	−1.00*
	IV	−.73*	72	−.14	−.97

* Significant at $\alpha \leq 0.10$

† Significant at $\alpha \leq 0.05$

‡ Significant at $\alpha \leq 0.01$

§ The r, intercept and slope shown below this heading are based on the data from all seven subjects. The slope and intercept are in terms of photobeam breaks per 0.1 hr for LA I and LA II, mean errors per reversal for SpDR and CDR, mean reinforcement ratios for PPS, and % correct responses for all other tasks.

¶ The r values shown below this heading are based on linear regressions done on the data of the three PCB subjects only. Regression equations for the four significant correlations are given in the text.

Block refers to blocks of consecutive sessions as described in the Methods, except for Task PPS, in which case the Blocks represent the eleven successive response criteria from 95% to 5% as also described in the Methods.

haviorally between the controls and the high PCB monkeys, it was evident that her body burden of PCB had exceeded her individual ZET value. Thus, 21 ppm of PCB in body fat at 4 months of age was sufficient to obtain some behavioral toxicity in AA18, and hence would be likely to produce effects in some appreciable proportion of a monkey population. If this appreciable proportion were to be approximately 50%, then 21 ppm would indeed be the ZET_{50} or the mean ZET. Therefore, despite the constraint mentioned above, we consider the best estimate of the mean ZET obtainable from the present data to be 21 ppm, the estimated peak body burden of AA18 at 4 months of age. However, it should be kept in mind that this estimate was derived from only three monkeys, and has a large and undeterminable confidence interval.

Any ZET estimates for PCBs, however rigorous statistically, must be interpreted in light of (1) the exposure parameters (dosage, route, duration, type of PCB, etc.), (2) the time of estimate of the body burden relative to the stage of exposure and (3) the time of estimate of the body burden relative to the stage of development of the organism. Relative to Point 1, infant monkeys brought to 21 ppm of PCBs at 4 months of age by chronic exposure *in utero* und through nursing will possibly exhibit different effects than monkeys brought to that concentration *in utero* only, or through nursing only, or via a single massive exposure. It should also be noted that the Aroclor 1248 to which the animals in this experiment were exposed is an extremely complex mixture of individual polychlorinated bi-phenyl compounds and it is impossible to establish, on the basis of these results, just which particular compounds, or metabolites thereof, are responsible for the effects reported. With regard to Point 2, for example, the use of peak PCB estimates will yield higher numerical ZET estimates than would the use of PCB body burdens either before or after the peak values.

Finally, with respect to Point 3, the interpretation of the ZET estimate must depend upon the nature and timing of the neurotoxicity of the PCBs. If the toxicity is acute (caused by the immediate action of PCBs on neural tissue), then the PCB levels present at the time of testing will be most appropriate for estimating ZET. If the toxicity is of the residual kind, possibly initiated by the presence of PCBs in brain during some critical developmental period, then PCB concentrations during the critical period would be the most appropriate for estimating the ZET.

The appearance of significant learning deficits in the present animals after essentially total clearance of PCBs from body fat is consistent with enduring or residual effects of the PCBs. The period of maximal vulnerability to the neurotoxic lesions is unknown for PCBs, but is probably prior to four months of age. If so, then the present mean ZET estimates obtained at the peak body burden at four months of age would be too high.

One other point should be considered here. PCBs in some samples of human milk fat have been reported at around 4 ppm ([17]; also unpublished data). During the present experiment, milk fat from the mother of one of the high PCB infants was sampled and assayed at 16 ppm of PCBs. If body burdens on the average are proportional to intake burdens, then subjects consuming milk fat at one fourth the PCB concentration consumed by other subjects should have body burdens of PCBs that are one fourth those of the other subjects. Given comparable, low level chronic exposures and vulnerability to PCBs in humans and monkeys, it is pertinent to note that the present estimate of the mean ZET (21 ppm) was about one sixth that of the body burdens of the present high PCB infants. This is suggestive that some human infants may be receiving chronic exposure to PCBs at concentrations in the vicinity of the mean ZET. While speculative exercises like this cannot substitute for hard data, they do suggest powerful reasons for immediate and continued research in this area.

From a regulatory standpoint, an important PCB level is the amount to be permitted in food. The present data, both behavioral and pathological, indicate severe toxicity with a continuous diet at 2.5 ppm of PCBs. Lower concentrations and less frequent intake of food contaminated with PCBs are now under study.

In conclusion, the present data primarily demonstrated a dose-effect relationship between PCB body burdens early in life and both immediate and later behavioral deficits. Surviving offspring were hyperactive in locomotor tests and deficient in learning various types of discrimination problems. The last observed learning deficits appeared to represent residual toxicity of the PCBs acting at some undetermined period in ontogenetic development. The data provided a very approximate estimate of 21 ppm in body fat at four months of age as the mean Zero Effect Threshold (ZET_{50}) for chronic PCB exposure, and the monkey which was observed at this level was clearly affected. As a regulatory level, the ZET_{50} would only protect half the population. The ZET_{001}, a clearly more acceptable, safe level, would be the value at about 5 SDs below the mean ZET. Since SDs cannot be estimated from the present data, the ZET_{001} cannot be specified. However, it seems plausible that the ZET_{001} might be as low as one tenth of the mean ZET, or about 2 ppm. Clearly, it appears unwise to tolerate exposure conditions resulting in infant body burdens as high as 21 ppm in body fat.

REFERENCES

1. Allen, J. R. and D.A. Barsotti. The effects of transplacental and mammary movement of PCBs on infant rhesus monkeys. *Toxicology* **6**: 331–340, 1976.
2. Aulerich, R. J., R. K. Ringer and S. Iwamoto. Reproductive failure and mortality in mink fed on Great Lakes fish. *J. Reprod. Fer.* **19**: 365–376, 1973.
3. Barsotti, D. A., R. J. Marlar and J. R. Allen. Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). *Fd. Cosmet. Toxicol.* **14**: 99–103, 1976.
4. Biros, F. J., A. C. Walker and A. Medbery. Polychlorinated biphenyls in human adipose tissue. *Bull. Envir. Contam. Toxic.* **5**: 317–323, 1970.
5. Bowman, R. E. Behavioral and neurological development as parameters in behavioral toxicology. *Proceedings, Workshop on Behavioral Toxicology*, DHEW Publication No. (NIH) 76–1189, 1976, 67–94.
6. Curley, A., V. W. Burse, M. E. Grim, R. W. Jennings and R. E. Linder. Polychlorinated biphenyls: distribution and storage in body fluids and tissues of Sherman rats. *Envir. Res.* **4**: 481–495, 1972.

7. DeLong, R. L., W. G. Gilmartin and J. G. Simson. Premature births in California sea lions: association with high organochlorine pollutant residue levels. *Science* **181:** 1168–1170, 1973.
8. Dobbing, J. Vulnerable periods in developing brain. In: *Applied Neurochemistry*, edited by A. N. Davison and J. Dobbing. Philadelphia: F. A. Davis, 1968, pp. 287–316.
9. Finklea, J., L. E. Priester, J. P. Creason, T. Hauser, T. Hinners and D. I. Hammer. Polychlorinated biphenyl residues in human plasma expose a major urban pollution problem. *Am. J. Publ. Hlth.* **62:** 645–651, 1972.
10. Fires, G. F. Polychlorinated biphenyl residues in milk of environmentally and experimentally contaminated cows. *Envir. Hlth. Persp.* **1:** 55–59, 1972.
11. Harlow, H. F. and F. A. Bromer. A test-apparatus for monkeys. *Psychol. Rec.* **2:** 434–436, 1938.
12. Holden, A. V. and K. Marsden. Organochlorine pesticides in seals and porpoises. *Nature* **216:** 1274–1276, 1967.
13. Holmes, D. C., J. H. Simmons and J. O'G. Tatton. Chlorinated hydrocarbons in British wildlife. *Nature* **216:** 227–229, 1967.
14. Hsu, I. C., J. P. Van Miller, J. L. Seymour and J. R. Allen. Urinary metabolites of 2, 5, 2′, 5′- tetrachlorobiphenyl in the nonhuman primate. *Proc. Soc. exp. Biol. Med.* **150:** 185–188, 1975.
15. Kolbye, A. C. Food exposures to polychlorinated biphenyls. *Envir. Hlth. Persp.* **1:** 85–88, 1972.
16. Kuratsune, M., T. Yoshimura, J. Matsuzaka and A. Yamaguchi. Epidemiologic study on usho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. *Envir. Hlth. Persp.* **1:** 119–128, 1972.
17. New York State Planning Commission (1977) Health Advisory Council, Report of the Ad Hoc Committee on the Health Implications of PCBs in Mother's Milk, January 7, 1977.
18. Nordberg, G. F. Effects and dose-response relationships of toxic metals. *Scand. J. Work Envir. Hlth.* **2:** 37–43, 1976.
19. Price, H. A. and R. L. Welch. Occurrence of polychlorinated biphenyls in humans. *Envir. Hlth. Persp.* **1:** 73–78, 1972.
20. Risebrough, R. W., P. Rieche, D. B. Peakall, S. G. Herman and M. N. Kirven. Polychlorinated biphenyls in the global ecosystem. *Nature* **220:** 1098–1102, 1968.
21. Shiota, K. Postnatal behavioral effects of prenatal treatment with PCBs (Polychlorinated Biphenyls) in rats. *Okajimas Fol. anat. jap.* **53:** 105–114, 1976.
22. Ulfstrand, S., A. Sodergren and J. Rabol. Effect of PCB on nocturnal activity of caged robins *(Erithacus rubecula L.). Nature* **231:** 467–468, 1971.
23. Van Miller, J. P., I. C. Hsu and J. R. Allen. Distribution and metabolism of ^{3}H-2, 5, 2′, 5′-tetrachlorobiphenyl in rats. *Proc. Soc. exp. Biol. Med.* **148:** 682–687, 1975.
24. Yobs, A. R. Levels of polychlorinated biphenyls in adipose tissue of the general population of the nation. *Envir. Hlth. Persp.* **1:** 79–81, 1972.