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REFERENCES

REVIEW OF STUDIES IN CHILDREN: POLYCHLORINATED BIPHENYLS, DIBENZO-P-DIOXINS AND DIBENZOFURANS

January 1997

Prepared for:
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OVERALL COMMENTS

Background

During the last decade, numerous papers have been published postulating positive associations between consumption of Lake Michigan fish and neurobehavioral effects in infants and young children or between body burdens of polychlorinated biphenyls (PCBs). Several comments and reviews have been written about these studies (Buck 1996; Colborn et al., 1996; Darvill et al., 1996; Guo et al., 1996; Gray 1996; Hanneman et al., 1996; Jacobson 1996; Paneth 1991 and 1996; Rice 1996; Schantz 1996a and 1996b; Seegal 1996). Additional similar studies have been conducted on three other groups of children in North Carolina, the Netherlands, and Taiwan. In Taiwan the infants were exposed in utero to high concentrations of polychlorinated dibenzofurans (PCDFs) because their mothers had consumed rice oil contaminated with PCBs, PCDFs and other organic chlorinated compounds. Both the mothers and the infants had clinical signs of poisoning in Taiwan, while the other groups of children were exposed to much lower levels of chlorinated organic compounds.

Such studies are difficult to undertake and all of the studies reviewed here have methodological problems. Presently no clear picture of any PCB effect emerges. In many of the different samples (maternal, cord blood, and milk) obtained from the study participants in the Netherlands, Michigan, and North Carolina the PCBs and/or dioxins were below the limit of detection even though the initial samples were collected in North Carolina and in Michigan over 15 years ago. In Taiwan, initially, only PCBs were measured and the PCB levels often were not particularly elevated and correlated poorly with clinical signs of poisoning. The PCBs also correlated poorly with the PCDFs when they were finally measured. Thus, it is not quite clear what the doses of PCDFs were that the symptomatic children received.

Quantitation of PCBs in Body Fluids

The quantitation of the PCBs in human serum, whole blood, plasma and milk is a fundamental problem in the studies in Michigan, North Carolina and the Netherlands. Particularly at the low levels in the various body fluids they are imprecise and much variation occurs. A difference between 4 and 6 ppb PCB, for instance, in whole serum is meaningless. This has been demonstrated in a number of publications. The variations that are introduced occur in serum and in milk because the lipid content varies, the recovery of material extracted from these samples varies, and the within laboratory (repeatability) relative standard deviations (RSD_r) and the reproducibility among laboratories varies. For PCB mixtures some uncertainty is also introduced by different means of quantitation of the PCBs.

Lipid Content

Phillips et al. (1989) measured PCBs in the serum of 20 volunteers after a 12-hour fast, 4 to 5 hours after a high fat breakfast, at midafternoon and again the next morning after a 12-hour fast. Nonfasting samples had 29% higher mean concentration ($p<0.05$) of PCBs than did fasting samples, 4.9 versus 3.8 ppb. Total lipids were 20% higher in nonfasting samples. Thus, meaningful comparison of analytical results requires standardizing collection procedures or correcting by total serum lipid levels. When PCB concentrations were corrected for total lipids and expressed on a lipid weight basis there were no significant differences among measurements. Differences between fasting and non-fasting means were reduced to 7% for PCBs (0.67 vs 0.63 μg/g lipid).

However, in all of the studies reviewed here the PCBs used for comparative analysis of serum PCBs have been on a whole serum (Michigan and North Carolina) or whole blood (Yu-Cheng) or plasma (Netherlands) basis and no mention is made of collecting fasting blood samples.

Repeatability, Reproducibility and Quantitation

Recovery of PCBs from spiked samples at serum PCB concentration between 10 and 30 ppb averaged 104.8% and 92.3% for concentration levels of 10 and 30 ppb, respectively (Burse et al., 1990). In another study the repeatability and reproducibility of PCB determinations in 6 collaborating laboratories was tested. The within laboratory (repeatability) relative standard deviations (RSD_r) of 18.8, 20.5, 10.2, and 14.1%, respectively, were noted for different laboratories. The reproducibility among laboratories was within the same range (Burse et al., 1989). All of these studies show that small differences in PCB concentrations in serum or milk have little meaning because of the variability introduced by sample collection and chemical analysis.

In North Carolina, Aroclor 1254 was used for quantitation and quantitation was done on only two peaks. In addition, accountability values were developed. These accountability values were used to continuously monitor recoveries and drifts in detector response. The mean percent accountability factors used to correct the DDE and PCB values were determined as follows: all sample analytical runs included spiked samples. All matrices, with the exception of formula, were spiked at the levels used for quality assurance validation work. The first 5 times a matrix was analyzed, 4 spikes were included (2 DDE and 2 PCB) in addition to other assay control materials and samples. An accountability value was determined by subtracting the baseline control value (parts per billion) from the level determined for the spiked sample (parts per billion) and dividing by the spiking level for the sample used.

After 10 accountability values had been determined for PCBs and DDE (2 values at each of the 5 spiking levels), these values were averaged and the resulting mean percent control samples were analyzed initially. Thereafter, for each matrix, the accountability factor was used to correct the values for PCBs and DDE in those assay runs used to generate the accountability factors. Each compound has its own set of accountability values and these values must lie between 60 and 120% to be acceptable. In succeeding assay runs, the spike alternated between PCBs and DDE, and rotated among the 5 spiking levels for each compound. As each new accountability value was determined, it replaced a previously determined value for that compound and spiking level. Accountability values were monitored to ensure that accountability factors were not dependent on spiking levels. The values for control samples, quality assurance blind samples, and reagent blanks also helped determine if the assay run was valid. No information is given in the papers reporting health effects on whether the impact of these manipulations was examined.

SUMMARY OF METHODOLOGICAL PROBLEMS OF THE FOUR COHORTS

The Michigan Studies

The Michigan studies consisted of a group of infants of which 242 were considered exposed because their mothers recalled eating Lake Michigan fish for many years in the past and 71 were considered not to be exposed because their mothers denied eating Lake Michigan fish. The PCBs were measured by the Michigan Department of Public Health in some samples of cord serum, serum from the mothers and in the mothers' milk.

Biomarkers

The PCB measurement information is published in two papers (Schwartz et al., 1983; Jacobson et al., 1984). An attempt was made to measure PCBs in 190 maternal and 198 cord sera of the 313 individuals (fish and non-fisheaters). In addition, milk from 138 nursing mothers who agreed to provide a milk sample were also analyzed. If a sample had been contributed from each subject there should have been a total of 313 samples for the sera and 138 for the milk samples since only 61% of the mothers nursed and 70.1% of those contributed a milk sample during the neonatal period. Of these 45 contributed a second milk sample. It is not clear in the various papers which of these milk samples came from mothers who ate Lake Michigan fish.

Two standards were used for the quantitation of the PCBs: Aroclor 1016 and Aroclor 1260. Aroclor 1016 was only quantitated in 6/190 maternal sera. PCBs were non-detectable or non-quantifiable in the other sera. Aroclor 1260 could only be quantified in 152/196 sera (152/313 participants). (Non-quantifiable refers to detectable levels below the quantitation limits of 5 ng/mL (ppb) for Aroclor 1016 and 3 ng/mL (ppb) for Aroclor 1260.)

Aroclor 1016 could only be quantified in 1/195 cord serum samples. Aroclor 1260 was quantified in 64/198 cord samples that were analyzed. Thus, for only 64 of the 313 infants could PCBs be determined in cord serum.

Of 138 milk samples analyzed, Aroclor 1016 was quantifiable in 42 samples during the neonatal period. Aroclor 1260 was quantifiable in 136/138 samples. For the 45 milk samples collected several months later, Aroclor 1016 was quantifiable in 12/45 samples and Aroclor 1260 in 41/45 samples.

The mean maternal serum PCB concentrations were reportedly 5.5 ppb for Aroclor 1260 and 1.6 ppb for Aroclor 1016. These means appear to be close to or below the limit of detection. The cord serum levels were lower since lipid levels are lower in cord blood. The

PCB serum levels are within the range of levels in the general population, particularly during the late 1970s and early 1980s when the samples were obtained. Thus, in the summary and evaluation of the reported correlations of adverse effects with PCBs in this group of children, the poorly defined exposure to PCBs is a major concern.

The Study Population

The assembled group of fisheating and non-fisheating mothers does not represent a random sample. The participating women came from 4 different hospitals in the area. Over 8,000 women were interviewed to arrive at a study group of 313 participants. It is unclear whether each hospital contributed the same number of participants, whether the hospitals were of equal size, served the same socio-economic groups or whether the refusal rate from each hospital was the same. About a third of those eligible for the study refused to participate. Eligibility for the study was based on fish consumption of a certain amount of fish in the past or present for the fisheating participants. This information was based on recall from the previous 6 years. In addition to the accuracy problems of recalling food consumption from the past a recall bias may have been introduced. When the children were 4 years old a subset of the mothers were asked again to detail their fish consumption. The correlation with the data collected 4 years prior was poor. Also, if fisheating was to be tested it is unclear why the number of fisheaters and non-fisheaters were not the same. As it turned out the PCB levels in fisheaters and non-fisheaters were actually similar and the hypothesis that fish contributed much to exposure was not proven. However, this piece of information is not explicitly explained in any of the papers. The two groups of children are simply combined.

In the majority of papers published on the Michigan group, the study methods are not well explained and important distribution data are rarely provided. It would be useful to know what the range of PCB levels in the different biological samples were for the non-fisheaters and the fisheaters, and how they compare. It would also be useful to know whether the mothers with the higher PCB levels were also the mothers who consumed alcohol, caffeine, smoked, and were heavier and older. These factors would affect PCB serum and milk levels.

The PCB milk levels were determined in two grab samples of a subset of the women and the type of milk sample was not the same for each woman. Since the levels in milk change over time and during a feeding period these grab samples are not representative of PCB levels in milk. The milk PCB levels can, therefore, not be used as surrogates for in utero PCB exposure. The authors fail to explain how they handled mothers who did not nurse or did not contribute milk samples.

Neurobehavioral Tests and Evaluation of Results

Most results from the different behavioral tests appear to be within the norm of the general pediatric population. However, many tests have not been validated or the investigators have modified them to meet their needs but their validity was not established in a larger, randomly selected pediatric population. Different examiners seem to have introduced inconsistencies when testing the children. An attempt was made to control for these inconsistencies. However, since these tests were performed over a period of about 18 months (time period of enrollment of new infants), it is unclear what the drift over time might have been. Exactly how the authors adjusted their data was not explained.

The statistical analyses in all the Jacobson publications were poorly explained; so poorly explained that it was difficult to determine what actually had been done. Various positive or negative correlations are reported for a variety of endpoints. Fish consumption and PCB serum or milk levels are not well correlated. It appears that fish consumption did not contribute much to PCB body burden. Furthermore, these correlations seem to be inconsistent, sometimes they are with fish consumption, sometimes with cord serum or human milk. Since these statistically significant differences do not support each other and since many endpoints were compared, the most logical explanation would be that they occurred by chance or that other unidentified factors were responsible. This could be elucidated further if distributions for the different parameters could be made available and if it could be determined whether there was a small subset in the larger study group that skewed the data and was responsible for the results. Furthermore, the participants differed substantially from the nonparticipants.

Since the differences in PCB levels are very small between different mothers and since in many mothers the PCB levels in maternal serum and in cord serum could not be detected or quantified, it does not make sense that PCBs would be responsible for the observed effects. It needs to be examined in more detail and in a more objective manner if other factors in this study population were causally associated with the reported subtle adverse effects. It was not determined if mothers who were older had higher PCB levels, were more likely to drink, smoke, and eat fish, actually represented a subset that was different from the rest of the study population. In some of the papers it appears that premature infants were included in the data. Again this would represent a different population that should not have been included.

Statistics

Although many of the comparisons were statistically significantly different, they often did not explain much of the variance nor were the statistical manipulations always appropriate. Mostly the authors examined straight line relationships and presented r*[1] as a measure of the strength of the straight-line relationship. However, the authors should have given R^2 to inform the reader of the percent of variance. Furthermore, the various authors performed in most of their studies many statistical analyses. With the high number of statistical tests chance alone would dictate that many statistically significant differences would be found even if no "true" differences existed.

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[1]*To quantify the strength of a linear relationship between X and Y, the first consideration would be the predictor of Y if X were not to use at all. The best predictor in this case would be the sample mean of the Ys, $\bar{Y}$. The sum of squares of deviations associated with the naive predictor of $\bar{Y}$ would be $SSY = \Sigma (Y_i-\bar{Y})^2$.

If variable X is of any value in predicting the variable Y, the residual sum of squares given by $SSY = \Sigma (Y_i-Y_{i,estimated})^2$ should be considerably less than SSY. If this is the case, the least squares model $Y_{estimated} = \beta_0+\beta_1X$ fits the data better than the horizontal line $Y_{estimated} = \bar{Y}$. A quantitative measure of the improvement in the fit obtained by using X is given by the square of the sample correlation coefficient. R^2 measures the strength of the linear relationship between X and Y in the sense that it gives the proportionate reduction in the sum of squares of vertical deviations obtained using the least-squares line $Y_{estimated} = \beta_0+\beta_1X$ relative to the naive model $Y_{estimated} = \bar{Y}$, the predictor of Y if X is ignored. The larger the value of R^2, the greater is the reduction in SSE relative to $\Sigma (Y_i-\bar{Y})^2$, and the stronger is the linear relationship between X and Y.

When assessing the strength of the linear association between X and Y, R^2 rather than r should be considered. For example when r = .3 R^2 = .09 that is only 9% of the variation in Y has been explained with the help of X. For the readers information, in this summary review the R^2 was calculated from the r values given in different papers. R^2 is not a measure of the magnitude of the slope of the regression line nor is R^2 a measure of the appropriateness of the straight-line model.

The North Carolina Studies

The reliability and validity of any epidemiological study is in large part dependent on the ability to accurately measure both the exposure and the disease or outcome of interest. In the North Carolina Cohort publications both the exposure and outcome measures are difficult to quantify in the best of circumstances. The authors fail to adequately discuss the methods used to quantify PCBs. While it is clearly beyond the scope of this type of publication to go into great detail regarding PCB measurement the authors should have given some information on quantitation and the difficulty with measuring PCBs.

The authors oly provided scanty information about how, where or when the human milk samples were taken. There are many important factors involved in collecting milk for the purposes of measuring lipid soluble compounds. Milk changes during the first weeks after delivery from colostrum to transitional milk during the first 2 weeks and to the milk excreted thereafter, with different concentration of lipids and PCBs. The lipid content also changes during the course of a feeding with the milk in the later part of the feeding having a higher lipid content. The proper way to collect milk is to completely empty one breast (by pumping) at each feeding for a 24-hour period no sooner than 2 weeks postpartum and to take a sample.

The outcomes of interest, the Brazelton Neonatal Behavioral Assessment, the Bayley's Scale and the McCarthy Intelligence Scale are all subjective measures of development and intelligence. The testing was done at three different sites by different testers over a long period of time. The authors do not disclose the number of testers for any of the testing. Because of the subjective nature of these tests they are at considerable risk of inter and intra-tester reliability problems. The information provided in the publications do not allow the reader to adequately assess whether the authors have properly addressed these issues within the context of the study methodology. Additionally, the differences in these scores detected among the children, irrespective of PCB exposure, are so small that it is very difficult to attribute any biological effect from any exposure, particularly one so difficult and so imprecisely measured as PCBs.

The biological significance of the reported findings are difficult to assess because of measurement problems with the independent and dependent variables and because of the small differences detected in the outcome measures. The differences detected in test scores can often be found in repeat testing, within a day or two, of the same child.

Finally, in addition to the above mentioned problems, the presentation of their data and the statistical methods utilized by the authors are confusing and in some cases inappropriate. The

over-manipulation of the PCB measurements to alleviate the problem of too many non-detectable measures is highly questionable and calls into question the overall reliability and validity of the study results.

The Yu-Cheng Studies

Yu-Cheng in Taiwan resulted from the ingestion of rice oil contaminated with chlorinated dibenzofurans, PCBs, and other chlorinated compounds. The toxic effects observed in the exposed populations were caused by chlorinated dibenzofurans. Thus, this population had different exposures than the Michigan, North Carolina or Netherlands children that have been studied. The PCB and PCDF body burdens in the Yu-Cheng children were much higher than those of the other groups. Many Yu-Cheng children had signs of intoxication: hyperpigmentation, chloracne like lesions, deformed nails, problems with dentition and dark pigmentation of the gums. The Taiwan health authorities established a registry of all people that either knew they had been exposed to the contaminated oil or had a PCB level above 10 ppb. Not everybody in the registry had symptoms and signs of poisoning nor were the PCBs necessarily a good surrogate for PCDF body burdens on a quantitative basis. Some children of comparison groups that were established occasionally also had the same symptoms and signs just not as frequently. Thus, some misclassification undoubtedly occurred. According to some accounts about 10% of the members of the registry had no clinical signs of poisoning. The levels of PCBs and PCDFs in blood varied a lot and initially PCDFs were not measured. The degree of exposure in many cases is therefore uncertain.

Apparently the first patients presented with illness in December of 1978. The assumption was made that it took about 6 months of ingestion of oil before signs and symptoms of illness occurred. In some studies children born between June and December of 1978 were assumed to have had in utero exposure and were included in the studies. However, the children born in June and July may have had little if any in utero exposure. Data from the plant supplying the oil such as dates and contaminated oil samples could not be found when the plant was inspected. However, high concentrations of PCBs were identified in the soil of the plant.

Recently Guo et al. (1996) presented data that showed that PCB blood levels measured in 61 of 69 mothers of these infants in 1980 - 1981 were fairly well correlated with PCB levels measured in 1992, R^2 = 0.57. On the other hand, the levels of 2,3,4,7,8 pentachlorodibenzofuran and 1,2,3,4,7,8-hexachloro-dibenzofuran showed a very poor correlation with the PCB levels measured in 1980 - 1981 with an R^2 of 0.013 and 0.010, respectively. Additional work is ongoing to elucidate this further.

Neurobehavioral Tests

Since many of the children had signs of poisoning, observer bias can not be ruled out. One of the tests used was the Rutter test. The Rutter test was a test developed in England. It is a questionnaire that was filled out by the parents and is therefore very subjective. Since the parents most likely assumed that something was wrong with the exposed children this would have had an impact on their evaluation. The test was developed for older children and not validated for young children, but in Taiwan it was given to children three years and older. Higher scores on the Rutter test suggest more problems. It should be noted that the results for the Yu-Cheng group and the comparison group were higher than for the children tested in England. Similarly, the evaluation by teachers and interviewers may have been influenced by preconceived notions.

The IQ tests were administered to groups of exposed and matched unexposed children. The matched children were usually children from the neighborhood that were identified by the parents of the Yu-Cheng children. Results varied over the years. In some years the differences were more pronounced than in other years. The results were not necessarily related to clinical signs of poisoning or PCDF body burdens.

PCDF Determinations

In the various studies reporting PCDF levels the limits of detection of the method is usually not given. In some cases although the children still have signs of PCDF poisoning many years after exposure, PCDFs were not detected. However, that may be a function of the limit of detection and inadequate sample collection (insufficient material). In the studies in the United States these chemicals are measured in serum, while in Japan and Taiwan they have been measured in whole blood. The preponderance of these chemicals is present in serum and not in red blood cells. Using whole blood would dilute the amount of chemical measured at least by a factor of 2 and recovery of the chemicals from the whole blood would also not be as efficient. In conclusion, the exposure in this population has not been well quantified because adequate analytical methods were not available at the time the outbreak occurred.

The Netherlands Studies

In these studies many parameters are better controlled than they were in the Michigan or North Carolina studies. However, this is also not a randomly selected group. The children were enrolled from two hospitals, one in Groningen and one in Rotterdam. There seem to be sufficient differences between the children from Groningen and Rotterdam to beg the question whether these two groups can legitimately be combined. The refusal rate and the

reasons for refusal are not given. The studies also differ from the other studies since for many of their correlations the authors use TEQ values which were converted from PCB serum levels and PCDD milk levels.

The toxicokinetics of different congeners of PCBs and PCDDs vary as does the distribution of these chemicals among different tissue compartments. The quantitation of some congeners is better than of others, adding additional variability. This would also be influenced by the amount of congener present in the different body compartments. Finally, for each mother analytical results were not available for all specimens and it is not stated how non-detectable values were treated.

Combinations of PCBs, PCDFs and PCDDs are not necessarily additive in their toxic effects but may be antagonistic and the data base on which the TEQ values are based is very limited. Therefore, the present TEQ system is very uncertain in its prediction of human health effects. Regulatory agencies who developed this system for convenience, have viewed the approach as an interim method. It is also important to recognize that the TEF/TEQ approach are order of magnitude estimates of relative potency. At present, toxicokinetics in different species are not considered. However, when comparing responses in different tissues or when comparing across different endpoints the TEF values rarely vary by more than a factor of 10 when they incorporate pharmocokinetic factors (Birnbaum and DeVito. *Toxicology*. 1995;105:391-401). The potency of the mono-ortho coplanar PCBs (105, 114, 118, 123, 156, 157, 167, 189) is 5-6 orders of magnitude lower than that of TCDD (Ahlborg et al. *European J Pharmacol*. 1992;228:179-199). Although the authors of the studies conducted in the Netherlands seem to have used the most recent TEQ values (Ahlborg et al. *Chemosphere*. 1994;28:1049-1067), the TEF values appear to be higher for the mono-ortho PCBs than the most recent recommendations (Birnbaum and DeVito. *Toxicology*. 1995;105:391-401). It is difficult to determine this since no ranges or means are given for the chlorinated organic compounds but it appears that the observed differences in the children in the present studies are based on less than one order of magnitude of TEQ values. If groups are constructed where the difference of exposure is less than one order of magnitude, any positive or negative correlations would not be meaningful because of the uncertainty inherent in the TEQ values.

Again distributions of the data are not clearly presented. There were apparently 24 children with clinical problems and there were 23 children with "hypotonia". Where these the same children? This information is not provided. The authors used mostly different methods than were used in the United States or in Taiwan to test the children. However, their tests suffer from similar ambiguity and subjectivity. Furthermore, differences between Rotterdam and Groningen were introduced by the evaluators. Alcohol consumption data and evaluation of the neurological behavior was collected

differently in the two towns. It is not stated in any of the papers, whether hypotonia and the other adverse findings were more prevalent in one or the other town. The authors also do not present any data where they compared the two towns to see whether they had statistical significant differences between the two groups unrelated to their TEQ values. It is also unclear how well they were able to control for confounders because of the variability that was introduced by the evaluators in the two towns.

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REVIEW OF INDIVIDUAL PAPERS

Introduction

In this section the individual papers are summarized. With the exception of the North Carolina study reviews most of the data presented in the summaries are taken verbatim out of the papers. Comments within the body of these summaries by the reviewers are given in parentheses. The summaries are followed by a critical review of the major methodological problems of each paper. In the North Carolina reviews, the authors' statements are in quotes and the reviewers specific comments follow these statements.

The intent of this entire review is to determine whether the research done so far is sufficiently robust to support claims that PCBs cause developmental and neurobehavioral effect in in utero exposed infants and children. Recently, Schantz (1996a) reviewed these same data. She pointed out that there were inconsistencies and methodological problems in a number of these studies. A number of experts in the study of PCBs or epidemiology, or both then commented on her paper. Most of these authors praised Dr. Schantz's review. Some felt that she had not gone far enough in her criticisms and pointed to additional problems. Most authors also recommended additional studies. However, none of the authors entertained the question whether such research can be done given the low level exposure to PCBs of potential study populations, their exposure to other pollutants and life style effects, and genetic variations. Furthermore, the range of environmental exposure is too small to identify a sufficiently highly exposed group for study.

Neurobehavioral or neurotoxic effects have not been reported in PCB exposed workers with much higher exposures in contrast to lead. Putting neurotoxicity aside, a dose or level of exposure that would cause acute or chronic toxicity of PCBs in humans has also not been convincingly demonstrated. Thus, not much evidence exists in humans that would support the notion that neurobehavioral effects following low level environmental exposure should be expected in children.

Synopses of Papers and Comments on Individual Papers

1. Fein G.G., Jacobson J.L., Jacobson S.W., Schwarz P. Intrauterine exposure of humans to PCBs: newborn effects. Environmental Protection Agency, EPA-600/3-84-060, 88 pp. May 1984.

The infant sample consisted of 164 males (52%) and 149 females (48%). All but two infants were white. Only one infant was below the tenth percentile on weight-for-gestational age. One severely premature newborn (GA = 26.9 weeks, birth weight = 909 gm) died.

Characteristics of the Sample at Birth (N = 313)

	Mean(SD)	Range
Infant characteristics		
Birth weight (gm)	3502(560.3)	909.1 - 5028.9
Gestation age (weeks)	40.4 (3.0)	24.3 - 45.9

95.8% began prenatal medical care by the fourth month of pregnancy, and the remaining 13 women by the sixth month. Among the women 77% reported consuming moderate to large quantities of Lake Michigan fish, while 23% reported consuming none. Women were asked if in the past (dating back to 1966) they ate greater amounts of Lake Michigan fish (e.g., the woman grew up in a family in which the father was a sports fisherman). If so, the amount and kind of fish consumed in the past, the number of years of consumption at that rate were recorded.

The following equation was used to estimate the mother's annual rate of consumption for each type of fish:

RELATIVE PCB FISH WEIGHT		NUMBER OF FISH MEALS/YEAR		0.23 KG (1 FISH MEAL)	=	PCB-KG PER YEAR
	X		X			

This measure is summed across species for each individual to determine annual fish consumption. To estimate cumulative exposure, annual PCB-fish consumption was multiplied by the years the mother reported eating contaminated fish at that rate. If she reported eating contaminated fish at a higher rate in the past, the number of years at the current and past rates were determined separately and then multiplied by their consumption rates as follows:

PCB PRESENT KG/ YEAR		NUMBER OF YEARS AT PRESENT RATE	+	PCB PAST KG/ YEAR	X	NUMBER OF YEARS AT PAST RATE	=	CUMULATIVE CONSUMPTION PCB-KG
	X							

Sampling Criteria: Exposed and Unexposed

Two inclusion criteria were used: (1) If reported cumulative exposure within a 6-year period was equal to 11.8 kg (26 pounds) or more of contaminated fish, the mother qualified. This criterion was based on evidence that PCBs are retained in body tissues over relatively long periods of time. A control group of newly delivered mothers who reported consuming no Lake Michigan fish was also selected.

Of the 8,482 women screened (i.e. 96 percent of all maternity patients in the four participating hospitals), about 4 percent or 343 ate PCB fish in sufficient quantities to qualify for inclusion in the exposed group. About 29% of the women screened reported eating *no* Lake Michigan fish, 4.6% or 114 of these women were invited into the study. (It was not explained how they were chosen.)

Of the 457 women invited to participate, overall 144 (32%) refused (a very high refusal rate; non-fisheaters 37.7%, fisheaters 29%). It is unclear what biases were introduced in the overall selection process.

Mean annual rate of PCB-fish consumption at the highest levels reported by the exposed mothers was 6.7 PCB-kg (14.7 PCB-pounds) per year (SD = 5.8 PCB-kg, range = 1.2-41.7). This rate is equivalent to about 0.6 PCB-kg per month or 2-3 salmon or lake trout meals (at 0.2 kg per meal). (It is not stated what the lower levels were.)

This selection procedure yielded a range of exposure levels. Their values depended on the particular measure of maternal fish consumption used. The fish consumption variables were positively skewed and were normalized by means of log transformations. One woman who reported eating 54.5 kg of contaminated fish in one year, was eliminated from the analyses.

Hospital personnel collected umbilical cord blood for every infant born in the participating hospitals at delivery. Cord blood specimens for newborns in the sample were thus available for laboratory analysis. Samples of maternal blood were collected on the second day following delivery. Sixty-one percent of the infants in the sample were breastfed, and of those, 70.3 percent of the mothers provided human milk samples between 1-16 weeks after delivery. A second milk sample was collected from 45 women who continued breastfeeding at 5 months.

In serum limits of detection were 5 ng/mL for Aroclor 1016 and 3 ng/mL for Aroclor 1260. All serum samples containing lipid levels below 200 mg/dL were omitted from the chemical analyses, since measurement techniques were judged to be insufficiently sensitive to detect PCB levels at lipid values of this magnitude. The

removal of three maternal sera and 43 cord sera did not alter the fundamental nature of the PCB distribution. Twenty-seven additional cord blood samples could not be analyzed for various reasons. Included in the PCB analyses were 198 cord and 196 maternal sera. The Ballard examination for fetal maturity was administered to 220 (66%) infants at 20-53 hours of age. The remaining 93 infants were not tested because maternal consent could not be obtained.

The NBAS (Neonatal Behavioral Assessment Scale) was administered to 287 (92%) newborns. Testing was performed on the third day after birth (i.e., at 48-72 hours). The testing of one premature was delayed until the infant reached the conceptual age of 37 weeks, the earliest age at which the NBAS is considered valid. The 44 NBAS items were reduced to seven summary clusters. The clusters generally resemble the seven clusters suggested by Lester, Als, and Brazelton.

To ensure sufficient variance in the case of dichotomous variables, such as marital status (married/unmarried) or parity (first born/later born), only variables were included for which incidence in each category exceeded 15%. Given this criterion, it was possible to assess the effects of 36 potential confounds.

The majority of the PCB levels matched to the 1016 standard were below quantification limits. Only one of 195 cord and five of 190 maternal sera were quantifiable. For many cord sera PCBs were also not quantifiable using the 1260 standard. Mean PCB levels in maternal sera quantitated as Aroclor 1260 were higher than in cord sera. Because human milk lipids fluctuate considerably, the same consistency does not appear for milk-values derived by the two methods. In the analyses of breast milk, measures that take lipid fluctuation into account are likely to be more reliable.

No relationship was found between cord serum PCB levels and maternal fish consumption, possibly because of the detection problems in cord serum analysis. A positive correlation was found between PCB levels in maternal serum and fish consumption. Mean PCB level in maternal serum was 6.1 ng/mL (SD = 3.7) among women who ate contaminated fish, and 4.1 ng/mL (SD = 2.7) among abstainers ($t = 3.83$, $p<0.0001$).

Maternal PCB-fish consumption was positively correlated with caffeine and alcohol consumption before and during pregnancy. Cord serum PCB level was positively associated with alcohol and caffeine consumption during pregnancy and maternal age. It was negatively associated with weight-gain during pregnancy ($p<0.05$-0.01). Birth weight and head circumference are significantly and negatively correlated with maternal PCB-fish consumption. Higher contaminated fish consumption predicted smaller birth size.

To examine dose-response relationships, PCB-fish consumption was divided into four categories. Mothers who reported having eaten Lake Michigan fish were divided into three approximately equal-sized groups: (a) high exposure (greater than 6.5 kg per year); (b) moderate exposure (3.5-6.5 kg); and (c) low exposure (1.9-3.4 kg). The fourth group consisted of mothers who did not eat Lake Michigan fish. Birth weight decreased in a dose-related fashion (the groups were about equal: 78, 82 and 81, respectively). Among the 4 groups the differences were very small, range for head circumference for most infants about 34.5 - 35.4 cm, birth weight for most infants somewhat below 3300 to about 3650 gram). Even after adjusting for potential confounds, the birth weight of the most highly exposed infants averaged 245 g less than that of the unexposed controls. (Since the birth weights and head circumferences of the term infants are quite close and within the normal range of birth weights of term infants the differences are more likely determined by the genetic make up of the parents and the prepregnancy weight of the mother and not necessarily by their fisheating habits. Furthermore, fisheating has a beneficial effect on the size of infants (Olsen et al. *Lancet*. 1986;2:367-369; Olsen et al. *Int J Epidem*. 1990;19:971-977).

Contaminated fish consumption predicts a shorter gestational period on both the last menstrual period measure and the Ballard examination. Gestational age decreases in a dose-related fashion, with the most highly exposed infants averaging gestational periods that are 6.3 days shorter than the nonexposed controls on both gestational age measures. (No ranges are given.)

With gestational age based on last menstrual period statistically controlled, fish consumption continues to predict lower birth weight and smaller head circumference, indicating that the affected infants are smaller-for-date. Fish consumption also predicts lower Ballard scores when birth size is held constant, F (1, 203) = 4.96, $p<0.05$, suggesting that the exposed infants' poorer Ballard scores are not attributable to their smaller size. (Thirty-nine percent of the infants were not tested. It was not stated how many were among the fisheating group.)

Two variables, maternal prepregnancy weight and sex of the infant, were found to function as suppressor variables in the present sample due to a tendency for cord PCB levels to be higher in the offspring of heavier mothers and in male infants. (It should be noted, see above, that fisheating was negatively correlated to higher prepregnancy weight. This was not statistically significantly different.) Since male infants tend to be larger than female infants (t (192) = 2.34, $p<0.025$, and t (192) = 5.31 $p<0.001$, for birth weight and head circumference, respectively, these variables initially masked the effect of cord PCB level on birth size. With maternal prepregnancy weight, sex of infant, and four potential confounding variables statistically controlled, cord serum PCB level predicts lower birth weight, smaller head

circumference, and a shorter period of gestation based on reported last menstrual period (Table 16 of the paper, only beta and F values are given in this table). The head circumference of the more highly exposed infants averages 0.3 cm less, and they are born on an average of 13.3 days earlier.

PCB-fish consumption predicts a linear combination of five NBAS clusters, F (5, 193) = 3.18, $p<0.01$, with a multiple correlation coefficient (R) of .28. The strongest relationships are with autonomic maturity, reflexes, and range of state. Each item in the autonomic and range of state clusters was examined in a separate regression analysis. PCB-fish consumption predicts two of the three autonomic items: motor maturity, F (1, 280) = 6.93, $p<0.01$; and amount of startle, F (1, 280) = 10.07, $p<0.005$; and one of the four range of state items: lability of states, F (1, 279) = 14.46, $p<0.001$. Motor immaturity (Scale points 1, 2, and 3) is defined as jerky, unbalanced, cogwheel-like movement in which flexor and extensor muscles seem to be competing. Of the highly exposed infants 11% are classified as immature and only 3% of the nonexposed controls. Fewer than six state changes during the NBAS examination indicate deficient lability of state (Als et al., 1979). Given this criterion, 42% of the highly exposed infants are hyporesponsive and only 17% of the nonexposed controls.

PCB-fish consumption is related to only one of these subgroups: reflexes associated with the mouth, F (1, 281) = 7.22, $p<0.01$. A second analysis differentiated reflexes which are abnormally strong or hyperactive from those which are abnormally weak or difficult to elicit. PCB-fish consumption is significantly related to a number of abnormally weak reflexes, F (1, 281) = 9.63, $p<0.005$.

The "worrisome" category describes a behavioral pattern that is sufficiently deviant to warrant concern. The Als et al. criteria for classifying each of the NBAS items were applied to the seven clusters used in the present study. Maternal fish consumption is highest among infants classified as worrisome on the three clusters associated with PCB exposure. On the range of state cluster, where two categories of worrisome behavior are defined, exposure is highest among the infants classified as flat or depressed.

Since small birth size and shortened gestation may be associated with neonatal behavioral deficits, it is possible that the behavioral effects are not consequences of exposure to PCB but by-products of PCB effects on birth size and gestational age. Partial correlation analyses indicate that the behavioral effects of contaminated fish consumption are not predicted by either birth size or gestational age based on last menstrual period. Controlling for the size and gestational age of the newborn does not appreciably alter the relation of contaminated fish consumption to NBAS performance.

The pattern is less clear for the Ballard examination. Although controlling for the Ballard has little effect on the relation of contaminated fish consumption with lability of states or reflexes, the effect on autonomic maturity is substantially reduced when the Ballard is held constant. Nevertheless, it does not seem appropriate to conclude that this effect is mediated by shortened gestation. (No reason is given by the authors why this is not appropriate.)

Evidence that the physical and behavioral effects of PCBs are independent suggests that some affected infants are small while others exhibit only behavioral deficits. The total impact of PCB exposure in the present sample can be estimated by examining the relation between adjusted fish consumption and a linear combination of birth size, gestational age, and behavioral variables. The multiple correlation between fish consumption and neonatal outcome emerging from this analysis is .34, F (7, 152) = 2.80, $p<0.01$.

An attempt was made to corroborate the behavioral effects associated with maternal PCB-fish consumption in analyses based on cord serum PCBs. Serum PCB level (adjusted for its four potential confounds) is not significantly correlated either with a linear combination of six NBAS clusters, F (5, 118) = 1.13, or with any of the individual clusters.

Other potential confounds, such as socioeconomic status, maternal smoking, parity, obstetrical complications, and exposure to PBBs are unrelated to maternal fish consumption and cord serum PCB levels. These variables, therefore, cannot account for the adverse neonatal outcomes associated with PCB exposure. With relevant confounding variables controlled, maternal fish consumption predicts adverse outcomes in three areas: birth size, gestational age, and neonatal behavior.

Intrauterine exposure to PCBs is most clearly associated with reduced birth size and a shorter period of gestation. The most highly exposed infants in this sample are about 200-250 g lighter than the nonexposed controls, whether exposure is measured by maternal fish consumption or cord serum PCB levels. Similar effects hold for head circumference and gestational age based on reported last menstrual period. All three measures decrease in a dose-related fashion with increasing levels of PCB exposure.

The birth weight effect found here is of the same order of magnitude reported by the Surgeon General (1979) for smoking during pregnancy; the infants of smokers are on the average 200 g lighter than those of nonsmokers.

The association of contaminated fish consumption with neonatal behavioral deficits is not corroborated by the cord serum measure of PCB exposure. This discrepancy may be due, in part, to the poor reliability of the cord serum measure. Cord samples were available

for only 48.7% of infants with the highest fish consumption exposure levels (>10 kg/yr.), compared with 65.3% for the remainder of the sample.

Critical Comments

Generally this study suffers from a poorly defined fisheating cohort, many missing values, poor data analysis and interpretation. Furthermore, the determination of exposure is flawed. In addition some of the numbers do not seem to add up.

The population of infants contained at least one if not more premature infants based on the range of weeks of gestation and birth weight. These infants should not have been included, since their behavioral tests would be different because of their immaturity. Only for one child was the test performed later because of immaturity.

The PCB exposure based on fish consumption data may not really represent the PCB body burden the women accumulated. Fish in the "past" 1970-1975? would have had higher levels, the women would have excreted a certain proportion of those PCBs. The weighting of the fish was arbitrary. The amount of the PCB in the fish depends on their size and the site where they were caught. A fraction of the PCBs is lost when the fish are cooked, the extent depending on the method of cooking. Also over time the PCB levels in fish in lake Michigan have decreased. The weights used for the PCBs in different fish species are not given anywhere, nor are they referenced. It is simply stated that "Dr. Swain of the EPA? helped in calculating the amount of PCB in the fish."

There was a 32% overall refusal rate when mothers were first enrolled. This may have introduced a selection bias. Furthermore, it is highly unusual to have less than a third controls (242 versus 71). If the 61% of breastfed infants pertains to all 313 infants then 190 infants were breastfed. Since only 70% of the 191 mothers supplied a milk sample, the total number of analyzed milk samples was 133 or 42% of the cohort. The authors state that 138 breast milk samples were analyzed. The discrepancies between these numbers may be the result of errors introduced by using or not using numbers after decimal points or they may be an indication of insufficient clean up of data entry errors by the investigators.

The milk samples were collected between 1 and 16 weeks of nursing. This presents an additional problem. The human milk secretion consists of colostrum during the first days after delivery, followed by transitional milk until about 2 weeks after delivery. Then mature milk is secreted. Colostrum is low in fat. Fat soluble contaminants, however, calculated on a fat basis are higher in colostrum than in mature milk. Because of these variations mature milk is the better choice for monitoring purposes. In addition, the timing of when the sample is taken during a feeding

period is important because levels of contaminants may decrease during a given feeding. The concentration of fat in milk from individual donors during a single feeding, during the day and between days fluctuates. Because of these physiological variations the PCB level and levels of other chlorinated organic chemicals can fluctuate during one feeding of up to a factor of five (Mes and Davies. *Chemosphere*. 1978;9:699; Wilson et al. *American J Dis Child*. 1973;125:814; Barnett et al. *Pest Monit J*. 1979;13:47; Focardi et al. *Riv Ital Ped*. 1984;10:286). For all of these reasons the predictive value of human milk levels of PCBs for human body burdens is limited unless rigorous (breast pump, totally emptying the breast and then taking a subsample and feeding the rest of the milk to the infant by bottle) and frequent sampling procedures are used. Even then this method of indirect body burden estimation lacks precision and can not be relied upon for small differences. For example, differences of one order of magnitude of PCB levels in a milk sample in the ppb or low ppm range (e.g. 1- 10 ppb or 100 ppb - 1 ppm) collected at one point in time could be indicative of the same or of different body burdens and levels of exposure. Based on one or two milk samples/person it would not be possible to make such a distinction.

To quantitate the PCB body burdens by calculating the amount of fish intake and weighting it by using mean? PCB levels in different fish species also introduces a great deal of uncertainty since the levels of PCBs in different fish of the same species vary depending on where the fish were caught and also what size they were. These variations can easily span over two orders of magnitude.

Although correlations between maternal and cord serum PCB levels were statistically significant with $r = 0.41$, $R^2 = .17$ and $r = 0.43$, $R^2 = .18$, since these samples are basically from the same persons these correlations are very poor. This may be a reflection of the variation in the analytical method if these samples were analyzed at different times. The correlations between quantitation on a fat basis and on a whole serum basis for either cord serum or maternal serum ($r = 0.93$, $R^2 = .87$) was high. Having a high correlation here is a given since only one analysis was done per sample and it is unclear why these calculations were even done.

If cord serum samples were available from all 313 infants and 43 samples were discarded because the lipid levels were below 200 mg/dL and 27 samples were discarded because they were inadequate then there must be some additional samples that were not analyzed since 313-43 = 270, 270-27 = 243 but not 198. It was stated in this document that 198 cord serum samples were actually analyzed. However, subtraction of 43 samples from the 242 infants of fisheating mothers would result in 199 cord samples while further deducting 27 blood samples would result in a total of 172 blood samples. It is really not clear how many cord samples of the control and the fisheating population were actually analyzed. This

very fundamental question would have to be addressed to interpret the data. It would also be important to know how many of the non fisheating mothers and infants had measurable levels of PCBs and what their ranges were in comparison to the fisheating population. The controls should also have been analyzed separately to determine what the correlation for the different endpoints was to PCB. It should have been determined whether women with smaller infants were the women who smoked, drank alcohol and consumed caffeine, and were perhaps also the older women. They may actually constitute a separate "risk-taking" population and would be different from the rest of the cohort. The authors did not explore whether major risk factors affected PCB levels in serum.

The authors state: "To estimate cumulative exposure, annual PCB-fish consumption rate was multiplied by the number of years the mother reported eating contaminated fish at that rate. If she reported eating contaminated fish at a higher rate in the past, the number of years at the current and past rates were determined separately and then multiplied by their respective consumption rates to yield a measure of cumulative exposure." This determination of exposure does not consider the toxicokinetics of the PCBs and groups individuals with quite varied exposure. Since PCB determinations were not made on many of the individuals in this study it is unclear how their PCB exposure relates to their fish consumption.

The Ballard Examination for Fetal Maturity was administered to 220 (70%) of the infants at 20-53 hours of age. It is not stated what this sample represents and how many non-fisheaters were included. It would have to be determined whether the infants that weighed less were over- or underrepresented in this sample. From the ranges of the weights of the infants given and the gestational age based on the last menstrual period some infants were obviously premature and should not have been included in these behavioral studies. Since data are always presented as group data, these potential shortcomings can not be evaluated.

A difference of 0.3 cm in the size of the head circumference is so small that it should not be interpreted as an observed effect, particularly since the measurements of the head circumference were not standardized, are imprecise and are affected after birth by the degree of swelling present. There would be less swelling in infants who were born by C-section. Interpretation of differences in head circumference would have to consider such variables. This does not seem to have been done in this study. Also variability exists between different people performing these measurements and it is unclear how many different people performed them.

2. Fein G.G., Jacobson J.L., Jacobson S.W., Schwartz P.M., Dowler J.K. Prenatal exposure to polychlorinated biphenyls: effects on birth size and gestational age. *J Pediatr.* 1984;105:315-319.

Methods

Study participants were 242 infants born between July 1980 and December 1981 to women who reported moderate consumption of Lake Michigan fish, and 71 infants born during this period whose mothers ate no Lake Michigan fish.

Women reported the amount of each species of Lake Michigan fish they consumed during the prior year and, if they ingested greater amounts at some time in the past, they reported consumption rates for that period as well. A weighted value was assigned to each fish species consumed, based on average contaminant levels reported for that species in Lake Michigan. Overall contaminated fish consumption was defined as the weighted sum of annual Lake Michigan fish consumption in the present or past, whichever was greater. The exposed group was limited to women who had consumed at least 11.8 kg contaminated fish over 6 years.

Cord and maternal serum samples were analyzed at the Michigan Department of Public Health by packed-column gas chromatography. Quantitation was provided by adapting the Webb-McCall method to a computer data system with Aroclors 1016 and 1260 used as calibration standards. Virtually all samples matched to the Aroclor 1016 standard were below the laboratory's detection limit. Thus, values based on Aroclor 1260 were used for data analyses. Serum PCB values were available for 196 mothers. Cord serum PCB and PBB values were available for 241 infants. (Please note: in other papers it is stated that 198 cord sera were analyzed for PCBs and PCBs were quantified in 64 cord sera.)

Gestational age was based on the mother's report of her last menstrual period and on the Ballard Examination for Fetal Maturity. The Ballard examination was administered at 30 to 42 hours after birth to 209 infants (163 fisheating mothers, 46 controls).

A comprehensive list of 73 potential confounding variables included data pertaining to demographic background, health history, pregnancy and delivery, obstetrical medication. Only those variables were included for which incidence in each category exceeded 15%. Given this criterion, it was possible to assess the effects of 37 potential confounding variables. The groups were compared on each of the outcome measures using analysis of covariance.

Results

The 242 women who reported eating Lake Michigan fish consumed 6.7 ± 5.8 kg/yr (mean ± SD). Median consumption was 5.0 kg/yr. equivalent to about two salmon or lake trout meals per month (range 0 to 4). These women reported having eaten Lake Michigan fish for an average of 15.9 ± 9.1 years. During pregnancy their consumption rates declined to 4.1 ± 4.4 kg/yr.

Maternal serum PCB concentrations for the sample as a whole averaged 5.5 ± 3.7 ng/mL, which is comparable to those for other midwestern American samples. Cord serum PCB levels averaged 2.5 ± 1.9 ng/mL. Although maternal fish consumption did not predict cord serum PCB levels, fish consumption predicted maternal serum PCB level ($r = 0.37$, $p<0.001$), which in turn predicted cord PCB level ($r = 0.41$, $p<0.001$). (Note: for $r = 0.37$, $R^2 = 0.13$, for $r = 0.41$, $R^2 = 0.17$ not much of a prediction, even though it is statistically significantly different.)

About two-thirds of the mothers invited to participate in the study agreed to do so. The participants were somewhat better educated than the refusers, 13.3 years of school (P) versus 12.7 (R), and higher in socioeconomic status, 39.3 (P) versus 35.7 (R). Ninety percent of the infants designated at medical risk by hospital staff were among the offspring of fisheating women, and all but one of their mothers agreed to participate. There is no evidence, however, that infants at risk were systematically underrepresented among the nonexposed mothers who agreed to participate according to the authors.

If the fisheaters are compared to the non-fisheaters, the percent of spontaneous deliveries, alcohol intake prior to pregnancy and during pregnancy, coffee intake, and cold medications were statistically significantly higher among fisheaters. (Note: it is unclear whether these variables occurred in different or in the same women, and how these observations relate to "problem" children. Smoking was not mentioned in Table 1 of the paper.)

Overall contaminated fish consumption predicted smaller birth weight and head circumference, and a shorter gestational period as estimated by the Ballard examination. Infants born to women who had consumed contaminated fish averaged 190 gm less in birth weight, 0.6 cm less in head circumference, and 4.9 days less in gestational age. The lower Ballard scores were related primarily to greater neuromuscular immaturity among the exposed infants. All four outcomes were related to overall contaminated fish consumption in a dose-dependent fashion. Note that the difference in weight and head circumference varies in different papers. The difference

in weight is 190 g in another paper and the difference in head circumference is given as 0.3 cm. Most likely different numbers of children were included. These differences are mentioned here as well.

Although fish consumption during pregnancy was highly correlated with overall fish consumption ($r = 0.79$, $R^2 = 0.62$, $p<0.001$), consumption during pregnancy did not predict either birth size or gestational age and was related only to neuromuscular maturity (18.6 ± 24 exposed during pregnancy versus 19.3 ± 2.5, $p<0.05$).

Infants with cord serum PCB concentrations at or above the laboratory's detection limit averaged 160 gm less in birth weight, 0.7 cm less in head circumference, and 8.8 days less in gestational age. Head circumference was significantly smaller ($p<0.01$) among newborn infants with detectable cord PCB levels, even when birth weight and gestational age were statistically controlled.

Exposed infants were 160 to 190 gm lighter than controls, a magnitude comparable to smoking during pregnancy. In contrast to smoking, however, which primarily affects birth weight, head circumference was most directly affected by PCB exposure. (This last statement is not really explained in the paper.)

Smaller birth size was predicted from a composite of present and past contaminated fish consumption but not from consumption during pregnancy alone. Socioeconomic status, maternal smoking, PBB exposure, and parity, were unrelated to exposure. It is therefore unlikely that they account for the adverse outcomes associated with contaminated fish consumption and cord serum PCB levels according to the authors.

Critical Comments

This study has several methodological problems. The non-fisheaters population is less than one-third the size of the fisheaters. It is unclear why there were only 71 "controls" which also differed in many aspects from the exposed mothers. Exposure is at times defined as ≥ 3.0 ng/mL n = 75, nonexposed n = 166 for cord serum levels; however, for the rest of the comparisons, exposure is consistent with eating more than 11.8 kg of fish over 6 years n = 242, nonexposed n = 71. It is unclear whether the 75 children with equal to or more than 3 ng/mL of PCBs in serum were the infants that were of smaller size and which group they were drawn from since in another paper (Schwartz et al. *AJPH* 1983;73:293-296) it appears that PCBs were also present in measurable amounts in children of non-fisheaters. In Schwartz et al. (1983) it is stated that PCBs were only measured in 198 cord serum samples and were nonquantifiable in 130 (65.7%) of these samples. However, in the paper under review here it is stated that PCB and PBB levels were available on cord serum for 241 infants.

The "control" and the "exposed" population are not well matched. The exposed population seems to have a higher percentage of "problem" pregnancies and reports more alcohol and caffeine consumption. Only two-thirds of those invited actually participated in the study. This may have introduced a selection bias. The data on fish consumption were based on recall by the mothers which may be very inaccurate particularly for events that occurred many years ago. The Ballard test was only administered to slightly more than half of the controls and to about 80% of the exposed. The smaller infants had a shorter gestation period. It is unclear which children were actually tested and whether all infants were term infants.

If the fisheaters are compared to the non-fisheaters, the percent of spontaneous deliveries, alcohol intake prior to pregnancy and during pregnancy, caffeine and cold medications intake were statistically significantly higher among fisheaters. All of these factors affect the size and maturity of infants. Furthermore, it has been shown in adults that a positive correlation exists between alcohol consumption and PCB blood levels (Kreiss et al. *JAMA*. 1981;245:2505-2509). Thus, alcohol would affect PCB serum levels as well as infant size and maturity.

Since no information is given about the distribution of PCB levels for the controls as compared to the fisheating group the reader can not judge whether the body burdens in the comparison population are similar or lower. The levels measured in the fisheating population are similar to levels found by other investigators in the general population during 1980-1981 when this cohort was established.

The statement "Although fish consumption during pregnancy was highly correlated with overall fish consumption ($r = 0.79$, $R^2 = 0.62$, $p<0.001$), consumption during pregnancy did not predict either birth size or gestational age and was related only to neuromuscular maturity (18.6 ± 24 exposed during pregnancy versus 19.3 ± 2.5, $p<0.05$)" suggests that these correlations may well have occurred by chance. Given that fish consumptions are compared, the correlation is moderate and not very impressive. It is difficult to understand this finding since it is the same group unless many of the women who ate fish discontinued this practice or some women started eating fish during pregnancy. The gestational age dictates neuromuscular maturity. It is not well explained how gestational age was controlled for in the data analysis. Since these correlations did not go in tandem with overall fish consumption and fish consumption during pregnancy, it must be assumed that they were unrelated to fish consumption.

This study lacks a critical analysis of the results to establish whether they are biologically plausible and an in depth consideration of alternative causes such as alcohol consumption and other risk-taking characteristics that are more prevalent in the

fisheating population. It also appears that the PCB levels in the exposed population do not differ in a meaningful way from that of the general population or for that matter in the "control" group. PCB quantitation is difficult and results of repeat analysis of the same sample may vary by between 50-25%. The exposure is poorly defined and the "control" population is small and quite different from the "exposed" population. Both these factors add additional concern that the data were overinterpreted.

3. Jacobson S.W., Jacobson J.L., Schwartz P.M., Fein G.G. Intrauterine exposure of human newborns to PCBs: measures of exposure. Chapter 22 In: *PCBs: Human and Environmental Hazards*, M. M. D'Itri and M. A. Kamrin, eds., Butterworth Publishers: Boston, 1983;311-343.

Methods

See also other papers by these authors.

Of the 457 women invited to participate, overall 144 (32%) refused.

70.3 percent of the nursing mothers provided breast milk samples between 1 and 16 weeks after delivery. A second sample of breast milk was collected at 5 months from 45 women who continued breastfeeding.

Detection limits for PCBs in serum were 5 ng/mL for Aroclor 1016 and 3 ng/mL for Aroclor 1260. Sera with lipid levels below 200 mg/dl were not analyzed, since measurement techniques were insufficiently sensitive to detect PCB. The removal of three maternal sera and 43 cord sera did not alter the PCB distribution according to the authors. Twenty-seven additional cord blood samples could not be analyzed for other technical reasons.

Results

Most of the PCB levels matched to the 1016 standard were below quantification limits. Only one of 195 cord and five of 190 maternal sera were quantifiable. PCB levels for a large proportion of the cord sera were also not quantifiable when the 1260 standard was used. When PCB concentrations were calculated on a fat basis, the mean PCB values were similar for the cord and maternal sera.

No relationship was found between cord serum PCB levels and maternal fish consumption. Mean PCB level in maternal serum was 6.1 ng/mL (SD = 3.7) among women who ate contaminated fish, as contrasted to 4.1 ng/mL (SD = 2.7) among those who abstained (t = 3.83, $p<0.0001$).

Critical Comments

It is unclear how many sera of the group of fisheaters and the group of non-fisheaters were analyzed. It is stated that 195 cord sera and 190 maternal sera were analyzed. There were a total of 313 children. It is also stated that 70 samples were not analyzed because they were low in lipids or because they were inadequate for other reasons. That would leave 243 samples. However, an additional 48 samples must also not have been analyzed. According to these calculations, 118 samples or more than one-third of the samples were not analyzed. Thus, whatever results are obtained from these analyses they would not be representative of the group. It is also unclear how many of the controls and how many of the exposed infants' sera were analyzed. The same dilemma exists with the maternal samples. It is anybody's guess what the data would have looked like if PCB levels had been available for everybody. It is unclear whether half of the limit of detection was substituted for nondetectable levels. This is even of greater concern since there was also a 32% refusal rate when the mothers were first enrolled. Thus, it is unclear what this entire study group represents and what selection bias may have been introduced. It was not determined whether the women who refused to participate had different characteristics from those that enrolled.

4. Jacobson J.L., Fein G.G., Jacobson S.W., Schwartz P.M., Dowler J.K. The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls across the human placenta and into maternal milk. *AJPH*. 1984;74:378-379.

Method

The study population consisted of 313 women and their infants delivered at four hospitals in Michigan. Cord and maternal serum were collected following delivery. Of the mothers, 61% nursed their infants and 70.1% of them contributed a milk sample between 1 and 16 weeks postpartum. Aroclor 1016 and 1260 were used as calibration standards. The Webb-McCall method was used for quantitation. Serum containing less than 200 mg/dL lipids were omitted because the method was insufficiently sensitive.

Results

Using Aroclor 1260 as the quantitation standard, the mean PCB level in 198 cord sera was 2.0 ng/mL (ppb) with 95% confidence limits of 0.1 - 7.2. The mean for 196 maternal serum samples was 4.7 ng/mL (ppb) with 95% confidence intervals of 1.1 - 14.3. The difference was statistically significant ($p<0.001$). When calculated on a fat basis this difference between maternal and cord serum was no longer statistically significantly different. The mean PBB concentrations for 230 cord sera was 0.3 ng/mL (ppb) with a 95% confidence interval of 0.0 - 1.7 and for the 205 maternal sera 1.7 with 95% confidence intervals of 0.0 - 9.7 ng/mL (ppb). On a whole milk

basis the PCB levels in 138 milk samples had a mean of 19.3 ng/mL with 95% confidence intervals of 5.4 - 63.1. On a fat basis the mean was 732.6 with 95% confidence intervals of 293.4-1827. The PBB levels on a whole milk basis had a mean of 3.6 ng/mL (ppb) with 95% confidence intervals of 0.0 - 23.0 and on a fat basis the mean was 105.1 with 95% confidence intervals 4.38. - 2089.8. Thus, the PBB and PCB levels were in the same order of magnitude.

Critical Comments

Although it is stated in the methods that the PCBs were quantitated on Aroclor 1016 and 1260, the results are only given based on the Aroclor 1260 quantitation. The measured levels are very low, many are close to the limit of detection and are consistent with levels in the general population during the time when the samples were collected. It is unclear why 95% confidence intervals were calculated or if they truly represent 95% confidence intervals. Normally the range, mean, median and standard deviation are given for data like these. If the distribution is skewed it should be described and if outliers are removed that should be stated. The number of nondetectable levels should also be given.

5. Jacobson J.L., Jacobson S.W., Schwartz P.M., Fein G.G., Dowler J.K. Prenatal exposure to an environmental toxin: a test of the multiple effects model. ***Developmental Psychology.*** **1984;20(4):523-532.**

Method

The sample consists of 242 infants born to women who reported moderate consumption of Lake Michigan fish and 71 infants whose mothers ate no Lake Michigan fish.

The exposed group was limited to women whose contaminated fish consumption totaled more than 11.8 kg over a 6-year period. Of the 8,482 women screened (about 96% of the maternity patients in the participating hospitals), 4.0% ate contaminated fish in sufficient quantities to qualify for inclusion in the exposed group. About 29% of the women screened reported eating no Lake Michigan fish; 4.6% of these women were invited to serve as controls.

Cord serum samples were analyzed. Values matched to the 1260 standard were, therefore, used in the analyses reported here. Any serum samples containing lipid levels below 200 mg/dL were omitted from the data analysis, because measurement techniques were judged to be insufficiently sensitive to detect PCB levels at lipid values of this magnitude.

The Ballard examination was administered at 30 to 42 hours after birth to the 173 infants for whom it was possible to obtain parental consent within this limited period of time (Ballard et al., 1979).

The Brazelton Neonatal Behavioral Assessment Scale (NBAS; Brazelton, 1973) was administered to 287 newborns. In all but four instances (98.6% of the cases), testing was performed on the third day after birth (i.e., at 48-72 hours). The 44 NBAS items were reduced to seven summary clusters

Control Variables

Thirty-six potential confounding variables were examined, including demographic background (e.g., socio-economic status, maternal age); maternal prepregnancy weight and height, sex of infant, parity and gravidity; stress during pregnancy; prenatal care and diet; weight gain during pregnancy; alcohol, caffeine, and nicotine consumption before and during pregnancy; delivery complications; obstetrical medication; age (in hours) at the Ballard and NBAS examinations; and cord serum levels of polybrominated biphenyls (PBBs). Contaminated fish consumption is correlated (at a $p < .10$ significance level) with caffeine consumption before and during pregnancy and with alcohol consumption before and during pregnancy (rs range from .10 to .17). Cord serum PCB level is correlated with alcohol and caffeine consumption during pregnancy, weight gain during pregnancy, and maternal age (rs range from .12 to .14).

Contaminated fish consumption predicts a linear combination of five NBAS clusters, $F(5, 193) = 3.18$, $p<.01$, with a multiple correlation of .28. The strongest relationships are with autonomic maturity, $F(1, 193) = 5.85$, $p<.025$; number of abnormal reflexes, $F(1, 193) = 3.97$, $p<.05$; and range of state, $F(1, 193) = 3.65$, $p<.06$. F ratios for the remaining three clusters are all less than 1.

Maternal contaminated fish consumption is highest among infants classified as worrisome on the three clusters associated with PCB exposure. On the range-of-state cluster, where two categories of worrisome behavior are defined, exposure is highest among the infants classified as flat or depressed.

High levels of contaminated fish consumption increase the likelihood of worrisome performance: autonomic maturity, reflexes, range of state.

Contaminated fish consumption predicts two of the three autonomic items: motor maturity, $F(1, 280) = 6.93$, $p<.01$, and amount of startle, $F(1, 280) = 10.07$, $p< .005$; and one of the four range of state items, lability of states, $F(1, 279) = 14.46$, $p<.001$. Scores below 4 on the motor maturity scale are classified as "worrisome" by Als and her associates (1979). These scores are assigned to infants who exhibit jerky, unbalanced, cogwheel-like movement in which flexor and extensor muscles seem to be competing. Eleven percent of the highly exposed infants are immature in this sense, as compared with only 3% of the nonexposed controls. Als et al. suggest that fewer than six state changes during the NBAS examination indicates deficient lability to states. Given this

criterion, 42% of the highly exposed infants are hyporesponsive, compared with only 17% of the controls. Contaminated fish consumption predicts number of abnormally weak reflexes, $F(1, 281) = 9.63$, $p<.005$.

Controlling for the size and gestational age of the newborn does not appreciably alter the relation of contaminated fish consumption to NBAS performance.

The effect of contaminated fish consumption on autonomic maturity is reduced substantially when the Ballard score is held constant.

Serum PCB level (adjusted for its four potential confounding variables does not predict a linear combination of NBAS clusters, $F(5, 118) = 1.13$. Unfortunately, cord serum values were not available on 36.7% of the infants in this sample. Moreover, cord values were obtained for only 48.7% of the infants with the highest fish consumption exposure levels (>10 kg/year), compared with 65.3% for the remainder of the sample.

When the behavioral variables are entered first, they explain 8.0% of the variance in contaminated fish consumption, $F(3, 204) = 5.91$, $p<.001$.

Discussion

Contaminated fish consumption predicts neonatal behavioral deficits, indicating (a) autonomic immaturity, that is, a greater propensity to startle, poorer motoric, reflex, and neuromuscular functioning and (b) depressed responsiveness, that is, a greater number of hypoactive reflexes and more limited lability of states. Although subtle when compared with deficits linked to certain other teratogens, these behavioral effects are in the worrisome range.

The present results must be interpreted with caution, however. Because neonatal deficits are frequently transitory, their long-term developmental implications are uncertain. Moreover, findings based on maternal recall need to be confirmed.

The behavioral deficits associated with fish consumption are not predicted by cord PCB level. This discrepancy may be due to the restricted range of the cord serum PCB values which as noted, were not available for many of the most highly exposed infants. Alternatively, it is possible that the behavioral deficits associated with fish consumption are due to the presence of toxins other than PCBs in the same contaminated fish. Toxaphene, for example, has recently been detected in fatty Lake Michigan fish in quantities comparable to those to PCBs (Everett, 1982).

In the present study, multiple regression analysis indicated that 12.2% of the variance in contaminated fish consumption is associated with measurable neonatal deficits.

Critical Comments

It is not stated in the paper how many of the infants tested were born to mothers that did or did not consume fish. The fisheaters were selected out of a group of 8,482 women of which 4% ate sufficient contaminated fish and 29% did not eat such fish. Four percent of this group was invited into the study as non-fisheaters. Since ultimately only a total of 313 children were studied, additional potential participants must have dropped out (4% of 8,482 = 339 children and 4.6 of 29% or 2,459 would be 113 children.) However, that was not explained in the paper. Furthermore, only a subset of the children received the Ballard examination and not all children were given the Brazelton test. Although with the Brazelton test, a worrisome performance was predicted by contaminated fish consumption it was not predicted by cord serum PCB levels. The authors suggest that this discrepancy may be due to the restricted range of cord PCB values which were not available for the most highly exposed infants. Alternatively, the behavioral deficits associated with fish consumption may be due to the presence of toxins other than PCBs in the same contaminated fish. Toxaphene was recently also identified in these fish according to the authors. Furthermore, the restricted range of PCB levels in cord serum most likely was caused by little exposure and little PCB contamination of the consumed fish.

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6. Jacobson S.W., Fein G.G., Jacobson J.L., Schwartz P.M., Dowler J.K. The effect of intrauterine PCB exposure on visual recognition memory. *Child Development*. 1985;56:853-860.

Note: the findings reported here are also in: New methodologies for assessing the effects of prenatal toxic exposure on cognitive functioning in humans. In: *Advances in Environ Sci and Tech*. 1988;21:373-388.

Introduction

Neonatal Behavioral Assessment Scale (NBAS) although recognized as providing a wealth of information regarding the status of the newborn, has uncertain predictive validity (Sameroff, 1978). Correlations between infant recognition scores and later vocabulary tests of IQ in four samples of children averaged .47 (range = .33-.66).

Methods

The sample consisted of 123 white, predominantly middle-class infants (69 males, 54 females). (It is unclear how this sample was selected.)

Cord serum samples were available for only 81 infants due to technical problems in the laboratory. Breast milk samples, collected after leaving the hospital, were provided by 67 of the 88 nursing mothers mostly within the first 2 months of nursing.

The 7-month-old infants were tested in front of an observation chamber containing a pivoting stimulus-presentation "stage." Two stimulus plaques were located 30.5 cm apart from center to center and about 30.0 cm from the infant's eyes. The chamber was illuminated by a GE 12-volt bulb. Infant fixation was judged from corneal reflections of the stimulus targets observed through a peephole located halfway between the two stimulus plaques. Fixation was recorded to the nearest tenth of a second on a digital electronic recorder.

The stimuli used consisted of two pairs of achromatic photos of women's faces and one pair of chromatic photos of babies' faces mounted on a 20.0 x 17.5-cm white poster board. One of three examiners administered the test. The infant was first exposed to a target photo, which appeared simultaneously in the right and left positions. After the infant fixated the target for a total of 20 seconds, the familiar target was paired with a novel target for two 5-second recognition periods, reversing left-right positions from one period to the next. Visual recognition was defined as the percent of total fixation paid to the novel target for each of three pairs of targets. An overall score for each infant was the mean percent of visual fixation to novelty over the three problems.

For the analysis of confounding variables only variables were included for which incidence in each category exceeded 15%. Given this criterion, it was possible to assess the effects of 30 potential confounds.

All variables that were correlated with PCB exposure at an alpha of less than .10 were considered. Three variables met this criterion for cord serum--socioeconomic status ($r = .22$, $R^2 = .05$), maternal age ($r = .22$, $R^2 = .05$), and parity ($r = .23$, $R^2 = .05$)--and three for contaminated fish consumption--pregravid maternal weight ($r = -.17$, $R^2 = .03$) and use of acetaminophen and antacids during pregnancy ($r = .15$, $R^2 = .02$ and $.17$, $R^2 = .03$, respectively). All potential confounds were entered first in each stepwise multiple regression analysis. Exposure level (cord serum PCB level or fish consumption) was entered at the final step of the regression, and a toxic effect was inferred if the incremental variance associated with exposure was significant after the effects of the potential confounds were removed.

Postnatal effects were assessed in a regression based on human milk PCB level, number of weeks of nursing, and weighting human milk level by weeks of nursing. This analysis included the nursing infants whose mothers had provided milk samples and the non-nursing infants, who were not exposed postnatally. The following 10 potential confounding variables associated with any of the three components of postnatal exposure were included in the regression analysis: socioeconomic status ($r = .15$, $R^2 = .02$), maternal age ($r = .21$, $R^2 = .04$), maternal height ($r = .23$-$.27$, $R^2 = 0.05$-$.07$), alcohol ($r = -.15$, $R^2 = .02$), smoking ($r = -.23$ to $-.25$, $R^2 = .05$-$.06$), caffeine ($r = -.19$ to $-.22$, $R^2 = .04$-$.05$), antibiotics ($r = .26$-$.34$, $R^2 = .07$-0.12), breast milk PBB level ($r = -.30$ to $-.32$, $R^2 = .09$-$.10$), maternal employment ($r = -.23$, $R^2 = 0.05$), and age at 7-month visit ($r = .16$, $R^2 = .03$). A postnatal exposure effect was inferred if the three components (human milk, weeks, and their interaction) jointly added to the explained variance when entered in the final three steps of the regression. (It is unclear whether for the non-nursing mothers the levels of the nursing mothers were used?)

Results

Percentage of total visual fixation to novel relative to familiar targets ranged from 28.3% to 77.5% with a mean of 57.3% (SD = 11.4%). r's ranged from -.06 to .04, $p>0.20$, $R^2 = .004$-$.002$. Visual recognition was unrelated to birth size, gestational age, and performance on the NBAS (correlation between fixation of familiar and unfamiliar pictures seems to be nonexistent?).

The percentage of unique variance explained by cord serum PCB level was 10.4. Maternal report of contaminated fish consumption also predicted poorer visual recognition memory, $\beta = -.20$, $F(1,112) =$

4.50, $p<0.05$; incremental R^2 = 3.8%. After controlling for the potential confounding variables, higher levels of PCB exposure continued to predict poorer recognition memory in the remaining infants, $\beta = -.39$, $F(1,68) = 10.83$ $p<0.005$, for cord serum, and $\beta = -.23$, $F(1,98) = 5.49$, $p<0.05$, for fish consumption. Postnatal exposure from nursing was unrelated to recognition memory performance at 7 months, $F(3,80) = 1.18$, N.S.

Brazelton Neonatal Scale. After each of the neonatal mediating variables and the relevant potential confounds were entered into the regressions, the unique variance associated with cord PCB exposure ranged from $F(1,71) = 8.69$, $p<0.005$, to $F(1,71) = 14.04$, $p<0.005$.

Preference for novelty decreased systematically with increasing levels of cord serum PCB. A post-hoc comparison based on the Newman-Keuls procedure (not explained in the paper) indicated that the infants with the highest cord serum PCB levels showed significantly less preference for novelty than infants in the lowest two groups, $p<0.05$.

Cord serum PCB levels ranged from 0.2-7.9 ppb. Infants with elevated cord serum PCB levels ($\geq$ 3 ng/mL or parts per billion) were three times more likely to score in the lower tail of the distribution (<M-1 SD) than non- or low-exposed infants.

According to the authors the present study links prenatal PCB exposure to deficits in visual recognition memory at 7 months in infants who appeared clinically normal at birth.

Critical Comments

It is not stated in the paper that the examiners were blinded to the exposure of the children. There were 69 males and 54 females and there were 92 fisheaters and 31 non-fisheaters. The selection of this subsample of 123 children out of 313 is not explained, it is not stated whether the number of males and females in the two groups, the fisheaters and non-fisheaters, were evenly distributed. Fourteen infants were less than 38 weeks old at birth. It is unclear whether these children primarily contributed to the deficits in visual recognition memory. The division of the infants into those with PCB cord sera below and above 3 ppb is arbitrary. In other papers 5 ppb was used as a dividing mark.

The overall results of visual fixation suggest that visual fixation was inconsistent. No information is given whether a difference between achromatic and chromatic faces was noted. It is also questionable that an infant familiarizes itself with a photo in 20 seconds. It would look at the new photo simply because there was motion and then look back at the old photo. A cynic might suggest that the time was set for only 5 seconds for that reason.

Interobserver variability is an additional problem. There were three examiners. Their performances could conceivably change over time. It would be impossible to adjust for this statistically. It would be useful to compare the three groups by examiner and determine what the differences are per examiner and whether most of the children with higher PCB levels were examined by the same person. Again no distributions are provided in this paper. How many children that were in the high and the low group actually had PCB determinations? On what basis were these children chosen? How did the PCB milk levels distribute over this group? How were the children evaluated for whom no PCB levels were available? In this paper claimed effects are poorly documented, the limitations of the visual fixation tests are not explained and it is unclear whether all infants had normal vision for their age, particularly if premature children were included.

7. Jacobson J.L., Jacobson S.W. Intrauterine exposure to environmental toxins: the significance of subtle behavioral effects. In: *Beyond the Individual Environmental Stressors*, pp. 125-137. EPA/600/D-89/235, PB90-143379, 1985.

Since random assignment to different exposure levels is not possible, human toxicity studies are necessarily correlational. The principal challenge in correlational research is the need to control for the myriad of potential confounding variables that could account for or "explain away" any observed effects. Since for each additional control variable at least 10 subjects need to be added to the analysis, the sample size required to control for all potential confounds is prohibitively large. We, therefore, adopted the following strategy. Data were collected on 36 potential confounding variables relating to demographic background, health history, pregnancy and delivery complications, maternal smoking, exposure to alcohol and caffeine, and PBB exposure. All potential confounds that were correlated with a given exposure measure at an alpha level less than .10 were controlled statistically in all analyses based on that measure.

Because the measures of exposure were continuous variables, step-wise multiple regression analysis was the primary statistical technique. The control variables were entered first in each regression to remove the variance in the outcome measure attributable to them. A toxic effect was inferred if the addition of the exposure measure at the final step of the regression significantly increased the variance explained in the outcome measure. In addition to the potential confounding variables, two suppressor variables--maternal prepregnancy weight and sex of infant--were used in the cord serum regressions to compensate for a tendency for cord serum PCB levels to be higher in the infants of heavier mothers and in male infants.

Contaminated fish consumption and cord serum PCB level predicted lower birth weight, smaller head circumference, and a shorter period of gestation. These outcomes are not predicted, however, by level of fish consumption during pregnancy. This discrepancy is surprising since the two fish consumption measures are highly intercorrelated, $r = .70$, $R^2 = .49$, and suggests that it is the mother's lifetime PCB accumulation, that puts the newborn at risk. A greater number of outcomes are predicted by overall fish consumption than by cord serum PCB level.

The authors interpreted their data as follows: in contrast to the physical size and gestational age effects, the behavioral deficits associated with overall fish consumption are also predicted by consumption during pregnancy. It is possible that lifetime exposure determines the effect on intrauterine growth, whereas exposure during pregnancy leads to subtle CNS damage. Alternatively, since the behavioral deficits are unrelated to cord PCB level, it is possible that toxins other than PCBs found in these same contaminated fish are responsible.

The mean birth weight of the more highly exposed infants, that is, those with cord serum levels at or above the laboratory's "detection limit," is 194 g less than that of the infants below this limit. Comparing infants whose mothers consumed relatively large quantities of contaminated fish (>6.5 kg per year) with the infants of non-fisheating mothers reveals a 245 g birth weight difference. Despite the 200-250 g deficit, however, only two of the infants had even moderately low birth weight (<2500 g) and none were below 1500 g.

According to the authors there are no established criteria for neonatal behavioral adequacy. Based on their assessments of a large number of newborns, Als, Tronick, Lester, and Brazelton, (1979) have suggested criteria for deficient performance on the Brazelton Scale, which can be adapted to the clusters used in the present study. In contrast to the birth size effects, the behaviors exhibited by the affected infants would elicit the concern of an experienced clinician. Behavioral deficits include a greater amount of startle; jerky, unbalanced cogwheel-like movement; abnormally weak reflexes; and poor responsiveness to stimulation. Because neonatal deficits are frequently transient and predictive validity has not been established for the Als criteria, it is not known whether these behavioral signs predict long-term developmental impairment.

Critical Comments

Behavioral effects were explained by the mother eating fish during pregnancy while this had no effect on birth weight and length of gestation. These two parameters were only affected by the prepregnancy fish consumption. The prepregnancy fish consumption

would have added to the mothers' body burdens and should also have had behavioral effects. One difficulty with the authors proposal that the body burdens were higher in women that ate a lot of fish prior to their pregnancy is that most of the PCB levels measured were very low. Furthermore, PCB cord samples were not measured in many of the infants or were non-detectable. Thus, in a large portion of the cohort, PCB exposure could not be adequately established. It is unclear whether a selection bias was introduced which might have affected the outcomes. Furthermore, if the body burdens were higher in females that ate a lot of fish prior to their pregnancy, then these higher body burdens should have had a more pronounced effect on the CNS if continued internal PCB exposure is postulated. It is unfortunate that all of the studies were done without better defining exposure or characterizing the PCBs that these women were exposed to. A reference for the statistical methods was not given. No distribution data were provided. Given the many missing data points and the uncertain information about fish consumption (recall by the mothers), the results reported here are not convincing.

8. Jacobson J.L., Jacobson S.W. Strategies for the longitudinal assessment of the effects of pre- and postnatal PCB exposure on human development. *Teratology*. 1985;31(3):6B.

This is only an abstract with little detail and is also reported in Jacobson et al. *Child Development*. 1985;56:853-860.

9. Jacobson S.W. Environmental toxins and infant development. Chapter 3 In: *Theory and Research in Behavioral Pediatr,* Volume 3, H. E. Fitzgerald, B. M. Lester, and M. W. Yogman, eds., Plenum Press: New York, 1986;107-146.

This article is a review in which several issues are addressed. Only the issues that are pertinent to the behavioral studies in infants as they relate to PCB exposure will be summarized here.

The author states that exposure to a toxic agent may have multiple effects in the same individual and different effects in different individuals, and that these effects may depend on the developmental status of an individual when exposed or assessed (Fein et al., 1983, Jacobson et al., 1984).

The author points out that the association of PCB level with low birth weight might have been spurious since the true cause of the low birth weight effect could be the alcohol consumed by the mothers of the high-PCB infants. To test for spuriousness PCB level was correlated with birth weight after statistically controlling for level of maternal alcohol consumption. Since the PCB effect on birth weight remained significant, it was inferred that PCBs are independently associated with low birth weight, over and above the effect of prenatal alcohol exposure.

Cord serum PCB levels were higher in the offspring of heavier mothers. The relation between PCB exposure and low birth weight was not evident at first because heavier mothers usually have heavier babies. When maternal prepregnancy weight was held constant, however, a significant negative relation emerged between cord serum PCB level and birth weight. The author also found significant correlations between PCB cord serum levels and alcohol consumption prior and during pregnancy, between caffeine consumption during pregnancy and cord PCB serum, and between fish consumption and alcohol and caffeine consumption.

The author also points out that since the unique variance associated with cord PCB exposure remained significant after each of the neonatal mediating variables and relevant potential confounds were entered into the regressions, it was concluded that the 7-month behavioral effects were not mediated by small birth size, gestational age, or neonatal behavioral performance (Jacobson et al., 1985).

As in most contemporary human teratological studies, multiple regression analysis was primarily relied upon. Potential confounds were entered first in each regression, with exposure level added as a continuous variable at the last step. A toxic effect was inferred if exposure explained a significant amount of unique variance in the outcome measure after the variance associated with the potential confounds was removed.

Testing for such a relationship requires a division of the sample into discrete exposure groups. "Outpoints" for these analyses were selected by dividing the frequency distribution for exposure level into four groups with equal numbers of subjects. The analysis of covariance (ANCOVA) was then run, in which the same potential confounds used in the regression analysis were statistically controlled. This analysis provided group means which were adjusted for the effects of potential confounds and suppressors (Fein et al., 1984; Jacobson et al., 1985).

In addition to the more general discussion, other facts were given in this paper about the Michigan cohort which can not necessarily be found in the original publications: PCB levels in the human milk samples averaged 1.0 part per million (ppm) on a fat basis, range = nondetectable to 3.0 ppm. Infants whose mothers were in the upper third of the distribution for contaminated fish consumption averaged 245 g less in birth weight than the nonexposed controls, 0.7 cm less in head circumference, and 6.3 days less in terms of gestational age. (Slightly different numbers are given in other publications.)

In contrast to the overall fish consumption, consumption during pregnancy did not predict either birth size or gestational age.

With two suppressor variables (maternal weight and sex of infant) statistically controlled, cord serum PCB level predicted lower birth weight, smaller head circumference, and shorter gestational age (Fein et al., 1984).

Seven clusters derived from the Brazelton (1973) Neonatal Behavioral Assessment Scale (NBAS) were examined. Consumption level of contaminated fish predicted a linear combination of five of those clusters, with a multiple correlation coefficient of .28. The strongest relationships were with autonomic maturity, reflexes, and range of state. Each of the items in the autonomic and range-of-state clusters was examined separately. Consumption of contaminated fish predicted (a) two of the three autonomic items, motor maturity and amount of startle, and (b) one of the four range-of-state items, lability of states.

Eleven percent of the more highly exposed infants received scores indicating jerky, unbalanced, cogwheel-like motor movements, compared with only 3% of the nonexposed; 33% exhibited at least four abnormal reflexes.

Cord serum PCB level was not significantly related to either a linear combination of NBAS clusters or any of the individual clusters. This discrepancy may be due to the fact that because of technical laboratory analytic problems, cord serum samples were not available for 36.7% of the infants in the sample. Moreover, cord values were obtained for only 48.7% of the infants with the highest levels of fish consumption exposure (more than 10 kg/year), compared with 65.3% for the remainder of the sample, so that the range of exposure levels available for the cord PCB analyses was restricted. Alternatively, it is possible that the behavioral deficits associated with fish consumption are due to the presence of toxins other than PCBs in these same contaminated fish.

None of the measures of prenatal PCB exposure were related to the Mental Development Index (MDI) or the Psychomotor Development Index (PDI) on the Bayley Scales. By contrast, higher cord serum PCB and maternal fish consumption levels both predicted poorer recognition memory performance. The infants performing poorly at 7 months had not necessarily exhibited these deficits at birth. Postnatal exposure from nursing was not related to recognition memory.

The clinical significance of the neonatal behavioral effects is even more difficult to interpret. Some neonatal deficits are transient, and although the Brazelton Behavior Scale is recognized as an excellent assessment of current newborn status, predictive validity has not yet been established (Sameroff 1978). Also, no established criteria exist for determining the behavioral adequacy or the severity of these behavioral disturbances. Although the predictive validity of the Als et al. (1979) criteria for the NBAS have also not been determined, the "worrisome" category describes

behavior sufficiently deviant to warrant the concern of the practicing clinician.

Critical Comments

This is a review and only some important points are gleaned from it and presented here. The authors are pointing out some of the uncertainties of their data and the possibility that there may be alternative interpretations for their results. Unfortunately, these points are not necessarily presented in the original papers where the data was initially published, nor are the methods they employed critically reviewed. What is totally unclear is how the authors held the prepregnancy weight of the mother steady and then were able to calculate a positive correlation between low birth weight and PCB cord serum levels.

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10. Jacobson J.L., Jacobson S.W. New methodologies for assessing the effects of prenatal toxic exposure on cognitive functioning in humans. Chapter 18 In: *Advances in Environ Sci and Tech.* 1988;21:373-388.

This chapter represents an overview of some of the studies. To select the sample, 8,482 mothers delivering infants in four western Michigan hospitals were screened between July 1980 and September 1981 for fish consumption. The questions about fish consumption were broken down by species so that more highly contaminated species could receive greater weight in the exposure estimate. Wayland Swain of the EPA Large Lakes Research Station assisted by weighting the various species according to their relative PCB content based on assays of fish performed by various investigators over a 10-year period. "Contaminated fish consumption" was defined as the weighted sum of annual Lake Michigan fish consumption in the present or past, whichever was greater.

The final sample of 313 women included 242 who ate Lake Michigan fish on a regular basis and 71 who did not eat these fish. Contaminated fish consumption among exposed mothers averaged 6.7 kg or about 14.7 pounds of fish per year (standard deviation (SD) = 5.8 kg, range = 1.2 to 41.7 kg per year). The women reported having eaten Lake Michigan fish for an average of 16.1 years.

PCB levels in the cord serum samples were low, with approximately two-thirds (180/286?) below the detection limit of 3 ng/mL (ppb).

Source	N	Mean	Low	High
Cord serum (ppb)	286	2.5	0.0	12.6
Maternal serum (ppb)	208	5.5	0.2	23.1
Breast milk fat (ppb)	138	812.7	185.7	2600.0

The data according to the authors showed a significant relationship between the amount of contaminated fish consumption reported by the mother and the level of PCBs in her serum.

196	Maternal fish consumption-maternal serum	(PCB 0.38*)
148	Maternal serum-cord serum	(PCB 0.42*)
91	Maternal serum-breast milk (fat basis)	(PCB 0.35*)

*$p<0.001$ (These correlations are not high for the types of samples that are compared even though they are statistically significant.)

Higher cord serum PCB levels were significantly related to smaller birth size and shorter gestation after controlling for potential confounding variables (Fein et al., 1984). Higher levels of maternal consumption of contaminated Lake Michigan fish were also significantly correlated with these outcomes. Length of gestation was assessed on the basis of both the mother's report of her last menstrual period and the Ballard examination. It was the mother's

lifetime fish consumption rather than her consumption during pregnancy that predicted the size and gestational age deficits (Fein et al., 1984).

Although these effects were statistically significant, their absolute magnitude was small. None of the infants of mothers who had consumed large quantities of fish weighed less than 1500 g. And few were actually preterm, that is, less than 38 weeks gestation. Newborns with detectable levels of PCBs averaged about 160 g (in other papers the difference in weight is given as 200 g) less at birth than infants with PCB levels under 3 parts per billion, a size deficit which is comparable to that associated with smoking during pregnancy (U.S. Public Health Service, 1979).

At birth <3 ppb = 3575 g ± 536, ≥ 3 ppb = 3412 g ± 538 (it is unclear whether these numbers were adjusted for sex). Higher levels of maternal contaminated fish consumption were associated with several neonatal behavioral deficits, including more jerky, unbalanced movement and startles, fewer state changes in response to external manipulation, and a greater number of abnormally weak reflexes. Although smaller birth size and shorter gestation were significantly related to both contaminated fish consumption and cord serum PCB level, the behavioral effects were not correlated with the cord serum measure. According to the authors, the finding that a greater number of outcomes were predicted by contaminated fish consumption suggests that the maternal report may actually provide a more reliable measure at these levels of exposure than cord serum. Alternatively, since the behavioral deficits were correlated with fish consumption but not with serum PCB level, it is possible that these deficits were due to other unknown toxins from the same contaminated fish.

In the Visual Recognition Memory Test of Fagan and Singer, the infant is seated on the mother's lap in front of an observation chamber containing two stimulus plaques. An observer watches the infant through a small peephole in back of the stage and records the infant's gaze direction on an electronic event recorder. The infant is initially shown two identical target photos, which appear simultaneously in the right and left positions until he or she has looked at them for a total of 20 seconds. The familiar target is then paired with a novel target for two 5-second recognition periods, reversing left-right positions from one period to the next. Because the normative response at this age is to spend more time looking at a novel stimulus, infants who recognize the original target will spend more time looking at the new one. A propensity to look longer at the unfamiliar stimulus, therefore, indicates the ability to recall the original target and to discriminate it from the new one. This recognition memory test has been found to predict IQ based on a picture vocabulary test at 4 and 7 years of age.

Preference for the novel stimulus was related to cord serum PCB level (Jacobson et al. *Child Development*. 1985;56:853-860). The most highly exposed infants spent about half the time looking at the novel stimulus. The infants with detectable cord serum PCB levels were almost four times more likely to score greater than 1 standard deviation below the sample mean. Multiple regression analysis showed that the recognition memory effect was not mediated by physical size or neonatal behavioral performance deficits. In contrast to prenatal exposure, PCB exposure from nursing was not related to any of the 5- and 7-month outcomes.

Critical Comments

In the paper it is stated "The finding that a greater number of outcomes were predicted by contaminated fish consumption suggests that the maternal report may actually provide a more reliable measure at these levels of exposure than cord serum. Alternatively, since the behavioral deficits were correlated with fish consumption but not with serum PCB level, it is possible that these deficits were due to other toxins from the same contaminated fish that were not measured by the analytical laboratory." This statement suggests that it is unclear why differences occurred among these infants. The fish consumption data is based on recall of past events which is unreliable. An additional uncertainty is introduced by the weighting of the fish for amounts of PCBs. No information is given in any of the publications by the authors on exactly how Dr. Swain determined what the PCB concentration in different fish species was and what his assumptions were. Furthermore, information on the size of the fish consumed by the mothers does not seem to have been collected nor is it known where the fish were caught. PCB concentrations in different fish of the same species vary, depending on their size and where they are caught. PCB levels have been decreasing since PCBs were banned. It is stated that the averaging of the PCB concentration in the fish was done over a ten year period, but it is unclear what ten year period was chosen and how the averaging was done. Different ways of cooking removes different amounts of PCBs. It is also unclear whether the assumptions used are based on whole fish or on the edible portion. Levels in the edible portion would be lower. Overall, reading the results suggests that the various correlations do not make much biological sense. Since many endpoints were compared, the observed differences most likely occurred by chance a possibility never entertained by the authors. Furthermore, age of the mother may be a confounder not properly evaluated, neither was the beneficial impact of breast feeding examined.

11. Jacobson J.L., Humphrey H.E.B., Jacobson S.W., Schantz S.L., Mullin M.D., Welch R. Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. *AJPH*. 1989;79:1401-04.

Methods

The study included two cohorts of children evaluated at age 4. The "fish exposure cohort" consisted of 236 children recruited in 1980-81 from four western Michigan hospitals. Annual Lake Michigan fish consumption was weighted by the PCB content of the species consumed, in the present or past, whichever was greater. All women consuming at least 11.8 kg over a six-year period (4.0% of those interviewed) were invited into the study. A small proportion (4.6%) of those who did not consume these fish were also recruited and constituted 22.7% of the final sample. Of those assessed as infants, 75.4% were seen again in the present study. The children evaluated at age 4 did not differ from those not retained in the sample in terms of cord and maternal serum PCB levels but were somewhat higher in socioeconomic status (SES) and maternal fish consumption and milk PCB levels. No further details are given in the paper.

The "farm exposure" cohort consisted of 87 children who had not been assessed as infants. Their families were enrolled by the Michigan Department of Public Health in long-term studies of exposure to PBB or PCB contaminated farm products. The PBB families had lived on or received food from quarantined farms; the PCB families had eaten milk and meat from farms whose animals consumed silage contaminated with PCBs.

Information regarding family SES and residence, maternal age, weeks of breastfeeding, maternal contaminated fish consumption during and before pregnancy, and child's contaminated fish consumption during the previous year was obtained by maternal interview at the four-year assessment. When compared with similar information obtained from the fish exposure cohort during infancy, test-retest reliabilities (Pearson r) ranged from $r = 0.68$, $R^2 = .46$ for maternal fish consumption to $r = 0.99$, $R^2 = 0.98$ for maternal age (median $r = 0.81$, $R^2 = .66$). The four-year maternal fish consumption report was also validated in relation to maternal serum PCB level obtained at delivery ($r = 0.37$, $R^2 = .17$, $P = 0.000$). Venous blood was obtained from 285 of the 323 children in the two cohorts.

Child's contaminated fish consumption was measured in terms of current annual kg consumed (log X + 1) with species weighted to reflect degree of PCB contamination. Weeks of nursing was transformed by recording seven values greater than 3 standard deviations above the mean to 1 point greater than the highest

observed value (no further details were given), as recommended by Winer (Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill; 1971).

Results

PCBs were detected in about half of the 4-year olds tested; PBBs, in relatively few samples; and DDT, in a large majority of the children. Although the mothers in the fish exposure cohort had eaten more Lake Michigan fish before and during pregnancy than their farm exposure counterparts, 7.6 kg/yr versus 1.2 kg/yr, PCB and DDT levels were similar in the two groups of children.

Blood lead levels were generally low, although 11 children had levels between 0.72 and 1.21 μmol/L (15-25 μg/dl). While cord serum level was unrelated to PCB body burden at age 4 ($P = 0.615$), maternal milk PCB level and weeks of breastfeeding jointly explained 59.7% of the variance in four-year PCB level ($P = 0.000$).

The Webb-McCall isomer identification method records chromatographic elution peaks, each of which is comprised of one or more specific PCB congeners. Peaks 203, 232/244, 332 and 372 were statistically more prevalent in the farm exposure cohort, suggesting that the farm children may have been exposed to sources of PCBs to which the fish cohort children were not exposed. (It is not explained what these peaks are, no systematic numbers are given.) Despite these differences, the overall pattern of elution peaks was highly similar in the two cohorts.

Serum extracts from 20 children with PCB serum levels in excess of 3.0 ng/mL were pooled into two composite samples--one for the boys ($N = 9$); the other for the girls ($N = 11$)--and analyzed at the U.S. Environmental Protection Agency by means of capillary column gas chromatography. The most prevalent congeners, Nos. 153, 138, 180, and 118 (2,2',4,4',5,5' and 2,2',3,4,4,'5' hexachlorobiphenyl, 2,2',3,4,4',5,5' heptachlorobiphenyl and 2,3',4,4',5 pentachlorobiphenyl) were also among the most prevalent in human milk and serum samples evaluated in prior studies.

Critical Comments

In this paper two groups of children from different studies (the Michigan Department of Public Health study on PBB which also included farm families with PCB exposures and the previously established cohort of fisheaters) were combined at age 4. No perinatal PCB data are available for the children exposed on the farms. It appears that the body burdens of DDT are as high as the PCB levels. Some children also had elevated blood lead levels. Overall the PCB and DDT levels are consistent with levels found in the general population. The children from the farm families also had low level PBB body burdens. This paper mostly defines exposure and does not relate exposure to health effects. Congener

identification in pooled serum samples was also done and differences between the two groups were detected.

12. Jacobson J.L., Jacobson S.W. Methodological issues in human behavioral teratology. In: *Advances in Infancy Research*, C. Rovee-Collier and L. P. Lipsitt, eds., ABLEX Publishing Corporation: Norwood, NJ, 1990;6:111-148.

In this paper the methodology used in the studies of the Michigan cohort is discussed and some pitfalls are pointed out. The authors discuss a variety of issues, the most important of these issues are presented here.

For example, it is stated: "We are currently collaborating with Robert J. Sokol of Wayne State University Medical School on a study of the effects of prenatal alcohol exposure on infant cognition. The principal focus of this study is the sensitivity to teratogenic exposure of a battery of new infant tests with predictive validity for cognitive functioning in childhood." Sampling criteria are based on data from previous studies (e.g., Jacobson and Jacobson, Dowler, Fein, and Schwartz, 1983; Streissguth, Barr, and Martin, 1983; Streissguth et al., 1984), which have found most alcohol-related deficits in infant performance at maternal drinking levels above 1.0 oz. of absolute alcohol per day (AAD) and no effects below 0.5 oz. AAD.

The authors also discuss their studies of the fisheating population. The participating parents of this population were higher in SES and education, more likely to be married, and older. The higher contaminated fish consumption and breast milk PCB levels among the participating mothers suggest that concern regarding exposure may have motivated some women to participate in the study according to the authors.

The threat to internal validity from refusal or attrition bias was assessed by comparing rates of illness or other disability in exposed and nonexposed participants and nonparticipants children.

Participant and Nonparticipant in Outcome by Level of Exposure

	High Exposure[a]		Low Exposure		
	Participants	Nonparticipants	Participants	Nonparticipants	F[b]
Congenital medical problems (%)	8.9	6.3	20.8	4.3	
Ill sincee birth (%)	11.1	30.0	13.9	6.9	
Hospitalized since birth (%)	24.4	40.0	30.7	31.0	
B. W.(gms)	3485	3224	3654	3833	<1
Visual recognition memory[c]	.53	.59	.60	.63	<1

Note: values are cell percentages were indicated, otherwise group means.
[a]Cord serum PCB level greater than or equal to 3.0 ng/mL or parts per billion.
[b]Tests the Exposure-by-Participation interaction.
[c]Proportion of time looking at the novel stimulus on Fagan and Singer's (1983) test.

Because postnatal PCB exposure from nursing is a function of both the contaminant level in the mother's milk and the amount of milk consumed by the infant, each nursing mother was given an extensive interview in person or by telephone regarding her infant's feeding patterns at 2, 4, 5, and 7-1/2 months. Mothers were asked for how many weeks since the last interview their infant fell into one of the following five categories: (a) exclusively breastfed, (b) primarily breastfed, (c) mixed, (d) primarily bottle fed (including solid foods), and (e) not breastfed. These categories were assigned weights of 1.0, 0.8, 0.5, 0.2, and 0.0, respectively. The number of weeks the infant fell in each category was multiplied by the appropriate weights and then summed. This detailed estimate of breast milk consumption was highly correlated with weeks of nursing. This analysis indicates that breast milk exposure can be measured simply by asking a mother when she weaned her infant and additional detail about her daily nursing patterns is not needed. Since the fisheating mothers in the Michigan cohort also consumed more alcohol than the comparison group, the authors' statements in this paper about alcohol consumption and other lifestyle factors are summarized here:

Barr et al. (1984) found decreased birth weight even in infants of alcoholic women who reported abstaining during pregnancy. Several studies have reported instances in which exposure to alcohol or caffeine prior to pregnancy predicts developmental outcome more

strongly than exposure during pregnancy (e.g. Barr et al., 1984; Jacobson 1983a and b; O'Connor et al., 1986). One explanation is that prepregnancy drinking patterns presumably continue in the early weeks after conception before pregnancy is recognized. Alternatively, prepregnancy drinking may be more predictive of teratogenic outcome, not because very early exposure is critical, but merely because it is measured more reliably than drinking during pregnancy. Pregnancy drinking may be more difficult to recall because it changes after pregnancy is recognized or may be reported less accurately because it is more stigmatized. There is also less restriction of range in the prepregnancy measure. Because prepregnancy drinking is usually correlated with intrapartum consumption, it may provide a more reliable surrogate measure for the exposure that occurs during pregnancy (Moron et al., 1985).

In the PCB research, infants were tested on the Bayley Scales of Infant Development at 5 months and Fagan's visual recognition memory test at 7 months. The Bayley, currently the most popular standardized infant test, is designed to assess neurobehavioral development across a broad range of competence. Although short-term test-retest reliability is good, providing evidence of concurrent validity, the Bayley has been criticized for not adequately assessing visual and auditory processing and for using tasks requiring motor proficiency to evaluate cognitive competence (Zelazo 1979).

The Fagan paradigm is based on the infant's well-documented preference for novel stimuli. After familiarization to a visual stimulus for a fixed period of time, the infant is shown the familiar stimulus together with a novel one. The infant's propensity to look longer at the novel stimulus provides evidence of his or her ability to recall the original stimulus and discriminate it from the new one. Predictive validity has been reported for cognitive functioning at 4 and 7 years (Fagan and McGrath 1981, Rose and Wallace 1985). Although no PCB or prenatal alcohol exposure effects on the broad-based Bayley Scales were found, the more focused Fagan test was sensitive to moderate levels of both these teratogenic exposures (Jacobson et al., 1985; Jacobson et al., 1983a and b). Visual discrimination and short-term memory processing are presumably needed for adequate Bayley performance, but these deficits were evident only when assessed in a task designed specifically to evaluate a limited domain of cognitive functioning.

In adopting tasks not designed specifically for use in teratological studies, it may be advisable to consider alternative approaches to data reduction. For example, Lester et al. (1982) recommended that data from the Brazelton Neonatal Scale be reduced to seven composite subscales, known as the Lester Clusters. These investigators' interest in the neurological integrity of the newborn led them to combine hypertonic and hypotonic responses on

the grounds that such responses are equally nonoptimal. Because most teratogenic agents are likely to be associated with either a hypertonic or hypotonic response, however, combining these two extremes is likely to mask teratogenic effects on tonicity. Certain modifications of the Lester Clusters for use in teratological studies (Jacobson et al., 1984) are, therefore, recommended.

In teratological studies, multiple regression is used to achieve statistical control, not to identify the best combination of variables that predict a given outcome. The focus is on the magnitude of the regression coefficient(s) associated with exposure rather than total variance explained by the model (R^2). Toxic effects are evaluated in terms of the statistical significance of the R^2 changes or "unique variance" associated with entering exposure at the final step of the analysis.

Critical Comments

In this article the authors discuss methodological issues in general and more specific issues such as the effect of alcohol on the fetus and the difficulty of obtaining an adequate history about alcohol consumption. These statements contrast with the analysis of their own data. The fisheating cohort definitely consumed more alcohol than the non-fisheating comparison group. However, the authors in their reports on the effects of PCBs on the fetus assume that they can control for this difference through statistical manipulations. Because of the uncertainties associated with the information on alcohol consumption, intake of caffeine and smoking, stepwise regression will not adequately control for these modifiers. Furthermore, some of the behavioral tests used by the authors to evaluate the children have not been standardized and are modified by different investigators to meet their individual needs. It is unknown whether these modified tests would be equally valid to evaluate the behavior of small children. Finally, in a table the authors illustrate how they in their Michigan cohort studies compared participants with non-participants to determine whether a bias may have been introduced (see above). The information given in the table is difficult to interpret. It is unclear over what time span the entries in the table extend. In participating and non-participating groups the number of ill infants and infants that have been hospitalized since birth is unexpectedly large. Among the participants more children seem to have congenital medical problems (whatever that is) than among the nonparticipants.

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13. Jacobson J.L., Jacobson S.W., Humphrey H.E.B. Effects of exposure to PCBs and related compounds on growth and activity in children. *Neurotox Teratol.* 1990;12:319-326.

Two cohorts of children were evaluated at age 4. The "fish exposure cohort" with 236 children recruited in 1980-81 whose mothers had in the present or past consumed at least 11.8 kg of contaminated fish over a 6-year period and a randomly selected small proportion (4.6%) of women who had not consumed such fish. The fisheaters constituted 77.3% of the final sample. Seventy-five percent of the children assessed as infants were seen again at age 4.

The "farm exposure" cohort consisted of 87 children who were also born in 1980-81 but had not been assessed as infants. Their families were enrolled in long-term studies of exposure to PCB or polybrominated biphenyl (PBB) contaminated farm products conducted by the Michigan Department of Public Health. The PCB families had eaten milk and meat from farms whose animals had consumed silage contaminated with PCBs.

Data were collected during two visits to the child's home: the first, at 4 years; the second, 3 months later. During the first visit the child was given the McCarthy Scales of Children's Abilities, a testing procedure with extensive informal interaction between child and examiner. The second visit consisted primarily of a series of reaction time tests, which involve sitting quietly in front of a video screen or other apparatus and require considerable concentrated attention and effort.

The mother was interviewed regarding demographic background, quality of intellectual stimulation provided in the home, perinatal medical history, smoking and alcohol consumption during pregnancy, familial stress, maternal employment, and the child's nursery school experience. She was also asked to complete the Peabody Picture Vocabulary Test-Revised (PPVT-R) and the Buss and Plomin Emotionality Activity Sociability (EAS) Temperament Survey for Children. Immediately following each of the home visits, the child examiner completed a 10-item Child Behavior Record (adapted from the Bayley Infant Behavior Record), which included a 9-point Activity Scale. Median interobserver reliabilities for examiner-rated activity were $r = .75$, $R^2 = 0.56$ and $.74$, $R^2 = 0.55$ for the first and second visits, respectively. Test-retest reliability, available for 35 children on the second visit, was moderate ($r = .44$, $R^2 = 0.19$).

Intercorrelations among the mother's EAS activity rating and the examiner activity ratings from the two home visits were extremely low (r's ranged from .13 to .23, R^2 0.017-0.05). The three ratings were transformed into standard scores and summed to provide a composite measure of activity, which was standardized to a mean of

0 and a standard deviation of 1.0. When compared with its three components, the composite activity rating was most highly correlated with two measures of behavioral impulsitivity obtained during reaction time testing and with the McCarthy Scales General Cognitive Index.

Prenatal exposure was established on the basis of cord serum PCB concentration. Postnatal lactation exposure was assessed in terms of maternal milk PCB level and duration of nursing. Venous blood was obtained from 285 of the 323 children. Specimens were obtained from 250 children at age 4; 35 children with small veins provided specimens at age 5. All 285 specimens were used to indicate child body burden at 4 years.

The cord and 4-year serum PCB measures and the alcohol, smoking, and PBB measures were positively skewed (skew >2.5) and were normalized by means of log X + 1 transformation.

Twenty-five control variables known or suspected to affect physical growth or behavioral development were assessed as potential confounders -- socioenvironmental -- maternal education, maternal age, marital status, number of children, parity, maternal employment, nursery school attendance, familial stress -- other demographic (sex of child, cohort); perinatal risk -- alcohol -- smoking -- delivery complications -- other environmental exposures (PBBs, DDT, lead). Inspection of the intercorrelations among these variables revealed that five socioenvironmental measures were moderately interrelated (median $r = .41$, $R^2 = 0.17$: range = .20-.61), as were the two alcohol ($r = .45$, $R^2 = 0.20$) and two smoking ($r = .74$, $R^2 = 0.55$) measures.

Control variables were selected for inclusion in multivariate analyses based on the premise that a potential confounder cannot be the "true cause" of an observed deficit unless it is related to both exposure level and outcome. Since influence on outcome was the criterion for determining which control variables to assess, any control variable that was related to an exposure measure (at $p<0.10$) was treated as a potential confounder in all analyses of its effects. In addition to control for confounding, five variables known to be strong predictors of child's physical size (maternal height and weight, paternal height and weight, and child's sex) were treated as covariates to increase statistical precision. The residual from a regression of child's weight on these covariates was used to provide an adjusted measure of child's weight from which the variance attributable to parental size and child's sex had been removed. Child's height and head circumference were adjusted in a similar manner.

Adjusted 4-year weight, height, and head circumference and the composite activity rating were each evaluated in three stepwise multiple regression analyses: one for prenatal exposure, based on

cord serum PCB level; one for postnatal exposure from nursing, based on maternal milk PCB level and weeks of nursing; and one for 4-year body burden, based on 4-year serum PCB level. The potential confounders of the exposure were entered in the first step of each regression analysis; the exposure measure (or measures), at the second step. A toxic effect was inferred only if the effect of exposure level remained significant (at $p<0.05$) after the effects of the potential confounders had been removed. If a toxic effect was indicated, dose dependence was tested by dividing exposure level into discrete groups and computing an analysis of covariance which adjusted for potential confounders. A dose-response relationship was inferred if the adjusted mean scores for the discrete groups declined monotonically with increasing levels of exposure.

Results

The fish consuming mothers smoked and drank more. There were no cohort differences in other perinatal risk factors. Farm exposure serum PCB levels were similar to those of the fish exposure children, presumably due to exposure from nonfish sources. Despite the difference in criteria for their recruitment, the two cohorts were similar in SES, many demographic characteristics, perinatal risk, and exposure to environmental contaminants. Their data were, therefore, pooled except in the analyses based on cord serum and maternal milk levels, which were not available for the farm exposure children.

Cord serum PCB levels for the children in this study ranged from 0 to 12.3 ng/mL (mean = 2.5, SD = 2.0), 4-year serum PCB levels, from 0 to 23.3 ng/mL (mean = 2.1, SD = 3.3). Maternal milk PCB levels (fat basis) ranged from 185.7 to 2600.0 ng/mL (mean = 835.9, SD = 388.4).

Prenatal PCB exposure was associated with lower weight at 4 years in a dose-dependent fashion. Children with cord serum PCB levels of 5.0 ng/mL or more weighed 1.8 kg less on the average than the lowest exposed children, after adjustment for covariates and potential confounders. The effect on child's weight was significant for girls [beta = -0.28, $F(1.52) = 4.52$, $p = 0.038$] but not for boys [beta = -0.16, $F(1.56) = 1.50$, $p = 0.226$]. Nevertheless, the magnitude of the deficit was similar for the two sexes (2.2 kg for girls, 1.7 kg for boys). None of the measures of PCB exposure were related to height or head circumference at 4 years. Although smoking during pregnancy had been associated with lower birth weight in this sample, it did not predict lower weight at 4 years [beta = 0.03, $F (1.222) = 0.19$, $p = 0.660$].

Thirty-one percent of the children with levels of 9 ng/mL or more were rated in the bottom tenth percentile for the sample as a whole, and none were in the top tenth percentile. Among this highest exposed group, 15.4% and 14.3% were rated "usually quiet

and inactive" on the Child Behavior Record at the first and second testing sessions, respectively, compared with only 5.4% and 7.8% for the lowest exposed children. Conversely, none of the highest exposed children were rated "in action during much of the period of observation" during the first testing session and only one received that rating for the second session, compared with 11.7% and 14.0% of the lowest exposed children for the two sessions, respectively.

The effect of maternal milk on activity was strongest in children of women with higher than average PCB levels who breastfed for at least 12 months.

Discussion

The 4-year weight deficit is associated with prenatal but not postnatal exposure, a pattern which also held for the size deficits seen in these children at 5 months postpartum.

In the present study, reduced activity was associated with both 4-year PCB body burden and its principal determinant, exposure to PCB-contaminated human milk.

The activity measure was relatively subtle, and its clinical significance is uncertain. None of the children received the lowest possible rating ("stays quietly in one place with practically no self-initiated movement"). The highest exposed children were about 2-3 times more likely to be rated "usually quiet and inactive" and less than half as likely to be "in action during much of the period of observation."

Critical Comments

It is impossible to determine which children of the fisheating cohort were included in this study. Originally there were 242 children from fisheating mothers and 71 children from non-fisheating mothers. In this study 75% of the original cohort were tested. If that is true then some of the non-fisheating group must have been added to the fisheaters. The fact that 31% of the children with the highest PCB levels were in the lowest percentile for activity is difficult to interpret since they represented only 4 children. There were only 13 children that had PCB blood levels above 9 ng/mL. Even if the two highest groups are combined the number of children that were "unusually quiet" would represent only a few children. The groups of highest exposed children are too small and the PCB levels too low to draw any conclusions. The authors have overinterpreted and perhaps misrepresented their data.

It is stated in the paper "Effects on activity are negligible unless the infant has breastfed for at least 1 year. Reduced activity is the only effect found to be associated with breastfeeding at general population levels of PCB exposure in the studies conducted to date." However, again this number of children

although not given can be presumed to be small. Only 45 mothers appear to have nursed their infants for 5 months and no information is given on food supplements given to infants that nursed for more than 6 months. Other confounders were not adequately evaluated. For instance, some of the children had body burdens of PBBs. The children also had measurable serum DDT and blood lead levels.

It is also highly unusual to suddenly enrich a cohort with another source of children. Finally, the age range of the children in the fisheating cohort is 1.5 years since they were recruited between July 1980 and December 1981. Testing children over such a time span could have introduced inconsistencies that go far beyond the median interobserver reliabilities for examiner-rated activity which were $r = .75$, $R^2 = .56$ and $.74$, $R^2 = 0.54$) for the first and second visits, respectively. Test-retest reliability, available for 35 children on the second visit, was poor ($r = .19$, $R^2 = 0.04$).

Intercorrelations among the mother's EAS activity rating and the examiner activity ratings from the two home visits were low (r's ranged from .13 to .23, $R^2 = 0.017$ to $R^2 = 0.053$, mean was not given). Overall these correlations suggest that there was a great degree of variability and inconsistency in the tests if different examiners were compared. The children apparently also performed quite differently at different times. Given these uncertainties no conclusions can be drawn from these data.

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Methods

The sample comprised 236 children (75.4%) recruited in 1980-1981 when 8,482 women delivered infants in four western Michigan hospitals were surveyed regarding their consumption of Lake Michigan fish. "Contaminated-fish consumption" was defined as the weighted sum of annual Lake Michigan fish consumption in the present or past, whichever was greater. All women consuming at least 11.8 kg during a 6-year period (4.0% of those interviewed) and a 4.6% random sample of those who did not consume these fish were invited into the study. The Lake Michigan fisheaters were overrepresented by a ratio of 3:1 in the final sample and on average ate the equivalent of two to three salmon or lake trout meals per month.

The children assessed at 4 years did not differ from those lost to follow up based on cord and maternal serum PCB levels, but had a higher socioeconomic status, higher maternal fish consumption and higher milk PCB levels. (Since cord and maternal PCB serum and milk levels were not available for some of the participants it is unclear how this determination was made.)

Prenatal exposure was based on umbilical cord serum PCB concentration. Postnatal exposure estimates were based on maternal milk PCB level and duration of nursing. Current body burden was defined as the PCB level in a serum sample obtained from the child at 4 years of age. Cord serum PCB levels were available for only 146 (62%) children. Of the 172 mothers who breastfed their infants (mean ± SD, 29.6 ± 29.0 weeks), 120 provided milk samples shortly after returning home from the hospital (between 1 - 16) weeks. Serum specimens were obtained from 178 children at age 4 and from 27 children at age 5. Of the pesticides screened only DDT was detected in sera.

Cognitive assessments. The McCarthy Scales of Children's Abilities were administered to each child in his or her home at age 4 (range 3.9-4.5 years). The McCarthy Scales were supplemented by the Beery Test of Visual-Motor Integration and the Peabody Picture Vocabulary Test-Revised. All five examiners were trained and supervised by one of us (S.W.J.). Interobserver reliability for the items in the McCarthy Scales requiring discretionary judgment ranged from 94% to 100% (median 99%).

Of the 236 children, 17 failed to cooperate with parts of the testing procedure. Their data were not included. The excluded children did not differ from the others in the sample in terms of their cord ($p = 0.97$) or 4-year serum ($p = 0.86$) PCB levels, their mothers' milk PCB levels were significantly higher than those of the other children ($p<0.001$).

Confounders examined

- Delivery complications (yes/no)
- Gravidity of child
- Maternal alcohol consumption before pregnancy†, during pregnancy†
- Maternal smoking before pregnancy, during pregnancy
- Polybrominated biphenyls (cord serum, maternal milk, and 4-year serum levels)
- DDT--5-yr serum level
- Lead--5-yr blood level
- Child's sex
- Age of child at testing
- Medications within 24 hours of testing‡
- Examiner

†Based on beer, wine and hard liquor consumption reported by the mother and summarized in terms of absolute alcohol per day.
‡Antihistamines, cough syrup, or seizure medication, coded present or absent.

Control variables were included in multivariate analyses based on the premise that a potential confounder cannot be the cause of an observed deficit unless related to exposure and outcome. The exposure, outcome, and control variables were checked initially for normalcy of distribution. Cord and 4-year serum PCB measures and alcohol, smoking, PBB, and DDT measures were positively skewed (skew >2.5) and were normalized by means of the following: log X + 1 transformation.

Each of the six McCarthy Scales was evaluated in three stepwise multiple regression analyses: one for prenatal exposure, based on cord serum PCB level; one for postnatal exposure from nursing, based on maternal milk PCB level and weeks of nursing; and one for 4-year body burden, based on 4-year serum PCB level. The potential confounders of the exposure were entered in the first step of each regression analysis; the exposure measure (or measures) was entered at the second step. A toxic effect was inferred only if the effect of exposure level remained significant ($p<0.05$) after the effects of the potential confounders had been removed. If a toxic effect was indicated, dose dependence was tested by division of exposure levels into discrete groups and computation of an analysis of covariance that adjusted for the potential confounders. A dose-response relationship was inferred if the adjusted mean scores for the discrete groups declined monotonically with increasing levels of exposure.

Results

Overall cord serum PCB levels averaged 2.5 ± 2.0 ng/mL (mean ± SD); maternal serum, 5.9 ± 3.6 ng/mL; and maternal milk, 835.9 ± 388.4 ng/mL. Postnatal exposure from nursing was the principal determinant of PCB body burden at 4 years of age. Mean 4-year serum PCB levels were 5.1 ± 3.9 ng/mL for children who were breastfed for at least 6 months, 1.2 ± 1.6 ng/mL for those who were breastfed for less than 6 months, and 0.3 ± 0.7 ng/mL for those who were not breastfed. Cord serum PCB level was unrelated to 4-year body burden. Cord serum PCB levels were 2.5 ± 2.1, 2.2 ± 1.6, and 3.0 ± 2.4 ng/mL for the three breastfeeding groups, respectively.

A higher cord serum PCB level predicted poorer performance on Verbal and Memory Scales. The Memory Scale, with the most highly exposed children scoring 6.6 points (0.66 SD) lower than the least exposed children. (Since PCBs could not be quantitated on many of the cord sera it is unclear how these scores were related to PCB cord serum or if only a subgroup of a few children were evaluated here or if half of the detection limit was used for children without PCB cord serum levels.)

Prenatal PCB exposure was associated with poorer performance on two subtests involving short-term memory: verbal memory, and numerical memory. There were no significant effects on the other subtests constituting these scales.

A higher PCB level in maternal milk predicted poorer performance on the McCarthy Memory Scale ($B = -0.27$, $p = 0.01$). The effect on the quantitative scale was not statistically significant ($B = -0.20$, $p = 0.07$). Duration of nursing, by contrast, was positively related to Memory Scale ($B = 0.23$, $p = 0.05$) and Verbal Scale ($B = 0.25$, $p = 0.04$) performance.

Although milk PCB level was related to Memory Scale performance in a dose-dependent fashion, a post-hoc Neuman-Keuls Test showed that only the most exposed group (>1.25 ppm) differed significantly from the other groups ($p<0.05$). The children in this group scored 9.2 adjusted scale points (0.92 SD) lower on average than the others in the sample.

The greatest quantities of contaminated milk were consumed by the infants whose mothers had PCB levels of at least 1250 ng/mL in their milk and who were breastfed for more than 9 months. Nevertheless, this group performed better on the McCarthy Memory Scale than children in the group with the highest PCB level who nursed for shorter periods. Because the memory deficit was apparently unrelated to amount of contaminated milk consumed, the effect of maternal milk PCB level on memory performance presumably reflected greater prenatal, rather than nursing exposure (the beneficial effect of nursing was apparently not considered here).

There were no significant effects of 4-year PCB body burden on the McCarthy Scales, VMI or PPVT-R.

Critical Comments

In this study 4-year-old children who were part of the "Michigan fisheating cohort" were tested with the McCarthy Memory Scale. It is claimed that children with higher cord PCB sera had slightly poorer short-term memory functioning (0.66-0.92 SD on the average). However, there was no correlation with length of breastfeeding and higher PCB milk levels. Since the poorer short-term memory was within one SD, they are within the normal range. Furthermore, levels of blood lead, serum DDT and PBB measured at 4 and 5 years of age would not be predictive of in utero exposure. The authors themselves established that PCBs measured at this age were not predictive of in utero exposure.

This study reconfirms that the exposure in this population is extremely low, not well defined. The differences between high and low exposure are meaningless since they are to some extent within the "noise" (variability) of the analytical method. Furthermore, the mothers of children with higher cord sera did not necessarily have higher PCB milk levels. This is curious since body burdens of mothers with higher milk levels should be higher and the fetus of those mothers should have had higher exposure. It is of course highly likely that the few milk samples that were collected during these longitudinal studies do not adequately predict body burdens. The differences observed in the memory tests have no clinical significance and no information was given on the expected variability of these memory tests in the general population. Thus, the associations observed most likely occurred by chance. Furthermore, the effect of breastfeeding on mental development was not adequately examined.

Many of the children (38%) had no cord PCB serum determinations. It is unclear whether all children were included in the analyses or only those that had PCB cord serum determinations. Also dividing children into subsets to determine whether a "dose response" relationship exists is arbitrary. A regression analysis should have been done. In conclusion, in the paper the methods are poorly described. It is unclear how the authors arrived at the results they presented.

15. Jacobson J.L., Jacobson S.W. *34th Conference of the International Association for Great Lakes Research*, June 2-6, 1991.

Two hundred and thirty-six children, recruited at birth on the basis of maternal consumption of Lake Michigan fish, participated in a longitudinal study. Prenatal exposure (umbilical cord serum PCB level) predicted poorer short-term memory function in four assessments: visual recognition memory at 7 months and verbal, quantitative, and pictorial memory at 4 years. Contemporary body

burden (assessed by 4-year serum PCB level) was associated with reduced activity level. These effects cannot be attributed to a broad range of confounding variables. Exposure from nursing was unrelated to cognitive function. These subtle deficits could have a significant impact on acquisition of reading and arithmetic skills in later childhood according to the authors.

Critical Comments

This is a review article and the reader is referred to the original articles which are:

Jacobson J.L., Jacobson S.W., Humphrey H.E.B. Effects of exposure to PCBs and related compounds on growth and activity in children. *Neurotox Teratol.* 1990;12:319-326.

Jacobson S.W., Fein G.G., Jacobson J.L., Schwartz P.M., Dowler J.K. The effect of intrauterine PCB exposure on visual recognition memory. *Child Development.* 1985;56:853-860.

16. Jacobson J.L., Jacobson S.W., Padgett R.J., Brumitt G.A., Billings R.L. Effects of prenatal PCB exposure on cognitive processing efficiency and sustained attention. ***Developmental Psychology.*** **1992;(28)2:297-306.**

Cognitive processing efficiency and sustained attention were evaluated in a 4-year follow-up of Michigan children exposed prenatally to PCBs. Because processing efficiency is not usually assessed in very young children, it was necessary to adapt the Sternberg (1969) short-term memory search task and to develop a new task for visual discrimination.

Methods

The sample consisted of 226, 4-year-old children who had been recruited in 1980-81 when 8,482 women delivering infants were surveyed. All women meeting an 11.8 kg criterion on a weighted measure of contaminated fish consumption over a 6-year period were invited, as was a 4.6% sample of women who did not eat Lake Michigan fish. Because many non-fisheaters also had elevated PCB levels from other sources, the non-fisheaters were not treated as a distinct control group. The children evaluated at 4 years were somewhat higher in socioeconomic status, maternal fish consumption and milk PCB levels.

Cord serum samples were available for only 143 children. Of the 167 mothers who breastfed, 118 provided milk samples. Four-year serum specimens were obtained from 177 children. Of the seven organochlorine pesticides tested, only DDT was detected in the children's sera.

The cognitive processing efficiency and sustained attention tasks were administered. The stimuli were presented and data recorded on an Apple IIe computer. The stimuli consisted of 10 drawings of familiar objects instead of the digits or letters typically used with older children. Prior to each test round, the child was shown a one-or three-item memory set. The one-item set always consisted of a fish; the three-item set, a star, a truck, and a horse. Sixteen stimuli, half of which came from the memory set, then appeared individually on the screen. The child was instructed to press a button in response to test stimuli from the memory set and to do so as quickly as possible. Each test stimulus was presented for 1,000 ms, with a 3,000-ms interstimulus interval. In the original Sternberg paradigm, a second response button is pressed in response to test stimuli not from the memory set, but this button was not used here because pilot testing indicated that 4-year-old children find it too confusing.

The visual discrimination task consisted of 24 problems using drawings from Kagan's (1965) Matching Familiar Figures Test. In each problem, the child was shown a criterion picture together with two others: one identical, the other differing in only a minor detail. The child was instructed to select the identical picture and was then asked to point to the discrepant detail. The time it took to make each selection was recorded from a stopwatch. A problem was considered correct only if the discrepant detail was identified. To make the data comparable to those provided by other processing efficiency tasks, mean RT was based only on problems receiving correct responses. Interobserver reliability among the four child examiners for recording RT on the stopwatch (Pearson r) averaged .90, $R^2 = .81$, across the 21 cases from the present sample who were assessed for reliability. Analysis of variance showed, however, that the observers' values varied by an average of 545 ms, $F(3,219) = 2.41$, $p = .07$, presumably because of differences in motor response time. RT was treated as missing for 18 children who did not respond correctly to at least four problems. Two other children refused to perform the task. (Note: it is not stated whether children were randomly assigned to the examiners or whether PCB values were available on the 20 children that did not participate. If that was the case then the number of children with PCB measurements would have been even further reduced.)

A 12-minute vigilance paradigm was adapted from Streissguth et al. (1984) for presentation on an Apple IIe computer. A criterion stimulus, a cat, appeared for 500 ms in one of three windows of a house at random intervals ranging from 4 to 48 seconds. The test was divided into three 4-minute blocks, with the cat appearing 14 times in each block. Two distractor stimuli, an apple and a butterfly, also appeared in the windows at random intervals. The child was instructed to "catch the cat" by pushing a button as soon as the cat appeared in the window. Data were not available for 11 children, because of equipment failure.

At the end of each of the home visits, the examiner completed a 10-item Child Behavior Record adapted from the Bayley Infant Behavior Record. Interobserver reliabilities (Pearson r) for 9-point scales assessing cooperativeness, emotional tone, activity, attention span, persistence in goal-directed effort, and endurance averaged .86 (R^2 = .52), .74 (R^2 = .55), .70 (R^2 = .49), .87 (R^2 = .76), and .86 (R^2 = .74), respectively.

Twenty-four control variables known or suspected to affect preschool cognitive functioning were assessed as potential confounders. Five socioenvironmental measures were moderately intercorrelated (median r = .47 (R^2 = 0.22), range = 35 to 67) as were the two maternal alcohol (r = .49, R^2 = .24) and two smoking (r = .73, R^2 = .53) measures.

Each of the child performance measures was evaluated in three separate hierarchical multiple regression analyses: one for prenatal exposure, based on cord serum PCB level; one for breastfeeding exposure, based on maternal milk PCB level and weeks of nursing; and one for current body burden, based on 4-year serum PCB level. A toxic effect was inferred only if the effect of exposure level was significant (at $p<0.05$) after adjustment for the effects of the potential confounders. Motor response time on the stopwatch were controlled by including dummy-coded variables for examiner in the first step of each regression of visual discrimination RT.

Results

The 4-year-old children had more difficulty recalling the individual stimuli. Despite the use of practice trials, the error rate averaged 6.5% on number correct and 10.2% on errors of commission, which was higher than the <5.0% reported by Keating et al. (1980).

Mean number of correct pictures selected (without regard to identification of the discrepant detail) was 15.5, or 65.4%, which is significantly better than chance, $z = 23.81$, $p<0.001$. Requiring correct identification of the discrepant detail, however, reduced mean number correct to 10.1, or 42.1%, indicating that the task was quite difficult for most of the children. As expected, performance deteriorated over the course of the vigilance task, which was designed to challenge the child's ability to maintain attention over time.

Vigilance RT, errors of omission, and errors of commission were each converted to standard scores and summed to provide a composite measure, on which higher scores indicated better sustained attention.

Higher cord serum PCB level was associated with a greater number of errors on the Sternberg memory task in a dose dependent fashion. The two highest exposed groups of children (≥ 3.0 ng/mL) received adjusted error scores that were two-thirds of an SD higher on the average than those of the lowest exposed children and were twice as likely to make 12 or more errors. The effect was statistically significant for number correct [beta = -.27, F (1,128) = 10.14, $p<0.01$] but not for errors of commission [beta = .15, F (1,128) = 3.29, p = .07]. The serum PCB levels had the following ranges: 0 - 1.49, 1.5 - 2.9, 3.0 - 4.9, 5.0 - 13. Only nine children were in the last group.

Maternal milk PCB level was associated with slower RT on the visual discrimination task. The effect was most evident at maternal milk PCB levels at or about 1.25 parts per million, with the reaction times of the highest exposed children averaging 1.2 SD slower than the least exposed group, after adjustment for confounding. In the present study, the greatest quantities of contaminated milk were consumed by the infants of mothers with milk PCB levels of at least 1,250 ng/mL who breastfed for more than 9 months. However, these children's mean visual discrimination RT was actually somewhat faster than that of other highly exposed children who had been breastfed for shorter periods. Because the processing efficiency deficit was, therefore, unrelated to amount of contaminated milk consumed, the effect of maternal milk PCB level presumably reflected greater prenatal, rather than breastfeeding exposure. (The authors do not seem to consider the beneficial effects of breastfeeding on performance.) Cord serum PCB level was also associated with poorer visual discrimination processing efficiency, although the effect was not statistically significant. A longer duration of breastfeeding was associated with better sustained attention on the vigilance task. Four-year serum PCB level was not related to any of the processing efficiency or sustained attention measures. DDT and blood lead levels were also measured in the 4-year-old children. PBB serum levels were also available; however, it is unclear at what age these measurements were made.

Discussion

The effects observed were modest, possibly owing to only moderate test-retest reliability and limited sensitivity in the assessment of exposure.

Prenatal PCB exposure was also associated with more short-term memory errors on the Sternberg. That this effect was seen despite extensive efforts to train the children to minimize errors attests to the robustness of the short-term memory deficit, which has also been found for visual pictorial memory in infants (S.W. Jacobson et al., 1985), auditory verbal and quantitative memory on the McCarthy Scales (J.L. Jacobson et al., 1990b).

Whereas short-term memory errors on the Sternberg were associated with higher cord serum PCB levels, visual discrimination processing efficiency related significantly only to maternal milk PCB level. Given that both these measures reflect prenatal PCB exposure, this inconsistency is presumably due to limitations in reliability of measurement.

Critical Comments

Only 226 children out of the original group of 313 children were tested. It is not stated why these particular children were tested and what the distribution was between fisheaters and non-fisheaters. Again the authors have inconsistencies in their data and their comparisons and statistical differences for different parameters do not follow any particular trend suggesting that these differences occurred by chance. This is supported by the fact that for many children there were no PCB determinations or they were below the limit of detection and it is not possible to evaluate these children's exposure. It is unclear how inclusion of these children affects the analysis. Furthermore, the authors did not control for the beneficial effects of extended breastfeeding on the brain. Finally, the behavioral tests that were performed were difficult for 4-year-old children and were actually adapted from tests developed for older children. These adapted tests have not been standardized. It is not clear what the results of these tests mean and how they would compare if results were obtained in a large cross section of the pediatric population of 4-year-old children.

The authors state that the deficits found were not attributable to PBB, lead or seven organochlorine pesticides measured at age 4. However, they were only able to measure DDT in the serum of the 4-year-old children. No prenatal exposure assessment for any of these chemicals was available to the authors. Since PCB levels in 4-year olds also did not correlate with the outcome measures, the effect of prenatal exposure to DDT, PBB and lead was not evaluated and cannot be dismissed based on data obtained in 4-year-old children.

17. Jacobson J.L., Jacobson S.W. A 4-year follow-up study of children born to consumers of Lake Michigan fish. *J Great Lakes Res*. 1993;19(4):776-783.

This is an overview of various studies conducted in the "Michigan" cohort. See Jacobson et al. *J Pediatr*. 1984;105:315-319. Jacobson et al. *Neurotox Teratol*. 1990;12:319-326. Jacobson et al. *Child Development*. 1985;56:853-860.

No new information is included.

18. Jacobson J.L., Jacobson S.W. The effects of perinatal exposure to polychlorinated biphenyls and related contaminants. Chapter 6 In: *Prenatal Exposure to Toxicants: Developmental Consequences*, H. L. Needleman and D. Bellinger, eds., Johns Hopkins University Press: Baltimore. 1994;130-147.

This is a review, the same data have been given in other publications: Jacobson et al. *J Pediatr.* 1990;116:38-45 and Jacobson et al. *Child Development.* 1985;56:853-860.

Although no effects were seen on the Bayley indices in the Michigan sample at five months, partial confirmation of the North Carolina finding came from analyses of Bayley Scale clusters derived using the data-reduction procedure proposed by McCall, Eichorn, and Hogarty (1977). Cord serum PCB level was associated with poorer performance on a cluster of items focusing primarily on fine motor coordination, $F(1,143) = 3.30$, $p<0.07$.

Cord serum PCB level and maternal consumption of contaminated fish were both associated in a dose-dependent fashion with poorer visual recognition memory at seven months (Jacobson et al., 1985).

Higher cord serum PCB level was associated with poorer performance on two of the five McCarthy Scales--Verbal and Memory. The strongest effect was on the Memory Scale, on which the most highly exposed children scored two-thirds of a standard deviation lower than the least exposed children after adjustment for confounding.

The duration of breastfeeding was positively related to Memory and Verbal Scale performance, apparently because the mothers who breastfed for longer periods also tended to provide more intellectual stimulation for their children.

The greatest quantities of contaminated milk were consumed by the infants of highly exposed mothers (≥ 1,250 ng/mL) who breastfed for more than nine months. However, this group performed better on the McCarthy Memory Scale than did other children who consumed highly contaminated milk for shorter periods. There were no significant effects of four-year serum PCB level on the McCarthy Scales.

Although limited in sensitivity, the cord serum and maternal milk PCB measures were high in specificity. A child with a high value on either cord serum PCB level (>5 ng/mL) or maternal milk PCB level (>1,250 ng/mL) was, therefore, categorized as "high"; a child who was not high on either measure but was moderate on cord (3.0 to 4.9 ng/mL) and/or maternal milk (1,000.0 to 1,249.9 ng/mL) was categorized as "moderate"; a child who was low on both measures was categorized as "low." This composite measure of prenatal exposure was then used in an analysis of covariance (ANCOVA) to predict McCarthy Memory score, after controlling for the confounders of both cord serum and breast milk PCB level. The adjusted mean score

for the highly exposed group was 0.7 SD lower than for the less-exposed group.

The composite measure of prenatal exposure was also used in a contingency table analysis. When compared with the sample as a whole, the most highly exposed children were more than twice as likely to score more than 1 SD below the sample mean and less than half as likely to score more than 1 SD above it. This analysis shows that the effects of prenatal exposure to PCBs on four-year memory function are not limited to a few individual children or outliers. It also illustrates that the Memory Scale decrement of 0.7 SD on the average substantially increases the likelihood that a child will score at the lower end of his or her reference group.

It was hypothesized that the deficits associated with this exposure would be subclinical and the cognitive deficits found in the present study are not readily evident to clinical observers.

Critical Comments

This is a review and the reader is referred to the original papers for detailed comments. However, it should be pointed out here as well that the exposure is poorly defined and it is unclear what these differences in subclinical behavioral effects mean. The children are all within the normal range since all observations are within 1 standard deviation from the mean. The division of high, medium and low exposure is arbitrary and may have been motivated by dividing the children into three equal groups. No information was given for normal ranges of the tests in the general population; however, the authors pointed out that their findings were subclinical. Their observations may actually have occurred by chance because of the data manipulation, the many uncertainties inherent in the data, and the small and inconsistent differences noted.

19. Jacobson J.L. Evidence for PCBs as neurodevelopmental toxicants in humans. Abstracts of the Thirteenth International Neurotoxicology Conference. *Neurotox*. 1995;16(4):752.

Evidence for the developmental neurotoxicity of PCBs at contemporary levels of environmental exposure comes primarily from two prospective longitudinal U.S. studies: the Michigan cohort and the North Carolina cohort. A reanalysis of the Michigan data based on a new, more reliable composite measure of prenatal exposure (derived from both cord serum and maternal body burden measures) confirmed results previously reported. Both studies found markedly greater vulnerability to in utero exposure. Between-study differences in the developmental outcomes affected may be due to regional differences in PCB congener pattern; greater dose concentrations when contaminated fish meals provide the primary source of exposure; or differences in socioeconomic status and/or assessment procedure.

Critical Comments

This is an abstract. The information given is insufficient for detailed evaluation.

20. Jacobson S.W. Methods for the assessment of neurodevelopmental effects in children. *Neurotox*. 1995;16(4):750.

(Abstract)

Prenatal PCB exposure was related to poorer FTII recognition memory in the Michigan and Taiwan cohorts; whereas prenatal alcohol exposure was not related to recognition memory but to slower processing speed on a new FTII measure and slower reaction time on the VExP. Poorer recognition memory and faster reactivity were detected in infants heavily exposed to cocaine. No processing speed deficits were seen in the Yu-Cheng infants. The specificity of these effects suggests that these substances operate on different sites of action in the CNS and/or differentially alter neuronal cellular and synaptic development. These new methods address questions more comparable to those studied in animal research.

Critical Comments

Too little detail is given in this abstract to evaluate the information.

21. Jacobson J.L., Jacobson S.W. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N Engl J Med*. 1996;338:783-789.

Methods

Two hundred and twelve children were examined, 68% of the 313 newborns studied in 1980-1981. The participants were similar to those lost to follow-up with respect to maternal consumption of Lake Michigan fish, duration of breast-feeding, and postnatal exposure to PCBs but were somewhat higher in prenatal exposure, socioeconomic status, and maternal age and education.

Because of limitations in the Webb-McCall method available during the early 1980s, PCBs were not detectable in 70% of the cord-serum samples and 22% of the maternal serum samples. Because placental transfer provides the sole source of fetal exposure to these compounds, which are in equilibrium in fat deposits throughout the body, maternal serum and milk concentrations provide alternatives to cord serum for evaluating prenatal exposure. To improve reliability and sensitivity in the assessment of fetal exposure, the values for cord, maternal serum and milk were converted to z scores and averaged together; serum values were included only if they exceeded the detection limit. For 11 children, no milk

specimen was available and values for cord serum and maternal serum were both undetectable; these children were assigned a prenatal exposure score at the 10th percentile of the distribution.
Each child was tested individually at home at a mean (± SD) age of 11.0 ± 0.2 years with the revised Wechsler Intelligence Scales for Children IQ test, the spelling and arithmetic subtests of the Wide Range Achievement Test -- Revised, and the word and passage-comprehension subtests of the Woodcock Reading Mastery Tests -- Revised. None of the eight examiners were aware of the children's exposure histories or any of the biochemical values. The interobserver reliability in recording the children's response times (r) ranged from 0.98 to 1.00.

One highly exposed child with an IQ of 63, who had been given a diagnosis of mental retardation, was excluded from the statistical analysis to avert undue influence of extreme scores.

Results

Maternal body burdens of PCBs (Table 1) were similar to or slightly above general population levels in the United States. The children who were breast-fed for extended periods accumulated substantial body burdens, and at four years of age many had serum concentrations similar to those of their mothers. By the age of 11, the serum concentrations had declined substantially ($p<0.001$), suggesting that there was little additional exposure after weaning.

Prenatal exposure to PCBs was associated with significantly lower full-scale and verbal IQ scores. An analysis of covariance (Fig. 1) indicated that the effect was primarily in the most highly exposed children -- that is, those with prenatal exposures equivalent to at least 1.25 μg per gram in maternal milk, 4.7 ng per milliliter in cord serum, or 9.7 ng per milliliter in maternal serum. The IQ scores of the most highly exposed group averaged 6.2 points lower than those of the other four groups, after adjustment for potential confounding variables ($P = 0.007$). The pattern of group differences in verbal and performance IQ resembled that shown for full-scale IQ.

The mean (± SD) age-equivalent level of word comprehension of the two groups with the highest exposures was 11.1 ± 1.7 years, after adjustment for confounding variables, as compared with 11.7 ± 1.7 years for the others ($P = 0.02$). No information was given about the age distribution in the different groups (the children were tested at age 11 ± 0.2 years) or the sex distributions. Furthermore, a difference between 11.1 and 11.7 years is meaningless. All cognitive outcomes that related significantly to prenatal exposure to PCBs also related to it significantly in these additional regression analyses. Among the other environmental contaminants assessed, only lead and mercury related significantly to poorer outcome after being controlled for confounding variables. A higher lead concentration when the children were four years of

age was associated with lower verbal IQ scores (P = 0.04) and verbal-comprehension scores (P = 0.04) and poorer word (P = 0.04), passage (P = 0.05), and reading (P = 0.03) comprehension; these effects were evident primarily in children with blood lead concentrations of at least 10 μg per deciliter (0.48 μmol per liter). A higher mercury concentration at 11 years of age was associated with poorer spelling (P = 0.006).

Eight of the 12 highly exposed children with low IQ scores were at least one year behind their peers in word or reading comprehension, and all but 1 were at least six months behind.

Critical Comments

Jacobson and Jacobson report that the IQs of 11 year old children were negatively affected by their in utero exposure to polychlorinated biphenyls (PCBs). The initial study population of 313 women was selected from 8482 pregnant women questioned about their Lake Michigan fish consumption for the previous 6 years. There were 242 who recalled eating enough Lake Michigan fish for inclusion and 71 who recalled eating no Lake Michigan fish. The selection process employed by the authors has been criticized elsewhere and does not constitute a random sample, limiting the ability to generalize the study results. The PCB levels previously reported for these women were similar to the general population in the early 1980s, regardless of fish consumption.

The present study population represents only 68% of the original overall study population and 69% of the fish-eating population. The authors report that the participants were similar to the 32% lost to follow-up with respect to maternal fish consumption, duration of breast feeding and postnatal exposure to PCBs but were higher in prenatal exposure, SES and maternal age and education. These differences are extremely relevant, particularly SES and maternal education, as they are related to the outcome measure of interest, I.Q. scores. A lost to follow-up rate of 32% in which there are significant differences between the two groups is not acceptable and severely compromises the validity of the present analyses.

Compounding this problem is that the cord serum, maternal serum and milk measures were "converted to z scores and averaged together." Apparently, this averaging of the z-scores was the measure of PCB determination used in the analyses to "improve reliability and sensitivity in the assessment of fetal exposure." The z-score method requires the computation of mean values and standard deviations of, for example, PCB measures in milk, for different categories of the covariates of interest (age for example) to adjust for differences (in PCB measures by age categories). A z test (large sample, e.g., >25) is done to compare the adjusted scores for the two groups. Because the adjustments use means and standard deviations of the measure of interest, any missing data

would have a significant impact on the z-score value. The authors do not adequately explain their methodology to determine exactly what they did; however, the z-score conversion is typically used when there is a small amount of missing data and performed on covariates of minor interest, as opposed to the situation here, where more than half the data are missing and they are the main measures of interest. If, in fact, the missing values were part of the z-score transformation, then it is possible that the mean values for 30% of the detectable cord serum, 78% of the detectable maternal serum and 45-65% of detectable PCB in maternal milk (there were only 138 milk samples collected on 313 women, but it is not known how many of the 138 are in the present analyses) were used as PCB measures for the missing cord serum (70%), maternal serum (22%), and breast milk (45-65%) measures which were nondetectable or unavailable. This use of z scores to create a composite PCB measure is grossly inappropriate with the distribution of missing values present in this data set and only serves to dramatically inflate or even artificially create PCB measures.

The authors performed one regression analysis for prenatal exposure to PCB and three for postnatal exposure to PCBs for each cognitive outcome. Well over 50 regression analyses were performed with various numbers of covariates in the model. With the high number of statistical tests and the small sample size, chance alone would dictate that many statistically significant differences would be found even if no "true" difference existed. The authors note that there was no relationship between postnatal exposure to PCBs and I.Q. scores, but report a negative effect on I.Q. for the prenatal measures. If one considers that the prenatal measure of PCBs was artificially created and inflated by using the z scores and that the cord and maternal sera were not adjusted for lipids this is not surprising. The postnatal PCB measures were PCB levels in maternal milk, which was adjusted for lipids, and the child's PCB serum level at 4 and 11 years of age (not lipid adjusted). Despite the fact that the serum was not lipid adjusted these postnatal measures most likely provide a more accurate measure of PCB exposure in these children.

It is not stated whether all "affected" children attended one or several different schools.

Lead exposure was assessed at 4 years of age and again at 11 years of age and mercury exposure at 11 years of age. A number of children had elevated blood lead and mercury levels. Separate regression analyses were done looking at blood levels and cognitive outcomes and were found to be related significantly to lower verbal IQ scores, verbal comprehension scores, and poorer word passage and reading comprehension. Higher mercury concentrations were associated with poorer spelling. Despite these reported findings, it does not appear that there was any attempt made to determine if the children with higher blood and mercury levels were the same children with higher PCB levels. It has been suggested that

elevated blood lead levels in early childhood can affect intellectual performance in later childhood (Needleman, 1990). In light of this, elevated blood lead levels should have been considered as a potential confounder in all of the analyses using PCB measurement as the dependent variable.

REFERENCE

Needleman H.L., Schell A., Bellinger D., et al. The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. *N Engl J Med.* 1990;322:83-88.

22. Jacobson J.L., Jacobson S.W. Prospective, longitudinal assessment of developmental neurotoxicity. *Environ Health Perspect*. 1996;104(S2):275-283.

The same information is also in Jacobson and Jacobson. In: *Advances in Infancy Research*. 1990;6:112-147.

A total of 21 children prenatally exposed to PCBs at relatively high levels are more than twice as likely to exhibit poor performance on the McCarthy Memory Scales at 4 years of age. Nevertheless, 12 of the highest exposed children performed in the normal range and 1 performed exceptionally well.

Jacobson et al. (1993) found alcohol-related deficits on the Bayley Scales only in the offspring of mothers over 30 years of age, suggesting that vulnerability may depend on physiological changes in the mother associated with a history of heavy drinking.

Critical Comments

The information in this paper has been presented in other articles and comments are made in the summaries of those articles. Apparently the authors have also published on alcohol related effects (Jacobson et al., 1993).

REFERENCE

Jacobson J.L., Jacobson S.W., Sokol R.J., Martier S.S., Ager J.W., Kaplan-Estrin M.G. Teratogenic effects of alcohol on infant development. *Alcohol: Clinical and Experimental Research*. 1993;17:174-183.

23. Schantz S.L., Jacobson J.L., Humphrey H.E.B., Jacobson S.W., Welch R., Gasior D. Determinants of polychlorinated biphenyls (PCBs) in the sera of mothers and children from Michigan farms with PCB-contaminated silos. *Arch Environ Health*. 1994;49:453-458.

The purpose of this study was to examine the degree to which maternal residence on a contaminated farm added to the established risk of PCB exposure from eating contaminated fish.

Methods

The farms were located in the lower peninsula of Michigan. All mothers with 6-12 year old children identified by the Michigan Department of Public Health by April 1988 were contacted and asked to participate. Of the 40 mothers contacted, 75% participated. The final sample consisted of 30 mothers and 44 children. The children were born between 1974 and 1981.

PCBs were quantified by adapting the Webb-McCall method to a computer data system, using Aroclors 1016 and 1260 as reference standards. The sum of 15 peaks was used for quantification of total PCBs. The limit of detection for PCBs was 3 ng/mL serum. Samples of whole blood were collected and analyzed for lead. The DL for lead was 2 μg/dl.

The relative importance of various predictor variables as determinants of PCB exposure was evaluated via hierarchical multiple regression. The regression models had power of 0.67-0.74 to detect a variance of 30% as significant (Cohen J. Statistical power analysis for the behavioral sciences. New York: Academic Press, 1977).

Results

Blood samples were obtained from 38 of 44 children and 28 of 30 mothers. PCBs were quantitated in serum samples from 42.1% of the children and 85.7% of the mothers. PBBs were not detected in the children but were present in trace amounts in 25% of the mothers. DDT was detected in 65.8% of the children and 92.6% of the mothers. Lead was detected but the levels were very low.

Years of residence on a silo farm and contaminated fish consumption were related significantly to maternal serum PCB levels. Together these two variables accounted for 29% of the variance in maternal serum PCBs.

	No. above DL*	% sample	>DL SD Mean (ng/mL)		>DL Range (ng/mL)
Children	16	42.1	6.8	5.2	3.1 - 23.3
Mothers	24	85.7	9.6	5.4	3.7 - 21.6

*Detection limit

PCB samples below the detection limit of 3 ng/mL were set to 1.5 ng/mL for the purposes of the analysis. Maternal serum PCB level and weeks of breastfeeding jointly explained 47% of the variance in the children's log serum PCB levels. Years of residence on a farm with a contaminated silo was also significantly related to the children's serum PCB levels, explaining an additional 8% of the variance. Contaminated fish consumption and age were not related significantly to the children's serum PCB levels.

As expected, weeks of breastfeeding explained a substantial amount of the variance in the children's log serum DDT level. There was no relationship between serum DDT and years of residence on a silo farm or between serum DDT and age.

Neither maternal estimates of contaminated beef and milk consumption nor actual PCB levels in scrapings from the inside of the silos were related to maternal or child serum PCB levels.

Discussion

The data clearly implicated human milk as the primary source of PCB exposure for the children even though they had already reached 6-12 years of age. Only 1 of the 13 children who were not breastfed had a serum PCB level above the detection limit, 16 of the 25 breastfed children had detectable serum PCB levels. Of the children, 66% and 93% of the mothers had detectable DDT levels.

Critical Comments

The PCB levels detected here are within the range of those found in the general population without any particular exposure.
Less than half of the children had detectable levels of PCBs while they were detectable in most of the mothers. Thus, the mothers appear to have had greater and/or larger exposure than the children. However, this can also be related to the serum lipids which usually would be higher in adults than in children. More children had detectable DDT levels than PCB levels. Measurable PCB levels were more prevalent among breastfed children. The sources of exposure to PCBs were not well identified since a large percent of the variance was not explained. This paper gives little new information. These children also had detectable DDT levels at age 4. It stands to reason that they would have had in utero DDT exposure as well. However, the prenatal DDT exposure was not

appropriately evaluated as a confounder in any of the studies performed on this cohort.

24. Schantz S.L., Jacobson J.L., Jacobson S.W., Humphrey H.E.B. Behavioral correlates of polychlorinated biphenyl (PCB) body burden in school-aged children. (Abstract) *The Toxicologist*. 1990;10:303.

This is a study of children whose PCB levels were reported in *Arch Environ Health* 1994;491:452-458. However, in this abstract a few more children are included and the mean PCB level is slightly higher. The range is the same. Not enough detail is given to evaluate this abstract. It would be important to know the age distribution in relation to the PCB serum levels.

25. Schwartz P.M., Jacobson S.W., Fein G.G., Jacobson J.L., Price H.A. Lake Michigan fish consumption as a source of polychlorinated biphenyls in human cord serum, maternal serum, and milk. *AJPH*. 1983;73:293-296.

Methods

The sample consisted of 242 women who reported moderate consumption of Lake Michigan fish over an average of 16.1 years (SD = 9.0, range = 1.0-40.0), and 71 women who reported no consumption of Lake Michigan sport fish. The principal measures of contaminated fish consumption were: 1) highest annual rate of Lake Michigan fish consumption; 2) rate of consumption during pregnancy; and 3) cumulative lifetime consumption. Each fish meal was assumed to consist of 0.2 kg of fish. All biological measures and two of the fish consumption measures (highest annual rate and consumption during pregnancy) were log transformed in the statistical analyses.

Between 1-16 weeks a milk sample was obtained from 138 women and another sample at 5 months from 45 women. The percentage of lipids in milk samples varies by the time of day and whether the sample was expressed before or after nursing. To calculate correlations involving milk values, quantitations on a fat basis were used. Exposure groups exhibited higher maternal serum levels compared with non-exposed controls.

Both fish consumption and maternal age were significant predictors of PCB levels for 193 maternal sera, yielding a multiple R of .39 ($p<0.01$). Only fish consumption predicted neonatal milk levels. (It was apparently not tested how maternal age related to fish consumption!)

Reported fish consumption levels and biological measures were compared for breastfeeding and non-breastfeeding mothers. No differences were found in reported levels of fish consumption, or in cord and maternal sera PCB levels. (Mean? PCB levels in non-fisheaters were around 4 ppb and in most fisheaters around 5-6 ppb

with a few outlier around 9 ppb based on a bar-figure in the paper. It is not explained what the bars represent.)

Contaminated fish consumption predicts PCBs in maternal milk as well as serum. Age is also a predictor of maternal serum PCB. (It was not clear whether age predicted fish consumption.)

Detectable PCB levels were found in maternal serum and milk samples whether from fisheating or from non-fisheating mothers. Eighteen (7.5%) of the human milk samples supplied by these mothers equaled or exceeded 1.5 ppm (fat weight basis).

Critical Comments

According to the authors, fish consumption and maternal age were significant predictors of PCB levels in maternal serum, yielding a multiple R of .39. Although the correlation may be statistically highly significant only 39% of the variance is explained by fish consumption and age, suggesting that there are other major past or present PCB sources that contribute to the overall exposure of this population. Possibly older participants may have eaten fish for longer periods of time and age and fish consumption would therefore be positively correlated. Fish consumption per se is a very uncertain measure of PCB exposure. The PCB measurements were only made on subsets of the cohort, either because samples were not secured or they were inadequate or levels of PCBs were below the limit of detection. For other samples they were detected but could not be quantified. The PCB serum samples for fisheaters and non-fisheaters seem to show little difference based on the information provided, although that is never explicitly stated. The amount of fish eaten and levels of PCBs in milk and in sera are only moderately correlated. It is unclear whether the people with nondetectable levels of PCBs or nonquantifiable levels of PCBs ate less fish or the same amount of fish. As a matter of fact, nowhere in this or in other papers by the same authors is information given on distribution. For instance, how many non-detectable and non quantifiable levels of PCBs were found in the samples from the fisheaters and in the samples from the non-fisheaters? How many samples were analyzed for the non-fisheaters and for the fisheaters? What were the differences between the groups? How many non-fisheaters nursed their infants? What was the distribution of the PCB milk levels for the fisheater and the non-fisheaters? Reading the various papers it appears that the PCB levels in the fisheaters and the non-fisheaters were quite similar. Thus, what is observed in the infants seems to be unrelated to PCB exposure from fish from Lake Michigan. Rather, lifestyle factors which include sports fish consumption, age of the mother, and physical constitution of the parents may be responsible for the effects noted in the infants. The correlations with slightly higher PCB levels can be explained by modifying factors of the mother such as age, alcohol consumption, and greater weight.

26. Rogan W.J., Gladen B.C., Wilcox A.J. Potential reproductive and postnatal morbidity from exposure to polychlorinated biphenyls: epidemiologic considerations. ***Environ Health Perspect.*** **1985;60:233-239.**

This paper, a presentation at the 1985 PCB meetings in North Carolina, is a review and discussion of laboratory and epidemiological data of reproductive effects in humans.

The discussion of toxicokenetics of PCBs is oversimplified and in some cases misrepresented. The excretion of PCBs from the body is not linear but follows first order kinetics. The lower the dose the slower the excretion and the higher the dose the faster the excretion. PCBs are distributed throughout the body but since they are lipophilic they are distributed proportionally to the amount of fat in cells; except for the brain where concentrations are lower than expected. The balance of PCB concentrations between various tissue compartments is a changing dynamic process.

The epidemiology of the effects of PCBs on human reproduction is reviewed and possible research ideas are presented. The authors discuss the teratology of PCBs that have been in heat exchangers, known from the two accidents resulting in Yusho and Yu-Cheng. The authors neglect to report that the teratological agent in those two accidents was not the PCBs per se but the polychlorinated dibenzofurans created when the PCBs were heated. A short paragraph on lactation as an indicator of exposure to the mother and as a vector of exposure to the infant is presented. The final section is on development of the infant. The importance of studying potential toxicity to the reproductive function, particularly in women is noted. The authors discuss the problems with the present analytical methods for compounds such as PCBs. Variation between labs, as well as within labs on duplicate samples is known to occur. The difficulty of interpreting a positive value is noted.

Comments

Since this is a review rather than presentation of a study, there are no critical comments.

27. Rogan W.J., Gladen B.C. Study of human lactation for effects of environmental contaminants: the North Carolina breast milk and formula project and some other ideas. *Environ Health Perspect.* 1985;60:215-221.

This paper was also a presentation at the 1985 PCB meetings. The authors reviewed the physiology of lactation, and the clinical course of breast fed children to identify adverse outcomes attributable to the environmental chemicals in question. Since the authors were involved with a large clinical study in which they measured PCBs and DDE in milk and gathered morbidity data they discussed the questions in the context of the design and conduct of that study. They also presented preliminary results.

A prospective cohort study was designed and PCBs were measured in maternal blood, cord blood, placenta, milk and formula. The association of PCBs and DDT to perinatal and developmental effects was examined. The determinants of maternal concentration of PCBs such as diet, occupation, cigarette and alcohol use, weight and age were reportedly measured but there is no discussion of exactly how this was done.

Data were gathered during physical examination of the child, medical history provided by the mother, and a review of medical records to confirm illnesses. A standard physical was made up with special attention to liver and spleen size and rashes. Several question regarding weaning, when and why were asked of the mother.

The effects on central nervous system were measured using the Brazelton Neonatal Behavioral Assessment Scale. For children 6 months to two years the Bayley Scales of Infant Development were used and at age three the McCarthy Scales of Children's Ability. Follow-up was extended to 5 years. The first baby was enrolled in April 1978 and the 856th in October 1982. Volunteers were recruited from 3 sources in North Carolina. Volunteers were found through hospital tour presentations, Lamaze and childbirth classes and by word of mouth.

During admission for delivery maternal blood, cord blood, placenta and a sample of whatever the infant was eating (colostrum or formula) were collected. The infant was examined, the Brazelton was performed and the obstetric and nursery records were reviewed. At six weeks the infant was seen again, a physical was performed, the medical history obtained and blood, milk or formula were collected. A similar visit occurred at 3 months (no maternal blood collected). At 6 months the Bayley's scales were done. Visits were also at 1 year, 18 months, 2 years and then yearly until age 5. The visits consisted of a history, physical, medical and hospital review and Bayley or McCarthy scales. Milk was collected as long as the mother was lactating.

Universal contamination of milk by PCBs and DDE were found. No relationship between age, race, occupation or cigarette smoking and PCBs were found. Regular alcohol use was associated with higher PCB levels. PCB levels declined over the course of lactation, values at 6 months were about 20% lower than in the beginning of lactation. The authors were unable to confirm the previously reported association between PCB and prematurity. The correlation between PCB level and birth weight was essentially zero.

Comments

This paper is primarily a review paper and does not provide sufficient information to evaluate the methodology and preliminary findings reported.

28. Rogan W.J., Galden B.C., McKinney J.D., et al. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichlorothene (DDE) in human milk: effects of maternal factors and previous lactation. *AJPH*. 1986;76:172-177.

This is the first report on the North Carolina cohort of mothers and infants. The authors introduce the topic of PCB use, environmental contamination and the two known PCB poisonings Yusho and Yu-Cheng. Animal data are reviewed and the lack of human data discussed. The results of the PCB and DDE analysis of human milk are presented in this publication. This review and all the others of the North Carolina cohort will be limited to the discussion of PCBs.

Study Design

Volunteers were recruited from hospital tours, Lamaze classes and from private and public prenatal clinics. No attempt was made to assemble a random sample. At or near term, a questionnaire was administered to the mother. Milk, colostrum or formula and blood, cord and maternal blood, and placenta were collected. Initial follow-up visits took place at 6 weeks, 3 months and 6 months postpartum. At each visit, formula or breast milk were collected. At the 6 week visit, another blood sample was taken from the mother. Children were seen again at 6 month intervals until age 2 and then yearly. Milk samples were collected as long as the women were lactating. In some cases an extra milk sample was taken at 9 months although no regular visit was scheduled. (No explanation for this is provided.) There is no discussion of the protocol for obtaining milk samples. Was the milk expressed or pumped, was a grab sample used or was a breast emptied at each feeding. These are important factors as the milk fat content of a given "feeding" changes over the course of it. This is a significant oversight in this report.

Chemical Methods

The specimens for chemical analysis were collected according to a protocol developed by the Chemistry Branch of NIEHS. This protocol is not described. The samples were shipped on dry ice to NIEHS where they were split, archived if possible and sent to a private laboratory in Wisconsin for analysis. The lab in Wisconsin performed lipid estimation and analyzed for PCBs and DDE by gas liquid chromatography. The exact methods are reported in another publication. (McKinney JD, Moore L, et al. Validation, extraction and cleanup procedures for polychlorinated biphenyls and DDE in human body fluids and infant formula. *J Assoc Off Anal Chem.* 1984;67;122-129.)

The authors state that "the PCBs in placenta are reported as "grams of chemical per gram of placenta." Serum is also reported without adjustment (as it is). The milk samples are reported as "fat adjusted, grams of chemical per gram milk fat." The authors give no further explanation on these measures or why they used these unusual measures.

Statistical Methods

The authors state that the chemical data were analyzed using analysis of variance applied to the logarithms of the original data. The data were skewed to the right and therefore log transformed. Descriptive statistics were also obtained on the log scale and then re-transformed. In some cases the levels of chemicals were below the limit of detection. In order to account for these unquantifiable observations, the authors report using techniques for censored data and present Kaplan-Meier estimates. The exact method used is not discussed, although, Kaplan-Meier estimates are typically used to calculate survival functions.

The authors report that "In order to examine whether or not the levels of chemicals depend on various characteristics of the mother it was necessary to use comparable levels for all women. Not all women provided samples at each visit, and not all women breast-fed. Therefore, the values from all samples from a given woman were combined and expressed as an estimated amount of chemical in milk at birth. Three steps were taken to accomplish this. First, any levels below the detection limit were changed to an estimated amount. The assumption was made that the chemical levels had a log normal distribution. The parameters were estimated and used as an estimate of the expected value conditional on being less than the quantitation limit. Second, levels were multiplied by a scale factor to make them all comparable to milk at birth. This scale factor is the median ratio of birth milk samples to the sample in question. It adjusts for the declining milk values over time and also for the fact that blood levels are substantially lower than milk levels. Third, all available levels were averaged. Cord blood and placenta samples were not used in this averaging as most of

them fell below the limit of detection and could not be reliably estimated." There is no further explanation of this estimated process or how the parameters for the non-detect levels were actually calculated, or what these parameters were. The authors report that the correlation between the estimated amount in milk and the actual amount (where the actual amount was available and above the quantitation limit) was 0.86. This "estimated chemical in milk at birth" is the measurement of PCB used in all statistical analyses. Because PCBs are difficult to measure, even in the best of circumstances, yielding within samples variation from 25-50%, manipulating the data by averaging and scaling does not seem prudent. Correlating this measure to the 'real' measure is relatively meaningless given the potential variation of 25-50% in PCB measurements.

Characteristics of Participants

Of the 931 children originally entered into the study, 856 children participated past the original neonatal contact. There were 6 sets of twins and 45 pairs of non-twin siblings. Ninety two percent of the women were white, 53% had a college education and 41% had a professional occupation. The median age was 27 (means are not reported) and 43% were primipara. Eighteen percent of the women smoked, 40% reported using alcohol at least once a week and 21% reported eating fish that was caught as opposed to purchased at least once during pregnancy. The authors do not explain whether these factors, alcohol consumption and smoking occurred prior to or during pregnancy. Eighty-eight percent of the women nursed to some extent. The median time of nursing was 29 weeks. Twenty-nine percent of women had previously breast-fed a child.

Chemical Levels

The results of PCB analysis of milk and blood are presented in Table 1 for PCBs and Table 2 for p,p'-DDE (dichlorodiphenyl dichloroethene). The authors present the number of samples, median, 95th percentile, maximum and percent in the non-detect range. As stated in the statistical methods section "the percentiles presented in Tables 1 and 2 are Kaplan-Meier estimates." There are several problems with these tables. First there is no indication as to whether the differences in the 'n' representing each visit are a result of a decline in the breast feeding (women stopped breast feeding) or whether a sample was not collected. The authors mention that these differences exist but do not elaborate. It is also clear that measures taken of maternal serum at birth and "estimated milk" at birth in some of the women who did not continue in the study were used in the analyses. There were 880 women who agreed to participate in the study, 71 dropped out and 12 of these did not give samples. (Why these women dropped out, and how they differed from the remaining group is not addressed.) Of the remaining 807 women they enrolled 856 children. The reported number of siblings is confusing and none of the

numbers presented add up to the 856 children. The authors report in Tables 1 and 2, 872 maternal sera at birth and 915 or 919 estimated milk samples at birth, this is from at most 856 pregnancies. These discrepancies are not readily deciphered by the reader.

PCB levels in milk at birth averaged 1.8 ppm (fat basis) and 2.5 ppm DDE. Levels were higher in milk than in serum and higher in maternal serum than in placenta. These differences reflect the differences in the fat content. PCBs in cord blood were almost always below the limit of detection while average levels for DDE were 3.95 ppb. The authors report that "correlations among the various samples taken from the same women were quite high" and refer the reader to Tables 3-4. The authors report that the correlation between milk and blood taken at different times were about 0.7-0.8 and for samples taken at the same time was about 0.7. Different times is not defined by the authors. Does it mean different visits, different times of the day, etc.?

Relation of Chemical Levels to Characteristics of Mother

Using the summary measure of estimated PCB amount in milk at birth the authors present the distributions for race, age and occupation using mean values and 95% confidence intervals. The number of individuals in each cell are not indicated nor are there any statistical tests for differences in mean levels to support the authors contentions; such as, the statement "showed effects of occupation." The use of confidence intervals with the presentation of mean values is highly unusual. Simple standard deviations would be much more informative and less confusing for the reader. The authors note that primipara women had 11% higher levels of PCB and women who consumed at least one drink per week had 13% higher PCB levels. The data for these increases are not presented nor are the results of any statistical testing to support these statements. Data on 45 pairs of non-twin siblings illustrate that PCB levels for the second recorded lactation are lower than for the first. This was noted for all but 7 of the pairs. The median percent change was a 26% drop for PCBs. The authors fail to note that PCB measurements may vary from 25-50% and again no statistical testing was reported. Levels of PCBs and DDE increase with age and DDE levels are 15% higher in smokers. PCB levels are higher in women consuming alcohol.

Discussion

The authors remind the reader that the population in this study was not randomly selected; however, the authors feel that it is reasonable to assume that the values found are typical. The values of PCBs reported here are somewhat higher than those reported in other studies. The authors discuss these other reports and their findings. The authors suggest that the reason for the higher levels in their participants is in the sensitivity of the

analytical method used. They state "the strength of the method is in the extraction and clean-up procedures designed to remove PCBs quantitatively from the sample matrix." The issue of quantifying PCBs is highly complex and in the best of circumstances yields results that can vary as much as 25-50% from the 'true' measure. The complexity and difficulties in PCB quantitation have not been adequately addressed by these authors.

The authors report that "one of the more striking of our findings is the demonstration of a substantial decline in the levels of both chemicals over the course of lactation..." These data, while alluded to by the presentation of the median levels and 95th percentiles for each visit, are not adequately presented anywhere in the tables or in the text and most importantly these changes are not statistically analyzed to determine whether the variation is within the normal range. In other studies a decline in blood levels but inconsistent or no declines in breast milk were noted.

The authors report that "besides previous lactation, the most important predictors of chemical levels were age and race." This statement is completely unfounded based on the presentation of data is this paper. Nowhere is there a discussion of statistical testing to confirm the validity of this. The authors report that the association of regular alcohol consumption with PCB levels was not expected and suggested they expected the opposite. The 'association' with alcohol was not tested for statistical significance and had the authors done their homework they would have expected it (Kreiss et al. *JAMA.* 1981;245:24).

The authors conclude with the "Our findings of clear declines with both current and prior breast-feeding indicate exposure of the child. For the infant, the PCB values at least are in a range that overlap exposures at which the most sensitive in vitro systems respond." Based on the presentation of data in this paper this statement is completely unfounded.

Comments

There are several problems with this paper, the most serious is the lack of any presentation or discussion of statistical testing to determine whether any of the differences found were variation with the normal range or were simply a result of chance alone. Other problems include:

1. The numbers don't add up. The abstract reports 868 women, the paper reports 856 in the original group and 807 in the final group. There are 931 children in the first group and 856 in the final group. By my estimate there should be 858 samples of at least maternal blood serum and there are 872 reported in Table 1. The numbers presented may be accurate but the reader has no way of knowing because of the poor presentation of the data.

2. The description of the chemical methods (while described adequately in a separate publication) should have included a discussion of the methodological problems when quantifying PCBs, which are considerable. The average reader would have no idea that PCB measures typically vary by 25-50%, in the best of conditions. Because the entire research issue hinges on the PCB measurements the reader should have been informed as to the rather significant problems with measuring PCBs.

3. The statistical analyses in this paper are extremely difficult to decipher. The choice of statistics is inappropriate and the explanation of results is incomprehensible. The manipulation of the data done "In order to examine whether the levels of chemicals depend on various characteristics of the mother it was necessary to use comparable levels for all women...." is so poorly described that the reader has no hope of understanding what they did or why, let alone of critically appraising it.

The lack of presentation of the statistical testing for the reported differences in PCB levels by age alcohol consumption and parity is highly questionable. The differences reported may be within the normal range of variation and may mean nothing.

The authors report that "the chemical data were analyzed using analysis of variance techniques applied to the logarithms of the original data." Nowhere in the text is there another reference to analysis of variance. This serves to confuse the reader and may lead some to erroneously interpret the results presented in the tables as results from analysis of variance.

Finally, because this report is the first in a series of reports on the North Carolina project, and the one the authors will cite as their report on methodology the problems with this paper take on an added dimension. A study of this magnitude merits a comprehensive in-depth discussion of the methodological issues, the authors approach to those problems and proper statistical testing and presentation of the data.

29. Rogan W.J., Gladen B.C., McKinney J.D., Carreras N., Hardy P., Thullen J., Tinglestad J., Tully M. Neonatal effects of transplacental exposure to PCBs and DDE. *J Pediatr.* 1986;109:335-341.

This is the second report for the North Carolina Cohort of women and infants. The authors discuss PCB and DDT use, environmental contamination and the two known PCB poisonings Yusho and Yu-Cheng. The authors state that they "began the project in 1978 to attempt to identify any morbidity that might be occurring in breast-fed children as a consequence of PCB or DDE contamination. The data from that study also allowed us to examine whether there were detectable effects of prenatal exposure to these compounds."

Methods

The North Carolina Breast Milk and Formula Project is a prospective birth cohort of 930 children born between 1978 and 1982 and followed through the present day. The authors report that some infants dropped out immediately but have at least partial information for 912 infants. The authors refer the reader to a previously reviewed paper (*AJPH*. 1986;76:172-177) for details of study design, description of the cohort and the levels of contamination. Three centers each contributed about 300 subjects, no mention of how these women were recruited is made, only that they were recruited at or near term and were disqualified only if they were unable or unwilling to participate for at least 6 months of follow-up. The authors made no attempt to randomize the sample.

During the hospitalization for delivery a questionnaire was administered to the mother. Samples of placenta, maternal and cord serum and milk/colostrum were collected following delivery. A physical examination of the infant was performed as well as the Brazelton Neonatal Behavioral Assessment Scales (BNBAS). Following discharge the authors examined the medical records of the mother and infant (purpose not stated). The BNBAS was done in the presence of the parents by staff trained in its use at centers certified by members of Dr. Brazelton's staff. The authors do not provide sufficient details regarding the staff who performed the BNBAS, such as the numbers and whether they were site specific. The authors do however, note that "the scores were monitored for drift over time and for intercenter differences." The authors report minor differences that were discussed among the examiners at meetings. Fifty-nine percent of the BNBAS examinations were done during the first week of life, 20% in the second week and 16% in the third week.

The children were seen again at 6,12,18 and 24 months of age and then yearly. Milk was collected from the mothers until lactation stopped. There is no mention by the authors of how milk was collected, either in this paper or the previous one where the methods are reported. Was a milk sample expressed at the visit, or was the sample collected at home by expressing or mechanical pumping? How large was the sample?

The results of the chemical analyses were blind to the mother and the examiner, except for mothers who had two children in the study, they were aware of the previous child's results. The authors go on to report that to get comparable levels on all women they combined the results from all available samples from each woman into a single overall summary of her body burden which was expressed as estimated concentration of PCBs in milk fat at birth. The computations involved in creating this average are given elsewhere and the authors cite their previous publication. As discussed in the previous review the appropriateness of the "estimated milk at birth" measures are questionable. Birth weight and head

circumference at birth were taken from the infant's chart, if not available they were obtained on physical examination. Jaundice was assessed as jaundice noted on the chart and if so, whether it was treated.

Brazelton Scale Summaries

The BNBAS yields results on 27 behavioral scales and 20 reflexes. The authors used the truncated scales used by Jacobson et al. (see Michigan Studies in this document), which consists of seven cluster scores: the response decrement cluster, the orientation cluster score, the alertness scale, the tonicity cluster score, range of state cluster score, the regulation-of-states cluster score and the autonomic maturity cluster score. The present authors also created two subscores for reflexes by counting the number that were hyper-reflexive (high) and the number that were hypo-reflexive (not elicited or low).

Statistical Analysis

Separate regression analyses were performed for PCB levels (expressed as the estimated milk at birth measure) and birth weight, head circumference and the BNBAS scores. Jaundice was analyzed by logistic regression. The following potential confounders were added to the regression model analyzing birth weight: infant's race, sex, mother's age, education, occupation, whether the mother smoked or had more than one drink a week, parity, maternal weight and the center at which she was enrolled. The regression model for head circumference included all of the above with the addition of birth weight. Covariates for jaundice were the center indicator and whether the infant was breast-fed. For the BNBAS scores the regression model also included mother's age, education, occupation, whether the mother smoked or had more than one drink per week and whether the mother consumed sport fish during pregnancy and whether she had general anaesthesia during delivery. The infant's race, sex and birth weight, whether the baby had jaundice, the age at which the BNBAS was administered and the number of hours since the infant had eaten were also in the model. The authors report that terms were also included to account for differences among the various centers and examiners.

Gestational age is conspicuously missing as a possible confounder as are indications of whether the birth was a normal vaginal or cesarean delivery. Apgar scores could have been used as measure of infant distress following delivery.

Results

The authors fail to report the number of infants that were given the BNBAS, or how this number relates to the overall number in the cohort. In the tables the numbers add up to 866, but in the methods it is reported that they had at least partial information

on 912 infants, suggesting that they had BNBAS results on 912 infants. This is a serious oversight on the part of the authors. The authors report no association between the estimated PCB in milk fat levels and birth weight. They report the familiar weight decrement associated with smoking and the male-female differences. The only other significant factor was maternal weight--larger mothers had larger babies. There was no association between head circumference and estimated PCB in milk fat. Head circumference did vary by the infant's birth weight, sex and the mother's education and occupation. There was no association between the estimated PCB in milk fat and hyperbilirubinemia. The authors report pronounced differences between breast-fed and bottle-fed infants with breast-fed infants having more jaundice.

For the BNBAS, only the tonicity and reflex scores were significantly associated by the estimate of PCBs or DDE in milk fat. The authors direct the reader to Table I to illustrate "the tonicity cluster score was affected by PCBs". Neither Table 1 nor the discussion in the text report any statistical testing (the results of the regression analysis) to support this statement. There is no way to determine whether the difference seen in Table I is normal variation or significantly different from what one would expect to see. The same problem exists with Table II in which the authors assert that "When we separated the abnormal reflexes into high and low, we found that both chemicals (PCBs and DDE) were associated with hyporeflexia.....Again, the PCB effect was present only at the highest doses whereas the DDE effect was a more gradual upward trend...." Statements such as these need to be accompanied by results of statistical testing and the associated significance level. In addition to these cluster scores, there were borderline effects of DDE on the regulation-of-states cluster with higher levels of DDE producing higher scores ($P = 0.06$) and of PCBs on the response decrement cluster with higher levels of PCBs producing lower scores ($P = 0.07$).

The authors go on to report their use of the four clusters of Als et al, without any explanation of what these might be in the previous methods section. They report that the results were similar. "The only cluster affected by the chemicals was the motor cluster which includes the number of abnormal reflexes, the general tone scale and the activity scale among others."

The authors report screening 54 potential confounders. The variables were chosen a priori as potentially relevant to the status of the child in the neonatal period. This list is not presented but said to be what others have used. Ten of these variables showed a relationship to PCBs or DDE at $p<0.10$: protein in urine during pregnancy, kidney or urinary tract infection during pregnancy, pneumonia or bronchitis during pregnancy, vaginitis during pregnancy, chronic thyroid problems, chronic arthritis, classification of the pregnancy as high risk, emergency cesarean section, low forceps or suction delivery and ABO incompatibility.

These 10 variables were added into the model but there was reportedly little change. None of the 10 factors were related to tonicity cluster score; however, infants who were delivered by cesarean section or with low forceps or suction had greater numbers of abnormal reflexes, but this reportedly did not change the significance of PCBs. The authors fail to provide the distribution of these 10 factors, such as how many cesarean sections there were and what the estimated PCB in milk fat at birth levels were in these women. This discussion of confounders is out of place in the results section, it belongs in the methods section. It is unclear as to which model these variables were used in or why they were not added to all of the regression models.

The authors present the results for infants who had their testing done prior to the 3 days recommended by Brazelton. The sample is halved, however, the authors state "the effects of PCBs on hyporeflexia remain significant.....the effect of PCBs on tonicity no longer achieves statistical significance, but it is unchanged in size."

Discussion

Direct comparisons of these results with the Michigan cohort, as the authors point out, are not possible because of the differences in chemical analytical methods. The authors note that their levels overlapped or were higher than those in women who consumed potentially contaminated fish. The authors suggest that because of this the North Carolina infants were probably at the high end of the non-occupationally exposed. Why this would be is not known.

The authors note the lack of an association seen between birth weight and PCBs or DDE as has been reported by several other authors, but have no explanation for this. The association between hypotonicity and hyporeflexia are seen by the authors as persuasive evidence of a potential fetotoxic effect of PCBs because they were able to document exposure analytically rather than inferring it from historical items as with the Jacobson study. The authors concede that there remains the possibility that even the measured amount of PCBs is a surrogate for some other agent, although unlikely, "because PCBs and DDE constitute the majority of persistent organochlorine compounds stored in humans and a potential confounding toxin would have to correlate strongly with both PCBs and the BNBAS results." They go on to discuss some of the potential mechanisms by which PCBs could have produced the reported effects.

Comments

This paper, the second report of the North Carolina cohort is plagued by the problems of their first report. They direct the reader to the first paper for study design and methodological issues which, as discussed, were inadequately presented. There is no discussion of the methodological problems and technical difficulties of measuring PCBs which are less of a problem for the measurement of DDE. Because this paper uses the estimated PCB in milk fat at birth as the measurement of PCBs it should have been fully described in this paper. The reader is at a disadvantage without this information. The other measurement of import is the BNBAS. These exams are based on the subjective impression of the examiner and therefore reliability of the testers becomes a great concern. The issue of inter-intra reliability is not adequately discussed. The authors state that "if some of the scales were missing for an infant we averaged the remaining ones." The appropriateness of this manipulation is not known. The authors fail to provide information on how many scales are missing for each infant and how many infants had missing scales.

In this paper the authors discuss the use of multiple regression analysis but do not present any tables of the standard information one would want to see from the results of a regression analysis. Beta coefficients, f statistics and p-values are not provided, let alone the sample size of each regression model. Additionally, simple distribution tables for the potential confounders tested in the regression analysis would have been informative.

The most important problem with the reported findings of this paper is that, while a list of potential confounders were analyzed, they are only as useful as their measurement permits. The measurement of maternal alcohol consumption, a true confounder, (related both to PCB level and results of BNBAS scores) is inadequately assessed. Simply determining whether a women had more than one drink per week does not provide the necessary information to statistically control for maternal alcohol consumption. Furthermore, hyporeflexia was associated with both DDE and PCBs while hypotonicity was only associated with PCBs. Since hyporeflexia and hypotonicity are clinically related, these separate associations with either DDE or PCBs are difficult to reconcile unless they occurred by chance.

30. Rogan W.J., Gladen B.C., McKinney J.D., Carreras N., et al. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichlorethene (DDE) in human milk: effects on growth, morbidity and duration of lactation. *AJPH*. 1987;77:1294-1297.

Methods

This is the third in the series of reports on the North Carolina cohort. The effect of PCBs in milk on growth and health were the focus of this report. The authors state that "about 900 families between 1978 and 1982" were enrolled in the study. The 3 previous reports are cited for information on study design, chemical analysis methods, characteristics of the cohort and findings on neonatal examination. Women who volunteered were predominately white (92%) and well educated. The children were seen at birth, 6 weeks, 3, 6, 12 and 18 months and then yearly until age five. Milk or formula was collected at each visit until 6 months, after that only milk was collected until the mother stopped nursing. Maternal serum, cord blood and placenta were also collected. The children were examined and a medical history was taken at each visit. The mother was also asked about weaning. Medical records were abstracted (it is not indicated for what). Of the 930 children whose mothers volunteered for the study, 858 participated beyond birth. Of the 858, 802 (93%) were still participating at one year of age.

PCB and DDE concentrations in milk at birth were estimated by combining all samples "as described elsewhere." The authors note that "concentrations decline over the course of lactation: for example, concentrations of PCBs at six weeks averaged 93 percent of concentrations at birth. To estimate a woman's concentration at any specific time point, the average decline was applied; values between visits were obtained by linear interpolation." As discussed in the previous reviews, these manipulations are of questionable validity.

Duration of nursing was calculated from reports by the mother with phases such as "mostly" breast-fed until cessation of lactation. The reason for weaning was assessed by a multiple choice question with an open-ended option. To examine the relation between PCBs and morbidity the authors needed to assign a dose to each child. Reportedly dose was determined by three conditions: the concentration of PCB in the fat of milk, the amount of fat in milk and the amount of milk consumed by the infant. The authors did not have enough data to deal with sustained differences in amount of milk fat among mothers and thus they used an average. PCB concentrations and duration information were combined to provide estimated amounts of PCBs consumed. Milk was assumed to average 2.5% fat over the entire lactation period. They assumed that children consumed 700 grams of milk daily as long as they were mostly breast-fed and half that amount afterward until breast-feeding stopped. For this calculation the time "mostly" nursed was

taken to be at most 9 months. There is no further explanation provided for this estimation.

Illnesses were counted as they were recorded in the child's medical records. No attempt was made to verify diagnosis. Illness reports were reviewed centrally and any necessary clarification was sought from field personnel. Individual illnesses were grouped into similar categories. Weights were obtained from the physical examination at each study visit, and when missed, weights were taken from medical records if possible. The authors contend that the observations concerning chemical exposures were double blind; however, women who had two children in the study knew the results from their first set of analyses during their second lactation. The number of women with two children in the study is not specified.

Results

The authors report that 88% of the children in the study were breast-fed and suggest that this number is so high because these women had a heightened interest in infant nutrition, by virtue of their volunteering for the study. The authors performed a multiple linear regression analysis in which duration of breast feeding (i.e., weeks mostly on the breast) was the dependent variable and mother's age, race, education, occupation, smoking, drinking, collection site, and estimated PCB and DDE concentrations in milk at birth were the independent variables. Table 2 presents the results of the regression analysis for all lactations and for first time lactation. The latter was done because levels of PCBs and DDE decline over the course of lactation and levels seen in a first lactation are higher than those seen in subsequent ones. The actual 'N' in each analysis is not presented, but if one does the math the "all lactation" model has 734 women and the "first lactation only" model has 469 women. The authors report that there is a decline in duration of lactation of about one week for each additional ppm of chemical based on the beta coefficient of -1.1. The authors fail to remark that the coefficient is not statistically significant, with the 95% confidence interval including 1, for both models suggesting that PCB body burdens do not explain any of the variation in length of breast feeding. The only significant predictors of longer length of lactation were older age, higher education, being a housewife or student and/or a nonsmoker.

The correlation between DDE and PCBs was 0.23. The authors observed that higher levels of DDE, but not PCBs, are associated with higher rates of lactational failure. However, the numbers are small and the correlation is not exactly linear.

The authors present their results of investigating the morbidity associated with PCB in Table 3. It shows the percent of children with upper respiratory, ear infections and gastroenteritis during three time periods (0-3,3-6,6-12 months). By looking within relatively short intervals the authors hoped to alleviate some of the potential confounding arising since illness can terminate breast-feeding. Within the intervals, the children are divided into those who never breast-fed, those who previously breast-fed but are now weaned and those breast-feeding at some time during the interval. Those who breast-fed during the interval are further subdivided into groups by estimated amount of PCBs consumed during the period. None of the diseases showed any evidence of harmful effects of PCBs. Analysis of weight gain showed the familiar lighter weight among breast-fed children but no effect of PCBs.

Discussion

The authors point out that the levels of PCBs seen in these women are as high as have been observed in the U.S. outside of specific high-exposure situations; however, they also point out the inappropriateness of comparisons among studies because of the differences in analytic procedures. The authors report that they found no evidence for an effect of chemical level on morbidity. They had hypothesized that the shorter duration of breast-feeding was more likely due to an inhibitory effect on lactation by the chemicals (DDE and PCBs) than to production of illness in the child and subsequent weaning. The authors state that this hypothesis is consistent with the lack of association between PCB levels and increased rates of illness. The authors postulate that the estrogenic properties of PCBs may suppress lactation and that this is the reason for early weaning. The data presented in this paper do not in anyway support this statement.

Comments

This paper, although titled "....Effects on Growth, Morbidity, and Duration of Lactation" deals very little with the issue of growth or morbidity. The main thrust of this paper is clearly the duration of lactation and women with 'lactation failure.' As stated by the authors the level of PCBs declines in breast milk over time and they describe how they adjust for this in their representation of PCBs in milk. They don't, however, indicate where these measures are used and in what analyses. It would be inappropriate to use repeated measures on the same individual in a regression analysis and there are no other statistical tests presented in the paper. Superfluous discussions of data manipulation in a paper with already too many topics only serves to confuse the reader. As in the previous papers by Rogan there are no simple tables outlining the distributions in the data with appropriate statistical testing for differences (i.e., t-tests for continuous data and chi-square for categorical data). The higher rates of lactational failure in women with higher DDE levels was

based on small numbers and no detailed information about the investigation of other potential reasons for lactational failure was reported.

31. Gladen B.C., Rogan W.J., Hardy P., Thullen J., Tingelstad J., Tully M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. *J Pediatr.* 1988;113:991-995.

This fourth report on the North Carolina cohort presents the results of the Bayley scales at 6 and 12 months. This paper reports 858 infants of whom had Bayley scores available at either 6 months or 12 months or both.

Methods

The authors refer the reader to previous articles for the methodology of the overall study. They briefly report that they followed a cohort of 858 North Carolina children with questionnaires, clinical examination, medical histories and developmental tests. The children were seen at or about birth and then at 1.4, 3,6,12,18,24,36,48 and 60 months. At these visits the authors collected samples and measured PCBs and DDE in the samples. They also measured PCBs and DDE in cord blood, placenta, maternal blood, and milk. No blood was drawn from the children. To measure the amount of PCB and DDE exposure prior to birth the authors "estimated the mother's body burden at the time of birth. The estimate was based on an average of all her samples (each scaled to account for differences between matrices over time) and was expressed as parts per million of PCBs in milk fat at birth." The reader is referred to the 1986 publication by Rogan for further details of the calculation. Unfortunately, this referred to publication offers no enlightenment to the reader. The authors go on to say "After birth the dose of interest was the cumulative amount of exposure to the time of the evaluation, because PCBs and DDE are largely stored by the child. This cumulative amount is a function of the concentration of the chemical in milk and the duration of breast feeding; we estimated the total number of milligrams consumed by methods detailed by Rogan et al....." As discussed in the previous reviews the validity of such data manipulations is questionable for measures that are by nature so imprecise. Exclusively bottle-fed children were counted as having no postnatal exposure because commercial formulas and cow milk are virtually uncontaminated.

The Bayley scales of Infant Development provide a mental development index (MDI) and a psychomotor development index (PDI) scaled like standard IQ tests. Testing was done at clinic visits in the presence of the parent(s). The testing was conducted over a period of about 6 years, and during those 6 years there were several examiners at each of three centers. (The number of different testers was not provided.) The authors state that they

did not carry out formal inter and intra-rater testing but that scores were monitored and any drift was discussed at meetings with study personnel. Because the testing spanned over 6 years and different personnel did the testing there should have been a rigorous protocol for maintaining integrity of the testing. The authors report that "testers were not specifically unaware of feeding method, but results of chemical analyses were not available at these ages." This is a confusing statement and leaves room for questioning the impartiality of the testers. For children who moved from the area the authors attempted to have Bayley testing done locally and the scores reported to them. (The number of children who moved was not provided, nor are the number of children for whom Bayley scores came from outside the 3 clinics.) The authors report obtaining both 6-month and 1 year scores for 706 children, 82 children had only 6-month scores and 14 had only 1 year scores. Three children had only partial scores. If the above numbers are added there are 53 children for whom they did not get scores. There is no explanation for this disparity in numbers, or where these children are. With 6% of the children missing tests scores the authors should have looked for potential differences between those with missing test scores and those who were successfully tested.

The authors report that they examined the relationship between mean Bayley scores and levels of PCB exposure. They identified potential confounding variables, a priori, both by their previous analyses of the data set and by consideration of those variables used in other studies. They used multivariate regression to control for confounding: the regression models used the Bayley index scores as the dependent variables and chemical levels and the potential confounders as the independent variables. Duration of breast feeding was handled in two different ways. For analyses with all children, feeding was grouped into bottle feeding or one of several categories of duration of breast feeding; for analyses limited to breast feeders, the number of weeks in which milk was the primary food was used.

Results

In Table II the authors present the mean PDI by PCB levels. There are 787 children reported in the 6 month scores and 720 in the 12 month scores. These numbers do not correspond with the numbers presented in the methods section, although the numbers were not terribly specific in the methods section. The differences in the Bayleys score between categories of PCBs are extremely small and in some instances are not even 1 point. The authors report that the "psychomotor index showed downward trends with increasing transplacental PCB exposure at both 6 and 12 months of age." This statement is an overstatement of the contents of this table. The mean PDI do not consistently go down with increase in PCB levels, and when they do it is not known whether this is a result of normal variation or whether it is significantly different. No statistical

testing is presented. A correlation coefficient and partial correlation coefficients (to examine potential confounding) looking at PCB levels as a continuous measure and test scores are notably absent from this analysis. These simple measures of association would have provided a quick overview of the explanatory impact of each of the variables. The PDI was not affected by DDE.

The authors present the results of the regression analyses in Table III. The verbiage below Table III is confusing and uses terms that were not used in the text. The two routes of exposure listed in the table are transplacental and milk. In the methods section the two measures are referred to as before birth and after birth. While a minor detail, it is important to properly label tables as they are often the first and only thing an interested reader may look at. The model includes all of the potential confounders but the table only presents the coefficients for the chemicals. It would have been interesting to see how each variable in each model contributed to the variation in Bayley's scores. Additionally, there are no numbers provided to indicate the number of children included in each of the models. The authors report that the 6 month PDI decreases 0.96 point for every increase of 1 ppm of PCBs. At 12 months the drop was estimated at 1.34 points per 1 ppm of PCB. Given the overwhelming difficulty of measuring PCBs with an error margin of 25-50% along with the subjective nature of the Bayley scales and poor measures of alcohol intake, interpreting the regression analysis as the authors have done is clearly inappropriate. The MDI scores were not related to transplacental or milk PCBs. However, the MDI was estimated to increase by 0.65 for every 1 ppm of DDE at 6 months. At 12 months the estimate was zero.

Discussion and Comments

The authors start out the discussion by stating that there is ample evidence that PCBs are neurotoxic. There is in fact limited evidence of the neurotoxicity of PCBs particularly in 1988 when this article was published. There are no data suggesting a dose-response curve, a necessary component of an association between a toxin and its effects. The authors present one study of the off-spring of rhesus monkeys that were fed high concentrations of PCBs. These monkeys produced off-spring that had difficulty learning mazes and had behavioral abnormalities both as infants and as juveniles. The authors do not indicate whether these results have ever been replicated. A single study has little value in supporting a statement such as "ample evidence." The authors mention the Yusho and Yu-Cheng poisonings as evidence of the neurotoxicity of PCBs but as noted by the authors these poisonings were not strictly PCBs but also involved highly toxic polychlorinated dibenzofurans. The authors also state that they are not aware of neurotoxic effects of DDE or DDT at low level exposure and they dismiss the effect on MOI observed at 6 months as a chance finding. Similar arguments could be made for the PCBs.

There is no known hypothesis for the action of PCBs on the nervous system, yet the authors present some possible mechanisms by which PCBs may act on the nervous system, specifically the "spinning syndrome" and the agonistic and antagonistic effect of PCBs on thyroid hormone. Neither of these possibilities have to date been convincingly demonstrated. The authors state that they are not aware of neurotoxic effects of DDE or DDT at low level exposure and they dismiss the effect on MDI observed at 6 months as a chance finding. Similar arguments could be made for the PCBs.

The authors note that "the issue of confounding is somewhat less problematic for this exposure than it is for other subclinical problems, such as iron deficiency and lead poisoning. Perhaps because much less is known about the determinants of maternal PCB exposure, no factor stands out as a major alternative explanation for the observed association." The fact that there is little known about maternal exposure is precisely what makes confounding much more problematic for PCBs than for lead or iron deficiency. Case in point is alcohol consumption. When this study was designed the investigators inadequately measured alcohol intake, possible because the effect of alcohol on the fetus was not well known, nor was the association between elevated PCB levels with increasing alcohol intake well known, although the results had been published (Kreiss et al. *JAMA*. 1981;245,24). Alcohol consumption was positively associated with log PCB levels when controlled for age, sex, fish consumption and serum cholesterol levels. Therefore, alcohol consumption is a significant confounder when examining neurotoxic effects of PCBs because maternal alcohol consumption is related to infant development and it is also related to PCB levels. The variation in maternal PCB levels are so small and the differences in the Bayley scores so slight it is difficult to interpret the data meaningfully, particularly with the lack of adequate control for the potential confounding effects of alcohol consumption.

32. Rogan W.J., Gladen B.C. PCBs, DDE, and child development at 18 and 24 months. *Ann Epidem*. 1991;1:407-413.

This fifth report on the North Carolina Cohort reports the results of the Bayley Scales at 18 months and 24 months of age. There were 676 children tested at 18 months of age and 670 tested at 24 months.

Methods

The authors refer the reader to the 4 previous publications for study methods. The authors report that "they saw" 788 infants at 6 months and 720 at 1 year. The authors report "very few refusals;" the number of refusals was not presented. They also report that "almost all of the families who were lost to follow-up had moved (or relocated)." There are no lost to follow-up numbers reported nor any data presented on how these children may differ from the

remaining group. The cohort started out at 858 and the 6-month Bayley was performed on 787 infants. What is not known is how many refused and how many were lost. These are important issues that were simply neglected by the authors.

The estimating and scaling procedure used to estimate the chemical concentration of PCBs and DDE for each women are reported by the authors. This reader still finds the descriptions confusing and continues to question the validity of such extreme data manipulation with measures (PCBs) that are so imprecise. The authors report that the correlation between the estimated measures and the real measures is .86, which is an R^2 of .73 for PCBs and 0.94 for DDE which is an R^2 of 0.88. If one considers the potential variation of 25-50% of PCB measures (in either direction) seen with repeated measurements on the same sample, creating estimates by averaging and scaling them to previous measurements is manipulating the data far beyond what it is capable of.

Postnatal exposure is defined as exposure exclusively through milk for the first year of life or so. The authors estimated this exposure by combining information on duration of breast-feeding and concentration of PCB in milk. The reader is referred to a previous publication for details on how this estimation was done. Feeding patterns were dealt with in two different ways according to the authors. For examining transplacental effects, they grouped the 712 children on whom they had any Bayley score into bottle-fed (10.8%) and those breast-fed according to duration: mostly breast-fed for 0 to 4 weeks and who were weaned by 9 weeks; medium for those mostly breast-fed 5 to 19 weeks and weaned by 19 weeks (19.7%); long for those mostly breast-fed for 5-19 weeks and weaned after 19 or mostly breast-fed for 20 weeks or longer and weaned after 49 weeks (23.2%). For children whose feeding pattern was ambiguous they were placed in the category representing a shorter duration. (The number of children with ambiguous feeding pattern was not given.) There were 558 children who were ever breast-fed, the median duration was 26 weeks. This number is confusing. Is this 558 of the 712 children for whom they had any Bayley scores or the 676 tested at 18 months or the 670 tested at 24 months, or even the 858 children who began the study. The authors state "To examine effects through milk, we limited attention to breast-feeder and adjusted for weeks breast-fed." As one progresses through the paper this statement becomes more confusing as it is clear that "transplacental PCB exposure" is the measure used in the analysis.

The Bayley consists of two sub-scales: the Mental Development Index (MDI) and the Psychomotor Index (PDI). The authors report that they had Bayley scores on 676 children at 18 months and 670 children at 2 years. There were missing PDI scores for 5 children at 18 months, no MDI scores for two children at 18 months and no PDI score for 10 children at 2 years. The authors report that testers were blind to chemical levels in the children. There is no

discussion of how, when, where and who was present during testing or the time span involved in testing all these children. There is no discussion of the number of testers or the inter and intra-tester reliability. These are extremely important factors when using subjective instruments such as the Bayley scales.

The authors report that they examined the relationship between Bayley scores and exposure to PCBs by linear regression. They adjusted for sex, race, actual age at examination, number of older siblings, maternal age, maternal education, maternal occupational grouping, maternal smoking, different examiners, and the mothers' "usual" level of alcohol consumption. The authors state that alcohol use was infrequent and the design of the questionnaire pre-dated the current interest in alcohol consumption during lactation and motor development.

Results

The crude means for PDI by categories of transplacental PCB exposure are given in Figure 1. The authors assume a linear relationship between exposure and the scores on the Bayley Scales. Confounding was controlled for in the regression model and the size of effects were also compared using regression analysis. There was no effect of PCBs or DDE acquired through milk on either the MDI or PDI. The transplacental PCBs effect on the MDI was small; however, the effect on the PDI at 24 months was larger and similar to that seen for the 6 and 12 month Bayley scores. The authors report that scores at the age of 18 months declined .38 for every increase of 1 ppm in PCBs, at the age of 24 months the decline in Bayley score was 1.16 ($p=.09$ for the coefficient at 24 months). The authors also report that the relationship between scores and PCB levels was not linear. They report that a more appropriate analysis with the transplacental exposures was one broken into categories and each category compared with the lowest category, although, the results of this analysis were similar. The only significant results were those for the 24 months PDI. The children in the two groups with the highest PCB exposure scored about 8 points lower. This difference was statistically significant using analysis of variance for heterogeneity of groups at the .05 level. At the 24 months PDI a positive DDE effect not consistent with data from other time points was significant at the 0.06 level. For DDE the positive effect appeared at all levels and no dose response relationship was evident.

Discussion

The authors report a persistent negative association between transplacental exposure to PCBs at four time points, for both linear and categorical analysis after adjusting for confounding factors. The authors report that the estimated magnitude of the PCB effect hovered around 1 point of decline in the PDI per 1 ppm increase in PCB concentration of PCB in mother's fat at birth at

all four time points. The authors go on to say "The effect appeared to be nonlinear, and was accounted for by a decline of about 8 points, occurring above a PCB concentration of 3.5 ppm in mother's fat at birth, about the 95th percentile in these data." At 24 months higher DDE exposure was associated with higher motor scores.

The authors report "There is no doubt that PCBs and similar compounds are toxic to the developing nervous system". They review the PCB poisonings in Asia and point to the problems observed in these children and results from the children of fish eating mothers in Michigan. The animal data are presented and possible mechanisms of effect are postulated.

The authors indicate that their data are observational rather than experimental and they could not rule out bias or uncontrolled confounding that could account for their findings; but they were not aware of any obvious covariates.

Comments

This paper has the same problems as the previous report on the Bayley at 6 months as well as the overall methodological problems. The authors report the association of 1 ppm increase of PCB with a decrease of one point in the PDI score. While the results of regression analysis may have yielded beta coefficients to indicate this association there is no reasonable translation into the real world of PCB measurement or Bayley score measurements for that matter, particularly since the relationship is not linear. Re-testing the same child with the Bayley's yields several point differences and PCB measurements on the same sample can vary by 25-50%. The results presented are severely limited by the inherent limitations of quantifying PCBs and the subjective nature of Bayley's testing. Similarly, the association of DDE levels with the MDI appears to be inconsistent and most likely represents a chance finding.

33. Gladen B.C., Rogan W.J. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. *J Pediatr.* 1991;119:58-63.

This is the sixth report on the North Carolina cohort of children. The authors report the results of the McCarthy Scales of Children's Abilities at 3, 4, or 5 years of age.

Methods

The authors refer the reader to the previous publications for information regarding the study methodology. They state that they enrolled 859 (previously 858) newborn infants between 1978 and 1982 and then followed them actively from birth to 5 years of age: information collected included questionnaires, medical records and

results of physical and developmental examinations. They also report collecting samples of maternal milk, maternal blood, cord blood, and placenta for chemical analysis.

The authors report that they attempted to have the children who had moved examined by a local psychologist. The number of children who had moved is not given. Scores were obtained on 645 children at 3 years, 628 at 4 years and 636 at 5 years of age. In 1988 the authors wrote to parents and asked for copies of report cards. They received at least one report card for each of 506 children. They used report cards from grade 3 or later. They obtained usable report cards for 366 children. The authors abstracted grades for English and mathematics from the report cards. Minor manipulations were performed to allow for uniformity of the grades (i.e., A=excellent, B=good, etc.). If there was more than one report card for a child the grades were averaged. The discussion of which children were included and who was not, and why is inadequate. There is no discussion regarding who did the testing for those children who did not move or how long it took all the children to move through this testing phase. There is no discussion of inter-intra tester reliability.

The authors report that prenatal exposure is a function of the concentration of chemical agent in maternal fat during pregnancy. To estimate this exposure the authors "....took all the samples that they had from a given woman, scaled to be comparable (with the milk sample collected at or near birth as the base) and averaged them; details have been given elsewhere." The only postnatal exposure for about the first year of life is breast feeding. "The chemicals accumulate in the child, so the dose of interest is the cumulative postnatal exposure. We estimate this dose by combining information on the duration of breast-feeding and the concentration of chemical in milk. The calculations for the estimated exposures are shown in detail elsewhere. About 88% of the children in this study were fed human milk."

Statistical Analysis

The authors report that McCarthy scores were analyzed by analysis of covariance. Transplacental exposure to PCBs were divided into 8 categories, and postnatal exposure through breast-feeding was divided into five. Transplacental and postnatal exposure to DDE were divided into 7 and 5 categories. Covariates included were the identity of the examiner, maternal age, race, occupation, education, smoking, drinking, child's gender, number of older siblings, and feeding pattern (bottle feeding or breast-feeding of short, medium or very long duration). Analyses of transplacental exposure included all children, those of postnatal exposure included only breast fed children. The English and mathematics grades were reportedly analyzed in the same way, except that the term in the models that identified the examiner was not included.

Results

The authors present the results of the McCarthy test scores by postnatal PCB in Table I. Unfortunately, the authors fail to provide a clear explanation of the table making it unnecessarily confusing for the reader. Additionally, there is an absence of any explanation of the meaning of the McCarthy test scores. They report that 83% of the original 859 children had taken at least one McCarthy examination. The authors report that those who were examined had the same maternal distribution of PCBs as those who did not; however, those who had McCarthy examinations were more likely to be breast-fed and to be breast-fed longer. No association was found between transplacental PCB exposure and McCarthy scores. The authors further report that for postnatal exposure, some variation by exposure was beyond that expected by chance, but the patterns again did not suggest cause and effect. For DDE, scores at all years showed similar patterns; higher scores were associated with the middle categories and the differences were marginally significant. Grades in English and mathematics had no statistically significant relationship to transplacental PCB or DDE exposure. The presentation of the regression analysis is limited to Fig 2. This figure is of interest as it demonstrates the nonlinearity of the relationship.

The quantitative McCarthy score varied among the transplacental DDE exposure categories at 3 years ($P = 0.06$), 4 years ($P = 0.06$), and at 5 years ($P = 0.05$). But a dose response relationship was not evident.

Discussion

The authors report that the previously seen delayed development in these children up to two years does not persist. They also failed to replicate the findings from the Michigan fisheater study between prenatal PCB exposure and score on the McCarthy Memory and Verbal scales at 4 years of age. They saw no consistent associations between exposure to PCBs through breast-feeding and the McCarthy Scales, nor did they see any significant associations with their measures of school performance.

The authors state that "laboratory experiments designed to evaluate the effects on the nervous system of prenatal exposure to PCBs changes in activity levels of the offspring are the most commonly reported effects. Evidence is also good that prenatal exposure affects learning, but motor function has not been so consistently affected." The two Asian poisoning episodes are presented as evidence of transplacental exposure resulting in developmental delays.

The authors note their negative findings and remind the reader that they can only "exclude effects within the resolving power of the measures that we used."

Comments

This final paper in the series of the North Carolina cohort is plagued by the same problems of the earlier reports. Additionally, there is a total lack of discussion or even a reference to the methods involved in using the McCarthy scales, or how it was done in this study. As this testing was done as long as 10 years after the initial study began, some discussion of attrition of the children should have been presented. The reason for using report cards is not fully explained. It appears that in evaluating their findings, the authors focus primarily on PCBs and dismiss the observed associations with DDE in this and most of their other papers.

34. Chao W.Y., Hsu C.C. Middle ear abnormalities in Yu-Cheng children. Dioxin '94. 14th international symposium on chlorinated dioxins, PCB and related compounds, Kyoto. *Organohalogen Compounds*. 1994;21:501-504.

From 1978 through 1979, a mass poisoning due to the contamination of rice bran cooking oil by PCBs and their thermally degraded compounds occurred in central Taiwan. A total of 118 Yu-Cheng children born between June 1978 and March 1985 during or after their mothers' consumption of contaminated rice oil agreed to participate in a clinical examination and follow-up. Another 118 children matched for age, sex, neighborhood, maternal age, and parental education and occupational class, were used as controls.

In the spring of 1993, out of this group of children 110 Yu-Cheng children (220 ears) and 96 matched control children (192 ears) were examined by otoscopy and tympanometry. The Yu-Cheng group included 57 boys and 53 girls; and the matched control group included 49 boys and 47 girls. The age of the children ranged from 8 years 1 month to 15 years 8 months. Appearance of the tympanic membrane was classified as normal, retracted, perforated, or adhesive conditions by otoscope examination . Middle ear pressure was further measured with Impedance Audiometry (Rion RS20).

By otoscopy, abnormal appearance of the tympanic membrane was noted in 49 Yu-Cheng children (76 ears); it is statistically significant from those (34 ears) of the control group as shown in Table 2 (X^2 = 14.8510, d.f. = 1, p = 0.00012). By tympanometry, it was further revealed that middle ear negative pressure (tympanogram B type and C type) was more prevalent in Yu-Cheng children (66/220) in comparison with those (28/192) of the control group.

Critical Comments

This short paper was presented at a conference. The authors give the number of Yu-Cheng children whose ears were affected; however, they do not give the number of control children. For the control children they only give the number of ears. In ear infections sometimes both ears are affected and sometimes only one ear, the statistical comparisons should have been made between the number of affected children and not between the number of ears. No information is given on how the abnormal findings in the ears relate to the age of the children in the two groups. Because of insufficient information this paper can not be critically reviewed.

35. Chen Y.C.J., Guo U.L.L., Hsu C.C. Cognitive development of children prenatally exposed to polychlorinated biphenyls (Yu-Cheng children) and their siblings. *J Formosan Med Assoc.* 1992;91:704-7.

During the summer of 1985, a field survey of all living children who were in utero during or after the period of oil contamination was performed. Seventy-four PCB-exposed women had children born between June 1978 and March 1985. One hundred and fifty-nine pregnancies were reported, 3 were ongoing, 5 miscarried, 8 aborted, 6 were stillborn, 6 were born alive but died later; leaving 131 living children. Of this group, 44 had older sisters or brothers that were born before the Yu-Cheng incident and had not been prenatally exposed to PCBs. The youngest of the elder siblings of Yu-Cheng children were carefully screened for PCB intoxication; 15 of them did not show symptoms or signs of PCB intoxication. These 15 prenatally PCB-intoxicated children were chosen to be the exposed subjects in this study, and the youngest of their elder unexposed siblings were the controls.

The WISC-R to assess the cognitive functioning of the subject children was used. The WISC-R, published in 1979, has been shown to be internally consistent and stable (1). The three subtests of the WISC-R scales were correlated with the Stanford-Binet Scale, with correlation coefficients of 0.84-0.89 for verbal intelligence quotient (VIQ); 0.55-0.66 for performance intelligence quotient (PIQ); and 0.83-0.89 for full scale intelligence quotient (FIQ).

Data on the subjects' cognitive development were collected yearly in August from 1985 to 1990. The WISC-R test was given to all children older than six years of age in their own homes. Each pair, the Yu-Cheng child and the control sibling, took the tests on the same day. Each tester held a bachelor's degree in either special education or psychology, and was trained by a senior clinical psychologist. The testers were unaware of the children's PCB exposure history. During each testing survey, random cross-validations of the testers were conducted to rule out variations in results introduced by individual testers. All data were analyzed using the SPSS/PC+ statistical package. The IQ scores for each Yu-Cheng child and his/her control sibling were compared using Student's paired / test.

Results

The age of the Yu-Cheng children was 5.69+/-1.66, and of the controls 8.62+/-1.28. There were 5 males and 10 females among the Yu-Cheng children, and 9 males and 6 females among the controls as of September 1, 1985. The Yu-Cheng children scored significantly lower ($p<0.05$) on the VIQ by 6.8 - 16.1 points; on the PIQ by 10.2 - 18.4 points; and on the FIQ, by 8.9 - 18.5 points, on the six measurements performed from 1985 to 1990. The differences in the WISC-R IQ scores for the different years of testing showed wide

ranges. In different years, different numbers of children were tested. It is unclear whether that pertains to the Yu-Cheng children only or also to their controls.

The WISC-R scores were normalized with the norm for Chinese children of each gender and age group (1). Therefore, the discrepancy in gender distribution and the age difference between Yu-Cheng children and their control siblings should not be a factor in the lower IQ scores among Yu-Cheng children according to the authors.

Critical Comments

The description of the selection of the Yu-Cheng children and their control siblings is unclear. It appears that infants presumed to have been exposed without clinical signs of intoxication were selected to represent the Yu-Cheng children. It is unclear what the assumption is based on that these children did indeed receive exposure. Based on a figure in the paper, the ranges between the controls and the exposed overlap, the number of children tested per year and the means of the IQ tests vary a great deal from year to year. It is not clear whether this is caused by the difference in the number of children tested, or whether the normalization for age and gender attempted by the authors was not as effective as they claim. No definitive conclusions can be drawn from this study.

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36. Chen Y.C.J., Guo Y.L., Hsu C.C., Rogan W.J. Cognitive development of Yu-Cheng ("Oil Disease") children prenatally exposed to heat-degraded PCBs. *JAMA*. 1992;268:3213-3218.

Methods

In early 1985, all living children born to women in the PCB registry maintained by the health departments responsible for the care of the Yu-Cheng cohort were identified. There were 128 Yu-Cheng children, born to 74 mothers. One hundred and seventeen Yu-Cheng children (born to 69 mothers) and neighborhood control children attended a clinical examination in April. A control group from families known to the exposed family was selected. These controls were matched for neighborhood, age (within 15 days for those under 1 year, and within 1 month for those older), sex, mother's age (within 3 years), parents' combined educational level (within about 3 years for the total), and parents' occupation (within one class of five classes from unskilled laborer to professional).

In addition to the Yu-Cheng children, the next older sibling if available was included as a second control. The siblings were born before the contaminated oil was available and only had exposure after birth. Out of 28 such older children 15 participated.

To evaluate the children's cognitive development, the Chinese fourth version of the Stanford-Binet (SB) test and the Chinese version of the Wechsler Intelligence Scale for Children, Revised (WISC-R) were used.

The fourth version of the SB test, translated and adapted to Chinese in 1977, is highly reliable in Chinese children. The Chinese version of the WISC-R, published in 1979, is internally consistent and stable.

The children were tested annually from 1985 through 1990. The SB test was used for children between 2.5 and 6 years of age, and the WISC-R was used for older children. The children were tested in their homes.

All Yu-Cheng children and control children are in these data at least once; 87 of the pairs were tested at the age of 4 years, 95 at the age of 5 years, 100 at the age of 6 years, and 87 at the age of 7 years. The older siblings were about 3 years older than the index Yu-Cheng children; none of the siblings were tested at the ages of 4, 5, or 6 years, and only four were tested at the age of 7 years.

Results

For each scale of the entire group of children tested other than the verbal subscale of the WISC-R, at the age of 6 years there is a consistent 5-point difference between the Yu-Cheng children and the control children ($p = 0.01$ by Wilcoxon's signed rank test). The mean IQ scores for the older siblings ($n = 4$) at the age of 7 years was 89.5 (SD, 23); the mean for the 14 Yu-Cheng children with older siblings when they were aged 7 years was 83.5 (SD, 13.1).

The serum PCB values soon after exposure were normally distributed on a logarithmic scale in the exposed mothers; the range was from 2 to 456 ppb; the arithmetic mean was 49.3 ppb, and the median was 25.5 ppb. The mean serum PCB value for 92 Taiwanese blood donors was 9.8 ppb, and the highest was 25.3 ppb. Although the levels in the exposed mothers were clearly higher than background, no relationship between PCB level in the mothers and developmental outcome of the children was noted. When the exposed children were divided into those with detectable PCBs ($n = 14$) and those without ($n = 7$), there was no consistent trend; the children with detectable PCBs had higher test scores as 4- and 5-year-olds and lower scores as 6- and 7-year olds, but the P values were very large because of the small numbers. Thus, these differences were not statistically significant.

Children from the same family do not strictly contribute independent observations, since their IQs are correlated. However, in analyses with only the oldest Yu-Cheng child from any family, the degree of the difference is similar but the P values are larger because of the small sample sizes (not statistically significant). The differences in the means on the SB test at ages 4 and 5 years are 6.5 points ($P = 0.01$) by Wilcoxon's signed rank test and 5.4 points ($P = 0.01$), respectively. The differences in the full scale IQ of the WISC-R at ages 6 and 7 years are 4.1 points ($P = 0.07$) and 5.6 points ($P = 0.02$), respectively.

Results of testing 21 exposed and 15 control children for PCBs and PCDFs have been reported elsewhere (1). Fourteen of the exposed children and six of the control children had detectable PCBs; the median was 1.0 ppb and the maximum was 77.8 ppb in the exposed children, while the median was 0 ppb and the maximum was 7.0 ppb in the control children. No child had detectable PCDFs. There was no relationship between levels in the mothers and in the children, but a strong relationship was not expected, since the children were tested at different ages.

There was no relationship between physical findings and developmental scores when the children were tested in 1985 (1). In addition, the authors were surprised by the relative mildness of the cognitive deficit that they identified in the present study. The authors also pointed out that about 10% of the cases in the Taiwan Health Department registry were asymptomatic even though

they allegedly have been exposed. A bias may have been introduced if the exposed child's parents were more likely to suggest impaired children.

Critical Comments

(Note: these comments should be reviewed by someone more familiar with IQ tests.)

This study raises a number of questions. In the paper 4 figures are presented which give the cumulative frequency in % of the IQ scores. The distribution of the scores for the controls as well as for the Yu-Cheng children suggests that the mean is less than 100. Some children with very low IQs seem to be included and the figures for both groups do not show a totally normal distribution. Among the controls as well as the Yu-Cheng children, many children with very low IQs are included and some geniuses seem to be present as well. These findings are rather remarkable for this small population. It is possible that the tests were not properly adjusted for the age groups or that the Chinese version of the tests give different results. It is not stated in the paper whether differences existed between testers or for individual testers over time. It is also not stated whether an equal number of controls and Yu-Cheng children were tested each time. The differences between the Yu-Cheng children and the controls were very small. Since not all children were tested each time it is unclear what the continuity of the effect is. Furthermore, it was not explained whether the same number of multiple children per family were tested among the controls and the Yu-Cheng children. Testing several children from the same family introduces an unknown bias, since children of the same family do not represent independent units. If the figures in the paper are examined, it is very obvious that the IQs of most children in the controls and in the exposed are to the left of 100 and do not show an even distribution.

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The second Yu-Cheng cohort is designed to establish two cohorts of later-born Yu-Cheng children: (1) 120 pairs of maternal Yu-Cheng children and their matched controls; (2) 100 pairs of paternal Yu-Cheng children and their matched controls.

The study period covered in this report is from October 1, 1991 to March 31, 1993. In the PCB registry, 140 children born to women and 109 children born to men of the Department of Health, Taiwan Province were identified. For each Yu-Cheng child, controls were selected, matched for neighborhood, age, sex, mother's/father's age, parental educational level, and occupation.

The main findings out of the first 2 years of fieldwork were summarized as follows: compared to their matched controls, the Yu-Cheng children were shorter in height by an average of 1.01 cm and less weighted by an average of 0.97 kg in the maternal descendants and 1.31 cm in the paternal side though they have caught up in weight. There were no differences in head circumferences, arm circumferences, and thickness of subcutaneous fat. Yu-Cheng children were reported to have more frequent upper respiratory infections and otitis media. They were reported by their mothers to have higher activity level, but no longer had increased physical habit or behavioral problems.

Later-born maternal Yu-Cheng children still scored 7 points lower on the Stanford-Binet Intelligence Test (SB-IQ) and 6-9 points lower on the verbal IQ, performance IQ, and full scale IQ of the Chinese version of the Wechsler Intelligence Scale for Children (WISC-R). The paternal Yu-Cheng children performed as well as their controls on the SS-IQ and WISC-R. The maternal descendant Yu-Cheng children fell significantly behind on the subscales of expressive language, conceptual comprehension, situational comprehension, self help, personal social development, and gross development (range of p values 0.05-0.09 assessed on the Chinese Children Development Inventory (CCDI).

Critical Comments

This is a short paper presented at a meeting. Few details are given. The difference in height is very small. Unless the birth dates were matched closely, which is usually not possible in these types of studies, it could simply be explained by slight differences (a few months) in age between the two groups. Rather than being given a mean difference in weight, information about the distribution of the weight among males and females separately would have been more informative for the reader. The differences in the

scores on the IQ tests can not be commented on. It is unclear how closely the children were matched for age, whether each group contained the same number of males and females. Each Yu-Cheng child had apparently 3 controls; however, only one control was tested for each Yu-Cheng child. It is not stated how this control was chosen.

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Chen Y.C., Guo Y.L., Hsu C.C., Rogan W.J. Cognitive development of Yu-Cheng ("Oil Disease") children prenatally exposed to heat-degraded PCBs. *JAMA*. 1992;268:3213-8.

38. Chen Y.J., Hsu C.C. Effects of prenatal exposure to PCBs on the neurological function of children: a neuropsychological and neurophysiological study. ***Dev Med Child Neurol.*** **1994;36:312-320.**

Introduction

Infants with congenital PCB poisoning are characterized by intrauterine growth retardation, brown staining of the skin and mucous membranes, widely open fontanelles, spotty calcification of the skull, natal teeth, and delayed developmental milestones.

Methods

One hundred and eighteen Yu-Cheng (prenatally PCB-exposed) children were identified who had been born to 74 women registered in the Taiwan Provincial Health Administration as victims of the 1978-79 Yu-Cheng incident. The children were born between June 1978 and March 1985. The children who served as controls were matched for residential area, sex, age, sibling order and socio-economic status. To obtain reliable results from WISC-R and P300, those children whose ages ranged from 7 to 12 years were selected. Of the 118 Yu-Cheng children 79 fell within this range. Twenty-seven pairs of children (Yu-Cheng and controls) were randomly selected from the 79 pairs of Yu-Cheng children and their controls for this study.

The study was conducted in February 1991. All children underwent neuropsychological assessment, which included cognitive testing, auditory event-related potentials (P300), and neurophysiological examination, including visual and short-latency somatosensory evoked potentials. The Chinese version of the WISC-R was administered to assess the cognitive function of the children.

P-VEPs (visual evoked potentials) were conducted to full-field stimulation. The stimulus was a black-and-white reversing checkerboard with each check subtending a visual angle of 50' of arc. The rate of pattern reversal was 2Hz. Disk EEG electrodes were placed on the scalp according to the 10-20 system at O2, Oz, O1 and Fz. The ground electrode was placed on the vertex (Cz position of the 10-20 system). The frequency band-pass was set at 1 to 200 Hz; 200 trials were averaged. Analysis time was 500 ms from the onset of stimulus. The peak latency of components N75, P100 and N145, the amplitude of N75, P100 and N145, and the amplitude of N75 and P100 were measured.

The mean value and SD of latencies and amplitudes for EPS and for WISC-R IQs in the Yu-Cheng group were compared with the control group using the Wilcoxon signed rank test. The relationship between the P300 and IQ scores of both groups was measured by a simple regression test and the Spearman rank correlation test.

Results

All the IQ scores were lower in the Yu-Cheng group than in the control group. The mean differences in WISC-R IQs between the two groups were -4.44 for VIQ, -4.14 for PIQ, and -5.66 for FIQ. The mean P300 latencies of the Yu-Cheng children showed apparent prolongation compared with their controls: 356.0±39.6ms vs 329.3±25.5ms at Cz; and 355.9±37.7ms vs 330.7±24.7ms at Pz ($p<0.01$ and $p<0.025$, respectively. The P300 amplitude showed a more significant reduction in the Yu-Cheng group than in the control group: 13.9±5.2μV vs 17.3±6.1μV at Cz; and 14.0±4.6μV vs 17.1±5.2μV at Pz ($p<0.025$ and $p<0.025$, respectively). The relationships between the findings of P300 at Cz and Pz, and FIQ in both groups were analyzed. In the Yu-Cheng group, the P300 latencies were inversely related to FIQ ($rs = -0.6$, $p = 0.003$ at Cz; and $rs = -0.5$, $p = 0.005$ at Pz). However, P300 amplitudes were not correlated with FIQ $rs = 0.3$, $p = 0.09$ at Cz and $rs = 0.3$, $p = 0.113$ at Pz).

No significant difference was found in the incidence of neurological deficits between Yu-Cheng children and the control group. Yu-Cheng children showed no apparent conduction abnormality in P-VEPs and SSEPs. Their visual acuity was normal. There were no significant differences in the latencies of N75, P100 and N145 between the Yu-Cheng and control children for both eyes. There was no significant reduction in the amplitude of N75 and P100 between the Yu-Cheng and control children.

The latency of P300 has been reported to be related to the solving of cognitive tasks, and also to be a useful method for evaluating the function of information processing (Brandeis and Lehmann 1986). The amplitude of P300 is related to the frequency of the occurrence of a target stimulus, reflecting concentration abilities (Sutton et al., 1965). A significant difference was found in latencies and amplitudes of the P300 between PCB-exposed children and their matched controls. Moreover, a significantly greater difference in P300 latencies was found for affected children with lower IQ scores compared with their matched controls.

Our results suggest that PCBs affect the cognitive process of the brain of PCB-exposed children and persist for a long period, and that the P300 may identify these subtle deficits of cognitive function. The differences for the WISC-R IQ scores for the two groups of 27 children that were tested was 4 points.

Critical Comments

It is not stated in the paper how well the children in the two groups were matched by age and sex. The sample size is small and there is variability within the two groups since the age range of the two groups may span 5 years. It is not explained how the authors adjusted for this variability or whether it is even

possible with this small sample size. Apparently the registry these children were drawn from contains individuals with varying degrees of exposure and about 10% have little or no exposure. It is not stated what the exposure of these children was or if any of them still had any clinical problems related to their past exposure. No inferences can be drawn from this paper.

39. Chen S.J., Lai T.J., Guo Y.L., Yu M.L., Hsu C.C. Behavioral development of Yu-Cheng children as compared to their matched controls. Dioxin '94. 14th international symposium on chlorinated dioxins, PCB and related compounds, Kyoto. *Organohalogen Compounds*. 1994;21:517-520.

The objective of this study is to compare the behavioral development in 118 Yu-Cheng children with their matched control children. The revised Chinese versions of Rutter's Behavior Rating Scales for Parents (1) and Werry-Weiss-Peters Activity Check List for Parents (2) was used to assess children's behaviors. Because the children were born over a seven-year period, they were of different ages in any round of follow-up. The scores were cumulatively combined from all eight follow-up years and analyzed by age.

The Rutter scale from age 3 years on was used because the percentage of questions not applicable to children 3-4 and 5+ years of age were negligible. The differences between the controls and the Yu-Cheng children after age adjustment were statistically significantly different for health, habit and behavior. The controls had lower scores for each of these items for almost all age groups. These data show that the Yu-Cheng children 0-8 years old had a mildly disordered behavior according to the authors. The effect persisted as the children aged and appeared to be similar in health, habit and behavioral subscores on the Rutter scale. At each age level the Yu-Cheng children had activity scores 1.85 to 6.89 points higher than those of the controls and the difference reached statistical significance for ages 4, 7, 8, 9, and 10.

According to the authors, the results could be due to unknown bias or confounding variables. Some of the mothers and some of the children still have visible physical signs, such as abnormal patterns of pigmentation, and hypoplastic nails. Such visible stigma may remind the parents that they were poisoned and therefore their children are "abnormal," and this perception of the parents may affect their reporting. The results could also be confounded by variables not measured in the study, such as temperament of the mother, child rearing attitudes or behaviors, and family and parental stresses. Data on home environment was collected (physical and mental) for the Yu-Cheng and control families in recent years and will soon be analyzed.

Critical Comments

This short paper was presented at a meeting. Insufficient information is given for evaluation. It appears that the children were tested repeatedly and that the number of tested children varied over the years. It is also not stated whether an equal number of controls and Yu-Cheng children were tested each time. These children were born in different years and the results are summarized by age. Thus, the same children may or may not have been included in each age group.

Both scales used in this evaluation depend on parental perception and as the authors point out may be biased since the parents of the Yu-Cheng children may have the perception that there has to be something wrong with their children.

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Introduction

In the Taiwan incident, more than 2,000 people who had consumed the contaminated oil for up to 9 months between June 1978 and October 1979 were included in the Yu-Cheng registry (1). In the first 3 years after the outbreak, babies born to exposed women had a high infant mortality rate (1). Surviving children had ectodermal defects, developmental delay, more behavioral problems, and higher activity levels (2-12). Followed from 1985 to 1991 with cognitive and behavioral tests, these children also had poorer cognitive development up to 7 years of age (7).

Methods

One hundred and thirty-two living children, born to exposed women between June 1978 and February 1985, were identified. One hundred and eighteen of them participated in a 6-year cognitive and behavioral follow-up study. For each Yu-Cheng child, a control matched on age (within 15 days for those under 1 year, and within 1 month for older children), sex, neighborhood, maternal age (within 3 years), and socioeconomic status was selected for the follow-up study.

There was no validated Chinese instrument for assessing children's behavior in 1985. The investigators had clinical experience with behavioral disorders in Chinese children. The items on Rutter's Child Behavior Scale A (13), validated in British children aged 9 to 12, appeared to be culture fair. The three sections concern health problems, habits, and behaviors. The questionnaire was modified so that all items allowed a "not applicable" answer, and the parents used this option for younger children in whom some of the behaviors asked about could not have occurred. Chinese parents reported a much higher frequency of behavioral problems in all children, including controls, than one would expect from the results in British children: 48% of the Yu-Cheng children and 25.5% of the control children aged 9 to 12 years met Rutter's criterion for a behavioral disorder, compared with 15.1% and 8.1% of the British boys and girls, respectively, of the same ages. Nevertheless, it seemed justified to use the Rutter scale from age 3 years on because less than 5% of the questions were not applicable to children outside the 9- to 12-year age group, and because there were simultaneous control children and so the "normal" values for the instrument did not have to be used. The test-retest reliability was assessed on 30 mothers with a 2-week interval between ratings, and the correlation between the scores on the two occasions was .82 (14).

The matched control was always evaluated on the same day as the Yu-Cheng child. The evaluations were conducted twice a year between fall 1985 and spring 1991, except for spring 1988.

Students' tests were used to compare the mean scores of Yu-Cheng children who had physical stigmata at birth or in the 1985 survey with the scores of those who did not, and to compare the scores of children who had detectable PCBs with the scores of those who did not.

Results

Because the children were born over a 7-year period, they were of different ages in any one round of follow-up. When children of different ages were combined and behavior scores analyzed by test time, the Yu-Cheng children were found to have scored 7% to 43% (mean = 23%) higher than the control children on the Rutter scale at every time point; all differences were statistically significant except for those in spring 1986 and fall 1987. If analysis was done by age and the scores from different time points were combined, the Yu-Cheng children scored 11% to 63% (mean = 28%) higher than their controls at each age.

There was no consistent trend toward decreased differences in scores of Yu-Cheng and control children as the interval between the exposure and year of birth increased. The difference between the Yu-Cheng and the control children did not decrease as the children grew older. At each round the Yu-Cheng children had a mean activity score 5% to 44% higher than the control children.

Children with physical signs had a higher mean score at some ages and a lower score at others. Cognitive scores, PCB detectability, maternal serum PCB levels, and breast-feeding mode did not have consistent relationships to Rutter or activity scores, either.

In utero exposure to PCBs and their heat-degraded contaminants is associated with mildly disordered behavior and increased activity level. The effects persisted as the children aged, and they appeared to be similar in children born up to 6 years after the mother was exposed.

Some of the children still have visible Yu-Cheng-related physical stigmata, such as abnormal patterns of pigmentation, hypoplastic nails, and teeth that are very susceptible to caries. Since both the behavior questionnaire and the activity checklist were filled out by a parent, the perception of the parent that his or her child is "abnormal" may contribute to the differences in reported behavior and activity. So far, a consistent relationship between either cognitive (15) or behavioral function in the children and the presence of the physical stigmata of the syndrome was not seen.

The data did not show consistent and significant associations between cognitive scores and behavioral scores for the Yu-Cheng children.

The finding of no consistent relationships between behavior/-activity scores and physical findings, cognitive scores, and serum PCB levels is consistent with a previous report in which inconsistent relationships between cognitive developmental scores and physical stigmata and serum PCB levels were found. It is possible that our measures of dose did not accurately reflect the children's in utero exposure.

Factors that were not measured could have caused residual confounding, these factors may include parity, temperament of the mother, child-rearing attitudes or behaviors, and family and parental stresses. There is, however, no a priori reason to believe that control families differed in stress levels, attitudes, or illnesses other than those caused by exposure.

Critical Comments

It is not stated how the children that were studied were selected. Since the data collected was provided by the parents, it is highly subjective and as the authors suggest may have been influenced by the parents perception of their children because they were or still are clinically affected by their past exposures. Even though the instrument used was determined to be "culture fair," it may not be appropriate for the younger age group tested here and parents in different cultures may have different criteria and expectations of their children. This difference is made obvious if the results obtained in Great Britain where the test was developed are compared to the results obtained in China among the controls. These data are biased to a great extent. This is supported by the fact that they could not be correlated to clinical signs of poisoning or levels of PCBs in body fluids. Thus, the results reported in this paper appear to be a product of the parents' biases rather than objective observations. There was also no correlation between cognitive and behavioral function in the children.

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Method

From the registry of cases maintained by Taiwan health officials, 132 children born to 74 mothers between June 1978 and March 1985 were identified and invited to participate in a clinical survey. One hundred and seventeen children attended. For controls, families were asked to provide names of children who lived in the same neighborhood and who were of similar age as the index child; 108 of 115 invited controls attended. Older siblings of the index children--some of whom had had household exposure to the oil, and some of whom escaped because the mother had been exposed at work--were also invited. At the examination, 77 exposed children, 80 controls, and 13 siblings provided spot urine samples, of which 75, 74, and 12, respectively, were usable.

Results

Mean values for urinary coproporphyrin and the pentaporphyrins and hexaporphyrins were modestly higher in the transplacentally exposed children than in the controls, with the most pronounced difference in the coproporphyrins; 8 (11%) of the 75 exposed children and 2 (3%) of the 74 controls had total porphyrins greater than 200 ($p = .051$). However, in a regression analysis excluding sibs and using total porphyrins as the dependent variable and exposed/control status, age, urine creatinine, and sex as the independent variables, the coefficient for the exposed/control term was not significant ($\beta = 6$, $p = .47$).

Using the simplest criteria for diagnosis of type B hepatic porphyria--a ratio of greater than one for uroporphyrins to coproporphyrins--4 of 75 exposed, 2 of 74 controls, and 1 of 12 sibs had type B. Exposed and control rates were not significantly different. None of the children had frank porphyria cutanea tarda.

Critical Comments

Results of this survey suggest that the total porphorins in this population are higher than in other populations that have been screened. This may be related to diet, exposures to other environmental chemicals or medicinal products. However, the findings do not suggest that the exposure to PCBs and PCDFs caused porphyria in this population.

42. Gladen B.C., Taylor J.S., Wu Y.C., Ragan N.B., Rogan W.J., Hsu C.C. Dermatological findings in children exposed transplacentally to heat-degraded polychlorinated biphenyls in Taiwan. ***British J Dermatol.*** **1990;122:799-808.**

The earliest cases of the poisoning outbreak in Taiwan was presented in December 1978. The illness was a form of chloracne, due to the ingestion of rice-bran cooking oil contaminated during manufacture with polychlorinated biphenyls (PCBs). The PCBs had been repeatedly heated and cooled and were contaminated with the highly toxic and acnegenic polychlorinated dibenzofurans (PCDFs). Distribution of the contaminated brand of oil was stopped in October 1979.

The offspring of female patients continued to be born affected, even though exposure ceased after the oil was recalled in the autumn of 1979. Taiwanese health department registry data up to February 1983 included 39 hyperpigmented children born to exposed mothers, of whom eight had died.

In January 1985, any of the children born in or after June 1978 were considered to have been at risk of transplacental exposure. There were 132 such living children, born to 74 mothers; they ranged in age from a few months to almost 7 years. Of these, 117 children from 68 families attended the clinic. The family was asked to suggest neighborhood control children and their mothers. The same questionnaires and examinations were given to these children. A total of 108 such children and 96 families attended the clinic.

Results

The transplacentally exposed children had a history of eyelid swelling and discharge, acne neonatorum, nail deformity, and hyperpigmentation at birth. Transplacentally exposed children were reported to have natal teeth or swollen gums at birth. Of the 57 hyperpigmented children, 46 were reportedly pigmented over their entire body.

Those children born shortly after direct exposure ceased were neither more nor less likely to exhibit the findings than those born long afterwards ($p>0.10$). In addition, if a woman had several children born after exposure, the first of these was neither more nor less likely to have these problems than were later children ($p>0.10$).

The main differences between the transplacentally exposed and control children were deformities of the finger- and toe-nails. The main finding was the 23 children with finger-nail deformities, of whom 22 also had toe-nail deformities.

Noticeable facial hyperpigmentation was three times more frequent in the exposed children than in controls ($P = 0.045$, Fisher's exact test). Hyperpigmentation of the genitalia was more frequent ($P = 0.0178$). Hyperpigmentation of the feet was also increased. The dentist who examined these children reported 43 transplacentally exposed, 33 controls and 11 siblings with oral pigmentation.

Acneiform lesions and/or scars at the time of the examination were twice as common in the transplacentally exposed children as in the controls ($p>0.10$); they were also seen in almost half of the older siblings. Of those with active lesions, seven of 11 exposed children, one of seven controls, no siblings, and five of 39 mothers had follicular keratoses. Other kinds of lesions showed no obvious link with exposure. Of those with either lesions and/or scars, 11 of 20 exposed, six of 10 controls, and all siblings had the problems confined to the face.

Discussion

The transplacentally exposed children appear to have an acquired neuro-ectodermal dysplasia with dental abnormalities, a growth deficit, developmental delay, and a behavior disorder (1).

About half of the older siblings with potential direct exposure that were seen had acneiform lesions and/or scars, compared to only 17% of the transplacentally exposed children. Nail changes were a much more striking indicator in these children than was acne. Another difference from direct exposure is the distribution of hyperpigmentation. In our group the finger-nails, gingiva/lips, and conjunctivae were not affected, but the genitals, face and feet were. Gingival hyperpigmentation was seen at much lower levels.

The PCBs were heat-degraded during use and thus were themselves contaminated by the PCDFs and by polychlorinated quaterphenyls. The authors stated that the mixture is toxic, but there is strong suspicion that the PCDFs play the major role in its toxicity.

Critical Comments

The findings of acneform changes in the skin, scars and hyperpigmentation of the skin, and mucosa and nail changes were more prevalent in the exposed children than in the comparison group. However, similar observations were also made in a smaller number of the comparison group. Thus, no clear-cut picture emerges. It is unclear whether some children included in the comparison group had also been exposed to the contaminated oil and whether exposure still occurred after the autumn 1979 when the oil was officially removed since earlier cases were not necessarily more severe. Furthermore, since hyperpigmentation, hyperkeratosis and nail changes were also present in the comparison group, potential exposure to higher than normal levels of arsenic would have to be ruled out.

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43. Guo Y.L., Lin C.J., Yao W.J., Hsu C.C. Musculoskeletal changes in Yu-Cheng children compared with their matched controls. Dioxin '92. 12th international symposium on dioxins and related compounds. University of Tampere, Tampere, Finland. *Organohalogen Compounds*. 1992;10:261-264.

This same information has been reported in more detail in:

Guo J.L., Lin C.J., Yao W.J., Ryan J.J., Hsu C.C. Musculoskeletal changes in children prenatally exposed to polychlorinated biphenyls and related compounds (Yu-Cheng children). *J Toxicol Environ Health*. 1994;41:83-93.

44. Guo Y.L., Lai T.J., Ju S.H., Chen Y.C., Hsu C.C. Sexual developments and biological findings in Yu-Cheng children. Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. *Organohalogen Compounds*. 1193;14:235-238.

The sexual developments by Tanner stages in boys and girls were not different between the Yu-Cheng and control groups. However, in the Yu-Cheng children aged 11 to 14, boys were shown to have a significantly shorter penis compared with their matched controls, but not in boys aged 10 or younger. The shorter statue in girls and shorter penis in boys were not related to the sexual development measured by Tanner stage.

Critical Comments

This report is a summary of a paper that was presented and gives little detail. The differences in the length of the penis were small, 0.6 cm, suggesting that this is not a very meaningful observation. Since sexual maturity and growth in general is variable and children over time tend to "catch up" the results of these single examinations cannot be meaningfully interpreted.

45. Guo Y.L., Chen Y.C., Yu M.L., Hsu C.C. Early development of Yu-Cheng children born seven to twelve years after the Taiwan PCB outbreak. *Chemosphere*. 1994;29:2395-2404.

Methods

The mass PCB poisoning in central Taiwan in 1978-79 was later called Yu-Cheng ("oil disease" in Chinese). When the PCB poisoning was discovered in 1979, a registry of 2,061 victims was set up by the Taiwan Department of Health. The inclusion criteria (Hsu et al., 1985) for the registry were: (1) consumption of brands of rice bran oil produced by the factory known to be the source of the contamination, and either (2) development of skin, eye, and other symptoms between January to October 1979, or (3) elevated serum PCB concentrations (higher than 10 ppb) if asymptomatic. About 10% of

the persons in the registry had a history of PCB exposure and had elevated serum PCB concentrations, but were asymptomatic.

In early 1992, all living children born to Yu-Cheng victims between July 1, 1985 and December 31, 1991 were identified. For every Yu-Cheng child, two children who matched the exposed child on neighborhood, age, sex, mother's age, parents combined educational level were chosen as controls. One of the two control children was used to assess development and behavior. The exposed parents and the subject children were visited in their homes.

The Chinese Child Developmental Inventory (CCDI), modified in 1978 from the Minnesota Child Development Inventory (MCDI), an instrument assessing the general developmental status of children aged six months to six years was used. CCDI has seven subscales: gross motor, fine motor, expressive language, comprehension-conceptual, situation comprehension, self-help, and personal social, and a summary scale, general development. The general development score consists of the most age-discriminating items derived from the seven subscales. The questionnaire consisted of 320 statements describing children's behaviors. The mother was asked by an interviewer whether the stated behaviors had ever been observed. Mothers were asked to respond to all questions regardless of the child's age.

Rutter's Child Behavior scale A (Rutter et al., 1970) was used to assess the behavior of Yu-Cheng and control children. The questionnaire was filled out by an interviewer. Rutter's Scale is a screening instrument to identify children likely to show problems in health, habits, and behaviors. Higher scores on this scale represent more problems. The questionnaire was modified so that all items allowed a "not applicable" answer, which could be used by the parents for younger children in whom some of the behaviors asked about were not expected to occur. The Rutter scale from age 3 years on is reported here because there were less than 5% "not applicable" answers for children 3 years or older. The interviewers were not aware of the child's exposure status.

Results

Sixty-three Yu-Cheng mothers' children, 48 Yu-Cheng fathers' children, and 3 children of intoxicated parents and their matched controls participated. Children of Yu-Cheng mothers scored lower than their controls in all CCDI subscales. The mean of the difference ranged from 0.8 - 2.3. The difference with 5.7 for general development was somewhat higher. Motor skills were not affected in this group of children. Children of Yu-Cheng fathers were not affected.

In children of Yu-Cheng mothers, girls were more affected than boys. Differences reached the level of statistical significance only among girls. The mean of the differences ranged from 0.8 - 3.9. The difference for general development was 7.3.

Both Yu-Cheng mothers' and Yu-Cheng fathers' children had Rutter's scores similar to that of their controls. In Yu-Cheng mothers' daughters, reduced scores in general development were seen in younger girls (one to three years) but not in girls of age four. This is only a one-time assessment, and the results may be due to chance or reflect a phenomenon that later-born Yu-Cheng girls will catch up with their controls.

The results in CCDI are compatible with reports in Yu-Cheng children born zero to six years after mothers' exposure. The data show a clear deficit in the general development category in the later-born Yu-Cheng children between 18 months and three years of age.

Critical Comments

It is not stated whether the interviewers were blinded to the exposure status of the children or whether different interviewers rated the developmental measurements the same. The number of interviewers is also not given. The questionnaires were answered by the mother and it would depend very much on her perceptions how these questions would be answered. Since all of these tests are very subjective, these small differences could be due to observer bias.

46. Guo J.L., Lin C.J., Yao W.J., Ryan J.J., Hsu C.C. Musculoskeletal changes in children prenatally exposed to polychlorinated biphenyls and related compounds (Yu-Cheng children). ***J Toxicol Environ Health.*** **1994;41:83-93.**

The children born to mothers exposed to PCBs and related compounds were called Yu-Cheng children. Local public health nurses were assigned to follow these families and to advise Yu-Cheng mothers not to breastfeed their children. One hundred and eighteen children born to 69 mothers were followed up yearly. For each Yu-Cheng child, one unexposed child was selected.

In February 1991, 73 of the 118 Yu-Cheng children and 69 of the control children (total of 55 pairs) were examined on their growth profile and structures of the joints. Joint structure was assessed by examination of joint laxity. Bone density and soft tissue composition were measured in 25 randomly selected subjects by dual-photon absorptiometry (DPA) using a LUNAR DP4 (Lunar Corporation, Madison, Wisconsin) to measure bone mineral density and soft composition for 25 randomly selected pairs of Yu-Cheng children and controls.

Yearly serum PCB levels had been previously determined from 1980 to 1986 in 61 of the 69 exposed mothers. Serum PCBs and PCDFs were determined in 14 of this group of Yu-Cheng children and a pooled sample of 10 control children. The detection limits of 2,3,4,7,8-pentachlorinated dibenzofuran (PnCDF) and 1,2,3,4,7,8-hexachlorinated dibenzofuran (HxCDF) averaged 100-200 ng/kg (parts per trillion, ppt) on a lipid basis and that of total PCBs averaged 0.5-1 μg/kg (parts per billion, ppb) on a whole weight basis.

Results

There were only 55 pairs of Yu-Cheng children and matched controls from the original cohort. The Yu-Cheng children were shorter in height compared to their controls by 3.1 cm. There was no difference in the weight, joint laxity, and total bone mineral density. The shorter statue, decreased total lean mass, and decreased soft content were statistically significant only in the first Yu-Cheng children born after PCB intoxication, but not in the second or later born children.

In the children included in this study, the maternal serum PCB levels ranged from 2 to 341 ppb around delivery, with a mean of 51.4, a standard deviation of 68.1, and a median of 24.8 ppb. When grouped by birth order after mothers' intoxication, the first children born after exposure were exposed to higher concentrations of serum PCBs in utero than the second and later-born Yu-Cheng children.

With a detection limit of 100-200 ng/kg lipid, the 9 detectable samples had average levels of PnCDF and HxCDF of 260 ng/kg lipid and 563 ng/kg lipid, respectively, as compared to the pooled serum levels of control children of 22 and 18 ng/kg lipid, respectively. The 6 detectable samples had average levels of PCBs of 2.99 μg/kg, with the controls 0.51 μg/kg.

A group of 113 Yu-Cheng patients had average blood levels of PCBs and PCDFs of 39 ppb and 76 ppt, respectively, on a whole blood basis from 1979 to 1981 (Kashimoto et al., 1985). With assumptions of hematocrit of 45 and lipid content of 0.35%, their values for total PCBs of 39 ppb in whole blood would be equivalent to 71 ppb in serum, approximately 40% higher than the average level of PCBs in our subjects' mothers around delivery. With the same assumptions, and a 1:3 ratio in contents of PnCDF : HxCDF, Kashimoto's PCDF value of 76 ppt would be converted to approximately 10,000 ng/kg of PnCDF and 30,000 ng/kg of HxCDF on a serum lipid basis. This level would provide an approximation of the Yu-Cheng mothers' serum PnCDF and HxCDF around their delivery of the subjects in this study. Although much lower than the mothers' levels years ago, our results in 14 serum samples of Yu-Cheng children indicated that serum concentrations of PCBs and PCDFs 12 years after exposure indirectly from the mothers were

still many times higher than those of a pooled serum sample of matched control children.

Critical Comments

It appears based on the measurements and calculations of the authors of this paper that the children in this study and their mothers had had much higher exposures to PCBs and particularly the more toxic PCDFs than background levels in the general population. It was not stated why the serum samples were only analyzed for PCBs 2,3,4,7,8-pentachlorinated dibenzofuran and 1,2,3,4,7,8-hexachlorinated dibenzofuran.

The differences in height, about 3 cm as a mean, and the perceived difference in lean mass of the children only occurred in the earlier born children and appears to be small. The authors state that they examined 55 pairs of children. They also state that they examined 118 Yu-Cheng children and 69 controls. These numbers are inconsistent. No explanation is given for this discrepancy.

47. Guo Y.L., Lai T.J., Chen S.J., Hsu C.C. Gender-related decrease in Raven's progressive matrices scores in children prenatally exposed to polychlorinated biphenyls and related contaminants. ***Bull Environ Contam Toxicol.*** **1995;55:8-13.**

Methods

There were 128 Yu-Cheng children born to 74 mothers. One hundred and eighteen Yu-Cheng children born to 69 mothers participated in long term follow-up together with 118 control children selected by matching for neighborhood age, sex, mother's age, parents' combined educational level, and occupation. The children were tested with Raven's Colored Progressive Matrices (CPM) and Standardized Progressive Matrices (SPM), the relatively culture-fair tests for cognitive development (Raven 1960). CPM were applied at 6, 7, and 8 years, and SPM were used at 9 years of age. CPM and SPM were adapted to Chinese and the reliability, standardized procedure of test administration, and background data in school-age children in Taiwan have been reported. The children were tested in their homes.

All children were tested at least once: 91 pairs at age six, 92 pairs at age seven, 82 pairs at age eight, and 67 pairs at age nine. Differences between scores of Yu-Cheng children and controls were calculated using Wilcoxon one-sample test. To examine whether later-born Yu-Cheng children were less affected by prenatal exposure, the differences in scores between exposed and control children were compared with the year of birth using regression analysis.

Results

Yu-Cheng children scored lower than their controls in CPM at ages six, seven, and eight; and borderline lower in SPM at age of nine. However, the differences of the means were small: 0.6-1.8 for the girls and from 1.9-3.6 for the boys. These differences were only statistically significant for the boys ($p = 0.05$ or less). Raven's Progressive Matrices indicate a person's ability to form comparisons and to reason by analogy.

Serum levels of PCDFs were not previously measured for the mothers of our subjects. However, Kashimoto et al. (1985) reported blood levels of PCBs and PCDFs of a group of 113 Yu-Cheng patients directly exposed during a similar period of time as our subject childrens' mothers as 39 and 0.076 ppb, respectively. In 1991, analysis of serum levels in a random subgroup of Yu-Cheng children in this study (Guo et al., 1994; Ryan et al., 1994) showed median levels of 106 ng/kg lipid, 160 ng/kg lipid, and 1.3 ng/kg whole weight for 2,3,4,7,8-PnCDF, 1,2,3,4,7,8-HxCDF, and total PCBs, respectively, which were much higher than those (19, 23, and 0.17, respectively) of a pooled serum sample of matched control children. No correlation was found between children's serum levels of toxicants and reduced CPM scores, this can be due in part to the small number of serum samples analyzed. There was also a lack of correlation between children's serum PCB/PCDF levels and mothers' levels at the time of pregnancy.

Critical Comments

The differences in the test results were quite small and were apparently unrelated to degree of exposure. The CPM/SPM scores were only statistically significantly different in the boys but not in the girls. It is not stated whether the testing was done by the same people in the boys and in the girls. Some information about PCB and PCDF lipid levels measured years after the poisoning occurred were given. These levels were still higher than those of controls, particularly the PCDF levels. Alternative reasons for why small differences in the test results were found in the presumably exposed children could be observer bias, inconsistencies in the number of children tested at different ages, low esteem, and lack of confidence in the exposed children.

48. Guo Y.L., Lambert G.H., Hsu C-C. Growth abnormalities in the population exposed in utero and early postnatally to polychlorinated biphenyls and dibenzofurans. *Environ Health Perspect.* 1995;103:117-121.

This is a review. Since many of the original articles have been reviewed, only the exposure information will be abstracted.

In the Yu-Cheng cohort, the adults were estimated to have consumed an average of 1g of PCBs and 3.8 mg of PCDFs during an average of 9 months of exposure to the contaminated oil.

However, Kashimoro et al. measured blood levels of PCBs and PCDFs between 1979 and 1981 in a group of 113 Yu-Cheng patients in a school for blind children in Taichung county, who had been exposed to the same contaminated rice oil as the children's mothers that underwent many of the behavioral tests. They had average blood levels of 39 ppb PCBs and 76 part per trillion (ppt) PCDFs on a whole blood basis. Assuming an average hematocrit of 45 and lipid content of 0.35%, their values for total PCBs of 39 ppb in whole blood would be converted to 71 ppb in serum, approximately 40% higher than the average level of PCBs in our subjects' mothers around delivery. If a 1:3 ratio in contents of PnCDF:1,2,3,4,7,8-hexachlorodibenzofurans (HxCDF) in the serum is assumed, Kashimoro's PCDF value of 76 ppt would be converted to approximately 10,000 ppt of PnCDF and 30,000 ppt of HxCDF on a serum lipid basis. If our subjects' mothers had similar PCDF and PCB serum ratios, their serum PnCDF level can be estimated as 6940 ppt, and HxCDF 20800 ppt on a serum lipid basis around the time of delivery of the Yu-Cheng children. The average serum level in 20 subjects' mothers in February of 1992, i.e., about 10 years after previous measurements, was 1507 ppt of PnCDF and 3583 ppt of HxCDF on a serum lipid basis, and 9.6 ppb of total PCBs on a whole weight basis (Guo L, Rogan W.J, Hsu C.C., unpublished data).

Critical Comments

These calculations illustrate that these patients had very high PCDF exposures and body burdens. Thus, the toxic effects observed were the result of this exposure rather then the result of the PCB exposures. It is not stated whether other PCDF congeners were assayed for.

49. Guo Y.L., Yu M.L., Hsu C.C., Lambert G.H. Neuro-endocrine developmental effects in children exposed in utero to PCBs: studies in Taiwan. Abstracts of the Thirteenth International Neurotoxicology Conference. *Neurotox.* 1995;16(4).

This is only an abstract. The information has apparently not been published yet.

50. Hsu C.C., Chen Y.C., Ko H.C. Yu-Cheng: studies in children. ***Environ Health Perspect.*** **1985;59:5-10.**

The information in this paper has been published elsewhere (Rogan et al. *Science*. 1988;241:334-336).

51. Hsu S.T., Ma C.I., Hsu S.K.H., Wu S.S., Hsu N.H.M. Discovery and epidemiology of PCB poisoning in Taiwan: a four-year followup. ***Environ Health Perspect.*** **1985;59:5-10.**

This is a review. Similar information is published in:

Hsu C.C., Yu M.L.M., Chen Y.C.J., Guo Y.L.L., Rogan W.J. The Yu-Cheng rice oil poisoning incident. *Dioxins and Health,* edited by Arnold Schecter, Plenum Press, New York, 1994. Chapter 20, 661-684.

Guo J.L., Lin C.J., Yao W.J., Ryan J.J., Hsu C.C. Musculoskeletal changes in children prenatally exposed to polychlorinated biphenyls and related compounds (Yu-Cheng children). *J Toxicol Environ Health*. 1994;41:83-93.

Oil samples collected from the outbreaks in Taichung, Hsinchu and Miaoli were heavily contaminated with PCBs (31 to 300 ppm). The period of PCB intake ranged from 3 to 9 months. The estimated average total PCB intake in each person varied from 0.77 to 1.84 g.

PCB blood levels in 613 patients in the first year of outbreak ranged from 3 ppb to 1156 ppb. Most (82.5%) blood levels ranged from 11 to 150 ppb with a peak frequency in 51 to 100 ppb (44.4%); 169 (27.6%) patients had PCB levels over 100 ppb.

The contaminated rice oils from Japan and Taiwan contained the same major components of PCDFs, the 2,3,4,6,7-penta-CDF and 2,3,4,7,8-penta-CDF.

Critical Comments

In this paper, additional PCB blood levels are given and the presence of the PCDFs in the rice oil is mentioned. Apparently the range of exposure in the population is rather wide and it is unclear whether some of the PCB levels that were measured represent background PCB levels. It is difficult to compare these levels since in Taiwan as well as in Japan, the levels in blood were sometimes measured in whole blood, while in the United States PCB levels are measured in serum, and in Holland they seem to have been measured in plasma. The number of peaks used for quantitation may also vary. None of the papers address the method of quantitation. It is unclear whether other PCDFs and perhaps PCDDs were looked for.

52. Hsu C.C. Follow-up study of intellectual and behavioral development of Yu-Cheng children. Symposium on health risk assessment on environmental, occupational and life style hazards, December 20-22, 1988, Taipei, Taiwan, Republic of China.

This report deals mainly with the cross-sectional findings of the first field work completed in 1986. At all age levels and on almost every assessment tool of intellectual development, there was a tendency for the Yu-Cheng children to score lower than their control children. The differences in favor of the control children reached statistically significant level in PDI of the BSID for those children below the ages of 30 months, in the spatial relationship subtest of the MCG for those above 5 years of age, and in PIQ of the WISC-R for those over 6 years of age. Temperamentally the Yu-Cheng children showed a tendency of being more intense in reaction, lower in threshold, more negative in quality of mood, and more distractible than their control children. The Yu-Cheng children also were rated as showing higher activity level. On the RBRSP, the Yu-Cheng children scored higher in somatic, habit and behavior problems.

Critical Comments

This is an abstract and no details are given. The information can therefore not be critically evaluated.

53. Hsu M.M.L., Chang J.B., Hsu C.C. Nail changes in PCB poisoning. Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. *Organohalogen Compounds*. 1993;14:251-252.

This is a short paper, the same information is provided in:

Gladen B.C., Taylor J.S., Wu Y.C., Ragan N.B., Rogan W.J., Hsu C.C. Dermatological findings in children exposed transplacentally to heat-degraded polychlorinated biphenyls in Taiwan. *British J Dermatol*. 1990;122:799-808.

Hsu M.M.L., Mak C.P., Hsu C.C. Follow-up of skin manifestations in Yu-Cheng children. *British J Dermatol*. 1995;132:427-432.

54. Hsu C.C., Hu H.F., Lai T.J., KO H.C., Chen Y.C. Behavioral development of Yu-Cheng children as compared to their matched controls. Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. *Organohalogen Compounds*. 1993;14:239-242.

Since August of 1986, the behavioral development of the 118 pairs of Yu-Cheng and his/her matched control child have been followed. The revised Chinese versions of Rutter's Behavior Rating Scales for Parents and Werry-Weiss-Peters Activity Check List for Parents were utilized. Each year, the behavior and activity questionnaires were

filled out by a parent under the instruction of a trained interviewer. The matched control was evaluated on the same day as the Yu-Cheng child.

Starting from 1985, Yu-Cheng children have been consistently rated by mothers to manifest higher activity level. The mean of the difference between the two children in each pair of the two groups was statistically significant each year. Data from the Teacher's Activity Check List which indicate that Yu-Cheng children have been rated by teachers to manifest higher activity level are also available. Since the two children of each of the 118 pairs have not been in the same class room, these data are only considered to be supplemental data.

Higher activity level and higher scores on the Rutter's Behavior Rating Scale have been consistently observed throughout the past seven years.

Critical Comments

These data are subjective and may have been influenced by the fact that the parents and teachers assume a priori that the children were affected. This perception that there might be something wrong with the exposed children may have influenced the parents judgement. Furthermore, the children's self esteem may have been affected. This may have influenced their behavioral development.

55. Hsu C.C., Yu M.L.M., Chen Y.C.J., Guo Y.L.L., Rogan W.J. The Yu-Cheng rice oil poisoning incident. *Dioxins and Health*, edited by Arnold Schecter, Plenum Press, New York. 1994;20:661-684.

This is a review article, the findings in infants are reviewed and summarized in the original articles (see references also).

Introduction

Eleven years after the Japanese Yusho incident, a similar tragedy happened in Taiwan in 1979. A Japanese-produced PCB mixture (Kanechlor 400, 500) was used as the heat-transfer medium in the process of deodorization and decolorization of rice oil. PCBs and their heat-degraded by-products, polychlorinated dibenzofurans (PCDFs), ter- and quaterphenyls (PCTs and PCQs), leaked into the rice oil and intoxicated 2,000 people. The initial clinical symptoms consisted of acne, pigmentation of the nails and skin, hypersecretion of the meibomian glands. The syndrome was referred to as Yu-Cheng, which translates to "oil disease."

Discovery and Epidemiologic Findings

On May 21, 1979, a local health bureau in Taichung county, central Taiwan, was notified of an outbreak of skin disease among staff and students boarded at the Hwei-Ming School for Blind Children. Similar cases involving 85 of 150 workers from a nearby plastic shoe factory were reported in early September, 1979. A common exposure, a rice bran cooking oil (C-rice bran oil) manufactured by a rice oil company in Changhua was identified. More cases from other companies and local households in both Changhua and Taichung counties were reported.

Samples of C-rice bran oil from the Hwei-Ming School and the oil store in Taichung, and blood from victims were analyzed and PCBs resembling Kanechlor 400, 500, were detected on October 6, 1979.

As of February 1983, 2,022 Yu-Cheng subjects were reported and included in the Yu-Cheng registry that was maintained by the Taiwan Provincial Health Department. More than 50% of the victims were less than 25 years of age. Generally speaking, the Yu-Cheng cohort is a young cohort of low socioeconomic status.

The only oil samples that were positive for PCBs/PCDFs were those from Taichung oil store, the School for Blind Children, and from victims' homes. No PCBs/PCDFs were detected in oil samples from Changhua oil stores and the oil company; perhaps the owners had removed all contaminated oil after the etiology of the illness had been publicly released. Even though no machine containing PCBs was found at the oil company, the high PCB levels from both soil samples at the site and blood samples from plant workers suggested that PCBs had been used in the plant recently. In addition, the detection of PCQs and PCDFs in the toxic oil further suggested that PCBs had been subjected to high temperatures.

Seven samples contained about 53-100 ppm total PCBs, one-tenth of the level in the Japanese Yusho incident, while one sample contained 405 ppm. The percentages of tetra-, penta-, hexa-, and hepta-chlorobiphenyls were 33.8, 47.1, 12.4, and 4.5, respectively, a pattern closely resembling Kanechlor 400, 500; and the most prominent congeners were 2,4,5,3',4'- (12.4%) and 2,3,4,3',4' - penta-CB (11.5%). There were about 0.01-1.68 ppm total PCDFs in the oil samples, with a constant PCDF/PCB ratio of about 0.1-0.3%. PCDFs with four, five, and six chlorines were present in the oil, and 2,3,4,8-tetra- and 2,3,4,7,8- penta-CDF were the major congeners. The PCQs to PCBs ratio varies in the reports on the Taiwan oil from about 39% to 180%.

Patients consumed the contaminated oil at an average rate of 1.4 kg per month (range 1.0-1.6 kg/month) for 2 to 3 (average 2.7) months before they became symptomatic, and for another 6 months before the oil was withdrawn. The PCB and PCDF concentrations in the oil samples were about 67-99 and 0.21-0.40 ppm, respectively. Thus,

the patients consumed on average 302 mg (range 196-457) of PCBs and 1.3 mg (range 0.5-1.9) of PCDFs before they developed symptoms, and about 1 g (range 0.7-1.4) of PCBs and 3.8 mg (range 1.8-5.6) of PCDFs total. The rice oil in Taiwan was withdrawn later. The Taiwanese patients consumed about ten times more contaminated oil than the Japanese patients; however, the PCB/PCDF concentration in the Japanese oil was ten times higher; therefore, patients of both countries consumed about the same amount of PCBs/PCDFs.

Total blood PCB levels have been measured by several research groups at different times. All persons tested in these studies had detectable PCB levels, and the mean levels ranged from 38 to 99 ppb. Rogan and colleagues collected blood samples from 21 children born to Yu-Cheng women during or after the outbreak and 15 age, sex, neighborhood-matched controlled children; 14 of the 21 exposed children and 6 of the 15 controls had detectable PCBs in blood, the mean PCB levels being 8.45 ppb (range 0.12-77.8 ppb) in the in utero-exposed children and 3.49 ppb (range 1.06-6.99 ppb) in the controls.

PCDFs and PCQs in Blood

Chen et al. also identified 2,3,4,7,8-penta- and 1,2,3,4,7,8-hexa-CDF as the major detectable PCDF congeners in 10 samples. Lundgren et al. identified the same congeners in 12 samples collected from different subjects in 1985, but at a lower concentration. In 1991, the two congeners were still detectable in 9 of the 14 blood samples drawn from the in utero-exposed children by Ryan et al., the 2,3,4,7,8-penta-CDFs ranged from 89 to 570 ppt, lipid, in the exposed children, and 22 ppt, lipid, in the pooled control sample; the 1,2,3,4,7,8-hexa-CDF ranged from 120 to 1590 ppt, lipid, in the exposed children, and 18 ppt, lipid, in the control sample.

Schecter et al. measured dioxin and dibenzofuran levels in six placentas of Yu-Cheng women obtained in 1984 and 1985, and one general population placenta from a U.S. woman. The Yu-Cheng placentas contained elevated levels of two congeners; 2,3,4,7,8-penta-CDF ranged from 820 to 12,560 ppt lipid, compared with 6.8 ppt lipid, in the nonexposed placenta; and 1,2,3,4,7,8-hexa-CDF ranged from 2,345 to 26,540 ppt lipid, compared with 8.7 ppt lipid, in the nonexposed placenta.

Increased eye discharge, swelling of eyelids, and disturbance of vision, were the major complaints at early stages; general malaise, numbness of limbs, pruritus, and headache and dizziness. Ten percent of the female victims were found to have abnormal menstruation. Decreased growth in height and weight of 30 elementary school students in Changhua were reported. These students had also ingested the contaminated oil.

Mucocutaneous pigmentation was the most common symptom, occurring in at least 90% of the patients; conjunctiva, gingiva, and buccal mucosa, nasal apex and ala, finger- and toenails. The next most common symptom was acneform eruptions, occurring in 75% of Wong's and 51% of Lu's patients. Deformities of finger- and toenails were found in 68% of Wong's patients, and in 38% of Lu's patients.

Both Lu and Wong (1984), Cheng et al. (1981) and Wong et al. (1982) tried to relate the total blood PCB level to the severity of skin symptoms, but neither group showed any consistent association. These negative associations plus the fact that neither report had any control or background rates for the skin lesions made the interpretation difficult. However, the very high rates of mucocutaneous pigmentation, the unique distribution pattern of acneform eruptions, and the unusual picture of hyperkeratotic plaques make a strong case that these symptoms are related to the ingestion of PCB- and PCDF- contaminated rice bran oil. Histologic examination of 21 skin biopsies showed hyperkeratosis, increased pigmentation of epidermis, and cystic dilatation of hair follicles. Except for the highly pigmented epidermis, the eruptions were indistinguishable from acne vulgaris.

The most common neurologic complaints were numbness and paresthesia of the extremities (36.1%) and headache and/or dizziness (34.8%). However, the incidences of the above-mentioned symptoms/signs in the general population were not reported. When compared with values for 63-150 nonexposed normal subjects, the exposed subjects were on average 4 m/sec slower than the controls in both sensory and motor NCV. Patients with blood PCB levels of 24 ppb or greater had significantly slower peroneal nerve motor NCV than those with blood PCB levels below 24 ppb. For 65 patients with known blood PCQ levels, blood PCQ level was negatively associated with median nerve sensory NCV.

Immunology

When compared with the normal Taiwan laboratory values, total leukocyte count of the patients was elevated (9650 ± 2800 versus 7053/mm^3 ± 1205) but with a normal differential, α_2-globulin was slightly increased and γ-globulin was slightly decreased. Delayed-type skin hypersensitive test using streptokinase/streptodornase solution was positive in 36% of patients compared with 79% of the Taiwan general population; this result suggests suppression of cellular immunity in the Yu-Cheng patients.

Serum immunoglobulin tests on a subset of 30 patients who had blood PCB levels above 15 ppb and 23 age, sex-matched controls showed significant decreases in IgA and IgM in the exposed (185 ± 88 versus 245 ± 70 for IgA, and 105 ± 58 versus 173 mg/100 ml ± 48 for IgM), suggesting suppression of humoral immunity. The 30 patients had two-thirds the percentage of T cells (42 versus 63%) of

controls, and the percentages of active T cells (11 versus 22%) and "helper" T cells (22 versus 37%) were also decreased.

Effect of heat-degraded PCBs on lymphocyte function still existed 3 years after the exposure.

Lu and colleagues studied urinary excretion of porphyrins and heme precursors in 69 blind students from the Hwei-Ming School and 20 healthy volunteers. The mean 24-hr excretion of uroporphyrin for the exposed students was 41.2 ± 24.6 mg/24 hr, compared with 13.6 ± 11.8 for the controls; levels of excretion of δ-aminolevulinic acid, a heme precursor, for the exposed and the controls were 1.0 ± 0.6 and 0.7 ± 0.3, respectively, and red cell δ-aminolevulinic acid dehydratase (δ-ALAD) activities were depressed. Excretion of coproporphyrin and porphobilinogen was not affected. The mean uro/copro ratio was 1.4 ± 1.3 in the exposed, and 0.5 ± 0.3 in the controls. Thirty-six of the sixty-nine exposed students had ratios above 1, while none of the 20 controls had a ratio that high. δ-ALAD activities of 23 exposed subjects from Changhua county were depressed in buffered solutions of different pH when compared with those of 20 healthy volunteers.

Of 49 children born to Yu-Cheng women between 1979 and 1985, the gestational age-adjusted birth weights for female and male exposed babies were respectively 83 and 87% of those of the "normal babies."

Critical Comments

This is a review and the papers summarized here have also been reviewed and commented on separately. The reader is referred to these summaries in this document. For instance, in the paper by Gladen et al. (1988), the controls as well as the exposed had rather high coproporphyrin levels, but a definite effect on urinary porphyrin levels in the exposed was not demonstrated

The Yu-Cheng patients were exposed to toxic levels of PCDFs. However, there was much variation among the exposures the individuals received. The concentrations of PCDFs and PCBs also varied in different oil samples. Attempts to correlate exposure and effects were only marginally successful. The reasons for this were multiple. The exposure was only assessed in a subset of individuals. Quantitatively, PCB exposure was a poor surrogate for PCDF exposure. Some of the disease endpoints were difficult to distinguish from background disease in the general populations. A certain amount of misclassification of cases and controls appears to have occurred as well, based on some information in this review and in other studies. In the immunology studies, it is not clear whether the controls were age matched and were also from an institution. Apparently, no information was collected on confounders that may have adversely affected the immune system.

REFERENCES

Cheng P.C., Chen C.J., Wong C.K., Chen P.H. Dermatological survey of 122 PCB poisoning patients in comparison with blood PCB levels [in Chinese; English summary]. *Clin Med (Taipei)*. 1981;7:15-22.

Gladen B.C., Rogan W.J., Ragan N.B., Spierto F.W. Urinary porphyrins in children exposed transplacentally to polyhalogenated aromatics in Taiwan. *Arch Environ Health*. 1988;43:54-58 (errata 348).

Gladen B.C., Taylor J.S., Wu Y.C., Ragan N.B., Rogan W.J., Hsu C.C. Dermatological findings in children exposed transplacentally to heat-degraded polychlorinated biphenyls in Taiwan. *British J Dermatol*. 1990;122:799-808.

Ju S.H., Chen Y.J., Chen Y.C., Hsu C.C. Follow-up study of growth and health of children born to mothers intoxicated by polychlorinated biphenyls [abstract]. *Pediatr Research*. 1992;28:93A.

Lu Y.C., Wong P.N., Dermatological, medical, and laboratory findings of patients in Taiwan and their treatments. *American J Ind Med*. 1984;5:81-115.

Rogan W.J., Gladen B.C., Hung K.L., Koong S.L., Shih L.Y., Taylor J.S., Wu Y.C., Yang D., Ragan N.B., Hsu C.C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science*. 1988;241:334-336.

Wong C.K., Chen C.J., Cheng P.C., Chen P.H.S. Mucocutaneous manifestations of polychlorinated biphenyls (PCB) poisoning: a study of 122 cases in Taiwan. *British J Dermatol*. 1982;107:317-323.

56. Hsu M.M.L., Mak C.P., Hsu C.C. Follow-up of skin manifestations in Yu-Cheng children. *British J Dermatol.* 1995;132:427-432.

In February 1991, the Yu-Cheng cohort (128 children born to 74 PCB-exposed mothers), their matched control cohort (115 children born to 69 mothers), and the older unexposed siblings, born before the PCB outbreak, from 15 exposed families, were invited for a comprehensive physical examination. Blood samples were taken for PCB and PCDF analysis. The control cohort, except for the 15 older siblings of exposed children, had been matched for neighborhood, age (within 15 days for those under 1 year old, and within 1 month for older children), sex, mother's age (within 3 years), parents combined educational level (within about 3 years for the total), and occupation (within one class of five classes, from unskilled laborers to professionals).

Eighty-eight exposed children and 86 control children were examined in the current study; 79 were from the Yu-Cheng child cohort, and the other nine were younger siblings of the cohort children who had been born since July 1985, about 6 years after the outbreak. Because the nine younger children were born to affected mothers, they qualified for inclusion in the exposed group. Among the 86 controls, 75 were from the matched control cohort established in 1985, and 11 were older unexposed siblings from exposed families. In total, 57 pairs were matched for age, sex and socio-economic status.

Any effects which might have been present could easily be mistaken for the usual dermatological changes related to age. Generalized pigmentation was not observed in this study. Local pigmentation in individuals may have resulted from different levels of sun exposure.

Nail changes were a prominent finding. The abnormalities were defined as follows: transverse groove(s)--at least one groove either longer than 2 mm or deep enough to retain some dirt after normal hand cleansing; irregular depressions--at least two small flattened or depressed areas which resulted in irregular reflections on the nail surface, or mild grooves not fulfilling the criteria of transverse groove(s); small pits--punctate pits smaller than 1 mm; koilonychia/flattening--whole nail plate flattening or concavity without surface irregularity; fine parallel transverse lines--multiple transverse, curved, fine ridges parallel to the distal contour of the lunula.

On the basis of the above classification, a marked difference between case and control groups was noted with regard to transverse grooves of the nails ($p<10^{-8}$). Transverse grooves occurred in both toe-nails and finger-nails, but koilonychia/flattening occurred principally in the toe-nails.

Discussion

In the present study, conducted 11 years after the Yu-Cheng outbreak, the skin or mucosal pigmentation and meibomian gland manifestations which had been observed previously were no longer present. It would appear that the skin manifestations of Yu-Cheng children, which were conspicuous in the initial stage of intoxication, have greatly improved, as have those in Yusho patients (10-year follow-up) and in Yu-Cheng patients (4-year follow-up).

The persistent nature of the nail changes suggests the possibility of intrauterine injury by PCBs. However, as far as the specificity of these nail changes is concerned, one or several transverse grooves, with or without an irregular nail surface, can also be observed in individuals with paronychia or a habit tic, and they may be indistinguishable from those in patients with congenital PCB exposure. Nevertheless, except for one child, none of our patients had any evidence of paronychia or a habit tic. In assessing nail changes attributed to PCB exposure, it is essential that abnormalities are strictly defined, and comparisons made with an appropriate control group.

According to a recent report of our collaborative study, limited data from nine serum samples from our Yu-Cheng children revealed that PCB and PCDF concentrations were still many times higher than those in a pooled serum sample from 10 matched control children (PCB, 2.99 vs 0.51 μg/kg lipid; PCDF, 260 vs 22 ng/kg lipid). However, these elevated serum PCBs and PCDFs did not positively correlate with the presence of nail changes.

Critical Comments

The described nail changes seem more prevalent in the exposed population. However, they are nonspecific and various fungus and chronic bacterial infections of the nails particularly in older children would have to be ruled out to determine how many of these changes were the result of the ingestion of toxic oil.

57. Ko H.C., Yao B.L., Chang F.M., Hsu C.C., Jacobson S.W., Jacobson S.L. Preliminary evidence of recognition memory deficits in infants born to Yu-Cheng exposed women. Dioxin '94. 14th international symposium on chlorinated dioxins, PCB and related compounds, Kyoto. *Organohalogen Compounds*. 1994;21:505-508.

This report presents preliminary findings on 11 infants recently born to Yu-Cheng women and 48 non-exposed controls, who were administered the Fagan visual recognition memory (VRM) test at 6.5 months.

The Fagan recognition memory test consists of 10 pairs of photos. The infant is initially shown two identical target photos until he or she fixates them for a total of 20 seconds. The familiar target is then paired with a novel target for two 5-second periods, reversing left-right positions from one period to the next. The normative response at this age is to spend more time looking at the novel stimulus.

Control mothers were more educated than Yu-Cheng mothers and more likely to be primiparous. Both characteristics were statistically significantly different.

Yu-Cheng offsprings showed significantly poorer recognition memory, as indicated by poorer novelty preference (55% for Yu-Cheng-exposed vs 65% for controls.

Critical Comments

These are preliminary findings and the number of exposed children that were tested (11) was small while the number of controls tested (48) was much larger. The controls seem to have been oversampled and it is not stated whether any matching was done between these two groups. I assume they were matched for age. Insufficient information is given in this abstract for proper evaluation. Since the mothers of the controls were primiparous more often and since they were better educated, these controls may have been inappropriate.

58. Lai T.J., Chen Y.C., Chou W.J., Guo Y.L., Ko H.C., Hsu C.C. Cognitive development in Yu-Cheng children. Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. *Organohalogen Compounds*. 1993;14:247-250.

(This is a short paper and gives few details.)

A description of the subjects of this study has been given previously. Tools of cognitive assessment were (1) Bayley Scale of Infant Development (BSID) for children less than 30 months old. (2) Stanford Binet Intelligence Test for those 30 months to 6 years old. (3) Wechsler Intelligence Scale for Children, Revised (WISC-R) for children 6 to 16 years old. (4) Raven's Colored Progressive Matrices for 5 to 9 year old children. (5) Raven's Standard Progressive Matrices for those ages 9 to 15. These were administered to all children at their homes every year except BSID was done every 6 months.

The mean age of Yu-Cheng children and their controls on September 1, 1985 was 3.6 ± 2 years. Each mean age of Yu-Cheng children and their reference older sibs on September 1, 1985 were 5.69 ± 1.66 and 8.62 ± 1.28 years old. (Please note: the last sentence was taken verbatim out of the paper and it is unclear what it means.) In the seven-year follow-up study of WISC-R between Yu-Cheng

children and their controls, there was a tendency for Yu-Cheng children to score lower in Performance IQ (PIQ), Verbal IQ (VIQ) and Full-Scale IQ (FIQ) by an average of 4 to 5 points. The differences of scores of the PIQ, VIQ and FIQ between Yu-Cheng children and their reference older sibs were decreasing in the later years, almost the same in the seventh-year data. Yu-Cheng children almost always had lower scores in the other measure tools as compared with their controls, and they were statistically significant in the occasional years.

Data on the WISC-R test revealed that Yu-Cheng children had consistently scored lower than their controls. In Verbal IQ, the differences between Yu-Cheng children and their controls were relatively small but tended to increase year by year and have been statistically significant since 1990, the sixth-year of fieldwork. It may either suggest a possibly delayed effect of prenatal exposure to PCBs on the verbal-related cognitive development of Yu-Cheng children or is purely due to other factors yet to be clarified. Yu-Cheng children had a tendency to catch up with their sibs in the cognitive development. There appeared to be a "learning effects" in the WISC-R IQ values for all children. The IQ scores increased with the age and year of testing. The learning effects were similar in all the children.

Critical Comments

This paper, an extended abstract, lacks clarity and cannot be evaluated. Furthermore, the authors do not really explain how they obtained their results, what confounders there might have been, and how meaningful their results are.

59. Lai T.J., Guo Y.L., Chen S.J., Yu M.L., Hsu C.C. Cognitive development in Yu-Cheng children. Dioxin '94. 14th international symposium on chlorinated dioxins, PCB and related compounds, Kyoto. *Organohalogen Compounds*. 1994;21:513-516.

The tools of cognitive assessments were (1) Bayley Scale of Infant Development (BSID) for those below 30 months of age, (2) Stanford Binet Intelligence Test for those from 30 months to 6 years, (3) Wechsler Intelligence Scale for Children, Revised (WISC-R) for those 6 to 16 years, (4) Raven's Colored Progressive Matrices (CPM) for those 5 to 9 years, and (5) Raven's Standard Progressive Matrices (SPM) for those 9 to 15 years old. These were administered yearly to all children at their homes except the BSID was done every 6 months.

The mean age of Yu-Cheng children and their controls on September 1, 1985 was 3.6 ± 2 years old. During the eight-year follow-up study of WISC-R between Yu-Cheng children and their matched controls, Yu-Cheng children scored lower in performance IQ (PIQ), verbal IQ (VIQ), and full scale IQ. However, the differences were not significant at the eight-year follow-up study.

CPM and SPM scores were also lower among Yu-Cheng children, but they were not statistically significantly different at the eight-year follow-up. In some other years, scores were also not always statistically significantly different.

Discussion

Data on the SPM and WISC-R test revealed that Yu-Cheng children had consistently scored lower than their controls. The eighth-year data of WISC-R revealed that Yu-Cheng children had a tendency to catch up with their controls in the cognitive development.

There seemed to be a similar "learning effects" in the WISC-R IQ values for all the children. The IQ scores increased with the year of examination and age.

Critical Comments

This is a short paper presented at a meeting. Insufficient detail is given for a critical evaluation.

60. Lai T.J., Guo Y.L., Yu M.L., Ko H.C., Hsu C.C. Cognitive development in Yu-Cheng children. ***Chemosphere.*** **1994;29:2405-2411.**

Method

Parents of 118 of the 127 Yu-Cheng children consented to participate in this 12-year follow-up study. For each Yu-Cheng child, a control child was assigned. Results from the Stanford-Binet Test and Wechsler Intelligence Scale for Children, Revised Form, will be presented. For the yearly field study, testers were trained by a senior psychologist (Ko HC). The Yu-Cheng child and his/her matched control child were tested on the same day, and random cross-validation was carried out. Paired two tailed t test was utilized to compare the difference of DQ/IQ score between the Yu-Cheng and the control child in each of the 118 pairs of subjects.

Results

Comparison of Mental Development Index (MDI) and Psychomotor Development Index (PDI) of the two groups of children showed that at each age level from 6 months to 30 months, the Yu-Cheng children scored lower than their controls. The means of difference between the two children in each pair for MDI and PDI ranged from 4 to 11 points. Comparing the two groups on Stanford-Binet IQs by age level from 2 through 5 years showed that the Yu-Cheng children scored lower in each of the 4 age levels. The means of the difference between the two children in each pair at different age levels ranged from 3 to 8 points.

Comparing the PIQs, VIQs, and FIQs of the two groups by year of test administration shows a steady trend similar to the BSID Developmental Indices and Stanford-Binet IQs. Yu-Cheng children scored lower in all of the 3 IQs of WISC-R in each year from 1985 through 1991. The means of the difference between the two children in each pair for each IQ in each year ranged from 1 to 7 points.

The comparison of the two groups on WISC-R PIQs, VIQs, and FIQs from ages 6 through 12 showed higher scores among the controls. However, not all scores are statistically significantly different for all years. Total PCDFs 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran are 2 to 132 fold higher than the controls.

Critical Comments

In addition to the test results, the authors also present results obtained in the same children in earlier years. The paper is not very clearly written and important information is omitted. For instance, did the investigators know who the Yu-Cheng children were? When the matching was done, was the birth order considered? If the controls were class mates, was the school performance of the controls known to those who chose them? In a study like this it would have been better if two controls at least had been used for each exposed child. The differences observed in the IQ tests between the matched pairs are not very impressive and it is curious that all individual control children appear to have had higher scores. Perhaps the controls came from different backgrounds. Also the individual differences are small.

61. Lambert G.H., Guo Y.L., Lai T.J., Hsu C.C., Garcia F., Taylor P., Schoeller D.A. Is PCB/PCDF induced long-term neurological dysfunction in the child dependent upon the ah receptor? Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. ***Organohalogen Compounds.*** **1993;14:263-266.**

Children from the Yu-Cheng cohort exposed in utero to PCB/PCDFs were recruited from the Yu-Cheng registry of 118 children. The caffeine breath test was conducted as a measure of P4501A2 activity and the Wechsler Intelligence Scale for children, Revised (WISC-R) was utilized as an assessment of neurological function. The results of the caffeine breath test were expressed as the percent dose of administered label exhaled over two hours after administering labeled caffeine (% dose exhaled over two hours).

In 1987, the WISV-R and the caffeine breath test were performed in fourteen of these Yu-Cheng children; and in 1991, thirty-four children were assessed by the WISV-R and had the caffeine breath test performed in 1992.

These caffeine breath test results did not significantly differ from the caffeine breath test results that were previously reported in control children. The VIQ, PIQ, and FIQ in the Yu-Cheng children in the 1987 study ranged from 68 to 120 with a median of 87 for the VIQ, 78 to 122 with a median of 101 for PIQ, and 72 to 123 with a median value of 94 for FIQ; and in the 1991 evaluation results ranged from 52 to 130 with a median value of 102 for VIQ, 81 to 147 with a median value of 115 for PIQ, and 49 to 141 with a median of 105 for FIQ.

To test the hypothesis, the correlation between the caffeine breath test and the VIQ, PIQ, and FIQ was examined by regression analysis. There was no correlation between the CBT and the VIQ, PIQ, and FIQ in the children studied in 1987 or 1991 and 1992 ($r^2<0.006$ for all comparisons).

The study hypothesis was confirmed by these results. The lack of any correlation between the P4501A2 activity and neurological function in these children indicate that their ongoing neurological deficits are not related to current P4501A2 activity induction nor ah receptor activation.

Critical Comments

This is essentially a negative study and supports the observation stated in many papers that the PCB and PCDF levels in serum are also poorly correlated with behavioral changes.

62. Lambert G.H., Mocarelli P., Hsu C.C., Needham L.L., Ryan J.J., Guo L., Brambilla P., Signorini B., Patterson D.G., Lai T.J., Garcia F., Ferrari E., Schoeller D.A. Cytochrome P4501A2 activity in dioxin exposed Seveso subjects as compared to polychlorinated biphenyl and polychlorinated dibenzofuran exposed Yu-Cheng subjects. Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. *Organohalogen Compounds*. 1993;14:253-256.

The comparison of P4501A2 activity in adults from two important cohorts exposed to PCDDs or PCDFs are reported. 1) The Seveso cohort exposed to 2,3,7,8-TCDD in 1976 with the exposure resulting in the highest serum levels of dioxin ever measured in the human. 2) The Yu-Cheng cohort exposed to polychlorinated biphenyls and polychlorinated dibenzofurans in 1978 which resulted in the most severe human health effects reported from exposure to the PHBs. In vivo P4501A2 activity was monitored by the [3-C-methyl] caffeine breath test a measure of cytochrome P4501A2 dependent 3-N demethylation.

The dose of labelled caffeine was 3 mg/kg up to a maximum dose of 200 mg. Two 15 ml aliquots of end tidal breath samples were collected just before the ingestion of caffeine and at 30, 60, 90,

and 120 minutes after the ingestion. The ratio of $^{13}CO_2$ to $^{12}CO_2$ in the breath as determined by differential mass spectroscopy was measured. The data were normalized for basal metabolic rate and expressed as % administered label exhaled over two hours. The serum samples are being analyzed by isotope dilution HRGC/HRMS.

The differential mass spectroscopy of the breath samples are completed for the Yu-Cheng population. The exposed subjects had caffeine breath tests ranging from 5.0 to 23.6% dose exhaled over two hours with a median of 10.2% dose exhaled over two hours. There were 37 control subjects for the Yu-Cheng subjects and their caffeine breath test ranged from 1 to 5.2 with a median value of 3.6% dose exhaled over two hours. The analysis of the caffeine breath tests for 28 Seveso subjects who did not smoke ranged from 1.8 to 11.0 with a median value of 4.32% dose exhaled over two hours. There are only 9 Seveso control subjects who did not smoke. Their caffeine breath test results range from 0.37 to 6.18 with a median value of 4.38% dose exhaled over two hours. The 105 Asian and North America control subjects previously studied had caffeine breath test results that ranged from 0.3 to 9 with a median value of 3.4% dose exhaled over two hours.

Critical Comments

No statistical analyses were presented. It appears that some of the Yu-Cheng patients have higher P4501A2 levels. No information is given about the body burdens of these patients or about the body burdens of the Seveso patients.

63. Lan S.J., Yen Y.Y., Ko Y.C. A study on development and growth of permanent teeth of Yu-Cheng babies. Symposium on health risk assessment on environmental occupational and life style hazards. Dec. 20 - 22, 1988 Taipei, Taiwan, Republic of China.

(More details on this subject are given in Shou-Jen Lan et al. *Bull Environ Contam Toxicol.* 1989;42:931-934.)

64. Lan S.J., Yen Y.Y., Ko Y.C., Chen E.R. Growth and development of permanent teeth germ of transplacental Yu-Cheng babies in Taiwan. *Bull Environ Contam Toxicol.* 1989;42:931-934.

This study will focus only on the growth and development of permanent teeth of babies exposed to PCBs transplacentally. Four primary schools were selected from high density areas of PCB intoxication. The exposure group consisted of poisoned mothers and transplacentally exposed Yu-Cheng babies born after 1979.

A reference group was selected based on the following criteria: (1) same sex, (2) age difference within three months, (3) same occupation of fathers, (4) similar family economic status, (5) indigenous residents of the area, (6) mothers not poisoned by PCB.

Each exposed case was matched against four randomly selected reference cases of the 18 exposed children (9 males and 9 females). A reference group of 72 (36 males and 36 females) were chosen but only 44 children (26 males and 18 females) joined the study. Panographs of the 62 participants were taken. The X-ray films were read by three dentists using single blind method.

A skillful and experienced dentist would easily observe any congenital absence of permanent teeth from children of 7 or 8 years old. The third molar is an exception so this tooth was not included in the study. All three dentists identified the same permanent teeth germ missing in all cases.

Among 9 transplacental Yu-Cheng girls, 4 were missing permanent teeth germ. Among the 18 girls in the reference group, none was missing permanent teeth germ due to congenital factors (0/18). Among 9 transplacental Yu-Cheng boys, one was missing permanent teeth germ due to congenital factors (1/9). Among the 26 boys in the reference group, one was missing a permanent tooth germ due to congenital factors (1/26).

Critical Comments

This study shows that transplacental exposure to PCDFs may result in loss of permanent teeth germs. The number of children examined was small and it is unclear how or why they were selected out of the group of transplacentally exposed children. Additional information on the prevalence of missing teeth germs in the general population in Taiwan would also be helpful since 1/26 control male children also had a missing permanent tooth germ.

65. Lan S.J., Yen Y.Y., Lan J.L., Chen E.R. Immunity of PCB transplacental Yu-Cheng children in Taiwan. ***Bull Environ Contam Toxicol.*** **1990;44:224-229.**

Methods

The exposed group was made up of transplacental Yu-Cheng children born to PCB-poisoned mothers after 1979. A control group matched on sex, age, paternal occupation, family economic status, resident area, school and classroom was also recruited. Each exposed case was matched with two randomly selected controls. The 19 exposed children (10 males and 9 females) 7.1 to 9.0 years old (8.4 ± 0.5), were selected to participate in this study. A control group of 38 children were selected but only 32 (16 males and 16 females) age 7.6 to 9.0 years (8.5 ± 0.4), agreed to participate. Because standard deviation of tests were so large, the Mann-Whitney U test was used for the statistical analysis.

Immunological and hematological tests. These examinations included T-cell, suppressor T-cell, helper T-cell, active T-cell, B-cell and WBC counting, leukocytes, differential and immunoglobulin IgG, IgA, IgM and complement C3, C4 and ANA (antinuclear antibody); flowcytometric study of cell surface makers; serological examination. Complement C3, C4 and immunoglobulin IgG, IgM, IgA in serum were analyzed with nephelometry by using the Beckman Array Protein System. ANA (Antinuclear antibody) was performed with the FIAX kit (Whittaker Bioproduct) using the Hep-2 cell line.

Results

All results were within the normal range. No significant difference for any immunological or hematological tests between the exposed and control group were noted. This result is different from previous studies of Yu-Cheng patients in Taiwan.

According to the authors these negative results can be explained by the fact that they examined these children many years after exposure, exposure was indirectly through the placenta and the age of their study subjects differed.

Critical Comments

The studies are straight-forward. However, the number of study participants is small and no information is given on the degree of exposure. The results also suggest, if immunological effects occurred at all they appear to be reversible.

66. Needham L.L. Historical perspective on Yu-Cheng incident. Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. *Organohalogen Compounds*. 1993;14:231-233.

Three general populations that have been exposed to PCDDs and PCDFs exist. These are the Yusho (1968, Japan), Yu-Cheng (1979, Taiwan), and Seveso (1976, Italy) populations.

All three of these episodes led to extremely high levels of PCDFs or 2,3,7,8-TCDD in the exposed populations. In fact, the toxic equivalency based on 2,3,7,8-TCDD per kilogram of body weight in selected chloracne cases showed remarkable agreement. Yet other adverse health effects in these populations differ.

Body Burden Levels (TEQ) in Chloracne Cases

INCIDENT	Levels (μg/kg bw)
Yusho*	3.0
Yu-Cheng*	2.0
Seveso+	3.0

*Exposure primarily to 2,3,4,7,8-PCDF and 1,2,3,4,7,8-HxCDF Levels from Ryan et al. *Fund Appl Toxicol.* 1990;15:722-731.

+Exposure to 2,3,7,8-TCDD. Calculated level from Needham et al. *Banbury Report.* 1991;35:229-257.

Critical Comments

This is a short paper presented at a meeting. In this paper data published elsewhere are reviewed.

67. Olafsson P.G., Bryan A.M., Stone W. Polychlorinated biphenyls and polychlorinated dibenzofurans in the tissues of patients with Yusho or Yu-Cheng: total toxicity. *Bull Environ Contam Toxicol.* 1988;41:63-70.

This short review addresses toxicity equivalency. Some levels of PCDFs and PCBs in tissue of Yusho and Yu-Cheng patients are given.

68. Rogan W.J. Teratogen update. PCBs and cola-colored babies: Japan, 1968, and Taiwan, 1979. *Teratology.* 1982;26:259-261.

In 1968, the use of rice bran cooking oil contaminated with polychlorinated biphenyls (PCBs) and other chemicals caused an outbreak of severe acne among residents of Kyushu, Japan.

In 1979, a very similar outbreak occurred in Taiwan.

Neither in Japan nor in Taiwan has there been a clear relationship between symptoms of those affected or fetopathy and dose. Blood levels of the chemicals drawn relatively soon after exposure have been difficult to interpret clinically. In addition, in both episodes the PCBs were used as heat transfer agents, and had undergone thermal degradation. The resulting mixtures are contaminated with the highly toxic PCDFs, as well as tri- and quaterphenyls. Thus, measurable PCBs may be only a surrogate for the toxic agent (most likely only on a qualitative basis).

Some follow-up information is available on the Japanese children. The growth disturbance tended to disappear a few years after exposure ceased.

Critical Comments

This is basically a review. The authors point out that the correlation between clinical symptoms and signs and PCB levels is poor. The authors point out that the PCDFs seem to be responsible for these outbreaks. Unfortunately, because the analysis for the PCDFs is more difficult and costly, not too many samples were analyzed for these chemicals. No samples were collected for PCDF analysis early in the outbreaks.

69. Rogan W.J., Gladen B.C. Dysmorphic and neurologic changes in children exposed transplacentally to polyhalogenated aromatic compounds. *Environ Health Perspect.* 1987;75:125.

This is a review of data also published in:

Rogan W.J., Gladen B.C., Hung K.L., Koong S.L., Shih L.Y., Taylor J.S., Wu Y-C., Yang D., Ragan N.B., Hsu C-C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science.* 1988;241:334-336.

Hsu S-T., Ma C-I., Hsu S-K., Wu S-S., Hsu N-H., Yeh C., Wu S-B. Discovery and epidemiology of PCB poisoning in Taiwan: a four year follow-up. *Environ Health Perspect.* 1985;59:5-10.

70. Rogan W.J. Gladen B.C., Hsu C-C. Persistent dysmorphic changes in children exposed transplacentally to polychlorinated biphenyls (PCBs). *American J Epidemiology.* 1987, p. 779.

This is an abstract. More detailed information is given in

Hsu S-T, Ma C-I, Hsu S-K, Wu S-S, Hsu N-H, Yeh C, Wu S-B. Discovery and epidemiology of PCB poisoning in Taiwan: a four year follow-up. *Environ Health Perspect.* 1985;59:5-10.

Rogan W.J., Gladen B.C., Hung K-L, Koong S-L, Shih L-Y, Taylor J.S., Wu Y-C, Yang D., Ragan N.B., Hsu C-C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science.* 1988;241:334-336.

71. Rogan W.J., Gladen B.C., Hung K.L., Koong S.L., Shih L.Y., Taylor J.S., Wu Y-C., Yang D., Ragan N.B., Hsu C-C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science.* 1988;241:334-336.

In 1985, 117 children born to affected women and 108 unexposed controls were examined. The epidemic was noted in May 1979. Cases were identified retrospectively from as far back as December 1978.

By 1983, 8 of 39 hyperpigmented children born to exposed mothers had died. In April 1985, a field survey was performed of all living children who were known to have been in utero during or

after the period of oil contamination. Seventy-four women in the health department's registry had living children born between June 1978 and March 1985. Use of these dates should identify any child with transplacental exposure, since the latent period during which oil was consumed but mothers were asymptomatic was about 6 months. The women reported 159 pregnancies in this time; 3 were ongoing, 5 miscarried, 8 were aborted, 6 were stillborn, and 5 born live later died, leaving 132 living children. Twenty-nine families had 1 eligible child, 34 had 2, 9 had 3, and 2 had 4. Controls came from 96 families who lived in the same neighborhoods. These 96 mothers reported 205 pregnancies in this period; 3 were ongoing, 8 miscarried, 4 were aborted, and 190 produced live births. Data was obtained on 115.

Exposed mothers reported lower birth weight (mean ± SE: 2749 g ± 46 g, n = 128; 3228 g ± 40 g, n = 115), hyperpigmentation, conjunctivitis, nail changes, and natal teeth in the children at birth. The largest difference in the medical histories as the higher rate of bronchitis.

The exposed children were smaller than controls, averaging 93% of control weight and 97% of control height, adjusted for age and sex. The gum hypertrophy or swelling noted by the mothers at birth was still apparent on examination. The differences in hyperpigmentation and nail deformities and pigmentation are large. The exposed children were delayed compared to controls in the age at which they performed tasks such as saying phrases and sentences, turning pages, carrying out requests, pointing to body parts, holding pencils, imitating drawn circles, or catching a ball. The neurologists had an overall clinical impression of developmental or psychomotor delay in 12 (10%) of the exposed compared with 3 (3%) of the control children, and of a speech problem in 8 (7%) versus 3 (3%).

Age-appropriate testing of cognitive development and behavioral assessment were performed in the home after the survey, using new controls matched for neighborhood, sex, age, sib order, and family socioeconomic status. Except for verbal IG on the Wechsler Intelligence Scale for Children (WISC), the exposed children always scored lower than the controls on the three developmental and cognitive tests. On the Rutter scales, the exposed children showed higher (that is, worse) scores on all three scales. There are no Taiwanese norms for the Rutter scales; both exposed and control children scored higher than would be expected based on the norms developed by Rutter et al.

The children of workers exposed to PCBs uncontaminated by polychlorinated dibenzofurns (PCDFs) do not show nearly so much toxicity, but the mothers achieve blood PCB levels that are comparable to those seen in the outbreaks. The most likely reason is the presence of the very toxic PCDFs in the cooking oil. The PCDFs are active at much lower doses. The oil in Taiwan had about

100 ppm PCBs, and about 0.1 ppm PCDFs. Although there has not been a human exposure to PCDFs in the absence of PCBs, it is reasonable to assume that much of the toxicity seen in both outbreaks is due at least in part to PCDF contamination.

Critical Comments

Some children in the exposed group were obviously affected by the contaminated oil; however, many children were included that appear to have been asymptomatic and it is totally unclear whether all children born between June and December of 1978 actually received exposure and how well exposure was defined for the overall cohort of these children. No information is given on the appropriateness of the control group. Since the Rutter test is based on the subjective impression of the parents or of the examiners and since these individuals could not be blinded, the perception that something might be wrong with the exposed children most likely introduced an observer bias. Finally, the toxic effects observed in the children were the result of exposure to PCDFs and not to PCBs.

72. Rogan W.J. Developmental follow-up of children exposed transplacentally to PCBs/PCDFs in Taiwan. Dioxin '91. 11th international symposium on chlorinated dioxins and related compounds. Abstracts of the symposium speakers, poster discussions, poster presentations, September, 1991, *NIEHS*.

(This is an overview of study results from in utero exposed children during the Yu-Cheng poisoning outbreak. The same information is published in the original articles on Yu-Cheng that have been reviewed.)

73. Rogan W.J. Environmental poisoning of children -- Lessons from the past. *Environ Health Perspect*. 1995;103(6):19-23.

This is a review. The results summarized here are given in more detail in:

Hsu S-T., Ma C-I., Hsu S-K., Wu S-S., Hsu N-H., Yeh C., Wu S-B. Discovery and epidemiology of PCB poisoning in Taiwan: a four year follow-up. *Environ Health Perspect*. 1985;59:5-10.

Rogan W.J., Gladen B.C., Hung K-L., Koong S-L., Shih L-Y., Taylor J.S., Wu Y-C., Yang D., Ragan N.B., Hsu C-C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science*. 1988;241:334-336.

Chen Y-C.J., Yue-Liang G., Hsu C-C, Rogan W.J. Cognitive development of Yu-Cheng ("oil disease") children prenatally exposed to heat-degraded PCBs. *JAMA*. 1992;268:3213-3218.

Chen Y-C.J., Yu M-L.M., Rogan W.J., Gladen B.C., Hsu C-C. A six-year follow-up of behavior and activity disorders in the Taiwan Yu-Cheng children. *American J Public Health*. 1994;84:415-421.

Rogan W.J., Gladen B.C., McKinney J.D., Albro P.W. Chromatographic evidence of polychlorinated biphenyl exposure from a spill. *JAMA*. 1983;249:1057-1058.

In the paper, information about other pollutants (mercury, lead, DDT, endrin, hexachlorobenzene) is also summarized.

74. Ryan J.J., Hsu C.C., Guo Y.L.L. Exposure of children whose mothers suffered from Yu-Cheng poisoning to polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. *Organohalogen Compounds*. 1993;14:243-246.

Details are published in Ryan et al. *Chemosphere*. 1994;29:1263-1278.

75. Ryan J.J., Hsu C.C., Boyle M.J., Guo Y.L.L. Blood serum levels of PCDFs and PCBs in Yu-Cheng children perinatally exposed to a toxic rice oil. *Chemosphere*. 1994;29:1263-1278.

Methods

In February 1991, a number of children and their mothers from the original first mother/children cohort (born up to mid-1985) had blood samples drawn. At this time about 12 years had elapsed since the mothers first consumed the toxic oil. This resulted in forty-five individual serum samples from exposed children and in two larger pools of about 30 mL each formed from the matched control children. About 85% of the individual children samples were derived from the first cohort. The average age of the children in the first cohort at this time was 9.6 years. The amount of serum available from the 45 children was small and averaged 2.3 g with a range of 0.12 to 5.0 g. The analyses were restricted to 36 samples with at least 1.5 g of serum or 4.0 mg of blood serum lipid.

The median amount of lipid present in these samples was about 8 mg and the average lipid content about 0.34%. Using a 3:1 signal to noise ration, this resulted in a median detection limit on a serum lipid basis of about 150 ng/kg (ppt); range 80 to 500 ppt.

The median detection level for individual PCB congeners was about 0.2 μg /kg (ppb) on a <u>whole</u> weight basis and for total PCBs about 1.5 μg/kg; range 0.5 to 10 μg/kg.

Detectable concentrations of 2,3,4,7,8-PnCDF and 1,2,3,4,7,8-HxCDF could be measured in 22 (49%) and 24 (53%) of the 45 sera samples. The PCBs could be detected in 20 of the 45 samples (44%); six

samples contained measurable PCB levels but non-detectable PCDFs. The PCB profile in the samples from exposed children is dominated (more than 80% of total) by the presence of PCB #'s 153, 138, 156, 180, and 170. This profile is similar to the so-called pattern A used for description of Yusho blood samples whereby the relative amount of PCB #118 (2,3',4,4',5-PnCB) is reduced and that of PCB #156 (2,3,3',4,4',5-HxCB) is increased. 2,3,4,7,8 PnCDF in 22 sera ranged from 89-1230 ng/kg lipid, 1,2,3,4,7,8 HxCDF ranged in 24 samples from 120-3040 ng/kg lipid and the PCBs in 20 samples ranged from 0.9 - 36 ng/kg on a whole weight basis. (The units given in the paper for the PCBs measured were ng/kg. Either the PCB levels in this population were exceedingly low or the paper contains an error and in reality the unit should be μg/kg.)

Comparison of the concentrations of the two PCDF congeners between the control (background or normal levels) and Yu-Cheng exposed group, and using only positive responses for the latter, shows that concentrations of 2,3,4,7,8-PnCDF are 10 to 15 times greater in the exposed and those for 1,2,3,4,7,8-HxCDF are 15 to 25 times greater. The level of PCBs in the control sample was 0.56 ppb on a whole weight basis and this value is 10 to 15 times lower than the average value from the exposed Yu-Cheng children.

Discussion

The blood PCDF levels from some earlier work on the Yu-Cheng population indicate that the blood lipid PCDF level soon after appearance of symptoms in the Yu-Cheng incidence in the early 1980s was about 15 and 40 ppb, respectively, for the two important PCDF congeners, decreased to about 3 and 10 ppb a few years later, and fell to less than 1 and 3 ppb in the late 80s.

Presently it is not possible to obtain a significant correlation between the PCDF or PCB exposure in these children's sera and any health effect, particularly the developmental component.

Critical Comments

It appears that some children in this exposed population had no measurable PCDF levels and very low PCB levels. This reinforces that in the epidemiological investigation, exposure was not well defined and some members of the group registered with the health department were not actually exposed to the contaminated oil or not all of the oil from the factory was contaminated.

76. Sunahara G.I., Nelson K.G., Wong T.K., Lucier G.W. Decreased human birth weights after in utero exposure to PCBs and PCDFs are associated with decreased placental EGF-stimulated receptor autophosphorylation capacity. *Molecular Pharmacology*. 1978;32:572-578.

In this study, data on EGF receptor properties were compared with the birth weights, and placental and blood determinations of selected PCBs and PCDFs taken from control and Yu-Cheng subjects.

Methods

Term placentas were obtained from eight nonexposed control and eight PCB-exposed (Yu-Cheng) patients. All control and Yu-Cheng subjects in this study were nonsmokers and gave birth to an offspring at the same hospital in Taiwan. The medical records of these subjects revealed a significant reduction in the birth weights between the unexposed Taiwanese (3.37 ± 0.13 kg) and the Yu-Cheng (2.86 ± 0.07 kg) subjects ($p<0.02$).

The placental tissue was dissected free of chorion, amnion, large blood vessels, and umbilical cord and minced with scissors. The EGR-stimulated receptor autophosphorylation was quantitated by cutting the appropriate band from the dried SDS-PAGE gels followed by liquid scintillation counting. P-labeled liquid scintillation analysis (80% efficiency) of excised 150- to 170-kDa SDS-PAGE bands permitted quantitative comparison between the samples from Yu-Cheng and the unexposed subjects.

Results

In Yu-Cheng subjects, the phosphorylation of the EGF receptor was decreased (150- to 170-kDa protein bands representing the intact and the proteolytically cleaved EGF receptor). Other than the EGF receptor, EGF-stimulated phosphorylation bands were detected at approximately 190 KDa and just above the front. The identify of these bands is not known. There was a significant reduction (>50%; $p<0.001$) in the average amount of placental EGF receptor autophosphorylation in the Yu-Cheng samples (1229 ± 365 dpm) compared to the controls (2764 ± 420 dpm). It was interesting that this decrease strongly correlated with the birth weight reduction seen in the offspring of the exposed mothers; Pearson $r^2 = 0.73$; $p = 0.003$). Addition of 2,2', 4,4', 5,5'-hexa-CB or 2,2',3,3',4,4',5-hepta-CB to solubilized membranes at a concentration of 1nM had no effect on EGF-stimulated phosphorylation of receptor.

When the binding kinetics were analyzed for all individuals in each group, the average high affinity EGF-receptor binding kinetics were found to be similar between the Yu-Cheng (K_d = 0.11 ± 0.02 nM, B_{max} = 784 ± 305 fmol/mg) and control groups (K_d = 0.10 ± 0.02 nM; B_{max}

= 788 ± 225 fmol/mg). The inter-individual variation in EGF binding properties or stimulation of receptor phosphorylation did not reflect sex differences in the fetuses.

To determine whether the inter-individual variability in ^{125}I-EGF receptor binding kinetics and EGF-stimulated receptor autophosphorylation levels may be due to the degree of exposure to the contaminated rice oil, analyses for selected PCBs and PCDF congeners in maternal blood and placental tissue to some of the control and Yu-Cheng subjects were also performed. GC-MS analysis of maternal blood and placental tissue samples revealed a high concentration of CB and CDF congeners in the Yu-Cheng subjects relative to the unexposed control subjects. Two toxic PCDFs (2,3,4,7,8-pentachloro- and 1,2,3,4,7,8-hexachloro-) were detected in blood and placental samples of Yu-Cheng subjects but not in controls. In general, placental tissue concentrations of the PCDFs were 25 times greater than in the maternal blood.

The total placental PCB quantities were significantly higher in the Yu-Cheng subjects (range of 6.46 - 30.6 ppb) than in the controls (range of 0.13 - 1.46 ppb). Pearson correlation analysis revealed that there was a significant correlation between the placental levels of total PCBs and EGF-stimulated receptor autophyosphorylation (r^2 = 0.80; p = 0.01). Various PCBs were analyzed including (2,3,3',4,4'-), (2,2',4,4',5,5'), (2,3,3',4,4',5-), (2,2',3,3',4,4',5-), and (2,2',3,4,4',5,5'-) congeners in placental specimens of the Yu-Cheng and control subjects. GC-MS analysis revealed that, of the different PCB congeners tested, the 2,2',4,4',5,5'-hexa- and 2,2',3,3',4,4'5-hepta- PCB congeners represented the highest concentrations with respect to total PCBs (37 ± 2% and 10 ± 2%, respectively). It was interesting that the decrease in the placental EGF receptor autophosphorylation levels was significantly associated with placental levels of these two PCB congeners.

There were no significant correlations between the ^{125}I-EGF receptor binding kinetics and the EGF-stimulated receptor autophosphorylation levels of the Yu-Cheng and the control individuals. No significant relation (p>0.10) was observed when placental PCDF concentrations were compared to birth weights, microsomal benzo[a]pyrene hydroxylase activities (a placental marker of human exposure to toxic polycyclic aromatics), the ^{125}I-EGF receptor binding kinetics, or EGF receptor phosphorylation levels. According to the authors, taken together these data suggest that total PCB concentrations may be a better indicator of effects of exposure to contaminated rice oil on placental EGF-stimulated receptor autophosphorylation and birth weights than PCDF concentrations.

Discussion

Since the physiological role of the EGF receptor in fetal development is not well defined, it is difficult to determine whether the decrease in placental EGF receptor autophosphorylation has a causative role in reduction of birth weight found with Yu-Cheng subjects. The mechanism by which exposure to a complex mixture of chemicals, as found in contaminated rice oil, results in alterations in EGF receptor-kinase activity is also not clear. It was interesting that the decrease in EGF-stimulated receptor autophosphorylation levels was correlated with the concentrations of total PCBs that have a low affinity for the Ah receptor, and not with the PCDFs (both 2,3,7,8- and 2,3,4,7,8- congeners), which have much higher binding affinities for the Ah receptor.

Critical Comments

The number of placentas tested was small. It needs to be determined whether all placentas of low birth weight infants have a decreased placental EFG receptor autophosphorylation. It should also be determined how the age of the mother affects placental EGF. In the paper it is not clearly stated which PCDFs were included in the correlation analysis between the EGF receptor and these chemicals.

77. Yen Y.Y., Lan S.J., Yang C.Y., Chen E.R., Ko Y.C. A follow-up study of PCB poisoned multipara mothers and their transplacental Yu-Cheng babies. Symposium on health risk assessment on environmental occupational and life style hazards, December 20-22, 1988, Taipei, Taiwan, Republic of China.

Details are published in Yen et al. *Bull Environ Contam Toxicol.* 1989;43:647-655. Another study which included more mothers was published in *Bull Environ Contam Toxicol.* 1994;53:633-641.

78. Yen Y.Y., Lan S.J., Ko Y.C., Chen C.J. Follow-up study of reproductive hazards of multiparous women consuming PCBs-contaminated rice oil in Taiwan. *Bull Environ Contam Toxicol.* 1989;43:647-655.

The complete reproductive outcomes of PCB-poisoned women were assessed in this study.

Methods

Only five multiparous PCB poisoned women with complete delivery history after PCB poisoning were included in this study. In the statistical analysis, every measurement of each Yu-Cheng baby was first normalized based on the mean and standard deviation (SD) of control population with same sex and/or gestation week ($Zi = (Xi - Mean)/SD$). Zi scores were then summarized and divided the square root of case number ($Z = \Sigma i/\sqrt{n}$) to derive the Z value.

Seven transplacental Yu-Cheng babies were followed in the baby health clinic. The growth curve of the body weight of children under the age of 6 years in Taiwan (Taiwan Provincial Maternal and Child Health Institute 1982) was used for comparison.

The total number of pregnancies of these five mothers was 17. Of these pregnancies, four fetuses aborted spontaneously (4/17 = 13.5%), one was stillbirth (1/17 = 5.9%), ten babies survived more than six months (10/17 = 55.8%), and two died before six months of age (2/17 = 11.8%). (The PCB blood levels in the five women in 1981 were 12, 31, 47, 201 and 688 ppb. There did not appear to be a correlation between PCB blood level and pregnancy outcome.)

In Taiwan, the spontaneous abortion and stillbirth rate for married women is 57/1000. The rate in this study was 294/1000. (A rate of 5.7% is abnormally low and most likely reflects under reporting.)

Adjusting for gestational week and sex, there was a significant difference in birth weight of the control population ($Z = 2.96$, $p<0.01$). But there was no difference in 1-minute and 5-minute apgar scores between Yu-Cheng babies and control population ($z = 0.54$ and $Z = 1.47$, respectively).

However, the placental weight of transplacental Yu-Cheng babies was not significantly different from that of the control population.

Body weights of the Yu-Cheng boys were within the normal range of the standard curves. The birth weight of transplacental Yu-Cheng girls was lower than that of the control population, their body weight improved and became equal to the standard of the Taiwan Provincial Maternal and Child Health Institute.

Critical Comments

This observational study of 5 women represents their case reports. The women do not appear to have been randomly chosen. The PCB blood levels varied widely and there is no correlation (dose response-relationship) with pregnancy outcomes. It is not stated whether these infants had hyperpigmentation or other abnormal findings observed in Yu-Cheng babies. It is unclear whether all or part of this group were simply women with problem pregnancies or whether their problems were the result of the PCB exposure. The background incidence of spontaneous abortions in the general population of Taiwan most likely reflects under-reporting (5.7%). Depending on the method of detection and the maternal age group, it can be as high as 50%.

79. Yu M.L., Gladen B.C., Rogan W.J. Some evidence for dose-response in polychlorinated biphenyls (PCBs) and dibenzofurans (PCDFs) teratogenesis. *American J Epidemiology*. 1990, p 763.

The authors used data from a project that began in 1985 in Taiwan, in which 117 children (mean age, 2.7 years in 1985) born to mothers in a PCB/PCDF poisoning registry and controls were followed, and they examined whether the developmental delay in the children was related to the severity of exposure to the mother or child. Blood samples from 38 children were analyzed; none had detectable PCDFs. More exposed children (14/21) than control children (6/15) had detectable PCBs; the highest median concentration was among the breast-fed exposed children (4.5 ppb), but bottle-fed exposed controls (0.44) and breast-fed controls (0.53) were about the same. There were no strong relations between either measure of exposure, and signs or symptoms in the children, or between symptoms in the mother and level in the child. The authors conclude that there is some evidence for dose-response in the expression of the syndrome in the children, but small numbers and the imprecision in measuring both IQ and exposure make quantification difficult.

Critical Comments

This is an abstract. Insufficient detail precludes any evaluation of the data. However, this abstract also suggests that a certain amount of misclassification of the cases may have occurred. These children either had originally very low PCB and PCDF exposure. If they had been exposed to toxic levels, they should still have measurable levels of PCDFs.

80. Yu M.L., Hsu C.C., Gladen B.C., Rogan W.J. In utero PCB/PCDF exposure: relation of developmental delay to dysmorphology and dose. *Neurotox Teratol*. 1991;13:195-202.

Method

In January and February of 1985, children with in utero exposure were identified using the registry of Yu-Cheng cases maintained by the Taiwanese Bureau of Disease Control. Public health nurses responsible for the care of the exposed women notified the authors of other exposed women whose children were not included in the registry; exposure status of all mothers was verified by questionnaire. Since the latent period during which oil was consumed but mothers were asymptomatic was about 6 months, children born from June 1978 to our enrollment time were considered to be potentially exposed transplacentally. A total of 132 such living children, born to 74 mothers, were located. The exposed children ranged in age from a few months to just under seven years. There were 23 under 1 year, 23 from 1 to 2 years, 23 from 2 to 3, 20 from 3 to 4, 12 from 4 to 5, 14 from 5 to 6, and 17 from 6 to 7.

The families were interviewed in their homes and got information on 128 exposed children. The questionnaire included information about developmental milestones. Also interviewed were mothers of 115 control children, matched for age and neighborhood, whose names had been suggested by the exposed mothers. No attempt was made to mask the interviewers to the case status of the family, since some mothers still had obvious chloracne. After the interview, families were invited to attend an examination. One hundred and seventeen exposed children and 108 control children attended. The neurologic examinations were adapted from those used in the Perinatal Collaborative Study and were used in two forms, one for prewalkers (19), one for walkers (20). The parent(s) were present during the exams, which were conducted by two Chinese-speaking child neurologists. It was not possible to completely blind the examiners. The examiners had the children attempt to perform age-appropriate tasks, such as catching a ball. These items were almost always observed directly by the examiners; they were accepted by report for a few uncooperative or very shy children (5% of the children on 3 items, at most 2% on the others).

Testing of cognitive development was conducted in the home at 6, 12, and 18 months after the initial survey. New controls were selected for neighborhood, sex, age, sibling order, and family socioeconomic status. The exposed and his/her control child were tested on the same day. The Bayley exam was given to children below 2.5 years, the Stanford-Binet from 2.5 to 6 years, and the Wechsler Intelligence Scale for Children (WISC) from 6 years on.

Assessment of the children's exposure was not ideal. Inquiries were made about dietary exposure to the contaminated oil at the initial interview, few women gave usable answers because these questions were about a period of time that was, at the interview, six years in the past. The women did respond more frequently to questions about their degree of symptoms. Serum PCB values were also available from the Taiwan Bureau of Disease Control on 34 of the 74 exposed mothers; 31 had detectable levels. Although some women had multiple samples, only the earliest was used for analyses. Blood from 36 children (5 cc) was analyzed for PCBs and PCDFs.

Developmental milestones reported by mothers were censored since some children were too young to have achieved the milestone in question; therefore, life-table approaches were needed. Exposed and control distributions were compared by the log-rank test; the test was stratified by the matched pairs. A child was considered relatively late on a particular item if the age at which it was achieved was greater than 125% of the mean age at which control children achieved it. The child was considered to be relatively delayed if he was relatively late on at least 25% of applicable items for which information was available. This definition is arbitrary and does not correspond to clinical delay. The

definitions were chosen so that the number of children in the relatively delayed group would not be too small for analysis.

For the developmental milestones recorded by the examiner, logistic regression techniques were used. If the age at which the milestone is achieved is assumed to have a logistic distribution, then the probability that a child of a certain age will have achieved it has a logistic regression on age. The slope and intercept parameters of the logistic regression can be transformed into the mean and standard deviation of the underlying logistic distribution. This procedure was used to estimate means for the two groups. In order to compare the groups, conditional logistic regression was used to account for the matching.

The instruments used to measure the children's intelligence or development varied with their age. This created difficulties in comparing groups of children, and so an adjusted developmental score was constructed. The tests used have, in theory, a mean of 100, but in practice this was not the case. In order to remove the variability attributable to these mean differences, a constant to all scores of a given test was added or subtracted; the constant was chosen so that the mean of the control group (over all time points) would be 100. This was done for the average of the two Bayley scores, for the WISC full IQ, and for the Stanford-Binet IQ. This adjusted score was then more comparable across ages. Combining scales which measure different things leads to some difficulties in interpretation, but it avoids the problem of the very small sample sizes that occur when the children are compared only with others of the same age.

For statistical comparisons involving body size, to adjust for age and sex, the curve for size versus age was estimated from the controls for each sex separately by fitting a 5th degree polynomial; each child's values were then expressed as percentages of this control mean.

Combining milestones as described above, 22% of the control children and 49% of the exposed showed evidence of relative delay by the author's definition; these percentages were similar across all ages.

The neurologic examiners also assessed whether children had achieved milestones. For milestones occurring at 18 months or more, the exposed children were uniformly delayed, although the differences were not statistically significant.

The Cognitive Testing

The exposed children were delayed at all three follow-up visits, although only the first and third were statistically significant ($p<0.05$). Delay agreed reasonably well. Those who were delayed according to parental report of milestones had adjusted

developmental scores 4 to 13 points lower at all three follow-up visits. Those with a clinical diagnosis of developmental delay at the initial visit scored on average 6 to 8 points lower on the adjusted developmental score at all three follow-up visits. For those with clinically detectable delays in speech, adjusted scores were 9 and 14-points lower at the first and third follow-up; only two of the affected children were tested at the second.

As a group, the exposed children were smaller than controls after adjusting for age and sex; their heights were 97 ± 1% and their weights 93 ± 1% that of controls. Their head circumferences and heights did not differ significantly. There was no relationship between body size and speech delay. The smaller children had lower adjusted developmental scores at follow-up than did the larger ones.

Exposed children who had certain neonatal findings related to Yu-Cheng (eye discharge and swelling, hyperpigmentation, and deformed nails) showed lower scores than those without. The children with a history of ever having fingernail or toenail deformity also scored lower, but the presence of nail abnormalities on physical examination at the initial visit was not consistently related to developmental score. The only other physical findings or historical reports significantly related to developmental score were intraoral pigmentation and facial pigmentation at the initial visit; like toenail abnormalities, only the second visit was affected significantly ($p<0.05$). There was no relationship between serum PCB levels in the mother and developmental scores in the child.

Of the 36 children for whom analyses were available, 20 had detectable PCBs. No PCDFs were found in any children. If PCBs are quantified by summing the values for the individual peaks, the median is 0.99 and the maximum is 77.8 parts per billion (ppb) in the exposed, while the median is 0 and the maximum is 6.99 ppb in controls. There is no relationship between the PCB levels in the mothers and the PCB levels in the children. Among exposed children, those with detectable PCBs had lower developmental scores than those without, particularly at the third examination.

Breast-fed exposed children had a median of 4.5 ppb, bottle-fed exposed had a median of 0.44, breast-fed controls had a median of 0.53, and bottle-fed controls had a median of 0. The breast-fed children scored only slightly lower at all three follow-up visits. When the comparison was limited to those with and without detectable PCBs to bottle-feeders to get a cleaner measure of prenatal exposure, it was again noted that those with detectable PCBs have lower scores.

Critical Comments

Since some of the children still apparently had signs of intoxication, it is difficult to understand the PCDFs were not identified in the children's serum. The limit of detection of the analytical method is not stated particularly in view of the fact that only 5 cc of blood were collected. With this small amount of blood, PCDF analyses was not possible. In analyzing the developmental information that was collected, a great deal of data manipulation took place. In addition, some arbitrary decisions were made. For instance, "the child was considered to be relatively delayed if he was relatively late on at least 25% of applicable items for which information was available." This definition is arbitrary and does not correspond to clinical delay. The definitions were chosen so that the number of children in the relatively delayed group would not be too small for analysis! This arbitrary definition of relatively delayed for statistical purposes is quite meaningless. It suggests that perhaps all of the children were in the normal range. In addition, observer bias cannot be ruled out.

81. Yu M.L., Hsu C.C., Guo Y.L., Lai T.J., Chen S.J., Luo J.M. Disordered behavior in the early-born Taiwan Yu-Cheng children. *Chemosphere*. 1994;29:2413-2422.

Methods

For the behavioral evaluation, 118 children born to Yu-Cheng mothers between July 1978 and June 1985 participated in a 12-year cognitive and behavioral follow-up study. For each Yu-Cheng child, a control matched on age (within 15 days for those under one year, and within one month for those older), sex, neighborhood, maternal age (within 3 years), and socio-economic status was selected for the follow-up study.

The Chinese version of Rutter's Child Behavior Scale A was used in assessing children's behavior. Rutter's Scale had been validated in British children of ages 9-12 and appeared culture fair to us from our clinical experience. The scale is a screening instrument to identify children likely to show some emotional or behavioral disorder. It consists of three sections: health problem, habit, and behavior statement. The Health Problem section consists of a checklist of eight health problems, such as complaints of headaches, complaints of biliousness, wets his/her bed or pants, has temper tantrums, etc., and the score can range from 0 to 16. The habit section consists of five questions on habits, for example, does the child stammer or stutter, does he/she ever steal things, does he/she have any eating difficulty, etc., and the score ranges from 0 to 10. The section of Behavioral Statement consists of eighteen brief statements concerning the child's behavior, such as very restless, squirmy, fights with other children, solitary, tends to be fearful of new things or new situations, etc.; the

score ranges from 0 to 36. The questionnaire was modified so that all items allowed a "not applicable" answer for younger children in whom some of the behaviors asked about could not have occurred. The Rutter scale from age 3 years on was used because the percentage of questions not applicable to children 3-4 and 5+ years of age were 3% and 0.5%, respectively. Higher scores on the scales represent more behavioral problems. The test-retest reliability was assessed on 30 mothers with a two-week interval between ratings, and the correlation between the scores on the two occasions was 0.82. The questionnaires were filled out by a parent with the instruction of an interviewer. Every Yu-Cheng child and his/her matched control were evaluated on the same day. The evaluations were conducted annually since fall 1985, and data collected from 1985 to 1991 are reported here.

Because the children were born over a seven-year period, they were of different ages in any one round of follow-up. The scores from all seven follow-up years were combined and analyzed by age. Paired t-tests were used to compare the mean Rutter score and the mean health, habit, and behavior subscores between Yu-Cheng and control children at each age.

Results

When the total Rutter scores from all test times were combined and analyzed by age, the Yu-Cheng children had mean score ranges from 8.7 to 16.5, and the mean differences between the Yu-Cheng and control children ranged from 1.8 to 2.4; all differences were statistically significant except for the 10-12 year olds (p was not given for most outcomes).

Discussion

The results shown in this paper could be due to unknown bias or confounding. Some of the mothers and some of the children still have visible Yu-Cheng related physical signs, such as abnormal patterns of pigmentation and hypoplastic nails. Teacher ratings on 20 pairs of children of maladaptive behavior at school were also available. The teachers' ratings are thus-far consistent with the parents' ratings.

With no measured serum PCB and PCDF level, it is hard to study the dose-response relationship between the amount of PCB and PCDF exposure and physical or mental findings. No relationship was found between maternal and child's physical symptoms and child's cognitive and behavioral scores. Blood samples were collected from the study children in spring 1991, and so far serum PCB and PCDF levels have been measured on 31 of the 107 collected samples. With the few available data, a relationship between serum PCB and PCDF level and behavior scores were not found. The data will be reanalyzed when more serum samples have been measured.

The biological mechanism by which PCBs cause behavioral toxicity is unclear.

Critical Comments

The results of this study raise many questions. The teachers, parents, and interviewers were not blinded but knew who the Yu-Cheng children were and may have assumed that these children were abnormal. Not only would that assumption have resulted in the reporting of more problems, but this attitude would also have modified the behavior of the children. This observation is strengthened by the fact that PCB and PCDF levels did not positively correlate, neither did physical signs with the effects found in the children. The authors also point out that their results could be due to some unknown bias.

82. Yen Y.Y., Lan S.J., Yang C.Y., Wang H.H., Chen C.N., Hsieh C.C. Follow-up study of intrauterine growth of transplacental Yu-Cheng babies in Taiwan. ***Bull Environ Contam Toxicol.*** **1994;53:633-641.**

Methods

This study included 78 PCB-poisoned women who had been pregnant or had given birth, after poisoning, between 1979 and 1986. Among a total of 184 pregnancies in the study group, there were 21 abortions or still-births, leaving 163 singleton births for analysis. Reproductive histories of these women were obtained by interview. The birth data of the transplacental Yu-Cheng babies were abstracted from medical records. The same data for 17 babies born by those mothers before the poisoning episode were also obtained.

The birth weight and gestational age of non-Yu-Cheng comparison babies were derived from the birth records of a teaching hospital in central Taiwan, the area where the PCB poisoning occurred. Data for 18,865 male and 17,054 female singleton babies born between 1977 and 1987 were collected (excluding 1980, which had incomplete birth records). In the analysis, the birth weight of each Yu-Cheng baby was first normalized based on the mean and standard deviation of birth weight of the control population with the same sex and gestational age to obtain an individual Z score. The scores were then summed and divided by the square root of the number of babies to derive the summary z value.

Results

One hundred and sixty-three singleton, live infants (86 boys and 77 girls) were born to 78 PCB-poisoned women between 1979 and 1986. The complete birth record was available for 130 live births (66 males and 64 females). The rates of low birth weight and prematurity among Yu-Cheng babies were significantly higher than

those of the control population (27.7% vs 6.3%, $p<0.01$, for low birth weight ; 24.6% vs 8.1%, $p<0.01$, for prematurity).

For the 17 babies born before the poisoning episode, adjusting for gestational age, there was no significant difference in birth weight compared to the control population. This indicated that these mothers did not tend to bear low birth weight babies before poisoning. However, there was one premature birth. The birth weight of Yu-Cheng babies improved in later pregnancies.

Except for male babies in 1979, the birth weight of Yu-Cheng babies born shortly after the poisoning episode was significantly lower than that of the control population. This situation improved starting in 1985, 6 years after the episode.

Another study reported a mortality of 110. However, even though these Yu-Cheng babies had a lower birth weight, their body weight increased sufficiently to catch up with the general population in early childhood (Yen et al., 1989).

Critical Comments

The birth records and birthweights of infants born to PCB and PCDF poisoned mothers were compared to records of control infants of a hospital. Obviously, infants of a number of poisoned mothers showed signs of toxicity. However, it is unclear how appropriate the comparison group was, since it was hospital based rather than population based. It is unclear whether the population group to which the poisoned mothers belonged also delivered at this hospital. If they were of a lower socioeconomic status, then their pregnancy outcomes could have been quite different. The authors fail to point out that the toxic agent in this poisoning episode were the PCDFs and not the PCBs.

83. Huisman M., Eerenstein S.E.J., Koopman-Esseboom C., Brouwer M., Fidler V., Muskiet F.A.J., Sauer P.J.J., Boersma E.R. Perinatal exposure to polychlorinated biphenyls and dioxins through dietary intake. *Chemosphere*. 1995;31:4273-4287.

Methods

From June 1990 until June 1992, a study population of 418 Dutch mother-infant pairs was recruited in the Groningen and the Rotterdam areas. In the sixth week after delivery, the 211 Groningen and 207 Rotterdam mothers were asked to complete a semi-quantitative food frequency questionnaire about their food habits during pregnancy. The food intake calculations were performed by dieticians using different computer programs (VOBEMA/Hanzehogeschool in Groningen, and Becel dietary program in Rotterdam). Both are based on the Dutch Nutrient Database and allow calculation of macronutrient intake (daily energy, protein, carbohydrate, and fat intake).

To estimate the dietary intake of 2,3,7,8-TCDD, dioxins, and planar PCBs, calculations were made using reference data for food products, as provided by the Dutch National Institute of Public Health and Environmental Protection (RIVM). The RIVM used the toxic equivalence factor (TEF) approach to calculate a toxic equivalent (TEQ) value for each food category. In Groningen, the calculated fat content per food category was multiplied by the TEQ values from the RIVM, whereas in Rotterdam, the calculated fat content per food item was used. In Groningen, the TEQ intake is the sum of the TEQ values of all food categories, whereas in Rotterdam, the TEQ intake is the sum of the TEQ values of all food items.

Maternal blood was collected in the last month of pregnancy and cord blood immediately after birth. In plasma samples only the four non-planar PCB congeners 118, 138, 153, and 180 could be analyzed due to the small volume of plasma. The sum of the four congeners (ΣPCB) in plasma was used in the statistical analyses. In the second and 6th weeks after delivery, the women who breast-fed their infants collected a 24-hour sample of breast milk. In breast milk, 17 dioxin and 3 planar PCB congeners were analyzed. In addition, twenty-three non-planar PCB congeners were measured in these milk samples. The analytical methods have been reported elsewhere.

Results

A higher intake of energy, protein and fat was found in Rotterdam than in Groningen. Ten questionnaires were randomly selected from each center and sent for processing to the other center. Analysis of these questionnaires revealed differences: the macronutrient intakes were on average estimated higher in Rotterdam than in Groningen. The analysis did not reveal a specific pattern of differences. However, it was not possible to re-assess all the questionnaires. The recorded intakes are thus subject to a considerable measurement error.

After adjusting for age, Quetelet index (weight/length), energy intake, alcohol consumption during pregnancy, and study center, a significant relation was found between the ΣPCB in maternal and cord plasma, and the dietary intake of planar PCBs. Nearly the same results are obtained if the dietary intake of planar PCBs is replaced by the 2,3,7,8-TCDD or the dioxin intake. In dietary intake surveys in Germany and in the Netherlands, a median daily dioxin intake of about 1.2 pg TEQ/kg body weight was found.

Although food is generally accepted as the major source of PCB and dioxin intake in the present study, the proportion of variance explained by food intake was low based on multiple regression analysis against the various congeners in human milk after adjusting for confounders.

In the present study, dairy products and industrial oils are the major contributors to the daily intake of dioxins and planar PCBs. Two modest dietary alterations will lower the intake of PCBs and dioxins, the replacement of the usually consumed cheese (48% fat) by low-fat cheese (20% fat), and the use of vegetable oils instead of contaminated fish oils in the preparation of foodstuffs by the food industry.

Consumption of low-fat cheese is estimated to result in a median reduction of 11% (range: 0-43%) of the PCB and dioxin intake. The median reduction due to abolition of the use of fish oils in industrial oils is estimated as 22% (range: 5-78%).

Critical Comments

In this paper the TEQ calculations in the macronutrients the women consumed at the two different centers were done differently. This created a considerable measurement error. In addition, the TEQ values from the Dutch government also have considerable uncertainty. This was not explained in the paper. In the present analysis, the food intake only explains a low proportion of the variance against the various congeners of dioxins and PCBs in human milk. These authors seem to have collected appropriate milk samples (24-hour samples at 2 and 6 weeks).

84. Huisman M., Koopman-Esseboom C., Fidler V., Hadders-Algra M., Van der Paauw C.G., Tuinstra L.G.M.Th., Weisglas-Kuperus N., Sauer P.J.J., Touwen B.C.L., Boersma E.R. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. *Early Human Development*. 1995;41:111-127.

Methods

The study was carried out in two widely separated and different areas in the Netherlands: Groningen, a semi-urban area in the northeast, and Rotterdam, a highly industrialized region in the southwest. Two hundred healthy pregnant women were planned to be included in each area: 50% who intended to breast-feed their infants for at least 6 weeks and 50% who preferred formula-feeding. The latter agreed to use Almiron M2 from one batch. The women and their children had to meet the following criteria: absence of serious illness and complications during pregnancy and delivery; caucasian race; first or second born term infants (37-42 weeks of gestation); no caesarian section; no forceps or vacuum extraction; availability of a maternal blood sample in the last month of gestation and of a cord blood sample.

Obstetrical data were evaluated according to the obstetrical optimality list. The neonatal neurological examination was scheduled between the 10th and the 21st day after delivery. For the assessment of the neonatal neurological condition, the comprehensive age-adequate neurological examination, as described by Prechtl (1980, 1977), was used. This technique, in contrast to the BNBAS (Prechtl 1977), has proven to be predictive for later major and minor neurological dysfunctions. The examination leads to a clinical diagnostic classification: normal, suspect, or abnormal. Furthermore, two clusters of items were formed: one describing postural tone, the other reflexes and responses. The latter cluster consisted of 11 items, the former consisted of 10 items. For each item score, 0 represents a low value, score 1 and intermediate value and score 2 a high value as may occur in healthy vigorous infants. By summation of scores, a postural tone and a reflex cluster score were calculated which could range from 0-17 and 0-22, respectively. A postural tone cluster score of ≤ 9 was considered to reflect a low muscle tone, and a reflex cluster score of ≤ 10 a low responsiveness. The cut-off points were arbitrarily chosen close to the median score.

Finally, the neurological findings were also interpreted in terms of optimality (Prechtl 1980). A neurological optimality score was calculated, consisting of 60 items for each of which an optimal range was defined. After giving a point for each item meeting these criteria, the neurological optimality score (NOS) was calculated by the summation of optimal items. It must be emphasized that optimal is not identical with normal, and non-optimal does not always mean abnormal. The neurological

examinations were carried out by M.H. in Groningen and by C.K.E. in Rotterdam. Both observers were unaware of the results of the chemical analyses of the plasma and milk samples but were not blind to the feeding status.

Maternal blood was collected in the last month of gestation and cord blood immediately after birth. Blood was collected in a vacuum system EDTA-tube; plasma was stored at -20° Celsius. Human milk was collected as a 24-hour sample in the second and sixth week, and if possible 3 months after delivery. This was achieved by emptying both breasts with an electric pump. Volumes were recorded and 10% aliquots were pooled and stored at -20° Celsius. The remaining milk was administered to the infants by bottle.

Plasma samples were analyzed for the four non-planar PCB congeners 118, 138, 153, and 180 only. The limits of determination for PCB 118, 138, 153, and 180 were 0.01 μg/l. The recovery of chlorinated biphenyl congeners added to the plasma before extraction, and determined as described above, was >95%. Control samples were analyzed to estimate the reliability.

The milk samples were analyzed for the seventeen 2,3,7,8-substituted PCDDs and PCDFs, which are usually found in biotic samples, three planar PCBs, and 23 nonplanar PCB congeners. Human milk was fortified with 16 ^{13}C-labelled PCDDs and PCDFs, and three ^{13}C-labelled PCB standards. The non-planar PCB congeners were measured by gas chromatography using electron capture detection.

To express the toxic potency of the mixture of dioxins and dioxin-like PCBs, the toxic equivalence factor approach was used. In order to calculate the toxic equivalent (TEQ) of each congener, concentrations of all 2,3,7,8-compounds and planar PCBs (PCB 77, 126, and 169) were multiplied by their TEF value. By adding up the individual TEQs, a dioxin TEQ and planar PCB TEQ score could be acquired. Mono-ortho PCB TEQ (PCB 105, 118, and 156) and di-ortho PCB TEQ (PCB 170 and 180) were calculated by multiplying the concentrations of their proposed TEF value. The total PCB/dioxin TEQ is a summation of the dioxin TEQ, the planar-, mono-ortho-, and di-ortho PCB TEQs.

To evaluate a postnatal effect, the sum (ΣPCB) of the four PCB congeners (PCB 118, 138, 153, and 180) was calculated as a measure of PCB exposure separately for cord plasma and breast milk. The ΣPCB_{cord} in cord plasma was used as a continuous independent variable, whereas the ΣPCB_{milk} in formula and human milk was used as a categorical independent variable (no, low and high postnatal exposure) in the statistical analysis.

The 5th, 50th, and 95th percentiles were used to describe the distribution of the concentrations. For a univariate comparison of the results in Groningen and Rotterdam, the chi-square test was

used and the Wilcoxon rank sum test performed at the 5% level. Both univariate analysis and logistic regression analysis were used to examine the relation between the NOS (Neurological Optimality Score), the postural tone cluster, the reflex cluster and levels of PCB and dioxin exposure, and the obstetrical variables. The NOS was dichotomized at the median of the pooled population. The postural tone cluster and the reflex cluster scores were dichotomized as (≤ 9, >9) and ≤ 10, >10), respectively. The PCB and dioxin values were logarithmically transformed. After adjusting for obstetrical variables, the effect of each chemical compound and total TEQ scores were examined. The results are reported as odds ratios (ORs) associated with doubling of concentrations, with 95% confidence intervals (CI), without any correction for multiple hypothesis testing.

Results

There were 104 breast-fed and 107 formula-fed infants in Groningen, and 105 breast-fed and 102 formula-fed infants in Rotterdam. In Groningen, the education of mothers and their partners was higher than in Rotterdam, as was the maternal alcohol consumption during pregnancy. Duration of gestation based on reported last menstrual period was also significantly different (40.6 ± 1.1 for Groningen vs. 40.1 ± 1.2 weeks for Rotterdam), but the difference was considered to be too small to carry biological significance. In Groningen, mean birth weight was slightly higher (3.56 ± 0.44 vs 3.47 ± 0.44 kg). No other difference was seen.

From the 418 children, 394 were neurologically classified as normal, 20 newborns as suspect, and 4 newborns as abnormal. The percentages of infants with a postural tone cluster score of ≤ 9 and a reflex cluster score of ≤ 10 were 43% and 22%, respectively. In Rotterdam, the optimality score was shifted to the left compared to Groningen. There appeared to be a systematic difference between the two observers in the assessment of nine items: stability of states; posture in supine position; abdominal skin reflex; active power; knee jerk; posture of head during traction test; Moro reaction, amplitude of abduction and extension; Bauer response; and Galant response. In Groningen, these items (except for active power) were more often considered optimal. Therefore, in the logistic regression model, an adjustment was made for the study center. The median NOS of the pooled population used as the cut-off point was 57; the score 57 or higher was considered optimal. The NOS of the 24 neonates who were clinically diagnosed as suspect or as abnormal were all but one below the median.

No significant relation was found between the NOS and PCB congeners in maternal or cord plasma. Plasma PCB levels affected neither the cluster scores for reflexes and responses nor for postural tone.

In human milk, logistic regression analysis with covariate adjustment showed significant effects on the NOS of some PCB and dioxin congeners: five PCDD, two PCDF, one planar PCB, two mono-ortho PCB, one di-ortho PCB, and seven nonplanar PCB congeners as well as the dioxin, mono-ortho PCB, di-ortho PCB, and total PCB/dioxin TEQ values. After adjusting for the study center, logistic regression analysis with the postural tone cluster score as the dependent variable demonstrated a significantly higher percentage of hypotonia with an increase in planar PCB TEQ (OR:1.64, 95% CI:1.03-2.63). No effect on the reflex cluster score was found.

After adjusting for the study center and for the ΣPCB_{cord} in cord plasma, a reduced neonatal neurological optimality and a higher prevalence of hypotonia was found in the highest exposed group at a turning point at 540 ng ΣPCB_{milk}/g milk fat. The percentage of breast-fed infants with a ΣPCB_{milk} content of ≥ 540 ng/g milk fat was 23%. In this group, the odds ratio for the NOS was 3.4 (95% CI:1.6-7.1). Such an odds ratio corresponds, for example, to an increase in the prevalence of non-optimality from 50 to 75%.

Our study does not confirm prenatal effects of PCBs which have been found in other studies. The combination of a high intrauterine and a high postnatal exposure might then result in neurological non-optimality as reflected by the decreased optimality score. Such a negative effect is not found in formula-fed infants with merely high plasma PCB levels. Breast-fed children exposed to higher planar PCB TEQ appeared to have a higher incidence of hypotonia. Since reflexes and responses were normal and the minor dysfunction mainly consisted of hypotonia, it is possible that the site of action is in the developing muscle.

Critical Comments

Many parameters are better controlled than they were in the Michigan and the North Carolina studies. However, this is also not a randomly selected study population and there seem to be sufficient differences between the participants in Groningen and in Rotterdam to beg the question whether they can legitimately be combined.

It is not explained whether the children with clinical problems are also children whose mothers had higher TEQ values in their milk and were the older women. Their seemed to be roughly 23 children with hypotonia and 24 children with clinical problems. It was not reported whether these children were identical or overlapped. The methods used to evaluate the children are different from the tools used in the United States. However, they are also subjective and summations and categorization of the different effects measured are arbitrary. Since alcohol consumption and the evaluation of the neurological behavior of the infants was different in Groningen

than in Rotterdam, the reader should have been informed whether there were more mothers in Groningen than in Rotterdam with higher TEQ values in their milk. It should also have been determined whether more children with hypotonia were clustered in one or the other hospital, since the study groups from the two hospitals seem to be very different.

The authors found no prenatal effect on the infants related to the chlorinated organic compounds. However, they analyzed plasma instead of serum as is done in the United States. It is unclear whether the results would be different had serum been examined instead. Furthermore, the toxic equivalency approach has many uncertainties since the relative toxicity of the individual congeners is based primarily on short-term in vitro cell and in vivo animal tests. Depending on the species and the types of tests performed, the toxicity of individual congeners may vary by at least one to two orders of magnitude. Thus, extrapolation based on these data to humans is extremely uncertain. In addition, the quantitation of the halogenated organic chemicals is not very precise and the neurological behavioral tests suffer from imprecision and subjectivity. Given these uncertainties, the small differences although statistically significantly different have no biological significance and seem to be within the noise level of the system that is the results of the chemical analysis, combined with the neurobehavioral testing and the TEQ approach.

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85. Huisman M., Koopman-Esseboom C., Lanting C.I., Van der Paauw C.G., Tuinstra L.G.M.Th., Fidler V., Weisglas-Kuperus N., Sauer P.J.J., Boersma E.R., Touwen B.C.L. Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. *Early Human Development*. 1995;43:165-176.

From June 1990 until June 1992, pregnant women were recruited for the study in Groningen and Rotterdam, based on their intention, to bottle feed formula or to nurse.

Social, obstetrical, and perinatal information was recorded in a questionnaire with 72 representative items. The number of items that fulfilled predefined optimality criteria was used as an obstetrical optimality score. All newborns underwent a neurological examination according to Prechtl. The optimality concept. *Early Human Development*. 1980;4:201-205.

A maternal blood sample was taken in the last month of pregnancy and cord blood was collected immediately after birth. Plasma samples were analyzed for four non-planar PCB congeners 118, 138, 153, and 180 only. The sum of the concentrations of the four PCB congeners 118, 138, 153, and 180 in plasma was used as a measure of prenatal exposure to PCBs. Postnatal exposure to PCBs and dioxins via breast milk was reflected by the levels of these compounds in a 24-hour sample taken in the second week after delivery. Contents of seventeen 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins and dibenzofurans, three planar PCBs, and 23 non-planar PCB congeners were determined in breast milk fat as well as in the formula milk fat. To express the toxicity of the mixture, the toxic equivalency approach was used.

At 18 months an age-specific neurological examination was conducted focusing on motor functions (grasping, sitting, crawling, standing, and walking) in a standardized free field situation. On the basis of this examination each toddler was classified as normal, mildly abnormal, or abnormal. "Mildly abnormal" signifies the presence of mild signs which do not necessarily lead to a handicapping condition, e.g., slight asymmetries, or mild hypo-, and hypertonia. The neurological findings were also evaluated in terms of optimality. A list of 57 neurological items was composed. A reduced optimality not always meant abnormal.

Chi-square, Student's t-test, and the Mann-Whitney U-test were used to compare groups. The effect of PCB and dioxin exposure on the neurological condition was investigated by multiple linear regression. The independent variables in the regression analysis were the logarithmically transformed PCB and dioxin levels, social, perinatal and obstetrical variables from the obstetric optimality list, and the study center. A P-value of 0.05 or less was considered significant.

Results

Four hundred and eight toddlers were classified as neurologically "normal." Nine children were categorized as "mildly abnormal." One toddler had a hypertonic syndrome which was diagnosed as "abnormal." In the normal group, the median neurological optimality score was 48 (range: 34-55), whereas in the group classified as mildly abnormal or abnormal, the median was found to be 42 (range 38-45). Three maternal blood samples were missing. No cord blood samples could be obtained from 36 mother-infant pairs. For the analysis of PCB 118 in cord plasma, nine samples were missing. In human milk, representative dioxin, planar and non-planar PCB congeners were available in 176, 194 and 195 milk samples, respectively.

Neither PCB nor dioxin exposure via human milk were associated with the neurological optimality score. The children of less educated fathers scored lower than the children of well educated fathers; the first-born children had a higher score than the second- or third-born children. The model also included a significant ($P = 0.011$) interaction between ΣPCB_{cord} and smoking of the father. The children of non-smoking fathers had the highest adjusted neurological optimality score in the presence of a low ΣPCB_{cord} value. In the case of a high ΣPCB_{cord} value, the optimality score was similar to that of children of smoking fathers. Nearly the same results are obtained if ΣPCB_{cord} is replaced by $\Sigma PCB_{maternal}$.

The size of the estimated prenatal PCB effect on the neurological optimality score is elucidated in the following example. A first-born toddler living in Groningen with a highly educated non-smoking father has an estimated neurological optimality score of 49.9 in case of a ΣPCB_{cord} value at the 5th percentile (i.e., 0.18) and a score of 47.9 in case of a ΣPCB_{cord} value at the 95th percentile (i.e., 0.86). Thus, the difference is only two points. Breast-fed children had a higher fluency cluster score compared to formula-fed children.

Critical Comments

The difference in the optimality score is small. Since there is a difference between the group aggregated in Groningen and the one aggregated in Rotterdam it should have been determined how the two groups compared. Results from almost 10% of the cord sera were missing and since plasma was analyzed, many more of the sera may have been below the limit of detection of the analytical method. The small effect on the difference in the neurological optimality score is meaningless.

86. Koopman-Esseboom C., Huisman M., Weisglas-Kuperus N., Van der Paauw C.G., Tuinstra L.G.M.Th., Morse D.C., Brouwer A., Sauer P.J.J. Effects of PCBs and dioxins during pregnancy and breast feeding on growth and development of newborn infants. A study design and preliminary results. Dioxin '92. 12th international symposium on dioxins and related compounds, Tampere, Finland. *Organohalogen Compounds*. 1992;10.

Methods

This study is part of a large cooperative survey on PCBs and dioxins in the Netherlands. The outline of the Rotterdam research plan is summarized in Tables 1 and 2

Table 1:
Biochemical parameters

	Human Milk	Maternal Plasma	Cord Plasma	Child Plasma
PCB 118,138,153,180	+	+	+	-
Dioxins (17 congeners)	+	-	-	-
Thyroid functions (TT3, TT4, TSH, FT4)	-	+	+	+
Vitamin A	+	+	+	+

Table 2:
Neurodevelopmental follow-up

	10 days	3 months	7 months	18 months
-Neurological examination Prechtl/Touwen	+	-	-	+
-Psychomotor development Bayley Scales of Infant Development	-	+	+	+
-Visual Recognition Memory Fagan Infantest	-	+	+	-
-Weight, Height, Head circumference	+	+	+	+

Preliminary data show accumulation of PCBs in mothers and their fetus and contamination of their milk. No significant correlations between in utero PCB accumulation and human growth parameters at birth, 10 days or 3 months were found.

Critical Comments

This is a short paper provided as part of a presentation. Insufficient detail is given for the evaluation of this material.

87. Koopman-Esseboom C., Brouwer M., Van der Paauw C.G., Tuinstra L.G.M.Th., Muskiet F.A.J., Sauer P.J.J., Boersma E.R. The Dutch PCB/dioxin study. Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. *Organohalogen Compounds*. 1993;14:81-84.

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(This is also published in Koopman-Esseboom et al. *Chemosphere*. 19194;28:1721-1732 and *Chemosphere*. 1994;29:2327-2338.)

89. Koopman-Esseboom C., Huisman M., Weisglas-Kuperus N., Boersma R.E., de Ridder M.A., Sauer P.J.J. Dioxin and polychlorinated biphenyl levels in plasma and human milk of women in relation to their living area in the Netherlands. *Pediatr Research*. 1994;36(1):21A.

(Abstract. More information is published in Koopman-Esseboom et al. *Chemosphere*. 1994;29:2327-2338.)

90. Koopman-Esseboom C., Huisman M., Weisglas-Kuperus N., Boersma E.R., de Ridder M.A.J., Van der Paauw C.G., Tuinstra L.G.M.Th., Sauer P.J.J. Dioxin and PCB levels in blood and human milk in relation to living areas in the Netherlands. *Chemosphere*. 1994;29:2327-2338.

Methods

The study group consisted of 418 healthy women recruited between June 1990 and June 1992. Women were asked by their obstetrician or midwife to volunteer for the study. Women were categorized into 3 subgroups: non-smokers, 1 to 10 cigarettes a day, or more than 10 cigarettes a day. All infants were born between the 37th and 42nd week of pregnancy. All women were Caucasian. It must be assumed that these women were the same women used in the study of infants. However, that is not explicitly stated in the paper.

One hundred and ninety-eight women who had lived for at least five years in Rotterdam city and the surrounding industrialized region in the western part of the Netherlands were selected. The other 211 selected women were living in Groningen and the surrounding area, a semi-urban and rural region in the northern part of the Netherlands.

The group of 198 women living in the western part of the Netherlands was subdivided into three subgroups. One hundred and nine women were living in Rotterdam; 49 women lived in Vlaardingen or Schiedam which is located on the northern edge of a highly industrialized zone containing a large waste incinerator and a number of large oil refineries and chemical industries; 40 women lived in Spijkenisse which is located on the southern edge of the same industrialized zone.

A blood sample was taken from the women in the last month of their pregnancy for the measurement of the PCB congeners 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180). Blood was collected in a vacuum system EDTA-tube.

In the second week after delivery, the women who nursed collected a 24-hour representative sample of milk by collecting before each feeding as much milk as possible from both breasts with a vacuum pump. An aliquot of 10% of each sample was pooled.

PCB congeners in plasma were measured by gas chromatography with electron capture detection (GC-ECD). The detection limit was 0.01 ng/g plasma.

The human milk samples were analyzed for the 17 most abundant 2,3,7,8-substituted PCDD and PCDF congeners and three planar PCB congeners (3,3',4,4'-tetrachlorobiphenyl (PCB 77), 3,3'4,4'5-pentachlorobiphenyl (PCB 126), and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169) by gas chromatography-high-resolution mass spectrometry (GC-HRMS). Twenty-three non-planar PCB congeners (IUPAC 28, 52, 66, 70, 99, 101, 105, 118, 128, 137, 138, 141, 151, 153, 156, 170, 177, 180, 183, 187, 194, 195 and 202) were measured by gas chromatography with GC-ECD.

To express the toxic potency of the mixture of dioxins and PCBs in milk samples, the international toxic equivalence factor (I-TEF) approach was used for the PCDDs and PCDFs and the WHO 1993 approach for the PCBs (1,2).

Results

After correction for covariates, a significantly higher level of PCB 118 in maternal plasma, and of dioxin-TEQ and 10 individual dioxin and PCB congener levels in human milk from the western industrialized areas compared to the more rural areas in the northern part of the Netherlands was found.

Critical Comments

The differences were small. The concentrations of the PCB congeners in maternal plasma overall ranged from 0.02 ppb to 2.3 ppb. The TEQ values for the "dioxins" in milk fat ranged from 11.1 - 76.4 ppt. In three of the subgroups the upper limit of the range was between 57 and 59 ppt. The study group was not a random sample of the general population and only 172 milk samples were available for analysis. The raw data from the different areas are quite similar and are borderline statistically significantly different except for PCB congener 118. The use of multiple regression analysis in this case is out of place since in this case this is not a health study but merely an effort to determine whether body burdens in different areas are different. Simple t tests are more appropriate and median should also have been given.

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(The information in this abstract is reported in detail in Koopman-Esseboom et al. *Pediatr Research*. 1994;36:468-473.)

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TT_3, total triiodothyronine
TT_4, total thyroxine
FT_4, free thyroxine
TSH, thyroid stimulating hormone

The study group consisted of 105 healthy mother-infant pairs recruited between June 1990 and February 1992, living in Rotterdam and the surrounding area. Only infants born at term (37 to 42 week of gestation) without congenital anomalies or diseases were included.

A blood sample was taken from the mothers in the last month of pregnancy (36th to 40th week) and from the umbilical cord for the measurement of the PCB congeners 118, 138, 153 and 180.
In the second week after delivery, the mothers collected a 24-hour representative sample of milk.

The human milk samples were analyzed for the 17 most abundant 2,3,7,8-substituted PCDD and PCDF congeners and three planar PCB congeners PCB 77, 126 and 169. Twenty-three nonplanar PCB congeners were measured also. To express the toxic potency, the TEF approach was used. It is not explained in the paper how this was calculated.

TT_4, TT_3, FT_4, and TSH were measured in maternal plasma during the last month of pregnancy, 9 to 14 days after delivery, in plasma of the umbilical cord, and in infants' plasma 9 to 14 days (infants at 2 weeks) as well as 3 months (infant 3 months) after birth. TT_3, TT_4, FT_4, and TSH were determined by chemiluminescence immunoassay, using standard Amerlite assay kits.

Data analysis was performed with the statistical software package SPSS/PC. Means, medians, ranges, and standard deviations are reported in terms of original distributions. Spearman rank correlation coefficients were measured between TT_4, TT_3, FT_4, TSH levels and individual PCB and dioxin congener levels as well as dioxin TEQ, PCB TEQ, and total PCB-dioxin TEQ levels. Because of

the large number of analyses, a p value <0.01 was estimated as being statistically significant. The Mann-Whitney test was used to analyze the significance of differences in thyroid hormone status between a low and high exposed breast-fed group; a p value <0.05 was estimated as being statistically significant.

Results

Of the initial 105 mother-infant pairs, 78 fulfilled all criteria and were included in the analysis. One mother appeared to have an autoimmune hypothyroidism. The levels of all the other mother-infant pairs were in the normal range for age-appropriate controls.

The mean level of the total PCB-dioxin TEQ in human milk was 74.86 pg TEQ/g fat (SD 26.19, range 30.85-154.21), the dioxin TEQ was 32.06 pg TEQ/g fat (SD 11.26, range 12.44-76.43), the planar-PCB TEQ was 19.95 pg TEQ/g fat (SD 8.54, range 6.39-51.11), and the nonplanar-PCB TEQ was 22.75 pg TEQ/g fat (SD 8.96, range 8.52-58.19).

Higher levels of total PCB-dioxin TEQ, dioxin TEQ, and both planar- and nonplanar-PCB TEQ in human milk were significantly correlated with lower maternal plasma TT^3 levels in the last month of pregnancy and with lower maternal plasma TT_3 and TT_4 levels in the second week after delivery. There was a trend toward a decline in the maternal plasma TT_4 level in the last month of pregnancy with higher planar-PCB TEQ levels ($r = -0.27$ ($R^2 = -0.07$), $p<0.05$). There were no significant correlations with maternal plasma FT_4 or TSH levels. The total PCB-dioxin TEQ, dioxin TEQ, and PCB TEQ levels demonstrated no significant correlations with TT_3, TT_4, FT_4, or TSH levels in umbilical cord plasma. However, higher TEQ levels were significantly correlated with higher plasma TSH levels in the infants in the 2nd week and 3rd month after birth.

The mean plasma TT_4 level was significantly lower, and the mean plasma TSH level was significantly higher in the high exposed group in the 2nd week after birth. In umbilical cord plasma and at 3 months, only the mean plasma TSH level was significantly higher in the high dioxin-exposed breast-fed group (8.5 ± 6.0 versus 11.6 ± 8.0 μIU/mL, $p<0.05$, and 1.6 ± 0.6 versus 2.3 ± 1.0 μIU/mL, $p<0.0004$). When the infants were divided into low and high total PCB dioxin TEQ-exposed breastfed groups (median = 72.43 pg TEQ/g fat), the mean plasma FT_4 level was also significantly lower in the high exposed group in the 2nd week after birth (24.6 ± 3.5 versus 23.0 ± 3.3 pmol/L, $p<0.05$).

Higher dioxin and PCB TEQ levels in human milk were significantly correlated with lower maternal plasma TT_3 and TT_4 levels and with higher infant plasma TSH levels in the 2nd week and 3rd month after birth. Twenty-two individual PCDD, PCDF, and PCB congener levels measured in human milk gave correlation coefficients with maternal

and infant thyroid hormone levels of the same order of magnitude as did the TEQ levels. Furthermore, only 78 mothers and their infants were tested. No information is given by the authors why the other mothers were not tested.

At the age of 3 months, no significant differences in the infants' plasma TT_3, TT_4, or FT_4 levels were found, although TSH was still significantly higher.

Critical Comments

The observed differences although in some cases statistically significantly different have no clinical meaning. The authors state that all thyroid function tests were within the normal range. They do not discuss the normal variations that are encountered during pregnancy and the early postpartum period. The authors developed TEQ values for non-planar PCBs but do not give information about the factors that they used for these PCBs, nor are they listed in the other papers. No conclusions can be drawn from the results of this study. The TEQ values in general as discussed in another paper are interim values which have been developed by governments to estimate the toxicity of these mixtures. However, they are very uncertain particularly if the toxicokinetics of different species are not taken into considerations. When toxicokinetics are considered, the uncertainty inherent in the TEQ values is about one order of magnitude. Given that the differences in the thyroid function tests are all within the normal range, associating slight elevation within this range with uncertain TEQ values is meaningless.

93. Koopman-Esseboom C., Huisman M., Weisglas-Kuperus N., Van der Paauw C.G., Tuinstra L.G.M.Th., Boersma E.R., Sauer P.J.J. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. ***Chemosphere*****. 1994;28:1721-1732.**

The study group consisted of 418 healthy mother-infant pairs recruited between June 1990 and June 1992. Two hundred and seven pairs lived in Rotterdam and the surrounding area. The other 211 pairs lived in Groningen.

To study the effects of postnatal dioxin and PCB exposure, a group of pregnant women was selected of whom 50% planned to breast-feed their infants and the other 50% to give formula. Formula contains negligible levels of dioxins and PCBs.

A blood sample was taken from the mothers in their last month of pregnancy (36th to 40th week) and a cord sample at delivery for the measurement of PCB congener levels 118, 138, 153, and 180. In the second and sixth week after delivery, the mothers collected a 24-hour representative sample of their milk.

(Details of the methods are also given in Koopman-Esseboom. *Chemosphere*. 1994;29:2327-2338, and will not be repeated here.)

Results

Results of three maternal plasma samples, 36 cord plasma samples were missing for the PCB 138, 153 and 180, and 45 for the PCB 118. The correlation coefficients between levels of the four PCB congeners 118, 138, 153 and 180 in maternal plasma and cord plasma vary from 0.52 to 0.74. Spearman rank correlation coefficients between these four PCB congener levels in maternal plasma and human milk are presented. The correlation coefficients are all significant, the corresponding congeners having the highest correlations. Spearman rank correlation coefficients between these four PCB congener levels in human milk and dioxin- and PCB-TEQ levels in human milk have been summarized. The correlation coefficients are highly significant. Spearman rank correlation coefficients between PCB congener levels in maternal plasma and dioxin-TEQ and PCB-TEQ levels in human milk are summarized. The correlation coefficients are less. In Figure II, a figure given in the paper, the 95% confidence interval for the regression line between the PCB 153 level in maternal plasma and the total-TEQ level in human milk is shown as well as the 95% predictive interval for the total-TEQ from the PCB 153 level for an individual subject. For instance, if the PCB 153 level in maternal plasma would be equal to the mean, 0.91 ng/g plasma, the upper limit of the 95% predictive interval of the total-TEQ level in milk would be 100.0 pg TEQ/g fat, the lower limit 39.8 pg TEQ/g fat. Since the predictive interval is wide, it is impossible to accurately predict the PCB and dioxin levels to which an individual fetus or breast-fed infant is exposed. (Even though the correlation coefficients are highly significant they are not optimal for these type of samples.) Paired human milk samples collected in the second and sixth week after birth were analyzed for dioxin- and PCB-TEQ levels. The Student's t test for paired samples showed no significant decrease in mean dioxin-, planar PCB- or total-TEQ level over a four-week period. However, the mean mono-ortho and di-ortho PCB-TEQ level were significantly decreased in this period.

The mean dioxin-TEQ level (30 pg TEQ/g fat) is higher than the dioxin background levels in Scandinavia (20 pg TEQ/g fat), Spain (13 pg TEQ/g fat) and the United States (17 pg TEQ/g fat).

PCB congener levels in maternal plasma are about five times higher compared to the levels in umbilical cord plasma. When expressed on a lipid base the levels are comparable.

The total-TEQ value and the contribution of individual dioxin and PCB congener levels to the TEQ value, depend on the TEF values used. According to the model used, the dioxins contribute 46%, the planar PCBs 24%, the mono-ortho PCBs 23% and the di-ortho PCBs 7% to the total-TEQ value (65.2 pg TEQ/g fat).

Correlation coefficients between the non-planar PCB congener levels (PCB 118, 138, 153 and 180) in maternal, and cord plasma and human milk are quite high within one biological sample as plasma or human milk (0.71 to 0.98), and between different biological samples. However, correlation coefficients between other PCB and dioxin congener levels in human milk differ considerably.

Critical Comments

The toxicokinetics of different congeners of PCBs and PCDDs vary as does the distribution of these chemicals among different tissue compartments. The quantitation of some congeners is better than of others adding additional variability. This would also be influenced by the amount of congener present in the different body compartments. Finally, for each mother analytical results were not available for all specimens and it is not stated how non-detectable values were treated. The authors of this paper expressed similar reservations. Combinations of PCBs, PCDFs and PCDDs are not necessarily additive in their toxic effects but may be antagonistic and dose response data are only available from in vitro and short-term laboratory animal studies, the present TEQ system is very uncertain in its prediction of human health effects. Regulatory agencies who developed this system for convenience have viewed the approach as an interim method. It is also important to recognize that the TEF/TEQ approach are order of magnitude estimates of relative potency. At present, toxicokinetics in different species are not considered. However, when comparing responses in different tissues or when comparing across different endpoints the TEF values rarely vary by more than a factor of 10 when they incorporate pharmocokinetic factors (Birnbaum and DeVito, *Toxicology*. 1995;105:391-401). The potency of the mono-ortho coplanar PCBs (105, 114, 118, 123, 156, 157, 167, 189) is 5-6 orders of magnitude lower than that of TCDD (Ahlborg et al. *European J Pharmacol*. 1992;228:179-199). Although the authors seem to have used the most recent TEQ values (Ahlborg et al. *Chemosphere*. 1994;28:1049-1067), the TEF values appear to be higher for the mono-ortho PCBs than the most recent recommendations (Birnbaum and DeVito. *Toxicology*. 1995;105:391-401). It is difficult to determine this since no ranges or means are given for the chlorinated organic compound but it appears that the observed differences for the TEQ values are based on less than one order of magnitude comparisons. With this much uncertainty it should be evaluated whether TEQ values should be used at all to predict subtle adverse effects in humans based on observations that are to some extent subjective.

94. Koopman-Esseboom C., Huisman M., Weisglas-Kuperus N., Boersma R.E., Touwen B.C.L., Sauer P.J.J. Results of the Dutch study on PCB and dioxin induced neurotoxicity in children. *Neurotox.* 1995;16(4).

(This abstract briefly describes studies published in more detail in Huisman et al. *Early Human Development.* 1995;43:165-176; Koopman-Esseboom et al. *Pediatr Research.* 1994;36:468-473; Huisman et al. *Early Human Development.* 1995;41:111-127.)

95. Koopman-Esseboom C., Weisglas-Kuperus N., de Ridder M.A.J., Van der Paauw C.G., Tuinstra L.G.M.Th., Sauer P.J.J. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. *J Pediatr.* 1996;97:700-706.

Methods

During the last trimester of pregnancy, women in the Rotterdam area were asked to volunteer for the study by their obstetrician or midwife, between June 1990 and February 1992. There is no information on the women who refused to take part in the study. Only first- or second-born term infants (37 to 42 weeks gestation) without congenital anomalies or diseases were included. Pregnancy and delivery had to be normal without signs of serious illness, or complications like a cesarean section, forceps, or vacuum extraction. Milk samples contained relatively high levels of both toxins. To study the effects of postnatal exposure to PCBs and dioxins, women were enrolled who intended to breastfeed for at least 6 weeks, as well as women who volunteered to formula-feed from one batch as a reference (Almiron M2, Nutricia NV, the Netherlands) during 7 months.

A blood sample was taken from the mothers in the last month of their pregnancy (36th to 40th week) and from the umbilical cord for the measurement of the PCB congener levels 118, 138, 153, and 180 by gas chromatography with electron capture detection. One maternal and 30 cord blood samples were missing for this analysis. In the second week after delivery the mothers who breastfed their infants collected a 24-hour representative human milk sample with a vacuum pump. Seventeen individual dioxin congener and 24 PCB congeners were measured by gas chromatography-high-resolution mass spectrometry. According to the toxic equivalent (TEQ) concept, the dioxin and dioxin-like PCBs (IUPAC No. 77, 126, 169, 105, 118, 156, 170, and 180) were added and summarized as the total PCB-dioxin TEQ level) as described in a previous article. The PCB congener levels 118, 138, 153, and 180 in human milk were also added and summarized as the PCB-milk-sum in comparison with the PCB-plasma-sum. Of the 105 human milk samples, 80 could be measured with sufficient accuracy for the total PCB-dioxin TEQ level, and 100 for the PCB-milk-sum.

The MDI and PDI of the infants was determined with the Dutch standardized version of the Bayley Scales of Infant Development, the BOS 2-30, at 3, 7, and 18 months of age. The Bayley scales were originally standardized for the Dutch population to a mean of 100 and a standard deviation of 15. All Bayley tests were performed at the infants' homes in the presence of the parent(s) by one examiner (C.K.E.), who was unaware of the infants' PCB and dioxin exposures.

Exposure. Data analysis was performed by using the Statistical Package for the Social Sciences (SPSS/PC, Cary, NC). In the analysis, the relationship between mean Bayley scores and the perinatal PCB/dioxin exposure was studied.

As a measure of prenatal exposure, the PCB-plasma-sum in maternal and umbilical cord plasma was examined, both after log transformation. Dioxin levels in human milk are known to be highly correlated with the dioxin levels in maternal plasma and in adipose tissue and are therefore a good estimation of the prenatal dioxin exposure. The total PCB-dioxin TEQ level in human milk was separately studied as an estimation of the prenatal dioxin exposure of the breastfed infants. Because it is assumed that breastfeeding per se has positive effects on the development of children, the length of breastfeeding was studied separately. This variable called "duration of breastfeeding in weeks," was divided into three categories: zero (formula-fed), short and long. In the multiple regression analysis this categorical variable was entered as a continuous independent variable with the value 0, 1, or 2. Linearity was used because the difference between category 1 and 2 is half the difference between category 1 and 3.

The postnatal PCB exposure and the total PCB-dioxin TEQ exposure were calculated separately as a multiplication of the PCB-milk-sum, respectively, the total PCB-dioxin TEQ level in human milk, and the duration of breastfeeding in weeks. Both variables were divided into three categories: low, medium, and high-exposed. Multiple regression analysis was used to study the effects of prenatal and postnatal exposure to PCBs and dioxins separately as well as combined.

Confounders. The socioeconomic, obstetric, and neonatal conditions were assessed by means of the obstetrical optimality scale. The following potential confounding variables were identified with univariate analyses: maternal smoking and alcohol usage (yes or no), parity (first or second), duration of gestation, birth weight, Apgar score, sex, duration of breastfeeding. Total triiodothyronine, total thyroxine, free thyroxine, and thyroid-stimulating hormone were measured in maternal plasma during the last month of pregnancy, in cord plasma, and in the infants' plasma in the second week, and in the third and eighteenth month after birth.

Results

Two hundred and sixty-eight women were involved in the study. After birth 231 mother-infant pairs fulfilled the inclusion criteria of the study protocol. A vacuum extraction or cesarean section was the main reason for excluding 37 women from the study. Six weeks after delivery 24 pairs were excluded from the study mainly due to the cessation of breastfeeding before this time. Of these 61 excluded pairs no PCB or dioxin analysis were performed nor were the infants tested with the Bayley scales. Of the remaining 207 infants, 105 were breastfed and 102 were formula-fed. Eighty of 105 breast milk samples could be measured.

Breastfed infants scored significantly higher on the MDI-7 ($P = .03$), the MDI-18 ($P = .01$), and on the PDI-7 ($P = .05$) compared with formula-fed infants. The PCB-cord-plasma-sum averaged 0.5 ± 0.3 ng/g; the PCB maternal-plasma-sum 2.2 ± 1.0 ng/g; and the total-PCB-dioxin-sum in human milk 66.6 ± 24.2 pg TEQ/g fat. The two measurements of prenatal exposure (the PCB-plasma-sum in maternal and cord plasma) were highly correlated ($r = 0.72$, $R^2 = 0.52$ $p<0.001$). Levels in formula were below the limits of detection.

There was a weak positive relation between the PCB and dioxin exposure levels and the education of the mothers and the HOME-score of the infants. The other possible confounders were not significantly related to exposure levels.

There was a significantly negative relation between prenatal PCB exposure, maternal plasma PCB levels, and the PDI score at three months of age. A doubling of the PCB-plasma-sum (e.g., 1 ng/g compared with 0.5 ng/g, or 2 ng/g compared with 1 ng/g), would result in a decrease by 3 points of the PDI-3 ($= -4.8 \times Ln2$). When the PCB-sum in cord plasma was used, only 175 cases could be analyzed. No effect of prenatal PCB exposure could then be found. This cannot be attributable to selection, because in this group the maternal PCB-plasma-sum still had a significantly negative influence on the PDI-3. When the total PCB-dioxin TEQ level in human milk was entered into the regression analysis, instead of the PCB-plasma-sum, as an estimation of the prenatal dioxin and dioxin-like PCB exposure of the breastfed infants, there was also a negative influence of this transplacental exposure on the PDI-3 outcome ($B = -7.4$, $SE = 4.0$, $P = .07$). The duration of breastfeeding is not significantly related to the PDI-3. Of the other potential confounders, only the gestational age was of a significantly positive influence ($P = .0001$). Examination of the postnatal exposure revealed no significant effect of the total PCB-dioxin TEQ exposure on the PDI-3 outcome. When the prenatal PCB exposure and the postnatal total PCB-dioxin TEQ exposure were entered together in the multiple regression analysis the results remained almost the same; a decrease by 3 points of the PDI-3 when

the prenatal PCB exposure would double, and no significant effect of the postnatal exposure.

At 7 months of age there was no significant effect of prenatal exposure on the PDI outcome. There was a significantly positive relationship with the duration of breastfeeding: infants being breastfed between 6 and 16 weeks would score 7 points higher and infants being breastfed between 17 and 30 weeks would score 14 points higher on the PDI-7 compared with formula-fed infants. This was a positive influence of breastfeeding itself. However, there is a negative influence of the postnatal PCB and dioxin exposure by breastfeeding on the PDI-7: infants who received a medium or high total PCB-dioxin TEQ exposure through breastfeeding had significantly lower PDI-7 scores (-10 points and -8 points, respectively), compared with formula-fed infants and breastfed infants who received a low total PCB-dioxin TEQ exposure (overall P = .05). The figure shows that infants being breastfed between 6 and 16 weeks (short), with a low total PCB-dioxin TEQ exposure, would score 7 points higher on the PDI-7, and infants being breastfed between 17 and 30 weeks (long), with a low total PCB-dioxin TEQ exposure would score 14 points higher on the PDI-7, compared with formula-fed infants. Infants being breast-fed for a short period with a medium or high total PCB-dioxin TEQ exposure would score 3 points (1 point lower, respectively) and infants being breast-fed for a long period with a medium or high total PCB-dioxin TEQ exposure would score 4 points (6 points higher, respectively) compared with formula-fed infants, which is of no significant difference. (The last sentence was copied verbatim and is unclear.) Furthermore, the PDI-7 is significantly negatively related to the number of older siblings ($p<0.0001$). When the prenatal PCB exposure and the postnatal total PCB-dioxin TEQ exposure were entered together in the multiple regression analysis the results showed the same trends; no effect of the prenatal exposure, and a negative influence of the postnatal exposure on the PDI-7 outcome, although of no significance (overall P = .09).

The PDI outcome at 18 months of age was neither significantly influenced by the prenatal or postnatal PCB and dioxin exposure, nor by the duration of breastfeeding, nor by the other confounders. The MDI score at 3 months of age was neither significantly related to the perinatal PCB or dioxin exposure, nor to the duration of breastfeeding. Gestational age was the only confounder with a significant relationship to the MDI-3 ($p<0.0001$).

The duration of breastfeeding is significantly positively related to the MDI-7. Infants who were breastfed for a short or long period would have an advantage of 2 and 4 points, respectively, compared with formula-fed infants. Neither the prenatal nor the postnatal PCB or total PCB-dioxin TEQ exposure have significant influence on the MDI-7 outcome. The MDI-18 was neither significantly influenced by the duration of breastfeeding, nor by PCB/dioxin exposure. However, there is a strong positive

relationship between the HOME score and the MDI-18 outcome ($p<0.0001$). Secondly, a higher educational level of the mother increased the MDI-18 score by 7 points ($P = .01$).

Instead of the postnatal total PCB-dioxin TEQ exposure, all analyses were repeated with the PCB-milk-sum multiplied by the duration of breastfeeding as an estimation of the postnatal exposure. The effect of this postnatal PCB exposure on the Bayley scores was not significant. There was no significant relationship between the thyroid hormone levels and the mental or psychomotor outcome at any age.

Critical Comments

The group of children was not randomly selected. The refusal rate and the reasons for refusal are unknown. In contrast to the studies conducted in the United States, plasma rather than serum was analyzed for PCBs. These authors calculated TEQ values as exposure levels which have a great deal of uncertainty. They also incorporated some PCB congeners into the TEQ values. The correlation analyses also included the dioxins. In this respect the studies are different from the studies conducted in the United States. The correlations made with the MDI and PDI were transitory, the negative impact of PCBs and dioxins was small and the MDI was positively correlated with breast feeding which in part offset the negative effect of the exposure to PCBs and dioxins. The authors claim that a doubling of the PCB-plasma-sum (e.g., 1 ng/g compared with 0.5 ng/g, or 2 ng/g compared with 1 ng/g), would result in a decrease by 3 points of the PDI-3 ($= -4.8 \times Ln2$). However, these differences in PCB levels are very small and within the noise level of the analytical method particularly if the blood samples were not standardized on fat content. In contrast to the Michigan and North Carolina studies, a small prenatal effect was observed only at three months. At 18 months neither PCB or dioxin exposure affected development. These observations also have no clinical significance.

96. Koppe J.G. Nutrition and breast-feeding. ***European J Obstetrics and Gynecology and Reproductive Biology.*** **1995;61:73-78.**

Methods

Thirty-four healthy well-nourished Caucasian women between the ages of 23 and 38 years (mean 29.2), who had the intention to breast-feed for at least 2 months participated in this study. After delivery, every subject was ad random assigned to one of the diets tested. Group A was assigned to the low fat/high carbohydrate/low dioxin diet. Group B was assigned to the high fat/low carbohydrate/low dioxin diet.

In both groups, the fat content of the milk was not decreased during the test diet. A significant change in dioxin concentration in milk fat after use of low dioxin containing test-diets was also found. In conclusion, it appears to be difficult to reduce dioxin concentrations in milk fat with short-term dietary measures.

Critical Comments

This paper seems to be an ancillary study. It is unclear whether the study participants were selected from the same group that is studied by Koopman-Esseboom et al., or whether it is an entirely different group. The third and fourth week after delivery was chosen for the intervention. An intervention of two weeks is much too short to affect dioxin milk levels as the authors themselves point out. Even long-term dietary intervention might not be readily detected since the initial levels are quite low and the analytical methods for the determination of dioxins are not very accurate.

97. Sauer P.J.J, Huisman M., Koopman-Esseboom C., Morse D.C., Smits-van Prooije A.E., Van de Berg K.J., Tuinstra L.G.M.Th., Van der Paauw C.G., Boersma E.R., Weisglas-Kuperus N., Lammers J.H.C.M., Kulig B.M., Brouwer A. Effects of polychlorinated biphenyls (PCBs) and dioxins on growth and development. *Human and Experimental Toxicology.* **1994;13:900-906.**

(This is a progress report of the study of 400 plus mothers and their infants. More details are given in Hinsman et al. *Early Human Development.* 1995;41:111-127 and Koopman-Esseboom. *Chemosphere.* 1994;28:1721-1732, *Pediatr Research.* 1994;36:468-472.)

98. Tuinstra L.G.M.Th., Huisman M., Boersma E.R., Koopman-Esseboom C., Sauer P.J.J. Contents of dioxins, planar and other PCBs in 168 Dutch human milk samples. Dioxin '95. 15th international symposium on chlorinated dioxins and related compounds, Edmonton, Canada. *Organohalogen Compounds.* **1995;26.**

Materials

Human milk samples were collected at the tenth (9-17) and forty-second (40-45) day after delivery. Before each suckling both breasts were emptied with an electrical breast pump. The obtained milk was mixed thoroughly and a 10% aliquot was taken to make up a 24-hour sample. This 24-hour sample was deep frozen (-20° Celsius) till analysis was performed. Human milk sample volumes ranged from 20-100 mls.

As the number of complete data sets for the second sampling was low, it was decided to use only complete data sets from the first sampling. Another reason was that for the MOPs (mono-ortho PCBs) and DOPs (di-ortho PCBs) content, a significant difference could be found between first and second sampling.

With the used TEFs, the contribution of the dioxins to the total TEQ seems to be less than 50%.

Dioxin planar and other PCB content in 168 Dutch human milk samples in TEQ (pg/g fat).

	dioxins	NOP*	MOP	DOP	total TEQ
mean	30.0	15.98	14.86	4.41	65.25

*non-ortho PCBs

Critical Comments

This is a short paper in conjunction with a presentation. Too little detail is given for an evaluation.

99. Tuinstra L.G.M.Th., Koopman-Esseboom C., Sauer P.J.J., Huisman M. Contents of dioxins, planar and other PCBs in human milk from the Rotterdam and Groningen area. Dioxin '93. 13th international symposium on chlorinated dioxins and related compounds, Vienna. ***Organohalogen Compounds*****. 1993;13.**

(This is a short paper. More details are published in Koopman-Esseboom. *Chemosphere*. 1994;29:2327-2338.)

100. Van den Berg M., Sinnige T.L., Tysklind M., Bosveld A.T.C. (Bart), Huisman M., Koopmans-Essenboom C., Koppe J.G. Individual PCBs as predictors for concentrations of non and mono-ortho PCBs in human milk. ***Environ Sci and Pollut Res*****. 1995;2:73-82.**

Thirty-two Dutch human milk samples were analyzed for PCBs with either HRGC-ECD or HRGC-LRMS in the NCI mode. Samples were collected from three different locations in The Netherlands: Amsterdam, Rotterdam and Groningen. Quantitatively, no differences could be observed between the three localities, while in addition the congener specific pattern showed a striking similarity for all individual samples.

Good linear relationships were observed between individual PCBs. Based on the results of this study, PCB 118 can be used to predict concentrations of the PCBs 105 and 126. PCB 153 can be used as a predictor for the PCBs 156, 157, 167 and 169, but also for the total toxic equivalencies (TEQs) of non and mono-ortho PCBs present in human milk. This method using certain PCBs as predictors for other toxicological relevant congeners, can be useful and cost

effective, e.g., for epidemiological studies. However, before applied a number of conditions should be met. These are:

1) A stable composition of the PCB matrix should be established.

2) A possible time dependent change in composition of the matrix should first be excluded when used over different time periods.

(No infants were studied.)

101. Weisglas-Kuperus N., Sas T.C.J., Koopman-Esseboom C., Van der Zwan C.W., De Ridder M.A.J., Beishuizen A., Hooijkaas H., Sauer P.J.J. Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. ***Pediatr Research.*** **1995;38(3):404-410.**

Methods

The total study group consisted of 207 healthy mother-infant pairs, recruited between June 1990 and February 1992, living in the Rotterdam area and was described in detail in a previous paper (1).

In a subgroup of infants, recruited between March 1991 and February 1992, white blood cell counts and immunologic marker analyses in cord and venous blood at 3 and 18 months of age were done. Because fresh blood is needed for these measurements, only infants born on a weekday and living close to the hospital were included in this part of the study. In 48 infants cord blood was analyzed. At 3 months of age, in 1 of the original 48 children the venipuncture was not successful, and a randomly chosen child was added to the study group. At 18 months of age fresh blood was available for analysis in 37 of the original 48 children, and 6 randomly chosen children were added. There were no significant differences between the total study group and the subgroup.

A blood sample was taken from the mothers in the last month of pregnancy and analyzed for four PCB congeners--PCB 118, 138, 153, and 180. In the second week after delivery, the breast-feeding mothers collected a 24-hour representative human milk sample. Seventeen individual dioxin congeners and 24 PCB congener levels were measured. The international toxic equivalence factor approach was used for PCDD and PCDF and the WHO 1993 approach for PCB. Of the 105 human milk samples, 80 could be measured with sufficient accuracy for the total TEQ level. As a measure of prenatal exposure, the PCB-plasma-sum of all individual mother-infant pairs was used. For the breast-fed infants the total TEQ level in human milk was separately studied as an estimate of the prenatal exposure. Postnatal PCB/dioxin exposure was calculated as a product of the total TEQ level in human milk, multiplied by weeks of breast-feeding.

Measures of immunologic effects. All parents were asked to complete a health questionnaire regarding their infant. The number of periods with rhinitis, bronchitis, tonsillitis, and otitis during the first 18 months of life was counted and used as an estimate of the health status of the infants.

Vaccinations against mumps, measles, and rubella were given to 205 of the 207 children at approximately 14 months of age. Humoral antibody production was measured at 18 months of age.

Monocyte, granulocyte, and lymphocyte counts were determined by whole blood fluorescence-activated cell sorter analysis combined with the white blood cell count. Absolute numbers of the following lymphocyte (sub)populations were determined: $CD4^+$ T-lymphocytes ($CD4^+CD3^+$), $CD8^+$ T-lymphocytes ($CD8^+CD3^+$), activated T-lymphocytes ($HLA\text{-}DR^+CD3^+$), as well as $TcR\alpha\beta^+$, $TCR\gamma\delta^+$, $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ T-lymphocytes, B-lymphocytes ($CD19^+$ and/or $CD20^+$), and NK cells ($CD16^+$ and/or $CD56^+/CD3^-$).

Data analysis. Data analysis was performed using the statistical software package SPSS Win 6.01. The relationship between immunologic parameters and PCB/dioxin exposure was studied in univariate analyses. In a first analysis, prenatal (PCB-plasma-sum and total TEQ) and postnatal (total TEQ multiplied by weeks of breast-feeding) PCB-dioxin exposure were studied in relation to the immunologic parameters. When the PCB-plasma-sum was significantly correlated ($p \leq 0.05$) with the outcome variable, analyses of the separate PCB congener levels 118, 138, 153, and 180 in maternal plasma were done. When the total TEQ was significantly correlated with the outcome variable, analyses of the dioxin and dioxin-like (planar, mono-ortho, and di-ortho) PCB congeners in human milk were done. Potential confounding variables at birth (birth weight, gestational age, sex, smoking and alcohol use during pregnancy, maternal education, and paternal occupation) and at 3 and 18 months of age (sex, nutritional status, duration of breast-feeding, maternal education, and paternal occupation) were selected, according to clinical and immunologic knowledge. Potential confounding variables were analyzed when the PCB/dioxin exposure was significantly correlated with the outcome variable.

The mean PCB-plasma-sum in the subgroup was 2.10 μg/L (SD 0.87, n = 55) and the mean total TEQ was 64.20 pg/g fat (SD 19.08, n = 19). There were no significant differences in the mean PCB-plasma-sum or total TEQ level between the total group and the subgroup.

There were no significant correlations between the number of periods with rhinitis, bronchitis, tonsillitis, and otitis during the first 18 months of life and prenatal (PCB-plasma-sum and total TEQ level) and postnatal (total TEQ level multiplied by the number of breast-feeding weeks) PCB/dioxin exposure. There were also no significant correlations between the specific antibody levels to

mumps, measles, and rubella at 18 months of age and pre- and postnatal PCB/dioxin exposure. The results of the white blood cell counts and the immunologic marker analyses in cord blood and venous blood at 3 and 18 months of age are all within the normal ranges in age-matched children.

At birth a higher total TEQ level was correlated with an increase in TcR$\gamma\delta^+$ T cells ($p = 0.03$). Correlation coefficients with the dioxin, planar, mono-ortho, and di-ortho PCB congeners only reached statistical significance for the dioxin TEQ level ($p = 0.01$). There were no significant correlations between the number of TcR $\gamma\delta^+$ T cells and the potential confounders.

At 3 months of age a higher total TEQ level was significantly correlated with a decrease in the number of monocytes ($p = 0.003$) and granulocytes ($p = 0.04$). A higher postnatal exposure was significantly correlated with a decrease in the total number of monocytes ($p = 0.03$), granulocytes ($p = 0.01$), and B-cells ($p = 0.05$). The monocyte count was significantly correlated with the dioxin TEQ level ($p = 0.01$), the mono-ortho ($p = 0.002$), and di-ortho ($p = 0.03$) PCB TEQ level. The granulocyte count was significantly correlated only with the total TEQ level. The number of CD19/20$^+$ cells was significantly correlated with the duration of breast-feeding ($r_s = 0.64$, $p = 0.003$).

At 18 months of age higher total TEQ and PCB-plasma-sum levels were significantly correlated with an increase in the number of CD8$^+$ T cells (PCB-plasma-sum, $p = 0.01$, total TEQ, $p = 0.002$). In maternal plasma PCB 118 ($r_s = 0.33$, $p = 0.03$), PCB 138 ($r_s = 0.32$, $p = 0.04$), PCB 153 ($r_s = 0.37$, $p = 0.01$) and PCB 180 ($r_s = 0.80$, $p = 0.002$) were significantly correlated with the number of CD8$^+$ T cells. In human milk correlation coefficients with the dioxin, planar, mono-ortho, and di-ortho PCB congeners reached statistical significance for the dioxin TEQ level ($p = 0.002$) and the planar ($p = 0.01$) and di-ortho ($p = 0.02$) PCB TEQ levels. A higher total TEQ level was also significantly correlated with an increase in the number of TcR$\alpha\beta^+$ cells ($p = 0.05$). Correlation coefficients between the number of TcR$\alpha\beta^+$ cells and the dioxin, planar, mono-ortho and di-ortho PCB congeners reached statistical significance for the dioxin TEQ level ($p = 0.009$) and the di-ortho ($p = 0.04$) PCB TEQ level. In addition, a higher dioxin TEQ level was also significantly correlated with more CD3$^+$ ($r_s = 0.61$, $p = 0.04$) and TcR$\gamma\delta^+$ ($r_s = 0.70$, $p = 0.01$) T cells. There were no significant correlations of the T cell markers at 18 months of age with postnatal PCB/dioxin exposure nor with the potential confounders.

Comparing the number of periods with respiratory tract infections and the leukocyte (sub)population at 18 months of age, there was a significant relationship only between the number of periods with bronchitis and the CD4$^+$ CD45RA$^+$ T-lymphocytes ($r_s = 0.33$, $p = 0.04$). Antibody levels to mumps were correlated with the total number of

lymphocytes (r_s = 0.32, p = 0.04), the $CD8^+$ (r_s = 0.33, p = 0.04) and $TcR\gamma\delta^+$ (r_s = .37, p = 0.02) T- and the B-lymphocytes (r_s = 0.32, p = 0.05). Antibody levels to measles were significantly correlated with the number of $CD8^+$ (r_s = 0.35, p = 0.03) and $TcR\gamma\delta^+$ (r_s = 0.34, p = 0.04) T-lymphocytes and with the NK cells (r_s = 32, p = 0.05). Antibody levels to rubella were significantly correlated with the number of granulocytes (r_s = 0.34, p = 0.04) and the $TcR\gamma\delta^+$ (r_s = 0.32, p = 0.05) T-lymphocytes.

Discussion

In this study two different effects of PCB/dioxin background exposure on the developing immune system of human infants were found. Prenatal PCB/dioxin exposure was associated with changes in T cell subpopulations in the blood. These changes were mainly seen at 18 months of age. At that age a higher prenatal PCB/dioxin exposure was associated with an increase in the total number of T cells as well as with an increase in the number of $CD8^+$ (cytotoxic), $TcR\alpha\beta^+$ and $TcR\gamma\delta^+$ T cells.

A higher prenatal as well as postnatal PCB/dioxin exposure was associated with lower monocyte and granulocyte counts only at 3 months of age. Unfortunately due to the nonparametric distribution of the white blood cell counts and the small numbers of cases left, multivariate analysis was impossible.

A higher postnatal PCB/dioxin exposure was associated with a decrease in the number of $CD19/20^+$ B cells. The correlation coefficient of the number of B cells with the duration of breast feeding was higher than with postnatal PCB/dioxin exposure. It is, therefore, presumed that the decrease in B cells was mainly an effect of breast feeding.

In this study there was no evidence of increased upper or lower respiratory tract symptoms or altered humoral antibody production in relation to PCB/dioxin exposure. Although there were differences in the leukocyte (sub)population between high and low PCB/dioxin-exposed infants, all values were within the normal range. Moreover, subtle changes in the number of blood leukocytes do not simply mirror alterations in the cell composition of lymphoid and nonlymphoid organs, nor do they simply reflect functional defects.

There is no evidence of clinical symptoms or direct changes in the humoral immunity response in infancy. The results of the white blood cell counts and immunologic marker analyses were all within the normal range, the described changes in the T cell lymphocyte population could persist according to the authors.

Critical Comments

There were some variations of different parameters studied in this subgroup of children that were within the range of normal. These associations appear to be inconsistent and meaningless from a clinical point of view and may well have occurred by chance. Repeat tests should have been done on the children to determine whether the low monocyte and granulocyte counts were persistent. Unrelated to any exposure to PCBS and PCDDs some people simply have lower granulocyte and monocyte counts then others. For this reason the laboratory gives ranges of normal values. The normal ranges of the laboratory performing these tests are not given. The authors may have overinterpreted their data. Confounders such as intercurrent viral infections, medications, and vaccinations were not examined in detail.

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Koopman-Esseboom C., Huisman M., Weisglas-Kuperus N., Van der Paauw C.G., Tuinstra L.G.M.Th., Boersma E.R., Sauer P.J.J. 1994. PCB, dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. *Chemosphere*. 28:1721-1732.

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CHEMICAL MANUFACTURERS ASSOCIATION

June 16, 1999

Dear PCB Panel and Industry Coalition (IC) Members:

Enclosed are the comments jointly submitted to ATSDR on their Draft toxicological Profile for Polychlorinated Biphenyls Update, dated December 1998.

Sincerely,

Elizabeth Festa Watson
Managing Director, CHEMSTAR
Manager, PCB Panel

enclosure

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June 8, 1999

Dr. Ganga Choudhary
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Atlanta, Georgia 30333

RE: Draft Profile for PCBs

Dear Dr. Choudhary:

The Chemical Manufacturers (CMA) PCB Panel and Chlorine Chemistry Council, the Utility Solid Waste Activities Group, and the National Electrical Manufacturers Association are submitting comments on ATSDR's Draft Toxicological Profile for Polychlorinated Biphenyls Update, dated December, 1998. The comments were prepared by Dr. Robert James, Dr. Nate Karch and Dr. Renate Kimbrough, three experts intimately familiar with the PCB studies.

As you are aware, the available database of studies on PCBs is vast. It has thus inevitably taken our experts slightly more time to review the Draft in the detail it deserves than originally provided by the Agency in its announced comment period. Because the comments are important to assuring that the Profile presents a complete and balanced presentation of the large amount of available literature, we trust that ATSDR will give them careful attention in revising the draft. Our review has found that ATSDR has taken an important first step in preparing a comprehensive update of the PCB profile. However, some sections of the profile contain incomplete, misleading, or inaccurate characterizations of the underlying literature. Considering its potential uses as a regulatory, risk management, and educational tool, we believe the profile should be as complete, accurate, and representative of the current state of PCB research as possible. To this end, we strongly urge ATSDR to substantially revise or completely rewrite sections of the PCB profile highlighted in our comments and re-issue the profile in draft form for final comments.

Among our comments are several suggestions to add studies currently not referenced. If the Agency needs copies of any of these studies, please let us know. Any requests should be sent to the CMA Panel Manager Elizabeth Festa Watson at 703-741-5629, or Elizabeth_Watson@cmahq.com.

Sincerely yours,

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Comments on the Draft ATSDR Toxicological Profile for Polychlorinated Biphenyls (PCBs) Update [ATSDR-#143]

June 8, 1999

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The Utility Solid Waste Activities Group

The National Electrical Manufacturers Association

The Chemical Manufacturers Association
Polychlorinated Biphenyls Panel

and

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I. EXECUTIVE SUMMARY

This document presents the joint comments of the National Electrical Manufacturers Association (NEMA), the Utility Solid Waste Activities Group (USWAG), the Chemical Manufacturers Association (CMA) Polychlorinated Biphenyls Panel, and the Chlorine Chemistry Council (CCC), on the *Draft ATSDR Toxicological Profile for Polychlorinated Biphenyls (PCBs) Update*, dated December 1998 (PCB profile). We appreciate the opportunity to present comments on the technical content and presentation of data in the draft PCB profile.

Toxicological profiles published by ATSDR have a broad impact. They are used as the basis of public policy through regulatory rule-making, are routinely consulted to support risk management decisions, and are risk communications tools used to inform and educate the public. With these uses in mind, we have commissioned widely recognized experts in PCB toxicology to review the profile. This Executive Summary (Section I) contains an overview of concerns brought to light by the review and suggestions of ways the PCB profile could be improved. Section II contains detailed discussions of key sections of the profile, focused on presentation of the current state-of-the-science. Section III consists of line-by-line comments on the text of the profile. Section IV consists of an appendix containing an additional, in-depth discussion of studies that evaluate neurological and neurodevelopmental effects in children exposed to PCBs *in utero*.

The PCB profile attempts to cover a huge body of literature. Our review finds it a useful guide to much of that literature. However, we have several overall suggestions and concerns regarding the presentation of the data within the document. Specifically, we are concerned that some sections of the profile contain incomplete, misleading, or inaccurate characterizations of the current state of PCB research. Most users of toxicological profiles are not intimately familiar with the underlying literature and rely on ATSDR to present representative summaries. We suggest, therefore, that some sections of the PCB profile be substantially revised or completely rewritten and that, after revising the document, ATSDR reissue the profile in draft form for final comments. The following paragraphs summarize our concerns and suggested improvements.

A. Controversial Hypotheses without Comprehensive Discussion of Relevant Literature

In several cases, the document presents controversial hypotheses in an unduly credulous fashion and without sufficient critical evaluation of the relevant scientific literature. Since much of the audience of the PCB profile will not be familiar with the underlying literature, it is essential that the profile present a critical, fact-based analysis of any hypotheses that are discussed. For example, the paragraph spanning pages 138 and 139 raises the hypothesis of a link between "xenoestrogens, including PCBs" and breast cancer. Having raised the issue, the profile fails to provide a comprehensive review of the literature investigating a link between breast cancer and exposure to PCBs. Substantial mechanistic, theoretical, and epidemiologic data demonstrate that such a link is highly unlikely. ATSDR should revise the profile to either present all the relevant information in sufficient detail that readers can accurately evaluate the data, or omit reference to this hypothesis entirely.

A similar problem arises on pages 122 and 123, where the PCB profile briefly reviews the data regarding the hypothesis that PCB exposure may induce endometriosis. In the process, the profile makes the bold statement on page 122 that "[e]ndometriosis is known to occur following exposure to dioxin and some dioxin-like chemicals." This is a misrepresentation of the evidence on the link between endometriosis and exposure to dioxin and some dioxin-like chemicals, and is not relevant to the health effects of PCBs. The otherwise reasonable discussion of the data on PCB exposure and endometriosis is clouded by this statement, which should be omitted from the profile.

B. Incomplete and Inaccurate Discussions of Certain Data Give the Appearance of Bias

Some places in the PCB profile present skewed, inaccurate discussions that give the appearance of bias. For example, on page 117 of the profile, the first paragraph under Section 2.2.2.4, Neurological Effects, was apparently written to convey the impression that substantial data conclusively link PCB exposure with neurological effects in adult humans. However, the paragraph contains misleading descriptions of the underlying data and well as inaccurate statements. The problems with this paragraph are detailed in Section II of the comments.

Because most readers of the PCB profile will not have access to all of the underlying literature, it is critical that the profile present scientific data in an unbiased, reliable manner. This paragraph and the Neurological Effects section should be completely rewritten to provide an accurate, representative summary of the underlying data. In addition, detailed comments in Section II identify other sections of the profile that should also be reexamined for their tone and content to ensure that data are presented in an accurate, unbiased manner.

C. Limitations of Studies are not Consistently Discussed

The PCB profile fails to present consistently the full range of caveats and limitations regarding the studies discussed. This is most noticeable in the discussion of the human literature, in which numerous confounding exposures exist and the adequacy of quantitative assessment of PCB exposure are constantly at issue. The profile discusses limitations in some places, but not in others. This is of particular concern whenever studies of fish-eating populations are discussed and presented. In some cases, studies of fish eating populations that provide no data on PCB exposure are cited (for instance, see the citation to Mergler et al., 1998 on page 117). Too often in the PCB profile, such studies are presented without a clear discussion of the following issues:

1. Whether PCB exposure was quantified in the study through sampling of human tissues or serum or through measurements on consumed fish;
2. Whether exposure to other contaminants of interest was quantified and adjusted for as potential confounders (fish are often contaminated with a range of persistent organochlorine compounds, other pesticides, and heavy metals); and
3. Whether other confounding factors relevant to the health endpoint of interest were accounted for in the study (for example, alcohol consumption).

Another example of the failure to communicate clearly the caveats and limitations occurs in the profile's presentations of the Jacobson studies. Limitations and questions regarding the

studies are mentioned in some places in the profile, but not every time the studies are discussed. Readers of the profile may obtain an inaccurate impression of the strength of these studies, depending on which part of the profile is read.

ATSDR should carefully review the PCB profile's presentation of human epidemiologic data to ensure that the appropriate caveats and limitations of such studies are presented clearly in each discussion of each study (and not just in a brief, passing reference somewhere in the general section). Given the numerous confounding factors associated with studies of fish-eating populations, studies without measurement of exposure to PCBs should not be included in this profile.

D. Important, Relevant Studies are not Cited

The profile fails to cite important, relevant studies on several topics. For example, the discussions of neurological and neurodevelopmental effects in animals fails entirely to cite the studies of some researchers and includes only a selection of studies from other researchers, resulting in omission of numerous important studies. Critical literature on the PCB-breast cancer hypothesis is also omitted. These and other omissions are detailed in comments in Sections II and III. The PCB profile should be revised to include the relevant missing literature.

E. Presentation of Data is often Disorganized and Lacking an Analytical Framework

Discussions of some groups of studies and data are disorganized, repetitive, and lack an analytical framework for evaluation. For example, this is particularly a problem in the discussion of the animal literature on neurobehavioral and neurodevelopmental effects (pp. 118-122 and 130-137), in which there is no organized discussion of important structure-activity relationships or mechanistic issues. Such a discussion would make clear the inconsistent nature of results on this topic to date and assist in placing these results in context with human data. ATSDR should revise this section of the PCB profile, and others as identified in Sections II and III of the comments, to present the data in an organized framework.

F. MRL Calculation is Based on an Animal Species More Sensitive to PCBs than Humans

Comparison of quantitative toxicologic data in animals and humans demonstrates that the Minimum Risk Level MRL calculation presented in the profile relies upon an inappropriately sensitive animal species. As discussed in detail in Section II, the rhesus monkey is clearly much more sensitive to every endpoint of PCB toxicity than humans. This is recognized in the PCB profile itself by stating that the MRL "*...is approximately 3 orders of magnitude below the low-end estimated dose for occupationally exposed individuals*" (pp. 204-205 of PCB profile). Therefore, an alternative animal-based MRL was derived for ATSDR's consideration in Section II.G.2 of this report.

G. Line-by-Line Changes

We identified numerous problems in the discussions of individual studies or problems of an editorial nature in our review of the draft profile. ATSDR should revise the profile to take these comments, presented in Section III, into account.

H. Appendix A: An In-Depth Discussion of Those Studies Evaluating the Neurological and Neurodevelopmental Effects in Children Exposed to PCBs *in utero*

Because the PCB profile relies heavily on studies evaluating neurological and neurodevelopmental effects in children exposed *in utero*, and particularly on the series of studies referred to as the "Jacobson" or "Michigan" cohort, a detailed discussion of the limitations of these studies is included as an appendix (Section IV).

I. Conclusion

Given the quantity and breadth of research on the toxicology of PCBs, ATSDR has taken an important first step in preparing a comprehensive update of the PCB profile. However, some sections of the profile contain incomplete, misleading, or inaccurate characterizations of the underlying literature. Considering its potential uses, we believe that the PCB profile should be as complete, accurate, and representative of the current state of PCB research as possible. To this end, we strongly urge ATSDR to revise substantially or completely rewrite sections of the PCB profile highlighted in our comments and reissue the profile in draft form for final comments.

II. INTRODUCTION AND GENERAL COMMENTS

The ATSDR Draft PCB Profile has been reviewed in detail and we have identified several sections that either present misleading information or are not supported by the data provided. We propose that the following major issues be re-examined and rewritten to reflect the available data. Given the substantial changes recommended in these comments, we propose that the PCB Profile be reissued as a draft and a second comment period provided to review the revised draft. We have also provided line-by-line comments in Section III, which identify problems with discussions of individual studies or problems of an editorial nature.

The major issues from the PCB profile for which we have provided detailed comments on in this section are as follows:

- ***Breast cancer.*** The profile briefly presents a speculative hypothesis about the role of organochlorine compounds, including PCBs, in the development of breast cancer without presenting the overwhelming data that refute this hypothesis.
- ***Reproductive effects.*** The profile mischaracterizes the cited studies on this endpoint.
- ***Endometriosis.*** The profile inappropriately presents a speculative hypothesis regarding endometriosis.
- ***Neurological effects (Human data).*** The profile presents a highly inaccurate and misleading summary of human literature on this endpoint.
- ***Neurobehavioral and Neurodevelopmental effects (Animal data).*** The PCB profile selectively cites results and fails to analyze the animal data.
- ***Developmental effects (Human data).*** The profile does not sufficiently qualify its discussion of the relevant human studies and omits significant relevant literature. Section IV, Appendix A presents a detailed critique of some of the human literature on potential developmental effects.
- ***Minimum Risk Level.*** The calculation of the MRL relies on data from an inappropriately sensitive animal species.

A. Breast Cancer

1. Overview

On pages 138-139, the ATSDR PCB Profile discusses the issue of environmental PCB exposure as a possible risk factor for breast cancer in women. This discussion occurs in a single, brief paragraph that is woefully incomplete, and inaccurate, giving the appearance of bias. The shortcomings of the discussion of possible breast cancer effects are several, and include:

- A large amount of relevant information was excluded;
- The few papers that were discussed were inadequately summarized;
- Important mechanistic data that contradict the hypothetical breast cancer-PCB link were not considered;

- Negative epidemiological data of considerable volume and strength were omitted; and
- Viewpoints of scientists who have outlined the significant limitations of the breast cancer-PCB hypothesis were not considered.

These shortcomings are discussed in some detail in the following paragraphs. Given the seriousness and breadth of the problems associated with the present ATSDR discussion of this issue, we feel that ATSDR should completely rewrite and update its analysis, or alternatively, strike it completely from the document so as not to mislead the general public.

Whether organochlorine compounds, including PCBs, might act as xenoestrogens and, via this potential mechanism, exert some effect on breast cancer incidence or mortality, has been discussed and evaluated for a considerable period of time. A large number of papers debating this issue, as well as studies attempting to develop epidemiologic evidence to test this hypothesis, have been published. Adding to this information is a considerable number of mechanistic studies analyzing the estrogenic and anti-estrogenic properties of organochlorine compounds, particularly PCBs. Thus, it is surprising to see this issue raised in the draft PCB profile but summarily discussed after reviewing only 10 papers, a number of which are only commentaries and provide no original data. A brief and less than exhaustive search produced more than 100 citations on this issue. Clearly, writers of the ATSDR PCB profile have not made a serious or meaningful attempt to consider and analyze the literature that is publicly available on this subject.

In addition to the troubling omission of much of the relevant literature, the ATSDR summary, particularly the concluding sentence of the paragraph on pages 138-139, is based on editorials or commentaries by Dr. Devra Davis, one of the initial proponents of this hypothesis. While her comments are of interest, they are generally directed at all organochlorine compounds, not specifically PCBs, and her research experience with these chemicals, and particularly PCBs, is very limited. In contrast, the large body of work by Dr. Stephen Safe was completely ignored despite the fact that his research interest has been almost exclusively PCB and TCDD toxicology. Furthermore, Dr. Safe has measured and identified the estrogenic and anti-estrogenic activity of both PCB congeners and their metabolites and has published several detailed, critical, and balanced reviews and commentaries on the hypothesis at issue. Likewise, the reviews and commentaries of Dr. Mary Wolff, another scientist who has contributed more basic research to this subject than Dr. Davis, were also ignored. Other examples of this selective use of the literature exist and make the current PCB profile indefensible against two major criticisms: 1) that of being inadequate as to the literature considered and evaluated; and 2) having a clear bias towards citing and reviewing papers by proponents of the breast cancer-PCB hypothesis while ignoring scientists whose papers provide substantial evidence that the hypothesis is not a viable one.

The breast cancer-PCB hypothesis appears to have originated from several observations: 1) the perception that breast cancer incidence has steadily increased in recent decades; 2) genetic and other known risk factors are believed to explain less than one-half of the current breast cancer cases; 3) many risk factors for breast cancer seem to be associated with increased estrogen levels; and 4) persistent organochlorine compounds, a number of which have some weak estrogenic or anti-estrogenic activity, are present at various levels in our environment

(Davis et al., 1993; Davis and Bradlow, 1995; Wolff et al., 1993). On the basis of these observations alone, it might be reasonable to propose and investigate the hypothesis that low levels of estrogenic, environmentally ubiquitous chemicals may contribute to the observed increase in breast cancer risk. However, at the present time, there are many simple trend observations that run contrary to this hypothesis and a considerable number of epidemiology and mechanistic studies that clearly refute it. For example,

1) The higher organochlorine levels in Japan (relative to Western countries) correspond to lower breast cancer rates than those observed in the U.S. Since the incidence of breast cancer increases among Japanese women who migrate to the U.S., the increase in breast cancer rates has to be caused by something other than organochlorine exposures (Adami et al., 1995).

2) Breast cancer has continued to increase despite significant declines in the breast milk and adipose tissue levels of PCBs and DDT (and its metabolites). These declines stem from the restricted use and/or ultimate ban of these compounds. The relationship between declining persistent organochlorine exposure and increasing breast cancer risk is counter to the proposed hypothesis (Safe, 1997).

3) The hypothesis itself ignores the complexity of PCB mixtures with respect to their congener compositions and the fact that a number of organochlorine compounds, especially coplanar PCB congeners, have significant anti-estrogenic activity as well. In fact, potency-times-dose analyses suggest that anti-estrogenic activity may well be the net result of environmental exposure, at least with regard to PCBs and TCDDs (Safe, 1995; Safe, 1997; Wolff and Toniolo, 1995; Wolff and Weston, 1997).

4) Initial case-control, correlational studies (those attempting to correlate blood and tissue levels of PCBs with the occurrence of breast cancer) were generally negative, but with somewhat variable results. However, two separate meta-analyses that combined the results of these smaller studies to obtain a larger, stronger analysis found PCBs had no effect on breast cancer incidence (Key and Reeves, 1994; Adami et al., 1995).

5) The proposed hypothesis for a link between PCB exposure and breast cancer has ignored the fact that the best tests of its validity are the results of cohort studies of women receiving high-dose occupational exposures and not low-dose, case-control or ecologic studies where the potential for confounding and bias is perhaps impossible to fully control. When the high-dose cohort studies are evaluated, there clearly is no risk of breast cancer following long-term exposure to PCBs that produced doses and body burdens several orders of magnitude higher than those in the individuals being studied in the case-control environmental studies (Brown, 1987; Taylor et al., 1988; Adami et al., 1995; Kimbrough et al., 1999). Probably no other observation or fact should carry as much weight in deciding the validity of the PCB-breast cancer hypothesis as this one, and the results are convincingly and unequivocally negative.

Despite the above facts and observations being well-documented in the PCB literature and other literature regarding persistent organochlorines, they were given little or no

consideration in the current ATSDR PCB profile. To demonstrate and emphasize that current epidemiology data do not support the organochlorine/breast cancer hypothesis (at least with respect to PCBs), and to a lesser extent to bring to light some of the numerous mechanistic studies that provide support for these negative findings, the information summarized in the last three points above is discussed in greater detail below.

2. Dose-Potency Estimates Suggest PCBs Could Not Increase Breast Cancer Risk Via The Proposed Xenoestrogen Mechanism

An increasing number of natural and synthetic chemicals are being recognized as having estrogen-like activity because of the recent interest in this hypothesis. What is frequently forgotten in this process of identifying chemicals with potential endocrine activity is that whether an effect will be seen in humans depends upon the dose or tissue levels of that chemical and its endocrine potency relative to the hormone it mimics. The analyses by Safe (1995) are not only informative, but go to the very heart of this issue, because an estrogenic effect can only be proposed if it has some possibility of occurring.

In a recent review analyzing the issue of organochlorines as environmental estrogens, Safe (1995) assessed the relative estrogenic activity (i.e., estrogen equivalents) of the chemicals women might be exposed to from their daily environment. Because the body produces estrogens and because women may add to their estrogenic load either by taking estrogenic drugs or by ingesting natural and synthetic estrogens contained in foods, Safe asked the obvious question – which types of estrogen-like chemicals would normally be expected to contribute the most estrogenic activity? To address this issue, Safe used the following equation to assess the total estrogenic activity one might be exposed to each day:

$$EQ = \Sigma([EC_i] \times EP_i)$$

Where,

EQ = the estrogen equivalents
EC_i = concentration of the individual chemical with estrogenic activity
EP_i = estrogenic potency of the chemical relative to some standard for estrogen activity

After reviewing the literature relevant to this subject, Safe notes that on average some 1,020-1,070 mg of estrogenic plant bioflavonoids and mycotoxins (e.g., quercetin, resperetin, and narigenin) are consumed daily. However, these chemicals are far less potent than estrogen and on a weight basis contribute only about 1/10,000th the activity of estrogen. By comparison, only a total of about 0.0025 mg of estrogenic organochlorine compounds are ingested with our food each day. But these compounds are even less potent, having about 1/1,000,000th the activity on a per weight basis as estrogen. For those women taking estrogens as part of their birth-control or post-menopausal therapy, Safe also identified daily doses and calculated the respective estrogen equivalents. For comparison purposes, Safe further estimated anti-estrogenic equivalents for those anti-estrogenic organochlorines using the same general procedure listed above (i.e., potency x dose = equivalent units of activity). The total and group estrogenic and anti-estrogenic equivalents that Safe derived are shown in Table 1. While this table ignores the contribution of normal blood levels of estrogen, two points are clear. First, after considering both the dose and

potency differences of the different types of estrogenic compounds that the general population is exposed to on a daily basis, the contribution made by organochlorine compounds (of which PCBs are only a fraction) is miniscule. By comparison, the total estrogenic activity of flavonoids in foods is almost 41 million (40.8×10^6) times greater than that coming from the organochlorine compounds; the estrogenic activity of many medications dwarfs that of the naturally-occurring flavonoids in foods. Second, the anti-estrogenic activity of one's daily persistent organochlorine exposure is approximately 50 times greater than that of the estrogenic component. This in turn suggests that the net effect of one's total persistent organochlorine exposure would, if anything, be anti-estrogenic and therefore inhibit estrogen-sensitive breast cancer.

Table 1: Mass Balance of Human Exposure to Environmental and Dietary Estrogens and Anti-estrogens.$^{\Psi}$

Source	Estrogenic equivalents (μg/day)
Estrogens	
Morning after pill	333,500
Birth control pill	16,675
Post-menopausal therapy	3,350
Flavonoids in foods	102
Organochlorine estrogens	0.0000025
Anti-estrogens	
TCCD/PCBs/Other persistent organochlorines	0.000120
PAHs in Foods	0.0050
Indole[3,2]carbazole*	0.00128

Ψ Adapted from Safe (1995)
* In 100 grams of brussel sprouts

To summarize, hypothesizing that persistent organochlorine compounds such as PCBs might have enough estrogenic activity to increase a women's risk for breast cancer, the proponents fail to consider two fundamental tenets of toxicology, those of dose and dose-response. As the evaluation by Dr. Safe reveals, it would be essentially impossible for small changes in one's daily exposure to any persistent organochlorine compound to produce any significant impact on one's total estrogen equivalent activity. Further undermining the possibility that persistent organochlorines contribute significantly to breast cancer risk, many of these same compounds have anti-estrogenic activity that would appear to be the more important factor in determining the net effect of these compounds. Lastly, Table 1 also highlights some clear and significant confounders that may be associated with ecologic or case-control studies that typically attempt to ascribe breast cancer risk to small differences in serum or adipose organochlorine levels. Since some foods and medications can provide a much larger dose of estrogenic compounds (both in terms of dose and aggregate potency units) than environmental organochlorines, they become a significant, unavoidable confounder to this type of epidemiology study. Further, it would appear to be impossible to measure the true impact of organochlorine compounds in the face of the far greater estrogenic and anti-estrogenic activity that results from

other ubiquitous environmental exposures. On this last issue it is noted that Adami et al. (1995), after reviewing both epidemiologic and mechanistic data, reached a similar conclusion:

> "Since organochlorines are only weak estrogens or antiestrogens, one has to consider much stronger endogenous or exogenous estrogenic stimuli, as well as the possible competition for estrogenic receptors. OCs and menopausal estrogen treatment, for example, are likely to have much more pronounced hormonal effects, albeit more time limited. The epidemiologic difficulty is then to discern in women the effects, if any, of weak, but prolonged, estrogenic or antiestrogenic exposure in the likely presence of intermittent stronger hormonal influences. Moreover, the chemicals considered are lipid-soluble, and reach human tissues predominately through the diet; they tend to be ingested together, perhaps in certain types of foods. Thus, the body burdens of many organochlorines are likely to be inter-correlated, and related as well to other lipid-soluble substances and to diet. This will complicate attempts to disentangle the independent effects, if any, of these exposures" (Adami et al., 1995).

3. Case-Control Studies Attempting To Correlate Organochlorine Blood and Tissue Levels To Breast Cancer Risk

Prior to 1994 there were five "correlational studies" that attempted to determine if exposure to persistent organochlorine compounds (as measured by tissue concentrations) was higher among women who developed breast cancer compared to those women who had not developed breast cancer. While this evidence by itself would not be sufficient to establish a causal relationship, those performing these studies believed that consistent and significant differences existed which provided suggestive evidence that persistent organochlorines might be an environmental factor to carefully evaluate. [Note: The potency analysis performed by Dr. Safe now indicates that this approach is probably fundamentally flawed.] As of 1995, seven "correlational studies" had measured organochlorine levels in the tissues of breast cancer patients and compared these to levels found in women not developing breast cancer (Wasserman et al., 1976; Unger et al., 1984; Mussalo-Rauhmaa et al., 1990; Falck et al., 1992; Wolff et al., 1993; Dewailly et al., 1994; Krieger et al., 1994). While conflicting observations have been reported in these studies, both positive and negative findings would be anticipated when the results are based on only a small number of cases, as was the rule among the first studies. For example, four of these studies examined 20 or fewer cases and two studies examined only 41-58 cases. In contrast, the largest of these studies, a study which found no relationship between breast cancer and PCBs, examined 150 cases.

Although the results of these early studies were generally negative, two groups of investigators have combined the studies' results into more robust data sets upon which meta analyses were performed and from which stronger and more reliable conclusions could be made. Key and Reeves (1994) analyzed six of the seven studies (Wasserman et al., 1976 was not mentioned in this review) and reported a combined rate ratio of 1.01 (99% CI = 0.92-1.10). In a more thorough analysis of the data available on this issue, Adami et al. (1995) also performed an analysis of all seven studies and derived a combined rate ratio of 1.03 (95% CI = 0.96-1.10). Thus, both combined studies, analyzing 301-310 cases, found that PCB blood and tissue levels

had no relationship to breast cancer.[1] Not only is the overall evidence, as of 1995, negative with respect to breast cancer and PCBs and DDT, but several reviewers have noted that the largest study, that of Krieger et al. (1994), is clearly the best study design. Not only did this study analyze 3-15 times more cases than any of the earlier studies, but because the serum PCB measurements were collected years before the cancer was diagnosed, it provided an analysis of what tissue concentrations were when the breast cancer might have been initiated rather than what concentrations were after the cancer had already developed (Adami et al., 1995).

Since 1995, additional studies on this issue have been published. Because no combined analyses have summarized the results of these studies, they are briefly discussed below on an individual basis. In addition, the study by Archibeque-Engle and colleagues is discussed because of its implications for studies of this type.

Archibeque-Engle et al. (1997). As indicated earlier, one proposed method of generating data to support or refute the hypothesis that organochlorine exposure is a risk factor for breast cancer is to determine whether breast cancer cases have higher residue levels of organochlorines in blood and adipose tissue compared to controls. To properly interpret the results of such studies, however, it is imperative to know how blood levels of organochlorines correlate with the overall organochlorine body burden, and in particular with organochlorine levels within the hypothesized target tissue. To this end, Archibeque-Engle and colleagues (1997) analyzed serum and breast adipose tissue from 36 Connecticut women for nine PCB congeners and eight organochlorine pesticides. Of the seventeen compounds analyzed, serum and breast adipose concentrations were only correlated for two (DDE and one PCB congener, BZ 153). However, while BZ 153 was detected in all 36 breast adipose tissue samples, it was absent from the majority of serum samples. Thus, the authors of this study suggest that serum organochlorine concentrations may be a poor indicator of total body burden and target tissue levels, and that studies exclusively employing serum or plasma measurements to test the hypothesis that organochlorine exposure is a risk factor for breast cancer should be interpreted with this potential limitation in mind. In contrast to the results of this study, however, evidence that lipid adjusted serum concentrations are adequate surrogates for adipose tissue concentrations also exists (Brown and Lawton, 1984; Patterson et al., 1988).

Hunter et al. (1997). In yet another study designed to test the hypothesis that higher blood levels of organochlorine compounds are associated with an increased risk of breast cancer, Hunter and colleagues (1997) measured DDE and PCBs in 240 women with breast cancer and 240 control women from the Nurses' Health Study. On average, women diagnosed with breast cancer, including estrogen-receptor-positive disease, had lower plasma levels of DDE and PCBs than did control women. In addition, there was no indication that women with the highest plasma levels of DDE and PCBs were more likely to have developed breast cancer than women with the lowest plasma levels. If anything, the data suggest just the opposite as women with the highest plasma levels had a non-significantly lower risk of breast cancer than those with the

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[1] Note: Both Key and Reeves (1994) and Adami et al. (1995) reported that there was also no relationship between tissue DDT levels and an increased risk of breast cancer; combined rate ratios and confidence intervals were 1.11 (99% CI = 0.97-1.26) and 1.08 (95% CI = 0.98-1.19), respectively.

lowest plasma levels. Therefore, this study does not support the hypothesis that DDT and PCB exposure are risk factors for breast cancer.

Lopez-Carrillo et al. (1997). This hospital-based study was conducted in Mexico and compared serum DDT and DDE concentrations in 141 breast cancer cases and 141 age-matched controls. The serum levels of DDE and DDT did not significantly differ between cases and controls, regardless of whether concentrations were reported on a lipid weight or wet weight basis. In addition, age-adjusted odds ratios for breast cancer did not increase among pre- or postmenopausal women as serum DDE concentrations increased. Therefore, this study fails to support the hypothesis that DDT exposure is a risk factor for breast cancer.

Moysich et al. (1998). This case-control study examined serum concentrations of DDE, hexachlorobenzene (HCB), and mirex, as well as several measures of PCB exposure (concentration of total PCBs, total number of detected PCB peaks, and concentrations of three PCB congener groups having various degrees of chlorination) in 154 postmenopausal breast cancer cases and 192 controls from the state of New York. Interestingly, Moysich and colleagues (1998) first attempted to group PCBs by enzyme induction or estrogenic activity, but discovered that most women had no detectable levels of congeners with such activity. The data collected in this study indicate that exposure to DDE, HCB, and mirex is not associated with an increase in the risk for postmenopausal breast cancer. In fact, the only statistically significant association between exposure to these compounds and breast cancer risk was an inverse one, where higher HCB levels were associated with a decreased risk of breast cancer. Neither higher serum levels of total PCBs, moderately chlorinated PCBs, or highly chlorinated PCBs, nor the number of PCB peaks detected, was associated with an increased risk for breast cancer among the total study population. Among the total study population, there was some evidence that the risk for breast cancer might be higher among women with detectable levels of less chlorinated PCBs compared to women without detectable levels. However, such evidence is highly suspect given that the risk was significantly elevated among those women with low, but not high, serum concentrations of the less chlorinated PCBs. Lastly, among those women in the study population who had at least one live birth but had never lactated, the data show marginally significant associations between breast cancer risk and serum concentrations of total and moderately chlorinated PCBs and the number of detected PCB peaks. The validity of these marginal associations is questionable, however, due to the very small number of cases and controls within each concentration stratum that had never lactated.

van't Veer et al. (1997). In a study that examined the possible link between DDT exposure and breast cancer, van't Veer et al. (1997) measured DDE in the adipose tissue collected from the buttocks of 265 postmenopausal breast cancer cases and 341 age-matched controls from Germany, the Netherlands, Northern Ireland, Switzerland, and Spain. Mean DDE concentrations were 9.2 percent lower among breast cancer cases than controls and there was no trend of increasing odds ratios for breast cancer with increasing adipose tissue DDE concentrations. This study therefore fails to support the hypothesis that DDT exposure increases the risk of breast cancer among European women.

These four recently conducted case-control studies (van't Veer et al., 1997; Lopez-Carrillo et al., 1997; Hunter et al., 1997; Moysich et al., 1998) each examined the concentrations

of organochlorine compounds in the blood or adipose tissue of breast cancer cases and matched controls. These studies, with the possible exception of that conducted by Moysich and colleagues, failed to find any evidence for an association between organochlorine concentration and breast cancer risk. Even Moysich et al. concluded that if an association existed between postmenopausal breast cancer and PCB exposure, then it was restricted to only those women in their study population who had given birth to at least one child but had never lactated (a seemingly biologically implausible suggestion relative to their remaining data). Furthermore, the positive associations for serum PCBs and breast cancer reported by Moysich and colleagues were only marginally significant and were, by the authors' own admission, based on unstable risk estimates due to the small number of cases and controls in each exposure stratum that had never lactated. The study by Archibeque-Engle et al. (1997) suggests that serum PCB measurements may not be predictive of total PCB body burden or breast adipose tissue PCB concentration, which further casts doubt on the validity of the marginally significant associations reported by Moysich et al. who examined PCBs in serum exclusively.

Taken *in toto*, these case control studies when combined with the results of the earlier meta-analyses offer no evidence that organochlorine/PCB exposure is a significant or otherwise meaningful risk factor for breast cancer.

4. Cohort Studies Of Occupationally Exposed Women Refute the Hypothesis That PCB Exposure Is A Risk Factor for Breast Cancer

Given that the ATSDR PCB profile discusses the occupational epidemiology studies elsewhere, it is surprising that the authors of the text discussing breast cancer failed to summarize and discuss the results of these studies. Because these women had exposures to PCBs that are several orders of magnitude higher than any found in the environment, and as they attained body burdens that were 100-1,000 times higher than those found in the general population, PCBs could not possibly increase the risk of breast cancer via environmental exposures and not have consistently produced a significant increase in breast cancer among those exposed occupationally.

As the major occupational cohort studies were reviewed by Adami et al. (1995), they will not be discussed at length here. The Adami et al. (1995) analysis is of additional interest because these scientists contacted the author (i.e., Sinks) of one study that did not contain breast cancer data in its final published form and obtained such information in order to strengthen their analysis of the major cohort studies. The studies analyzed by Adami et al. (1995), and a description of each study contained in their combined analysis is provided in Table 2. As the results from Table 2 reveal, long-term, high-level occupational exposure to PCBs is not associated with breast cancer. In general, the studies have reported a deficit in breast cancer incidence.

Table 2: Summary Analysis of Occupational Studies of PCBs and Breast Cancer			
Study	**Study Description**	**Obs/Exp Ratio**	**95% Confidence Interval**
Brown, 1987	Retrospective cohort study; mortality of 1,318 women employed in capacitor manufacturing plants in NY and MA.	0.77	0.35-1.46
Sinks, 1994*	Retrospective cohort study; mortality of 846 women employed in capacitor manufacturing plants in IN.	0.51	0.06-1.85
Bertazzi et al., 1987	Retrospective cohort study; mortality of 1,556 women employed in capacitor manufacturing plants in Italy.	1.01	0.11-3.63
Nicholson et al., 1987	Retrospective cohort study; mortality of 521 women employed in capacitor manufacturing plants in NY.	1.33	0.43-3.10
Summary Analysis		**0.84**	**0.50-1.33**
Adapted from Adami et al. (1995) * personal communication			

The only large cohort study of persons working in PCB capacitor manufacturing plants that has been published since the Adami et al. (1995) analysis that provides information on breast cancer is the recent study by Kimbrough et al. (1999). Among the 2,544 hourly women workers and 469 salaried women workers in the cohort, the SMRs and 95 percent confidence intervals for breast cancer in these two groups were 0.82 (0.53-1.21) and 1.04 (0.38-2.26), respectively.

In summary, the strongest and most convincing epidemiologic evidence is provided by the occupational cohort studies that are consistently negative with respect to breast cancer. As such, these studies refute the hypothesis that the weak estrogenic activity of PCBs might somehow influence the incidence of breast cancer among women. When viewed together with the largely negative case-control or correlational studies involving environmentally exposed women, the epidemiological evidence provides little or no support for the hypothesized association between PCBs and breast cancer.

5. Important Mechanistic Data Were Ignored in the Discussion of the Breast Cancer Issue

The "xenoestrogen" hypothesis that suggests organochlorines may have significant estrogenic activity is not particularly relevant to PCBs. While some congeners and lower chlorinated mixtures may be weak estrogens at high doses, this hypothesis ignores the fact that many of the more persistent, coplanar PCB congeners in fact have anti-estrogenic activity. Furthermore, based on assays with hydroxylated PCB congeners identified in human serum, the metabolism of PCBs apparently decreases rather than increases estrogenic activity. Finally, and as indicated earlier by Safe in his 1995 dose-response analysis of all environmental estrogens,

the weak estrogenic or anti-estrogenic activity of PCB congeners is not likely to have any measurable or significant impact on circulating estrogen levels in the general population.

Since the hypothesized link between breast cancer and PCBs fully rests on the ability of some congeners to bind the estrogen receptor and exert estrogen-like activity, the profile should provide a limited discussion of the anti-estrogenic activity of some congeners and PCB mixtures to ensure a balanced presentation. In addition, the potential implications of such anti-estrogenic activity for the development of breast cancer via the "xenoestrogen" hypothesis should be discussed. This would appear all the more important given that the epidemiological data do not implicate PCBs or other organochlorines as risk factors for breast cancer. It would be appropriate for the discussion to be general in nature, as the relationships of PCB structure to estrogenic or anti-estrogenic activity are still being defined and activity of PCB congeners is variable and dependent upon the response (cell growth, reporter gene activity) and the cell system utilized. Thus, unqualified statements about a congener's or PCB mixture's estrogenic/anti-estrogenic activity are difficult to make with any degree of confidence. Nonetheless, the general concept that certain PCBs may exert anti-estrogenic activity and/or antagonize the estrogenic effects of other congeners is noteworthy, as is the fact that the net activity of some PCB mixtures (especially the more highly chlorinated mixtures which pose the greatest threat in terms of bioaccumulation) may be anti-estrogenic despite the mixture containing some weakly estrogenic congeners. The writers of the profile are encouraged to consult the following references on this issue: Krishnan and Safe, 1993; Bergman et al., 1994; Soto et al., 1995; Connor et al., 1997; Gierthy et al., 1997; Kramer et al., 1997; Moore et al., 1997; Safe and Zacharewski , 1997; Safe 1998; and Ramamoorthy et al., 1999.

The discussion of a possible association between PCB exposure and breast cancer in the ATSDR PCB profile is incomplete and misleading. Either ATSDR should rewrite this section to reflect the current state of knowledge regarding the hypothesis that PCB exposure is a risk factor for breast cancer, including the issues outlined above, or this discussion should be omitted entirely. If this section is to be rewritten, given the extent of the changes required, we propose that the PCB profile be reissued in draft form for additional comments.

B. Reproductive Effects

Numerous inaccuracies are present in the discussions on p. 122 of studies of potential reproductive effects in humans. In the discussion of the studies by Mendola and coworkers, the profile neglects to mention that the findings are based on an index of estimated PCB exposure, not on actual exposure measurements. In the description of Mendola et al. (1997), the PCB profile inaccurately reports the results of the study of menstrual cycle length. A statistically significant shortening of the cycle was associated with the highest PCB exposure indices (moderate and high) and with the consumption of more than one fish meal per month, but not with consuming contaminated fish for seven years.

Other inaccuracies are present in the discussion of native populations in the Arctic on p. 122. The profile cites Ayotte et al. (1995) as reporting levels of PCBs up to 150 µg/L in blood. The study does not contain that value. The value is reported in the next cited article, Wormworth (1995), which is a narrative piece describing the research by Dewailly and coworkers on

populations in the Arctic. This article is not a research report and contains no data, and is apparently also the source for the statement in the ATSDR profile that this level of PCB exposure can cause chloracne. Wormworth (1995) makes this statement without references, and it cannot be supported in the general literature. Therefore, ATSDR should correct the citation for PCB blood levels of 150 µg/L, reference the original data, and not rely on the secondary citation by Wormworth (1995). In addition, ATSDR should provide support for the statement that these high PCB blood levels are associated with cloroacne.

The sentence referring to Gerhard et al. (1998) states that blood concentrations of PCBs in women with repeated miscarriages were significantly increased compared to the general population. However, Gerhard et al. (1998) makes no such statement. PCB blood levels in the study group of women with repeated miscarriages were compared to a "reference level" of undefined origin instead of comparison with a reference range with a mean and variance. Twenty-two percent of the cases had PCB levels above the reference level, while the majority of cases did not (78%). Whether this percentage is statistically elevated is not discussed in the paper. The interpretation of these findings is greatly hindered by the failure of the authors to describe the origin of the reference level or provide statistics for the data in their Table 1.

We suggest that ATSDR re-examine the literature cited in the discussion of reproductive effects and rewrite this section to reflect the data actually reported in the cited studies. Statements about alleged associations, particularly those made regarding high PCB blood levels and chloracne, need to be fully supported and referenced. With the proposed changes in this and other sections, we feel that ATSDR should resubmit the PCB profile as a draft for a final comment period.

C. Endometriosis and PCB Exposure

The profile mentions a possible link between PCB exposure and endometriosis in only two places (pp. 122 and 123), and correctly notes that the animal and human evidence is negative. However, on p. 122, the profile makes a general, unreferenced statement regarding dioxins and "dioxin-like chemicals":

> "Endometriosis is known to occur following exposure to dioxin and some dioxin-like chemicals" (ATSDR, 1998, p. 122).

This statement mischaracterizes the data that exist regarding the relationship between exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or other structurally related compounds and induction of endometriosis. A comprehensive review on this topic in 1995 concluded that:

> "Associations between organochlorine exposure and endometrial cancer or endometriosis have even more limited empirical basis [than between such exposures and breast cancer]. The hypothesis that human exposure to environmental levels of organochlorines would favor an estrogenic overactivity leading to an increase in estrogen-dependent formation of mammary or endometrial tumors is not supported by the existing in vitro, animal and epidemiological evidence" (Ahlborg et al., 1995).

Since the publication of that review, the possible relationship between TCDD exposure and endometriosis (and endometrial cancer) has been examined in both animals and humans. The animal studies have produced conflicting results, with TCDD enhancing the development of experimentally-induced endometrial lesions in some studies (see, for instance, Johnson et al., 1997), and inhibiting development in others (Yang and Foster, 1997). One small case-control study of women with endometriosis found a greater percentage of cases than controls with detectable TCDD levels (18% versus 3%), but no relationship between TCDD level and severity.

The evidence with respect to "dioxin-like" compounds is generally negative. The term "dioxin-like" is not a precise term, but is often applied to other dioxin and furan compounds with 2,3,7,8- chlorine substitution and coplanar PCB compounds with lateral substitution. Assuming that definition, one study showed enhancement of experimentally-induced endometriosis in mice given 2,3,4,7,8-pentachlorodibenzofuran, but no activity of the coplanar compound PCB 126 (Johnson et al., 1997). A study of rhesus monkeys given Aroclor 1254 (which contains coplanar PCB compounds) for six years found no increase in incidence or severity of endometrial lesions compared to controls (Arnold et al., 1996).

The statements in the PCB profile should be edited to either delete reference to dioxins entirely, or, at a minimum, to reflect accurately the mixed nature of the data on the possible relationship between TCDD and endometriosis in animals, the lack of data on TCDD and endometriosis in humans, and to emphasize the unequivocally negative data in animals on PCB exposure and endometriosis. We suggest that given the magnitude of changes proposed, ATSDR reissue the PCB profile as a draft for final comments.

D. Neurological Effects in Humans

This section of the profile, beginning on p. 117, contains numerous misleading or inaccurate statements regarding the human evidence for potential effects of PCBs and should be completely revised. The problems are illustrated in the first paragraph of the section, each sentence of which is at best, uninformative, and at worst, completely inaccurate. The first sentence of this paragraph in the profile (p. 117) states that

> "[n]eurotoxic effects have been shown to occur in populations such as Native Americans and Eskimos that eat fish from waters contaminated with PCBs." (ATSDR 1998, p. 117)

However, no references are given and no data are provided to support this statement, either in this paragraph or in any subsequent paragraph. While studies of numerous fish-consuming populations have been conducted, the implication that they provide specific information regarding the effects of PCBs is misleading.

The second sentence of the PCB profile's first paragraph in Section 2.2.2.4 (Neurological Effects) states that

> "[t]hese effects were noted in an aging population of Great Lakes fish-eaters, and ortho-substituted PCBs were the strongest predictor of behavioral outcomes (Schantz et al., 1996a)." (ATSDR 1998, p. 117).

This is a misrepresentation of the cited study (Schantz et al., 1996a), which reports *no results* related to behavioral outcomes. The cited article describes the design and formulation of a cohort study of aging fish-eaters, the procedures used to evaluate and classify exposure, and the neurological testing planned for the cohort. In their final sentence in their paper, Schantz et al. (1996a) conclude that, based on animal data,

> "[w]e ***predict*** that the serum concentration of *ortho*-substituted PCBs will be the strongest predictor of behavioral outcomes in this population" [emphasis added] (Schantz et al., 1996a).

The first results from this cohort have just been published (Schantz et al., 1999). The authors note that the results from cognitive testing are going to be published later and report the results of motor function tests:

> "[t]hese findings suggest that PCB/DDE exposure from Great Lakes fish has ***not*** significantly impaired hand steadiness or visual-motor coordination in this sample of older adults" [emphasis added] (Schantz et al., 1999).

The first paragraph of the ATSDR PCB profile continues to misrepresent the published literature by stating that

> "***[s]imilar*** effects have also been reported for a population along the St. Lawrence River (Mergler et al., 1998). In this population, fish-eaters performed poorly on tests that required cognitive flexibility, word naming, auditory recall, and complex motor tasks. Unfortunately, fish contaminated with PCBs are also contaminated with heavy metals, pesticides, and other chemicals, making it difficult to ascertain whether the neurotoxic effects seen in these studies were due to PCBs" [emphasis added] (ATSDR, 1998, p. 117).

The effects are not "similar" to anything since a single study reporting effects has yet to be referenced in this paragraph of the ATSDR profile. The statements, while technically true, misrepresent this study. Mergler et al. (1998) specifically are concerned with effects of mercury, manganese, and lead in fisheaters. This is reflected in the study design in which blood levels of lead, manganese and mercury were determined, but *no measurements of PCB levels* were made. While the study provides no information regarding the potential effects of PCBs, it does illustrate the variety of confounders that must be controlled for in any study of fish-eating populations.

ATSDR then cites Seegal (1995) regarding the relative neuroactivity of various PCB congeners, and states that

> "[s]everal studies have focused on effects in the developing nervous system; however, effects are seen in adults as well, mainly changes in levels of biogenic amine neurotransmitters, e.g., dopamine" (ATSDR 1998, p. 117).

This statement is highly misleading and implies that these types of effects have been seen in humans, when in fact they have not. The Seegal statements refer to animal studies. The mixed and contradictory effects on brain neurotransmitters observed in animals are discussed elsewhere in these comments.

Finally, ATSDR concludes the paragraph with a statement based on the study of Humphrey et al. (1983), that "[a] 19% prevalence of numbness was reported among farm families who consumed dairy products and beef that were contaminated with PCBs." This isolated statement regarding a subjective complaint is uninformative when made, as it is, completely out of context. It would be preferable to know, for example, what other exposures and endpoints were examined, whether alcohol consumption was considered, the age range of the subject population, and what comparison population was used. In addition, consideration should be given to the fact that the general population rates for reported numbness or tingling vary widely, from about 5 percent to over 20 percent (Croft et al., 1993; Derogatis, 1993; Lipscomb et al., 1992). Without a more in-depth discussion of this finding, its significance, if any, is not apparent.

In the next paragraph, ATSDR briefly describes a study by Corrigan et al. (1998), in which brain tissue (caudate nucleus) from patients with Parkinson's disease had higher levels of PCBs 153 and 180, DDE, and dieldrin than that from controls. The profile should note that the study was extremely small, with samples from only eight Parkinson's patients and seven controls. In addition, the controls were only controls in the sense that they did not have Parkinson's disease and were in the same broad age group. The authors point out, for example, that they were unable to control for rural/urban living. This study should be considered a pilot study. In addition, the profile neglects to mention that two similar studies found no PCB residues in cortical brain tissue from Parkinson's and Alzheimer's patients and their nonneurological controls (Fleming et al., 1994; Corrigan et al., 1996).

ATSDR should redraft this section of the profile to correct the misleading and inaccurate statements regarding the evidence for neurological effects in humans from PCB exposure. Overall, the weight of evidence does not support human neurological effects from exposure to PCBs. We propose that the ATSDR PCB profile be reissued as a draft for final comments, given the magnitude of necessary corrections.

E. Neurobehavioral and Neurodevelopmental Effects of PCBs: Animal Data

The presentation of data on the neurological and neurodevelopmental effects of PCBs in animals is overly simplistic and suffers from selective citation of results, lack of organization, and a complete lack of analysis. The comments that follow illustrate some, but not all, of the

problems with the PCB profile's presentation of these data. These comments focus on Chapter 2, Health Effects, and specifically, on the discussions of neurological and developmental effects in the section on oral exposure.

1. Organization and Coverage

The data on neurobehavioral and neurodevelopmental effects of PCBs in animals cover a wide range of endpoints, including structural, neurophysiological, neurochemical, and neurobehavioral effects. Within the general category of neurobehavioral endpoints, literally dozens of individual endpoints have been studied, representing reflexes, learning and memory, spontaneous behavior, and other categories of effects. The data also address different species, routes and timing of exposure, including the issue of the relative importance of pre- and post-natal exposures. Finally, studies done to date have extensively examined hypotheses regarding mechanism of action and structure-activity relationships among PCB congeners. None of these areas are discussed in an organized way in the PCB profile, and many of them are ignored completely. The number of articles cited in the profile represents only a small fraction of the relevant literature.

One organizational problem with the profile is the lack of a clear rationale for the presentation of data on the neurological effects in animals dosed during the perinatal period. A few of the studies on the neurological effects in animals exposed perinatally are discussed in Section 2.2.2.4, Neurological Effects. Section 2.2.2.6, Developmental Effects, also discusses data from studies of perinatally-exposed animals, but includes very little of the relevant data discussed in Section 2.2.2.4; conversely, it also contains many similar studies which are not discussed in Section 2.2.2.4. There is no apparent logic for the selection of studies covered in each section.

There is no apparent organization of studies within either of these sections by endpoint, dosing regimen, species, or any other factor. Studies by researchers in the same groups on the same topics using similar protocols are separated by pages. For example, on page 121, the discussion of effects on brain neurochemistry focuses on the work of the Seegal group on dopamine decreases. However, Morse et al. (1996a; Seegal is a co-author), which also discusses brain neurotransmitter changes, is presented on page 118. More importantly, the finding by Morse et al. (1996a) that no effect on dopamine levels was observed is left in isolation. This observation should be presented in concert with the other relevant work on this topic, where the inconsistency is obvious and relevant. Other studies examining a similar endpoint are discussed elsewhere, or not at all.

2. Lack of Analysis

The influence of the structure of PCBs on their biologic effects is recognized elsewhere in the PCB profile. However, the sections discussing neurological and neurobehavioral effects of PCBs clearly do not reflect such an understanding. The discussions do not highlight the divergent effects and conflicting results that exist in the literature (decreases, increases, and no change in neurotransmitters; decrements and improvements in performance on learning and memory tests, etc.). In some cases the observed differences in results correlate with variations in

structure of PCB congeners. The lack of discussion of the relationship between structure and neurobehavioral or neurodevelopmental effects is a serious deficiency in the current draft PCB profile.

The lack of a sophisticated approach to the neurobehavioral and neurodevelopmental data is further illustrated by the absence of any discussion of the research on potential mechanisms of action underlying the observed changes in neurobehavioral performance. An analysis and discussion of any of the proposed mechanisms correlate with some, but not all observed neurobehavioral changes. The lack of an analysis of possible neurobehavioral mechanisms in the current draft PCB profile is a serious deficiency, since a lack of understanding of the underlying mechanisms for these changes affects our judgment of the consistency and reliability of the observations and our understanding of how they might relate to human experience.

ATSDR also failed to discuss any of the issues inherent in interspecies comparison of neurodevelopmental endpoints. Different species experience critical phases of neurological growth and development at different times. These growth and development differences affect the validity of extrapolation from one species to another.

ATSDR needs to include discussion and analysis of a number of issues related to the data on neurobehavioral and neurodevelopmental effects in animals. These issues include, but are not limited to, differences across animal species, potential mechanisms of actions, and possible structure-activity relationships. Because these issues were not addressed in sufficient detail, we recommend that the section be rewritten to include this analysis. Furthermore, the depth of analysis would suggest that a reissue of the draft following the necessary revisions is warranted.

3. Dose and Extrapolation Issues

The NOAEL/LOAEL table (Table 2.2) and the accompanying figures in the ATSDR PCB profile inappropriately group data from studies of commercial PCB mixtures with data from studies of individual PCB congeners. ATSDR fails to acknowledge the critical nature of structure-activity relationships in PCB effects by presenting the data for individual congeners through the identification of number of chlorines only (e.g., "hexachlorobiphenyls"), rather than with identification of the specific congener. Since the degree of both acute and chronic toxicity varies considerably among congeners, this presentation is almost useless in providing information that could assist in the assessment of exposures reasonably anticipated to be experienced by humans. ATSDR needs to re-assess the available data considering the specific consequences analyzed, possible structure-activity relationships, and which compounds are most relevant for human exposure.

4. Omission of Relevant Data

Substantial numbers of studies and results from cited studies were omitted in ATSDR's review. As discussed above, studies of neurochemical endpoints, including changes in dopamine levels, are sprinkled throughout Sections 2.2.2.4 and 2.2.2.6. Most of the cited studies by the Seegal group discuss an observation of decreased dopamine levels in treated animals (see pages 120 and 121). However, as discussed above, contrary data published by Morse et al. (1996a) are

discussed in a separate location in the PCB profile. Additional research on brain dopamine levels by Eriksson and Fredriksson (1996), which also showed no effect, was never mentioned in the review. Finally, Seegal's own work showing that, under some circumstances, administration of PCBs resulted in *increased*, rather than decreased, brain dopamine levels, is never cited (Seegal, 1996a; Chu et al., 1995).

ATSDR should rewrite and include the following relevant studies or results from numerous research groups:

- Studies investigating brain neurotransmitter levels and neurobehavioral effects by Dr. Lee Meserve's group (see, for example, Juarez de Ku et al., 1994 and Corey et al., 1996) are not cited. Juarez de Ku et al. (1994) is cited in the profile, but only in relationship to the findings on thyroid hormone levels and not with respect to brain neurotransmitter findings. A discussion of these studies with respect to neurotransmitter effects should be included in Section 2.2.2.4 of the profile.

- The extensive work on neurobehavioral changes in rats by Dr. Susan Schantz's group is represented by a single citation to one study; at least three other studies from this group are highly relevant (Schantz et al., 1995a, 1995b, 1996b). Significantly, in some of these studies, specific PCB congeners produced *improved* performance on learning and memory tasks.

- Tilson and Kodavanti have published extensively on the effect of PCBs on protein kinase C (PKC) and calcium transport in rat brain cells (see, for example, Kodavanti et al., 1993, 1994, 1995, 1996a, and 1996b; Kodavanti and Tilson, 1997). Only three of these studies are mentioned in the profile, they are not mentioned until the Relevance to Public Health Section of the profile (Section 2.5), which does not provide a complete picture of the group's results and conclusions.

- The early work of Tilson's group on rats with "spinning syndrome" from PCB exposure are not mentioned (see, for example, Tilson et al., 1979; Agrawal et al., 1981), nor is the related work on spinning syndrome by Chou et al. (1979) presented.

- Other miscellaneous relevant studies were omitted. For example, Bernhoft et al. (1994), which reported no effect of PCB treatment during gestation on the learning behavior of rats, was omitted.

In at least two cases, critical research and publications are omitted from a discussion with the result that the findings discussed seem more reliable and consistent than they are. One example of this is the brief citation in Section 2.5 (pp. 220-221) regarding the finding of Angus and Contreras (1994) that Aroclor 1254 decreases dopamine levels in rat pheochromocytoma cells (PC12 cells). However, relevant work by Seegal is not included. Seegal and coworkers have published extensively on the pattern of results for PCBs using this experimental model and have concluded that it is not a reliable *in vitro* model of the changes in dopamine observed *in vivo* (Seegal 1996a). It is very important in a summary section such as Section 2.5, Relevance to Public Health, to include the most reliable data on this topic when available.

Another example of omission occurs in the discussion of the findings of changes in muscarinic receptor density reported by Eriksson et al. (1991). ATSDR summarizes these findings in isolation in Section 2.5, Relevance to Public Health (p. 221), but neglects to add that these authors were unable to replicate these results in later experiments. Eriksson and coworkers have shifted their focus to other endpoints and mechanistic avenues for the observed neurobehavioral changes and no longer credit changes in muscarinic receptors with a significant role in toxicity (Eriksson and Fredriksson 1996a, 1996b). The presence of a superficial reference to this finding in the summary section "Relevance to Public Health" demonstrates a lack of analysis and understanding of the relative significance of various findings in this area.

ATSDR needs to revise any discussion of neurobehavioral and neurodevelopmental effects in animals to include all relevant data. We have identified a number of omissions above, which should be included in the profile. In addition, we recommend that specific studies not be cited in summary sections, as this gives unwarranted weight to these data. Given the extent of the changes proposed, we suggest the ATSDR PCB profile be resubmitted for public comment as a draft prior to finalization.

5. Issues in the Presentation of Data

Pantaleoni et al. (1988) is cited with different one-sentence descriptions on pages 131, 133, and 224. This comprehensive study examined the role of gestational versus lactational exposure on a variety of behavioral endpoints. However, the characterizations of the study in the profile are incomplete and, in two cases, misleading. On page 133, the profile description reads:

> "Neurobehavioral alterations...were observed in offspring of rats treated by gavage with 2 mg/kg/day Fenclor 42 on postnatal days 1-21..." (ATSDR, 1998, p. 133)

This description makes it sound as though rats treated by gavage on postnatal days 1-21 gave birth to offspring that later demonstrated deficits. In actuality, the dosing by gavage was to nursing dams and the changes were observed in the nursing offspring who were being exposed to PCBs solely through lactation, rather than gestationally.

On page 224 (in Section 2.5, Relevance to Public Health), the profile cites Pantaleoni et al. (1988) as providing evidence of "[n]eurodevelopmental deficits have also been reported in offspring of rats treated with relatively low doses of PCBs during gestation and lactation." However, the doses used in Pantaleoni et al. (1988), in any of the treatment groups, are not "relatively low." In most of the experiments performed in this study, a total dose of 40 mg/kg or more was administered over a few days. As comparison to Table 2.2 shows, this is not a particularly low dose. Why this study would be cited in particular in the summary "Relevance to Public Health" section is not clear and we recommend that it be removed, because reference to a specific study in the summary section gives it unreasonable significance.

An unpublished study, Freeman et al., is cited in two places (p. 118 and 122) in Section 2.2.2.4. No details were reported other than that the study is a one year study of rats given diets containing Aroclor 1016, 1242, 1254, or 1260, and that no signs of neurotoxicity were observed.

If an unpublished study is to be cited, details on the study's design, protocols, and results should be included in the PCB profile.

ATSDR refers to data in other sections of the profile, sometimes in misleading ways:

- ***Section 2.5: "Relevance to Public Health."*** In the conclusion to the sub-section on Neurological Effects within Section 2.5, the profile states:

  > "It is important to mention, however, that experimental evidence in animals indicates that exposure *in utero* and through suckling may lead to neurobehavioral deficits in the offspring. Studies regarding exposure *in utero* and through lactation in humans have provided inconclusive results." (ATSDR, 1998, p. 221)

  This statement is correct in noting that the human studies present conflicting and inconclusive results. However, the first cited sentence should be revised to reflect the more complicated picture presented by the animal data. Depending on the congener administered and experimental conditions, PCB exposure *in utero* has resulted in both deficits and improvements in neurobehavioral effects (Bowman et al., 1978, 1989, 1990; Schantz et al., 1989, 1995).

- ***Section 2.11.2: "Reducing Body Burden."*** This section contains many speculative statements. In particular, the last statement of the section, "PCB-exposed lactating females should be counseled about this possibility and may choose not to breast feed their infants" contradicts the recommendations of health agencies around the world. For example, Brouwer et al. (1998), state that the "current evidence does not warrant altering the previous WHO recommendation for promotion/support of breast feeding."

F. Developmental Effects

The PCB profile presents extensive discussions of various studies of developmental effects in humans; specific comments on these discussions follow in this section. However, the profile omits two series of studies that are relevant and should be reviewed. The studies of the Dutch cohort, published by Huisman, Koopman-Esseboom, and others over the past several years, are an important body of literature. While these studies suffer from problems with the underlying population selection and reliance on the TEQ method for exposure calculations, nonetheless they should be reviewed in the profile. Likewise, studies by Winneke and colleagues of a cohort in Germany should also be included.

Our general comments regarding the PCB profile's discussions on developmental effects are more concerned with the manner in which this subject is discussed than with serious technical insufficiencies. The major point that we wish to stress is that there are serious limitations associated with the Jacobson (Michigan fisheater) studies and ATSDR gives such cursory descriptions of these limitations in much of the text that a lay reader might easily misunderstand or underestimate their significance. For example, the initial discussion of the studies that begins on page 125 consists of more than three pages of descriptive text that is only occasionally interspersed with sentences that point out some of the flaws and problems

associated with the studies. In contrast, the discussion on page 260 that we believe is more appropriate, places a nearly equal emphasis on the studies' findings and flaws. By doing so, it conveys to the reader that such studies form an insufficient basis upon which to make conclusions regarding the developmental effects of PCBs and that definitive conclusions rest with future studies or studies in progress. Studies of questionable validity should neither be emphasized nor totally discounted in the profile, but care should be taken not to validate such studies by devoting an inordinate amount of text to their discussion.

As indicated by the extensive quotations presented herein, many objective scientific reviewers feel as though the studies implicating PCBs as neurodevelopmental toxicants frequently fail to control for important confounders and lack internal and external consistency. As such, and as alluded to on page 260 of the draft profile, the validity of the studies has been diminished to the point where the studies' findings should be viewed with skepticism, especially in light of the lack of confirmation provided by the study of the North Carolina cohort. In Appendix A, Section IV, we have provided detailed discussions of each Jacobson paper, the limitations of each study, and the comments about these papers that have been made by prominent scientific reviewers. This Appendix further underscores the reasons why the profile should not validate these studies by devoting an inordinate amount of text to their discussion. Such validation is presently unwarranted and awaits the results of ongoing and future studies of the hypotheses raised to date.

Although the current draft PCB profile does mention a number of the scientific limitations of the Jacobson studies, it does not convey that there is considerable doubt among many scientists as to the veracity of the findings (particularly on pages 125-129). Such doubt is due in large part to the fact that, as pointed out by Schantz (1996), discrepancies exist between the findings of the Jacobson studies and those of Rogan, Gladen, and colleagues. The following statements by the World Health Organization and a number of prominent scientists who have reviewed the literature clearly illustrate the inconclusive nature of the Jacobson studies (Paneth, 1991; WHO, 1993; Expert Report on Polychlorinated Biphenyls, 1994; Swanson et al., 1995; Schantz, 1996; Buck, 1996; Seegal, 1996b; Kimbrough, 1997; Middaugh and Egeland, 1997; and Borak and Israel, 1997). ATSDR needs to re-evaluate their reliance on and discussion of the Jacobson studies and provide a more detailed and balanced summary of the limitations. The extent of the criticism of these studies suggests that they are not considered reliable by the scientific community.

Table 3: Criticisms of Jacobson Studies	
Topic	**Quote and Source**
Methodology	"The results of this study are far from conclusive. There are three general areas of methodological concern: exposure assessment and its validity, selection of exposed and control samples, and comparability of the exposed and control samples." (Paneth, 1991)
	"A number of methodological concerns have been raised about the Jacobson study, including issues related to exposure assessment, sample selection, and control of potential confounding variables." (Schantz, 1996)
	"Unfortunately, the absence of a strong epidemiologic design in the Michigan study makes it difficult to interpret the results. Control of confounding, as discussed in the commentary, is not the only threat facing the study results. Potential sources of bias in this study include selection and information bias coupled with varying attrition rates over time in relation to status." (Buck, 1996)
	"Criticisms of the Jacobsons' studies are divided into three major areas: (1) the selection procedures used to identify exposed and control subjects; (2) the interpretation of analytical PCB data; and (3) the statistical comparisons of exposed and control subjects...." (Seegal, 1995b)
	"In the Michigan study reports, the methods were not well explained, and distribution data such as the range of PCB levels in the different biological samples of non-fisheaters and the fisheaters were rarely provided." (Kimbrough, 1997)
	"Given these methodologic issues, we think that this study provides little evidence that in utero exposure to low levels of PCBs affects intellectual function." (Middaugh and Egeland, 1997)
Sample selection	"Several methodologic flaws cast doubt on the validity of the Jacobsons' findings. They studied only 212 children (47 percent) of the 452 mothers originally invited to participate. This group was a highly selected subgroup of the initial study population of 8482 women. The authors did not adequately explain these inconsistencies in sample size and did not provide the numbers of exposed and unexposed children for whom results were available. For example, descriptive statistics were given for 178 children according to PCB concentrations in maternal milk in Figure 1, yet PCBs were measured in only 113 samples of maternal milk. No data were presented to support the value of PCB concentrations in breast milk as an adequate measure of prenatal exposure." (Middaugh and Egeland, 1997)
	"The study begins with a marvelous initial idea--that of surveying a large group of new mothers about their Great Lakes fish eating habits. In ends with the use of state-of-the-art psychological assessments, analyzed with sophisticated statistical tools. But in between, too many uncertainties cloud the picture. The central difficulty is the nature of the exposed and control populations: How comparable are they on characteristics other than fish-eating behavior? How certain are we that fish-eating history is a good measure of PCB exposure? Why should serum levels and ingestion histories give different results, and how is this to be interpreted?" (Paneth, 1991)
Data limitations	"...PCB levels were not available for a large portion of the study participants. The PCB levels in fisheaters and non-fisheaters was similar. Because of this the two groups of children were combined in some reports..." (Kimbrough, 1997)
	"...Since the levels in milk change over time and during a feeding period, these grab samples are not representative of PCB levels in milk and can not be used as surrogates for in utero PCB exposure. The authors failed to explain how they handled mothers who did not nurse or did not contribute milk samples." (Kimbrough, 1997)

Table 3: Criticisms of Jacobson Studies

Topic	Quote and Source
Statistics	"This study describes important biological effects of PCB ingestion on reproduction and on development. These include...poorer performance on a variety of neurobehavioral tests at birth, at seven months, and at four years. Yet if several measured variables differed substantially between the exposed and the unexposed mothers (four of them, ingestion of alcohol, caffeine, and cold medicines, and prepregnant weight, are diet or ingestion related), how many unmeasured differences between the groups might account for the findings?" (Paneth, 1991)
	"Jacobson et al. (1985) examined visual recognition memory in 7-month-old infants of women who had consumed contaminated Lake Michigan fish. The authors reported a statistically significant correlation between cord serum PCB levels and impairment of visual recognition memory. It should be mentioned, however, that interpretation of these test results is extremely difficult. In view of the variability associated with the measurements of fixation time (no standard deviations were reported), it is unclear whether any of the group means are statistically different. Moreover, the clinical meaning of the differences noted is not known." (WHO, 1993)
	"The statistical analyses were poorly explained, and it was difficult to determine what actually had been done. Fish consumption and PCB levels were not well correlated. ... Although many of the comparisons were statistically different, they often did not explain much of the variance nor were the statistical manipulations always appropriate." (Kimbrough, 1997)
	"Data on two important risk factors and potential confounders, alcohol ingestion and cigarette smoking appear to be inconsistent. Although 37 percent of the mothers smoked before and 28 percent during pregnancy, virtually none drank during pregnancy (shown in Table 1 of the article). In contrast, data from the Behavioral Risk Factor Surveillance Survey System suggested that a high proportion (14 to 21 percent) of women of childbearing age in Michigan consumed alcohol frequently. Furthermore, the method used to control for potential confounders may not be adequate. The authors stated that a variable's "association with either exposure or outcome can be used as the criterion for inclusion" in a model. However, standard epidemiologic analytic methods do not advocate the use of this method for model development." (Middaugh and Egeland, 1997)
	"The manner in which cohort data have been manipulated and transformed raises doubts. There are actually fewer exposure data than seem at first apparent, and most of the analyses depend on proxy measures or transformed data rather than direct measurements of PCB levels. Likewise, the methods used to estimate, manipulate, and transform the data are not adequately described." (Borak and Israel, 1997)
	"It is unclear whether the Jacobson study truly reflects the biological toxicity of PCBs. Instead, the reported findings might result from the manner in which the raw data have been manipulated and transformed." (Borak and Israel, 1997)
Confounding/bias	"In the studies by Schwartz et al. (1983), Fein et al. (1984), and Jacobson et al. (1984a), the influence of important variables, such as smoking and alcohol use, were not studied extensively enough. The Brazelton test was used in these studies. However, this test was never intended to be used to evaluate neurological conditions. The value of this test to predict behavioural abnormalities in infants is small. The Public Health Council of the Netherlands (1985) concluded, therefore that the reported changes could not be interpreted [sic] by the Brazelton test. The important confounding factors "smoking" and "alcohol" were not studied or not well studied, while it is known that these factors can result in such changes. Furthermore, there was an indication that women consuming fish also consumed more alcohol and coffee and used more medical drugs than those who were not fish eaters." (WHO, 1993)

Table 3: Criticisms of Jacobson Studies

Topic	Quote and Source
Confounding/bias (con't)	"Outcomes may reflect an inadvertent selection bias: The authors do not describe what information was provided to participating parents about the study findings or their child's performance, and it is not possible to determine the characteristics of the children who dropped out of the study. Thus, we cannot determine whether interactions between researchers and subjects may have influenced some subsets of children to withdraw from the study, thereby biasing results." (Borak and Israel, 1997)
Causal association	"Maternal contaminated fish consumption was also associated with several adverse behavioral outcomes on the NBAS (32). Infants of women who ate the most fish exhibited motor immaturity, poorer lability of states, a greater amount of startle, and more abnormally weak (hypoactive) reflexes. However, the other, more direct measure of exposure, umbilical cord serum PCB level, was not related to any adverse behavioral outcomes on the NBAS. This suggests that the adverse behavioral outcomes observed on the NBAS should be interpreted with caution. They may be related to other contaminants present in the fish or to some other aspect that differed between the fish-eaters and not-fish-eaters." (Schantz, 1996)
	"Drs. Jacobson and Jacobson (Sept. 12 issue) reported that low-level exposure to polychlorinated biphenyls (PCBs) in utero is associated with lower IQ scores (by an average of 6.2 points) among school-age children. The results seem implausible given the fact that Taiwanese children who were exposed prenatally to levels of PCBs that were 10 to 20 times higher and to levels of certain congeners of polychlorinated dibenzofurans that were 100,000 times higher than background levels, the IQ score was only 5 points lower than that in unexposed children." (Middaugh and Egeland, 1997)
	"Results from human epidemiologic studies (Fein et al., 1984; Jacobson et al., 1984b, 1985, 1990a,b; Rogan and Gladen, 1985; Rogan et al., 1986a,b, 1991; Flack, 1992) considered to be inclusive in the present review have been used as evidence to support the claim that present environmental exposures are producing irreversible toxicity in the human population. These claims, in our opinion, should not be considered as being supported by acceptable evidence obtained from humans." (Swanson et al., 1995)
	"Based on the above analysis, and considering the marginal significance of the observations, the information reported by Fein et al. (1984)...and Jacobson et al. (1990a,b) do not meet the criteria for the establishment of a causal association for an effect of PCBs on growth and behavior in human populations." (Expert Report on Polychlorinated Biphenyls, 1994)
Risk assessment	"...from a more basic risk assessment point of view, because of the lack of correlation of fish consumption with fetal cord PCB levels and the above-mentioned concerns related to the subjects selected, control of potential confounders, and PCB analytical procedures, these studies [Jacobson studies] fail to conclusively determine whether PCBs are responsible for the observed deficits. Indeed, if one considered comments by Paneth, Swanson et al., and those expressed by an expert panel, the current epidemiological data even fail to support the conclusions that fish-borne contaminants, rather than uncontrolled variables associated with a lifestyle based on consumption of fairly high levels of sport-caught fish are responsible for the association between fish consumption and physical and cognitive dysfunctions." (Seegal, 1996b)

In response to the results being reported in the "Jacobson studies," other scientific investigations have been initiated to examine whether a link exists between PCB exposure and altered neurodevelopment. To date, no conclusive confirmation of the reported results in the "Jacobson studies" has been provided. The following comments reflect the view of many in the scientific community regarding the lack of evidence for PCB-induced neurobehavioral deficits (Expert Report on Polychlorinated Biphenyls, 1994; Buck, 1996; Kimbrough, 1997).

Table 4: Reviewers Comments on Level of Evidence for PCB-Induced Neurobehavioral Effects in Humans	
Topic	**Quote and Source**
Sample selection	"All of the studies have methodological problems. All but the Yu-Cheng children in Taiwan were not directly selected from the general population, but from hospitals, physicians' offices and by word of mouth. ... The PCB body burdens in all studies except that of the Yu-Cheng children were similar to those of the general population. Furthermore, in many of the different samples (maternal blood, cord blood and milk) obtained from study participants in the Netherlands, Michigan and North Carolina, the PCBs were below the limit of detection. ..." (Kimbrough, 1997)
Test validity	"Some tests used to evaluate the neurobehavior of infants and children were modified to accommodate specific needs or to facilitate statistical analyses. However, neither the original nor the modified tests were validated on a representative group of the pediatric population. ..." (Kimbrough, 1997)
PCB sampling	"Furthermore, variability of quantification of low concentrations of PCBs in human serum, whole blood, plasma and milk is a fundamental problems in the studies in Michigan, North Carolina and the Netherlands. Variability occurs in serum and in milk because the lipid content varies and because of other variations in the analytical methods. Thus, small differences in PCB concentrations in serum or milk are meaningless. In none of the studies was the lipid content of serum considered when PCB levels were evaluated." (Kimbrough, 1997)
Exposure assessment, confounding	"In all studies, PCB exposure was poorly defined. The variations noted in the neurobehavioral tests were not convincingly linked to PCBs. In the studies conducted in Michigan, North Carolina, and the Netherlands the exposures of the study populations are consistent with the exposures of the general populations. Quantification of low levels of PCBs are not very precise. Genetic make-up, other environmental factors and the home environment will affect neurobehavior in infants and children. These major confounders or covariates cannot be successfully separated from any postulated PCB effects. Because of these limitations studies like the ones reviewed here should not and cannot be done, and do not provide any useful information." (Kimbrough, 1997)
	"The studies of female populations exposed to PCBs through the general environment evaluated reproductive effects, ranging from abortions, premature deliveries, reduced birth weights, decreased head circumference, and possible neurological effects; however, none of the studies adequately addresses the confounding effects of simultaneous exposures to other chemicals or various social/lifestyle factors, such as prescription and nonprescription drug use, or socioeconomic status. Consequently, conclusions of causal relationships between these effects and exposure to PCBs per se are tenuous at present." (Expert Report on Polychlorinated Biphenyls, 1994)
Causal association	"Current data remain inconclusive at best" (Buck, 1996)
	"Numerous papers have been published postulating associations between consumption of Lake Michigan fish and growth and neurobehavioral effects in infants and young children, or between these effects and in utero exposure to polychlorinated biphenyls (PCBs). Studies were also conducted on three other groups of children in North Carolina, the Netherlands, and Taiwan. ..." (Kimbrough, 1997)

ATSDR needs to include a discussion of the overall conclusion of the level of evidence for PCB-induced neurobehavioral effects in humans. We have summarized some of the literature that could be included in this analysis. The general consensus appears to be that there are insufficient data to attribute neurobehavioral effects observed in humans to their PCB exposure. With the addition of the proposed discussion and revisions to other sections of the

profile, it is suggested that the ATSDR PCB profile be reissued as a draft for a second round of comments.

G. ATSDR's Minimal Risk Level

As described in detail in Appendix A of the PCB profile, and to a lesser extent on pages 204-205 and in a footnote to Table 2.2, ATSDR derived a chronic-duration oral exposure MRL of 0.02 µg/kg-day for PCBs (Aroclor 1254). This MRL is based on a LOAEL for decreased antibody response in rhesus monkeys of 5 µg/kg-day which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) and rounded upward. It is our opinion that this MRL is inappropriately derived from an overly sensitive animal model and is thus a lower dose than is warranted to protect human health. Indeed, the PCB profile itself recognizes the fact that significant species differences in sensitivity to PCBs exist (see the bottom of page 202 and the beginning of page 203). The draft profile even places the *"...MRL in context..."* by stating that it *"...is approximately 3 orders of magnitude below the low-end estimated dose for occupationally exposed individuals"* who were without any evidence of impaired health (see the bottom of page 204 and the beginning of page 205). Therefore, in the following paragraphs, we evaluate the scientific merit of ATSDR's MRL approach and discuss alternative means of MRL derivation that we believe are more reflective of the existing scientific data.

1. Evaluation of the Monkey as a Basis for Aroclor 1254 MRL Development

The purpose of developing a chronic-duration MRL is to identify an allowable dose that is likely to be without appreciable risk of adverse noncancer health effects over a duration of exposure greater than 365 days. This MRL must be protective and must consider potentially sensitive individuals within the population. In view of this, there is an understandable bias toward conservatism in setting a MRL to "err on the side of safety." At the same time, it is important that the MRL not be overly conservative. If the MRL is set too far below actual human response levels, risk estimates using this MRL become exaggerated leading to unwarranted fear and concern, distorted priorities in terms of contributors to public health risks, and wasted and/or misallocated resources. Therefore, the goal in MRL development should be more than simply selecting the lowest dose that can be found in the most sensitive species available. It should instead represent a reasoned judgment of the likely dose-response relationship for the chemical in humans.

There have been several studies of potential health effects of humans exposed to PCBs. Three types of study populations have been examined: 1) individuals exposed occupationally to PCBs; 2) individuals with environmental exposure to PCBs; and 3) individuals mistakenly ingesting rice oil contaminated with PCBs and other compounds. The ability of the last group to provide insight into PCB toxicity is obscured by the fact that the rice oil was also contaminated with the more potently toxic polychlorinated dibenzofurans (PCDFs). In fact, several lines of evidence indicate that the symptoms exhibited by individuals with rice oil poisoning ("Yusho," in the case of the poisoning incident in Japan in 1968, and "Yu-Cheng," for the 1979 incident in Taiwan) were due to the PCDFs rather than PCBs (Masuda and Yoshimura, 1984; Masuda et al., 1985; James et al., 1993). Studies of individuals exposed environmentally to PCBs are

potentially useful, but are often compromised by low or equivocal PCB exposures, as well as concurrent exposure to other environmental contaminants. In contrast, some worker populations (e.g., workers in capacitor and transformer manufacturing facilities) had substantial job-related exposures to PCBs lasting up to 20 years or more. Studies of the health of these workers were often detailed and included physician examinations. Although levels of PCB exposure of these workers were not measured precisely, they can be approximated based on historical industrial hygiene surveys. This permits an assessment of the PCB effects likely to be observed in humans within the range of plausible human exposure, and the dosages (or body burdens) at which these effects are likely to appear.

The ability of the current MRL for Aroclor 1254 to reflect dose-toxicity relationships in humans can be assessed by comparing the responsiveness of the experimental model upon which it is based, the monkey, with humans. The monkey clearly shares a great many anatomical and physiological similarities with humans, but this does not necessarily mean that it is similarly responsive to a particular chemical. When available, empirical comparisons of potency provide an important test of the validity of the model. The following sections compare PCB doses observed to produce specific effects in the monkey versus clinical observations in humans for the same effect. This section begins with the effect upon which the MRL for Aroclor 1254 is based, immune system effects, but also provides a general assessment of the monkey model by including comparative responsiveness to other effects as well.

a) Immune system effects

The MRL for Aroclor 1254 is based on immune effects observed in rhesus monkeys. Tryphonas et al. (1989, 1991a, 1991b) fed female rhesus monkeys diets containing 5, 20, 40, or 80 μg/kg-day, and found diminished IgG and IgM antibody responses to sheep red blood cell (SRBC) antigen. A statistically significant effect on IgM occurred at the lowest dose, 5 μg/kg-day, while an effect on IgG was seen at doses of 40 μg/kg-day or higher. Based on these observations, a dose of 5 μg/kg-day was considered to be the LOAEL for immune effects in monkeys.

There are no studies in humans which have evaluated potential immune effects of PCBs in the same way as the Tryphonas monkey studies, but there is abundant information regarding the functional immune status of PCB exposed individuals. In one study, responsiveness to immune challenge with mumps and trichophyton antigens was compared between PCB-exposed workers and non-exposed controls (Emmett et al., 1988a, 1988b). These antigen challenge tests are instructive because, like the SRBC test used in the monkey studies, interaction of the three principal cells of the immune system (macrophages, T-lymphocytes, and B-lymphocytes) is required. No significant effects on responsiveness were noted, despite the fact that the capacitor workers had PCB serum levels much greater than those in the monkeys in the Tryphonas studies.

If PCBs in concentrations of 10 ppb in serum result in loss of immune competence, as suggested by the monkey studies, individuals with substantially higher serum PCB concentrations should be at demonstrably greater risk of morbidity and mortality from infectious disease. This clearly is not the case. Taylor et al. (1988) conducted a mortality study of over 6,000 capacitor workers, including a group of workers in direct exposure areas with a geometric

mean serum PCB concentration of 302 ppb. No increase in mortality from infectious disease was observed in these workers. Although Kimbrough et al. (1999) calculated SMRs for 92 underlying causes of death, only selected causes of death were reported in their Table 4. Unfortunately, infectious disease was not among them. Levels of circulating immunocytes were often included among the battery of clinical tests in studies of the health of PCB-exposed workers. Among these studies (Fischbein et al., 1979; Baker et al., 1980; Maroni et al., 1981a, 1981b; Chase et al., 1982; Smith et al., 1982; Stark et al., 1986), none found an association between PCB exposure and leukocyte or differential blood counts.

A distinction must be made between statistical changes in one or more immune system parameters and clinically relevant compromise of the immune system. The immune system is complex and redundant. Perturbations in one or a number of immune parameters (such as circulating immunoglobulin levels) do not necessarily indicate immune impairment, and the results of studies showing apparent alterations that are limited in size or number must be interpreted with caution. From the Tryphonas studies, it appears that signs suggesting significant immune abnormalities may have occurred at 80 µg/kg-day (or approximately 100 ppb PCBs in serum), but it is unclear at what PCB dosage frank immune dysfunction might occur in these monkeys. Studies in humans indicate that PCB blood concentrations in this range, and much higher, have no demonstrable adverse effect on immune function. Under the circumstances, it is difficult to argue that a MRL based on a 5 µg/kg-day LOAEL in monkeys is needed to protect humans against immunotoxic effects of PCBs when such effects cannot be demonstrated in humans, even among individuals with blood concentrations orders of magnitude higher.

b) Oculodermal effects

In the study by Tryphonas et al. (1991a, 1991b), oculodermal effects were observed in female rhesus monkeys ingesting 5, 20, 40, or 80 µg/kg-day of Aroclor 1254 in the diet. Signs and symptoms included ocular exudate, inflamed and prominent tarsal (meibomian) glands, and distorted or discolored nail beds. PCB serum concentrations at steady-state, achieved after about 10 months of treatment, were (from the lowest to the highest dosage group) 10.4, 32.1, 68.1, and 105.1 ppb. Oculodermal symptoms were evident at the lowest dosage, and this was reported to be the most sensitive clinical effect associated with PCB ingestion in these animals (Tryphonas et al., 1991a). In another study, rhesus monkeys fed Aroclor 1254 in the diet in concentrations producing dosing rates of 100 and 200 µg/kg-day experienced acne-form lesions, hair loss, and erythema and swelling of the eyelids (Barsotti et al., 1976). Using the relationship between ingested dose and PCB blood levels observed in the studies of Arnold et al. (1990) and Tryphonas et al. (1986), the serum PCB levels in these monkeys can be projected to be about 150 ppb at the time symptoms appeared, after one to two months of treatment.

From these studies, it appears that oculodermal toxicity from Aroclor 1254 in rhesus monkeys is associated with serum PCB concentrations of 10 ppb. At the same time the monkey studies were being conducted, several reports appeared in the literature describing the background serum PCB concentrations that existed in the general population (See Table 5).

Table 5: Serum PCB Concentrations in U.S. Populations without Occupational Exposure to PCBs

Study	No. Subjects	Year	PCB Level (ng/ml or ppb)		
			Arithmetic mean	Geometric mean	Range
Sahl et al., 1985a, 1985b	738	1982-84	5	4	<1-37
Welty, 1983	59 (OH subjects) 40 (WV subjects)	1983	5.8 6.7	4.4 5.0	1-45 1-23
Condon, 1983	990	1983	4.9	4.2	2-30
Reid and Fox, 1982	138	1981	3.6	—	<3-43
Humphrey, 1983	418	1980	—	6.6	<3-60
Drotman et al., 1981	17	1979	7.5	5.8	2-30
Chase et al., 1982	19	1979	12	—	10-27
Vernon et al., 1981	7	1979	4.9	4.2	2-11
Kreiss et al., 1982	1,631	1978-79	7.7	6.4	<1-57
Baker et al., 1980	110	1977	18.8	—	6-79
Hovinga et al., 1993	95	1989	—	6.8	2-42.1
Humphrey, 1983	29	1973	17.3	15	<5-41
Source: ATSDR, 1995					

The results of these studies showed average blood or serum PCB concentrations around 10 ppb, with some individuals having concentrations as high as 60-80 ppb. With almost everyone in the U.S. having serum PCB levels similar to those producing discolored and disfigured nails and eye swelling and discharge in monkeys, these effects of PCBs would be difficult to miss if humans were as sensitive as monkeys. Studies of individuals exposed environmentally to PCBs reported no evidence of oculodermatologic abnormalities (Stark et al., 1986; Stehr-Green, 1986a, 1986b), and studies of PCB-exposed workers have found dermal symptoms (namely, chloracne) only in individuals with much higher serum PCB levels. One study (Baker et al., 1980) examined workers with serum PCB levels ranging from 17 to 75 ppb and found no dermal effects. Another study carefully evaluated transformer workers with serum PCB concentrations as high as 300 ppb (Emmett et al., 1988). They describe their observations as follows:

> "A detailed physical examination was performed. A thorough skin examination was performed by the same dermatologist under the same conditions. For each body region we systematically recorded: the presence and degree or absence of erythema, scaling, hyperpigmentation and elastosis; and the number of closed comedones, open comedones, inflammatory papules, pustules, cysts and milial cysts. In addition, for the facial region we recorded the presence of hypertrichosis, atrophic scarring, xanthelasma, scleral abnormalities, conjunctival abnormalities including hyperemia, meibomian gland secretion, palpable meibomian glands, lid margin or palpebral changes, superficial corneal

> pigmentation, oil precorneal film and acrus senilis ... The presence of one or more comedones at any body site was higher in the exposed group. There was no significant difference for the number and types of facial lesions including those of chloracne or for other physical examination variables. Although we systematically searched for mucocutaneous symptoms and signs of Yusho including dark brown nail pigmentation, follicular hyperkeratosis, increased palm sweating, and hyperpigmentation of the skin and mucous membranes, we did not find them." (Emmett et al., 1988)

Chloracne, a dermal effect similar to some of the symptoms displayed in PCB-treated monkeys, has been observed in PCB-exposed workers, but serum PCB levels of at least 150 to 200 ppb are required (Gaffey, 1983), and some workers have had serum PCB levels of 1,000 ppb or more without dermal symptoms (Ouw et al., 1976; Maroni et al., 1981a, 1981b). Thus, while the monkeys are arguably valid qualitative predictors of the dermal effects of PCB exposure, they appear to be at least 15-20 times more sensitive than humans.

c) Other endpoints

In addition to comparisons of dose-response relationships for immune and oculodermal effects, the general appropriateness of the monkey as a model for PCB toxicity in humans can be evaluated through examination of other toxicological endpoints. Available information regarding these endpoints is summarized below.

Gastrointestinal effects. Severe hypertrophic, hyperplastic gastritis has been observed in monkeys treated with 2.5 ppm in the diet (Allen, 1975), and a dosage approximately 2-fold higher (200 µg/kg-day) produced erosion, ulceration, collagen necrosis in the oral cavity, and hypertrophic/mucinous gastropathy in the stomach of female rhesus monkeys (Tryphonas et al., 1986). Among the many clinical studies of PCB-exposed workers with equivalent or higher PCB exposures, no gastrointestinal abnormalities have been reported.

Serum cholesterol. Arnold et al. (1993a, 1993b) found significantly diminished serum cholesterol levels among rhesus monkeys receiving 40 or 80 µg/kg-day Aroclor 1254. At least five studies have examined serum cholesterol and other lipids in PCB-exposed workers and compared them with controls (Baker et al., 1980; Chase et al., 1982; Smith et al., 1982; Emmett, 1985; Emmett et al., 1988). None found a significant increase or decrease in serum cholesterol among PCB-exposed workers.

Reproductive effects. Arnold et al. (1995) conducted breeding experiments with male and female monkeys treated with 0, 5, 20, 40, or 80 µg/kg-day Aroclor 1254. After 37 months of exposure, females were bred with an untreated male. During the study, two of the monkeys in the high dose group had to be euthanized because they developed a severe wasting syndrome associated with the PCB exposure. Other monkeys (including some controls) also had to be euthanized for reasons unrelated to PCB treatment. The reproductive outcomes are summarized in Table 6. As this table demonstrates, PCB exposure appears to result in increased adverse reproductive outcomes, including decreased numbers of live births, increased suspected resorptions, and

perhaps increased risk of post-partum death. Evidence of these effects appeared at the lowest PCB dose, 5 µg/kg-day.

Table 6: Reproductive Outcomes Among Female Rhesus Monkeys Administered Aroclor 1254 for Approximately 3 Years Prior to Mating

Dose	Number of...						Total Incidence Rate
	Pregnancies (Available)	Live Births	Post-Partum Deaths	Abortions	Suspected Resorptions	Stillbirths	
0	11(16)	9	0	1	0	1	0.182
5	10(16)	5	1	1	2	2	0.500
20	4(15)	1	1	3	0	0	0.750
40	6(14)	4	1	1	1	0	0.333
80	5(15)	1	1	1	3	0	0.800

Source: Arnold et al., 1995.

As noted above, the serum PCB levels associated with this dose are only marginally higher than the average background PCB levels in the general population, and are within the range of serum PCB levels observed in individuals without occupational PCB exposure. These kinds of severe reproductive effects would be difficult to miss in humans with comparable or greater serum PCB levels. However, among women exposed occupationally to PCBs the only effect that has been observed is a slight decrease in infant birth weight (Taylor et al., 1989). In studies of women with lesser PCB exposure, no consistent effect on infant birth weight has been observed, with some studies finding slightly lighter babies among women with greater exposure, some finding no effect, and still others finding heavier babies associated with increased PCB exposure (Longnecker et al., 1997). Also, studies of birth outcome have found no increased risk of spontaneous abortion or stillbirth attributable to PCB exposure (Longnecker et al., 1997). These comparisons indicate that monkeys are more sensitive to the reproductive effects of PCBs than humans.

Lethality. The most convincing data for demonstrating that monkeys are particularly sensitive to PCBs is lethality. In Barsotti et al. (1976), one of nine monkeys treated with either 100 or 200 µg/kg-day died from toxicity during the course of the study. In another study, Tryphonas reported,

> "The results suggest that severe potentially fatal PCB toxicity can develop in rhesus monkeys following ingestion of Aroclor 1254 at 200 µg/kg/day for a period of 27 months or longer." (Tryphonas et al., 1986)

In a later study, three monkeys had to be euthanized due to severe PCB toxicity (Tryphonas et al., 1991a, 1991b). While the authors did not make clear what PCB dosage these animals received, the highest treatment group was 80 µg/kg-day, and the blood PCB concentrations in these animals after 55 months of treatment was 285 ppb (mistakenly reported in the paper as being in ppm). In contrast, studies of PCB-exposed workers find no evidence of increased mortality, even among groups of workers with average serum PCB concentrations of 400 ppb or more, and individuals with serum PCB levels as high as 3,250 ppb (Lawton et al., 1985; James et al., 1993).

d) Conclusions regarding comparative toxicity of PCBs between monkeys and humans

In view of the anatomical and physiological similarities between humans and monkeys, there may be a basis to predict that monkeys could serve as qualitative indicators of PCB toxicity in humans. There is ample empirical evidence, however, that monkeys are much more sensitive to PCB toxicity than humans, and therefore, are not a good animal model from a quantitative perspective. Monkeys treated chronically with relatively low doses of PCBs exhibit a variety of adverse health effects, including changes in immune system parameters, oculodermal effects, gastrointestinal lesions, reproductive impairment, hematological abnormalities, and death. None of these effects, except chloracne, have been observed in the numerous clinical studies of PCB-exposed workers despite vastly higher PCB serum concentrations. Available data suggest that monkeys are more responsive to PCBs than humans and respond at doses that are at least an order of magnitude lower. Therefore, we consider the monkey an inappropriate species for deriving an ATSDR MRL, and suggest that data from a more appropriate species be sought as the basis for MRL derivation.

2. Derivation Based on Studies of Another Animal Species

Table 7 summarizes health effects observed from sub-chronic and chronic administration of Aroclor 1254 in rats, mice, rabbits, and guinea pigs (studies in mink suggest that this species is as (or more) sensitive to PCBs than monkeys and would therefore also be an inappropriate model). For the purposes of identifying a LOAEL or NOAEL for Aroclor 1254, only effects observed at the lower end of the dose range (namely, 5 mg/kg-day or less) have been tabulated, with only a few exceptions. From the information presented in Table 7, it appears that the most sensitive toxic endpoint is thyroid function. The existing data do not support identification of a NOAEL for this effect, but indicate a LOAEL of about 0.10 mg/kg-day. The appropriate uncertainty factors for derivation of a MRL from the LOAEL of 100 µg/kg-day for thyroid toxicity include the following factors: 10 for sensitive individuals; 3 for interspecies extrapolation; and 3 for use of a LOAEL instead of a NOAEL. Larger values for the latter two of these three uncertainty factors were not considered necessary since occupational exposures of 0.070-0.140 mg/kg-day (Kimbrough, 1995) did not result in thyroid insufficiency or any other endocrine dysfunction (James et al., 1993). With a total uncertainty factor of 90 (rounded to 100), the MRL for Aroclor 1254 would be 1.0 µg/kg-day.

Table 7: Summary of Effects Observed at the Lowest Dosages of Aroclor 1254*			
LOAEL mg/kg-day	**NOAEL mg/kg-day**	**Effect**	**Reference**
0.09	—	Decreased serum T3 and T4 levels in Sprague-Dawley rats treated for 5 months.	Byrne et al., 1987
0.10	—	Decreased serum thyroxine in Fisher 344 rats treated for 15 weeks	Gray et al., 1993
0.10	—	Increased femur density in Fischer 344 rats treated for 10 weeks	Andrews, 1989
0.125	—	Transient effect on auditory startle in offspring of rats exposed from mating through weaning	Overmann et al., 1987
0.18	—	Marked thymic atrophy in New Zealand rabbits treated for 8 weeks	Street and Sharma, 1975
0.25	0.05	Decreased serum corticosterone, DHEA, and DHS in Sprague-Dawley rats treated for 5 months.	Byrne et al., 1988
0.25	—	Altered thyroid ultrastructure and increased serum T3 in Osborne Mendel rats treated for 4 weeks	Collins and Capen, 1980a
0.25	—	Altered thyroid follicular structure in Holtzman rats treated for 5 weeks	Kasza et al., 1978
1.0	—	Altered auditory threshold for high-intensity stimuli in offspring of rats dosed from gestation day 6 through postnatal day 21	Herr et al., 1996
1.0	0.1	Increased liver weight, hypertrophy, and vacuolar degeneration in Fisher 344 rats treated for 15 weeks	Gray et al., 1993
1.0	0.1	Cortical tubular protein casts in Fisher 344 rats treated for 15 weeks	Gray et al., 1993
1.0	—	Transient reduction in total and free serum thyroxine in offspring of rats treated on gestation days 6-21.	Goldey et al., 1995
1.0	0.5	Increased liver weight and serum cholesterol in Fisher 344 rats treated for 4 days.	Carter, 1985
1.25	—	Increased liver weight and triglyceride concentrations in Sprague-Dawley rats treated for 35 days	Bruckner et al., 1977
1.25	—	Decreased body weight gain in Fisher 344 rats treated for 104-105 weeks.	NCI, 1978
1.3	0.13	Decreased motor coordination of pups from Wistar rats treated for 42 days	Overman et al., 1987
1.5	0.39	Lipid accumulation in hepatocytes in Sherman rats treated for 186 days	Linder et al., 1974
1.5	0.32	Decreased litter size in Sherman rats treated for 129 or 186 days pre-mating	Linder et al., 1974
2.5	—	Vacuolated thyroid follicular cells and decreased T4 in Osborne Mendel rats treated for 4 weeks	Collins et al., 1977
2.5	—	Decreased thyroid function in pups from Osborne Mendel rats treated for 42 days	Collins and Capen, 1980c
2.5	1.25	Alopecia, facial edema, and exophthalmia in Fisher 344 rats treated for 104-105 weeks	NCI, 1978
2.5	—	Decreased survival in Fisher 344 rats treated for 104-105 weeks	NCI, 1978
3.13	—	Serum thyroxine and brain choline acetyltransferase activities depressed in offspring of rats exposed during pregnancy and lactation	Juarez de Ku et al., 1994

Table 7: Summary of Effects Observed at the Lowest Dosages of Aroclor 1254*			
LOAEL mg/kg-day	**NOAEL mg/kg-day**	**Effect**	**Reference**
4.0	1.0	Altered auditory threshold for low intensity stimuli in offspring of rats dosed from gestational day 6 through postnatal day 21.	Herr et al., 1996
4.0	1.0	Transient reduction in motor activity and auditory deficits at a low frequency (i.e., 1 kHz) in offspring of rats gavaged from gestation day 6 through postnatal day 21	Goldey et al., 1995
4.88	0.49	Increased liver weight and necrosis in BALB/c mice treated for 6 months	Koller, 1977
5.0	—	Increased liver weight and porphyria in Sherman rats treated for 2 months	Goldstein et al., 1974
5.0	2.5	Decreased fetal weight for Sprague-Dawley rats treated for 10 days during gestation	Spencer, 1982
5.0	—	Altered brain levels of glial fibrillary acidic protein and synaptophysin in offspring dosed on gestation days 10-16	Morse et al., 1996b
5.0	—	Reduced the concentration of dopamine and its metabolites in various brain regions of adult rats treated for 30 days	Seegal et al., 1991
8.0	4.0	Altered auditory startle response in offspring of rats gavaged from gestation day 6 through postnatal day 21	Goldey et al., 1995
10.0	—	Mild hypothyroid effects and lowered hippocampal cholinergic transporter levels in offspring of rats dosed from gestation day 6 through postnatal day 21	Zahalka et al., 1995 (abstract)
15.0	7.5	Effect on flavor aversion of adult rats exposed 5 days per week for 4 to 6 weeks	Nishida et al., 1997
500	250	Transient depression of spontaneous motor activity in mice given a single oral dose	Rosin and Martin, 1981.
* Excluding studies in Mink and Monkey; Information taken from ATSDR, 1995 and selected articles			

3. Relevance of Aroclor 1254 to Environmental Exposure

The derivation of an MRL for PCBs on the basis of a study of Aroclor 1254 raises one additional issue. The toxicity of PCBs varies substantially from one congener to another. The mixture of congeners present in Aroclor 1254 is not the same as the mixture of congeners encountered by people in the environment due to differences among congeners in the rate of degradation, environmental transport properties, and bioaccumulation. Since environmental exposures are to a different mixture than the mixture used as the basis for the MRL, interpretation of actual exposures in light of the MRL may be difficult. This issue should be discussed in the profile.

4. Conclusions Regarding the Aroclor 1254 MRL

There are several issues we have raised for ATSDR to consider regarding the derivation of the MRL for PCBs based on Aroclor 1254. We suggest that ATSDR reexamine the available data and either rederive an MRL or provide additional support for the current value. It is proposed that the PCB profile be reissued as a draft to provide the public with the opportunity to comment on the revisions.

III. SPECIFIC LINE-BY-LINE COMMENTS

The following are line-by-line comments regarding specific issues with the PCB profile, including editorial comments.

1. **Page 2, lines 5 and 8:** The "12" designation does not indicate that the molecule contains 12 carbon atoms; it is coincidental. Aroclor terphenyl products were designated by a 5400 series number, but they only contained 18 carbon atoms per molecule. Further, Aroclor PCB production ended in August 1977, not October. The last shipments to customers were in October.

2. **Page 4, top:** Somewhere early in the text of this document, the authors should establish the equivalence of ppm = mg/kg and ppb = µg/kg. The document switches back and forth and for the lay reader this change in units is not easy to follow. Also, in line 16, there is an unnecessary semicolon after "widely."

3. **Page 4, 2nd paragraph:** While this paragraph attempts to discuss environmental exposures in general terms, it is written so simplistically as to be misleading. While the first sentence discusses possible routes of exposure (i.e., waste sites, fish, well-water), the implication of this sentence is that exposure = dose which is simply not true. Studies by Stehr-Green, Needham, and other scientists, even after examining heavily contaminated areas such as Paoli, PA, or New Bedford, MA, have shown that elevated PCB levels in the environment, particularly those in soil, do not translate into a measurable dose for those living near these sites. Furthermore, PCBs are not volatile and have very limited water solubility; so, once again these exposure routes will typically have no measurable impact on one's body burden of PCBs. There simply is no documented evidence that any person's body burden has been elevated above normal background levels by breathing air or consuming contaminated well water near hazardous waste sites. Because this paragraph is both speculative and alarmist, it should be removed from the document or rewritten to indicate the limited amount of PCBs one might absorb from most environmental sources.

4. **Page 5, 7th line from bottom:** There is no documented evidence that breathing indoor air in buildings containing PCB-containing parts contributes measurably to one's body burden. Here and on page 6 (Section 1.5, last sentence of 1st paragraph) ATSDR uses the term "electrical parts" although we believe they meant "electrical equipment." A more important problem with this statement is that it suggests that electrical equipment can be expected to contain PCBs, which is not true, and so misinforms and perhaps alarms the public concerning the typical use or failure of electrical equipment in buildings. PCBs have not been used in the manufacture of electrical equipment for more than two decades and many older capacitors and transformers have been removed in the interim.

5. **Page 6, 6th line of Section1.5:** Delete "and" after the word *exposure*. In the 9th line, delete the word "usually."

6. **Page 7, top, lines 4-6:** The implication that PCBs may play a role in causing breast cancer is not supported by the literature. This misperception by the authors of the draft profile is not surprising since they failed to review most of the literature on this subject. Because this sentence is alarmist, speculative, and inaccurate, it should be deleted or alternatively, restated to reflect that the literature does not support such an association (see our general comments on this profile). This statement or something similar is repeated throughout the document and should be addressed in all locations.

7. **Page 8, 3rd paragraph:** This entire paragraph speculates as to possible harmful effects that have yet to be confirmed. As such, it is misleading and may easily be misinterpreted by the general reader. The first sentence implies that occupational exposure decreases birth weight even though the small change reported was not a statistically significant one. Statistics are used in scientific studies to prevent one from concluding that observations are real when in fact they may be due to chance. Because no statistically significant change was recorded, it is inappropriate to imply that it may be real when no evidence exists.

   The remaining sentences apparently refer to the Jacobson studies. These flawed studies have been criticized by the scientific community (see comments above). Therefore, the mention of these studies is not warranted in an executive summary portion of the document like Section 1.5. See our general comment section and Appendix A for additional comments that support our concern for the manner in which this subject has been addressed.

8. **Page 10, lines 7-8:** What basis is there to speculate that PCBs can be carried home from the fiber, semiconductor, or sawmill industries? We are aware of no literature that can be cited to support such a statement. PCB-containing electrical equipment is handled according to specific regulations and it is highly unlikely that anyone working at a facility where PCB-containing electrical equipment is used would ever "carry home" PCBs.

   We note that the last few sentences of this paragraph are accurate – i.e., if it is possible for employees to have contact with hazardous substances at their place of work they may be so informed by their safety officer. The information would seemingly eliminate any need for speculation with regard to whether PCBs may be transported into the home environment.

9. **Page 10, lines 4-6 from the bottom:** In two successive sentences the ATSDR document contradicts itself. The first sentence reads – "*Although these tests can indicate whether you have been exposed to PCBs to a greater extent than the general population, they do not predict whether you will develop harmful effects.*" This statement is true and factual. The next sentence then goes on to state that these tests "*...can suggest that you have an increased risk of developing harmful effects compared with the general population.*" This statement is contradicted by the first sentence and is misleading to the point of being false. The term "*risk*" as used here is no doubt intended to mean some low-level, theoretical, mathematical probability associated with the animal cancer data on these compounds. But the general public will typically view a health risk as something that, regardless of its true probability of being manifest, is relatively likely to happen. For this reason, the second sentence should be removed. Instead, readers should be told to consult a health expert on the subject so that their blood levels may be placed into proper perspective. We note that other than chloracne

(found only in persons with very high PCB blood levels), no other adverse effect has been causally attributed to PCBs (see ATSDR PCB Profile references James et al., 1993; Kimbrough, 1995; and other epidemiology reviews on this subject). Given this, how could a person with minor elevations in serum PCB levels be at an "increased risk?"

10. **Page 12, top:** The statement as written implies that the 3 ppm tolerance level applies to all the listed foodstuffs, while in actuality there are different levels for different kinds of foods. The different FDA levels are also missing from Table 7-1 of the PCB profile that lists the regulations and guidelines applicable to PCBs.

11. **Page 12, 3rd paragraph:** What is the source of the warning about acute (30 minute) high level ($\geq$ 10 mg/m^3) exposure to PCBs? Whatever the source, it is inaccurate. Early studies of air levels in capacitor manufacturing plants found that levels of up to 10 mg/m^3 of air, while irritating to the eyes and mucous membranes, were well tolerated otherwise. Such concentrations frequently occurred in plants in the first several decades of PCB use but were not immediately dangerous to life or health (e.g., See the exposure data in Fischbein et al., 1979. The upper end of exposure was to 11 mg/m^3 of air and yet no identifiable disease, let alone effects that endangered life and health, were observed in this study.). In short, several of the epidemiology studies cited in the ATSDR PCB profile rebut this assertion.

12. **Page 15, 3rd paragraph:** PCBs are no longer produced in Japan or Germany; the discussions of that production should be in the past tense.

13. **Page 15, 4th paragraph, 2nd line:** Delete "may." What else could the toxicity depend on?

14. **Page 16, line 10:** Should read 2,3,7,8-TC<u>DD</u>.

15. **Page 16, line 12:** Insert semicolon after "citizens."

16. **Page 16, line 14:** "Between" should read "among."

17. **Page 16, line 22:** "to PCB" should read "of PCB."

18. **Page 16, 5th line from bottom:** To a large extent this sentence is confusing *toxicity* with *potency*. However, in some cases the toxicities of lower chlorinated PCB mixtures (e.g., Aroclor 1221) will differ from higher chlorinated mixtures (e.g., Aroclor 1260). On the other hand, in many instances there are no differences in toxicity (the type of adverse effects produced), there are only differences in potency (the dose required to produce it).

19. **Page 17, first sentence of 1st paragraph:** Insert "heat-degraded" before *Kanechlors*.

20. **Page 21, 2.2.1.1:** Why are Brown (1987) and Brown and Jones (1981) the only cohort epidemiology studies specifically mentioned here? At a minimum, Sinks et al. (1992) should be included, as well as the new Kimbrough et al. (1999) study of GE workers. Gustavsson and Hogstedt (1997) could also be discussed. Frankly, since acute lethality is the focus of this section (see the next paragraph and the animal data), the first paragraph of this section

should simply state that no acute lethality in humans has ever been caused by exposure to PCBs. They are not acutely toxic (with the possible exception of irritation) at any human exposure level that would reasonably be anticipated today.

21. **Page 25, 1st paragraph**: Too much emphasis is placed on symptom reporting that was collected in cross-sectional studies. Capacitor manufacturing plants used large amounts of volatile degreasing solvents (e.g., TCE or TCA), thus exposures to these solvents may have caused or significantly contributed to pulmonary symptom complaints (see discussions by Emmett et al., 1988; James et al., 1993).

22. **Page 25, 2nd paragraph:** Any suggestion that PCBs adversely affect pulmonary function based on Emmett (1988b) should be dropped. Since this effect is eliminated by controlling for smoking, this uncontrolled observation can not be stated to have been associated with PCBs. Similarly, the Warshaw data is not internally consistent with itself and is probably an error as discussed in Lawton et al. (1986). ATSDR should not discuss findings which are removed when better controls for confounding are included, or which are later shown to be incorrect by subsequent studies. It becomes too confusing and possibly misleads the reader. This document should attempt to summarize what is known about the human health effects of PCBs and eliminate the many speculations and tangential information that is currently provided in this document.

23. **Page 26, 1st paragraph:** This paragraph should either be deleted or a complete analysis of all mortality studies should be provided. Overall, there is no evidence to suggest that PCBs might increase cardiovascular mortality. Furthermore, the current discussion is flawed. For example, the last sentences suggest that groups have elevated risks, but the ATSDR PCB profile lists SMRs of 0.17 and 0.33, both of which indicate a decrease not an increase in the incidence of a disease.

    Here and elsewhere in this document the PCB profile restates the findings of a single study reporting an elevation. The single positive observation, flawed or not, is reported in isolation while ignoring similar and frequently stronger studies that report no such association. This produces an unbalanced discussion that is not in keeping with the goal of an ATSDR toxicological profile.

24. **Page 26, GI Effects:** This document discusses unsubstantiated symptom reporting which is provided without statistical comparisons to a control group.

25. **Page 27, hematological effects:** Why report the isolated PMN finding of Lawton et al. (1985), which the authors themselves did not believe and which has not been observed in another study? For many well-known reasons, spurious or chance observations are frequently reported in epidemiology studies. The goal should be to analyze all of available data so as to eliminate isolated, spurious findings from further consideration by focusing on what is consistently found and confirmed in other studies.

26. **Page 28, hepatic effects:** ATSDR does not indicate that any of the changes in serum liver enzymes levels were statistically significant. As they were not, it should be so stated.

27. **Page 28, 10th line from bottom:** Observed serum enzyme level increases were "too small." Too small for what? We believe that what was meant was "too small to implicate liver injury," but this omission affects the overall conclusion one might be expected to reach from this paragraph.

28. **Page 30, lines 3-5:** The evidence for hepatotoxicity is not weak- it is nonexistent. Anyone familiar with the nature of these sometimes sensitive tests, some of which can be elevated by diseases, drinking alcohol, etc. would disagree with the current ATSDR characterization. If occupational exposure was in fact hepatotoxic, then the numerous clinical studies that have been published would have had no trouble documenting it.

    The fact that the draft profile makes note of liver enzyme induction, an effect known to have occurred in capacitor workers and one that may account for elevated serum enzymes, is further evidence that the PCB profile's suggestion of "weak evidence of hepatotoxicity" is unfounded. According to the draft profile, liver enzyme induction could be solely responsible for the "nonsignificant" serum enzyme elevations that the profile states were reported.

29. **Page 31, top:** Temperatures of 55-138°C are far too low to cause any formation of PCDFs, let alone significant formation. Morita et al. (1978) showed that a temperature of 300°C was required for a week with excess oxygen to cause measurable formation, and short-time formation is maximal at about 650°C.

30. **Page 34, Section 2.2.1.4:** The symptoms discussed are not necessarily or solely caused by an effect on the CNS. In any event, without statistical comparisons to a control group, the reporting of such symptoms is meaningless. Any population of about 300 individuals would likely contain persons reporting these same symptoms. Furthermore, the cross-sectional studies that these symptoms are taken from are well known to provide unreliable indications of the true disease incidence. Given the preceding, we believe that the listing of symptoms without control data impresses upon the lay reader that systemic effects might be occurring, even though a trained toxicologist might not reach the same conclusion. Where symptoms are reported out of context, they should be placed in context or removed. The way things currently stand, there is no clear indication as to whether any organ effects were seen and whether such was the case should not be left to the readers' imagination. For example, these symptoms are taken from studies that found no clinical evidence of toxicity, but this important information was omitted from each section discussing symptoms.

31. **Page 35, genotoxic effects:** Why are effects that are only seen in those studies confounded by exposure to benzene, formaldehyde, or to combustion products from a fire reported in this document?

32. **Page 36, lines 12 and 17:** With regard to the rectal cancer, it is impossible for the number of expected deaths to be the same in the original study and in the update 7 years later! [Note the increase in the expected number for the liver cancers.] To state they are the same is to state no more person-years of exposure or latency were accumulated in the update, which itself

makes no sense. The longer the follow-up, the greater the years of follow-up and the larger the expected number of deaths in all categories.

33. **Page 36, bottom and page 37, top:** Tironi et al. (1996) have updated the Bertazzi et al. (1987) study.

34. **Page 38, 3rd paragraph, last line:** This is an overstatement of the conclusions of the Loomis study. The only hint of an association was the teasing out of the melanoma results based on the internal dose-response analysis. There was no excess in the "unteased" results.

35. **Page 38, last paragraph:** The Bahn study is not a complete cohort study but is preliminary data reported in a letter to the editor. This distinction should be made as it is listed with other, larger cohort studies. As it stands, its placement in the draft profile implies that it is published cohort study.

    Similarly, on the next page, Davidorf and Knupp (1979) is summarized as though it contains information relevant to assessing occupational PCB exposure. It does not.

36. **Page 40, top, line12:** The NIOSH (1990) study is cited as if it were a separate study from Sinks et al. (1992), but in fact it is merely the preliminary report of the same study and cohort.

37. **page 40:** The recent cohort mortality study by Kimbrough et al. (1999) should now be added to this discussion.

38. **Page 41, 4th line from bottom:** The risk estimate presented by Finley et al. (1997) is a vast overestimate of the true risk. Not only did they rely on unvalidated toxicity equivalency factors to estimate the concentrations of Ah receptor active PCBs, they used a cancer potency factor (CPF) for dioxin, which the same authors argue in a separate publication is a vast overestimate.

39. **Page 42, 3rd paragraph:** This study by Whysner is not a lethality study. Why is it summarized here? It should be moved to the genotoxicity and mechanism sections.

40. **Page 44:** The NOAEL/LOAEL table (Table 2.2 in the PCB profile) and the accompanying figures group data from studies of commercial PCB mixtures with data from studies of individual PCB congeners and fail to acknowledge the critical nature of structure-activity relationships in PCB effects (see detailed comments above).

41. **Page 97, 1st paragraph:** The Stehr-Green studies are of persons living near a waste site. As such, the inhalation route of exposure is the most probable for those not directly visiting the site. Why is this paper put in with the oral exposure studies?
42. **Page 100, hepatic effects:** The correlations between serum ALT and serum lipid levels is spurious as it is caused by the partitioning of lipophilic PCBs into higher blood levels of lipids. This is indicated earlier in the document. The correlation described here is not an effect on the liver and should be deleted!

43. **Page 101, 7 lines from bottom:** This text should be removed. Induction may also increase the elimination of mutagens and carcinogens. In animal studies, the induction of liver enzymes by PCBs prior to exposure to a carcinogen has consistently decreased the carcinogenic effects of the procarcinogen.

44. **Page 106, endocrine effects:** The Mendola study is not one of effects that can be attributed to PCBs, as a number of persistent contaminants bioaccumulate in fish.

45. **Page 107, 2nd paragraph:** The draft profile suggests that PCBs may induce breast cancer and begins by citing 4 articles. The last paper, that of Welp et al. (1998) states in the abstract the following: "[e]xposure to chlorinated hydrocarbon pesticides, chlorinated solvents, and polychlorinated biphenyls may be risk factors, although the evidence is insufficient." The first article cited is the review by Ahlborg et al. (1995), which stated: "[t]he hypothesis that human exposures to environmental levels of organochlorines would favor an estrogenic overactivity leading to an increase in estrogen-dependent formation of mammary or endometrial tumors is not supported by the existing *in vitro*, animal and epidemiological evidence." Why weren't these cautionary and contradictory conclusions also indicated in the draft profile's text? The writers of the profile must differentiate between speculative hypotheses and fact or hard evidence.

The last sentence of this paragraph states that the epidemiology studies "suggest" PCBs may play a role in the induction of breast cancer, and then cites three papers by proponents of this theory. Why wasn't the far more thorough epidemiology analysis that was performed by Adami et al. (1995) also considered here? The Adami et al. (1995) study analyzed all available epidemiology studies and actually reached the opposite conclusion stated in the draft PCB profile.

The next two sentences discuss fisheater studies as though fisheating was a reliable, unconfounded exposure surrogate for PCBs. Since it is not, fisheater studies should be deleted. The fact that the fisheater studies found nonsignificant elevations and decreases, depending upon what coast one lived on, reflects the biases and confounding associated with studies of this type. Here and elsewhere the reported findings of "fisheater" studies should be deleted unless ATSDR can clearly demonstrate that these are valid studies of PCB-induced effects and not of a compilation of environmental factors, for which the actual etiologic agent responsible for the observed change is not known.

See our general comments for numerous ways in which additional information can be added to the present analysis that is provided in this paragraph. As it is currently written this paragraph is incomplete, biased, and inaccurate. The conclusions or suggestions made here are simply not supported by the scientific literature.

46. **Page 114, Section 2.2.2.3:** Once again a fisheater study with unknown relevance to PCBs is reviewed in the profile as though it were a study specifically of PCB effects. In fact, the authors of Svensson et al. (1994) note in their discussion that the consumption of fatty acids from fish at the levels expected in the high-consuming fish eater is, in itself, associated with

decreases in NK cell levels. The results of this study can not be attributed to PCBs and it should be removed from the profile.

47. **Page 117, top:** As stated in our general comments, the monkey is not a reliable species from which an MRL may be derived because it is more sensitive to PCBs than man.

48. **Page 117:** This section of the profile contains numerous misleading or inaccurate statements regarding the human evidence for potential effects of PCBs and should be completely revised. Relevant studies or results from numerous research groups are not covered. Some studies of fish eating populations that provide NO data on PCB exposure are cited. Additionally, there is a lack of a clear rationale for the presentation of data on the neurological effects in animals dosed during the perinatal period (see detailed comments above).

49. **Page 118, Jacobson paragraph:** The Jacobson studies are seriously flawed and can not be relied upon. This issue was clearly noted in the previous ATSDR PCB profile and should be reiterated here. Strangely, this ATSDR PCB profile concludes the paragraph on Jacobson by citing scientists who have criticized the Jacobson studies, but the draft profile fails to mention these criticisms. The statements by the World Health Organization and a number of scientists who have reviewed the available literature clearly illustrate the inconclusive nature of the Jacobson studies (Paneth, 1991; WHO, 1993; Expert Report on Polychlorinated Biphenyls, 1994; Schantz, 1996; Buck, 1996; Seegal, 1996b; Kimbrough, 1997; Middaugh and Egeland, 1997). The draft profile should be rewritten to note and explain the serious flaws in the Jacobson studies rather that discussing them as though the whole of the scientific community accepted their findings without reservation (see our comments above and Appendix A).

50. **Page 118:** Studies by researchers in the same groups on the same topics using similar protocols are separated by pages. Brain neurotransmitter changes are discussed on this page and then later on page 121 (see detailed comments above).

51. **Page 118 and Page 122:** An unpublished study, Freeman et al., is cited and no details of the report are provided (see detailed comments above).

52. **Page 122, Section 2.2.2.5:** The second paragraph of this section discusses fisheater studies as though the results of such studies can be directly attributable to PCBs. A number of chemicals are known to bioaccumulate in fish including heavy metals and organochlorine pesticides. Because fish consumption is confounded by multiple chemical exposures, the results of fisheater studies can not be fully attributed to PCBs. If the discussions of fisheater studies are kept in the ATSDR profile, they should include such information for the sake of the lay reader. For example, this caveat is stated clearly on page 138 at the end of the first paragraph of the cancer section, "[i]*n all of these studies, it is difficult to account for the presence of other toxic compounds invariably present.*"

53. **Page 122:** Numerous inaccuracies are present in the discussions of studies of potential effects in humans (see detailed comments above).

54. **Page 125:** The profile omits two series of studies that are relevant and should be reviewed. The studies of the Dutch cohort, published by Huisman, Koopman-Esseboom, and others over the past several years, are an important body of literature. The German studies by Winneke and others should also be reviewed and included (see detailed comments above).

55. **Pages 125-128, Section 2.2.2.6:** While the Jacobson studies are discussed here with some mention of the problems associated with their experimental design, the studies themselves receive considerable discussion while the limitations of these studies tend to receive only brief mention. Given the considerable uncertainty that surrounds these studies, perhaps their collective results should be summarized in a paragraph that is followed by a paragraph devoted to their numerous limitations. This would be a more accurate and fair portrayal of these studies.

56. **Pages 131, 133, and 224:** Pantaleoni et al. (1988) is cited with different one-sentence descriptions on each page. The characterizations of the study in the profile are incomplete and, in two cases, misleading (see detailed comments above).

57. **Page 136, Section 2.2.2.8:** We believe the second paragraph discussing Swedish fisheater studies should be deleted. Its inclusion here implies these results are relevant to understanding the possible carcinogenicity of PCBs in humans, but this assertion cannot be justified scientifically. By far the best measures of the potential carcinogenic potential of PCBs in humans are the large, major cohort studies. The findings in all other studies are of such limited value (and inherently variable) in a causation assessment that their discussion only creates confusion and heightens the lay reader's concern beyond that which is justified.

58. **Page 137:** The study by Oakley et al. (1996) establishes a possible mechanism for DNA damage via oxidative stress under unusual circumstances, especially the high concentration of cupric chloride. However, it is not clear what relevance these conditions have for human exposure to PCBs.

59. **Page 137, 14 lines from the bottom:** The sentence that begins, "[*t*]*he NOAEL for genotoxic effects in mice has been suggested that PCBs may induce breast cancer...,*" clearly has some kind of editorial error that needs to be corrected. The sentences that follow appear to be a repeat of an earlier section discussing the xenoestrogen-breast cancer issue. Thus, the last half of the page should be removed from the genotoxicity section.

60. **Page 138, last paragraph:** This paragraph is the second repeat in three pages of information that is already provided on page 107. This redundancy should be removed from the document. The negative animal data provided on page 140 contradicts the proposed hypothesis, and given the negative animal and human data on this issue, one wonders why the ATSDR PCB profile has given it so much attention.

61. **Pages 138-139:** The ATSDR PCB profile discusses the issue of environmental PCB exposure as a possible risk factor for breast cancer in women. This discussion occurs in a

single, brief paragraph that is woefully incomplete, biased, and inaccurate (see detailed comments above).

62. **Pages 138-139:** In hypothesizing that organochlorine compounds, such as PCBs, might have enough estrogenic activity to increase a women's risk for breast cancer, the proponents of this hypothesis failed to consider the relative magnitude of estrogenic and antiestrogenic exposures from various sources (see detailed comments above).

63. **Page 139, 2nd paragraph:** This discussion of a correlational study by Hardell is misleading. To state that such data are suggestive of an immunosuppressive effect of PCBs that is related to NHL is an affront to scientific interpretation and causation methodology. Nothing in this study provides information that would indicate the levels found are immunosuppressive. More importantly, if this were true then the high-dose PCB cohort studies, studies of individuals with PCB tissue levels that are 100-times or more greater than those in Hardell, would have surely identified PCBs as a risk factor for NHL. This discussion is yet another example of the draft profile not critically evaluating the conclusions of some papers against an often plethora of contradictory data that exist in other studies. This provides an inconsistent picture for the lay reader, and in this case, may raise concerns that simply are not justified.

    The text should be edited to report what Hardell found, higher levels of PCBs in NHL patients. Care should be taken not to overinterpret the data. We note that at the end of this paragraph the draft profile states that "*[t]hese studies do not show that PCBs caused NHL.*" Again, the inconsistency of the interpretations being conveyed will confuse the lay reader.

64. **Page 139, 3rd paragraph:** This small paragraph on Yusho/Yu-Cheng should be removed. The inapplicability of these incidents because of the PCDF exposures has already been discussed. The gratuitous inclusion and then dismissal of these incidents is unnecessary here.

65. **Page 145, 6 lines from top:** This sentence suggests that younger animals have been shown to be more sensitive to the carcinogenic effects of PCBs. We are aware of no such studies. If no scientific justification can be demonstrated, this should be deleted or at least made clear that is an assumption for which no supportive data exist.

66. **Page 151, Section 2.2.3.4:** The novelty of the reported findings should be discussed here. The reported findings are completely at odds with the remaining epidemiology studies and animal studies have not identified any peripheral neuropathy.

67. **Page 195, last two sentences of 2nd paragraph:** There are a number of difficulties with the TEF approach including the fact that Ah-receptor antagonism is found among PCB congeners (see ATSDR comments in last paragraph of page 247). This should be added to the discussion of the problems associated with this approach.

68. **Page 197, 11 lines from the bottom:** This sentence states that most non-neural toxic and biochemical effects occur by the Ah-receptor mechanism. Even if the paper cited reached this conclusion, there is insufficient scientific evidence to make such a claim. For example,

both coplanar (Ah-receptor dependent) and non-coplanar (Ah-receptor independent) PCB congeners cause hepatotoxicity (see Biocca et al., 1981 which is cited in the draft profile). The effects of the ryanodine receptor, and the possibility that this pathway contributes to non-coplanar congener toxicities, is just being identified and explored. In addition, cytochrome P-450 induction of the phenobarbital-like enzymes clearly uses a receptor mechanism separate from that of the Ah-receptor. Thus, there are ample reasons why this simplistic statement is wrong. Because the Ah-receptor was the first receptor mechanism identified it has received most of the press to date. This does not mean that it is the major mechanism. The fact that PCB mixtures frequently do not behave like dioxin, and in fact can antagonize the effects of dioxin, is sufficient evidence that this conclusion is an erroneous one.

69. **Page 204-205:** The MRL which is based on a LOEL for monkeys was divided by an uncertainty factor of 300. The MRL appears to be derived from an overly sensitive animal model, which ATSDR states that "is approximately 3 orders of magnitude below the low-end estimated dose for occupationally exposed individuals" (see detailed comments above).

70. **Page 205, 6 lines from the top:** This statement lends more credence to the Jacobson studies than is presently warranted. Since the Jacobson studies were flawed in their initial development, the fact that Dr. Jacobson continues to follow his cohort and report findings does not represent "increasing evidence." We suggest this unsubstantiated statement be deleted.

71. **Page 217, Immune Section:** The first sentence suggests that studies of fire-exposed individuals and fisheaters are reliable PCB exposure studies that are unconfounded by exposures to other chemicals, when in fact they are not. Because these studies are so confounded, they do not provide possible exceptions to the better controlled capacitor worker studies of Emmett et al. (1988a, 1988b). The references to these two studies should be removed from this sentence.

72. **Page 220, 2nd paragraph, 5 lines from top:** The reference here to the Jacobson studies is once again overstated. The caveats and limitations indicated elsewhere in the profile should be reiterated here.

73. **Pages 220-221:** Critical research and publications are omitted from a discussion with the result that the findings discussed seem more reliable and consistent than they are (see detailed comments above).

74. **Page 224, 1st paragraph, 7 lines up from bottom:** Elsewhere in the document the Jensen (1987) article is cited as evidence that the levels in Jacobson were underestimated and may have in fact been higher than found in the general population. Given the problems associated with the Jacobson findings and the analytical techniques these researchers used, this sentence is at best premature and should be deleted.

75. **Page 225, 2nd paragraph, last two sentences:** DNA adducts are not a clastogenic response as these sentences suggest.

76. **Page 233, 2nd and 3rd paragraphs:** The Mendola and Gerhard studies do not provide the kind of evidence that one would use to determine if a chemical causes endocrine effects. These studies are mentioned elsewhere so their inclusion here is not justified. We recommend that these paragraphs be deleted, otherwise the lay reader is left with the impression that more credence is attached to these studies than they warrant.

77. **Pages 233-234, last paragraph of 233 through first two paragraphs of 234:** Once again the xenoestrogen/breast cancer theory is discussed. As there is now considerable information that contradicts this hypothesis, its discussion should occur only once in the final document. These paragraphs should be deleted here because it does not adequately or accurately address the topic, i.e., endocrine modulation.

78. **Page 236-237:** This long paragraph on the Jacobson studies is unnecessary. While it at least lists some of the limitations of these studies, the results and limitations of these studies are discussed elsewhere (e.g., see ATSDR statement in last paragraph of page 245). We suggest a simpler summary of this area and mention of additional studies that are underway in this important area. Once the results of these studies are available, we might be able to determine if children are more susceptible. At present, however, even the Jacobson studies suggest that childhood exposures are not critical. Perhaps the ATSDR profile should separate childhood susceptibility from *in utero* susceptibility in order to clarify the actual issue that has been raised with PCBs by the flawed Jacobson studies.

79. **Page 252, last sentence of section:** This statement contradicts the recommendations of health agencies around the world (see detailed comments above).

80. **Page 260:** Many objective scientific reviews disagree with Jacobson, believing that the studies that implicate PCBs as neurodevelopmental toxicants fail to control for confounders and lack internal and external consistency.

IV. APPENDIX A: An In-depth Discussion of Those Studies Evaluating the Neurological and Neurodevelopmental Effects in Children Exposed to PCBs *In utero*

Because the ATSDR PCB profile repeatedly attempts to rely upon these studies, and in particular upon the series of studies referred to as the "Jacobson" or "Michigan" cohort, a more detailed discussion of the limitations of these studies that were briefly referred to above in SectionVI is appropriate.

A. The Jacobson (Michigan Fisheater) Cohort

The Jacobson cohort consists of individuals selected after interviews with 8,482 women who delivered babies in four hospitals near Lake Michigan during the years 1980 to 1981. The initial interview process was performed with the intention of determining how much and what kinds of Great Lakes fish they had consumed during the period leading up to conception and delivery of their children. Following various interview and screening procedures, the final participants, who initiated the series of studies in this cohort, consisted of a total of 313 mothers and their children; 242 considered "PCB exposed" and 71 considered unexposed. In a series of cross sectional studies the physical status at birth and neuromuscular development from birth through childhood were subsequently evaluated in these children and reported on by Jacobson and coworkers.

1. Jacobson et al. (1983)/Schwartz et al. (1983)

The establishment of the Jacobson cohort began with reports by Jacobson et al. (1983) and Schwartz et al. (1983). These investigators reported the measurements of exposure used to compare 242 infants born to mothers who consumed moderate amounts of Lake Michigan fish to mothers who did not. *In utero* PCB exposure was estimated based on two criteria: 1) reported consumption of Lake Michigan fish; and 2) PCB levels in umbilical cord serum. These two criteria were used to establish the PCB-exposed and non-exposed groups for later investigations into purported PCB-induced effects in newborns, infants, and young children.

In the Jacobson et al. (1983) report, the authors state that the less chlorinated PCB congeners were quantitated against an Aroclor 1016 standard and the heavier congeners against an Aroclor 1260 standard. The detection limit for the Aroclor 1016 standard was 5 ppb and the detection limit of the Aroclor 1260 standard was 3 ppb. These relatively high detection limits (relative to the low ppb PCB levels common to environmentally exposed individuals) resulted in most of the serum samples yielding nondetectable or nonquantifiable serum PCB levels. Thus, it becomes quite disturbing to find these investigators (i.e., Jacobson an coworkers) providing dose-response relationships in their later studies that include serum PCB concentrations that are below these detection limits. Yet, Jacobson and coworkers never provide an explanation as to how this was possible.

The first two studies of this series of reports, Jacobson et al. (1983) and Schwartz et al. (1983), described the cohort selection procedures and the methods used to estimate fish consumption rates and, consequently, PCB exposure. The following are shortcomings identified

in these two initial reports, shortcomings so serious as to limit the interpretations one might wish to attribute to these and subsequent studies.

a) The primary determinants used to characterize PCB exposure status–consumption of Lake Michigan fish and cord serum levels–were inaccurate and unreliable.

Fish Consumption

The determination of the magnitude of PCB exposure defined by fish consumption relied solely upon the memory of mothers one day after delivery. Thus, recall bias could not be avoided. Mothers were required to remember the total amount, the source, and the specific species of fish consumed for a number of years prior to the infant's birth, as well as the rate of consumption for each of those years for up to 16 years pre-delivery.[2] Recalling how many meals of lake trout versus brook trout they had eaten the five or six years previous would likely have strained the memory of even the most conscientious respondent. The PCB content of each species was estimated by the authors and simply weighted for PCB content relative to five common Lake Michigan species (Jacobson et al., 1983). As a result, not only was the rate of fish consumption crudely estimated (i.e., recall bias and poor memory), but the amount of PCBs in those fish was also poorly quantitated. Given this level of uncertainty, any estimates of PCB exposure reported by these authors must be viewed as simply gross approximations. The authors acknowledged this obvious shortcoming of the methodology and noted, *"maternal recall may be inaccurate, and attempts to adjust for contaminant levels of the particular species of fish consumed yield at best only rough approximations of exposure"* (Jacobson et al., 1983, emphasis added). It is of interest to note that the investigators did not pursue evaluating maternal PCB exposure based on the fish species weighting scheme, even though a correction for the type of species consumed is warranted. So, despite the authors' own acknowledgment that *"maternal recall may be inaccurate,"* maternal fish consumption rates were developed based solely on each person's own recall of past events, and this inadequate measure of PCB exposure was used throughout the series of studies as one of the two primary estimates of *in utero* PCB exposure.

A demonstration of just how inaccurate maternal recall (and consequently estimates of PCB exposure) was in this study can be found in the recent report by Hovinga et al. (1992). These investigators reported that for the time period which included the birth dates of the children in the Jacobson cohort (1982 through 1989), the average level of PCBs in serum of Lake Michigan fish eaters was 19 ppb. Schwartz et al. (1983) reported that in women who claimed to have consumed from six to 51 meals of fish per year, serum PCB levels were approximately only 6 ppb. This disparity suggests that either the serum PCB measurements of Jacobson may be in error or, just as likely, that the fish consumption estimates provided by the respondents in the Jacobson cohort were both inaccurate and exaggerated.

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[2]Paneth (1991) highlighted the fact that these studies are based on a "promising database" – i.e., interviews with women who recently delivered babies. However, Paneth notes that *"the published reports do not provide critical features of any large survey–the number of interviewers, their training, and the instrument used."* Thus, one cannot comment on the quality of the information obtained during these interviews.

In the Jacobson cohort, women who claimed to consume no Lake Michigan fish had PCB serum levels of approximately 4 ppb. Kimbrough (1995) has reported that the median PCB serum level in the general U.S. population now ranges between 2 ppb and 7 ppb. Therefore, both the exposed and unexposed groups of women in the Jacobson cohort would be considered typical of the general population and, based on serum levels alone, did not experience an unusual exposure to PCBs. Women considered in the "highest annual rate," those claiming to have consumed 52 to 183 meals per year, had blood levels of only 9.5 ppb. These women, based on their measured PCB blood levels, would also be considered indicative of having only background exposures, and are certainly not consistent with the blood PCB levels reported by Hovinga et al. for typical Lake Michigan fish eaters.

Given the preceding, it is difficult to see how one could conclude that any of these women, whether self-reported fisheaters or not, were different from one another in terms of their PCB body burden predicated solely on the criteria of fish consumption rates. Based on this realization alone–that these women were alike in terms of their PCB tissue levels–findings of adverse effects which in later studies were attributed to "fish consumption" cannot be considered a result of PCB exposure. As pointed out by Paneth (1991), *"estimated cumulative PCB ingestion correlated only modestly with maternal PCB levels. The correlation coefficient of 0.29 indicates only 8 percent of the variance in serum PCB is associated with estimated PCB ingestion."* Based on this limited correlation, and recognizing that self-described fish eaters had PCB serum levels more indicative of background exposures and not Lake Michigan fish consumption, one must question whether these women accurately recalled their fish ingestion histories. Mausner and Kramer (1985) point out that for prospective studies like these, *"it is essential that individuals be correctly classified with regard to exposure to the hypothesized risk factor."* As can be seen from the above analysis, this was not the case with the Jacobson cohort, and the validity of the subsequent studies suffers because of this oversight. Given that the authors suggest several correlations exist between fish consumption[3] and adverse neurodevelopmental effects in subsequent papers, the magnitude of this serious flaw in estimating PCB exposure cannot be overstated.

Cord Serum

The major problem with the use of cord serum levels (while in theory the best estimate of *in utero* exposure) is an analytical one. Cord serum PCB samples were collected in only 63% of the newborns whose mothers participated in the study (198 of 313). Of the 198 cord serum samples available, fully two thirds (68%) were nondetectable or nonquantifiable for PCB content. Thus, cord serum levels of PCBs could be reported for only 20% of the original group of newborns (64 of 313). Since the exposure metric (cord serum PCB level) could have only represented a small fraction of the population, a response of the whole population cannot be

[3] It is interesting to note that although determined in the original study, maternal serum PCB concentration, which one would assume accurately reflects total PCB exposure, was never used as an index of maternal exposure in later studies. Rather, fish consumption, a much less precise measurement, was used as an index of maternal PCB exposure.

stratified on the basis of this exposure variable. However, this is precisely how the authors later manipulate these limited results.

The authors arbitrarily suggest that the quantitation limit, 3 ng/ml (ppb), represents the "exposed" children and those whose cord serum levels were below the quantifiable limit were "unexposed" (Fein et al., 1984). Just how many of the children defined as "exposed' based on blood levels were actually controls when originally selected according to reported maternal fish ingestion rates is never indicated in these studies. Thus, is appears that dose-response relationships later portrayed in the studies published by Jacobson (based on ingestion rates versus blood levels) use different populations of the cohort with exposure or nonexposure classifications varying with the surrogate measure of exposure. Furthermore, any corollaries with serum PCB levels were the result of a dichotomous relationship that was based simply on the sensitivity of the analytical methods available in the mid-1980s. As the authors used this metric to substantiate an association between PCB levels exceeding the quantitation limit and adverse effects, no cause-and-effect relationship could have been implied with these limited data. Therefore, associations later reported by these investigators (Fein et al., 1984; Jacobson et al., 1985; Jacobson et al., 1990a; Jacobson et al., 1990b) based on this measure of exposure must be considered an artifact of their selection criteria and cannot be given much credence based on the following considerations:

- The selection of 3 ppb as the cut-off between exposed and nonexposed was arbitrary and based on analytical limitations rather than biological or behavioral (i.e., fish ingestion) considerations. Would the reported associations between cord serum and adverse effects described by these investigators still have existed if the quantitation limit of their study had been 0.5 ppb, 1 ppb, 2 ppb, or 4 ppb?

- The cord serum levels, while not corresponding to fish consumption, did correlate with maternal PCB serum levels. As previously discussed, the maternal serum levels were, for the most part, typical of background exposure and not consistent with Lake Michigan fish consumers. This implies that simply being in the quantifiable range does not in itself constitute an unusual exposure.

- Based on fish consumption, 77% (242/313) of the participants were exposed, while using cord serum levels equal to or exceeding the quantitation limit (3 ppb), only 32% of the offspring (64/198) of the participants were considered exposed. It is unclear from the published data whether some of those cord serums levels above the quantitation limit are from non-fish eaters, and when the 3 ppb cut-off is used a larger percentage of formerly exposed infants (when defined by reported fish consumption) now become unexposed infants. As this measure provides no indication of fish consumption and no known relationship to the authors' original premise (i.e., that high consumers of fish may be at some risk), its use is a tenuous one. For example, what other potential risk factors were high in women with measurable PCB levels? Were DDT and mercury levels also elevated in these women?

- Use of the quantitation limit as a definition of exposure means that for the entire original population of 313 newborns, there was only a 1 in 5 chance (64/313) of hitting a detectable PCB level. Consequently, being categorized as an "exposed individual" when actual PCB measurements were used was only 20% in contrast to the 77% likelihood found when reported fish ingestion rates were applied.

- The fact the fish consumption rates and measurable PCB levels end up selecting different "exposed and unexposed" populations indicates that these measures have little in common, yet the authors make attempts to correlate study findings to both PCB exposure indices. In fact, they tend to report the exposure metric that seems to correlate best with the neurodevelopmental endpoint they are attempting to measure at that particular moment.

- Because Jacobson and coworkers define exposure on the basis of detectable serum PCB levels, their results should have been comparable to and confirmed by the Rogan studies. But the series of studies performed by Rogan and coworkers yielded decidedly different results (e.g., see the comparative discussions provided in Expert Report on Polychlorinated Biphenyls, 1994; Schantz, 1996).

A careful analysis of this study must conclude that there is an unacceptably high level of uncertainty with the exposure category used in subsequent studies. Some 68% of the blood samples were not quantifiable as to PCB levels, and the status of "exposed" is ultimately determined in subsequent papers either by an analytical technique with serious limitations or by a self-reported ingestion value that is highly susceptible to recall bias. In short, the range of measurable levels is so small and carries such uncertainty with it that any reported correlation between an adverse effect and cord serum levels must be viewed with a great deal of skepticism. As others have noted (Expert Report on Polychlorinated Biphenyls, 1994), perhaps the best example of the unreliability of the dose-response relationships reported for the Jacobson cohort is the negative association between birth weight and head circumference and cord serum PCB levels that were reported in the initial studies (Fein et al., 1984). These particular endpoints and measurements are objective ones as they are based on physical measurements of infant size, but the negative relationship between exposure and infant size that Jacobson and coworkers report has never been reproduced in any subsequent similar investigation that was performed by other scientists. Thus, an additional concern for the exposure assessment approach taken in this series of studies is that it is not a scientifically rigorous or reliable one (as evidenced by the fact that the first potential adverse effect recorded, and probably the most objective measurement reported by these authors, has never been repeated or confirmed).

One additional indication of the inability of these investigators to adequately characterize *in utero* exposure can be found in the reported correlations between the key exposure parameters. According to the authors themselves (Jacobson et al., 1985), *"cord serum PCB levels [are] the more direct measure of prenatal exposure"* relative to any other biological measure (e.g., maternal serum and breast milk). It should be noted that Schwartz et al. (1983) had reported the regression analysis for four biological measures of PCB body burdens that were based on

reported fish consumption and stated, *"None of the correlations between fish consumption and cord serum PCBs were significant."* Thus, the authors ultimately admit that fish consumption as a measure of PCB exposure does not correlate with the actual measure of PCB exposure–serum blood levels–and their own statistical analyses further demonstrate that fish consumption is an inadequate determinant of prenatal PCB exposure. Thus, it is easily argued that no reported correlation between fish consumption rates and any effect measured in these studies can be considered to be reliable (Expert Report on Polychlorinated Biphenyls, 1994), i.e., based on their own analyses Jacobson and coworkers should have discontinued using fish consumption in any of their future studies as a measure of PCB exposure.

While the researchers continued to monitor the neurodevelopmental progress of this group of children, all of their subsequent analyses are flawed due to their inability to satisfy a basic tenant of toxicology: they failed to adequately demonstrate that the children they classified as "exposed" to PCBs had in any way experienced a dose of PCBs which was quantitatively different from those considered "unexposed." Despite the inadequacies of their own dose metrics, the authors of these studies nonetheless later contended that *"the effects of contaminated fish consumption on birth size and gestational age were corroborated in analysis based on the cord serum measure"* (Jacobson et al., 1984). The authors have clearly over-interpreted these results and overstated the biological significance of findings based wholly on these poorly developed exposure categories. Thus, it is not surprising that other scientists reviewing these studies have questioned their validity; for example:

> "There are a number of inconsistencies in the proposed dose-response relationship between exposures to PCBs and the effects reported. The authors suggested that PCBs in the fish were the cause of the reported effects although no correlation was observed between fish consumption and concentrations of PCBs in umbilical cord blood serum..."
>
> "The above factors all detract from the verification of a dose-response relationship between PCBs and the effects observed. The deficiency in the criteria for a dose-response relationship, a basic requirement for establishing causality (Hill, 1965; Fox, 1991), and the lack of substantial differences between the concentrations of PCBs and in the general population, indicate that the effects reported on human development in the populations studied were not causally related to exposures to PCBs (Expert Report on Polychlorinated Biphenyls 1994)." [emphasis added]

and,

> "A number of methodological concerns have been raised about the Jacobson study, including issues related to exposure assessment, sample selection, an control of potential confounding variables..."
>
> "The possibility that MeHg [i.e., methylmercury] may explain some or all of the effects observed in the Michigan fish exposure cohort must be considered. Methylmercury is a well-recognized developmental neurotoxicant, and small

amounts are present in Lake Michigan fish. Furthermore, the effects observed in nonhuman primates exposed to low levels of methylmercury in utero are very similar to those observed in the children of the Michigan cohort." (Schantz, 1996)

b) The exposed and unexposed groups were not well matched in terms of numbers of participants in each "exposure" group.

In prospective studies, the ability to detect statistically significant differences between groups of participants is dependent upon the number of controls relative to cases. This important consideration was apparently ignored by the authors of the Jacobson studies. As noted by the Agency for Toxic Substances and Disease Registry (1995), it is unclear why the investigators chose an exposed sample population three times larger than the control sample population instead of a similar number of individuals in both groups. This decision greatly limited the statistical power of the subsequent studies. Simply increasing the number of controls to equal the "exposed" mothers would have increased the statistical power by 50%. If the authors had selected two controls for every woman considered exposed, the statistical power would have increased 2.25 fold from the method selected (Paneth, 1991).

There are additional questions regarding the selection process employed by the investigators which are critical given the investigators' decision to assign exposure in a non-random manner. In these instances, it is important to compare the exposed group to a nonexposed group which is as alike as possible in potentially confounding characteristics. With only 71 controls and numerous confounding variables, this was virtually impossible (Paneth, 1991). This situation could have been avoided by simply matching the control group with the exposed group based on characteristics that are likely to be confounding. As suggested by Paneth (1991), this is the only way one can have confidence that *"the control group will reflect all the differences that exist in the population between high consumers and non-consumers of Great Lakes fish."* Unfortunately, the investigators chose not to follow this important design constraint. Since the control group was so small, it is not possible to get an adequate picture of the characteristics which make up the general population, and as Table 3 (which is reproduced below) clearly demonstrates, the exposed and control populations were poorly matched for a number of important confounders. Due to this serious oversight, confounding can never be completely eliminated from their subsequent studies of this same cohort.

Considering that of the women originally interviewed to participate in the study some 2,485 reported eating no Great Lakes fish, one must seriously question why the authors only enrolled 71 controls for these studies. The selection of fewer controls than exposed only weakens the power of the study and its ability to describe fully the range of variation among nonexposed women. The table below is taken from Jacobson et al. (1983). It illustrates the considerable range of values reported for some measurements. As the exposed population was much larger than the unexposed population, one wonders whether the distribution of extreme values is the same for both the exposed and unexposed subgroups given the considerable range of values that are being reported in Table A-1.

Table A-1: Characteristics of the Sample at Birth (N = 313)			
Assessment Parameter	**Mean**	**(SD)**	**Range**
Infant characteristics			
Birth Weight (g)	3502.7	(560.3)	909.1-5028.9
Crown-heel length (cm)	51.1	(2.5)	28.5-56.0
Head circumference (cm)	35.1	(1.4)	30.5-41.3
Ponderal index[a]	2.7	(0.3)	1.9-5.6
Gestational age (weeks) [b]	39.9	(2.3)	26.9-45.9
Apgar scores			
1 - minute	7.9	(1.3)	1.0-9.0
5 - minute	8.9	(0.8)	2.0-10.0
Postnatal complications score[c]	0.9	(0.1)	0.2-1.0
Family characteristics			
Socioeconomic status (SES)[d]	39.3	(13.5)	14.0-66.0
Maternal age	26.1	(4.6)	18.0-42.0
Maternal education (years)	13.3	(1.9)	10.0-19.0
Marital status (married)[e]	283.0	(90.4)	—
Obstetrical complications score	0.84	(0.1)	0.6-1.0

[a]Based on the following equation (Miller and Hassanein, 1971):

$$\frac{\text{birth weight (g)} \times 100}{\text{crown-heel length (cm)}^3}$$

[b]Based on obstetrician's estimated date of confinement
[c]Based on PCS (Littman and Parmelee, 1978)
[d]Based on Hollingshead index (n.d.)
[e]N is presented rather than mean; percent of women married in parentheses
[f]Based on OCS (Littman and Parmelee, 1978)

c) Investigators assumed Lake Michigan fish only contain PCBs.

Of the numerous bioaccumulative chemicals found in Lake Michigan, Jacobson and co-workers focused upon only one, PCBs. In the late 1970s and early 1980s, fish from Lake Michigan were contaminated with a number of organochlorine compounds, particularly pesticides like DDT and with organo-metals like mercury, which is a well-known human neurotoxicant. Despite the occurrence of these contaminants, Jacobson and coworkers only quantitated PCB body burdens at birth. In subsequent reports on the children comprising this cohort, Jacobson and coworkers reported significant correlations between adverse effects and "fish consumption." Given that fish-eaters had background PCB serum levels that were inconsistent with other reports of fish consumers, it is difficult to conclude that any reported association could be attributed to PCBs. The reported correlations might also be shown to exist for the serum levels of other chemicals common to these women, but as these confounders were not measured they could not be addressed.

Important statements made by Jacobson et al. (1984) about their study include the following:

"Several available measures of PCB contamination are examined. Each of these presents its own set of problems. Since maternal and cord blood are low in lipids, PCB are difficult to recover. . . . Maternal reports of fish consumption provide an alternative measure of exposure. They summarize the history of the respondent's exposure in contrast to biological measures, which are limited to a single point in [time and are] susceptible to short-term fluctuations. However, maternal recall may be inaccurate, and attempts to adjust for contaminant levels of the particular species of fish consumed yield at best only rough approximations of exposure." [Note: The bracketed words above were added to the sentence as part of this sentence was missing in the copy of the paper available for review.]

"The sample was selected to over-represent women who had consumed Michigan fish during some time in their lives. The majority (77%) reported consuming moderate to large quantities of lake Michigan fish, while the rest (23%) reported consuming none of these fish."

"Of the 8,482 women screened (i.e., 96% of all maternity patients in the four participating hospitals), about 4 percent or 343 ate PCB-contaminated fish in sufficient quantities to qualify for inclusion in the exposed group. About 29.3 percent of the women screened reported eating no lake Michigan fish; 4.6 percent or 114 of these women were invited to serve as controls."

"The fish consumption variables were positively skewed and were normalized by means of log transformations."

"Estimation of PCB levels in sample extracts were based on comparison of peaks on the chromatogram with respective peaks of known concentrations in the reference standards. The Aroclor 1016 portion of the chromatogram was used to estimate the quantity of lower chlorinated congeners, while the 1260 portion was applied to measure levels of more highly chlorinated congeners and isomers. Limits of detection were 5 ng/ml for Aroclor 1016 and 3 ng/ml for Aroclor 1260 in the sample extracts."

"The majority of the PCB levels matched to the 1016 standard were below quantification limits. This was particularly true for the cord and maternal sera analyses. Among these samples only one of 195 and five of 190 cases were quantifiable. Since comparisons with the 1260 standard yielded considerably fewer values below the quantification limit, 1260 values may provide a more reliable measure of body burden."

"PCB levels for a large proportion of the cord serum samples were not quantifiable, even when the 1260 standard was used."

"No relationship was found between cord serum PCB levels and maternal fish consumption, possibly because of the detection problems in cord serum analysis described earlier." [emphasis added]

"Mean PCB level in maternal serum was 6.1 ng/ml (SD = 3.7) among women who ate contaminated fish, as contrasted to 4.1 ng/ml (SD = 2.7) among those who abstained ($t = 3.83$, $p < 0.0001$)."

Criticisms of the Michigan studies of Schwartz et al. (1983) and Jacobson et al. (1984) made by Kimbrough (1997) include:

(1) PCB levels were not available for a large portion of the study participants;

(2) PCB levels among fish eaters and non-fish eaters were similar (because of this the groups of children were combined in some reports);

(3) The methods are not well explained;

(4) The distribution data, such as the range of PCB levels in the fish eaters and controls, were rarely provided;

(5) Grab samples used to determine PCB levels in milk are not representative of PCB levels in the milk of the entire population and cannot be used as surrogates for *in utero* exposure;

(6) The authors failed to explain how they handled mothers who did not nurse or did not contribute milk samples;

(7) The statistical analyses were poorly explained;

(8) Fish consumption and either PCB serum or PCB milk levels were not well correlated; and

(10) While many of the comparisons were statistically significant, the authors many times did not explain much of the variance associated with the correlation they observed. Nor were the statistical manipulations conducted by the authors always the correct ones.

2. Fein et al. (1984)

This study consisted of 242 infants born between July 1980 and December 1981 whose mothers reportedly ate Lake Michigan fish and 71 infants born during the same period whose mothers ate no fish. The measures of exposure used in this study were "contaminated fish consumption," defined as a weighted sum of the annual Lake Michigan fish consumed in the past or present (whichever was greater), and maternal serum and cord serum PCB analyses. The 242 women who reportedly ate Lake Michigan fish consumed fish at a yearly average rate of 6.7 ± 5.8 kg of fish per year, but during their pregnancy this rate declined to 4.1 ± 4.4 kg per year. The

"No relationship was found between cord serum PCB levels and maternal fish consumption, possibly because of the detection problems in cord serum analysis described earlier." [emphasis added]

"Mean PCB level in maternal serum was 6.1 ng/ml (SD = 3.7) among women who ate contaminated fish, as contrasted to 4.1 ng/ml (SD = 2.7) among those who abstained ($t = 3.83$, $p < 0.0001$)."

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median consumption rate was 5.0 kg/yr which approximates two fish meals per month. These women also reported their duration of fish consumption as 15.9 ± 9.1 years.

Maternal serum PCB measurements for the whole group were reported to be 5.5 ± 3.7 ng/ml (ppb), an average level that was comparable to ambient serum levels for any unexposed US population. Cord serum levels were lower and averaged only 2.5 ± 1.9 ppb. These "average" cord serum levels were based on 198 samples, 68% of which were below quantitation limits, so this exposure measurement is of questionable reliability. Analyses showed that the reported maternal fish consumption rate did not correlate with cord serum PCB levels, and the correlation between maternal fish consumption and maternal serum PCB levels was also very weak (r= 0.37). Thus, fish consumption explained little of the measured differences in maternal serum PCB levels (i.e., according to Paneth [1991] fish consumption accounts for 8% of the variation observed in measured PCB bloods levels of the participants).

While the major developmental measurements taken in this study were the baby's birth weight and head circumference, they also measured neuromuscular maturity. These authors reported that based on overall consumption of contaminated fish, infants born to non-fish eaters had significantly higher neuromuscular maturity scores than did infants born to fish eaters; however, based on cord serum PCB levels, there was no such difference (see Table A-2). Thus, the question remains as to whether the reported difference in neuromuscular maturity scores is real given that the better measure of PCB exposure, i.e. cord serum levels, did not significantly differ between non-fish eaters and fish eaters.

Table A-2: Neuromuscular Maturity Scores*	
Overall Contaminated Fish Consumption	
Non-Fish Eaters (n=71)	*Fish Eaters (n=242)*
19.96 ± 2.48	18.52 ± 2.44**
Cord Serum PCB Level	
< 3 ng/mL (n=166)	*≥ 3 ng/mL (n=75)*
19.95 ± 2.42	19.00 ± 2.40
* Adjusted for sex of infant, type of delivery, maternal weight gain during pregnancy, and maternal age. ** Significantly different from controls.	

As can be seen in the table below, the infants and mothers comprising the exposed (fisheating) group differed significantly from the nonexposed (nonfisheating) group in such important variables as type of delivery, alcohol consumption before and during pregnancy, and use of cold medications. Variables that were significant at the $p < 0.10$ level were pre-pregnancy weight and caffeine consumption during pregnancy. Thus, the fisheating and nonfisheating groups differed in factors that might affect the birth size and maturity of the infants other than just PCB exposure. Finally, it should be noted that the fish contained other organochlorine compounds as well as methyl mercury, and yet the potential effects of these compounds were not considered or controlled for in this study.

The importance of Table A-3 is that it illustrates that one of the most important difficulties faced by this longitudinal study is the fact that the 242 fisheating (exposed) mothers differed substantially from the 71 nonfisheaters (control) mothers in a number of important characteristics. Exposed mothers weighed 4.1 kg less before pregnancy, a 1/3 standard deviation in pregnancy weight. Pre-pregnancy weight is one of the strongest determinants of birthweight. Exposed mothers were almost twice as likely to consume alcohol before pregnancy, and more than three times as likely to consume alcohol during pregnancy. Both groups differed in caffeine consumption and in cold medication use during pregnancy. There was a striking difference in the proportion of deliveries that were not spontaneous; the rate among exposed was almost 50% higher than controls. These differences raise the possibility that women who eat large quantities of Great Lakes fish are a culturally distinct subset of the area population, and that these and other differences between the exposed and control populations may be associated in some way with behaviors or characteristics that lead to less favorable pregnancy outcomes and infant development. In short, if several *measured* variables differed substantially between exposed and nonexposed mothers (that is, if ingestion of alcohol, caffeine, cold medicines, and pre-pregnant weight are diet- or ingestion-related as are their different fish consumption rates), then as Paneth (1991 asks - How many *unmeasured* variables exist that also differ between the two groups and might influence or account for the findings?

Table A-3: Control Variables Yielding Differences for Exposed vs. Non-Exposed Infants

	Exposed	Non-Exposed	p value
Overall contaminated fish consumption[1]			
Maternal pre-pregnancy weight (kg)	62.0 ± 11.6	66.1 ± 14.7	<0.10
Type of delivery (% spontaneous)	67.4	77.5	<0.05
Alcohol prior to pregnancy (%)	54.2	28.2	<0.001
Alcohol during pregnancy (%)	22.7	7.0	<0.01
Caffeine[2] prior to pregnancy (%)	40.1	28.2	<0.10
Caffeine[2] during pregnancy (%)	22.7	12.7	<0.10
Cold medications during pregnancy(%)	28.6	13.2	<0.01
Cord serum PCB level[3]			
Maternal age (yr)	27.1 ± 5.3	26.0 ± 4.2	<0.10
Weight gain during pregnancy (kg)	12.7 ± 4.6	13.8 ± 4.6	<0.10
Type of Delivery (% spontaneous)	63.2	73.5	<0.10

[1] Fish consumption: exposed defined as ≥ 11.8 kg over 6 years, n=242; non-exposed n = 71.
[2] Equivalent of > 2 cups of coffee per day.
[3] Exposed defined as ≥3.0 ng/ml, n=75; non-exposed n=166.
Adapted from Fein et al. (1984)

In their Environmental Health Criteria Document for PCBs, the World Health Organization (1993) had the following criticisms of the Jacobson and Fein studies:

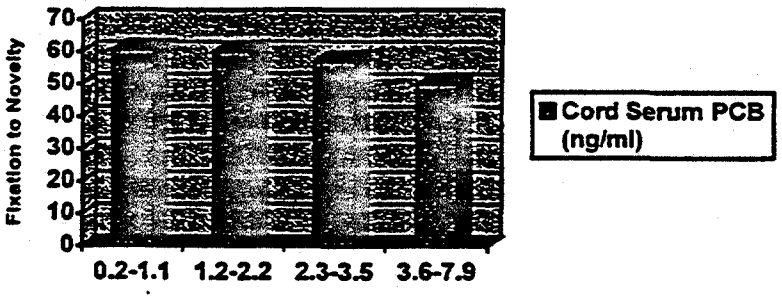
> "In the studies by Schwartz et al. (1983), Fein et al. (1984), and Jacobson et al. (1984a), the influence of important variables, such as smoking and alcohol use, were not studied extensively enough. The Brazelton test was used in these studies. However, this test was never intended to be used to evaluate neurological conditions. The value of this test to predict behavioural abnormalities in infants is small. The Public Health Council of the Netherlands (1985) concluded, therefore that the reported changes could not be interpreted by the Brazelton test. The important confounding factors 'smoking' and 'alcohol' were not studied or not well studied, while it is known that these factors can result in such changes. Furthermore, there was an indication that women consuming fish also consumed more alcohol and coffee and used more medical drugs than those who were not fish eaters." [emphasis added]

3. Jacobson et al. (1985)

This is a follow-up study of Fein et al. (1984). In this particular study, 123 infants were administered Fagan's test of visual recognition at 7 months. Cord serum PCB level and maternal reported fish consumption were purportedly associated with lower preference for a novel stimulus. Postnatal exposure from breast-feeding was not related to visual recognition memory.

Unfortunately, no raw data on neurobehavioral performance were provided in the article. Data was instead presented in histogram form (see Figure 1 on page 857), with no standard deviations provided.

Figure 1. Visual recognition memory scores. (Adapted from Jacobson et al., 1985).



An important flaw with this study is that the authors purportedly find a dose-response relationship for blood PCB concentrations that could not even be detected, based on their own reported detection limits, as will be discussed in detail below (under discussion of Jacobson et al., 1990a study). The authors listed PCB levels as low as 0.2 ng/mL (see Figure 1). The cord

serum PCB levels are obviously at levels at or below their reported limit of detection which was 3 ppb for Aroclor 1260 and 5 ppb for Aroclor 1016. The authors did not discuss how they overcame this problem of accurate quantitation. Recently, these authors (Jacobson and Jacobson, 1996a) admitted that these results were unreliable:

> "In the Michigan study, the relation between cord serum PCB level and the Fagan measure appears dose-dependent (Figure 3; Jacobson et al., 1985) although the values for the two groups below the detection limit are uncertain." [emphasis added]

and,

> "Given the limitations in the methodology then available for assessing cord serum PCB level, it is difficult to determine the actual levels at which the infants were exposed." [emphasis added]

Another important confounding variable is attributable to the apparent high variability and imprecision of the visual memory score. The representative score of the entire study cohort (n = 123) was 57.3 ± 11.4% (with this standard deviation being quite high) (range of 28.3 to 77.5%). Considering the variability in the composite mean score and the fact that this composite score is quite similar to the mean score reported for infants with cord PCB levels up to 3.5 ppb (below the reported limits of detection), the existence of a true dose-response relationship between infant PCB exposure and visual recognition memory must be questioned. The authors failed to provide in this paper standard deviations or error bars in Figure 1 of the paper (relating to the percentage of fixation to novelty scores for the infants). The authors make no attempt to evaluate the contribution of other organochlorines or other chemicals which are known to be present in fish from Lake Michigan. Also, the meaning of the purported relationships between PCB exposure and decreased visual recognition may be meaningless in terms of clinical effects. As noted by the authors, the cohort was described as a "clinically normal population:" *"The present study demonstrates the sensitivity of Fagan's visual recognition memory test in detecting performance deficits in a clinically normal population. Since developmental delay does not necessarily lead to poor functioning in the mature organism (Grant 1976), the potential of this test to predict long-term damage from toxic exposure needs to be confirmed in research assessing prenatally exposed infants at older ages."* [emphasis added]

It should be noted that the percentage of variance explained by cord serum on visual recognition scores was only 10.4. Further, maternal report of consumption of contaminated fish had a R^2 of only 3.8%.

In their Environmental Health Criteria Document for PCBs, the World Health Organization (1993) had the following criticism of the Jacobson et al. (1985) study:

> "Jacobson et al. (1985) examined visual recognition memory in 7-month-old infants of women who had consumed contaminated Lake Michigan fish. The authors reported a statistically significant correlation between cord serum PCB levels and impairment of visual recognition memory. It should be mentioned,

1.2 ± 1.6 ppb for children breastfed for less than 6 months; and 0.3 ± 0.7 ppb for children that were not breast fed. PCB body burden at 4 years was unrelated to cord serum levels. A statistically significant association was observed between prenatal PCB exposure (cord blood PCB concentrations) and verbal and memory scores. Division of subjects into four exposure groups based on cord blood PCB concentrations showed the relationship with lower McCarthy verbal scores and McCarthy memory scores to be dose-related after adjustment for confounding variables including maternal age, gravidity, and examiner. No other significant associations between cord blood PCBs and cognitive test results were observed.

A statistically significant negative correlation was observed between PCB levels in breast milk and verbal memory and numerical memory test scores (i.e., higher PCBs levels were associated with poorer performance in these tests). The duration of nursing (originally intended by the authors to be a partial measure of total PCB exposure from breast feeding) and verbal scale and memory scale test scores were also significantly correlated, but the correlation was positive (infants who breast fed longer had higher scores). This correlation decreased (they do not indicate if it was still significant) when control variables ostensibly related to quality of maternal stimulation were included in the analysis, and the authors attribute this positive effect of nursing to greater intellectual stimulation by the mothers. When subjects were subdivided based on PCB levels in breast milk, there were no significant differences among the groups in terms of memory-scale performance, except that the highest PCB concentration group had scores significantly lower than the others. No significant associations between PCB body burden in 4-year olds and performance on any of the tests was observed when control variables were included in the analysis.

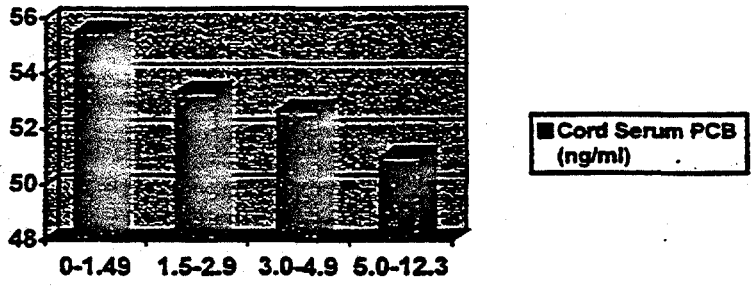
The authors interpret their findings as indicating that *in utero*, but not postnatal, exposure to PCBs had detrimental effects on short-term memory development. This is supported, they argue, by the correlations observed between cord blood PCB levels and memory performance tests at 4 years of age. The absence of correlations between poor test performance and body PCB burden at 4 years of age, or with any indicator of exposure during breast feeding other than PCB levels in breast milk, are taken as meaning that postnatal exposure is of no consequence. The correlation between PCB levels in breast milk and memory test scores is postulated to arise from PCB breast milk levels being a function of maternal PCB levels, which are in turn correlated with PCB exposure *in utero* during pregnancy. Thus, in this case, the PCB breast milk levels are thought to be more important as a reflection of prenatal, rather than postnatal, exposure.

A critical weakness of the study is its focus on PCBs as the potential causative agent for any effects that are observed. The authors made a modest attempt to address the possibility of other environmental contaminants as causative agents or confounders by looking for polybrominated biphenyl (PBBs), lead, and selected chlorinated pesticides in the blood samples from children at 4 years of age. Only DDT, PBB, and lead were reportedly found, although the detection limits for the other analytes were not specified. Lead, PBB, and DDT levels (presumably actually DDE levels, or a sum of DDT and metabolites, though this is not described in the report) were included in the analysis as potential confounders for regressions involving body burdens at 4 years of age. Attempts to control for confounders at 4 years of age is not relevant to *in utero* exposures—attempts to control for these confounders by Jacobson were done

too late and thus are not relevant. No significant associations between PCB exposure and any of the cognitive tests were identified in these regressions. On the other hand, significant associations were found between test scores and PCB cord blood levels and with PCB levels in breast milk. For these regressions, only PBB levels were included in the regression as confounders. As such, all of the positive findings resulted from analyses in which there was essentially no consideration of other environmental agents as causative factors.

Another important flaw in this study, as was also the case for Jacobson et al. (1985) (and is the case for future Jacobson studies) is that this study's authors purport to find dose-response relationships for blood PCB levels that could not even quantitatively be measured (i.e., they report a dose-response relationship at a level that was below the limit of detection mentioned in earlier studies). As can be seen on page 42 of the study (see Figure 2 below), there were groups of children with cord serum levels ranging from 0-1.49 ng/ml and from 1.5-2.9 ng/ml. Supposedly, the limit of detection for the cord blood PCB methodology used was *3.0* ng/ml, based on information provided by Jacobson in his earlier studies. The authors even state that the mean cord blood PCB level for the group was 2.5 ng/ml, a level that reportedly could not even be detected by the authors' analytical method.

Figure 2. McCarthy Verbal scales scores. (Adapted from Jacobson et al., 1990).



Strangely, this inconsistency was recently conceded by Jacobson and Jacobson (1996a), although they failed to explain how they were able to overcome this flaw in their data set:

> "In Michigan, two-thirds of the cord serum samples were below the laboratory detection limit of 3 ng/mL, which rendered uncertain the reliability of many of the reported values." [emphasis added.]

and,

> "In Michigan, cord serum PCB level was associated with poorer performance at age four years on the McCarthy Verbal and Memory Scales (Figure 4; Jacobson et al., 1990). As with the Fagan test, the effects appear to be dose-dependent, although the values for the two groups below the detection limit are uncertain." [emphasis added]

This inconsistency has also been noted by the Seegal (1996b) as well as by Schantz (1996) in their reviews of the PBC/neurodevelopmental literature:

> "...this authors must add one potentially important additional issue related to the analysis and interpretation of PCB concentrations in fetal cord samples. In Jacobson et al. (1983), the authors state that fetal cord serum PCB concentrations expressed either in terms of Aroclor 1016 or Aroclor 1260 standards, were largely "detectable, but not quantifiable." In a footnote in Table 2 in Jacobson et al. (1983), the detection limit for Aroclor 1016 was stated to 5 ng/ml, while the detection limit for Aroclor 1260 congeners was 3 ng/ml. Despite these statements describing limitations in the detection thresholds, subsequent research articles state that a dose-response relationship was observed between fetal cord serum PCB levels and important neurobehavioral endpoints, at PCB levels that were not quantifiable. ... Because of the dependence of many of the Jacobsons' variables on measures of fetal cord PCB concentrations, many of which were below the published detection limits and were not originally correlated with fish consumption, the ability of the authors to conclude that there were 'dose-dependent' relationships between these measures and fetal cord serum PCB concentrations must be questioned. Given the Jacobsons' earlier statements concerning a significant relationship between developmental indices and fetal cord serum PCB levels, divided into subjects with levels less than 3 ng/ml and subjects with greater than 3 ng/ml, it is perplexing why statements concerning dose-response were made in later articles." (Seegal 1996b)

and,

> "Several of the early articles list the detection limit for PCBs in umbilical cord serum as 3.0 ng/ml (Fein et al., 1984; Schwartz et al., 1983), yet dose-response graphs in later articles include several groups below 3.0 ng/ml [e.g., Jacobson et al., 1990a, 1990b; Jacobson et al., 1992]. The detection limit for PCBs in cord serum is not given in any of these more recent articles. Were the samples reanalyzed using a more sensitive technique? The method for partitioning these children into these groups needs to be clarified." (Schantz 1996)

A review by Paneth (1991) discusses the three general areas of methodological concern in the Jacobson studies: (1) exposure assessment and its validity; (2) selection of exposed and control subjects; and (3) the comparability of exposed and control samples. Overall, Paneth concludes: *"In the final analysis, the study's findings, striking though they may be, cannot be viewed as conclusive."*

Assessing Exposure

Paneth points out shortcomings in the methodology used by Jacobson et al.'s group in determining the exposure to PCBs in their studies:

> "... published reports do not provide critical features of any large survey—the number of interviewers, their training, and the instrument used."

and,

> "The investigators estimated PCB consumption from the number and species of fish that the mothers reported eating. Using EPA measurements of PCB in Great Lake fish, they assumed that different species had different amounts of PCB. A meal of lake trout, for example, contained five times more on the PCB scale than a meal of brook or steelhead trout; recalling which species they'd had eaten five or six years before may have strained the dietary memories of some mothers, especially since most analyses are based on fish consumption histories covering the previous six years."

Selecting Cases and Controls

First, Paneth (1991) discusses the attrition between sampling and participation of the group and the fact that the attrition was not random and it was not unbiased. Paneth also points out that *"The key contrasts in this study are between the most highly exposed 4 percent of mothers and those who reported never eating Great Lakes fish. The 5654 mothers with intermediate levels of Great Lakes fish consumption—two-thirds of those surveyed—were not studied further."*

Next, Paneth (1991) goes on to discuss the shortcomings of the manner in which Jacobson et al. selected their controls:

> "The investigators made two unusual decisions in selecting their control sample. The first was to restrict its size to just a third of the exposed sample (114 compared to 343 before attrition). This greatly limited the study's statistical power."

> "The second unusual decision was to choose a random, rather than a matched, sample... In studies of reproductive and neurodevelopmental outcomes, this is critically important, since a variety of socio-economic and other maternal characteristics powerfully influence such variables as birthweight and cognitive function... Proper control in the analysis depends on finding controls who have confounding characteristics similar to those of the cases. With as few as 71 controls, and a host of potentially confounding variables, this can sometimes be impossible. The common solution when it is not feasible to select a large control group, but when a large pool (2,485) of potential controls is available, is to try to make the control sample as comparable to the exposed group as possible. This is done by matching the control group to the exposed group on characteristics that are likely to be confounding. Matching takes advantage of the large array of characteristics of individuals in the source population... But unless investigators actually do such matching—as they did not do in this study—the control group will reflect all the differences that exist in the population between high consumers and non-consumers of Great Lakes fish."

Laboratory Data

Paneth (1991) points out the problems with the lab data in the study: (1) attrition of available samples for mother-infant pairs; and (2) problems with the correlation between PCB levels and fish ingestion histories.

> "First, virtually all of the PCB measurements obtained were 'detectable but not quantifiable.' ... estimated cumulative PCB ingestion correlated only modestly with maternal PCB levels. The correlation coefficient of 0.29 indicates that only 8 percent of the variance in serum PCB is associated with estimated PCB ingestion. But even this modest correlation undoubtedly overestimates the real correlation between the two variables, since the middle two-thirds of the data were excluded as noted above, and since assessing only the extremes of a distribution almost invariably exaggerates a correlation coefficient. Mean maternal serum PCB was 6.1 ppb (± 2.7) in women who reported no fish consumption. Exposed and control mothers thus overlapped substantially in serum PCB measurements."

> "The correlation between estimated cumulative PCB ingestion and infant serum PCB was 0.09, indicating virtual independence of these two measures. Fish consumption correlated weakly with breast milk values: 0.21 for samples obtained in the neonatal period, 0.10 for samples obtained at 5 months. Thus, the serum and milk measurements provide only weak support for the validity of dietary assessment as a measure of PCB exposure."

> "Neonatal neurobehavioral measures, however, were associated only with PCB ingestion history; cord PCB levels were not associated with these outcomes. But at age four, it is cord PCB level that is associated with cognitive measures; PCB ingestion history is not mentioned at all. Clearly, some of the associations found depended on the type of exposure measure used, and this vitiates the strength of the findings."

Comparing Cases & Controls

Paneth (1991) also points out the differences between the cases and controls in the Jacobson et al. studies. These differences between groups likely had a significant effect on the reported outcomes of the studies. Differences noted by Paneth (1991) include: (1) fish eaters weighed an average 4.1 kg less prior to pregnancy, with pre-pregnancy weight being one of the strongest determinants of birth weight; (2) fish eaters were about two times as likely to report alcohol use prior to pregnancy and more than three times likely to report use during pregnancy; (3) both groups differed in caffeine consumption prior to and during pregnancy; (4) both groups differed in use of cold medicines in pregnancy; (5) there was a great difference in the proportion of deliveries that were not spontaneous (rate in fish eaters almost 50 percent higher). With respect to these differences, Paneth suggests: *"These differences raise the possibility that women who eat large quantities of Great Lakes sport fish are a culturally distinct subset of the population, and that these differences may be associated in some way with behaviors or other*

characteristics that lead to less favorable outcomes of pregnancy and infant development." Paneth also questions: *"... if several* measured *variables differed substantially between the exposed and the unexposed mothers (four of them, ingestion of alcohol, caffeine, and cold medicines, and pre-pregnant weight, are diet or ingestion related), how many* unmeasured *differences between the groups might account for the findings?"*

Schantz points out some of the same flaws identified by Paneth et al. (1991).

"PCB exposure measures used in the study included the mother's estimated total lifetime Lake Michigan fish consumption, the infant's umbilical cord serum PCB level, the maternal serum PCB level at birth, and the breast milk PCB level at birth and 5 months for women who breast fed. The Michigan fish consumption.... Average fish consumption for the 242 women who reported eating Lake Michigan fish was 6.7 kg/year, equivalent to about two salmon or Lake trout meals/month. The women reported eating Lake Michigan fish for an average of 16 years (14). Maternal serum PCB concentrations averaged 5.5 ng/ml and umbilical cord serum PCB concentrations averaged 2.5 ng/ml (69). The concentration in milk fat was 812 ng/ml in the first week after birth and 769 ng/ml at 5 months. Those who know the PCB literature will recognize that these levels are not much above the background levels typically detected in the United States and other industrialized nations (43). Cumulative fish consumption was only moderately correlated (r = 0.21-0.29) with PCB levels in maternal serum and milk (69)."

and,

"Head circumference was significantly smaller even after controlling for birth weight and gestational age. As the authors point out, the size deficits they observed are comparable to those associated with smoking during pregnancy (35)."

and,

"Maternal contaminated fish consumption was also associated with several adverse behavioral outcomes on the NBAS (32). Infants of women who ate the most fish exhibited motoric immaturity, poorer lability of states, a greater amount of startle, and more abnormally weak (hypoactive) reflexes. However, the other, more direct measure of exposure, umbilical cord serum PCB level, was not related to any adverse behavioral outcomes on the NBAS. This suggests that the adverse behavioral outcomes observed on the NBAS should be interpreted with caution. They may be related to other contaminants present in the fish or to some other aspect that differed between the fish-eaters and not-fish-eaters."

and,

"Neither maternal fish consumption nor umbilical cord serum PCB level was related to scores on the Bayley Scales."

and,

"Children from the cohort were reassessed when they reached 4 years of age (36-38)... neither the quantity of breast milk consumed nor the child's current serum PCB level related to any of the outcomes."

and,

"The finding from the tests of cognitive processing efficiency were not as clear. The authors reported that prenatal exposure to PCBs was associated with less efficient visual discrimination processing and more errors in short-term memory scanning (38). However, in each case only one of the two exposure measures correlated with the outcome. Reaction time on the visual discrimination task was correlated with maternal milk PCB level, but not with umbilical cord serum PCB level. These inconsistencies suggest that these findings should be interpreted with caution. No effects on sustained attention were observed."

and,

"The physical growth and activity level of the children in the PCB fish exposure cohort were also assessed at 4 years (37)... there was no relationship between prenatal PCB exposure and height or head circumference at 4 years."

and,

"Paneth (56) has cited three areas of methodological concern: validity of the exposure assessments, selection of the exposed and control samples, and comparability of the exposed and control samples. One concern he raises with regard to exposure assessment deals with the method of estimating maternal fish consumption. As outlined above, the investigators estimated maternal fish consumption from the number and species of fish the women reported eating during the previous 6 years. Species that were known to be more contaminated were weighted more heavily. For example, a meal of lake trout received five times more weight than a meal of brook of steelhead trout. Recalling which type of trout they had eaten 5 or 6 years previously may have been difficult for some women. Problems with obtaining accurate retrospective dietary information may be one factor contributing to the relatively low correlation between PCB body burdens and fish consumption that were observed in this study (69)."

and,

"... the investigators did obtain umbilical cord serum, maternal serum, and maternal milk for direct measurement of PCB exposure. Unfortunately, attrition problems and a lack of analytical sensitivity weaken these data. Umbilical cord serum samples were obtained from only 198 (63%) of the infants and the PCB

concentrations in well over half of the obtained samples were not quantifiable (69)."

and,

"Nearly 100% of those sampled had detectable levels of PCBs in their milk fat, and the mean PCB concentration in milk fat as well above the limit of detection (69)... Unfortunately, the problem of attrition creeps in again, weakening this approach. Neonatal milk samples were obtained from just 138 (44%) of women in the sample (69). The published articles do not discuss whether the 138 women who provided milk samples differed from those who did not provide milk samples on any important demographic or health characteristics."

and,

"The published articles also do not make clear which indices-cord serum, milk fat of fish ingestion- the authors consider to be the best, or most appropriate, measures of exposure. The earlier articles report the relationship of fish ingestion and umbilical cord serum PCB levels to neurodevelopmental and anthropometric end points, but make no mention of neonatal milk PCB levels (14.32). In contrast, the later articles focus on cord serum and neonatal milk PCB levels (36-38), and fish ingestion in not mentioned at all. Some of the associations between exposure and outcome depended on which exposure measure was used (e.g. (32.38). Thus, it seems critically important to spell out the strengths and weaknesses of each approach, as well as the reasons for selecting one measure of exposure over another."

and,

"Paneth goes on to argue that another area of concern is the selection of the control sample. As he details, two unusual decisions were made in selecting the control sample for this study. First, its size was restricted to just one-third the size of the exposed sample. This decision greatly limited the study's statistical power. By choosing as many controls as cases, the investigators would have increased their statistical power by 50%, and by choosing twice as many controls as cases they would have increased it by 225% (56). Selecting a smaller control sample also put increased weight on the characteristics of the women in that sample. The second unusual decision was to choose a random rather than a matched sample. In any study in which exposure is not randomly assigned, it becomes very important to select controls that are as similar as possible to exposed individuals with regard to potentially confounding characteristics. This is done by selecting a control group that is matched to the exposed group on as many potentially confounding characteristics as possible. Matching is particularly important in studies of neurodevelopmental outcomes because they can be influenced by a wide variety of socioeconomic and maternal factors."

and,

"The final, and ultimately most important, issue raised by Paneth is that the fish-eating mothers in this study did, in fact, differ from the non-fish-eating mothers on several important characteristics. The average age, educational level, and socioeconomic status of the two groups were very similar. However, the two groups differed on several important health-related characteristics. The fish-eaters weighed, on average, 4.1 kg less than no fish-eaters before pregnancy (14). As Paneth points out, prepregnancy weight is a strong determinant of birthweight, one of the end points that was affected in these children. The fish-eaters were more likely to report using alcohol and caffeine both before and during their pregnancies, and they were more likely to use cold medications during their pregnancies. They also had fewer spontaneous deliveries than the non-fish-eaters."

and,

"...It is not clear that all of the potential confounding variables were controlled in all of the analyses. Their approach was based on the premise that a control variable cannot be the true cause of an observed effect unless it is related to both exposure and outcome."

and,

"It is not clear why they chose to include additional predictors of body size in those analyses. Various other investigators have reported a positive relationship between blood or milk PCB levels and alcohol consumption [e.g., (9.47.61)]. Thus, alcohol consumption would seem to be an important variable to include as a covariate in all analyses, particularly given the well-established relationships between alcohol consumption, birth weight, and cognitive outcome. A related issue is that the investigators apparently did not include as potential confounders dichotomous control variables for which the incidence in each category did not exceed 15% (14)."

and,

"Related to these concerns is the fact that each published report lists a different set of control variables that are related to the exposure measures and it is never clearly spelled out why they differ. For example, Jacobson et al (32) list only alcohol and caffeine use as confounders correlated with fish consumption. No mention is made of maternal prepregnancy weight, type of delivery, or use of cold medications, variables that were identified as confounders related to fish consumption in another article from the same research group (14). On the other hand, Jacobson et al. (32) list alcohol and caffeine use, maternal weight gain during pregnancy, and maternal age as potential confounders correlated with umbilical cord serum PCB level, whereas Fein et al. (14) list maternal age,

maternal weight gain during pregnancy, and type of delivery as potential confounders for that exposure measure. In the later publications, maternal age and gravidity are controlled for in the analyses using cord serum PCB levels, and no mention of alcohol, caffeine, maternal weight gain, or type of delivery is made (36-38). The investigators allude to the fact that the confounds associated with a particular exposure measure can change over time due to attrition (41), but these inconsistencies are never fully explained."

Schantz (1996) also notes problems with the Jacobson series of studies:

"A related issue has to do with the statistical analysis of these data. It is difficult for the reader to evaluate the differences between groups because most of the graphs do not include error bars and the authors do not always clearly indicate in the text on the graphs which groups differed statistically. In the follow-up studies conducted when the children were 4 years of age, the highest exposure group contained just 10 children (Jacobson et al., 1990a, 1990b; Jacobson et al., 1992). Thus, it becomes very important to know whether or not significant differences were limited to that most highly exposed group. If so, we would be wise to interpret these findings very cautiously until such a time as they are replicated in a larger cohort of exposed children."

The Expert Report on Polychlorinated Biphenyls (1994) evaluating PCB toxicity also comments at length on the multitude of problems that plague the Jacobson studies. Some of these comments are as follows:

(i) "There are a number of inconsistencies in the proposed dose-response relationship between exposures to PCBs and the effects reported. The authors suggested that PCBs in the fish were the cause of the reported effects although no correlation was observed between fish consumption and concentrations of PCBs in umbilical cord blood serum. ... The concentrations of PCBs in cord serum at birth, as reported by Fein et al. (1984), were correlated with decreased cognitive performance in the children at 4 years of age; however, there was no correlation between concentrations of PCBs in cord serum and maternal fish consumption (the proposed source of PCBs). In addition, the possible confounding effects of socioeconomic factors, smoking tobacco, alcohol consumption, and other lifestyle factors known to affect cognitive performance were not adequately addressed. ... Also, the number of inconsistencies in the reported analyses for PCBs in maternal and cord blood sera, and in breast milk, affect the confidence of the overall evaluation of possible dose-response relationships. It appears that analyses for PCBs were not conducted on serum samples with lipid concentrations less than 200 mg/dl. This decision could bias the interpretation of subsequent correlations with adverse effects in an undetermined manner since the PCBs in blood are associated with blood lipids. ... The deficiency in the criteria for a dose-response relationship, a basic requirement for establishing causality (Hill 1965; Fox 1991), and the lack of substantial differences between the concentrations of PCBs in the study and in the general population, indicate that the effects reports on human

development in the populations studied were not causally related to exposures to PCBs."

(ii) "The second factor affecting the strength of the proposed association is that the women from the elevated fish consumption group also reported significantly greater consumption of alcohol, caffeine, tobacco, and cold remedy prescription use during pregnancy than those consuming less fish. ... All these confounding factors seriously detract from the biological plausibility of an association between the amount of exposure to PCBs, as indicated by the concentrations of PCBs in blood, and effects on infant development."

(iii) "A third factor affecting the strength of the proposed association is the lack of plausibility and consistency of association that are required (Hill 1965; Fox 1991) to establish a causal relationship. Greater umbilical cord serum concentrations of PCBs were marginally associated with cognitive performance parameters (e.g., poorer verbal and memory scores on the McCarthy Scales performance tests and lower scores in the verbal and numerical memory subtests) in the same children 4 years later (Jacobson et al., 1990a). Scores on such tests are affected by socioeconomic status of the subjects and the age at which the tests were conducted. Scores for other components of the McCarthy Scales performance tests (perceptual performance, quantitative, motor, and general cognitive index) were unrelated to *in utero* exposure to PCBs. Also, no correlation was observed between fish consumption and concentrations of PCBs in umbilical cord serum. As a result, no correlation can be made between contaminated fish and scores on the McCarthy Scales performance tests. These factors seriously weaken the plausibility of associations between the effects reported by Fein et al. (1984) and exposures to PCBs."

"The arguments for plausibility of association are also weakened by observations that, even though postnatal lactational exposures to PCBs are far greater than those experienced *in utero*, the children exposure to PCBs via lactation for longer periods have significantly greater scores on both the Memory and Verbal Scales tests. ..."

"The plausibility of the results of the Jacobson et al. (1990a) study is further eroded by the report that certain marginal deficits observed in some clusters of the McCarthy tests were associated with greater concentrations of PCBs in the serum of the mother, but not with greater exposures to PCBs through lactation. Furthermore, it was not apparent that the scores obtained in the tests of any of the children were outside the ranges of normal since no such ranges were given. Also, a total of approximately 38 behavioral and neurological tests were conducted on the children, even though the results of only 2 tests were reported to be affected. Some of the association based on chance alone would be expected from this large number of tests. In addition, the assessment of the test results was based on a "clustering" approach, which is not a standard procedure in evaluating neurological test results from children. ..."

"The plausibility of the proposed association is further degraded by inconsistencies in the information reported in different publications of the same studies. Jacobson et al. (1990a) reported, based on Fein et al. (1984), that prenatal exposure to PCBs through mothers consuming fish was associated with decreased birthweights. The same children, assessed 4 years later, had greater serum concentrations of PCBs (attributed mainly to exposures during lactation) than those from mothers consuming lesser quantities of fish, and were also reported by Jacobson et al. (1990b) to have reduced activity levels. This result, however, is not in accordance with the findings of Jacobson et al. (1990a) that the children who had longer lactational exposure had no cognitive deficits when compared to children with short lactational exposure. In fact, prior to the controlling of confounding variables, the children with longer lactational periods tended to have greater scores on the McCarthy Scales performance tests (Jacobson et al., 1990a). ..."

(iv) "The fourth factor affecting the strength of the proposed associations is that the criteria of consistency of observation that is an essential component in the establishment of causality of association based on epidemiological data (Hill, 1965; Fox, 1991) is not met by the studies reported by Fein et al. (1984) and Jacobson et al. (1990a, 1990b)."

5. Jacobson et al. (1992)

This is another in a series of studies of the neurobehavioral status of children born to mothers who ate Lake Michigan fish contaminated with PCBs. It follows closely the study published by these authors in 1990 (Jacobson et al., 1990a) analyzing McCarthy Scales performance of 4-year olds — in fact, the tests described in this study were administered to the same children 3 months after the McCarthy Scales tests. The study group was 226 4-year old children from mothers whose fish ingestion fell into one of two categories: 1) ingestion of 11.8 kg or more weighted measure fish consumption over a 6-year period (fish with greater contamination levels were assigned greater weight); and 2) women who ate no Lake Michigan fish. These two groups were not equally represented, but instead the study group was constructed such that more highly exposed children were intentionally over-represented.

The authors postulate that lower performance by PCB-exposed infants in previous tests might be due to lower cognitive processing efficiency or to difficulties with sustained attention, and they attempt to evaluate both of these in relationship to three measures of PCB exposure — cord serum PCB levels at birth, parameters associated with PCB exposure during breast feeding (PCB levels in breast milk and duration of breast feeding), and body burden of PCBs at 4 years of age. Because cognitive processing efficiency is not usually measured in children as young as 4 years, a method previously developed (Sternberg visual search and recognition memory test) was modified to make it simpler (e.g., pictures were used as stimuli instead of digits or letters, and the subject responds by pressing one button instead of choosing between two). In addition to the modified Sternberg memory trials, a visual discrimination task was given to the children (the timed ability to identify which picture is different from another and in what way), as well as a

vigilance test to evaluate sustained attention. Not all children could be tested due to equipment failures and to lack of cooperation by some of the subjects.

To simply analyze, the authors reduced the parameters for performance on the Sternberg memory test to two – reaction time and total errors. Performance on the vigilance test was reduced from reaction time, number correct, and errors of commission to a composite vigilance test score. The visual discrimination test, like the Sternberg memory test, produced a score for reaction time and for accuracy. A limited group (37) of children were re-tested one month after the original testing to determine the stability of testing results. Due to equipment failure, data for some tests were available for only 16 or 22 of these children. Inter-test reliability was limited, with r values ranging from 0.38 to 0.77. When the results of the cognitive processing and vigilance tests were compared with the results of McCarthy Scales tests administered 3 months earlier, there was little or no correlation between previous results and reaction time measurements for either the Sternberg memory or visual discrimination tests. Moderate correlations with accuracy components of these tests were observed, however, as well as with the vigilance composite score.

A stepwise multiple regression analysis was conducted for test score results and: 1) cord serum PCB level; 2) breast milk PCB levels and weeks of breast feeding; and 3) PCB body burden at age 4 years. Twenty-four other variables regarded as potential confounders were also included in the analysis (e.g., socioeconomic status, maternal age, maternal education, maternal vocabulary, maternal smoking, etc.). After controlling for these variables, higher PCB cord serum levels were found to be associated with more total errors in the Sternberg memory trials, but not with reaction time. When the authors divided the children into different "dose" groups based on cord serum PCB levels, this effect appeared to be dose-related. Maternal milk PCB levels (but not cord serum PCB levels or weeks of breastfeeding) were associated with decreased reaction time, but there was no association with the number of errors. Longer breastfeeding was associated with better scores on the sustained vigilance task.

As with their analysis of McCarthy Scales scores published previously, the authors regard cord serum PCB levels as indicative of *in utero* exposure, breast milk PCB levels as indicative of both *in utero* and postnatal exposure during breastfeeding, and duration of breastfeeding and 4-year body burden as being indicative primarily of postnatal exposure. With this in mind, they interpret the diminished accuracy on the Steinberg memory trials (associated with PCB cord serum levels) and the decreased reaction times in the visual discrimination tests (associated with breast milk PCB levels) to be related to *in utero* PCB exposure. They suggest in the Discussion section that the reaction time deficit in the visual discrimination test did not show up in the Sternberg memory test because the latter test *"may have been simplified to the point that they placed too little demand on the child's problem-solving resources."* They offer no explanation for why the two potential measures of *in utero* exposure (PCB cord serum levels and breast milk levels) don't provide consistent associations (i.e., why they don't have significant associations with the same effects), other than to attribute this to *"limitations in the reliability of measurements."* Consistent with their 1990 report, they conclude that *in utero*, but not post-natal, PCB exposure is associated with subtle deficits in short-term memory and processing efficiency that may impair learning.

As with other studies in this series, the associations are targeted almost exclusively to PCBs, although the source of PCB exposure (contaminated Lake Michigan fish) is known to include other chemical contaminants. While some effort was made to include other chlorinated environmental contaminants in this analysis, this was only done for regressions involving 4-year body burden data. This information was not included in regressions involving PCB cord serum and breast milk data, where the significant associations were reported. Also, in this study, it is difficult to assess how the arbitrary selection of PCB cord blood groups (0-1.49 ppb; 1.5 - 2.9 ppb; 3.0 - 4.9 ppb; and 5.0 - 13.0 ppb), affects the apparent dose-relatedness of the effect. That is, if the dose groups are assigned differently, do the results and conclusions change?

Relevant quotes from Jacobson et al. (1992) include:

"The present study is the first to use assessment of cognitive processing efficiency in an evaluation of developmental neurotoxicity and among the first to adapt this type of paradigm for preschool-aged children. ... When compared with standardized IQ tests, these paradigms provide greater specificity in identifying cognitive deficits and in the present study also demonstrated good internal consistency. The effects observed were modest, however, possibly owing to only moderate re-test reliability and limited sensitivity in the assessment of exposure." [emphasis added]

and,

"As predicted, the deficits in processing efficiency and short-term memory function were specifically associated with an *in utero* exposure; neither was related to amount of contaminated breast milk consumed or current PCB body burden."

and,

"Whereas short-term memory errors on the Sternberg were associated with higher cord serum PCB levels, visual discrimination processing efficiency related significantly only to maternal milk PCB level. Given that both of these measures reflect prenatal PCB exposure, this inconsistency is presumably due to limitations in reliability of measurement. The packed column gas chromatographic analysis used here, although state of the art in the mid-1980s, provides relatively low resolution and high detection limits. ... environmental PCB exposures typically also entail exposures to polychlorinated dibenzofurans (PCDFs) and dibenzo-p-dioxins (PCDDs), highly toxic by-products in the combustion and manufacture of PCBs, which are present only at trace levels and could not be evaluated. In light of the recent refinements in analytic methodology, replication of the present findings is warranted using more sensitive analytic techniques." [emphasis added]

and,

> "It should be emphasized that the cognitive deficits reported here and elsewhere are subtle at these levels of exposure; there is no evidence of mental retardation or gross impairment. Given the importance of short-term memory and processing efficiency in mastering reading and quantitative operations, however, these deficits, although subtle, could have a significant impact on the acquisition of basic cognitive skills in school." [emphasis added]

Schantz (1996) had the following criticism of this study:

> "The authors reported that prenatal exposure to PCBs was associated with less efficient visual discrimination processing and more errors in short-term memory scanning (Jacobson et al., 1992). However, in each case only one of the two exposure measures correlated with the outcome. Reaction time on the visual discrimination task was correlated with maternal milk PCB level, but not with umbilical cord serum PCB level, whereas the number of errors on the short-term memory scanning task was correlated with umbilical cord PCB level but not with maternal milk PCB level. These inconsistencies suggest that these findings should be interpreted with caution. No effects on sustained attention were observed." [emphasis added]

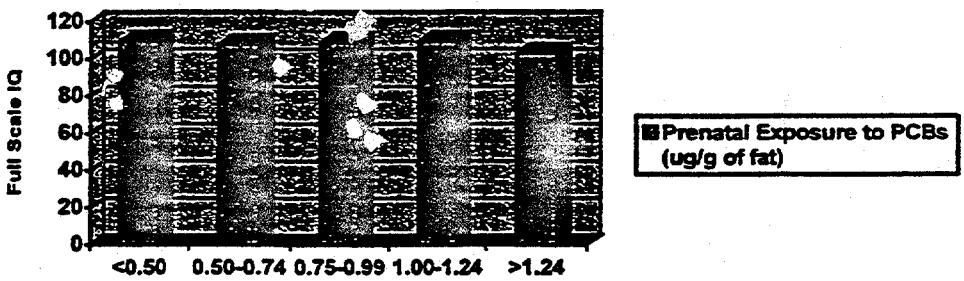
6. Jacobson and Jacobson (1996b)

The latest in the series of studies of the Michigan cohort is the recently published study of Jacobson and Jacobson (1996b), in which IQ tests were administered to the children, who were at the time of this follow-up, approximately 11 years of age. Other tests administered include the spelling and arithmetic subtests of the Wide Range Achievement Test-Revised and the word and passage-comprehension subtests of the Woodcock Reading Mastery Tests-Revised. In this study, 212 children (63% of the 313 newborns studied originally in 1980-1981) were evaluated. Blood samples were analyzed for PCB content at age 11 also. DDT, as well as other organochlorine insecticides were analyzed in blood samples, as was lead. Of the pesticides, only DDT was detected. Mercury was analyzed for in hair samples at this time, too. Maternal body burdens of PCBs were reported to be similar to or slightly above concentrations of the general population. Authors reported that by age 11, the serum PCB levels had declined substantially, suggesting that there was little additional exposure after weaning.

As will be discussed later by Middaugh and Egeland (1997), while there are IQ scores included in this figure for 178 children, based on data provided in Table 1 of the paper, only 113 samples of maternal milk were available. Unlike previous studies, in this study Jacobson and Jacobson created what they call a "composite prenatal-exposure score" to classify subjects based on prenatal PCB exposure. In order to come up with this "composite score," the values for cord and maternal serum and milk were converted to Z scores and averaged together. Serum values were only included if they exceeded the detection limit. PCBs were not detected in 70% of the cord serum samples and 22% of the maternal serum samples, and thus, these samples were not included in the analysis. For 11 children, no milk specimen was available and values for cord

and maternal serum were undetectable, so these children were assigned a prenatal-exposure scores at the 10th percentile of the distribution. The composite scores were divided into five groups for dose-response analysis based on the *a priori* cutoff points used in their four-year follow-up study for the PCB levels per gram of fat in milk: <0.5 µg, 0.5-0.74 µg, 0.75-0.99 µg, 1.0-1.24 µg, and ≥1.25 µg. Thus, it would appear that the exposure classification for many of these children is based on either maternal serum and/or milk PCB concentrations since a majority of the cord serum levels were undetectable (and thus excluded from analysis), even though Jacobson and Jacobson (1997) state that of three measures (cord and maternal serum and milk PCB levels) maternal milk levels are the most removed from the fetus.

Figure 3. Scores for Full-Scale IQ according to prenatal exposure to PCBs (expressed in terms of the fat concentration of maternal milk). (Adapted from Jacobson and Jacobson 1996).



While Jacobson and Jacobson (1996b) conclude that *"prenatal exposure to polychlorinated biphenyls was associated with significantly lower full-scale...IQ scores,"* there appears to be no dose-response relationship with respect to full scale IQ, based on prenatal exposure to PCBs (based on the composite prenatal-exposed score) (see Figure 3).

The numerical data was not provided for this figure, and error bars were not included in the graphs in the paper. [Note: In the recent Jacobson and Jacobson (1997) article in Teratology, error bars are provided for the Full-Scale IQ scores. It is obvious that there is considerable overlap between the IQ scores for the various groups: *estimated* Full-Scale IQ scores based on conversion of error bars from graphs to standard deviations–<0.5 µg/g – 109 ± 7.5; 0.5-0.74 µg/g – 107.8 ± 11.9; 0.75-0.99 µg/g – 108.9 ± 10.5; 1.0-1.24 µg/g – 109.0 ± 10.7; and ≥1.25 µg/g – 102.6 ± 12.2.]

While similar graphical data are not provided for Verbal and Performance IQs scores, the authors state that they found that the pattern of group differences were similar to those of the Full Scale IQ score. What is important to note from these data is that even among the most highly exposed children (i.e., those with prenatal exposures greater than 1.24 µg/g of fat (maternal milk), their average Full-Scale IQ scores are all above normal IQs (with normal being about 100 points). On this issue Jacobson and Jacobson state on page 785 (bottom of column 1) that – *"Although the test scores were normalized to a mean of 100 ± 15, the population mean for the Weschler IQ test had risen to 108 since its most recent standardization in 1974." The means of the Full-Scale, Verbal, and Performance IQ scores for the entire cohort (211 children tested,*

with one was dropped due to very low IQ score) were 107 ± 12, 106 ± 13, and 107 ± 13, respectively, very similar to 'normal' IQ scores."

Jacobson and Jacobson (1996b) reported that, in their opinion, prenatal exposure to PCBs was associated with poor verbal comprehension and freedom from distractibility (numerical data not provided). They also report that the effect was primarily seen in the most highly exposed children – i.e., those with prenatal exposures equal to at least 1.25 µg/g PCB in maternal milk, 4.7 ng/mL in cord serum , or 9.7 ng/mL in maternal serum. On academic achievement tests, prenatal exposure was reportedly significantly associated with poorer performance on all three word-comprehension subtests (antonyms, synonyms, and analogies), although data were not provided. They also reported that the more highly exposed children "lagged behind their peers" in word comprehension by an average of 7.2 months, and that the mean age-equivalent level of word comprehension of the two groups with the highest exposures was 11.1 ± 7 years, compared to 11.7 ± 1.7 years ($p = 0.02$). However, data were not provided to support these statements, so it is not certain if there was any dose-response relationship for this effect (among all the exposure groups). Further, these levels are very close to each other, Z score of 0.35.

Confounders were present in this study. Lead and mercury related significantly to poorer outcome after control for other confounding variables. A higher lead concentration when children were 4 years of age was associated significantly with lower verbal IQ score, verbal-comprehension scores, and poorer word, passage, and reading comprehension. These effects were reportedly evident in children with blood lead levels above 10 µg/dl. A higher mercury concentration at 11 years was associated significantly with poorer spelling.

The authors admit that lack of a known biologically plausible mechanism by which an effect on neurobehavior might occur, *"The mechanism responsible for this heightened intrauterine vulnerability is not known..."* Jacobson and Jacobson also remarked that *"There was no evidence of gross intellectual impairment among the children we studied."*

Middaugh and Egeland (1997) in a letter to the editor discussing the recent Jacobson and Jacobson (1996b) study had the following criticisms of this study:

> "Drs. Jacobson and Jacobson (Sept. 12 issue) reported that low-level exposure to polychlorinated biphenyls (PCBs) in utero is associated with lower IQ scores (by an average of 6.2 points) among school-age children. The results seem implausible given the fact that Taiwanese children who were exposed prenatally to levels of PCBs that were 10 to 20 times higher and to levels of certain congeners of polychlorinated dibenzofurans that were 100,000 times higher than background levels, the IQ score was only 5 points lower than that in unexposed children."
>
> "Several methodologic flaws cast doubt on the validity of the Jacobsons' findings. . . . For example, descriptive statistics were given for 178 children according to PCB concentrations in maternal milk in Figure 1, yet PCBs were measured in only 113 samples of maternal milk. No data were presented to support the value

of PCB concentrations in breast milk as an adequate measure of prenatal exposure."

"Data on two important risk factors and potential confounders, alcohol ingestion and cigarette smoking appear to be inconsistent. Although 37 percent of the mothers smoked before and 28 percent during pregnancy, virtually none drank during pregnancy (shown in Table 1 of the article). In contrast, data from the Behavioral Risk Factor Surveillance Survey System suggested that a high proportion (14 to 21 percent) of women of childbearing age in Michigan consumed alcohol frequently. Furthermore, the method used to control for potential confounders may not be adequate. The authors stated that a variable's "association with either exposure or outcome can be used as the criterion for inclusion" in a model. However, standard epidemiologic analytic methods do not advocate the use of this method for model development."

"Given these methodologic issues, we think that this study provides little evidence that in utero exposure to low levels of PCBs affects intellectual function." [emphasis added]

7. Jacobson and Jacobson (1997b)

In this recent article by Jacobson and Jacobson (1997b), these authors apply their new "composite" score (see discussion above) for prenatal exposure to data from some of their previous studies. The correlations between fish consumption and PCB body burden were described as "moderate" due to the mother's inability to recall fish consumption accurately and the fact that they were exposed to PCBs from other sources. Jacobson and Jacobson remark that the new composite measure was supported by their finding that it correlated more strongly with maternal PCB-contaminated fish than any of its components (cord or maternal serum or milk levels), however, see the contradictions of this statement that were raised in Section 1.1.1.

Jacobson and Jacobson (1997b) report that the relationship of the composite exposure score and the McCarthy Verbal, Quantitative and Memory Scales is stronger than previous exposure surrogate, the cord blind PCB measure. However, when one reviews Figures 1 and 2 of this article, there is no true dose-response relationship between the new composite score and the McCarthy General Cognitive Index or the Memory Scale. Instead these figures indicate there is no change in response until the highest exposure group is reached, and the authors still fail to provide error bars for the histograms they provide. As the highest exposure group contains few individuals, the changes reported may reflect some spurious change caused by the limitations of group size rather than indicating a true PCB-related effect. Finally, as this paper recasts previously reported data using a new exposure index, and so does not represent new findings, the same problems inherent to earlier studies are also associated with this report.

B. The North Carolina or Rogan Cohort Studies

1. Rogan et al. (1986a, 1986b)

The North Carolina Breast Milk and Formula project is a prospective birth cohort study of 930 children born between 1978 and 1982. The purpose of the study was to determine how perinatal exposure to "background" levels of PCBs (or DDE) affected child development. Because some infants dropped out of the study, neonatal information was available for 912 infants. When the infant was born, samples of placenta, maternal cord and serum were collected as were samples of milk/colostrum. The Brazelton Neonatal Behavioral Assessment Scales (BNBAS) tests were administered within the first three weeks after birth. The children were seen again at 6 weeks, at 3, 6, 12, 18, and 24 months, then yearly. Covariates included in the analysis of birth weight included the infant's race and sex, maternal age, education and occupation, as well as alcohol consumption, smoking, maternal weight, and previous pregnancies. Covariates for head circumference were the same as for birth weight, plus the covariate of birth weight. Covariates for the BNBAS analysis included the mother's age, education, and occupation, and smoking and drinking indicators, as well as whether the mother consumed sport fish during the pregnancy or had general anesthesia during delivery. The infant's sex and birth weight were included.

The details of the study design, a description of the cohort and the levels of chemical contamination were published by Rogan et al. (1986b). Samples of breast milk, cord serum, maternal serum (birth and 6 wks), and placenta were analyzed for PCB content. 88% of the cord serum PCB levels were below quantitation limits (i.e., <4.27 ppb). Because the investigators were unable to determine PCB levels in a significant number of cord serum samples, breast milk concentration at birth was chosen as the value to be used as an indication of *in utero* exposure. This value was calculated by combining all the values of samples from a given women at various time points after parturition and then estimating the amount of chemical in breast milk at birth. This correlation was used to determine the concentration for each woman, and in turn, this value was used as the exposure level for her child. The mean calculated (estimated) concentration of PCBs in milk at birth was 1.74 ppm with a maximum of 15.83 ppm.

Based upon breast milk PCB and DDE levels, exposure was characterized as being "*probably as high as is encountered in the general population.*" However, the reported breast milk levels appeared higher-than-average, leading to the authors' fourth suggestion that they were "*probably at the high end of nonoccupationally exposed* [persons]."

There was no association between PCB (or DDE) levels and birth weight, head circumference, or hyperbilirubinemia. Only BNBAS test scores related to tonicity and reflexes were correlated with PCB and DDE levels. When analysis was restricted to testing conducted within the first three days after birth, as recommended for this test, PCBs were significantly associated only with hyporeflexia. Attributing hyporeflexia solely to PCBs is inappropriate because DDE was also significantly correlated with hyporeflexia. An additional consideration, as the authors note, is that the results of these tests are generally not strongly predictive of later findings and therefore do not discriminate between transient and lasting behavioral abnormalities. Important remarks made by the study authors include the following:

> "The multiple regression analyses indicated that there was no association between PCB or DDE levels and birth weight. ... There was also no association between head circumference and PCB or DDE levels."

and,

> "The exposure of these infants was probably as high as is encountered in the general population... Direct comparisons of levels in our subjects with those in other studies are difficult, because chemical analytical methods differ, but our levels overlapped or were higher than those in women who consumed potentially contaminated fish. Thus these North Carolina infants were probably at the high end of the nonoccupationally exposed, more on the basis of geographic location than anything specific. The generally higher levels of organochlorine compounds seen in the southeastern United States is unexplained."

and,

> "The only neonatal effects that we could demonstrate were hypotonicity and hyporeflexia on the BNBAS examination. The question, then, is whether such effects are lasting or whether they disappear after the newborn period. The BNBAS has been described as a snapshot of the newborn infant, and it has not generally been strongly predictive of later findings."

Remember also that in their Environmental Health Criteria Document for PCBs, the World Health Organization (1993) had the following criticisms of using the Brazelton test on infants (although these comments were applied to the Jacobson studies they are also relevant here):

> "The Brazelton test was used in these studies. However, this test was never intended to be used to evaluate neurological conditions. The value of this test to predict behavioural abnormalities in infants is small. The Public Health Council of the Netherlands (1985) concluded, therefore that the reported changes could not be interpreted [*sic*] by the Brazelton test."

2. Gladen et al. (1988)

In a follow-up study of the children studied in Rogan et al. (1986a), Gladen et al. (1988) [and later Rogan and Gladen (1991) (see below)] measured infant cognitive and motor development by administering the Bayley Scales of Infant Development. The participants consisted of a volunteer sample of 858 infants, of whom 802 had Bayley Scales scores at 6 or 12 months of age. Tests were administered at 6 and 12 months of development. A number of covariates were used in the regression model (see Table 1 on page 992 of the paper).

Gladen et al. (1988) identified a decrease in Psychomotor Development Index (PDI) but not in the Mental Developmental Index (both components of the Bayley Scales of Infant Development) at 6 and 12 months of age. The PDI assesses both fine and gross motor coordination. The decrease in the PDI was associated with transplacental exposure to PCBs, and

the lower scores appeared to be dose-dependent (fetal exposure extrapolated from milk fat-associated PCBs). This effect was only minor however as was pointed out by the investigators who state:

> "Clinically, the relatively small differences we observe in developmental test scores are undetectable; differences of this magnitude can easily be seen when a single child is retested."

Exposure to PCBs was through breast feeding was not related to the Bayley scores. As will be shown later follow-up studies in this cohort, these deficits did not persist past age two.

3. Rogan and Gladen (1991)

This is another follow-up study of the North Carolina cohort children. This study reports the findings of the evaluation at age 18 and 24 months. In this study, 676 children were tested at 18 months of age and 670 were tested at 24 months of age. Again, authors examined the relationship between Bayley scores and exposure to PCBs and DDE by linear regression, adjusting for sex, race, age at examination, number of older siblings, maternal age, maternal education, maternal occupational grouping, maternal smoking, different examiners, and the mothers' usual level of alcohol consumption.

At 18 and 24 months, adjusted scores on the PDI of the Bayley Scales were 4 to 9 points lower among children in the top 5th percentile of transplacental PCB exposure, and this effect was significant at 24 months. There was no significant effect on PDI at 18 months of age. Authors reported no consistent effect from exposure to PCBs (or DDE) through breast milk. The effects of transplacental PCBs on the MDI were also small. However, the authors note that the effects on the PDI at 24 months were larger and similar to those seen at 6 and 12 months of age.

Rogan and Gladen (1991) concluded that there was a *"small delay in motor maturation attributable to transplacental exposure to PCBs that is still detectable at 24 months. There is no evidence of an effect from the larger but later exposure through breast milk..."* In the Discussion section of the paper, the authors admit that *"It is not possible to rule out uncontrolled confounding, or some other bias..."*; thus, the authors themselves recognize the small changes they have observed are not necessarily the result of PCB exposure but may stem for other factors not identified and adequately controlled for in this study.

4. Gladen and Rogan (1991)

Again, this study represents another follow-up study of the North Carolina cohort first studied by Rogan et al. (1986). The cohort at the time of this study was 5 1/2 to 10 1/2 years of age. In this study, a volunteer sample of 859 children, 712 of whom had been examined with the McCarthy Scales of Children's Abilities at 3, 4, or 5 years, with 506 sending report cards. McCarthy scores were analyzed by analysis of covariance. Covariates included were identity of the examiner, maternal age, race, occupation, education, smoking, drinking, child's gender, number of older siblings, and feeding pattern (bottle fed or breast-feeding of short, medium, long, or very long duration).

Authors found in this follow-up that the effects reportedly detected in their earlier studies were no longer evident at age 3, 4, or 5 years. Neither transplacental nor breast feeding exposure to PCBs (or DDE) was related to McCarthy scores at ages 3, 4, or 5. Similarly, the authors found no significant relationship between PCB or DDE exposure by either route and performance in school (based on report card grades). The negative results of this follow-up study are discussed by the authors:

> "Neither transplacental nor breast-feeding exposure to PCBs or DDE affected McCarthy scores at 3, 4, or 5 years. There was no statistically significant relationship between poorer grades and PCB or DDE exposure by either route."

and,

> "The deficits seen in these children on the Bayley Scales of Infant Development through 2 years of age are no longer apparent."

and,

> "In these data the association of prenatal PCB exposure with delayed development, seen previously up to 2 years of age in these children, does not persist. We were also unable to confirm an association, seen in a similar study in Michigan, between prenatal PCB exposure and scores on the McCarthy Memory and Verbal scales to 4 years of age. We saw no consistent associations between exposure to either PCBs or DDE through breast-feeding and the McCarthy Scales, nor did we see any significant associations with our measures of school performance." [emphasis added]

and,

> "Thus, despite the difficulties posed by negative findings, we find the results from this large and reasonable well documented cohort to be reassuring."

With respect to the findings of this study, Schantz (1996) remarks:

> "Despite the early deficits in psychomotor performance on the Bayley Scales, there was no indication of a relationship between PCB exposure and scores on the McCarthy Motor Scale. This scale is not an exact analogue of the Bayley Psychomotor Scale, but it is similar in that it uses common, age-appropriate tasks to assess motor function. These findings suggest that the initial delay in psychomotor development associated with transplacental PCB exposure does not persist beyond 2 years of age. There was no indication of a relationship between PCB exposure and scores on the McCarthy Memory Scale. Thus, these authors were not able to confirm the relationship between prenatal PCB exposure and short-term memory deficits reported by Jacobson, Jacobson, and colleagues." [emphasis added]

> "In summary, Rogan and Gladen did not confirm the Jacobson's findings of decreased birth size and weight, or impaired short-term memory function, but did find evidence of a delay in psychomotor development in their most highly PCB-exposed children. The study appears to be methodologically sound in most respects. The sample was large enough to provide good statistical power and attrition was low. A comprehensive list of covariates that might be expected to influence developmental outcomes was included in the regression models..." [emphasis added]

and,

> "Decreased birth weight and head circumference and deficits in short-term memory functioning were observed in the Michigan cohort, but not in the North Carolina cohort. It has been suggested that these differences may be due to differences in exposure. Unfortunately, different analytical techniques were employed in the two studies, making it impossible to directly compare exposure levels. The reported exposure levels for the North Carolina cohort were nearly double those for the Michigan cohort, but there is good evidence that the analytical technique used in the North Carolina study overestimated PCB exposure by a factor of about two. If the North Carolina data are corrected for this overestimation, the actual exposure levels are very similar to the exposure levels in the Michigan study, not lower than the Michigan levels as has been previously suggested. Thus, it is unlikely that the differences in the level of PCB exposure can account for the differences in outcome. ... A related possibility is that the effects observed in the two Michigan cohorts were associated with other, unmeasured contaminants that were present in fish and covaried with PCBs. That is, perhaps PCBs were merely a marker for some other highly lipophilic compound such as methylmercury or chlorinated dibenzodioxins. This seems plausible when one considers the effect on birth weight. The effect seen in the Michigan cohort was of a size (160g) that should have been easily detectable in the larger North Carolina cohort, yet there was not even a suggestion of an effect on birth weight in that cohort. ... Finally, it is possible that inadequate control of confounding variables such as maternal prepregnancy weight, alcohol consumption, and smoking in the Michigan study resulted in spurious findings." [emphasis added]

C. The Oswego, New York, Cohort

This another prospective epidemiological study that is on-going (Oswego Newborn and Infant Development Program) and consists of children who had maternal exposure to PCBs from exposure to Lake Ontario fish (high-exposure group n = 152; low-exposure group n = 243; control group n = 164). It should be noted again, however, that there are other important developmental contaminants in Lake Ontario fish including dibenzodioxins, dieldrin, lindane, chlordane, cadmium, mercury, and mirex. In this preliminary report by Lonky et al. (1996), no effect of PCB exposure on physical development was found, but children in the high exposure group tested with the Brazelton Neonatal Behavioral Assessment Scale reportedly had more

abnormal reflexes and less mature autonomic responses. There apparently was no significant effect on other NBAS cluster scores: orientation, motor, range of state, or regulation of state.

Table A-5: Group Means and Standard Deviations for NBAS 12-24 hr (T1) and 25-48 hr (T2) Cluster Scores for # Abnormal Reflexes and Autonomic Scores

	High Fish		Low Fish		No Fish Control	
Cluster	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
# Abnormal Reflexes	4.09 ± 2.39	3.34 ± 2.20	3.50 ± 2.22	2.10 ± 1.85	3.62 ± 1.92	1.92 ± 1.77
Autonomic	5.90 ± 1.27	5.49 ± 1.19	6.14 ± 1.19	6.26 ± 1.12	5.96 ± 1.36	6.32 ± 1.18

* For number of abnormal reflexes higher scores reflect poorer performance. For Autonomic scores, higher scores indicate better performance.

None of the Z-scores for these clusters scores were calculated to be significant. It should be noted that this is the first report of this cohort and it is based only on assessments made in the first 48 hours after birth. Limitations of this study's findings are noted by the authors:

> "...the importance of these findings is mitigated by several unknowns at this time. First, while the NBAS has demonstrated utility in studies of high-risk infants and in studies of effects of obstetric medication and effects of maternal substance abuse (Brazelton et al., 1987), evidence for the predictive validity for later behavior of NBAS scores in non at-risk populations is limited. ... we are at this time not well prepared to hypothesize what the long-term significance of the present NBAS findings might be."

> "Relationships between NBAS scores and total PCBs, PCB congener patterns, DDE, HCB, lead, and mercury in cord blood of babies and hair of the mother in high fish and no fish control groups awaits analysis."

There was no significant difference between PCB groups and controls on the Ballard Neuromuscular scores (Fein et al., 1984 reported significant differences between infants of non-fish eaters and infants of fish eaters on this score). While the authors did not report significant differences between groups for alcohol consumption, it appears that there was quite a difference between groups for beer consumption: no fish control group, 4.32 ± 16.4 units; low fish group, 7.69 ± 28.7 units; and high fish group, 10.51 ± 46.5 units. Given these differences, and the differences in medication and caffeine use reported in the Michigan fisheater study, it is possible that use of medications during childbirth, a factor that is appears was not controlled for by the authors, may have been responsible for some of the changes among populations that were observed.

D. Summary and Conclusions

A series of cross sectional studies were initiated in the early 1980s that sought to investigate the developmental effects in humans as a result of environmental exposure to PCBs. This series of studies, commonly referred to in the literature as the "Jacobson" or "Michigan Fisheater" studies, have consistently reported to have found both physical and cognitive deficits in the more highly exposed individuals. The North Carolina cohort, a much larger cohort with reportedly higher PCB serum levels, failed to confirm the physical changes and permanent cognitive effects reported by Jacobson. As the Jacobson studies have been criticized for several methodological shortcomings (World Health Organization, 1993; Expert Report on Polychlorinated Biphenyls, 1994; ATSDR, 1995; Swanson et al., 1995; Schantz, 1996; Seegal, 1996b; ATSDR, 1997; Kimbrough, 1997), no causal association between PCB exposure and developmental effects can be established based on the results of these studies alone (Expert Report on Polychlorinated Biphenyls, 1994). Accordingly, the Expert Report on Polychlorinated Biphenyls evaluated the findings reported by Jacobson against the criteria for establishing causation and reached the following conclusions:

Regarding the Strength of the Proposed Association They Identified Four Limitations

(i) There are a number of inconsistencies in the proposed dose-response relationship between exposures to PCBs and the effects reported. The authors suggested that PCBs in the fish were the cause of the reported effects although no correlation was observed between fish consumption and concentrations of PCBs in umbilical cord blood serum. ... The concentrations of PCBs in cord serum at birth, as reported by Fein et al. (1984), were correlated with decreased cognitive performance in the children at 4 years of age; however, there was no correlation between concentrations of PCBs in cord serum and maternal fish consumption (the proposed source of PCBs). In addition, the possible confounding effects of socioeconomic factors, smoking tobacco, alcohol consumption, and other lifestyle factors known to affect cognitive performance were not adequately addressed. ... Also, the number of inconsistencies in the reported analyses for PCBs in maternal and cord blood sera, and in breast milk, affect the confidence of the overall evaluation of possible dose-response relationships. It appears that analyses for PCBs were not conducted on serum samples with lipid concentrations less than 200 mg/dl. This decision could bias the interpretation of subsequent correlations with adverse effects in an undetermined manner since the PCBs in blood are associated with blood lipids. ... The deficiency in the criteria for a dose-response relationship, a basic requirement for establishing causality (Hill 1965; Fox 1991), and the lack of substantial differences between the concentrations of PCBs in the study and in the general population, indicate that the effects reports on human development in the populations studied were not causally related to exposures to PCBs.

(ii) The second factor affecting the strength of the proposed association is that the women from the elevated fish consumption group also reported significantly greater consumption of alcohol, caffeine, tobacco, and cold remedy prescription use during pregnancy than those consuming less fish. ... All these confounding factors seriously detract from the biological plausibility of an association between the amount of exposure to PCBs, as indicated by the concentrations of PCBs in blood, and effects on infant development.

(iii) A third factor affecting the strength of the proposed association is the lack of plausibility and consistency of association that are required (Hill 1965; Fox 1991) to establish a causal relationship. . . . These factors seriously weaken the plausibility of associations between the effects reported by Fein et al. (1984) and exposures to PCBs. . . .

. . . Also, a total of approximately 38 behavioral and neurological tests were conducted on the children, even though the results of only 2 tests were reported to be affected. Some of the association based on chance alone would be expected from this large number of tests. In addition, the assessment of the test results was based on a "clustering" approach, which is not a standard procedure in evaluating neurological test results from children. . . .

. . . The plausibility of the proposed association is further degraded by inconsistencies in the information reported in different publications of the same studies. Jacobson et al. (1990a) reported, based on Fein et al. (1984), that prenatal exposure to PCBs through mothers consuming fish was associated with decreased birthweights. The same children, assessed 4 years later, had greater serum concentrations of PCBs (attributed mainly to exposures during lactation) than those from mothers consuming lesser quantities of fish, and were also reported by Jacobson et al. (1990b) to have reduced activity levels. This result, however, is not in accordance with the findings of Jacobson et al. (1990a) that the children who had longer lactational exposure had no cognitive deficits when compared to children with short lactational exposure. In fact, prior to the controlling of confounding variables, the children with longer lactational periods tended to have greater scores on the McCarthy Scales performance tests (Jacobson et al., 1990a). ...

(iv) The fourth factor affecting the strength of the proposed associations is that the criteria of consistency of observation that is an essential component in the establishment of causality of association based on epidemiological data (Hill, 1965; Fox, 1991) is not met by the studies reported by Fein et al. (1984) and Jacobson et al. (1990a, 1990b).

Given the fact that the Expert Report found the Jacobson studies provided only a weak and frequently implausible association, with inconsistent or deficient dose-response relationships, and is an association for which consistency with other studies cannot be demonstrated, it is not

surprising the Panel concluded a causal association cannot be established for the purported effects:

> "Based on the above analysis, and considering the marginal significance of the observations, the information reported by Fein et al. (1984), Rogan et al. (1986a,b) and Jacobson et al. (1990a,b) do not meet the criteria for the establishment of a causal association for an effect of PCBs on growth and behavior in human populations." (Expert Report on Polychlorinated Biphenyls, 1994; emphasis added)

As indicated by the extensive quotations by other scientists that were provided in the summarization of this literature, it is clear that a considerable number of scientists have reviewed these studies and have noted the lack of consistency, the failure to control for confounders, the experimental design problems inherent to the Jacobson studies, and the fact that the North Carolina cohort and other studies have failed to confirm the findings initially reported by Jacobson. Therefore, like other scientists, it is the conclusion of this review that no causal (or even suggestive) association currently exists between PCB exposure and permanent neurodevelopmental effects in humans.

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MORTALITY AMONG WOMEN WORKERS EXPOSED TO PCB

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This study covers the mortality rate of workers of an industrial plant in the town of Monza that since 1946 has been manufacturing condensers and large phase advancing conductors that are impregnated with PCB. The workers manufacturing such products are largely women. The first environmental measurements of PCB levels in the plant were carried out in 1954, after three cases of chloracne were noticed among the workers operating the autoclaves. The exposure period of the workers had been rather short: between four and seven months. The measurements revealed levels of "Aroclor 1254" between 5,200 to 6,800 micrograms per cubic meter.

Subsequent determinations in 1977 showed concentrations in the environment that were much reduced with respect to the preceding years (between 48 and 275 micrograms per cubic meter). Furthermore, in the same year and later in 1982, additional determinations of that substance were made on the work surfaces and on the palm of the hands of the workers, in order to evaluate another possible absorption avenue, the one through the skin, in view of the well known liposolubility of PCB. The results, obtained with the same analytical method in both years, showed a reduction in the exposure during that period of time, but also a persistence, since the utilization of PCB had been terminated in 1980.

The population under study is composed of all the workers in every department of the plant, including the administration department. Such decision was made in view of the ubiquitous contamination of the surfaces in the plant, including those in the offices located in the same building. The minimum working period for inclusion was one week, since it was not possible to obtain reliable data for shorter periods. Short exposures are of interest *a priori*, in view of two cases of chloracne observed in workers exposed for periods of just a few months. Therefore, our study includes all workers with at least one week of actual working period from 1946 to 1982, for a total of 1556 women who added up to a total of 44,328.5 person-years. Their mortality rate was studied for the period 1954-1991. For each worker we have collected information, with regard to vital statistics and to working statistics, through the company records, whereas their present conditions (whether dead or alive) were ascertained through a search in the vital statistics offices of the cities of residence and birth. For the dead workers, the cause of death was requested as reported in the forms of the National

Institute of Statistics, and the initial causes were codified according to the VIII Revision of the International Classification of Illnesses and Causes of Death [I.C.D.].

Table I Mortality Rate of Women Workers Exposed to PCB.

Cause of Death (I.C.D.)	Observed	Expected	SMR	IG 95%
All causes	47	34.4	137	100-182
Malignant tumors (140-209)	19	16.1	118	71-184
Lymphatic and hemopoietic tumors (200-209)	5	3.5	141	46-330
Lymphatic (200-202)	4	2.3	177	48-153
Circulatory system diseases (390-458)	3	4.5	67	15-195
Respiratory system diseases (460-519)	2	1.1	188	21-681
Hepatic cirrhosis (571)	2	1.1	184	21-662
Accidents and traumas (800-999)	12	3.7	327	169-571

[SMR is the ratio of observed over expected cases times 100]

Table II. Characteristics of Deaths Caused by Lymphatic-hemopoietic Tumors.

Type and Seat of tumor (Revision I.C.D. 8)	Age at Start of Employment	Year of Start of Employment	Length of Exposure	Latency	Age at Death
Lymphosarcoma (200)	24	1968	0.7	2	26
Hodgkin lymphoma (201)	19	1949	12.0	15	34
Hodgkin lymphoma (201)	17	1960	1.0	1	18
Other neoplasia of lymphatic system (204)	16	1950	17.0	33	49
Lymphatic leukemia (204)	34	1962	6.0	26	60

Note: All data are expressed in years.

Follow-up reached the 99% completion level: only 16 workers could not be traced. The analysis considers them "alive" for the purposes of the study. We calculated then the specific rates for each cause, by age and by calendar period, and compared them with those of the general population of Monza (about 100,000 people), where the plant is located. Such point of reference was chosen in order to indirectly decrease the influence of eventual confusing factors of micro environmental, socioeconomic and cultural types. Furthermore, while the number of individuals in the population of Monza is relatively low, it is not so low to cause the reference rates to be unreliable.

For the purpose of evaluating the exposure of a single individual, in view of the ubiquitous nature of the contamination in the working environment, we have utilized the length of the working period in the plant as the exposure index.

The results (Table I) indicate an increase in the female population of mortality rates for "All causes", with a SMR of 137. We also observed a modest increase in "Malignant tumors" with a SMR = 118. This increase, in particular, is manifested in the "Lymphatic and hemopoietic tumors". For these the SMR is 141, with 5 cases observed versus 3.5 cases expected. Four

of these five neoplasia were of lymphatic origin versus 2.3 expected, with a SMR of 177. When we examine in detail these 5 neoplasia (Table II), we can notice that 3 out of 5 cases appeared in individuals who had an exposure longer than 5 years and a latency greater than 10. All dates of beginning of employment were before 1969. Considering the non-neoplastic causes, we can observe that the SMR for "Diseases of the Cardio-Circulatory System" is clearly reduced, with a value of 67; instead the risks of "Diseases of the Respiratory System", with a SMR of 188, and "Cirrhosis", with a SMR of 184, appear to have increased: both are based on two cases only. The most abnormal piece of data in this population is the high mortality rate for "Accident and Traumas", having a SMR of 326.6 with high statistical significance. We are faced with 12 observed cases versus 3.7 expected, and on close examination we note that two are "electrocutions", one of which surely happened in the work place, whereas most deaths were due to traffic accidents. With regard to traffic accidents we could hypothesize that the workers had more opportunities to travel and to utilize means of transportation. But when we make the comparison with the male population for the same period under examination in the present study, we can note some differences that are shown in Table III.

Table III. Comparison Between Mortality Rates of Women and Men Exposed to PCB,

Cause of Death (I.C.D.)	Female			Male		
	Obs/Exp	SMR	IC 95%	Obs/Exp	SMR	IC 95%
All causes	47/34.4	137	100-182	45/55.7	81	59-108
Malignant tumors (140-209)	19/16.1	118	71-184	20/18.4	109	67-168
Digestive system tumors (150-159)	2/2.2	92	10-333	10/5.1	195	94-359
Lymphatic and hemopoietic tumors (200-209)	5/3.5	141	46-330	3/1.5	202	41-591
Cardio-circulatory system diseases (390-458)	3/4.5	67	13-195	10/16.8	59	28-109
Respiratory system diseases (460-519)	2/1.1	188	21-681	2/2.0	98	11-355
Hepatic cirrhosis (571)	2/1.1	184	21-662	3/3.1	98	20-285
Accidents and traumas (800-999)	12/3.7	327	169-571	9/9.0	100	40-189

* $p < 0.05$

First of all, under "Death from all Causes" males clearly show a decrease in risk, which may be attributed to the well known *"Healthy Workers Effect"*, not evident in the female population. There are also differences with regard to the "Digestive System Tumors", that are high in the male population and comparable to the reference population in the female group, and in the last three sets of causes (Respiratory System Diseases, Hepatic Cirrhosis, and Accidents and Traumas) that, as we have seen, have quite high SMR values for the women but values close to 100 for the men. "Malignant Tumors" tend to have slightly higher values also for the men, whereas lymphatic and hemopoietic tumors carry a decidedly elevated risk. In conclusion, we can state that working women present elements of selectivity in comparison with the general female population. Sufficient information is not available to permit to identify the factors of such a selectivity. In our case, if we observe the mortality rate, a qualifying factor is certainly the workers' exposure. The generally higher mortality rate of

our population is however mainly due to the excess in deaths due to "Accidents and Traumas". This piece of data is interesting because it compels us to consider risk factors that are not exclusively inside the working environment, but also those socioeconomic factors that are tied to the working conditions of women. With regard, on the other hand, to the elevated risk for tumors of the lymphatic and hemopoietic tissues, such risk is consistent with data from experimental and epidemiology studies, and is evident, as noted above, also in the male population of our investigation.
It is not however possible, at least because of problems of population size and of lack of data on internal exposure, to define a *per se* biologically plausible correlation between such an increase and exposure to PCB.

Epid Prev 1996: 20: 200-202

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CERTIFICATION

This is to certify that the attached translation, namely:

- Mortality among women workers exposed to PCB,

is a true and accurate translation from Italian into English to the best of my knowledge and belief.

Allen Cooperman
1/21/99

ep

MORTALITÀ DI LAVORATRICI ESPOSTE A PCB

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MORTALITY AMONG WOMEN WORKERS EXPOSED TO PCB

Lo studio riguarda la mortalità dei lavoratori di un'industria di Monza che dal 1946 ha prodotto condensatori e grassi conduttori di rifasamento impregnati in PCB. Gli addetti a questa produzione erano in larga maggioranza donne.
Le prime misurazioni ambientali dei livelli di PCB nella fabbrica furono eseguite nel 1954, dopo segnalazione di tre casi di cloracne, tra i lavoratori addetti alle autoclavi con un periodo di esposizione piuttosto breve (da 4 a 7 mesi). Tali rilievi evidenziarono valori di "Aroclor 1254" da 5.200 a 6.800 $\mu g/m^3$.
Le indagini successive, eseguite nel 1977, mostrarono concentrazioni ambientali notevolmente ridotte rispetto alle precedenti (tra 48 e 275 $\mu g/m^3$); inoltre nello stesso anno e nel 1982 furono effettuate altre misurazioni della sostanza sulle superfici di lavoro e sul palmo delle mani dei lavoratori, per la valutazione di un'altra possibile via di esposizione, quella cutanea, data la nota liposolubilità dei PCB. I dati, ottenuti con lo stesso metodo analitico nei due anni, evidenziarono una riduzione delle esposizioni nel corso del tempo, ma anche una sua persistenza, dato che l'utilizzo di PCB era cessato nel 1980.

La popolazione studiata è costituita dagli addetti a tutti i reparti della fabbrica, compresa l'area amministrativa: tale scelta è stata fatta tenendo conto della contaminazione ubiquitaria delle superfici nell'industria, inclusi gli uffici, siti nello stesso fabbricato. Il minimo periodo di attività lavorativa stabilito è di una settimana, poiché per periodi inferiori non è stato possibile ottenere dati attendibili. Esposizioni brevi sono a priori di interesse, dati due casi di cloracne osservati in lavoratori esposti per pochi mesi. Sono stati inclusi nello studio, quindi, tutti i lavoratori con almeno una settimana di impiego dal 1946 al 1982, per un totale 1.556 femmine che hanno contribuito a produrre 44.328,5 anni-persona. La loro mortalità è stata studiata nel periodo 1954–1991. Per ciascun dipendente sono state acquisite informazioni di tipo anagrafico e relative alla storia lavorativa attraverso i registri di fabbrica, mentre lo stato in vita è stato accertato con una ricerca presso gli uffici anagrafici dei comuni di residenza o di nascita. Per i lavoratori deceduti è stata inoltre richiesta la causa di morte come riportata su modulo ISTAT e la causa iniziale è stata codificata secondo l'VIII Revisione della Classificazione Internazionale

Tabella I. Mortalità di lavoratrici esposte a PCB.

Causa di morte (I.C.D.)	*Osservati*	*Attesi*	*SMR*	*IC 95%*
Tutte le cause	42	34,1	1,57	[illegible]
Tumori maligni (140-209)	17	14,1	1,16	[illegible]
Tumori linfatici ed emopoietici (200-209)	5	3,5	1,41	[illegible]
Linfatico (200-202)	4	2,3	1,77	[illegible]
Malattie apparato cardiocircolatorio (390-459)	3	4,5	0,67	[illegible]
Malattie apparato respiratorio (460-519)	2	1,1	1,88	[illegible]
Cirrosi epatica (571)	2	1,1	1,84	[illegible]
Accidenti e traumatismi (800-999)	12	3,7	3,27	[illegible]

Tipo e sede di tumore (ICD 8ª revisione)	Anni alla assunzione	Anno di assunzione	Durata di esposizione	Latenza	Casi osservati
Linfosarcoma (200)	24	1968	0,7	2	26
Linfoma di Hodgkin (201)	19	1969	12,0	15	34
Linfoma di Hodgkin (201)	17	1960	1,0	1	18
Altre neoplasie della serie linfatica (202)	16	1950	17,0	33	49
Leucemia linfatica (204)	34	1962	8,0	26	60

Nota: tutti i dati sono espressi in anni

delle Malattie e delle Cause di Morte.
Il follow-up ha raggiunto il 99% di completezza: solamente 16 lavoratrici non sono state rintracciate. Nell'analisi esse sono state considerate "vive" alla fine dello studio. Sono quindi stati calcolati i tassi specifici per ogni causa, per età, per periodo di calendario, e confrontati con quelli della popolazione della città di Monza (circa 100.000 abitanti), dove sorge l'impianto. Tale riferimento è stato scelto per diminuire indirettamente l'influenza di eventuali fattori di confondimento di tipo macroambientale, socioeconomico e culturale. Inoltre la numerosità della popolazione di Monza è ridotta, ma non tanto da rendere inattendibili i tassi di riferimento.
Al fine della valutazione dell'esposizione individuale, considerata la contaminazione ubiquitaria degli ambienti lavorativi, è stata adottata come indice di esposizione semplicemente la durata del periodo di impiego nell'azienda.
I risultati (Tab. I) indicano un'aumentata mortalità nella coorte femminile per "Tutte le cause" con un SMR di 137. È osservabile anche un modesto aumento per "Tumori maligni" con SMR=118. Tale aumento è sostenuto in particolare da "Tumori linfatici ed emopoietici" per i quali l'SMR è di 141, con 5 casi osservati contro 3,5 attesi. Quattro di queste cinque neoplasie erano di origine linfatica contro 2,3 attese, con un SMR di 177. Osservando nel dettaglio queste 5 neoplasie (Tab. II) possiamo notare che 3 dei 5 casi compaiono tra coloro che hanno un'esposizione superiore a 5 anni e latenza maggiore di 10. Tutte le date di assunzione erano precedenti al 1969. Considerando le cause non neoplastiche possiamo riscontrare un SMR per "Malattie dell'apparato cardiocircolatorio" chiaramente ridotto, con un valore di 67; sembra invece aumentato il rischio per "Malattie dell'apparato respiratorio" con un SMR di 188 e per "Cirrosi" con un SMR di 184, entrambi basati su due casi. Il dato più abnorme in questa coorte è l'elevata mortalità per "Accidenti e traumatismi" che presenta un SMR di 325,6 con significatività statistica. Si tratta di 12 casi osservati contro 3,7 attesi e, osservando nel dettaglio, possiamo notare che due di essi sono "elettrocuzioni", di cui una sicuramente avvenuta in ambito lavorativo, mentre la maggior parte dei decessi è attribuibile ad incidenti del traffico. Per quanto riguarda gli incidenti del traffico, si può a questo proposito ipotizzare che le lavoratrici abbiano più frequenti opportunità di viaggiare e di usare mezzi di trasporto.
Effettuando, invece, il confronto con la coorte maschile per lo stesso periodo considerato in questo studio, si notano alcune differenze che possiamo osservare in Tabella III: prima di tutto nella "Mortalità totale" i maschi mostrano una netta diminuzione di rischio attribuibile al noto "Healthy Workers Effect", non evidenziabile, invece, per la coorte femminile; differenze sono presenti anche per i "Tumori dell'apparato digerente" che si mostrano molto elevati nella coorte maschile, mentre sono paragonabili al riferimento in quella femminile, e per gli ultimi tre raggruppamenti di cause che sono "Malattie dell'apparato respiratorio", "Cirrosi" e i noti "Accidenti e traumatismi" che, come abbiamo visto, presentano SMR particolarmente elevati nelle donne e hanno, invece, valori vicini a 100 nella coorte maschile. Per quanto riguarda i "Tumori maligni", sono leggermente tendenti all'aumento anche nella coorte maschile, con un rischio decisamente elevato per tumori linfoemopoietici.
In conclusione possiamo affermare che le donne che

Tabella III. Confronto mortalità femmine/maschi esposti a PCB.

Cause di morte (I.C.D.)	Femmine OM	Femmine SMR	Femmine I.C. 95%	Maschi OM	Maschi SMR	Maschi I.C. 95%
Tutte le cause	47/34,6	137	101-182	45/55,7	81	59-108
Tumori maligni (140-209)	19/16,1	118	71-184	20/18,6	109	67-166
Tumori apparato digerente (150-159)	7/7,2	97	39-199	10/5,1	195	94-359
Tumori linfatici ed emopoietici (200-209)	5/3,5	141	46-330	3/1,5	202	41-591
Malattie apparato cardiocircolatorio (390-458)	5/4,5	67	15-195	10/10,8	93	45-169
Malattie apparato respiratorio (460-519)	2/1,1	188	21-681	2/2,0	98	11-353
Cirrosi epatica (571)	2/1,1	184	21-662	3/3,1	98	20-285
Accidenti e traumatismi (800-999)	12/3,7	327	169-571	9/8,0	113	52-214

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lavorato presentano elementi di selezione rispetto alla popolazione generale femminile. Non sono però disponibili informazioni sufficienti ad identificare i fattori di tale selezione. Nel nostro caso, studiando la mortalità, un fattore qualificante era senz'altro l'esposizione lavorativa. L'aumentata mortalità generale della nostra coorte è però principalmente motivata dall'eccesso riscontrato a carico delle morti per "Accidenti e traumatismi". Questo dato è interessante perché impone di considerare i fattori di rischio non esclusivamente all'interno dell'ambiente di lavoro, ma anche i fattori socio-economico-culturali legati alla condizione lavorativa delle donne. Per quanto riguarda, invece, l'elevato rischio per tumori a carico del tessuto linfoemopoietico, esso è consistente con i dati di studi sperimentali ed epidemiologici, oltre a comparire anche, come abbiamo visto, nella coorte maschile della nostra indagine.

Non è tuttavia possibile, se non altro per problemi di numerosità e di mancanza di dati di esposizione interna, definire un'associazione di per sé biologicamente plausibile tra questo aumento e l'esposizione a PCB.

EVALUATION OF THE TOXICOLOGY OF PCBs

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With assistance from

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Washington, D.C.

March 1, 1989

CONTENTS

C O N T E N T S
(continued)

I. EXECUTIVE SUMMARY

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Polychlorinated biphenyls (PCBs) are a class of chemical compounds that were widely used as lubricants and fluids in electrical equipment, among many other uses, until concerns about their potential hazards from environmental exposures arose in the 1970s. This report sets out an assessment of the available scientific literature concerning the hazards, particularly cancer, resulting from human exposure to PCBs. It was prepared under the guidance of a panel of internationally recognized experts in medicine, toxicology, and epidemiology and has been subjected to scientific peer review. (The curricula vitae of the panel are set out in Appendix A.) The panelists have unanimously endorsed the findings presented in the study.

The study focuses on whether low-level environmental exposure to PCB is likely to cause cancer in humans. The panel observes that other possible human impacts from low-level environmental exposures to PCBs are of far lower likelihood than any possible carcinogenic effects. Health effects, other than carcinogenicity, that have been observed following exposure of laboratory animals to PCB mixtures include liver and stomach lesions and reproductive effects. The panel notes that these effects, as well as skin lesions (i.e., chloracne) that have been reported in humans following exposure to very high levels of PCBs, occur at higher doses than those associated with risk levels of concern for potential carcinogenic effects. Thus exposure levels that pose no significant risk of cancer should not be of concern with regard to other health effects.

A variety of epidemiologic studies have examined whether PCB-exposed workers have incurred a greater likelihood of cancer mortality than the general population. The report summarizes the literature in the field. The panel concludes that the body of epidemiological evidence does not demonstrate a causal relationship between PCB exposure and any form of cancer. This conclusion is confirmed by reviews of several other expert groups, including the EPA, FDA, and the World Health Organization. In light of the long-term and widespread usage of PCBs in numerous industrial settings and the extensive exposure of workers in some cases, it is likely that evidence of carcinogenicity would have already been revealed by the studies if PCBs were in fact a potent human carcinogen.

Experiments have also been conducted with laboratory animals to estimate the potential impacts of PCBs on humans. In such studies the subject animals are chronically exposed over a long time period to high does of PCBs through, for example, the addition of PCBs to the animals' food. The studies show that, of the PCB mixtures that have been adequately tested, only certain mixtures -- commercial mixtures of PCBs containing approximately 60 percent chlorine by weight (Aroclor 1260 and Clophen A60) -- produce a statistically significant increase in the incidence of malignant liver tumors in rodents. In a study conducted by the National Cancer Institute, Aroclor 1254 (approximately 54% chlorine by weight) was not found to be carcinogenic. Other Aroclors have not been tested in acceptable animal bioassays for carcinogenicity. Clophen A30, which closely matches Aroclor 1242 in composition (approximately 42 percent chlorine by weight), has been tested; it causes an increase in benign (non-cancerous) liver tumors and is less potent than Clophen A60 when both mixtures were tested in bioassays of identical design.

Based on the fact that no statistically significant increase in malignant tumors has been seen in animal studies with lower chlorinated PCB mixtures, the panel concludes that, if the lower chlorinated PCB mixtures are carcinogenic at all, their potencies are far less than those of mixtures with 60 percent chlorine content.

The panel points out that animal studies have limitations as a reliable indicator of effects in humans. Because of interspecies differences in factors such as absorption, metabolism, and elimination of a test substance, effects that are observed in animals may not occur in human populations, or may occur with different frequencies. Moreover, in order to apply the results from animal studies to humans, it is necessary to make adjustments to account for the fact that environmental exposures of humans are many orders of magnitude less than those to which the rodents were exposed in the animal studies. There are substantial experimental data from which it can be inferred that the carcinogenic effects, if any, from PCB exposure arise only after a certain threshold exposure has been exceeded. Thus, at the low doses that are typical from human environmental exposures to PCBs, the panel concludes that no cancer risk may exist.

Despite the limitations of the animal data, it is common for regulatory agencies to estimate potential risks to humans by extrapolating observations in highly exposed animals to humans. The typical extrapolation procedure also assumes the absence of a threshold and a direct proportion between risk and exposure at low doses. Such an application of the animal data reflects a policy decision to adopt a conservative estimate of potential human risk from exposure, and it is the approach taken in this report. Even if this approach were applied to the data on PCBs, however, the panel concludes that it is necessary, at the least, to recognize the differences in potency among the various types of PCB mixtures. The report defines the appropriate adjustments for the various mixtures.

In summary, while the data show that some carcinogenic effects are observed in rodents that are exposed over a long term to high doses of 60 percent chlorinated PCB mixtures, these animal data have uncertain implications for human exposure. Moreover, no statistically significant increase in malignancies are seen in animals exposed to high levels of lower chlorinated PCB mixtures. Thus, even if the animal data were deemed relevant to the environmental exposure of humans, at the least, adjustments must be made for lower potency of the lower chlorinated PCB mixtures. In any event, the panel concludes that the evidence does not demonstrate a causal relationship between exposure to PCBs of any type and any form of human cancer.

II. INTRODUCTION

II. INTRODUCTION

Polychlorinated biphenyls (PCBs) are a class of chemical compounds that were widely used as lubricants and as fluids in electrical equipment, among many other uses, until concerns about their potential hazards arose in the late 1970s.

This report examines the available scientific data regarding the potential carcinogenicity of commercial PCB mixtures. It has been prepared by a group of independent experts who have been engaged to assist Texas Eastern and subjected to scientific peer review. The curricula vitae of the members of the group are set out as Appendix A.

This analysis is confined to an examination of the data regarding the potential tumorigenicity of PCBs because this is the toxicity endpoint of primary concern with regard to low-level, environmental exposures to PCBs. Other health effects demonstrated in animal models occur at higher doses than the doses associated with risk levels of concern for potential tumorigenic effects. Thus, the exposure levels that pose no significant risk for tumorgenicity from chronic exposure should not be of concern for other health effects.

III. SCIENTIFIC EVIDENCE RELATED TO THE TUMORIGENIC POTENTIAL OF PCBs

III. SCIENTIFIC EVIDENCE RELATED TO THE TUMORIGENIC POTENTIAL OF PCBs

A. Summary and Conclusions

This report provides a summary of epidemiological and toxicological data related to the potential tumorigenic effects of PCBs. The data reveal that there is insufficient evidence to classify PCBs as human carcinogens, i.e., the available epidemiological data do not show a causal relationship between PCB exposures and human cancer. This conclusion is supported by reviews conducted by several expert panels (e.g., EPA 1988, ATSDR 1987).

The data also show that certain mixtures of PCBs, those associated with Aroclor 1260,[1/] produce excess tumors[2/] of the liver in rodents in long-term feeding studies at very high doses. Because there is some equivocal evidence that other mixtures of PCBs may produce excess tumors in experimental animals, we cannot reject the hypothesis that other mixtures of PCBs (those associated with other Aroclors) are animal tumorigens. Nevertheless, it will be shown that, if these other mixtures of PCBs are tumorigens, they are certainly of lower tumorigenic potency (tumorigenic risk per amount of exposure) than those associated with Aroclor 1260.

1/ As will be discussed herein, there are a variety of different types of PCBs. Aroclor 1260 was a tradename for a particular PCB mixture containing 60 percent chlorine by weight. Lower chlorinated Aroclor mixtures (for example, Aroclors 1248 and 1242, 48 and 42 percent chlorine, respectively) were also widely used.

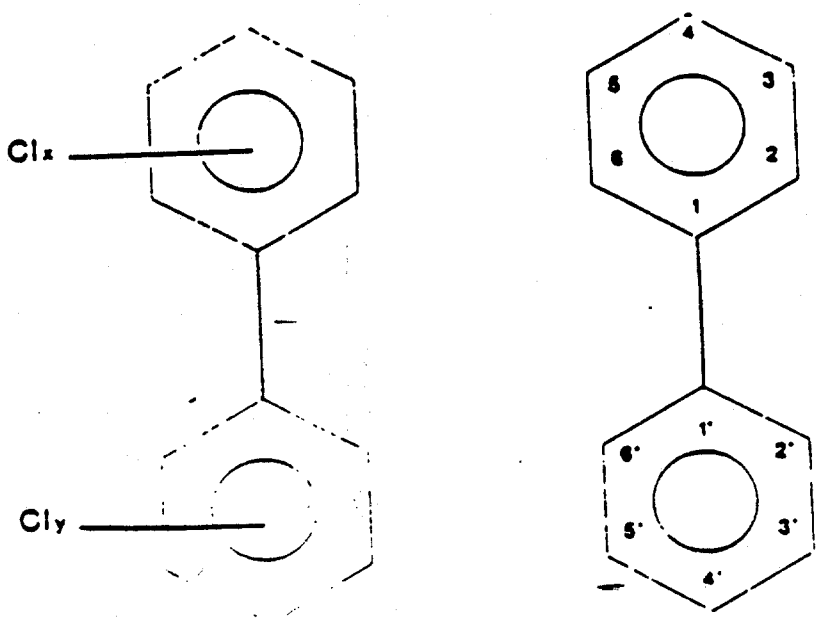
2/ The excess tumors may be either benign (non-cancerous) or malignant (cancerous). It is conservatively assumed by regulatory agencies that both types should initially be counted equally in estimating carcinogenic potency. PCBs are therefore referred to as tumorigens, i.e., capable of producing either type of tumor.

In the concluding part of this report, we shall present estimates of the tumorigenic potencies of the mixtures of PCBs associated with Aroclors 1260, 1254, 1248, 1242, and 1232. These potency values are included in this report because of their use in estimating the potential carcinogenic risk that might result from human exposure to these mixtures.

Before presenting a review of the data related to carcinogenicity, a discussion of the chemical compositions of various sets of PCBs is presented.

B. Chemical Composition of Aroclors

Polychlorinated biphenyls (PCBs) are mixtures of chemically related compounds. The various PCBs all share the same basic biphenyl (12-carbon) structure (figure 1) with a varying number of chlorine atoms. Up to ten of the carbon atoms of the biphenyl molecule can chemically bond (attach) to chlorine atoms. If only one chlorine atom is bonded to the biphenyl molecule, the product is referred to as monochlorobiphenyl. It is possible for the single chlorine atom to bond to carbon atoms in different positions in the biphenyl structure, and each such bonding creates a new chemical (figure 1). There are thus several different forms of monochlorobiphenyls that may have different properties. These different structures are referred to as monochlorobiphenyl isomers. Similarly, different isomers may be created when two chlorines are present (dichlorobiphenyls), when three chlorines are present (trichlorobiphenyls), etc., depending on the location of the chlorine atoms with reference to the carbon atoms in the biphenyl structure. PCBs that have different numbers of chlorines, e.g., 5 chlorines (pentachlorobiphenyls) and 6 chlorines (hexachlorobiphenyls), are called congeners. The various PCB mixtures are referred to as sets of congeners, even though some of these sets contain PCBs related to each other as isomers.



$$\left(\begin{array}{l} x + y = 1\text{-}10 \\ x,y \leq 5 \end{array} \right)$$

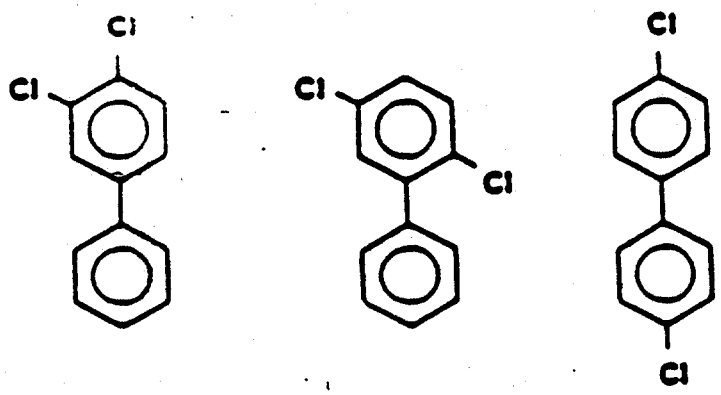


Figure 1. Structure and nomenclature of PCBs. Top left: Biphenyl structure with notation of variable number of chlorines. Top right: Biphenyl structure with numbering of carbon atoms for designating isomers. Bottom: Three isomers of dichlorobiphenyl: 3,4-dichlorobiphenyl, 2,5-dichlorobiphenyl, and 4,4'dichlorobiphenyl.

Individual chlorinated biphenyls can be separated from a mixture by a technique called gas chromatography, and their structures can be confirmed using mass spectroscopy. Figure 2 is a schematic that was produced from the results of such a procedure for Aroclors 1260, 1254, and 1242. When the results of such a separation are represented in a graph (i.e., a chromatograph) as in figure 2, the horizontal location of the bar (labelled "Peak Number") is an indication of the specific PCB congener, or congeners if the separation is not complete. (The relation between these Peak Numbers and PCB congeners by chemical designation is described in Appendix B.) The height of the bar is a measure of the amount (i.e., mass fraction) of each of the various chlorinated biphenyls that are present. Such chromatographs are sometimes called fingerprints, because they can be used to determine the degree of similarity between two mixtures of chemicals even when all of the components of the mixture have not been identified.

Although the correlation is imperfect, as a general rule the number of chlorine atoms per biphenyl molecule increases with Peak Number. Most of the congeners in Aroclor 1260 have more than 4 chlorines, and the average is about 6 chlorines. The chlorine content of Aroclor 1254 is lower than for Aroclor 1260, averaging about five chlorines, and that of Aroclor 1242 lower still, averaging about three chlorines. Thus, although all three are mixtures of PCBs, the actual chemical composition of the mixtures (i.e., the fingerprint) is quite different. Even so, it is readily apparent from figure 2 that the spectra of congeners (i.e., the chemicals present) in the three mixtures overlap (i.e., have some chemicals in common for the mixtures), especially for Aroclor 1260 and Aroclor 1254. Aroclor 1260 has relatively little overlap with Aroclor 1242. Because the chemicals that comprise these mixtures are different, the chemical, physical, and toxicological properties of each mixture would also be expected to differ.

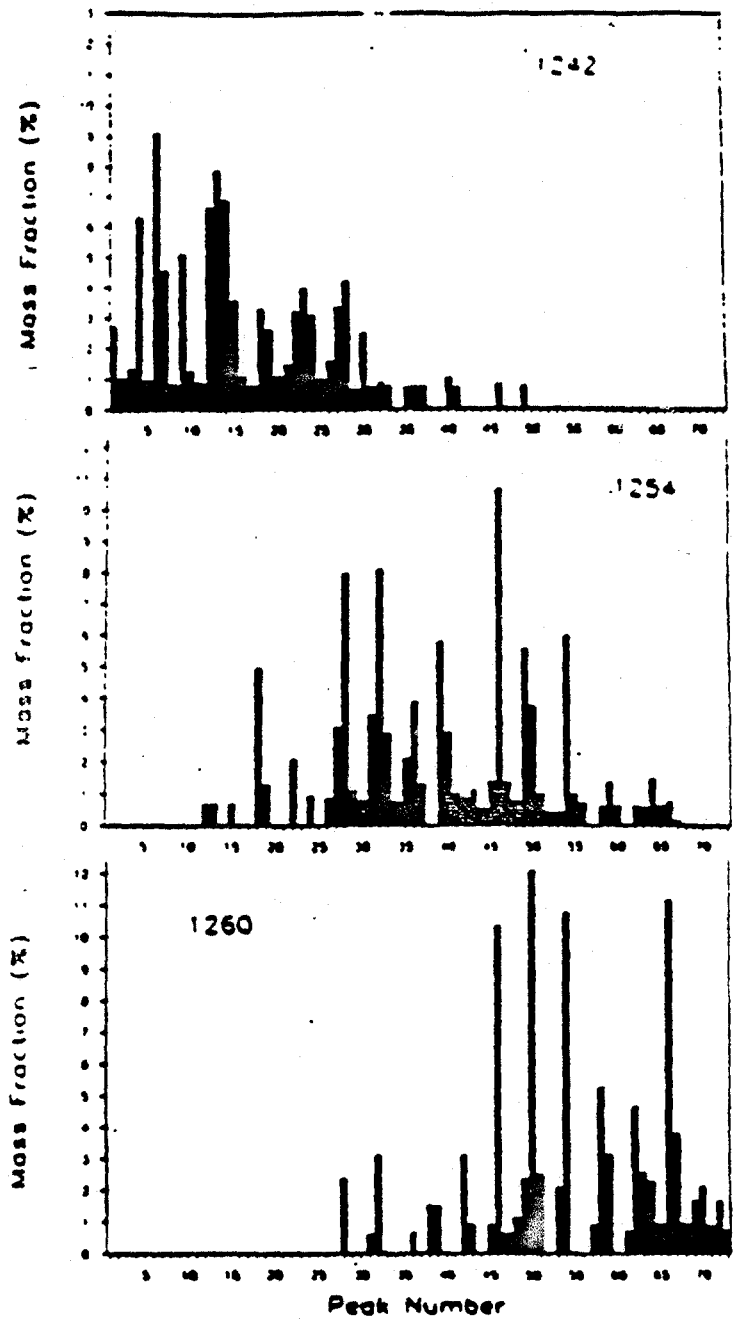


Figure 2. Distribution of mass fractions of PCB congeners in Aroclors 1242, 1254, and 1260.

Two other commercially produced PCBs -- Clophen A30 and Clophen A60 -- are relevant to this discussion. These substances were produced in West Germany and have compositions similar to the Aroclors. Specifically, Clophen A60 is approximately identical to Aroclor 1260, and Clophen A30 is similar to Aroclor 1242. The importance of these similarities will be discussed later.

C. Epidemiological Data

1. General Considerations

The study design that is most commonly used to examine the occurrence of cancers in PCB-exposed populations is a type of cohort study (mortality study) in which death from cancer (all cancers and site-specific[1/] cancers) is the endpoint of interest. In the majority of these studies, the cancer mortality of the population exposed to PCBs is compared to cancer mortality rates of the general population. Standardized Mortality Ratios (SMRs) are then determined by dividing the number of cancer deaths (either total or site specific) in the exposed group by the number of deaths that would be expected by applying rates developed from a reference population. The choice of the reference population (e.g., U.S. average, regional, or state) is critical for the analysis because the normal occurrence of cancer or a particular type of cancer may vary with the population selected. It is often preferable to use a local population (i.e., from the same region or locality as the study population) as the reference population to account for possible unknown confounding variables that could

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1/Refers to the anatomical site (e.g., liver) at which the cancer was identified.

influence the mortality experience of the study. For example, if drinking water contaminants increased the cancer mortality of the study (and local) populations, the use of cancer mortality rates based on the U.S. population would not account for this, whereas the use of local rates would.

Statistical analyses are generally conducted on the findings to determine whether any observed increase in the general or site-specific mortality rates in the exposed cohort is statistically significant.[1/] It should be noted that the probability that one or more comparisons will be found to be statistically significant by chance alone increases with the number of comparisons that are made (e.g., number of site-specific cancer mortality rates in the study cohort vs. the number expected based on reference population rates) (Daniel 1983).

If a statistically significant increase in the overall or site-specific rate of cancer mortality is found in the study cohort, one must assess whether the observed difference might have resulted from bias in the manner of data collection. This involves evaluating the ability of the investigators to determine who was exposed (and to what extent) and the methods used for identifying cases of cancer mortality. One must also determine whether the finding might have resulted from the effects of uncontrolled or "confounding variables." This is done by assessing the degree to which investigators accounted for other risk factors (e.g., smoking) in the study design and analysis.

[1/] The level of statistical significance, or p-value, is generally used to determine this. Traditionally, if a p-value is less than 0.05, chance (although always a possibility) is considered to be an improbable explanation of the results. Conversely, if the p-value is greater than 0.05, chance is considered to be a likely explanation for the observed effect, e.g., cancer.

In the particular case of examination of epidemiological data related to cancer endpoints, there are a number of specific methodological issues that should be considered. These include:

Latency Period: For many cancer endpoints, a long interval may occur between exposure and detectable tumor development. Thus, any cohort study should have a sufficiently long period of follow-up to account for this factor.

Misclassification: It is necessary to classify accurately the exposure status of humans to the substance of interest over time. Important differences in interpretation of epidemiological findings may be obscured if persons are misclassified with regard to exposure.

Pathological Verification of Disease: It is important that investigators clearly state the methods used for identifying disease. There is considerable uncertainty associated with the diagnoses based on death certificates. These should be confirmed using pathology records whenever possible. This is especially important in identifying primary cancer sites because of the tendency of malignancies to metastasize, i.e., to spread to organs other than the site affected by the substance of interest.

Confounding Variables: Exposure to an agent, such as PCBs, may be associated with other possible determinants of cancer risk. For example, cancer rates differ on both a regional and community basis. These differences may in some cases be attributable to qualitative and quantitative variations in the

spectrum of environmental agents (e.g., chemicals in air and water) to which local populations are exposed. Exposure associated with personal habits (e.g., smoking) and past occupations may also account for an observed increase in cancer mortality in a study population. If information is available on the potential confounding variables, it may be possible to adjust for their effects in the analysis. In many cases, however, the relevant confounding variables may be unknown or difficult to measure, complicating the determination of conclusions regarding causation.

After assessing the possible effects of the above factors in an individual epidemiological study, it is generally necessary to apply certain additional criteria in making a judgment as to causality — i.e., whether exposure to the agent was the cause of the observed health effect. These criteria are frequently presented and discussed in general textbooks of epidemiology (e.g., Rothman 1982, Mausner and Kramer 1985). Most authors base their discussions of the subject on the nine criteria that were noted by Hill (1965) as especially important for consideration when reaching a judgment on the causal nature of an association. The current practice among epidemiologists is to adopt a set of criteria that represent a modification of those originally presented by Hill. Mausner and Kramer (1985) identified the following criteria to evaluate the likelihood that an association is causal:

Strength of the Association: This criterion refers to the degree to which the incidence of the disease is elevated in the exposed population as compared to the control population. In mortality studies, the strength of the association is indicated by the magnitude of the Standardized Mortality Ratio (SMR).

Statistical analysis of the data is necessary to determine the likelihood that an observed association between exposure to an agent and the subsequent development of disease (e.g., as indicated by an elevated SMR) is a chance outcome or is indicative of a true association.

Dose-Response Relationship: This refers to the criterion that the risk of developing the disease usually increases as the exposure increases. The demonstration of such a dose-response relationship increases the likelihood of a causal association.

Consistency of the Association: This criterion refers to the repeated observation of an association in different populations under different circumstances (e.g., under different patterns of exposure).

Temporally Correct Association: Exposure to the suspected causative agent must precede the effect in time. Also, with respect to cancer, a sufficient latency period (i.e., period between exposure and the development of the disease) is necessary for the association between exposure to the agent and the development of disease to be biologically plausible.

Specificity of the Association: This criterion requires that exposure to a causative agent should lead to a unique effect. While the observation of specificity is strong evidence for causal association, its absence is of less significance. Some agents (e.g., smoking) have been strongly linked to multiple effects.

Coherence With Existing Information: This criterion usually refers to the extent to which a causal interpretation is biologically plausible given the current state of scientific knowledge. The likelihood of an association is stronger if it is supported by experimental evidence.

As noted by Rothman (1982), there is no rigid rule to specify when a causal relationship has been established. Any conclusion regarding the likelihood of a causative association is ultimately based on individual, expert judgment. The more criteria that are met for an exposure in question, the greater the likelihood of a causal association. The observation of a statistical association in one or more epidemiological studies usually is insufficient, by itself, to establish causation.

2. Review of Studies

The potential biological effects of human exposure to PCBs have been examined in several populations, primarily workers exposed through occupational activities. As noted previously, EPA (1988) and the Agency for Toxic Substances and Disease Registry (ATSDR 1987) have concluded that the available data are not sufficient to demonstrate that PCBs cause cancer in humans. Nevertheless, the available data, and the strengths and weaknesses of the studies, are reviewed in this section.

The majority of these studies involve occupationally exposed cohorts, and most include only rough measures of exposure (e.g., duration of employment, employment category). The studies differ in the extent to which investigators were able to account for possible confounding variables. As noted above, in some cases such variables could account for any observed increases in cancer mortality in the study population.

a) Brown and Jones (1981); Brown (1987)

Brown and Jones (1981) reported the results of a retrospective mortality study of 2,567 electrical capacitor workers in two plants located in the United States. An update of this study was subsequently published by Brown in 1987. The type of PCB mixture used in the plants varied over the years and included Aroclors 1254, 1242, and 1016. (The latter is a purified version of Aroclor 1242.)

In the initial study (Brown and Jones 1981), the cohort was followed until January 1, 1976, and included all workers with at least 3 months of employment (after 1940) in areas where there was potential PCB exposure. The expected number of deaths in the cohort was determined using age-adjusted U.S. mortality rates (white males and white females) for the appropriate time periods. The total mortality in the study cohort was lower than expected (163 observed vs. 182 expected), as were the total number of cancer deaths (39 observed vs. 44 expected). The Standardized Mortality Ratio (SMR = [observed deaths/expected deaths] x 100) for cancer deaths in the study cohort was 89. Thus, there were fewer cancer deaths among those exposed to PCBs than would be expected in a general, non-exposed population.

With regard to site-specific cancer mortality, Brown and Jones (1981) reported a greater than expected number of deaths due to rectal cancer (4 observed vs. 1.19 expected) and cancer of the liver, gallbladder, and biliary passages (3 observed vs. 1.07 expected). These findings, however, were not statistically significant which strongly suggests that the observed increases are chance occurrences.

In the update of the original study, Brown (1987) followed the mortality experience of the

original cohort through 1982. Once again, the total mortality in the study cohort was lower than expected (295 observed vs. 317.6 expected), with an SMR of 78.

During the additional observation period no additional deaths from cancer of the rectum were observed, resulting in a lowering of the SMR from 336 to 211 (4 observed vs. 1.9 expected). Two more deaths from the disease categories that include cancers of the liver, gallbladder, or biliary passage were reported; these sites were not analyzed individually for statistical significance. This resulted in a statistically significant excess in mortality when the observed number of deaths from these different categories were combined (5 observed vs. 1.9 expected). But the grouping of the 5 cases of liver, gallbladder, and biliary tract cancer into one category (and thus treating them as a single disease) is questionable.[1/] The etiology of the cancers also suggests that they should be considered separately. The evidence for an association between exposure to some environmental agents (e.g., mycotoxins, hepatitis B virus) and an increased risk of developing hepatocellular (liver cell) carcinoma is relatively strong, whereas that for cancer of the hepatobiliary tract (bile ducts found within the liver) is much less compelling (for example, see Zimmerman 1978). Moreover, none of the 5 cases that were grouped by Brown (1987) were identified as a primary carcinoma of the liver, suggesting that liver

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1/The International Classification of Diseases (ICD) code (Eighth Revision) for deaths from cancer at each of these sites is different, indicating that ICD considers them different diseases.

might merely be the common site of metastasis for cancers from sites of unrelated origin.

An analysis of the data did not show an increase in risk with an increase in latency (time since first employment) or any indication of a dose-response relationship (as measured by length of employment) among the deaths from cancers of the liver, gallbladder, or biliary tract. In discussing the study findings, Brown noted that, due to the small number of deaths and the variability of specific cause of death (i.e., within the category including mortality from malignancies of the liver, gallbladder, and biliary tract), it is difficult to interpret the significance of the findings with regard to PCB exposure.

b) Bertazzi et al. (1982, 1987)

Bertazzi and coworkers (1982) reported preliminary results of a retrospective mortality study of production workers in a capacitor manufacturing facility who were employed for at least 6 months between 1946 and 1970. During the early years of production, workers were primarily exposed to PCB mixtures containing 54% chlorine (Aroclor 1254 and Pyralene 1476) that were later replaced by mixtures containing 42% chlorine (Pyralene 3010 and 3011). Mortality was observed between 1954 and 1978 and compared to local rates. The authors reported a statistically significant increase in cancer mortality among males. The observed excesses in cancer deaths in males were primarily attributed to malignancies of the lymphatic and hematopoietic (blood forming) tissues and the digestive system.

In the update of this study (Bertazzi et al. 1987), the cohort was expanded to include non-production workers. The investigators also

decreased the minimum period of employment (after 1946) needed for inclusion in the cohort from 6 months to 1 week. The mortality experience of the cohort was followed from 1946 to 1982.

The short minimum exposure period is a major flaw in the study design. By defining the cohort in this manner, the authors could attribute cases of cancer mortality to PCB exposure that were likely due to other causes or factors. For example, of the 12 cases of mortality that were identified in females, only four were fully characterized with respect to parameters such as length of exposure, and one of these had an exposure period of only three months. It is possible that the remaining eight cases include women who were exposed for very limited periods, making it much less likely that the cancers were associated with work-related exposures.

The total number of deaths in the cohort by 1982 was 64 (30 men and 34 women). Bertazzi et al. used both national and local mortality rates (adjusted by age, sex, and year) to determine the expected number of deaths in the study cohort. Total mortality (i.e., from all causes) was not elevated for males, but there was a statistically significant increase in overall cancer deaths (as indicated by the Standardized Mortality Ratio [SMR]) and in cancers of the gastrointestinal tract, based on either national or local mortality rates.

In females, statistically significantly increased SMRs were observed only when local mortality rates were used to determine expected numbers of cause-specific deaths. Significant excesses were observed for the categories of deaths due to malignant tumors (cancers) (SMR = 226) and deaths due to hematologic neoplasms (cancers of the blood system) (SMR = 377). The local mortality rates

for females in the age group of concern (generally less than 45 years old), however, are associated with a high degree of uncertainty because of the relatively few deaths that occurred among women of this age group in the town (population 150,000).

Bertazzi et al. (1987) reported that when the data were analyzed by duration of exposure, latency, and the year of first exposure, no pattern or trend in mortality was observed for any category of cancer mortality in males or females. They also noted that, in some cases, an examination of the employment history of cancer victims tends to reduce the probability of an association with PCB exposure, in particular with regard to the males with the excess of digestive system cancer (6 observed vs. 1.7 [national] or 2.2 [local] expected). Upon closer analysis of these cases, the authors state that one individual with stomach cancer had been hired at an advanced age and received a very short exposure. Furthermore, two of the individuals (one with stomach cancer, one with pancreatic cancer) had been security guards with no history of direct PCB exposure. This suggests that only 3 of the observed cases may be in people who had any significant exposure to PCBs, and only one individual (with pancreatic cancer) was exposed for more than one year.

The findings of the epidemiological study conducted by Bertazzi et al. (1987) are not indicative of a causative link between exposure to PCBs and the subsequent development of cancer in humans. In the cancer mortality cases that were identified, no dose-response relationship was observed and no pattern was observed with regard to latency and disease. As noted by the authors, some of the male cancer mortality cases had little or no opportunity for direct PCB exposure. Finally,

interpretation of the study results is constrained by the small number of deaths that had occurred in the study cohort, the short minimum exposure period required for inclusion in the study cohort, and the use of relatively unstable local mortality rates as a standard of reference.

c) Gustavsson et al. (1986)

Gustavsson et al. (1986) reported the results of a study of the mortality and cancer incidence among a cohort of 142 male Swedish capacitor manufacturing workers during the period of 1965 to 1982 (with cancer incidence followed through 1980). The workers had been employed for a period of at least six months between 1965 and 1978 and had been exposed to Aroclor 1242 (or equivalent). Airborne PCB levels were measured at 0.1 mg/m^3 in 1973, with possibly higher levels in the 1960's.

A total of seven cancer deaths were identified in the cohort, which was not significantly different from the expected number (5.4), calculated using national statistics. There was also no tendency towards an increase in the mortality or cancer incidence in the most highly exposed subgroup of 19 workers. Although the results indicate no increase in cancer mortality in the study cohort during the study period, the results are not conclusive because of the small cohort size and brief follow-up period.

d) Bahn et al. 1976

Bahn et al. (1976) reported a statistically significant ($p < 0.001$) increase in deaths due to malignant melanoma (2 observed vs. 0.04 expected) in a small group (31) of research and development employees believed to have been heavily exposed to PCBs. The major pathways of exposure were not

identified by the authors. The workers were exposed to Aroclor 1254, among other chemicals, during various periods between 1949 and 1957. Although the authors suggest that PCB exposure may account for the observed excess of malignant melanoma, the small size of the study cohort and the fact that individuals were exposed to other toxic and potentially carcinogenic compounds during their employment makes it impossible to attribute the excess cancer cases to any specific agent.

Bahn et al. reported their findings in the form of a letter. They have never been presented in the form of an epidemiological study with data that can be independently evaluated and published in a journal for peer review. Therefore, these findings are difficult to evaluate as part of the "weight of evidence" regarding the carcinogenicity of PCBs in humans.

A letter by Lawrence (1977) questioned whether the study demonstrated any adverse effects from exposure to PCBs due to concommitant exposure of workers to other, possibly carcinogenic, chemicals. In response, Bahn et al. (1977) maintained the assertion of a "possible association" between PCBs and malignant melanoma, but agreed that the data were not conclusive.

e) Studies in Populations Following the Accidental Ingestion of PCBs, PCDFs, and Other Contaminants

Kuratsune et al. (1986) reported on the results of mortality studies of Japanese "Yusho" patients who had ingested contaminated rice oil in 1968. The oil was contaminated with Kanechlor 400 (similar in PCB composition to Aroclor 1248) as well as polychlorinated dibenzofurans (PCDFs) and polychlorinated quaterphenyls (PCQs). The

composition of the Kanechlor 400 involved in this incident had been altered (i.e., there was a much higher concentration of contaminants than the commercial grade mixture) as a result of its use in a heat exchanger. It is also probable that additional contaminants were generated during the use of rice oil in cooking.

In the 887 males who were included in the cohort, statistically significant increases in mortality from all malignancies (33 observed vs. 15.5 expected), liver cancer (9 observed vs. 1.6 expected), and lung cancer (8 observed vs. 2.5 expected) were reported, based on national rates. The use of local rates decreased the SMR for liver cancer from 560 to 390, which was still statistically significant. No SMR based on local mortality rates was calculated for lung cancer. No significant excesses in cancer mortality were observed for the 874 female patients included in the cohort.

There is evidence that confounding factors could have influenced the findings of Kuratsune et al. (1986). It has been reported that 70% of the identified Yusho patients are from two prefectures that have reported the highest incidence of liver cancers in Japan (Kuratsune 1986), suggesting the possible existence of local factors that have not been identified. For example, as reported in Ikeda et al. (1986), the rate of mortality from liver cancer was substantially different for the Yusho patients in Fukuoka prefecture than in Nagasaki prefecture. This led the authors to conclude that "Such a remarkably uneven geographic distribution of livear [sic] cancer deaths makes it hard to consider the observed increased risk of liver cancer as simply due to the poisoning." Kuratsune et al. (1986) were also unable to control for possible confounding

factors such as smoking habits (especially important with regard to the observed excess of lung cancers), drinking habits, and occupational exposures.

The liver cancer diagnoses on which Kuratsune et al. rely were obtained from death certificates, without any confirmation of pathology through tissue examination. The cases were thus not restricted to primary liver cancers, but also would have included cases in which the liver was a site of metastasis for cancers originating at other sites. The significance of the elevated incidence of liver cancer is thus subject to question.

Many investigators also believe that exposure to PCDF congeners is the primary cause of the symptom pattern observed in Yusho (Miyata et al. 1985, Kashimoto et al. 1985, Masuda 1985). They support this contention by reference to the high degree of toxicity (primarily to the liver) of certain of the PCDF congeners in laboratory animals. These toxic PCDF congeners (including 2,3,7,8-tetrachlorinated; 2,3,4,7,8-pentachlorinated; and 1,2,3,4,7,8-hexachlorinated dibenzofuran isomers) were identified in Yusho oil and in the tissues of Yusho victims (Miyata et al. 1985). As further evidence that PCDFs were the agents most likely responsible for the severity of Yusho symptoms, Kashimoto et al. (1985) and Hara (1985) refer to the relatively mild symptoms observed in PCB-exposed workers who had serum PCB levels similar to those observed in Yusho victims, but without detectable levels of PCDFs.

A second major outbreak of disease caused by ingestion of contaminated rice oil (called Yu-Cheng in Chinese) occurred in central western Taiwan in 1979. The oil that was responsible for this incident contained PCBs, PCDFs, and PCQs that were comprised of congeners similar to those identified in Yusho

specimens (Miyata et al. 1985). No data were located regarding the incidence of cancer mortality in Yu-Cheng victims. It is possible that there has been an insufficient number of deaths in this group for any meaningful analysis of mortality data.

3. Conclusions Regarding Epidemiology Data

Although several studies have investigated the possibility of an association between PCB exposure and human cancer, the results do not support a causal relationship. The primary reasons for this conclusion are:

(i) Strength of the Association: When the excess cancer cases observed by Bertazzi et al. (1987) and Brown (1987) are examined closely, the relationship of the excess cancers to PCB exposure appears doubtful. For example, 2 of the 6 cases of digestive system cancer that were identified in the male subcohort by Bertazzi et al were in individuals whose jobs involved little or no direct PCB exposure, and a third case was in a worker who began employment at an advanced age. Furthermore, any perceived linkage between any chronic effect and employment is dubious because the cohort includes individuals with only one week of employment. None of the 5 excess liver, gallbladder, or biliary tract cancers observed by Brown (1987) was identified as primary liver cancer, thus rendering suspect the identification of the liver as the target organ. The findings of statistically significant increases in liver cancer among male Yusho victims (Kuratsune et al. 1986) cannot be attributed to PCBs because of concurrent

exposure to high concentrations of other toxic contaminants (e.g., PCDFs).

(ii) Dose-Response Relationship: In studies in which excess cancers were observed, there is no relationship between degree of PCB exposure and cancer risk. For example, in the follow-up study by Brown (1987), 4 of the 5 excess liver or biliary tract cancer cases were observed in the lowest exposure group, with none in the highest exposure category. Bertazzi et al. (1987) were also unable to identify a dose-response relationship between PCB exposure and increased cancer risk.

(iii) Consistency and Specificity of the Association: There is no consistent pattern of associations among the various studies, either with respect to the type of human cancers observed or the nature and extent of PCB exposures.

(iv) Temporally Correct Association: For some of the cases identified by Bertazzi et al. (1987), it appeared that there was little or no opportunity for exposure before development of disease. Also, no pattern of increased risk with an increase in latency was reported by Brown or Bertazzi et al.

(v) Coherence With Existing Information: Experimental data do not suggest that PCBs are a causative agent for cancer in mammals at sites other than the liver. The evidence that PCBs are causative agents for liver cancer in humans is inadequate. A statistically significant increase in mortality from cancer

of the liver, gallbladder, or biliary tract (combined) was observed in one occupationally exposed cohort (Brown 1987); however, none of these cases were identified as primary liver cancer. There was no confirmation, by tissue analysis, of the Yusho liver cancer victims identified by Kuratsune et al. (1986). These cases were also not restricted to primary liver cancers.

There is insufficient evidence to show a causal relationship between PCB exposure and the subsequent development of any form of cancer. In light of the long-term and widespread usage of PCBs in the workplace and, in some cases, the extensive exposures of workers, it is likely that evidence of carcinogenicity in humans would have been observed in the various epidemiological studies discussed above if PCBs were in fact potent carcinogens.

D. Animal Studies

1. Introduction

The numerous human studies are insufficient to show that PCBs cause cancer in humans. When data from human exposure are inadequate to assess the potential hazards from a substance, experiments with laboratory animals are often performed to identify potential adverse effects that might occur in humans. While animal studies have been accepted as a general indicator of possible effects in humans, not all effects observed in all animals will occur in humans. A chemical-specific evaluation may indicate the data from animals is inappropriate, especially when the effects are observed in only one species of animal and cannot be duplicated in other species. For this reason, consistent results from studies in several species are required to justify convincingly that it is proper to

extrapolate results of studies of laboratory animals to humans.

Although laboratory animal testing is, in general, a useful tool for predicting the impact of an agent on humans, the limitations of these tests must be acknowledged. For some chemicals or chemical-specific effects (e.g., tumor forming potential), there can be considerable uncertainty with regard to the applicability of test results in predicting human response. The most obvious and important reason for this is the fact that such animals are physiologically different than the human species. No matter how convincing the results from animal studies, a question always remains about their relevance to human populations because of interspecies differences in factors such as absorption, metabolism, and elimination of a test substance.[1/] In addition, some types of tumor responses (e.g., the rodent liver tumors that are the only clear animal response produced by any of the PCBs) are much less certain predictors of human cancer than are other types of tumor responses.[2/] Finally, all studies must be critically evaluated with respect to the quality of test design and conduct.

Although the above limitations associated with animal tests, such studies are frequently used for regulating

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1/For example, metabolic differences may undermine the validity of extrapolating from animals to man if the carcinogen is a metabolite of the original chemical and the animals used in the bioassay differ substantially from humans in their production of that metabolite.

2/There is a high and variable incidence of liver tumors in various strains of commonly used laboratory mice, as well as a high spontaneous incidence in the livers of rats of preneoplastic cells (i.e., cells in an altered state that may have carcinogenic potential) that can be stimulated by promoting agents to produce tumors (Nutrition Foundation 1983, Schulte-Herman et al. 1983).

environmental carcinogens. The use of animal test data in this fashion, even when available human data do not suggest a problem (as in the case of PCBs), is based on the policy goal of regulators of providing maximum assurance of public health protection in the absence of complete scientific certainty.

For purposes of this report, the animal data on PCBs will be used to determine potential health risk because data from human exposures show no demonstrable health effects other than chloracne. Therefore, the animal data are presented on the most sensitive endpoint of concern with respect to PCBs: rodent tumorigenicity. In choosing this endpoint as the most sensitive, the conservative assumption is made (as it is by regulatory agencies) that all of the different commercial PCB mixtures are tumorigenic, even though this has not been demonstrated in laboratory or epidemiological studies.

There are effects other than tumorigenicity that have been observed in animals exposed to PCBs at relatively low exposure levels. These include reproductive effects such as reduced birth weight and hyperactivity in the offspring of exposed monkeys (Barsotti and Van Miller 1984, Bowman et al. 1981) and altered menstrual cycles in exposed monkeys (Allen et al. 1979), as well as induction of hepatic microsomal enzymes (enzymes produced by liver cells) in rats (Litterst et al. 1972). Tumorigenicity, however, is the most sensitive endpoint for low-level, environmental exposures to PCBs. Therefore, protection of public health based on tumorigenic risk is protective of adverse effects for other sensitive potential endpoints, such as reproductive effects. Other PCB-related effects would have to be considered if we were concerned with short term, high-level exposure to PCBs.

2. Animal Studies Regarding the Tumorigenicity of Commercial PCBs

The PCB mixtures that have thus far been tested in acceptable animal chronic bioassays for tumorigenicity

include Aroclor 1260, Clophen A60, Aroclor 1254, and Clophen A30. Each of these bioassays is discussed below.

There are no acceptable bioassays concerning the carcinogenicity of Aroclors 1248 or 1242. While there are some animal studies on Aroclor 1242; Aroclor 1248; and Kanechlors 300, 400, and 500 (Japanese commercial mixtures), which qualitatively add to the body of knowledge concerning the potential tumorigenicity of PCBs, these studies are not conclusive and cannot be relied upon for quantitative determinations. This is primarily because of inadequacies in the design (e.g., insufficiencies in study length, numbers of test animals, dose levels tested) of the studies that have been conducted to date. Cancer bioassays conducted by Industrial Bio-Test Laboratories (IBT) have generally been considered invalid by regulatory agencies (cf. Garmon 198[illegible] therefore, this series of chronic animal studies on Aroclors 1242, 1254, and 1260 will not be used for this quantitative analysis.[1/]

In light of the limited number of studies the cancer potency factors for the various Aroclor mixtures must be calculated from the sets of animal data for Aroclor 1260, Clophen A60, Aroclor 1254, and Clophen A30. The significance of the difference in tumorigenic potency among these congeners, as well as the procedure used for adjusting these data for use in risk assessments, is discussed later.

1/Both the FDA and EPA consider these studies to be invalid because of severe procedural and record-keeping deficiencies. Also, the results of a re-evaluation of the original data by Calandra (1976) used terminology that does not conform to current practice for diagnosing hepatocellular proliferative lesions (tissue in which liver cells are dividing at an abnormally fast rate).

The analysis presented in this report follows the current regulatory practice of treating PCBs as complete carcinogens[1/]. Several factors, however, demonstrate that some of the carcinogenic effects of PCBs are due to promotion rather than initiation:

1. A substantial number of experiments have shown that PCBs do not cause direct genetic effects in several assay systems (e.g., as reviewed by ATSDR 1988).

2. PCBs have been shown to cause the promotion of liver tumors in rodents initiated by other compounds (e.g., Kimura et al. 1976; Nishizumi 1976, 1979; Tatematsu et al. 1979; Preston et al. 1981).

Other compounds that act as promoters have threshold doses (e.g., a dose below which no effect is observed) that have been demonstrated experimentally (e.g., as reviewed in Butterworth and Slaga 1987 and Schulte-Hermann 1985). It is believed that a threshold exists for all chemicals that act solely as promoters.

These points have important implications for carcinogenic risks from exposure to PCBs at very low dosages, such as might arise from environmental

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[1/] The process of carcinogenesis is generally regarded as a multistage process. It is considered to consist of, at a minimum, an initial stage in which the genetic material of a cell is permanently altered (initiation) followed by later stages (that may occur many years later) in which the initiated cell undergoes changes which are not fully understood, but which include cell division (promotion). A complete carcinogen is a substance which acts as both an initiator and a promoter in that it can, by itself, cause an increase in tumor formation.

exposures. If the tumorigenic effects of PCBs in laboratory animals are solely or primarily due to promotion, the potential tumorigenic risk will be greatly overstated at very low dosages. Studies with other promoters indicate that the carcinogenic effects of promoters are, at least to some degree, reversible and that a threshold exposure level must be exceeded to produce any effect on carcinogenesis. Thus, the no-threshold, linearized multistage model, which assumes that any level of exposure has some risk, will overstate the risk, especially at low doses (exposures). If PCBs were solely promoters, no tumorigenic risk whatsoever would be expected from doses (exposures) that are below the threshold. It is thus very possible, even if the animal data are reliable indicators of effects in humans at high doses, that no risk would result from low-dose environmental exposures to humans.

a) Aroclor 1260

There are two cancer bioassays of Aroclor 1260: Norback and Weltman (1985) and Kimbrough et al. (1975).

Norback and Weltman initially exposed 70 male and 70 female Sprague-Dawley rats to dietary concentrations of 100 ppm Aroclor 1260 for 16 months, 50 ppm for 8 subsequent months, and control diets for 5 months. The control group consisted of 63 male and 63 female control rats. At months 1, 3, 6, 9, 12, 15, and 18, four controls and six PCB-treated rats had partial hepatectomies (removal of the liver) in order to observe sequential morphological changes and progression to neoplasms[1/]. One set of rats was

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1/The term neoplasm refers to a new and abnormal formation of tissue, which can be in the form of a tumor. A neoplasm may be benign (not spreading into surrounding tissues) or malignant (i.e., cancerous).

sacrificed at 24 months; at 29 months, terminal sacrifices on all remaining rats were completed. Sequential observations showed that an increase in the size of some liver cells (centrilobular cell hypertrophy) was present at 1 month; small organized regions of changes in liver cells (foci of hepatocyte alterations) were seen at 3 months; larger areas of liver cell (hepatocyte) alterations were observed after 6 months; benign, i.e., non-cancerous, tumors (neoplastic nodules) appeared at 12 months; and malignant tumors (trabecular carcinoma and adenocarcinoma)[1/] were apparent later (after 15 and 24 months, respectively).

The total incidence in Norback and Weltman of trabecular carcinoma was 23% (21/93) with 2/46 and 19/47 in males and females, respectively. Adenocarcinoma appeared at an incidence of 26% (24/93) of which 24/47 occurred in females and 0/46 in males. Neoplastic nodules were observed in 8% (7/93) of the Aroclor 1260 animals (5 males and 2 females). Neoplastic nodules were observed in one female control animal, resulting in a total incidence of 1% (1/81) for neoplastic nodules in controls. No other hepatocellular neoplasms (liver tumors) occurred in the control group. Bile duct hyperplasia (excessive proliferation of normal cells), cysts, and adenofibrosis (benign tumor containing connective tissue) were seen in 38%, 8%, and 9% of the treated animals, and 5%, 1%, and 4% of the control animals, respectively. Although hepatocellular neoplasms were present in 96% of the treated females and 15% of the

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1/Adenocarcinoma refers to a malignant tumor arising from glandular tissue (in this case the liver); trabecular carcinoma refers to a specific type of liver cancer.

treated males, the neoplasms did not metastasize or cause increased mortality relative to controls.

An analysis of the data using Fisher's Exact Test shows the incidence of carcinoma in the females was statistically significantly greater than control females; however, this was not so for males. The incidence of total liver tumors (carcinomas and neoplastic nodules) in males and females was statistically significantly greater than their respective control groups.

Kimbrough et al. (1975) also performed a rodent bioassay for Aroclor 1260. Initially, 200 female Sherman strain rats were fed 100 ppm of Aroclor 1260 for 21 months. Dietary exposure was discontinued for six weeks before all exposed animals were sacrificed. The initial control group consisted of 200 female rats. Malignant tumors (hepatocellular carcinomas) were observed in 26 of the 184 surviving PCB-exposed rats, and benign (non-cancerous) tumors (neoplastic nodules) of the liver were observed in an additional 144 of the exposed rats. Only 1 of the 173 surviving control animals developed hepatocellular carcinoma while none of the control rats developed neoplastic nodules. Analysis of this data using Fisher's Exact Test shows the incidence of carcinoma in exposed rats was statistically significantly greater than in controls.

Kimbrough et al. (1975) also reported the incidence of tumors in organs other than the liver. A number of organ sites showed lower tumor incidence in PCB-treated animals than in the controls. If the total number of tumors at all sites is summed, however, the lower incidence of certain tumor types in the PCB-treated animals as compared to controls was more than counterbalanced by the increase in liver and other tumors compared to control animals.

b) Clophen A60

Clophen A60, the German commercial equivalent of Aroclor 1260, was tested in a cancer bioassay in Wistar rats by Schaeffer et al. (1984). Male Wistar rats received dietary concentrations of 100 ppm Clophen A60 over a period of 832 days. Malignant tumor (hepatocellular carcinoma) incidence in the treated group at 48% (61/126) was statistically significant compared to 0.76% (1/131) in controls. Benign tumors (neoplastic nodules) of the liver were also statistically significant at 49% (62/126) in the Clophen A60 group compared to 3.8% (5/131) in the control groups.

It is important to note, however, that there was a statistically significant lower survival in control animals compared to the Clophen A60 group, i.e., the animals that were exposed to PCBs tended to live longer than animals that were not exposed (controls). This lower survival of the control animals may have led to a lower tumor incidence in controls than might have been seen if survival among the controls had been equivalent to that of the exposed animals, because tumor incidence generally increases with age. When comparing animals with the same length of survival, however, there is still a statistically significant increase in liver tumors in the Clophen-exposed animals versus controls.

c) Aroclor 1254

The National Cancer Institute (NCI 1978) and Kimbrough and Linder (1974) conducted bioassays on the carcinogenicity of Aroclor 1254 in rats and mice, respectively.

The NCI study protocol consisted of 24 male and 24 female Fischer 344 rats that received diets containing either 0, 25, 50, or 100 ppm Aroclor 1254

for 104 to 105 weeks. There was a small, dose-related increase in the incidence of combined benign (adenoma) and malignant (carcinoma) tumors. There was a larger increase in the incidence of nodular hyperplasia.[1/] Although the occurrence of the liver lesions in these rats was not statistically significant, none of the benign or malignant changes in the liver (including hyperplastic nodules, adenomas, or carcinomas) were observed in control animals. Additionally, four adenocarcinomas and one carcinoma of the gastrointestinal tract observed in treated rats may have been treatment related, according to NCI, because the historical incidence of these tumors in this laboratory is only 6/600 in males and 2/600 in females. In this bioassay, however, few sections of the stomach had been evaluated. NCI (1978) concluded that the high incidence of hepatocellular proliferative lesions in male and female rats were related to treatment, but that Aroclor 1254 was not carcinogenic in this bioassay.

A re-evaluation of the NCI data by Morgan et al. (1981), which focused only on the tumors of the gastrointestinal tract, revealed greater numbers of stomach tumors than originally reported. These tumors were not statistically significantly greater than controls and did not appear to be dose-related. When compared with the incidence of historical controls (includes all control animals of this strain from past studies), the total incidence of adenocarcinomas of the stomach in all dose groups

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[1/] Hyperplasia is the condition in which normal appearing cells are proliferating at an excessive rate. A nodule is a small aggregation of these cells.

combined (6/144) was significant. It is important to note, however, that the historical controls may not have been examined in a manner as sensitive to detecting tumors as that used by Morgan et al.

In a subsequent paper, Ward (1985) reported the results of the same re-evaluation by Morgan et al. including data concerning proliferative lesions of the liver, as well as the glandular stomach. This re-evaluation showed a statistically significant increase in benign (i.e., non-cancerous) tumors (hepatocellular adenomas) in male rats exposed to 100 ppm Aroclor 1254 compared to controls. The original NCI bioassay had only reported one hepatocellular adenoma in high-dose males; Ward reported seven adenomas. Ward also showed a dose-related trend in hepatocellular adenomas. This difference may be due to a disagreement in pathological evaluations of tissues.

In a study by Kimbrough and Linder (1974), 9/22 (41%) male BALB/cJ mice fed 300 ppm Aroclor 1254 for 11 months developed tumors of the liver (hepatomas). A similar group receiving the treated diet for only 6 months, followed by control diet for 5 months only had a 4% (1/24) incidence of hepatomas. No hepatomas were observed in 58 control mice. Additionally, all PCB-treated mice had enlarged livers and adenofibrosis of the liver. A major limitation of the study was the high early mortality with subsequent autolysis (tissue degeneration following the death of an animal), thereby eliminating over 50% of the original mice from the final results.

In sum, Aroclor 1254 has not been shown to be carcinogenic in animal studies. There is some evidence that there was a treatment-related increase in non-cancerous changes (hepatocellular proliferative lesions) in rats in the NCI (1978)

study; however, the response was weak (and not statistically significant). No conclusions can be drawn from the re-evaluation of these data by Ward (1985) and Morgan et al. (1981) because of uncertainties associated with their analyses. The findings of Kimbrough and Linder (1974) suggest a treatment-related increase in benign liver tumors in mice treated with Aroclor 1254. The interpretation of these results are limited by previously noted study inadequacies.

d) Aroclors 1248, 1242, and 1232

At present, there are no studies concerning the tumorigenicity of Aroclors 1248, 1242, or 1232 from which reliable carcinogenic potency factors could be derived. Mammalian carcinogenicity bioassays of acceptable quality (e.g., sufficient duration, number of test animals, and test doses) have not been conducted on these Aroclors. Moreover, these PCB mixtures may not be of sufficient tumorigenic potency to cause an observable increase in tumor incidence when tested in a standard rodent bioassay. There are, however, two primate studies (one on Aroclor 1248 and the other on Aroclor 1242) that describe modifications and lesions of the gastric mucosa. Although these studies do not show tumorigenicity as an endpoint, they may be qualitatively significant in light of the stomach adenocarcinomas observed in Aroclor 1254-exposed rats (NCI 1978, Morgan et al. 1981, Ward 1985).

Two studies, Allen et al. (1973) and Becker et al. (1979), reported PCB-induced changes in the stomach, but no increase in stomach tumors. The severity of the effect was correlated with the duration and level of exposure and was observed at relatively low concentrations (0.12 mg/kg/day in the

Becker et al. study). Neither of these studies was designed to examine carcinogenesis nor can they be used for cancer potency estimation of Aroclor 1248 or Aroclor 1242. When considered along with the results of the NCI bioassay, these results suggest that the stomach cannot be discounted as a potential target organ for PCB. It is important to note, however, that there has been no reported incidence of stomach tumors in bioassays of Aroclor 1260, Clophen A60, or Clophen A30. Further, the incidence of stomach tumors in Aroclor 1254-exposed animals was not significantly greater than in controls, was not dose related, and was so low that even if stomach tumors were considered, they would have no effect on the tumorigenic potency estimates derived in this document.

e) Clophen A30

Clophen A30, a German commercial PCB mixture similar to Aroclor 1242, was also tested in the previously-cited study by Schaeffer et al. (1984). Male Wistar rats received dietary concentrations of 100 ppm Clophen A30 over a period of 832 days. Liver cancer (hepatocellular carcinoma) incidence in the treated group was 3% (4/130), while in the control group the incidence of hepatocellular carcinoma was 0.76% (1/131). Non-cancerous tumors (neoplastic nodules) of the liver were 29% (38/130) and 3.8% (5/131) in the Clophen A30 and control groups, respectively. The incidence of neoplastic nodules but not the incidence of hepatocellular carcinoma (malignant tumors), in the Clophen A30 group was statistically significantly increased compared to controls.

f) Kanechlors 300, 400, and 500

Three rodent studies of Kanechlors 300, 400, and 500 add qualitative support to the variation of tumorigenic potency among the PCB mixtures. These include rat studies by Kimura and Baba (1973) and Ito et al. (1974) and mouse studies by Ito et al. (1973).

Kimura and Baba (1973) exposed male and female Donryu rats to Kanechlor 400; initial exposure was 38.5 ppm in diet but was increased to the very high dose of 616 ppm to keep pace with body weight gain. When severe body weight loss was observed, the dose was reduced to 462 ppm. The total Kanechlor consumption in females ranged from 700 to 1,500 mg and in males from 450 to 1,800 mg. Non-cancerous (adenomatous) nodules were observed in 6/10 of the females consuming more than 1,200 mg of Kanechlor 400; no such lesions were observed in the males. EPA (1988) concluded that this study was too short and the exposure level too high (treated animals received doses exceeding the maximum tolerated dose) to provide a good experimental basis for the determination of the carcinogenic potential of Kanechlor 400.

In a second rat study, Ito et al. (1974) exposed male Wistar rats (via feed) to 100; 500; or 1,000 ppm of either Kanechlor 300, 400, or 500 for 28 weeks to one year. Nodular hyperplasia was observed in all of the Kanechlor 500 dose groups and in the 100 and 1,000 ppm dose groups exposed to Kanechlor 400 and Kanechlor 300. The incidence of this nodular hyperplasia increased with dose as well as with percent chlorine content. EPA (1988) stated that this study does not demonstrate tumorigenicity, but it cannot be considered evidence of non-tumorigenicity because of the short duration and small number of subjects per group limit the ability of the study to

detect tumorigenicity. EPA also concluded that the nodular hyperplasia, which appeared as early as 40 weeks, further precludes considering this study a negative finding. In addition, this study did not include female rats, which in light of the Kimura and Baba (1973) study results, may be more sensitive than males.

In a series of mouse studies by Ito et al. (1973), male mice were exposed to either Kanechlor 500, 400, or 300 in feed at concentrations of either 500, 250, or 100 ppm for 32 weeks. Although liver weight increase in all treatment groups was greater than controls, liver cancer (hepatocellular carcinomas) and increase in the number of liver cells (nodular hyperplasia) were induced in only the high dose (500 ppm) group exposed to Kanechlor 500. Forty-two percent (5/12) of the high-dose Kanechlor 500 group showed hepatocellular carcinomas, while 58% (7/12) showed hyperplastic nodules. Amyloid degeneration[1/] of the liver was observed in mice fed Kanechlor 500 or 400 at 250 ppm or 100 ppm, but not in the 500 ppm groups; however, according to the authors, the effects seen in the Kanechlor 500 group (nodular hyperplasia and hepatocellular carcinomas) could have masked any amyloid degeneration. Some mice fed Kanechlor 300 in the 500 ppm, 250 ppm, and 100 ppm dose groups also showed amyloid degeneration. None of the controls showed hepatocellular carcinomas, nodular hyperplasia, or amyloid degeneration. For evaluating carcinogenicity, interpretation of this study is

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1/This is a type of tissue or organ degeneration that is characterized by the deposition of a starchlike substance (amyloid) in the tissues.

limited by several factors including short study duration (52 weeks), lack of data on female mice, the small number of mice per dose group, and a lack of dose-response.

In conclusion, the Kanechlor data seem to indicate tumorigenic potential of these mixtures in rodents. The limitations of study design suggest that these data should not be used to derive a cancer potency factor; however, they do qualitatively support the liver as a site of action for PCBs in rodents.

g) Conclusions Regarding Animal Data on Tumorigenicity

There is clear evidence indicating that some of the highly chlorinated commercial PCB mixtures are tumorigenic in some animals. The responses are mostly limited to the livers in rats and mice, although there is a suggestion that some PCB mixtures may also affect the stomach of rats and monkeys.

There is uncertainty as to whether or not Aroclors 1248, 1242, and 1232 are tumorigenic in animals. Because there are no valid cancer bioassays for these mixtures, a comparison with other commercial PCB mixtures based on comparative composition is the only basis for evaluation. The best evidence for comparison comes from the study by Schaeffer et al. (1984) in which male rats were exposed to either Clophen A60 or Clophen A30. As previously explained, the Clophen A60 rats showed a 48% incidence of hepatocellular carcinoma, while the Clophen A30 rats showed only a 3% incidence of hepatocellular carcinoma that was not statistically significant. Although these results are not evidence for tumorigenicity for the lower-chlorinated Aroclors or Clophens, the data can be used to derive a

preliminary and conservative estimate of relative cancer potency. If we assume Clophen A60 parallels the cancer potency of Aroclor 1260 and Clophen A30 parallels that of Aroclor 1242, then we can conclude that the cancer potency of Aroclor 1242 is much lower (at least 16 times lower) than that of Aroclor 1260. The data for Aroclor 1254 qualitatively indicate an even lower potency than Aroclor 1260 than indicated by the Clophen data. These data, however, are not as well suited for use in quantitative estimation of cancer potency as the Clophen data because the data for Aroclor 1254 are from a different strain of rats than the data for Aroclor 1260.

It must be emphasized that reliance on the rodent liver-tumor data to estimate effects in humans may be conservative. As previously noted, the relevance of liver tumors in rodents to humans has been questioned because of the high and variable incidence of liver tumors in various strains of mice (e.g., Butler and Newberne 1975, Nutrition Foundation 1983, Clayson 1981) and the high spontaneous incidence in the livers of rats of preneoplastic cells that can be induced by promoting agents to produce tumors (e.g., Ogawa et al. 1981, Ward 1983, Schulte-Hermann et al. 1983).

Indeed, in a review of proliferative hepatocellular (liver) lesions of the rat, EPA (1986) has stated that, although neoplastic nodules are increased in animals receiving carcinogens and some neoplastic nodules may have "malignant potential," others may only be "hyperplastic" lesions and still others may regress following cessation of exposure. Thus, EPA (1986) stated that "the exact contribution of neoplastic nodules to the overall incidence of hepatocellular tumors in the rats is unclear at this

time." Nonetheless, despite the skepticism that must surround reliance on observations of hepatocellular tumors in rats as indicators of tumorigenic effects in humans, the standard regulatory practice is to assume these data are accurate predictors of carcinogenic potency in humans. This approach must be seen as possibly resulting in exaggeration of the hazards of PCBs.

E. Cancer Potency Differences Among PCB Congeners

1. Importance of Differences Among Mixtures of PCB Congeners

In light of the limitations in the available animal test data, a cautious approach would be to classify the various Aroclors as potential animal tumorigens. The considerations that lead to this position may be briefly summarized:

(i) There are no valid test data on "Aroclors" other than Aroclor 1260 and Aroclor 1254.

(ii) It is not clear which specific congeners are responsible for Aroclor 1260-induced tumorigenicity.[1/] Figure 2 reveals that all commercial PCB products have some congeners in common. Thus, it is possible that all Aroclors contain some tumorigenic congeners.

(iii) The tests of Aroclors 1260 and 1254 involved different strains of rats, and the different

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[1/] There are strong reasons to believe that substantial differences exist in the toxicity and tumorigenicity of various PCB congeners.

outcomes could possibly reflect differences in experimental design.

(iv) Clophen A30 produces excess benign tumors in the rat liver, thereby suggesting a tumorigenic response for a mixture of congeners similar to that associated with Aroclor 1242.

For the above reasons the possibility of animal tumorigenicity cannot be ruled out for Arcclors other than Aroclor 1260. Nevertheless, the available data reveal clear differences in tumorigenic potencies among these sets of congeners. (By "potency" we refer to the incidence of tumors, i.e., risk, associated with a specific PCB dose.) These potency estimates are critical to an evaluation of PCB risks and are discussed in the next section.

There is some evidence that, for certain noncancer endpoints, the biological activity of PCBs increases with increasing chlorine content (see section III.E.3 for discussion). Studies by Ito et al. (1973) and Koller (1977) have shown that the degree of liver cell proliferation and pathologic alterations is much higher in mice chronically exposed to commercial PCB mixtures of higher chlorine content than to those with lower chlorine content. Hepatic microsomal enzyme induction potency also increases with increasing chlorine content (Litterst et al. 1972). PCBs containing 54% or greater chlorine content appear to be the most potent at inducing these effects. The differences in the ability of various PCB mixtures to ellicit biological changes other than cancer may be important indicators of differences in tumorigenic potency as well. Some of these endpoints, such as liver cell proliferation, may be associated with cellular events that might affect the rate of tumor formation.

Thus, the observed differences in carcinogenic potency and in other biological effects among the PCB mixtures may be correlated, but a definitive causal relationship has not been established.

2. Relative Potencies of Aroclors

The most compelling evidence for potency differences among the commercial PCBs is derived from the studies of Clophen A60 and Clophen A30. These products were tested in experiments of identical design and yielded quite different outcomes. As previously shown, the incidence of hepatocellular carcinoma in the Clophen A60 rats was 16 times greater than the incidence in the Clophen A30 rats (Schaeffer et al. 1984). Combining the incidence of both hepatocellular carcinomas and neoplastic nodules yields a smaller difference in cancer potency between Clophen A60 and Clophen A30. Specifically, the tumorigenic potency of Clophen A30 proved to be at least 10 times less than that of Clophen A60. This comparison is based on the highly conservative assumption that the excess benign tumors observed in the Clophen A30 experiment should be given equal weight to the malignant tumors produced by Clophen A60. If the benign and malignant tumors are weighted differently, the potency difference is even greater.

Because of the strong chemical similarities between Clophen A60 and Aroclor 1260 and between Clophen A30 and Aroclor 1242, the data on the Clophens can be used to estimate the potency of (untested) Aroclor 1242 relative to that of Aroclor 1260. Specifically, we propose to assign a potency of 0.1 to Aroclor 1242 relative to 1.3 for Aroclor 1260 (see section III.F.2.b for discussion).

The potency difference observed for Clophens is supported by the results of the experiments involving Aroclors 1260 and 1254. Although the difference in potency between Aroclors 1260 and 1254 appears to be even greater than that between Clophens A60 and A30, it must be

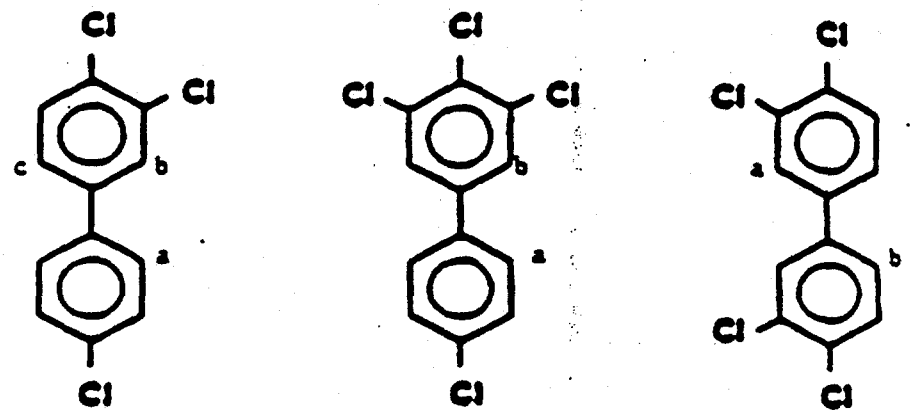
recognized that the two Aroclors were assayed in different rat strains, whereas the two Clophens were tested in identical experiments. The difference in potencies between Aroclors 1260 and 1254 nonetheless suggests that our reliance on the potency differential between the Clophens is highly conservative, i.e., highly overestimates the tumorigenic potency of the less chlorinated Aroclors.

3. Information on Mechanism of PCB-Induced Toxicity Supports Potency Differences

The role of structure on the potencies of PCB isomers and congeners has been extensively investigated (Safe 1984). The most potent compounds, namely 3,3',4,4'-tetra-; 3,3',4,4',5-penta-; and 3,3',4,4',5,5'-hexachlorobiphenyl are all coplanar (i.e., flat) in structure and bind with high affinity to the aryl hydrocarbon (Ah) receptor.[1/] These compounds, however, are either not detectable or are present in only trace levels in the lower chlorinated PCB mixtures.

Several studies have demonstrated that the responses caused by 11 monoortho analogs (i.e., specific congeners) of the coplanar PCBs resemble those described for the higher chlorinated commercial PCBs. This group of 11 congeners (see figure 3), although they are not coplanar, bind with low to moderate affinity to the Ah receptor but are much less potent than the coplanar PCBs. These 11

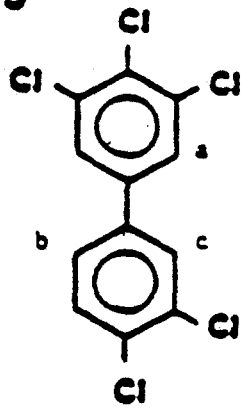
1/The Ah receptor is a protein molecule that binds a variety of chlorinated hydrocarbons such as PCBs, polychlorinated dibenzofurans, and polychlorinated dibenzo-p-dioxins. Experiments have shown: (1) the strength of the binding varies among the isomers of each of these classes of compounds and (2) inbred strains of animals that have high levels of this receptor are more sensitive to some of the toxic effects of these chemicals than strains that have low levels of the receptor.



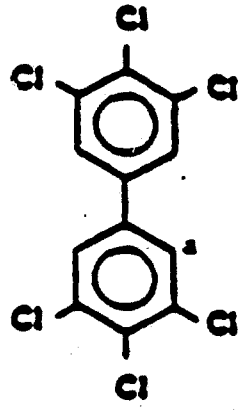
a: 2,3',4,4'
b: 2,3,4,4'
c: 2,4,4',5

a: 2',3,4,4',5
b: 2,3,4,4',5

a: 2,3,3',4,4'
b: 2,3',4,4',5



a: 2,3,3',4,4',5
b: 2,3',4,4',5,5'
c: 2,3,3',4,4',5'



a: 2,3,3',4,4',5,5'

Figure 3. Monoortho substituted tetra-, penta-, hexa-, and heptachlorobiphenyls. Letters a, b, and c are alternate positions for chlorine substitution. For example in the first structure, the molecule is 2,3',4,4'-tetrachlorobiphenyl if a chlorine atom is in the "a" position.

PCBs, however, have been identified in the commercial Aroclors and are a major contributor to the activity of these mixtures.

The tumorigenic potencies of individual PCBs have not been determined; however, mechanistic studies indicate that PCBs and related halogenated aryl hydrocarbons act as tumor promoters. Moreover, at least in the skin model for carcinogenesis using hairless mice,[2/] the observed structure-activity relationships confirm the role of the Ah receptor in this process. If one accepts the hypothesis that the mechanism of PCB tumorigenicity involves interaction with the Ah receptor, the structure-toxicity relationships that are also dependent on relative binding affinities for the Ah receptor protein can also be used to estimate tumorigenic potencies of individual PCBs and PCB mixtures.

F. Estimation of Potencies of PCB Mixtures

1. EPA Approach to Estimating Cancer Potency Factors

EPA (1986) has developed specific guidelines for risk assessment involving carcinogens. These guidelines require the derivation of a cancer potency factor (CPF) through the application of a mathematical model to extrapolate the observed dose-response data to very low doses at which humans are exposed (typically, hundreds of thousands of times lower than those used experimentally). It is not known whether the model is accurate; in fact,

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2/The skin of (genetically) hairless mice has been used as a model system for evaluating the potential of some chemicals to promote cancer. Usually, the cancer initiator is either injected or painted on the skin, followed by repeated applications of the suspected promoter. Appearance of skin lesions, including tumors, is recorded.

according to EPA, its use is designed to produce an upper limit on risks. The true risk, according to EPA (1986), is likely to be lower and could be zero.

EPA's CPFs are derived using the 95% upper bound of the slope of the linearized, multistage model for extrapolation to low doses. This model is based on certain assumptions about the action of carcinogens that may or may not be appropriate for PCBs. Furthermore, this model is one of the more conservative extrapolation models, i.e., it usually estimates a higher CPF than other models. Even though the analysis that follows is based on EPA's CPF, a further review of the scientific data may justify a different procedure for extrapolating to low doses.

EPA (1988) has calculated a CPF for PCBs of 7.7 $(mg/kg/day)^{-1}$ based on the Norback and Weltman (1985) study.[1/] Prior to this, EPA (1984) had determined the CPF for PCBs to be 4.34 $(mg/kg/day)^{-1}$ based on the Kimbrough et al. study. Both of EPA's CPFs are based on studies in which rats were exposed to Aroclor 1260, and the agency has suggested that this CPF should be used for all PCBs. EPA (1988) has published, however, a "preliminary calculation" indicating a CPF of 2.6 $(mg/kg/day)^{-1}$ for Aroclor 1254 based on the 1978 NCI bioassay data. EPA states that, although the Aroclor 1260 data are the best for estimating the cancer potency of PCBs as a whole class of compounds, it is appropriate to ask whether existing data on other PCB mixtures are adequate for making separate cancer potency estimates. Citing limitations in the data for calculating separate

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1/The units on CPF are "risk per unit dose", where dose is expressed in mg/kg body weight/day. Multiplying "risk per unit dose" by the "estimated lifetime average daily dose" (in units of mg/kg body weight/day) yields an upper-bound estimate of lifetime risk.

cancer potency estimates for each PCB mixture, EPA has made a policy choice to use the CPF from Aroclor 1260 to characterize the upper limits on risks for all other PCB mixtures. As discussed below, EPA's approach is not supported by the available scientific information.

2. Modification of EPA Approach Based on Relative Potency Adjustment

EPA's cancer potency estimate for PCBs should be modified in two significant ways: a) the EPA potency estimate (based on Aroclor 1260) should be changed to reflect the lower potencies of Aroclor 1254, Aroclor 1248, Aroclor 1242, and Aroclor 1232; and b) the interspecies extrapolation factor used by EPA should be changed from a dosage per surface area scaling factor (i.e., $mg/m^2/day$) to a dose per unit body weight scaling factor (i.e., mg/kg/day).

a) Interspecies Scaling

The interspecies scaling (i.e., extrapolation) of dose is necessary to compensate for differences between humans and laboratory animals for such factors as size, lifespan, and basal metabolic rate. The most commonly used measures of dose are milligrams of chemical per kilogram of body weight of the animal per day (mg/kg/day) and milligrams of chemical per square meter body surface area per day ($mg/m^2/day$). Debate over the choice of dosage unit has centered on the appropriate measure for body size (kg body weight or m^2 body surface area) and on the temporal descriptor (per day or per lifetime) (cf. Hoel et al. 1975, Crump et al. 1980, Food Safety Council 1980, Allen et al. 1987). For carcinogenic compounds, both scaling factors have been used in risk assessment by different federal agencies, and both scaling factors were considered valid when

reviewed by the Office of Science Technology and Policy (OSTP 1985). For example, the EPA uses $mg/m^2/day$ while the Food and Drug Administration uses mg/kg/day. (EPA recently published a notice in which, among other matters, it requested comments on whether to modify its approach.)

The use of $mg/m^2/day$ as a scaling factor tends to give higher risk estimates per unit of dose than does mg/kg/day. (Risk is presumed in the linear no-threshold model to be directly proportional to dose.) For example, in extrapolating from mouse to man, the use of $mg/m^2/day$ will result in a risk estimate (per unit of dose) that is approximately 12 times greater than the estimate obtained using mg/kg/day. In extrapolating from rat to man the risk estimate is approximately 7 times greater when surface area scaling ($mg/m^2/day$) is used as opposed to mg/kg/day.

There are a number of reasons why extrapolation should be undertaken on a body weight basis. First, consider the basis of the surface area scaling factor. Hoel et al. (1975) proposed the use of dosage units in $mg/m^2/day$ on the basis of studies of the acute toxicity of anticancer drugs in humans and animals. In these studies, the acutely toxic level was similar in mouse, rat, hamster, dog, monkey, and man when dosage was expressed as $mg/m^2/day$. This finding is not unexpected. In many cases toxic substance are detoxified by the metabolic processes of the organism. The body surface area of an animal is an indirect measure of the animal's basal metabolic rate. It is this relationship between body surface area and metabolic rate that explains the interspecies similarity in dosages when expressed on a surface area basis. But this relationship for acute toxic effects does not

necessarily apply for other effects. The relationship between dose and body surface area, a priori means very little when considering chronic effects such as cancer.

In contrast, the Scientific Committee of the Food Safety Council (1980) favored the use of body weight as the basis for extrapolation. The council explained that "with long experience of the value of extrapolation on body weight basis, we recognize this as the most satisfactory procedure." Crump et al. (1980) and Allen et al. (1987) determined, based on an analysis comparing the carcinogenic potency of 13 chemicals in humans and rodents, that the unit of dosage measurement giving the closest correlation between species was mg/kg/day.

A similar conclusion was reached by Crouch (1983), after examining a large data set on chemicals that had been tested for carcinogenicity in more than one species. Some of the chemicals in this data set had also been studied epidemiologically in humans. Crouch (1983) found that he could derive a range of scaling factors to extrapolate among species, strains, or sexes, but argued that a body-weight scaling factor value of 1 (i.e., mg/kg/day) should be chosen for general extrapolation from rodents to humans.

In the absence of good evidence for the use of a more complex procedure, we believe that the use of mg/kg/day is the most appropriate basis for interspecies dosage comparison. In addition to its relative simplicity, this procedure appears to have the best empirical support (Crump et al. 1980, Allen et al. 1987).

b) Potency Differences

Based on the above discussion of interspecies scaling factors and the earlier discussion of observed potency differences, it becomes possible to derive potency factors for each commercial PCB mixture. EPA derived a potency factor of 7.7 per mg/kg/day for Aroclor 1260 based on the study by Norback and Weltman (1985). In deriving a potency value for this report, we preserve EPA's conservative linearized multistage low-dose extrapolation model, but modify the interspecies scaling procedure by about six-fold,[1/] as discussed above. This leads to a potency factor of 1.3 per mg/kg/day for Aroclor 1260.

As noted earlier, Clophen A60 is at least ten times more potent than Clophen A30 in studies of identical design. Because Aroclor 1260 is similar in composition to Clophen A60 and Aroclor 1242 is similar to Clophen A30, it is reasonable to assume that Aroclor 1242 should exhibit a potency no more than one-tenth that of Aroclor 1260. We thus assign a potency to Aroclor 1242 of 0.13 per mg/kg/day. It should be noted, however, that there are no studies that show a statistically significant increase in tumors for any mixture of PCBs other than Aroclor 1260 and Clophen A60. The potency of the less chlorinated mixtures of PCBs may thus be appreciably less than our estimate.

A simple interpolation procedure can be used to assign potencies to the other Aroclors. The

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1/The potency value is based on a study in rats. EPA's estimate is 7.7 per mg/kg/day. We used actual body weight data from the Norback and Weltman study rather than the generic factor of 7.

procedure assumes that potency declines proportionally with chlorine content. This can be approached either through an analysis by percent chlorine content of a particular Aroclor type or through an analysis by average chlorine number by Aroclor type. For example, if potencies of 1.3 and 0.13 are assigned to Aroclor 1260 (60% chlorine) and Aroclor 1242 (42% chlorine), respectively, then potencies can be assigned to Aroclors 1254, 1248, and 1232 based on their respective percent chlorine: 54%, 48%, and 32%. Similarly, the potencies of Aroclor 1260 and Aroclor 1242 can be used as the basis for interpolation of potency factors for Aroclors 1254, 1248, and 1232 from the average number of chlorine atoms in each Aroclor.

Because commercial PCBs are complex hydrocarbon mixtures, which are not completely identical with respect to specific isomer content, chlorine number can vary within Aroclor type. In order to derive a potency value based on actual chlorine number, rather than percent chlorine, an average value for chlorine number per Aroclor type must be determined. Several approaches have been used to derive the average chlorine number: a probabilistic (pseudo-stochastic) approach in which chlorine number is calculated based on the relationship between percent chlorine and chlorine number in individual PCB congeners; an approach calculating the average chlorine number based on weight percentages of congeners in commercial PCBs; and an approach calculating the average chlorine number based on data present in an ENVIRON (1987) report listing amount (percent mass fraction) of specific congeners in Aroclors 1260, 1254, and 1242. Slight variations in the average chlorine number per Aroclor type are seen among these three different approaches. When these alternative

values of chlorine number are each used to derive a cancer potency estimate, however, virtually no difference in potency exists. Therefore, the values for cancer potency are virtually the same no matter which approach is used to derive average chlorine number.

The tumorigenic potency values derived for various Aroclors using a percent chlorine approach and an average chlorine number approach are presented in table 1. All potencies except the potency for Aroclor 1260 (that provides the basis for the other estimates) are rounded to one significant figure. It is evident that the cancer potency values derived using a percent chlorine approach are virtually identical to those using an average chlorine number approach. Because it is our hypothesis that the cancer potency of PCBs varies with chlorine number, it is probably more accurate to rely on the mean chlorine number.

It is important to note that the TPFs in table 1 have been developed by combining the incidence data for benign and malignant tumors. Some individuals have suggested that only malignant tumors should be used to estimate cancer potency.— If the data on the incidence of animals with malignant liver tumors (carcinoma or adenocarcinoma) only, i.e., excluding neoplastic nodules, were used, the potency factor for Aroclor 1260 would be 0.98 per mg/kg/day. Based on an analysis of malignant tumors only, Clophen A60 is about 13 times more potent than Clophen A30. Hence, the potency of Aroclor 1242, which is similar in composition to Clophen A30, should exhibit a potency no more than one-thirteenth that of Aroclor 1260, if malignant tumors only are considered. This would reduce the potency value of Aroclor 1242 to 0.075 per mg/kg/day. These values are slightly lower than those estimated in table 1.

TABLE 1

Alternative Estimation of Tumorigenic Potency (TPF) Factors For Various PCB Mixtures -- Benign and Malignant Tumors Combined[1/]

PCB Mixture	% Chlorine	TPF $(mg/kg/d)^{-1}$ Based on % Chlorine	TPF $(mg/kg/d)^{-1}$ Based on Mean Chlorine Number
Aroclor 1260	60	1.3	1.3
Aroclor 1254	54	0.9	0.8
Aroclor 1248	48	0.5	0.4
Aroclor 1242	42	0.1	0.1
Aroclor 1232	32	0.08	0.07

1/Based on a comparision of data from Clophens A60 and A30, combining benign and malignant tumors as discussed in the text.

3. Uncertainties and Limitations

There are several important limitations that tend to contribute to the conservative nature of this analysis:

1. All environmental PCB mixtures are assumed to be potential animal tumorigens, even though data are available to support such a conclusion only for those closely resembling Aroclor 1260.

2. Those PCB mixtures that are known or potential animal tumorigens are assumed to have the potential to cause tumors in humans, notwithstanding the absence of evidence of a causal relationship from all available epidemiology data.

3. A linear, no-threshold, low-dose extrapolation model is used to estimate potencies for all PCBs, notwithstanding the fact that PCBs do not exhibit many of the characteristics of carcinogens for which such models were developed. Indeed, it is possible that there is a threshold dose that must be exceeded before PCBs could pose any cancer risk, whatsoever.

G. Conclusions

Although some workers have been exposed to high levels of PCBs for long periods of time, several studies of such populations have not provided information establishing that PCBs cause cancer in human beings. If PCBs were potent human carcinogens, it is likely that such an increase in cancers among these several worker populations would have been observed. Therefore, based solely on data from exposure of people, it is not possible to conclude that PCBs are carcinogenic to humans.

Of the PCB mixtures that have been tested in animals, those that are 60% chlorinated (i.e., Aroclor 1260 and Clophen A60) are carcinogenic in rats. Tests in other species have not been adequate to demonstrate or rule out carcinogenicity. PCB mixtures that are less chlorinated (i.e., Aroclor 1254, 54% chlorinated, and Clophen A30, 42% chlorinated) were not carcinogenic in rats, but the latter increased the incidence of non-malignant tumors. Two conclusions can be reached from these data:

1. Highly (60%) chlorinated PCBs are carcinogenic in one animal species.

2. Less chlorinated PCBs are either not carcinogenic or are substantially less potent than the more highly chlorinated mixtures.

Guided by these conclusions, it is possible to make several conservative hypotheses:

1. If 60% chlorinated PCBs are carcinogenic in one mammalian species, it is assumed they may be carcinogenic in others, including humans, i.e., Aroclor 1260 and Clophen A60 may be carcinogenic in humans. It must be noted that this is a conservative assumption, based on current regulatory practice.

2. Because there is some overlap in the congener composition of the commercially available PCB mixtures and because no data exist to determine unequivocally which of the congeners are responsible for the animal carcinogenicity, other mixtures of PCBs may be viewed as carcinogenic. Again, this is an assumption that is consistent with current regulatory practice.

3. If all PCBs are assumed to be tumorigenic in humans, the maximum potencies of less chlorinated PCBs can be estimated from the available bioassay data.

These hypotheses form the basis of the estimates of human carcinogenic potency that are derived in this report. As is discussed herein, however, these estimates constitute a conservative upper bound on cancer potency. In the case of PCBs, the carcinogenic risks to humans from environmental exposure are almost certainly less than our estimates and, in fact, could well be zero. EPA (1986) has also acknowledged the conservative nature of upper-bound cancer potency estimates.

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