

Workshop on Human Health Impacts of Halogenated Biphenyls and Related Compounds

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A workshop on the Human Health Impacts of Halogenated Biphenyls and Related Compounds was held to assess the state of current research on these chemicals and to make recommendations for future studies. Participants discussed results from laboratory animal experiments on PCBs, PBBs, dioxins, and dibenzofurans which demonstrate a common mode of toxicological action while also revealing large variations in toxicological potency both within and between these chemical families. These variations demonstrate the importance of congener-specific analyses in future studies of effects of exposure to these compounds. Results from epidemiological studies of environmentally exposed adult and pediatric populations from the U.S., Japan, and Taiwan and occupationally exposed cohorts from around the world were considered. It was concluded that available evidence did not demonstrate serious adverse effects such as cancer, in exposed adult cohorts but did provide indications of possible neurobehavioral effects in children exposed *in utero*. In addition, workshop participants described newly developed markers of exposure and techniques for assessing endocrinological, immunological, and neurological effects and suggested these be applied to epidemiological studies of the effects of polyhalogenated compounds. Other recommendations included identification of other cohorts and development of a large registry of exposed individuals; performance of detailed studies of reproductive function and outcomes in exposed populations; and follow up of neurobehavioral effects in offspring of exposed women.

Introduction

The focus of this workshop was to determine the current status of knowledge concerning the toxicity of halogenated biphenyls and related compounds to humans; summarize ongoing or planned research aimed at increasing our knowledge about the human toxicity of these chemicals; and make recommendations for future research that can best provide the information that is needed. To accomplish this goal, 78 scientists representing many fields of study participated in a workshop on the Human Health Impacts of Halogenated Biphenyls and Related Compounds on November 8 and 9, 1989, in Ann Arbor, Michigan. The invited participants listened to prepared talks, had extensive discussions, and a selected group met together to make consensus recommendations.

Introductory speakers addressed the current understanding of the mechanistic basis for the toxicity of the polyhalogenated biphenyls and related compounds (PHBs) based on animal and *in vitro* studies and also

provided an overview of current knowledge about the human toxicity of these compounds. Subsequent sessions dealt with environmentally exposed populations; occupationally exposed populations; pediatric populations; and biological measurements and markers of exposure. The final half-day was devoted to developing the recommendations that are provided at the end of this report.

The workshop was initiated by the Division of Cancer Etiology, National Cancer Institute, and was supported jointly by the National Cancer Institute (NCI), Agency for Toxic Substances and Disease Registry (ATSDR), Environmental Protection Agency (EPA), and National Institute of Occupational Safety and Health (NIOSH). The program was developed and the conference organized by Lawrence J. Fisher, Director, Institute for Environmental Toxicology, Michigan State University, and David Schottenfeld, Chairman, Department of Epidemiology, School of Public Health, The University of Michigan.

Session I: Overview

Steven Safe (Texas A&M) discussed the large body of evidence showing that a number of chemical families, including the polychlorinated dibenzodioxins (PCDDS), polychlorinated dibenzofurans (PCDFs), polychlorinated

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biphenyls (PCBs), and polybrominated biphenyls (PBBs), all have similar toxic effects in animals and appear to act via the same receptor mechanism (1). The constellation of biological effects produced by these chemicals in experimental animals includes induction of drug-metabolizing enzymes, weight loss, thymic atrophy, and reproductive dysfunction. The proposed mechanism of action is initiated by interaction of the chemical with a cytosolic receptor (the Ah receptor), followed by movement of the toxicant-receptor complex to the nucleus, interaction with responsive genetic elements resulting in altered gene expression, and ultimately the manifestations of toxicity.

These chemicals are all inducers of enzymes responsible for metabolism of xenobiotics. Two specific enzyme activities, aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD), are often used as markers of exposure to PHBs. Studies have shown there is a correlation between the *in vitro* potencies of these compounds as inducers of AHH and EROD activities and their *in vivo* toxic effects; e.g., weight loss (2,3). This has led to the suggestion that AHH and EROD induction activities measured *in vitro* can be used as surrogates for toxicity of the chemicals *in vivo*.

Although many of the PHBs elicit a similar pattern of responses, their relative potencies may vary over many orders of magnitude, even within the members of the same family, e.g., different congeners of PCDDs. The dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is considered the most toxic of this class of chemicals and is used as the standard for comparison. In general, the coplanar PCB molecules that are sterically most similar to TCDD exhibit the highest toxicity in laboratory animals. The relative potencies of the congeners vary somewhat with the assay system; however, the order of potency of the coplanar PCBs is 3,3',4,4',5-penta CB > 3,3',4,4',5,5'-hexa CB > 3,3',4,4'-tetra CB (2).

Relative potencies in enzyme induction assays have been used in conjunction with congener-specific analyses to provide estimates of the total activity of environmentally relevant mixtures of PHBs. For example, a mixture of PCBs and PCDFs could be assigned a total activity by multiplying the concentrations and relative potencies of all the congeners present and then summing these values. An alternative approach is to assess the biological activity of the mixture directly using the enzyme induction assays. The total activity assessed by either method has been used as a surrogate for estimating the toxicity of the mixture under investigation and for some mixtures the toxicities have also been determined. However, the extrapolation from enzyme induction assays to toxicity in humans or animals remains to be validated.

Recent studies suggest that specific PHBs can act as 2,3,7,8-TCDD antagonists of some Ah receptor-mediated responses in rodent and mammalian cells in culture (4). These antagonists, exemplified by 1,3,6,8-substituted PCDFs and the commercial PCB mixture, Aroclor 1254, typically exhibit low Ah receptor agonist

activity and moderate affinity for the Ah receptor. For example, cotreatment of C57BL/6 mice with subeffective doses of Aroclor 1254 and effective dose (ED) 80-100 doses of 2,3,7,8-TCDD resulted in partial antagonism of 2,3,7,8-TCDD-mediated AHH and EROD induction, cleft palate, and immunotoxicity. These interactions generally require antagonist/agonist ratios of > 1000 and are both species and response dependent.

Another important finding from nonhuman studies is that the PCB congener distribution in environmental samples (e.g., food fish) is quite different from the composition of the parent commercial mixtures, such as the Aroclors. It appears that selective enrichment of several congeners, including the more toxic coplanar PCBs, occurs in some environmental samples. The explanation for these observations is not fully known and more analytical work is necessary to validate these conclusions.

These results obtained from experiments in animal and *in vitro* systems, coupled with limited high resolution analytical data, emphasize the importance of congener-specific analyses of PHBs in assessing their potential adverse effects in humans. More research on nonhuman models is needed to assess the toxicity of the large number of PCB congeners not yet studied, elucidate basic mechanisms of action and interaction, and provide the data necessary to fully interpret results from studies of human cohorts (5).

Renate Kimbrough (U.S. EPA) discussed exposures and risks in human populations. Although there have been a large number of people exposed to PHBs, quantitative estimates of exposure have not been well developed and relationships between exposure and adverse health outcomes have been difficult to document (6). There is a background exposure as evidenced by measurements in the early 1980s of the general U.S. population showing PCB serum levels averaging 5 to 7 ng/mL and adipose levels averaging 0.9 to 9.4 mg/kg. There is great interindividual variation, and extrapolating from serum levels to total body burden requires great care.

A number of populations have been identified and classified according to type of exposure, e.g., heavy fish eaters, those exposed through ambient air, those exposed *in utero*, and various worker groups. Above-background serum PCB levels have been measured in a number of these populations, with workers showing the highest levels—up to 3500 ng/mL in serum.

Serum PCB levels appear to increase with age, and there is some evidence of a correlation with high cholesterol levels and alcohol consumption (without clinically significant liver dysfunction). In women, PCB levels decrease with increasing lactation, as the chemical is concentrated and excreted in milk.

Studies of various PHB-exposed populations to date have not shown a clear increase in mortality or cancer incidence. There is some evidence of adverse reproductive outcomes although the effects appear to be small. Since the validity of epidemiological studies depends strongly on good measures of exposure and establish-

ing a dose response, better and more consistent measures of exposure will be needed before the relationship between PHBs and human toxicity can be fully understood (7).

Discussion

In response to questions, it was emphasized that interactions among PHBs are species specific, and the results from animal studies cannot be generalized to humans. There are a number of levels at which antagonism of PHB effects by lower potency PHB congeners can occur: e.g., binding to receptor, accumulation of complexes in nucleus and posttranscriptionally. The mechanism of action for these antagonistic effects has not yet been elucidated. A related issue is the difficulty of distinguishing between differential sensitivity and antagonism when a mixture of PHBs is being evaluated.

Another issue raised was possible differences in congener distribution in tissues related to route of exposure. Although this has not been thoroughly investigated, current data suggest that route of exposure is not an important variable in the disposition of PHBs.

Session II: Human Environmental Exposures

The presentation by Masanori Kuratsune (Nakamura Grakuen College, Japan) was devoted to studies of an environmentally exposed cohort in Japan. This population, known as the "Yusho" cohort, consists of individuals who were exposed to rice oil contaminated with PCBs, PCDFs, and related compounds over a 6 to 8 month period in 1968. Using the enzyme induction assay and extrapolating from results in laboratory animals, it was calculated that about 75% of the toxicity of the mixture in the rice oil was due to PCDFs and most of the remainder to PCBs, with a very small contribution from PCDDs (8).

Approximately 1800 people were exposed and have been followed during the past 20 years (9). The initial severe chloracne has declined significantly, but some of the exposed individuals still show evidence of dermal toxicity. A calculation of the time required for nearly total elimination of PCDFs from the patients' bodies was made on the basis of results from repeated analyses of stool samples from the same individuals. The calculated value was about 58 years.

Mortality in the population at the end of 1983 was no different from the nonexposed population. However, there were some excess liver and lung cancer deaths in males. The excess of liver cancer deaths was suspected to be due to PHB exposure, although the present evidence is not conclusive. One confounding factor was the higher liver cancer incidence in the provinces where the exposure occurred, as compared to the country as a whole. In addition, the data included some liver cancer deaths occurring relatively soon after exposure and not likely to be a consequence of PHB exposure. Last,

confirmatory autopsies were not performed in several cases, casting doubt on the diagnoses for these cases. Women exposed during the Yusho incident have not shown excess cancer mortality (10).

Chen-Chin Hsu (National Cheng-Kung University, Taiwan) discussed the Taiwan or "Yucheng" incident, which occurred in 1978-1979, which also involved ingestion of contaminated rice oil and affected almost 2000 individuals. In comparison to the Yusho incident, the exposures were lower in most individuals. However, effects on the offspring born to exposed pregnant women were evident. The most striking was hyperpigmentation, and many offspring were described as cola-colored. In addition, neuroectodermal toxicity was noted in fetuses of exposed mothers (11).

Investigators focused on detection of possible behavioral effects caused by *in utero* exposure, and 118 children, ranging from 1 to 8 years of age, were studied in detail in 1985. They were compared to nonexposed children and also to siblings who were exposed directly rather than transplacentally. The results of these studies indicated lower overall intelligence scores, hyperactivity, and greater behavioral problems in the children born to PHB-exposed mothers compared to children in referent groups. These were subtle effects, and there was no evidence of mental retardation. The results suggest that *in utero* exposure may cause deleterious effects not observed when exposure occurs after birth (11). Follow-up of these children indicates that the differences from the control group are increasing with time, suggesting that the early effects may reflect permanent changes.

Harold Humphrey (Michigan Department of Public Health) discussed some Michigan populations that represent some of the largest cohorts exposed to PHBs. These include about 4000 Michigan farmers exposed to PBBs in Michigan during a contamination incident in 1973-1974, over 200 members of farm families exposed to PCBs that were used to line their silos, and about 600 Lake Michigan shoreline residents who ate large amounts of fish contaminated with PCBs and a number of other chemicals including *p,p'*-dichlorophenyl dichloroethylene (DDE), chlordane, and mirex. Data on all of these cohorts have been collected since the early 1970s, and members of each group continue to be followed so that possible long-term effects can be detected (12,13).

In addition to collecting interview and medical record data from members of these cohorts, periodic measurements of serum and adipose PHB levels have been made. These studies of PHB-exposed populations have shown a correlation of blood and adipose PBB and PCB levels with exposure, but no convincing evidence of toxicity due to these exposures has been found (13). More intensive study is needed to determine if subtle, adverse effects have occurred in members of each cohort or their offspring.

Walter Rogan (NIEHS) described some other PHB-exposed populations, including 5000 people exposed to dioxin at Quail Run, Missouri, from 1971 to 1983 (14),

about 600 people exposed to a mixture of dichlorodiphenyl trichloroethane (DDT), DDE, and PCBs in Triana, AL (15), and those exposed to PCBs and their combustion products in Binghamton, New York (16). The largest available cohort, consisting of about 20,000 individuals, is from Seveso, Italy (17). This population, including a highly exposed group of about 700, was exposed to dioxins as the result of an industrial accident in 1976. Up to the present, the only adverse dioxin-related effect that has been observed as a result of the Seveso incident is chloracne. However, a number of measurements used in other epidemiological studies of PHB exposure have not been performed on this population. Because of the size of this cohort, it represents a good opportunity to test for effects that are suggested by the results obtained in other studies, but which have not yet been examined.

Discussion

A number of issues were raised with respect to interpreting the results of studies of these environmentally exposed populations. One central problem is how to combine these cohorts in a scientifically valid manner to obtain a large enough population to establish correlations, with a high degree of confidence, between exposure and adverse effects. Related to this central issue are a number of questions that must be answered: (a) How comparable are exposure measures (e.g., serum levels) performed by different laboratories or at different times in the past? (b) How comparable are methods of surveillance and classification of adverse health effects? (c) How comparable are the control groups? (d) How applicable are results derived from one ethnic group to other ethnic groups? (e) How can measures on one type of sample (blood) be converted to measures of other types of samples (fat)? (f) Can groups be combined if different mixtures of PHBs were involved in the exposure?

Session III: Occupationally Exposed Populations

A presentation by Laurie Piacitelli (NIOSH) described a NIOSH registry of individuals occupationally exposed to 2,3,7,8-TCDD or hexachlorodibenzodioxins (18). A total of 6583 workers, who were employed at 14 plants between 1942 and 1983, are in the registry. At present, 1469 of these workers are deceased. Both a retrospective cohort mortality and a medical and reproductive outcome study are being performed using this cohort.

Serum samples were collected from 281 workers in two plants and 260 unexposed referents. Workers had a significantly higher mean lipid-adjusted serum 2,3,7,8-TCDD level (252 parts per trillion [ppt]) than the referent group (7.8 ppt), even though worker samples were collected 15 to 37 years after last exposure. To estimate serum levels in workers at the time of last occupational 2,3,7,8-TCDD exposure, extrapolations

were performed assuming a 7-year 2,3,7,8-TCDD half-life. The mean extrapolated level was 2658 ppt for one plant and 866 ppt for the other, confirming that the occupational population had substantial exposure. The serum 2,3,7,8-TCDD levels significantly correlated with years of exposure (19). A matrix for estimation of occupational exposure is being developed from historical data on industrial processes, fluid and tissue analyses, and industrial hygiene practices. Analysis of the mortality study is underway and will be completed in fiscal year 1990.

A second large occupational registry, described by Eric Johnson (NIEHS), is international in scope and is a combined effort of NIEHS and the International Agency for Research on Cancer (IARC) (20,21). The populations that are part of this registry are from 12 countries and include both manufacturing and user cohorts that were exposed to phenoxy herbicides, chlorophenols, and hexachlorophene. There are almost 20,000 individuals enrolled in this registry at the present, with a total of about 30,000 anticipated. Mortality and cancer incidence analyses are being conducted and are almost complete. This population should be available to investigators for the study of other end points, such as reproductive effects and serum dioxin levels, sometime in the future.

The third presentation in this session, by John Brown, Jr. (General Electric), described results from a study of worker populations from two plants that manufacture capacitors. The major exposures were to the Aroclors (PCBs) that were part of the dielectric fluid in the capacitors. The Aroclors contained significant levels of coplanar PCBs. For example, Aroclor 1254 contained 38 ppm of 3,3',4,4',5-PCB. PCDFs were also found as contaminants in capacitor fluid samples but at very low levels. Because of the automated nature of the process, only about 200 out of a total of 2500 workers in these plants could be classified as highly exposed. Results from this study have not demonstrated acute (i.e., chloracne) or chronic toxicity, although there was some evidence of induction of microsomal enzymes (22,23). Two additional results were that half-life of the PCBs in exposed workers was slowing with time and was 6 to 12 years at the time of last measurement and that higher chlorinated congeners appeared to be retained in the body preferentially and have extremely long half-lives (24-26).

Discussion

Questions were raised regarding the possibility of establishing a PCB occupational registry using a number of different cohorts. It was suggested that there are over 750 workers who have shown blood serum PCB levels above 10 ppb. This cohort could be increased in size if workers from small plants were identified and included. The creation of a PBB-exposed worker registry was also suggested. The half-life data obtained in the capacitor worker study led to requests for studies to clarify the time course of PHB tissue distribution

and redistribution following exposure. As with the environmentally exposed populations, a number of problems need to be resolved before different cohorts can be combined to increase the power of the epidemiological results.

It was also pointed out in the discussion that comparison of occupationally and environmentally exposed populations is complicated by the differences in congener mixtures to which the two types of populations were exposed. The workers were exposed to commercial mixtures, while environmentally exposed populations (e.g., fish eaters) were exposed to mixtures that had quite different congener distributions than those present in the commercial products. More study is needed to estimate what effect these exposure differences have on the potential for toxic responses.

Session IV: Pediatric Populations

Walter Rogan (NIEHS) started this session with a description of a study performed on the offspring of mothers who were exposed to background levels of PCBs. It was carried out in North Carolina and initially enrolled about 900 mothers exhibiting a range of levels of PCBs in milk. Offspring born from 1978 to 1982 were studied for 5 years. Attempts were made to correlate child development with degree of PCB exposure. Both on the Brazelton scales as newborns and on the Bayley scales as 1-year olds, children in the top 5% of transplacental exposure, as determined by milk levels, showed delay in maturation of motor abilities (27,28). The magnitude of the effects of prenatal exposure were small; a 1-ppm increase in PCB milk level in the mother was associated with a 1-point reduction in the Bayley score of cognitive function. There appeared to be no relationship between measured development and exposure through breast milk.

A second pediatric study, described by Joseph Jacobsen (Wayne State University), was performed on the offspring of 242 mothers who regularly consumed Lake Michigan fish contaminated with PCBs and other compounds and 71 mothers who did not eat these fish. Measurements were made of both physical and behavioral characteristics of children as well as cord serum, maternal serum, and breast milk PCBs levels (29). Results indicated decreased birth weight and head circumference as well as reduced gestational age in the offspring of the most heavily exposed women when compared to women in the same locale who did not consume fish (30). In addition, exposed infants exhibited poorer central nervous system function at 7 months of age, as indicated by poorer performance on a widely used test of infant visual recognition memory (31). Results from this study also showed that transplacental exposure was more effective in producing these effects than postnatal exposure through breast milk (32,33).

Discussion

In response to questions, it was evident that differ-

ences in the analytical procedures used in the two studies for determining PCBs make it difficult to make comparisons between the two cohorts. In addition, it was suggested that environmental lead exposure in infants might also contribute to abnormal central nervous system development in early childhood. However, it was noted that there are some differences in the effects observed in these studies and those observed with lead. For example, lead affects mainly mental scores, while the present studies show primarily motor effects. A related issue was whether careful studies of heavy metal levels in the mothers or the food supply had been performed in the above studies of PCB effects. Heavy metal analyses had not been included, leaving open the question of a possible contribution of exposures to contaminants other than PCBs. In a different vein, concern was expressed about possible impacts of sample selection and attrition on the results of the study of Michigan women.

Session V: Biological Measurements and Markers of Exposure

In contrast to other sessions in the workshop, Session V addressed techniques that might be useful for answering a number of outstanding questions about the human toxicity of the compounds of concern. George Lucier (NIEHS) examined the issue of the comparative sensitivity of humans and animals to the effects of the polyhalogenated biphenyls and related compounds. A comparison was made between PHB effects seen in rat livers and those observed in human placentas collected in connection with the Yucheng incident in Taiwan (34). In human placentas, the PHBs showing the highest affinity to the Ah receptor were the PCDFs. Although enzyme induction was detected only in placentas from exposed individuals, induction in these tissues was not correlated with PCDF concentrations. Results from rat liver studies showed that the enzyme induction response saturates at low concentrations, while incidence of cancer continues to increase above these concentrations (35). Thus, the enzyme induction does not appear to be a marker for carcinogenicity. Effects on receptor systems involved in cell proliferation (e.g., epidermal growth factor [EGF], glucocorticoid, and estrogen receptors) were generally similar in the placenta and rat liver and dose-responses for these effects were similar to those for carcinogenic responses (36,37). Quantitative and qualitative comparisons of the rat and human data suggested that humans were at least as sensitive as rats to several biochemical effects of PHBs (38). However, these conclusions are based on a number of critical assumptions, and further research is needed to establish relationships between PHB exposure and functional changes.

George Lambert (Loyola University Stritch School of Medicine) discussed human P-450I enzyme activity as a biomarker and described the use of caffeine (labeled with stable isotope) breath test to monitor P-450I activity in humans. He indicated that P-450I activity can

predict toxic potential of PHBs in animals but that this relationship has not been established in humans (39). Studies of PBB-exposed individuals from Michigan showed greater P-450I enzyme activity compared to controls, as measured by the breath test. There was a good correlation between the rate of caffeine metabolism; i.e., P-450I activity and PBB serum levels in adults (40). However, exposed prepubescent children did not show evidence of enzyme induction as measured by the breath test. Similar results were found with the Yucheng populations. The caffeine breath test promises to be a valuable technique for assessing exposure and enzyme induction in adults but may not be suitable for studying such effects in prepubescent children.

Walter Piper (University of Michigan) discussed possible PCB-related effects on the endocrine system that might be measured in humans. A basis for this work is research showing that steroid synthesis is P-450-mediated and reports indicating that various hormone levels are affected by TCDD exposure in laboratory animals. Up to now, there has been insufficient study of the time course of these responses. Preliminary studies, such as those of adrenocorticotrophic hormone (ACTH) response to TCDD exposure in the rat indicate a rapid response, as early as one day after treatment. Other work showed a TCDD-induced decrease in luteinizing hormone lasting about 3 days and followed by a return to normal levels. Based on current data, the half-life for P-450-mediated reactions is from 1 to 5 days. More detailed studies of the effects of PHBs on endocrine systems in animals may provide valuable information about the mode of action of these chemicals in humans. In addition, hormone levels and responses may be useful as markers of exposure to PHBs. Measurements of such levels can be made in a number of different body fluids including blood, urine, and saliva (41,42).

Roderick Nairn (University of Michigan) presented the immunosuppressive effects produced by PHBs and discussed their possible use as markers of exposure. Early reports from studies of PBB-exposed farmers indicated some immunological effects, but the studies used relatively crude measures of immune function (43). More sensitive methods have become available, such as using antigenic probes and cell-sorting analysis to examine subsets of lymphocytes, and a careful re-examination of exposed populations using this newer methodology is now appropriate. In addition, immunological responses have the potential to be very important as biomarkers of exposure because they are very sensitive, quite specific, and often persist for long periods of time. More work needs to be done in developing applications of available immunological techniques to the study of PHB exposures.

The last speaker, Joseph Arezzo (Albert Einstein College of Medicine), described new quantitative techniques for assessing both peripheral and central nervous system function in humans. There are a variety of such tests that have become available, some of which can be self-administered (44-47). For example, new

equipment is available to test distal sensory function. This can be used to assess peripheral neuropathies. Vibration testing can assess large fiber function, and thermal testing can be used for small fiber assessment. Results from these and other recently developed techniques can be combined to provide an accurate assessment of the presence or absence of neurological deficits as well as diagnostic information about the type of effect and the possible site of action (48). Such studies could be valuable in assessing PHB toxicity as well as possibly indicating that exposure at toxicologically significant levels has occurred.

Workshop Recommendations

1) Perform congener-specific analyses of tissues and fluids obtained from exposed and referent populations. Correlate exposure history with presence of specific congeners and biomarkers of exposure. Measure binding of specific congeners to Ah receptors in selected human tissues.

Rationale: It is clear from laboratory studies that older analytical methods measuring total levels of PHBs; e.g., incompletely separated PCB mixtures, do not provide sufficient information to make valid comparisons among exposed populations. Because the biological potency of individual congeners varies widely and because some congeners may act as antagonists, the potential toxicity of any PHB mixture must be assessed on a congener specific basis.

2) Make detailed studies of reproductive function and outcomes in exposed and referent populations.

Rationale: Reproductive effects (decreased fecundity and skeletal abnormalities) are seen consistently in lower animals exposed to PHBs. Such effects are observed in wildlife populations exposed to PHB mixtures similar to those involved in human exposures.

3) Examine the feasibility of establishing registries large enough to detect relatively rare events. To accomplish this, it is important to look for groups of exposed individuals that may not yet have been identified. It is also important to establish means of standardizing exposure and effect measures.

Rationale: Most of the individual cohorts are too small to detect effects that occur at low incidence. It is not clear whether combining various cohorts will be feasible or appropriate, but any such combination will require comparable measures of exposures and effects.

4) Neurobehavioral effects previously observed in the offspring of exposed individuals should be followed up. This follow-up should include congener-specific analyses, careful studies of possible confounding variables, and biochemical measurements. In addition, new neurological measurement techniques should be applied to these populations.

Rationale: Several cohorts of transplacentally exposed offspring of PHB-exposed parents have been identified and studied. The results of the studies suggest this exposure is linked to abnormal motor and behavioral development. Thus, these cohorts are impor-

tant resources and should be examined more carefully to gain maximum scientific benefit.

5) Perform careful heavy metal analyses of exposed and referent populations.

Rationale: A number of behavioral effects associated with PHB exposure in children are similar to those caused by heavy metals. It would, therefore, be useful to investigate whether heavy metals, especially lead, contribute to the effects observed with PHB exposure.

6) New techniques of neurotoxicological, immunotoxicological, endocrinological assessment should be applied to exposed and referent populations of both adults and children. Results in humans should be compared to those in other species.

Rationale: New quantitative techniques for studying PHB effects in humans have become available. With these techniques it is possible to determine if the same effects found in animals occur in humans and, if so, the relative sensitivity of humans compared to these other species.

7) Continue to monitor cancer incidence and mortality in exposed populations using appropriate and consistent diagnostic parameters.

Rationale: The latency period for appearance of cancers in humans due to PHB exposure is unknown and may be decades. Many of the more highly exposed populations will be reaching the expected limit of this latency period in the next 10 years, and intensive monitoring is needed to evaluate the possible link between PHB exposure and cancer.

8) Explore the idea of establishing a PHB epidemiological research center or centers to facilitate the study of available cohorts.

Rationale: At present, studies of cohorts are performed on an *ad hoc* basis with a minimum of coordination. As a result, studies which could be done cooperatively are performed separately, often with a greater cost and a loss of data. This problem could be minimized with the establishment of such centers. An additional research priority that could be facilitated by such a center is the standardization of biological and chemical measurements performed on PHB-exposed populations.

REFERENCES

1. Safe, S. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanism of action. *CRC Crit. Rev. Toxicol.* 13: 319-394 (1984).
2. Leece, B., Denomme, M. A., Towner, R., Li, S. M. A., and Safe, S. Polychlorinated biphenyls: correlations between *in vivo* and *in vitro* quantitative structure-activity relationships (QSARs). *J. Toxicol. Environ. Health* 16: 379-388 (1985).
3. Safe, S. Determination of the 2,3,7,8-TCDD toxic equivalent factors: support for the use of the *in vitro* AHH induction assay. *Chemosphere* 16: 791-802 (1987).
4. Bannister, R., Davis, D., Zacharewski, T., Tizard, I., and Safe, S. Aroclor 1254 as a 2,3,7,8-tetrachlorodibenzo-p-dioxin antagonist: effects on enzyme induction and immunotoxicity. *Toxicology* 46: 29-42 (1987).
5. Safe, S. Polychlorinated biphenyls (PCBs): mutagenicity and carcinogenicity. *Mutat. Res.* 220: 31-47 (1989).
6. Kimbrough, R. D., and Jensen, A. A. Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products. Topics in Environmental Health, Vol. 4. Elsevier Science Publishers, Amsterdam, 1989.
7. Kimbrough, R. D. Human health effects of polychlorinated biphenyl (PCBs) and polybrominated biphenyls. *Annu. Rev. Pharmacol. Toxicol.* 27: 87-111 (1987).
8. Tanabe, S., Kannan, N., Wakimoto, T., Tatsukawa, R., Okamoto, T., and Masuda, Y. Isomer-specific determination and toxic evaluation of potentially hazardous coplanar PCBs, dibenzofurans and dioxins in the tissues of "Yusho" PCB poisoning victim and in the causal oil. *Toxicol. Environ. Chem.* 24: 215-231 (1989).
9. Kuratsune, M. Yusho, with reference to Yu-Cheng. In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products (R. D. Kimbrough and A. A. Jensen, Eds.), Topics in Environmental Health, 2nd ed., Vol. 4. Elsevier Science Publishers, Amsterdam, 1989, pp. 381-400.
10. Kuratsune, M., Nakamura, Y., Ikeda, M., and Hirohata, T. Analysis of deaths seen among patients with Yusho. *Chemosphere* 16: 2085-2088 (1987).
11. 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. and Hsu, C. -C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science* 241: 334-336 (1988).
12. Landrigan, P., Wilcox, K., Silva, J., Humphrey, H. E. B., Kauffman, C., and Heath, C. Cohort study of Michigan residents exposed to polybrominated biphenyls: epidemiological and immunological findings. *Ann. N.Y. Acad. Sci.* 320: 284-294 (1979).
13. Humphrey, H. E. B. Chemical contaminants in the Great Lakes: the human health aspect. In: Toxic Contaminants and Ecosystem Health: A Great Lakes Focus (M. S. Evans, Ed.), John Wiley and Sons, 1988, pp. 153-165.
14. Hoffman, R. E., and Stehr-Green, P. A. Localized contamination with 2,3,7,8-tetrachlorodibenzo-p-dioxin: the Missouri episode. In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products (R. D. Kimbrough and A. A. Jensen, Eds.), Topics in Environmental Health, 2nd ed., Vol. 4. Elsevier Science Publishers, Amsterdam, 1989, pp. 471-484.
15. Kreiss, K., Zack, M. M., Kimbrough, R. D., Needham, L. L., Smrek, A. L., and Jones, B. T. Cross-sectional study of a community with exceptional exposure to DDT. *J. Am. Med. Assoc.* 245: 1926-1930 (1981).
16. Schecter, A., and Tiernan, T. Occupational exposure to polychlorinated dioxins, polychlorinated furans, polychlorinated biphenyls, and biphenylenes after an electrical panel and transformer accident in an office building in Binghamton, NY. *Environ. Health Perspect.* 60: 305-313 (1985).
17. Reggiani, G. M. The Seveso accident: medical survey of a TCDD exposure. In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products (R. D. Kimbrough and A. A. Jensen, Eds.), Topics in Environmental Health, 2nd ed., Vol. 4. Elsevier Science Publishers, Amsterdam, 1989, pp. 445-470.
18. Fingerhut, M. A., Marlow, D. A., Halperin, W. E., and Honchar, P. A. The NIOSH Occupational Dioxin Registry: A Status Report. Proceedings of the Fifth International Conference on Dioxin, Bayreuth, Federal Republic of Germany, September 16-19, 1985. National Institute of Occupational Safety and Health, Cincinnati, OH, 1985.
19. Sweeney, M. H., Fingerhut, M. A., Connally, L. B., Halperin, W. E., Moody, P. L., and Marlow, D. A. Progress of the NIOSH cross-sectional medical study of workers occupationally exposed to chemicals contaminated with 2,3,7,8-TCDD. *Chemosphere* 19 (1-6): 973-977 (1989).
20. Johnson, E. S., Winkelmann, R., L'Abbe, K. A., Kogevinas, M., Sarracci, R., Bertazzi, P. A., Bueno de Mesquita, H. B., Coggon, D., Green, L. M., Kauppinen, T., Littorin, M., Lynge, E., Mathews, J. D., Neuberger, M., Osman, J., and Pearce, N. Phenoxy acid herbicides and contaminants: description of the International Register of Workers. *Am. J. Ind. Med.* 18: 39-45 (1990).
21. Kogevinas, M., Saracci, R., Bertazzi, P. A., Bueno de Mesquita, H. B., Coggon, D., Green, L. M., Johnson, E. S., Kauppinen, T.,

- L'Abbe, K. A., Littorin, M., Lynge, E., Mathews, J. D., Neuberger, M., Osman, J., Pearce, N., and Winkelmann, R. Exposure information in the IARC International Register of Persons Exposed to Phenoxy Herbicides and Contaminants. In: *Dioxin '90 EPRI-Seminar, Vol. 1* (O. Hutzinger and H. Fiedler, Eds.), Eco-Infirma Press, Beyreuth, Germany, 1990, pp. 293-296.
22. Lawton, R. W., Ross, M. R., Feingold, J., and Brown, J. F., Jr. Effects of PCB exposure on biochemical and hematological findings in capacitor workers. *Environ. Health Perspect.* 60: 165-184 (1985).
 23. Lawton, R. W., Ross, M. R., and Feingold, J. Spirometric findings in capacitor workers occupationally exposed to polychlorinated biphenyls (PCBs). *J. Occup. Med.* 28: 453-456 (1986).
 24. Brown, J. F., Jr. Polychlorinated biphenyl (PCB) partitioning between adipose, tissue and serum. *Bull. Environ. Contam. Toxicol.* 33: 277-280 (1984).
 25. Lawton, R. W., Brown, J. F., Ross, M. R., and Feingold, J. Comparability and precision of serum PCB measurements. *Arch. Environ. Health* 40: 29-37 (1985).
 26. Brown, J. F., Jr., Lawton, R. W., Ross, M. R., Feingold, J., Wagner, R. E., and Hamilton, S. B. Persistence of PCB congeners in capacitor workers and Yusho patients. *Chemosphere* 19: 829-834 (1989).
 27. Rogan, W. J., Gladen, B. C., McKinney, J. D., Carreras, N., Hardy, P., Thullen, J., Tinglestad, J., and Tully, M. Neonatal effects of transplacental exposure to PCBs and DDE. *J. Pediatr.* 109: 335-341 (1986).
 28. Gladen, B. C., Rogan, W. J., Hardy, P., Thullen, J., Tinglestad, J., and Tully, M. Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethane transplacentally and through human milk. *J. Pediatr.* 113: 991-995 (1988).
 29. Jacobson, J. L., Humphrey, H. E. B., Jacobson, S. W., Schantz, S. L., Mullin, M. D., and Welch, R. Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. *Am. J. Public Health* 79: 1401-1404 (1989).
 30. Fein, G. G., Jacobson, J. L., Jacobson, S. W., Schwartz, P. M., and Dowler, J. K. Prenatal exposure to polychlorinated biphenyls: effects on birth size and gestational age. *J. Pediatr.* 105: 315-320 (1984).
 31. Jacobson, S. W., Fein, G. G., Jacobson, J. L., Schwartz, P. M., and Dowler, J. K. The effect of PCB exposure on visual recognition memory. *Child Dev.* 56: 853-860 (1985).
 32. Jacobson, J. L., and Jacobson, S. W. New methodologies for assessing the effects of prenatal toxic exposure on cognitive functioning in humans. In: *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus* (M. Evans, Ed.), John Wiley and Sons, New York, 1988, pp. 373-388.
 33. Jacobson, J. L., Jacobson, S. W., and Humphrey, H. E. B. Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. *J. Pediatr.* 116: 38-45 (1990).
 34. Lucier, G. W., Nelson, K. G., Everson, R. B., Wong, T. K., Philpot, R. M., Tiernan, T., Taylor, M., and Sunahara, G. I. Placental markers of human exposure to polychlorinated biphenyls and polychlorinated dibenzofurans. *Environ. Health Perspect.* 76: 79-87 (1987).
 35. Sloop, T. C., and Lucier, G. W. Dose-dependent elevation of Ah receptor binding by TCDD in rat liver. *Toxicol. Appl. Pharmacol.* 88: 329-337 (1987).
 36. Sunahara, G. I., Nelson, K. G., Wong, T. K., and 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. *Mol. Pharmacol.* 32: 572-578 (1987).
 37. Sunahara, G. I., Lucier, G. W., McCoy, Z., Bresnick, E. H., Sanchez, E. R., and Nelson, K. G. Characterization of 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated decreases in dexamethasone binding to rat hepatic cytosolic glucocorticoid receptor. *Mol. Pharmacol.* 36: 239-247 (1989).
 38. Lucier, G. W., Sunahara, G. I., and Wong, T. K. Placental markers of human exposure to polychlorinated dibenzofurans and polychlorinated biphenyls: implications for risk assessment. In: *Complex Mixtures and Cancer Risk* (H. Vainio, M. Sorsa, and A. J. McMichael, Eds.), IARC Scientific Publications, Lyon, France, 1990, pp. 55-64.
 39. Lambert, G. H., Humphrey, H. E. B., and Schoeller, D. The effects of polybrominated biphenyls (PBB) on the human cytochrome P-450 system (P-450). *Pediatr. Res.* 21(4): 258 (1987).
 40. Campbell, M. E., Lambert, G. H., Kalow, W., Humphrey, H. E. B., Long, M., Tang, B. K., and Spieldberg, S. P. Comparison of a caffeine urine test with the caffeine breath test: two noninvasive markers of polycyclic aromatic hydrocarbon-inducible cytochrome P-450 activity. *Clin. Pharmacol. Ther.* (in press).
 41. Piper, W. Role of heme in endocrine function. *Sem. Hematol.* 25: 330-335 (1988).
 42. National Research Council, *Biologic Markers in Reproductive Toxicology*, National Academy Press, Washington, DC, 1989.
 43. Bekesi, J. G., Holland, J. F., Anderson, H. A., Fischbein, A. S., Rom, W., Wolff, M. S., and Selikoff, I. J. Lymphocyte function of Michigan dairy farmers exposed to polybrominated biphenyls. *Science* 199: 1207-1209 (1978).
 44. Arezzo, J. C., Schaumburg, H. H., and Spencer, P. S. Structure and function of the somatosensory system: a neurotoxicologic perspective. In: *Toxicology of the Eye, and Other Special Senses* (A. W. Hayes, Ed.), Raven Press, New York, 1985, pp. 41-54.
 45. Arezzo, J. C., Schaumburg, H. H., and Laudadio, C. Thermal sensitivity tester: device for quantitative assessment of thermal sense in diabetic neuropathy. *Diabetes* 35: 590-592 (1986).
 46. Moody, L., Arezzo, J. C., and Otto, D. Evaluation of workers for early peripheral neuropathy: the role of existing diagnostic tools. *Sem. Occup. Med.* 1(3): 153-161 (1986).
 47. Bove, F., Litwak, M. S., Arezzo, J. C., and Baker, E. L. Quantitative sensory testing in occupational medicine. *Sem. Occup. Med.* 1(3): 185-189 (1986).
 48. Arezzo, J. C., and Schaumburg, H. H. Screening for neurotoxic disease in humans. *J. Am. Coll. Toxicol.* 8(1): 147-155 (1989).