Title of Proposal: Human Health Consequences of Polybrominated Biphenyls (PBB's) Contamination of Farms in Michigan.

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I. INTRODUCTION AND BACKGROUND

The polybrominated biphenyls (PBB) are a group of organic compounds widely used in recent years as flame retardants. There is little information available on the human toxicity of PBB. Such compounds, however, are closely related in chemical structure to polychlorinated biphenyls (PCB) which have been shown to be capable of causing abnormalities of skin, liver, metabolism, and nervous system. In addition, PCB's have recently been shown in rats to cause liver tumors, thus raising the possibility by inference that PBB's may also be carcinogenic. In fact, since PBB's have slower rates of metabolic degradation and more prolonged retention times in fat than PCB's, it is distinctly possible that such compounds have even greater carcinogenic potential.

In 1973 and 1974, between 10,000 and 12,500 Michiganresidents, principally farm families and their neighbors, were exposed to grain, meat, dairy, and poultry products that had been contaminated with Firemaster PB-6, a commercially available PBB mixture consisting principally of hexabrominated biphenyl. The events leading to exposure began in the summer of 1973 when 10-20 fifty-pound bags of the PBB mixture were inadvertently substituted for magnesium oxide, a cattle feed supplement, and distributed in feed to farmers in many areas of Michigan. Decreased milk production, loss of appetite, skin changes, hair loss, and marked loss of weight developed between August and October 1973 among dairy cattle which received this contaminated feed, and many of the cows died. There was also a high incidence of stillbirths and neonatal deaths in calves born to such herds. Analysis of the feed in April 1974 by the U.S. Department of Agriculture detected the presence of PBB in large amounts. Soon thereafter, analyses of milk and adipose tissue (fat) from affected cattle showed PBB levels as high as 400 and 4,600 parts per million (ppm) respectively. PBB was detected also in poultry, swine, and sheep that had consumed the contaminated feed, and in eggs.

Human consumption of food products from the contaminated animals began shortly after initial contamination (summer 1973) and continued until quarantine of affected herds and flocks was established in May 1974. At that time, the maximum limit of PBB allowed in milk or in meat fat was set at 1.0 ppm and in eggs at 0.1 ppm, and 183 farms were quarantined. In November 1974, it was judged that human exposure to PBB was still occurring and thus the maximum allowable limits were decreased to 0.3 ppm for milk and meat fat and to 0.05 ppm for eggs, and another 229 farms were quarantined, additional farms being quarantined in subsequent months. As the result of these quarantines, at least 21,000 cattle,

5,000 swine, 1,300 sheep, more than 1,500,000 chickens, 40 million eggs, and thousands of pounds of dairy products were destroyed.

To determine whether or not persons exposed to PBB-contaminated food products had absorbed any of the chemicals or had suffered any acute adverse health effects, the Michigan Department of Public Health (MDPH) undertook a series of toxicologic and clinical studies in the summer and fall of 1974. These studies showed that blood levels of PBB were significantly higher in a sample of 110 persons from quarantined farms as compared to a control group of 104 persons from non-quarantined premises (Table 1); even in the non-quarantined farms, however, there was evidence of some low-grade contamination with PBB. At the same time, there was no evidence that any consistent pattern of acute illness had occurred among the exposed groups, and in particular no disease similar to that seen in the Yusho episode where persons in Japan were exposed to rice oil contaminated with PCB (1).

Table 1

Distribution of PBB Blood Levels, Michigan 1974

	Quarantined Farms				Nonquarantined Farms			
	Adults		Children		Adults		Children	
PBB Blood Levels (ppm)	No.	%	No.	%	No.	%	No.	%
0	3	3.7	-	-	21	28.4	-	-
0.002 - 0.019	43	52.4	8	28.6	52	70.3	29	96.7
0.020 - 0.090	19	23.2	10	35.7	1	1.4	1	3.3
0.100 - 0.490	11	13.4	3	10.7	0	0	0	0
0.500 - 2.260	6	7.3	7	25.0	0	0	0	0
Total	82	100.0	28	100.0	74	100.1	30	100.0

II. NEED FOR LONG-TERM EVALUATION

Despite the absence of detectable acute illness in persons in Michigan exposed to PBB, the suspected long persistence of PBB in the human body raises the possibility of chronic internal exposure and suggests the need for longer term follow-up of at least the more heavily exposed

individuals. While the precise nature of any delayed or chronic illness that might develop in these people cannot be predicted from the small amount of toxicologic data currently available, it is not unlikely that PBB will behave like comparably halogenated PCB residues (2), with perhaps more pronounced long-term effects because of slower degradation and excretion (3). Among such effects may be metabolic diseases and chronic neurologic syndromes, precipitated by events causing sudden release of PBB from adipose stores, such as acute weight loss, high fever, or surgery.

Of particular concern is the possibility of increased cancer risk in PBB-exposed persons. Several studies in which Sherman rats were fed PCB for a prolonged period revealed development of liver lesions classified as adenofibrosis (4, 5, 6). More recently a prolonged (6 to 11 months) PCB feeding study with BALB/cJ male mice demonstrated the induction of adenofibrosis of the liver in all of the experimental survivors with hepatomas in 9 of 22 mice fed PCB for 11 months (1,7). Such hepatomas represent potentially malignant lesions, a certain percentage of which can metastasize and be transplanted. In her review of the polyhalogenated polycyclic compounds, Dr. Kimbrough noted that the distinction between adenofibrosis and adenocarcinoma is difficult. However, studies were cited in which the coexistence of this lesion and carcinomatous lesions in rat livers were interpreted to indicate that the adenofibrosis lesion is in fact a precursor of cancer (7). Another study showed that feeding Japanese PCB (Kanechlor 500) to male dd mice for 32 weeks produced liver tumors histologically characterized as nodular hyperplasias and well differentiated hepatocellular carcinomas (8). It was noted that PCB also promoted the induction of tumors by another compound, benzene hexachloride. The hepatocarcinogenic effect of PCB has recently been demonstrated in female Sherman rats fed Aroclor 1260 at 100 ppm for 21 months (9, 10). When sacrificed at 23 months, it was found that experimental rat livers had areas of hepatocellular alteration (182/184), neoplastic nodules (146/184), and hepatocellular carcinomas (26/184). Finally, Allen and Norback have reported that ingestion of PCB for 3 months at levels less than 10 times greater than that occurring in human food samples produced hypertrophy, hyperplasia and dysplasia of the gastric mucosa of male Rhesus monkeys (2). These abnormalities were interpreted as the result of chronic irritation by the chemical. Although neoplastic transformations were not observed, it was noted that chronic irritation has been associated with cancer of the stomach and oral mucosa.

III. PURPOSE OF STUDY

In the light of these experimental findings, it would appear prudent to establish and maintain long-term health surveillance with particular emphasis on neoplastic disease among persons in Michigan exposed in

1973 and 1974 to PBB. The object of this proposal is to define and recruit such a cohort of PBB-exposed individuals in Michigan and to arrange for their long-term periodic followup, primarily with respect to incidence of cancer.

IV. METHOD OF STUDY

The major components of the study will be: (1) selecting participants; (2) conducting interviews to enroll subjects, to determine history of PBB exposure, and to obtain brief past histories of major medical events; (3) collecting and testing blood specimens for PBB levels; (4) periodic surveillance of participants, especially for the development of cancer; and (5) analysis of data.

(1) Selection of participants. The problem of PBB contamination has involved approximately 500 farms in nearly one-half of the counties in Michigan (Figure 1). The MDPH and the Michigan Department of Agriculture (MDA) have ranked these farms according to the PBB levels in each type of produce which formed the basis for quarantine (Appendix). It is estimated that an average of 5 persons live and/or work on each of these contaminated farm premises. Thus, a total of approximately 2,500 persons have been exposed to PBB on these farms either as the result of handling contaminated grain and/or by consumption of contaminated meat or dairy products. In addition, preliminary investigations suggest that each of the quarantined farms delivered meat, milk or eggs to 3 to 4 additional five-member families or to 7,500 to 10,000 persons. This represents a total exposed population of 10,000 to 12,500 persons.

The present study will focus on the approximately 4,000 persons in this population who had contact with the most heavily contaminated farms. The following two groups will be included:

(1) Farm families: all members of the household or households (as defined by 1970 U. S. Census) who operated a quarantined farm in 1973 and/or 1974.

(2) Recipient families: all members of a household who did not themselves operate a quarantined farm, but who received dairy products, meat, or eggs, from a contaminated farm premise. Each farmer will be asked the names of 3 families whom he supplied directly, and 1 such family will be chosen for each farm family selected. Members of recipient families will be identified on their data forms according to the contaminated farm premise from which they received their produce.

The actual selection of farm and recipient families will be accomplished by proceeding down the ranked list (Appendix) from the most heavily contaminated premise to the least, selecting 1 recipient family for each

farm family that is chosen. For logistic reasons, this selection process may concentrate on particular geographic areas in Michigan where clusters of contaminated farms are located.

In terms of indicating dose-response relationships, it should be noted that while the study will look at the most heavily contaminated farms, we would expect that there will be a wide range in the actual PBB exposure of persons on these farms or consuming produce from these farms. The previous study of the acute toxicity of PBB made the point that some persons on the highly contaminated farms had serum levels of PBB approaching zero, presumably because of small or nil individual contact with contaminated grain or food products. Furthermore, the inclusion in the proposed study of persons who did not actually reside on contaminated farms, but only received their products, may increase the opportunity for inclusion of individuals who had little or no contact with contaminated materials.

(2) Enrollment interviews. As soon as the study farms have been identified, the farmers involved will be contacted by letter regarding the study. The purposes, benefits, and possible risks of participation in the study will be fully explained to them. An appointment for an initial interview will be made for each farmer and his family.

During the interview, the long-term nature of the project will again be carefully explained, and signed informed consent will be obtained from each adult and from the parent or guardian of each minor child. Each participant will then be enrolled using a questionnaire on which will be recorded (Appendix A) identifying information including name, address, telephone number, and social security number; brief past medical history focusing on major medical events and chronic conditions; and a retrospective history of PBB exposure. Recipient families will be identified as noted above, and enrolled in the same fashion.

As part of this initial enrollment, an effort will be made to contact and enlist the aid of each participant's personal physician in establishing a prospective system for recognizing and recording major illnesses and medical conditions. Physicians will be kept regularly informed of study findings and test results.

(3) Determination of PBB blood levels. At the time of the enrollment interview, a venous blood sample will be collected from each person enrolled in the study. Primary analysis of these specimens will be conducted by gas chromatography in the MDPH Laboratories (Appendix B). In addition, aliquots from 10% of the specimens will be submitted to CDC for confirmatory testing both by gas chromatography and in some instances by mass spectrometry. The proportion of specimens tested at CDC will decrease to 1% after the first 6 months of the study.

We anticipate that it will be possible, on the bases of PBB blood levels, to establish several exposure groups, each containing a statistically

adequate number of subjects (if followed over a substantial number of years). The extent to which serum PBB levels measured 2-3 years post-exposure will be useful measure of PBB exposure is unknown; however, based on previous work, it seems not unlikely that persistent levels of PBB will be found, materially aiding the classification of exposed persons into exposure categories.

(4) Follow-up evaluation. A follow-up letter will be sent each year to each participating family. This letter will describe the progress of the study, and participants will be asked to complete a brief health questionnaire to be returned to MDPH. Like the initial evaluation, this questionnaire will inquire specifically about symptoms relating to chronic conditions, particularly cancer and metabolic and neurologic conditions. All diagnoses of cancer will be confirmed by review of clinical specimens and pathologic materials.

Intensive effort will be made to reach any family that does not respond to the annual questionnaire. Follow-up telephone interviews will be attempted, and missing persons will be sought by review of post office records, driver's license records, Social Security records, death certificates, and by interviews of friends and neighbors.

(5) Analysis of data. The incidence of cancer and other disease entities will be assessed at regular intervals in the study group. It is proposed that observed incidence be compared with data from the ongoing NCI-supported SEER cancer registry in Iowa to determine departures from expected incidence. It is thought that the Iowa and Michigan groups will be comparable in terms of demographic data and in background patterns of cancer mortality. (Retrospective assessment of these similarities will be undertaken through review of 1970 U.S. Census data and of data on cancer mortality by county collected by NCI)

Although this proposal focuses on problems of initial cohort identification and less on periodic followup, it is anticipated that annual surveillance of the study population will need to be continued for perhaps 20 to 30 years to adequately gauge the possible effects of PBB exposure on cancer incidence. The animal experiments cited above, as well as general experience with chemical carcinogenesis, predict long latent periods between initial chemical exposure and eventual cancer development, particularly if levels of chemical exposure are low. Although little PBB carcinogenic activity may therefore be expected in the initial phases of followup, it is estimated that under ideal surveillance conditions, roughly 20,000 person-years of observations will accrue over the first 5 years of the study with an expected incidence of perhaps 0.3 cases of hepatic cancer.

V. CDC PARTICIPATION

It is proposed that this study be administered by CDC through sub-contract with MDPH. Following approval and funding by NCI, final contracting

arrangements will be undertaken by CDC with MDPH, the project to begin as soon as such arrangements are complete. It is anticipated that, in addition to final negotiation of the subcontract, CDC resources will be used in this study in 2 areas:

1) Epidemiologic support will be provided both from CDC headquarters in Atlanta and from CDC's Epidemic Intelligence Service Officer assigned to MDPH. CDC epidemiologists will assist in developing the final questionnaires and consent forms for use in the study, and to some extent in helping guide the actual field work in Michigan. Close contact with the study's progress will also be maintained through contract progress reports submitted to CDC by MDPH on a quarterly basis.

2) Laboratory support, as indicated in the preceding proposal, will be furnished by CDC's Toxicology Laboratory. By measuring PBB levels by both gas chromatography and mass spectrometry on a sample of serums collected by MDPH, CDC will provide independent confirmation of laboratory results. Should results in the 2 laboratories differ significantly, further arrangements for additional laboratory work may need to be negotiated.

The CDC professional personnel to be involved in the epidemiologic and laboratory phases of the project are:

Function	Name	Level
Project Officer	Philip J. Landrigan, M.D.	05
Project Advisor	Clark W. Heath, Jr., M.D.	06
EIS Officer, Michigan	D. Michael Shasby, M.D.	04
Toxicology Advisor	Renate Kimbrough, M.D.	GS-14
Chemist	John Little, Ph.D.	GS-13
Chemist	Virlyn Burse, B.S.	GS-11

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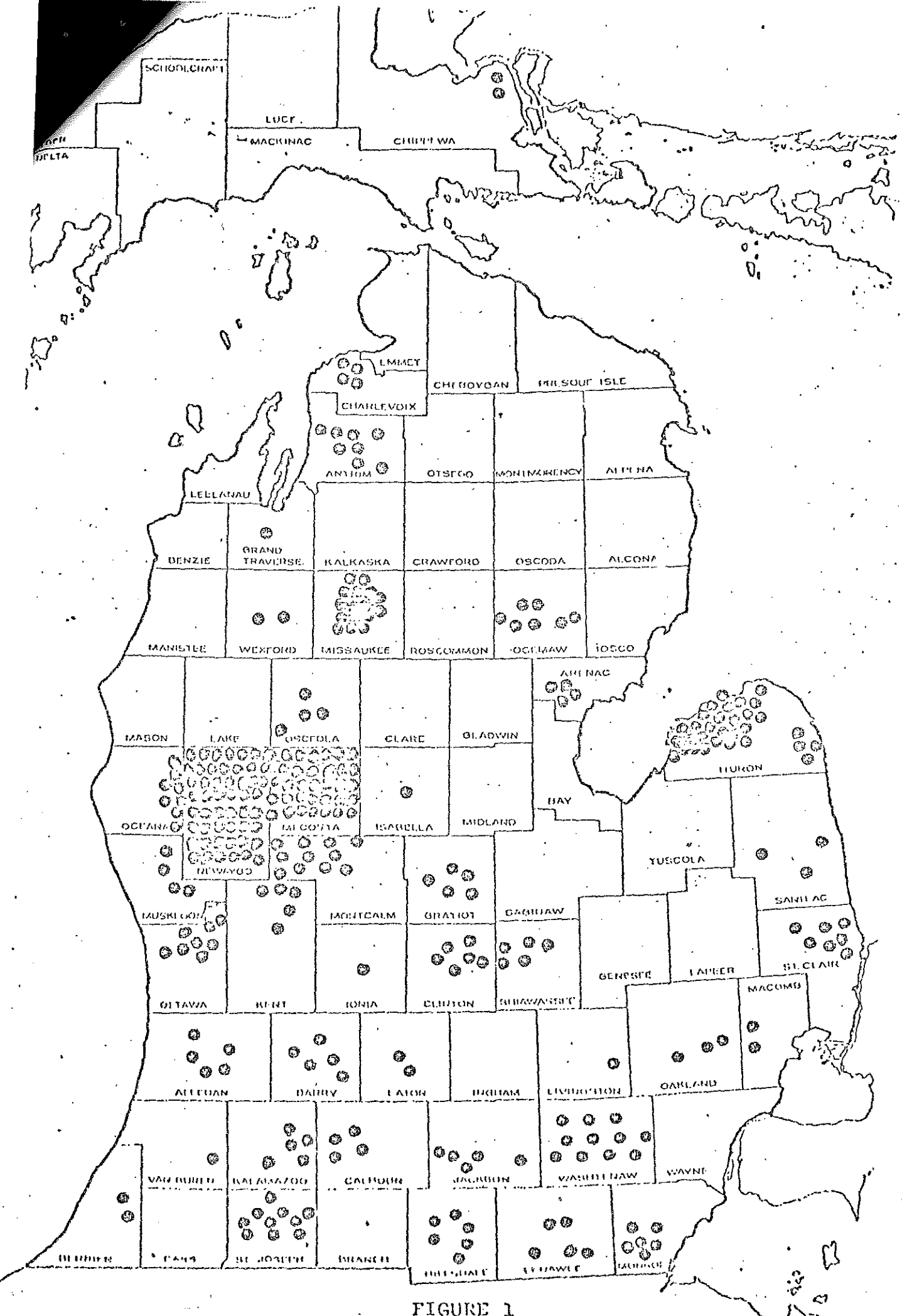
SCHOOLCRAFT
LUCE
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CHIPPEWA
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EMMET
CHEBOYGAN
PRESQUE ISLE
CHARLEVOIX
ANTRIM
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MONTMORENCY
ALPENA
LEELANAU
BENZIE
GRAND TRAVERSE
KALKASKA
CRAWFORD
OSCODA
ALCONA
MANISTEE
WEXFORD
MISSAUKEE
ROSCOMMON
OGEMAW
IOSCO
ARENAC
MASON
LAKE
OSCEOLA
CLARE
GLADWIN
HURON
OCEANA
MECOSTA
ISABELLA
MIDLAND
BAY
NEWAYGO
TUSCOLA
SANILAC
MUSKEGON
MONTCALM
GRATIOT
SAGINAW
GENESEE
LAPEER
ST. CLAIR
MACOMB
OTTAWA
KENT
IONIA
CLINTON
SHIAWASSEE
ALLEGAN
BARRY
EATON
INGHAM
LIVINGSTON
OAKLAND
VAN BUREN
KALAMAZOO
CALHOUN
JACKSON
WASHTENAW
WAYNE
BERRIEN
CASS
ST. JOSEPH
BRANCH
HILLSDALE
LENAWEE
MONROE

FIGURE 1

Appendix B

LABORATORY TECHNIQUES (MDPH)

Clotted blood specimens will be received for serum separation and PBB testing by the MDPH Environmental Epidemiology Laboratory in cooled red top vacutainer tubes (no preservative added). Tissue specimens, at least 0.5 g (no preservatives used), will be received in cooled, clean glass containers. Unused portions of collected specimens will be preserved at -20°C. The following is an outline for the extraction-gas chromatographic procedure used at MDPH for PBB determinations:

1. 2 ml aliquot of serum or macerated tissue suspension.
2. Add 1 ml of methanol - mix.
3. Add 2 ml of a hexane/ethyl ether mixture (1:1) and mix on a roto rack for 10 minutes.
4. Centrifuge mixture at 2200 rpm for 2 minutes and transfer extract to a Mills tube.
5. Repeat above extraction twice.
6. Reduce final extract to a low volume by evaporation in a water bath utilizing a micro Synder column.
7. Clean up extract by passing through a micro Fioricil column (1.6g) using a benzene/hexane (5:95) solvent. Collect 10 ml of eluate.
8. Reduce final eluate to a low volume (5 ml) by evaporation with a nitrogen gas stream.
9. Inject 5 ml samples of eluate into a dual column gas chromatograph equipped with an electron capture dector. The 6' x ¼" columns are

packed with 1% OV17 on 100/120 mesh Gas Chrom Q or 5% OV210 on 80/100 mesh Supelcotort. A column temperature of 205-210°C and carrier gas (nitrogen or helium) flow rate of 100 ml/min. are used.

Appropriate reagent blanks, spiked sheep blood standards for recovery efficiency determination, and UV degradation PBB confirmation will be used. Selected specimens will be sent to CDC for confirmatory testing by gas chromatography; one in 4 of these specimens will be further analyzed by mass spectrometry for the possible presence of furans, dioxins, or other toxic compounds that may coexist with PBB.

share such information with the mothers under their care.

NOTE: The participation of employees of Federal agencies in developing these recommendations in no way implies the endorsement of the recommendations by the Federal agencies.

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3

The Short Term Effects of PBB on Health

Michigan Department of Public Health

SUMMARY

A study was made of 165 persons who lived on quarantined farms (exposed group) and 133 persons who lived on nonquarantined farms (control group) to determine the immediate effects of PBB on health. A history of illness was obtained from all and physical examinations and selective laboratory work on as many as possible. PBB blood levels were obtained on 110 exposed persons and 104 from control persons. The following conclusions could be reached from these data:

1. There is no disease or symptom that occurred consistently in the "exposed" group or that affected most of them.
2. Complaints of illness in adults were not significantly more frequent in the exposed group than the control group.
3. Adults having the highest PBB blood levels did not show significant increase in complaints of illness. There was no detectable direct relationship between PBB blood level and complaints of illness.
4. There were no significant increases in complaints of illness in exposed children.
5. Family members tended to have similar levels of PBB in the blood.
6. Physical examinations and laboratory tests of exposed persons revealed no unexpected or unusual abnormalities.
7. Detection of any long term effects of such exposure must await a long term study of the problem. No long term effects can be detected at this point.

BACKGROUND

In 1973, some cattle from Michigan dairy herds showed signs of sickness, to have excessive abortions, to have a marked fall in milk production, and even to die. It was not until some 9 months later that the cause was suggested to be polybrominated biphenyl (PBB), a fire retardant used in a wide variety of manufactured products which had been accidentally mixed with cattle feed. As a result some 450 farms were quarantined and thousands of cattle and other animals were destroyed.

The question then arose as to the possible short and long term effects on human health of exposure to PBB from eating contaminated meat or drinking contaminated milk. There was no previous experience with PBB exposure of humans, and therefore effects, if any, were unknown. In an initial attempt to find answers to questions about short term effects, the state health department began, in the summer of 1974, a study involving a group of "exposed" persons from quarantined farms and a number of "unexposed" persons. The study included a written questionnaire to collect historical information on diseases, symptoms and conditions plus physical examinations and laboratory tests on those persons willing to cooperate in the study.

Data were collected for a group of persons presumably exposed to PBB through ingestion of farm products. (The definition of an exposed person was one who lived or worked on a quarantined farm for more than 6 months since May 1, 1973.) In addition, a control group of persons was selected living on farms in the same geographic area which had not been quarantined, and who were therefore presumably not exposed.

A total of 298 persons participated in the study. 133 questionnaires (85 adults - 48 children) were completed for the control group; 165 questionnaires (104 adults, 61 children) for the exposed group. PBB blood

values were not obtained on all participants because some were unwilling to visit the clinic for a physical exam and because blood was not drawn from small children. As a result (see Tables 1 and 2), 104 PBB blood values were measured from the control group (74 adults, 30 children), and 110 blood values were measured from the exposed group (82 adults, 28 children).

TABLE 1

NUMBER OF PERSONS INCLUDED IN THE STUDY

Group	Adults	Children	Total
Quarantined Farms	104	61	165
Nonquarantined Farms	85	48	133
Total	189	109	298

TABLE 2

NUMBER OF PBB BLOOD VALUES OBTAINED FROM STUDY PARTICIPANTS

Group		Obtained		Not Obtained		Total
		No.	%	No.	%	No.
Quarantined Farms	Adults	82	78.8	22	21.2	104
	Children	28	45.9	33	54.1	61
Nonquarantined Farms	Adults	74	87.1	11	12.9	85
	Children	30	62.5	18	37.5	48

PBB VALUES

The blood values for PBB were higher in the exposed group than in the unexposed group. Some individuals in both groups had none detected, but 56 persons (51%) in the exposed group had levels of 0.02 ppm or higher whereas only 2 persons (2%) in the unexposed group had such levels. The highest level detected in the exposed group was 2.26 ppm; the highest level in the unexposed group was 0.06 ppm. Table 3 shows a breakdown of the levels for adults and children for the two groups.

Table 3

PBB Blood Levels*	Quarantined Farms				Nonquarantined Farms			
	Adults		- Children		Adults		- Children	
	No.	%	No.	%	No.	%	No.	%
0	3	3.7	-	-	21	28.4	-	-
0.002 - 0.019	43	52.4	8	28.6	52	70.3	29	96.7
0.02 - 0.09	19	23.2	10	35.7	1	1.4	1	3.3
0.1 - 0.49	11	13.4	3	10.7	0	0	0	0
0.5 - 2.26	6	7.3	7	25.0	0	0	0	0
Total	82	100.0	28	100.0	74	100.1	30	100.0

*measured in parts per million (ppm)

HEALTH CONCERNS OF EXPOSED AND UNEXPOSED FAMILIES

The basic question was whether or not any illnesses, ailments, or complaints could be identified which could be attributed to PBB ingestion. To help answer this question a set of 24 variables was selected consisting of symptoms, illnesses and complaints that were suggested by symptoms experienced in a related polychlorinated biphenyl (PCB) Japanese experience, plus the reported initial complaints from Michigan persons after PBB exposure. These are listed in Table 4.

Table 4

SELECTED CONDITIONS AND COMPLAINTS INVESTIGATED IN THE ADULT STUDY POPULATION	
Complaint	Complaint
Numbness	Thyroid Trouble
Balance Problems	Headache
Nausea	Fatigue
Stomach Pain	Irritability
Appetite Change	Anxiety
Weight Change	Depression
Liver Trouble	Pink Eye
Hepatitis	Rash
Fainting	Sores
Loss of Power	Acne
Blurred Vision	Skin Color Change
Light Sensitivity	Hair/Fingernail Change

Symptoms were not considered to be significant for consideration unless more than 10 individuals (5%) in the entire study complained of them. The ten symptoms meeting this requirement were: increased frequency and severity of balance problems, increased severity of rashes, increased frequency and severity of headache, increased frequency and severity of fatigue, and increased frequency and severity of anxiety. None of these complaints occurred in more than 12% of the study subjects. This means that there were no complaints occurring consistently in either group. The remaining analyses are to determine whether or not any of these complaints were associated with exposure to PBB or increased PBB levels in the blood.

Upon analysis, none of these symptoms was statistically significant. This means that the evidence presented is not sufficient to indicate a true difference between the 2 groups. In other words, the exposed and non-exposed groups have essentially the same frequency of these conditions. However, it is also true that the proportion of positive responses for each of the variables is greater in the exposed group than in the control group. Therefore, further analysis was made.

HEALTH CONCERNS BY PBB BLOOD LEVEL

Theoretically, if the PBB level had any effect or influence on the conditions reported, there should be more conditions or complaints from these persons with the highest PBB levels.

To determine whether or not this was the case, the complaints of the 17 adults whose PBB blood levels were the highest (between 0.1 and 2.26 ppm) were compared statistically with the complaints of the remainder of the study group, regardless of whether they were in the exposed or unexposed groups. The comparison showed that none of the complaints was significantly more frequent in those with the highest PBB levels. Six complaints were reported in a greater proportion of those with the highest PBB levels and four

in a lower proportion as follows:

Observed in a greater proportion	Observed in a lesser proportion
Severity of anxiety	Severity of rash
Frequency of anxiety	Frequency of balance problems
Severity of tiredness	Severity of headaches
Frequency of tiredness	Frequency of headaches
Severity of balance problems	
Frequency of numbness	

A further attempt was made to determine whether or not there was a relationship between the frequency of complaints and the PBB blood levels by dividing the entire study group (disregarding whether they were "exposed" or "unexposed") into three groups: those with zero levels, those in the middle range (0.002 to 0.020), and those with the highest levels (above 0.020). If there is an effect related to PBB blood levels one would expect to see the lowest frequency of complaints in the zero group, the highest frequency in the highest group, with the middle group falling in between. The results are shown in Table 5.

For all but one complaint, the high group showed the greatest proportion. For the first five complaints listed the proportion was about the same in the low and middle groups. For the last five complaints listed, the proportion was higher in the low than the middle group. The proportion of subjects complaining of increased frequency of headaches was a little higher in the low group than in the high. Unless the frequency with which these complaints are found increases considerably with continued observation no implication of a significant effect on health due to PBB will be warranted.

In reviewing the data, it should be kept in mind that all but 1 of the persons in the high group are from the exposed group. Because these people knew they had been exposed to PBB the results can be biased. Similarly, almost all (21 of 24) persons in the zero group were from the control group.

Table 5

NUMBER OF POSITIVE RESPONSES FOR SELECTED SYMPTOMS, CONDITIONS AND COMPLAINTS IN THE STUDY POPULATION BY PBB BLOOD LEVEL VALUE

Symptom, Condition or Complaint	PBB Blood Levels[1] (ppm)					
	0.0		0.002-0.020		greater than 0.020	
	No.	%	No.	%	No.	%
Severity of rash	1	4.2	5	5.2	4	11.4
Frequency of tiredness	3	4.8	4	4.1	9	25.7
Severity of balance problems	1	4.2	5	5.2	5	14.3
Frequency of balance problems	1	4.2	5	5.2	4	11.4
Severity of headaches	2	8.3	8	8.3	4	11.4
Severity of anxiety	2	8.3	6	6.2	7	20.0
Frequency of anxiety	2	8.3	3	3.1	6	17.1
Severity of tiredness	3	12.5	6	6.2	9	25.7
Frequency of numbness	1	4.2	2	2.1	2	5.7
Frequency of headaches	5	20.8	9	9.4	6	17.1
Number of people	24		97		35	

*Denominator used is 96 due to one missing observation

Of the symptoms with a significant number of positive responses, the severity and frequency of rash is probably the most obvious physical condition. Therefore, the relationship between reports of increased severity and frequency of rash in the exposed group and the increased severity and frequency of other symptoms was examined. Rash severity was compared with the severity of 15 other symptoms, only one of which (sores) showed a significant correlation. Rash frequency was compared with the frequency of 9 other symptoms, of which 5 (balance problems, fainting, weakness, irritability, and sores) showed a significant correlation. (Table 6)

These data can be interpreted to mean that if a person reports an increase in the severity of rash, he is likely to report an increase in severity of sores, Similarly, if a person reports an increase in the frequency of rash, he is likely to report an increase in the frequency of balance problems, fainting, weakness, irritability and sores. Although these correlations are statistically significant, only 3 persons reported both increased rash severity and sore severity, while no one reported an increase in rash frequency and increase in the frequency of all 5 of the other symptoms.

CHILDREN

Data for children were analysed in a similar way. The only symptom which appeared to be significant was stomach pain for which there were 5 responses. However, 2 of these children were from the exposed group, and 3 from the control group. Of these 5 children, 3 had zero PBB blood levels, and the other 2 had values of 0.002 ppm and 0.004 ppm.

Table 6

SIGNIFICANT CORRELATION BETWEEN RASH SEVERITY & 9 OTHER SEVERITY SCORES		SIGNIFICANT CORRELATION BETWEEN RASH SEVERITY & 15 OTHER FREQUENCY SCORES	
Variable	Correlation Coeff.	Variable	Correlation Coeff.
Sores	0.400	Balance	0.247
		Fainting	0.326
		Weakness	0.273
		Irritability	0.351
		Sores	0.351

FAMILY CLUSTERS

Upon analysis of the data to this point, it seemed appropriate to try to determine the extent, if any, of clustering of PBB values within family units. Intraclass correlations were therefore calculated for 154 persons in 50 farm families, 25 from the exposed group and 25 from the control group.

The correlations were 0.672 for the exposed farms and 0.324 for the control farms (an absolute correlation is 1). Both correlation coefficients are statistically significant, and explain 67% of the variation in PBB values for exposed families and 32% for control families. Interestingly, the zero PBB values did not appear to cluster in farm families. For only 2 families, with 2 persons each, were all family members recorded as having PBB values of zero. This appears to indicate that persons on control farms had some relatively minor exposure to PBB from a variety of sources not common to all members of a farm family.

The 27 persons with PBB values greater than 0.1 ppm were distributed among only 7 families, with 22 of the 27 persons coming from 4 families. This strongly indicates that these persons were exposed to PBB from a common source.

CLINICAL AND LABORATORY FINDINGS

Physical examinations and laboratory tests were performed on 158 persons (85 exposed, 73 control) at special clinics in Big Rapids and Grant, Michigan, during the fall of 1974. Of the adults exposed to PBB 14 reported the onset of more than one symptom suggesting anxiety during the exposure period, when their cattle seemed inexplicably ill and milk production was down. Only 4 such symptoms were noted during this period by the control adults. A papular dermatitits was recorded for 3 exposed and no control adults. While the statistical comparison of these conditions showed no clear difference between exposed and control groups, it should be noted that these symptoms were mainly experienced in association with the exposure and cattle illness period.

Physical examinations did not show any unusual abnormalities, in either the exposed or control groups. No one had an enlargement of the heart, liver or spleen. Examinations of the nervous system revealed no excess of disorders in exposed persons. All of the reported skin eruptions had faded away by the time of these examinations. At examination, no significant abnormalities were found in exposed children.

Laboratory examinations of urine and complete blood counts have not revealed consistent abnormalities related to exposure or blood PBB levels. Blood analysis was limited to blood cell counts because chemical analysis of blood from a sample of exposed persons in June, prior to this study, was judged by toxicologists to be non-contributory, i.e. showed no findings of diagnostic significance. Some elevated and some reduced white blood counts were reported, but they were not in excess of that which would be expected from a normal distribution of the population. Likewise, urinalyses showed no evidence of unusual or unexpected abnormalities among either the exposed or control groups.

DISCUSSION

If PBB exposure caused short term human illness, the data would be expected to reveal an illness or a group of symptoms common to a significant number of those exposed. In fact, the data do not show such a pattern, leading to the conclusion that PBB, thus far, has not been shown to be the cause of any identifiable human ailments.

This is not to say that PBB exposure has caused no ailments, but only that there has been no consistent pattern of illness or symptoms which occurred excessively in exposed persons, and which could, therefore, be attributed to PBB. Unquestionably, farm family members who encountered a long-unexplained illness in their cattle, and who were faced with quarantine and destruction of their herds, could be expected to exhibit some situational stress and related symptoms. However the data fail to indicate that PBB exposure has caused human ill health up to the present time. In addition, while the numbers are too small to be meaningful, it should be noted that 3 babies have been born to women exposed to PBB throughout their pregnancy, and all appear to be normal and healthy.

The question of possible long term, delayed, chronic effects on human health remains unanswered. In an attempt to find answers to this question, a proposal for continuing surveillance and study for any late, toxic, carcinogenic, or teratogenic (causing birth defects) effects of PBB has been developed in conjunction with the Federal Center for Disease Control and the Food and Drug Administration. This proposed study would involve some 6000 persons over a 5 to 15-year period. A protocol for that study is appended.

COHORT STUDY OF MICHIGAN RESIDENTS EXPOSED TO POLYBROMINATED BIPHENYLS: EPIDEMIOLOGIC AND IMMUNOLOGIC FINDINGS

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INTRODUCTION

The polybrominated biphenyls (PBB) are a group of halogenated aromatic hydrocarbon compounds employed until recently as flame retardants in the plastics industry.[1] PBB have low acute toxicity,[2] but are fat-soluble, biologically persistent,[3] and capable of causing chronic injury to the liver,[4] kidneys,[5] skin,[5] and lymphatic tissue.[6] In addition, PBB have been found in chronic rat feeding studies to induce neoplastic liver nodules.[4]

In 1973, several hundred pounds of PBB were introduced into cattle feed as the result of a shipping accident in Michigan.[7] Thousands of dairy animals[8] died or were destroyed, and widespread human exposure resulted from contact with contaminated feed and from consumption of PBB in dairy foods. While human exposure was most intense on quarantined farms,[9] and in chemical workers,[10] low-level exposure was widespread throughout the state, and in October, 1976, a survey of randomly selected samples of human breast milk showed that 51 (96%) of 53 samples from the lower peninsula of Michigan contained detectable levels of PBB.[11]

To determine the effects of PBB exposure on human health, the Michigan Department of Public Health (MDPH), the Center for Disease Control (CDC), the National Institutes of Health (NCI and NIEHS), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA) have established a cohort of persons with varying levels of PBB exposure. The members of this cohort have been examined systematically to determine whether or not they have experienced an increased incidence of acute or subacute illness, of biochemical aberrations, or of alterations in the outcome of pregnancy in relation to their PBB exposure. In addition, the health status of the cohort is to be followed prospectively over the next several decades to determine whether they will experience an increased incidence of chronic disease, particularly of cancer. In this report, we shall summarize the epidemiologic and clinical data collected on the Michigan cohort in the first 4 years since the onset of PBB exposure. In addition, we shall present the results of an immunologic study conducted in 1977 to evaluate peripheral lymphocyte function in the most heavily exposed subset of the cohort.

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METHODS

Selection of Participants

Four categories of Michigan residents were invited by mail and by interview to participate in this prospective cohort study:

Farm Residents on Quarantined Farms

Invited to join this group were the 2,248 persons who were members of families residing on quarantined farms at the time of those farms' quarantine for PBB contamination; included here were 110 residents of quarantined farms who had participated in a 1974 MDPH pilot study of PBB exposure.[9] Quarantine was established when any single specimen of meat, eggs, or milk from a farm exceeded quarantine action limits; the number of specimens submitted for PBB testing varied from farm to farm. In May, 1974, the maximum allowable limit for PBB in milk or meat fat was set at 1.0 parts per million (ppm), and in eggs at 0.1 ppm, and 183 farms were quarantined. In November, 1974, these quarantine action limits were decreased to 0.3 ppm for milk and meat fat, and to 0.05 ppm for eggs, and another 229 farms were quarantined. Additional farms have subsequently been placed under quarantine. The survey participation rate in Group 1 was 95.6% (TABLE 1).

TABLE 1

SURVEY PARTICIPATION RATES BY ENROLLMENT GROUP (MICHIGAN PBB STUDY, 1976–77)

Group	Invited (No.)	Enrolled (No.)	(%)
1. Quarantined farm residents	2248	2148	95.6
2. Farm product recipients	1495	1421	95.1
3. Chemical workers (and families)	322	251	78.0
4. Pilot study control participants	60	57	95.0

Farm Product Recipients

Invited into this group were 1,495 persons who had not themselves resided on quarantined farms, but who had received meat, eggs, or dairy products directly from quarantined premises in 1973 or 1974. The participation rate in Group 2 was 95.1% (TABLE 1).

Chemical Workers

This group includes workers, who had been exposed occupationally to PBB in a chemical manufacturing plant in Michigan, as well as the members of these workers'

families. The total number invited was 322; the participation rate was 78.0% (TABLE 1).

Pilot Study Participants

Invited to join this group were 60 persons who had been identified in the MDPH pilot study[9] as being resident on farms with low-level PBB contamination; they had served as a control group in the pilot study. The participation rate in this group was 95.0% (TABLE 1).

Finally, a small number of additional persons were enrolled into the cohort who were not originally invited to participate:

(1) 331 self-referred persons who had either resided on farms identified as being contaminated by PBB in levels below quarantine limits or who had eaten food produced on such farms (Group 5); and

(2) 337 self-referred volunteers who had no direct connection with contaminated farm premises (Group 6).

Field Studies

All persons enrolled in the study were visited in their homes over a 12-month period between September, 1976 and August, 1977, by trained interviewers from MDPH. Repeated visits (up to 4 per household) and repeated mailings were made in an attempt to reach families who could not initially be contacted, or who refused in the first instance to participate in the cohort study. After informed consent had been obtained for each person, a questionnaire was administered which sought data on potential sources of PBB exposure, as well as on the occurrence since 1973 of 17 symptoms and conditions considered possibly related to PBB contact. This symptom list was developed from anecdotal data on the possible human toxicity of PBB[8] as well as from published information[12] on the human toxicity of the polychlorinated biphenyls (PCB). Symptoms and conditions sought were weight loss, fatigue, headaches, skin rash, changes in skin pigmentation, changes in the nails, heart disease, gastrointestinal complaints (nausea, vomiting, diarrhea, or abdominal pain), liver disease, Reye's syndrome, diabetes mellitus, thyroid disease, peripheral neuropathy, convulsions, joint disease, benign tumors, and cancer. Data were obtained also on tobacco and alcohol consumption. Detailed information, including birth weight data and data on any congenital malformations, was sought on the outcome of all pregnancies since 1963.

Toxicologic Laboratory Studies

A venous blood sample for PBB analysis was requested of each study participant, and 3,639 were obtained. Samples were analyzed for PBB concentration at MDPH using gas chromatography with electron capture detection; the limit of detection for PBB was 1 part per billion (ppb).

Quality control on PBB analysis was conducted jointly by MDPH and the Toxicology Branch, Clinical Chemistry Division, Bureau of Laboratories, CDC. In addition to internal quality control at MDPH, which consisted of repeated analyses of quality control specimen pools, external surveillance was provided by (a) repeat analysis at CDC of 13% of samples, and (b) blind insertion by CDC of quality

evaluation specimens into the regular system of specimen collection. For 466 serum samples analyzed in duplicate at MDPH and CDC, mean PBB values were 16.9 and 16.2 ppb respectively; the coefficient of correlation between paired results was 0.9982.

Immunologic Studies

To evaluate peripheral lymphocyte function in the members of the cohort identified previously as having highest serum PBB values, an immunologic investigation was undertaken by MDPH and CDC in collaboration with the University of Michigan (UM) in October, 1977.

Invited to participate were all 41 cohort members shown previously to have serum PBB values $\geq$ 300 ppb; 34 of these 41 (83%) agreed to participate. Invited as a comparison group were all 7 persons shown to have a serum PBB concentration less than 1 ppb, as well as a random sample of 59 of the 915 enrollees with serum values of 1–9 ppb; 56 (85%) of these 66 persons agreed to participate.

Fasting early morning venous blood specimens, taken from 8–10 persons per day, were collected in heparinized vacuum tubes over a 3-week period in participants' homes and at nearby field stations. Members of both exposure groups were included each day. Samples were coded in the field and then transported at ambient temperature by automobile to the UM laboratory where test procedures were begun 3–5 hours after venipuncture. To examine for any possible decrement in lymphocyte function during transport, blood samples were obtained in the field from 1–3 MDPH staff members each day (total, 9 persons, 33 samples) from whom blood samples had previously been taken in the UM laboratories and tested immediately.

Mononuclear cells were obtained by centrifugation through a ficoll-isopaque gradient.[13] The mononuclear cell layer was removed and washed twice in Hanks' balanced salt solution (HBSS). Cells to be used for T and B lymphocyte enumeration were resuspended in HBSS, counted, and diluted to a final concentration of 4×10^6 mononuclear cells/ml. Cells to be used for lymphocyte transformation were resuspended in RPMI-1640 (GIBCO, Grand Island, N.Y.), and counted and diluted to a concentration of 10×10^6 cells/ml. These procedures gave greater than 99% cell viability as determined by exclusion of 1% trypan blue dye.

B cells were enumerated by detecting complement receptors by means of complement-coated zymosan particles according to the methods of Mendes *et al.*[14] Two-hundred lymphocytes were counted in duplicate specimens using a phase microscope; cells that had three or more zymosan particles attached were recorded as B cell rosettes.

T lymphocytes were enumerated by determining the number of cells that formed rosettes spontaneously with sheep erythrocytes according to the method of Jondal *et al.*[15] Two-hundred lymphocytes were counted in duplicate specimens using a phase microscope. Cells that had three or more erythrocytes attached were recorded as T cell rosettes.

Peripheral blood lymphocytes were studied for transformation to three mitogens, phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) in wells of flat-bottomed microtiter plates. Each mitogen was run in quadruplicate wells that contained 10^5 lymphocytes/well in a total volume of 0.2 ml RPMI-1640 with 20% autologous plasma.

Microtiter plates were placed in a 5% CO_2 humidified incubator at 37°C. After incubation for 96 hours cells were pulsed with 1 microcurie (μCi) of ^{3}H-thymidine and

TABLE 2

MEAN AGE AND PERCENTAGE MALE AND FEMALE BY ENROLLMENT GROUP (MICHIGAN PBB STUDY, 1976-77)

Group	Mean Age (yrs)	Percentage Male	Percentage Female
1. Quarantined farm residents	28.0	52.6	47.4
2. Farm product recipients	28.3	48.4	51.6
3. Chemical workers (and families)	22.6	51.6	48.4
4. Pilot study control participants	33.7	50.0	50.0
5. Low-Level PBB farm residents	27.9	53.1	46.9
6. Volunteers	29.4	50.8	49.2

harvested 6 hours later with an Otto-Hillier cell harvester. Cells were then washed with saline, dried, resuspended in scintillation fluid and counted. Data were expressed as mean counts per minute (cpm) per 10^5 lymphocytes.

EPIDEMIOLOGIC RESULTS

Members of the Michigan cohort ranged in age from 1 to 89 years (median, 24 years) at the time of enrollment; 2,317 were male and 2,220 female (8 of unspecified sex). Age and sex distributions were approximately comparable in the 6 groups (TABLE 2).

Analysis of data on the prevalence of symptoms showed that fatigue, headaches, paresthesias, and joint problems were the conditions most frequently reported. For nearly all conditions the volunteer group (Group 6) had the highest prevalence, followed closely by the group who had resided on farms with low-level PBB contamination (Group 5) (TABLE 3).

Data on serum PBB levels showed a range of values from 0–1900 parts per billion (ppb); 106 values were greater than 100 ppb. Examination of results by age-group showed that children aged 10 years or less tended to have the highest serum PBB concentrations. Above age 10, there was no significant gradient by age ($F = 1.81$, $p = 0.11$). Males had significantly higher values than females ($\chi^2 = 256.5$; $p < 10^{-10}$).

TABLE 3

PREVALENCE RATES (%) FOR SELECTED SYMPTOMS BY ENROLLMENT GROUP (MICHIGAN PBB STUDY, 1976-77)

Symptoms and Conditions	Group 1	2	3	4	5	6
Fatigue	36.4	32.4	22.0	15.8	41.4	54.4
Rashes	8.1	7.5	6.4	1.8	14.5	3.1
Joint pains	25.1	26.4	18.9	11.1	32.0	39.9
Hepatitis	1.5	1.8	1.6	0.0	3.3	3.6
Diabetes mellitus	1.9	2.2	2.4	1.8	2.7	2.4
Benign tumors	4.2	5.4	5.8	1.8	5.5	9.8
Cancer—all sites	0.4	0.5	0.4	0.0	0.6	0.6
Number of subjects	2428	1421	251	57	331	337

Serum PBB levels showed a strong tendency to cluster by families (intra-class coefficient of correlation = 0.85).

Examination of serum PBB levels by enrollment group showed that highest values were found in the chemical workers and in the members of their families (TABLE 4) followed by the residents on quarantined farms (Group 1). In the chemical worker group, the workers themselves had a serum PBB level of 108.7 ppb (range, 0–1,240 ppb), significantly higher than that in members of their families. Volunteers (Group 6) had the lowest serum PBB levels.

Paired serum samples, one collected in 1974 and the other in 1977, were available for 148 members of the cohort. These data indicate that serum levels were generally stable over the 3-year period. The mean change in serum ppb value was −16 ppb, and the coefficient of correlation (r) between the 2 sets of values was 0.96.

Simultaneous paired specimens of blood and adipose tissue were obtained on 221 Michigan residents, 19 of whom were members of the cohort. A high coefficient of correlation (r) was seen between these 2 sets of values (0.951), and in the 132

TABLE 4

SERUM PBB LEVELS* BY ENROLLMENT GROUP
(MICHIGAN PBB STUDY, 1976–77)

Group	Number	Serum PBB Range	Mean	Median
1. Quarantined farm residents	1750	0–1900	26.9	4.0
2. Farm product recipients	1114	0–659	17.1	3.0
3. Chemical workers (and families)	216	0–1240	43.0	4.5
4. Pilot study control participants	44	1–13	3.5	2.0
5. Low-level PBB farm residents	242	0–24	3.5	2.0
6. Volunteers	273	0–111	3.2	1.0
Total	3639	0–1900	21.2	3.0

*PBB levels in parts per billion (ppb).

instances where both values were above detection limits the ratio of adipose to serum PBB concentrations was 362.8:1.0 (FIGURE 1).

An evaluation of dose-response relationships was undertaken by dividing the cohort into seven segments on the basis of serum PBB levels. No positive associations were found between serum concentrations of PBB and reported symptom frequencies (TABLE 5). Symptom-prevalence rates (excluding volunteers) were slightly higher in persons with no detectable PBB in serum than in those with measurable quantities. Relationships between symptom prevalence rates and serum PBB level were also examined within each enrollment group, and no positive trends were found; in all groups, including chemical workers and quarantined farm residents, highest prevalence rates occurred in persons with lowest serum PBB levels.

Since 1973, 65 children have been born to women in Michigan with potential exposure to PBB (TABLE 6). Serum PBB concentrations in the 52 women examined at the time of delivery ranged from not detectable (<1 ppb) to 1150 ppb (mean, 26.2 ppb). Cord serum PBB levels at birth in 58 infants ranged from not detectable to 104 ppb (mean 3.2 ppb). The mean ratio of maternal/cord serum PBB values for the 13 maternal-infant pairs where both had detectable values was 7.04:1.0 (range, 1.5–

10.3). Breast milk samples were obtained from 32 women, and PBB values (fat basis) ranged from 32 to 93,000 ppb (mean 3614 ppb). The mean ratio of serum to breast milk PBB values in the 21 women where both concentrations were detectable was 122.0:1.0 (range, 62.6–256.7).

Immunologic Results

Total leucocyte counts did not differ significantly among the high PBB, low PBB, and MDPH staff groups. The mean percentage of circulating lymphocytes was significantly elevated in the high PBB exposure group as compared to the low ($p < 0.0001$). However, this increase was found only in children less than 12 years of age who made up a greater proportion of the high than of the low exposure group.

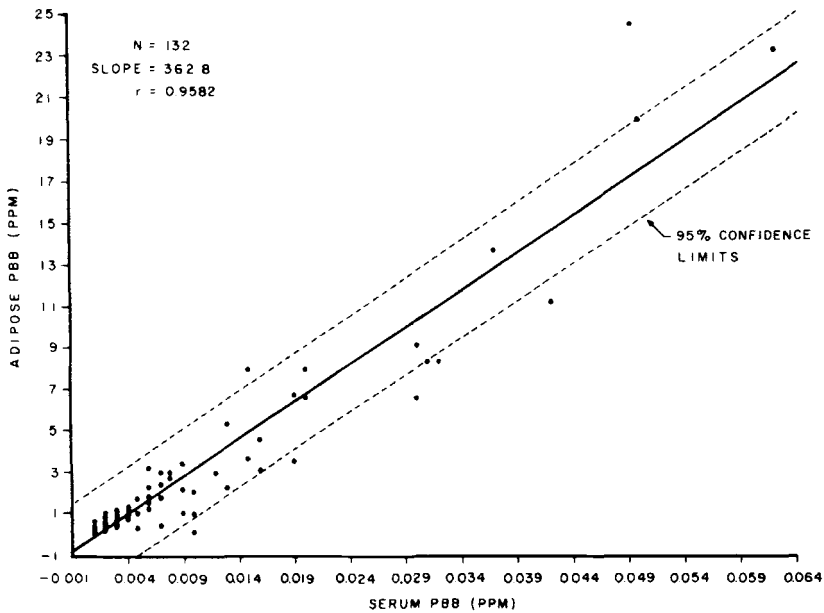

FIGURE 1. Correlation between simultaneous paired serum and adipose PBB levels. Michigan PBB Study, 1976–1977.

The high exposure group had no depressions in absolute numbers of T or B lymphocytes, or in responses to any of the 3 mitogens as compared to the group with low PBB exposure (TABLE 7). However, 15% of persons from the 2 exposure groups were found to have 2 or more abnormalities in measures of *in vitro* lymphocyte function.

Significant differences were found (TABLE 7) between results obtained in the field on MDPH staff and results obtained on these same individuals in the nontransported samples taken at the UM laboratory; this discrepancy suggests the possible existence of a "transportation effect."

TABLE 5

PERCENTAGE INCIDENCE OF REPORTED SYMPTOMS AND CONDITIONS* SINCE 1973 BY SERUM PBB† LEVEL (MICHIGAN, PBB STUDY, 1976–77

Symptoms and Conditions	0	1	2–3	4–9	10–19	20–99	100+	Mean
Fatigue	46.5	40.1	40.7	38.1	37.8	28.7	27.4	38.3
Rashes	9.0	8.5	8.4	7.2	8.3	6.3	4.1	7.8
Joint pains	42.9	32.7	29.8	26.4	28.4	21.6	17.1	28.6
Hepatitis	4.5	1.4	1.8	1.4	1.3	0.0	0.0	1.4
Diabetes mellitus	4.7	2.9	2.4	1.8	1.9	3.3	0.0	2.4
Benign tumors	2.7	7.5	5.6	4.3	5.3	3.8	2.6	5.3
Cancer-all sites	0.0	0.9	0.5	0.5	1.0	0.4	1.8	0.6
Number of subjects*	89	716	941	892	316	276	126	3,356

*Excludes volunteer subjects.
†PBB concentrations in parts per billion (ppb).

DISCUSSION

The finding in this study of elevated serum PBB levels in families from quarantined farms and in Michigan chemical workers confirms earlier reports of increased PBB absorption in these groups.[9–11] Although a number of exposed individuals reported symptoms, as has been the case in previous studies,[16,17] it was noteworthy that symptom prevalence rates bore no relationship to serum PBB levels.

TABLE 6

PBB CONCENTRATIONS* IN MATERNAL SERUM, CORD SERUM, AND BREAST MILK† (MICHIGAN PBB STUDY, 1976–77)

		PBB Concentrations			
Specimen	Number	Range	Mean	Median	Average Ratio to Maternal Serum (and Range)
Maternal serum	52	0–1,150	26.2	2.5	—
Cord serum	58	0–104	3.2	1.0	7.04 (1.5–10.3)
Breast milk	32	32–93,000	3614	225	122.0 (62.2–256.7)

*PBB concentrations in parts per billion (ppb).
†Breast milk PBB values expressed on fat basis.

TABLE 7

LYMPHOCYTE FUNCTION TESTS BY EXPOSURE GROUP
(MICHIGAN PBB STUDY 1977)

	Low Exposure Group		High Exposure Group		p-Value (Student's t-Test)	MDPH Staff Bloods Drawn in Lab		MDPH Staff Bloods Drawn in Field		p-Value (Paired t-Test)
	n		n			n	Mean ± 1 S.D.	n	Mean ± 1 S.D.	
Mean serum PBB level, ppb	(51)	2.8	(32)	787		(8)*	1.88 ± 1.55	(8)*	1.88 ± 1.55	
(range)		<1 11		188 2560			(<1 5.0)		(<1 5.0)	
T and B Cell Quantitation										
% T cells (mean ± 1 S.D.)	(56)	61.4 ± 10.2	(34)	67.2 ± 11.8	.015	(9)*	72.9 ± 8.1	(33)*	65.0 ± 13.0	.15
% B cells (mean ± 1 S.D.)	(56)	5.01 ± 2.36	(34)	5.11 ± 2.51	.857	(9)*	6.4 ± 2.5	(33)*	5.6 ± 3.5	.40
Lymphocyte Function Tests										
Maximal blastogenic response of peripheral blood lymphocytes (in scintillation counts per minute) to:										
PHA (mean ± 1 S.D.)	(54)	38224 ± 16803	(31)	45797 ± 19349	.056	(9)*	63170 ± 16308	(31)*	38101 ± 20742	.001
PWM (mean ± 1 S.D.)	(54)	41936 ± 21871	(31)	43505 ± 16523	.731	(9)*	86763 ± 25814	(31)*	36766 ± 15636	.0001
Con A (mean ± 1 S.D.)	(54)	44721 ± 21731	(31)	54359 ± 26750	.074	(9)*	65858 ± 37265	(31)*	44737 ± 23217	.025

*Same individuals constitute both groups.

Symptom frequency was much more closely related to mode of entry into the cohort, with highest frequencies occurring in volunteers (Group 6) and in persons from farms that were contaminated by PBB at levels insufficient to result in quarantine (Group 5). These observations suggest that factors other than PBB absorption may have been responsible for the production of symptoms and that selection factors may have played an important role. Also, however, it may be necessary to examine samples of the commercial PBB mixture dispersed in Michigan to determine whether it contained other toxins in addition to PBB, possibly in concentrations not correlated directly with PBB levels.

The failure of this investigation and of a previous immunologic study[18] to demonstrate dose-related depression of lymphocyte function in persons exposed to PBB suggests that there may be no causal relation between the two. If, however, an association does exist, our failure to detect it could have resulted from any of several possibilities. One is that the methods used in this study were insufficiently sensitive. Another is that the 3–5 hours' transportation time required here may have depressed cell function in both of our groups to a point where intergroup differences were no longer evident. A third is that persons in both study groups (as well as MDPH field staff) may have had exposure to PBB at concentrations above a threshold level for lymphocyte depression; hence all may have been equally depressed. A final possibility is that recovery of immunologic function may have occurred in the exposed population in Michigan in the 8 months which elapsed between this and the previous, more strongly positive study.[18] Further investigation will be required to distinguish among these possibilities, and to explore the clinical significance of the immunologic abnormalities observed in members of the two exposure groups.

PBB is a highly lipophilic compound and extremely persistent in the human body. Persons exposed to PBB may be at potentially increased risk of developing delayed reactions, particularly cancer, since PBB fed to rats, has produced neoplastic liver nodules.[4] Because of the potential for delayed disease, MDPH and the federal agencies plan to continue to follow the Michigan cohort for at least another 10–15 years through repeated contact with the exposed persons and their family physicians. Also, within the next year, a cohort of approximately 2000 Iowa farm residents without known exposure to PBB is to be recruited and interviewed using procedures identical to those used in Michigan; this group will be followed prospectively as a comparison cohort.

Summary

Polybrominated biphenyls (PBB) were dispersed widely in Michigan by a 1973 shipping accident in which PBB was introduced into cattle feed. Human exposure resulted principally from ingestion of contaminated dairy food products. To determine whether PBB exposure has or will cause acute or chronic illness, a prospective cohort study of 4545 persons has been undertaken. Three exposure groups were sought: all persons living on PBB-quarantined farms; persons who had received food directly from such farms; workers (and their families) engaged in PBB manufacture. Enrollment rates were 95.6, 95.1 and 78.0%. Also enrolled were 725 persons with low-level PBB exposure. All were queried concerning 17 symptoms and conditions related possibly to PBB. Venous blood was drawn on 3639 and analyzed for PBB by gas chromatography. Mean serum PBB levels were 26.9 ppb in quarantined farm families, 17.1 in recipients, 43.0 ppb in workers, and 3.4 ppb in the low exposure groups. No associations were found between serum PBB levels and symptom prevalence rates. To evaluate peripheral lymphocyte function, T and B cell quantitation and *in vitro*

responses to 3 nonspecific mitogens were studied in 34 persons with highest PBB levels (mean, 787 ppb), and in 56 with low values (mean, 2.8 ppb). No statistically significant differences in lymphocyte number or function were noted.

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