A Search for Polychlorinated Biphenyls in Human Milk in Rural Colorado

by E. P. Savage, J. D. Tessari, J. W. Malberg, H. W. Wheeler, and J. R. Bagby
Institute of Rural Environmental Health
Department of Microbiology
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, Colo. 80521

Background

JENSEN (1966) first reported residues of polychlorinated biphenyls (PCB's) in tissues of fish and birds. Since then PCB's have been identified in a number of different substrates, and the Food and Drug Administration has reported them in fresh fruits, vegetables, fish, poultry, eggs, milk, and in other food commodities. PCB's have also been reported in human adipose tissue and in human milk. RISEBROUGH et. al. (1971) reported PCB's in 16 human milk samples. DYMENT et. al. (1971) in a study conducted in Texas were unable to find PCB's in human milk samples from New Guinea and Texas. These reports prompted us to question the presence of PCB's in human milk in areas of rural Colorado. Samples were collected from women living in and near Fort Collins and Greeley, Colorado.

Fort Collins, located along the eastern slope of the Rockies approximately 60 miles north of Denver, has a population of approximately 45,000, and is primarily an agriculture and university community. Greeley, located approximately 30 miles southeast of Fort Collins and 50 miles northeast of Denver, has a population of about 45,000, and is also primarily an agricultural and university community. Both communities have some light industrial and manufacturing development.

Methods

A total of 39 human milk samples were collected from lactating mothers by manual expression and stored in 30 ml glass tubes fitted with plastic screw caps lined with teflon or aluminum foil. An epidemiological questionnaire was completed on each participant showing age, occupation, birthplace, race, years lived in Colorado, and if they had ever lived in communities larger than 50,000 population.

Aroclor samples for standards were obtained from the Monsanto Chemical Co., St. Louis, Missouri. Standard iso-octane solutions of Aroclor 1254 and 1260 were prepared for gas chromatographic analysis so that 1 microliter of solution contained 500 picograms of Aroclor.

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A MicroTek 220 gas Chromatograph equipped with a nickel-63 electron capture detector, and Coulson Conductivity Detector was used for analyses. Columns used were: 1.5% OV-17/1.95% QF-1, 4% SE-30/6% (OV-210 and 3% OV-1). Solid support material was Chromosorb W, high performance DMCS 100-120 mesh. Temperatures for the Electron Capture Detectors were 300°C, injection ports and transfer lines 245°C, Columns 200°C, and Inlet 240°C. Thin layer chromatography equipment consisted of: 8" x 8" glass plates, 8½" x 4½" x 8½" developing tank, desaga/brinkmann standard counting board, desaga/brinkmann standard model applicator, spotting pipettes, glass desicator, and ultra violet light.

The extraction procedure, which was a modification of those described by GIUFFRIDA et. al. (1966) and CURLEY and KIMBROUGH (1969), consisted of three parts: (1) the fat was isolated from the milk, (2) the PCB's were extracted from the fat, and (3) the extract was cleaned up. In our modification, samples of 15 grams of mother's milk were placed into clean glass centrifuge bottles. Approximately 1 gram of glass wool was added to each sample. The purpose of the glass wool was to adhere to the coarse precipitate of the milk solids. After addition of 100 ml of acetone, the milk samples were shaken for two minutes and centrifuged at 1000 rpm for 2 minutes. The acetone layer was then decanted into a one liter separatory funnel. After decanting, a volume of 25 ml of acetone was added to each sample and the extraction procedure was repeated three more times. All four extractions were then transferred into a liter separatory funnel. The portion of the milk sample that was coagulated was then extracted with 2-25 ml portions of n-hexane and centrifuged for 2 minutes at 1000 rpm. The two portions were then transferred into the one liter separatory funnel and 50 ml n-hexane, and 125 ml of a 2% sodium sulfate solution was added to the funnel. The separatory funnel was shaken manually for 2 minutes. The layers were then allowed to separate and the lower acetone-aqueous layer was drawn off and discarded. Another 125 ml of 2% sodium sulfate solution was added to the separatory funnel, shaken manually and allowed to separate. The lower aqueous layer was again discarded and the upper layer was poured into a 500 ml concentrator flask. The solvent flask was transferred to a rotary evaporator and the solvent extract was removed under water aspirator suction at 37°C until the solvent volume was reduced to approximately 2 ml. The sample extract was then taken through the Florisil procedure as described in the manual of Analytical Methods.

The samples were analyzed by gas liquid chromatography. These analyses were followed by the TLC procedure for PCB's as prescribed in the manual of Analytical Methods.

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Results

The age of study subjects ranged from 19 to 33 years. Their median age was 26 years.

The occupations of study participants were: Two secretaries, one medical technician, two nurses, one student, two beauticians, one editor, two teachers, one electronic technician, and 27 housewives.

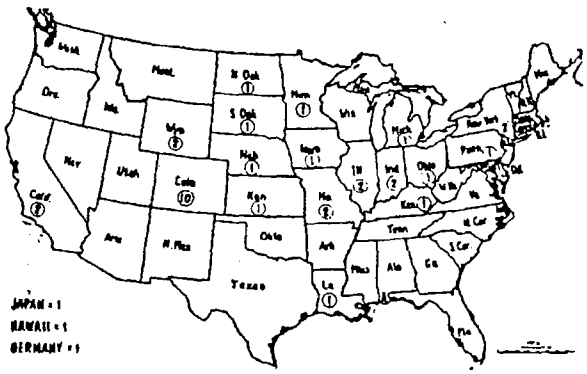


Figure 1. Place of birth of participants.

The place of birth of the study participants is shown in Figure 1. Eleven were born in Colorado and two each were from New York, California, Indiana, Illinois, Missouri, and Wyoming. One was born in each of 12 additional states, plus Germany and Japan. Two participants did not give their place of birth.

TABLE 1

Number of Years Study Participants Had Lived in Colorado.

No. Years	No. Participants
0 - 5	21
6 -10	4
11 -15	1
16 -20	4
21 -25	5
26 +	1
Unknown	3
Total	39

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The number of years the study participants had lived in Colorado is shown in Table 1. Over 56 per cent had lived in Colorado less than 5 years and of this group more than 25 per cent had lived in the state one year or less. Only 9, approximately 25% of the study participants, had not lived in communities of 50,000 or greater at some time during their lives.

TABLE 2

Epidemiological Data for Lactating Mothers Positive for PCB Residues

Colorado - 1972

Age	Birthplace	Occupation	Race	Years in Colorado	Lived City Larger than 50,000	Levels of PCB (ppm)
25	Colorado	Housewife	W	25 years	Yes	0.1
20	Colorado	Housewife	W	20 years	No	0.05
26	Michigan	Housewife	W	3 years	No	0.04
22	Germany	Housewife	W	3 years	Yes	0.04
19	N. Dakota	Housewife	W	7 months	No	0.04
25	Ohio	Secretary	W	2 years	Yes	0.04
20	Colorado	Housewife	W	19 years	Yes	0.04
23	Japan	Housewife	O	1 year	Yes	0.04

The epidemiological data for the eight participants with milk samples positive for PCB's are shown in Table 2. The levels of PCB's ranged from a low of less than 0.04 ppm to a high of 0.1 ppm. The range of age of the positive mothers was from 19 to 26 years. Three were born in Colorado, one each in Germany, Japan, Michigan, Ohio, and North Dakota. Seven were causasian and one oriental. Five had lived in a city of 50,000 population or greater at some time during their lifetimes and three had not. Seven of the women were housewives, and one was a secretary. It is of interest that the two highest levels of PCB's were found in two housewives who had never lived outside of Colorado.

Discussion

The median age of the women positive for PCB's was 22½ years, as compared to a median age of 26 years for all women in the study group. However, this figure is not statistically significant due to the low number of positives.

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The length of time that those positive for PCB's had lived in Colorado is of interest. Seven of the eight had lived in the state a year or longer. Two had never lived outside of the state and one had lived in another state for only one year. There were four other study participants who had never lived outside of Colorado who were negative for PCB's.

This study indicates that PCB's are found at very low levels in human milk in Colorado. The highest level was 0.1 ppm in a 25 year old housewife who has lived her entire life in the state. Six of the positive samples were at the low level of our detection cabability at 0.04 ppm or less.

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