Hexachlorophene Concentrations in Human Milk

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Until recently, it was the practice in many nuseries to regularly bathe newborn infants with hexachlorophene-containing solutions (KASLOW et al., 1973). With the discovery of the hazard of central nervous system toxicity, the Food and Drug Administration (F.D.A.) in cooperation with the Committee on Fetus and Newborn of the American Academy of Pediatrics issued a recommendation that the routine use of hexachlorophene for total body bathing of infants be discontinued (ANON. 1971). A renewed interest in the pharmacology and toxicology of this compound has resulted. In the past, most studies have been concerned with the absorption of hexachlorophene through intact or burned skin (CURLEY et al., 1971; CARROLL et al., 1967), but hexachlorophene can also be absorbed from the gastro-intestinal tract (KIMBROUGH 1971; KIMBROUGH and GAINES 1971).

As it has been the practice at some hospitals to instruct nursing mothers to wash their nipples with hexachlorophene-containing preparations as well as to wash their babies after discharge with these products, it was decided to perform hexachlorophene assays on human milk samples obtained before and after the F.D.A. recommend-ations were issued in December, 1971. To our knowledge these are the first reported determinations of hexachlorophene in human milk.

MATERIALS AND METHODS

Six human milk samples were obtained during the first six months of 1971 and five samples were obtained during March, 1973 from white, urban middle-class nursing mothers. Samples were frozen in glass bottles until analysis.

The method of analysis is a variation of methods used by Shafik (personal communication) and ULSAMER (1972), modified to eliminate the formation of emulsions and the saponification of triglycerides during sample cleanup. After thawing, samples were shaken vigorously for 30 seconds and two 5-gram aliquots taken. To one aliquot 10 mg of hexachlorophene in 25 ml of pH 11 sodium hydroxide was added. The samples were quick-frozen, lyophillized, homogenized with acetone at 3000 rpm for 30 seconds, and centrifuged at 2500 rpm for 5 minutes. The clarified acteone was decanted and the residue washed twice with 2.5 ml acetone which was added to the supernatant. The acetone was evaporated at 60°C with a gentle flow of prepurified nitrogen and 2.5 ml of acetone and 2.5ml of 0.01N potassium hydroxide were added. The solution was washed with three 5 ml portions of hexane and adjusted to pH 4.5 with 0.25N acetic acid. Five ml of chloroform

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was added to the acidified solution and shaken. The mixture was centrifuged (2 minutes at 2500 rpm) and the chloroform layer transferred. The water layer was reextracted with 2.5 ml chloroform and 1.2 ml of acetone and the combined extract evaporated to dryness with a nitrogen flow at 60°C. One ml benzene was added, the sample placed in a 70°C bath and the derivatizing reagents (0.1 ml acetic anhydride and 0.1 ml pyridine) added. After 15 minutes the benzene was evaporated with nitrogen at 60°C. The residue was taken up in 1 ml of hexane and the solution passed through a silica column (0.5 g heat-activated silica gel in a 6 mm i.d. column). The sample tube was rinsed with 2 additional 1 ml portions of hexane which were also added to the column which was eluted with two 5 ml portions of benzene. The eluate was evaporated (nitrogen 60°C) to less than 5 ml and its volume adjusted to 5 ml.

The gas chromatograph was equipped with a radioactive nickel electron capture detector and was used with a 6 foot x 4 mm i.d. glass column packed with 2% OV-210 on 100/120 mesh chromosorb W. The column was run at 230°C, the injection block at 250°C, and the detector at 280°C. The flow rate of prepurified nitrogen was 60 ml per minute. The retention time of diacetylhexachlorophene (the derivative prepared by our procedure) under these conditions was 8 minutes. Aliquots (5 ml) of sample were injected. A calibration curve was run (5,10,20,30 and 40 pg of diacetylhexachlorophene) before the sample injections and another standard was run immediately after the sample injections to check detector sensitivity. The detection limit was 2 ppb; peaks smaller than this are reported as "trace". Recovery of hexachlorophene from human and cows milk was 60%$\pm$ 5% at these low levels.

RESULTS AND COMMENT

Eleven human milk samples from eleven donors were analyzed (Table 1).

TABLE 1
HEXACHLOROPHENE CONCENTRATIONS IN HUMAN MILK

	Sample No.	Hexachlorophene Concentration (ppb)
Milk donated in 1971 before F.D.A. restrictions	1	Trace
	2	2.1
	3	4.6
	4	5.8
	5	7.7
	6	9.0
Milk donated in March, 1972 after F.D.A. restrictions	1	None detected
	2	None detected
	3	Trace
	4	Trace
	5	8.3

The hexachlorophene concentrations found in the six samples collected before the F.D.A. restrictions went into effect ranged from "trace" (< 2 ppb) to 9.5 ppb with a mean of 5.7 ppb (± 2.9 ppb standard deviation). The five milk samples obtained after the restrictions were in effect contained 8.3 ppb in one, "trace" levels in two, and undetectable levels in two specimens. Using the Wilcoxon Rank Sum test, no significant difference was found between these groups. Regretably, information concerning the specific history of hexachlorophene use of these donors was not available. None of the 20 specimens of commercial cows' milk tested contained any detectable hexachlorophene.

These concentrations of hexachlorophene in human milk are considerably lower than concentrations measured in infant blood at the time of discharge from the hospital (CURLEY et al., 1971) which ranged from 9 to 646 ppb with a mean of 109 ppb. They are also lower than concentrations found in the blood of adults which ranged from 1 to 89 ppb with a mean of 28 ppb. Since transfer of hexachlorophene across membranes depends upon the concentration of the non-ionized form only and since weak acids such as hexachlorophene are less ionized at the pH of milk (about 6.6) than at the pH of plasma(7.4), one would not expect hexachlorophene to be concentrated in milk.

The data suggest that the population was previously exposed to low levels of hexachlorophene resulting in low parts per billion residues in human milk. As dietary concentrations below 100 ppm had no effect on rats (KIMBROUGH and GAINES 1971) and the biological half-life of hexachlorophene in rabbits is only approximately 18 hours (WIT and VAN GENDEREN 1962), we conclude that human milk concentrations in the low ppb range probably do not constitute a hazard. It would be of interest, however, to study nursing mothers who for appropriate medical reasons must continue to use topical preparations containing hexachlorophene.

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