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We report here on the effect of a vegetarian diet on organochlorine concentration in human breast milk, and the role of dairy fats within the vegetarian diet. Since the pioneering work by Laug et al. in 1951, the presence and concentration of organochlorines in breast milk have been well established through direct measurement (1). There are also a small number of studies investigating the possible factors determining residue concentrations, but nearly all of these studies are univariate; i.e., they consider only one factor at a time. A notable exception is Bradt and Herrenkohl (2), who used a multivariate approach. In two of the three statistical procedures reported below, we follow Bradt and Herrenkohl, and we use regression analysis to investigate the possible effects of diet, while controlling for other factors previously identified. The organochlorines considered here are: DDE, p,p'-DDT, dieldrin, heptachlor epoxide, oxychlordane, β-BHC, TNC, HCB, and AR-1254 (PCB). All were banned or under restricted use prior to the study, so that there is relatively little exposure through direct spraying of food crops.

Factors identified in the literature include the following. (i) Direct Exposure: From DDT recently sprayed for malaria control (3); house dust, where pesticides are used for household pest control (4); use

of nonpersistent pesticides in the home (2); occupational exposure to DDT by agricultural applicators, in a study of DDT and DDE in body fat (5) [although this study was not on residue levels in breast milk, it is of interest because of a possible correlation between organochlorine concentrations in breast milk and in body fat(6)]; current cigarette consumption (2). (ii) Lactation: A long term factor measured by the number of children previously breastfed, leading to a secular decline in organochlorine concentrations in breast milk (2,7,8). While there is evidence of considerable short term fluctuation in organochlorine concentrations in breast milk (9,10), lactation has been identified as a principal route of excretion of organochlorines (8,9), with the appearance of eventual decline in concentration depending on the specific organochlorine involved (11). (iii) Age: The role of age is somewhat unclear. Savage reported that the age of his high residue group was slightly younger than that of his low residue group (7); Wilson et al. also found DDT levels in breast milk diminishing with age (12); however, Tables 28-30 of (7) suggest that breast milk concentrations of dieldrin, heptachlor epoxide, and oxychlordane may increase with maternal age; also Wasserman found organochlorine concentrations in body fat generally to increase with age, at least up to 45 (13). (iv) Region: Savage found regional differences in the United States to be one of his most important factors, previous lactation being the other (7). Region may to some extent be a proxy for variables of direct exposure, but where there are major regional differences in the volume of organochlorines released to the environment, regional variables may still be important (14). (v) Fat Content: Nearly all the organochlorine residue in breast milk resides in

the fat fraction of the milk, and the percentage of fat varies from one to eight percent, with fore milk (milk taken early in the nursing period) low in fat and hind milk (taken late) rich in fat (12).

Diet, and more specifically the role of a vegetarian diet on organochlorine concentrations in breast milk, has received relatively little attention, although possible food chain effects have been mentioned several times (7,12). In 1958 Hayes reported that vegetarians had about half the levels of DDT and DDE in their body fat, compared with people from the general population (5). Bradt and Herrenkohl found that total DDT in breast milk increased with the number of calories in the diet and suggested that "Ingesting a greater than average amount of food per day could increase the amount of DDT ingested and hence the amount of DDT stored"(16). On the other hand, Wilson et al. (12) found no correlations between the concentration of total DDT in breast milk and the number of days per week the donor ate meat or fish, but the variation in diet was rather small (personal communication with Wilson).

The role of a vegetarian diet is suggested by the fact that the organochlorines under study are lipophilic and long-lived, with a tendency to bioconcentrate in the food chain. Table 1 illustrates that organochlorines are found more frequently in foods higher in the food chain; moreover, that the frequency of detection is not much different in dairy products than in meat, fish, and poultry. In recent years organochlorines have been removed from fats and oils during processing, so that low frequencies of detection for these foods are not unexpected. Banning or restriction of use was already evident by the time of the FDA survey in 1974, by the low frequencies of detection of the pesticides in food crops.

To investigate the possible role of a vegetarian diet on organochlorine concentrations in breast milk 46 vegetarian nursing mothers were questioned as to their diet, other possible routes of exposure, and previous lactation, and then matched against controls drawn from the 1,436 women studied by Savage. For each vegetarian, a category was defined by region, urbanization, occupation of the mother, the number of children previously nursed, the number of siblings, and smoking habits, the categorization reflecting the level of detail known about the full sample of 1,436. A number of women from the full sample fit each such category, and from this number one was chosen at random for each matched pair. None of the vegetarians were current smokers, there was minimal occupational exposure to the mother or father, and the controls were selected to reflect these facts.

Whole milk extracts from the vegetarians were analyzed for ten organochlorines, by EPA, using the same method as the previous study of 1,436 from which the matched pairs were drawn (7). The sensitivity limits defined on a whole milk basis are, in parts per billion: p,p'DDT, 5; o,p'-DDT, 5; DDE, 2.5; dieldrin, 2.0; heptachlor epoxide, 1.0; oxychlordane, 1.0; β-BHC, 2.5; TNC, 1.0; HCB, 0.5; PCB, 50. Values below these sensitivity levels on a whole milk basis were reported as trace, and amounts below detection were reported as zero.

For each organochlorine, the relative numbers of observations reported as above zero and above trace are shown in Table 2. As all the values for o,p'-DDT were reported either zero or trace this organochlorine was dropped from further statistical analysis. As can be seen from columns 7 and 8 of Table 2, the 46 matched pairs drawn from the

entire sample of 1,436 tend to have lower values than the entire sample itself. For regression analysis, trace, a reported range of possible values, was taken to be one-half the level of sensitivity on a whole-milk basis. The relatively large numbers of values reported trace (column 2 minus column 5, and column 3 minus column 6 in Table 2) are expecially high for PCB, and make statistical inference tenuous for this chemical (16).

A simple procedure which obviates part of the statistical difficulty associated with qualitative trace values is the nonparametric sign test (17). Because the fat content is not controlled in the selection of the matched pairs, the concentrations were adjusted to a fat basis (nanogram organochlorine per gram fat in milk). As can be seen in Table 3, for 7 out of 9 organochlorines the vegetarians had significantly lower concentrations on a fat adjusted basis than did their matched counterparts. However, the concentrations of HCB were significantly higher in vegetarians under the sign test while PCB levels did not appear to differ between vegetarians and matched pairs. The sign test performed on concentrations on a milk basis, uncontrolled for fat content, yields the same pattern of results, but with less statistical significance.

A closer look at the difference between vegetarians and women with normal diets is afforded by pooling the two groups and performing a regression analysis with control variables explicitly introduced (Table 4). The dietary or food chain variable (DIET) is defined to be one for a vegetarian and zero for a woman on a normal diet. The regions are the same as those defined by Savage, but in the present analysis the

coefficients are specified in terms of differences from Savage's Midwest region (18). FAT is the percent fat content of milk; milk from the vegetarians contained, on average, more fat (3.4 percent fat) than milk from mothers in the general population (3.0 percent). AGE is the age of the mother at the time the milk sample was taken; the average age of the vegetarians (26.9 years) was a little higher than the average age of the women from the general population (25.8 years). BRFD is the number of children previously breast-fed. Table 4 summarizes the regression analysis for 35 vegetarians and 46 mothers from the general population, 11 vegetarians being dropped due to incomplete information.

As can be seen in Table 4, being a vegetarian is associated with lower concentrations of the seven agricultural organochlorines with five of the coefficients significant. Again, the exceptions are PCB and HCB, which have positive though statistically insignificant coefficients. The total concentration of the seven agricultural organochlorines is also significantly lower for the vegetarians. As a general pattern, the larger the number of children previously breast-fed, the lower the concentration of the organochlorines. Women who have breast-fed more children tend to be older than those who have breast-fed fewer children (the correlation between these two variables is 0.47) but exclusion of AGE does not affect BRFD or the other variables notably. As expected, because nearly all the organochlorine content is in the fat fraction of the milk, the last variable, percent fat is constantly strong and positive. A test that fat content is not the sole determinate of organochlorine concentration in breast milk is given by the statistical significance of DIET in Table 4. Direct regressions of the organochlorine concentration

in milk fat, not reported here, also indicate that diet and other factors influence concentrations in milk fat.

More detailed data are available for the vegetarians considered by themselves (see Table 5). The vegetarians were asked the frequency of their consumption of foods falling into the classes of dairy products, grains, and legumes, and whether or not the food was organically grown (without pesticides). The questionnaire of the vegetarians offered a sharper definition of the extent of previous lactation, in terms of the total months of previous nursing (MONTHS). Direct exposure (EXP) is a composite of exposure from farm residence, household use of pesticides, and use of professional exterminators (19). For DAIRY each of the following was scored zero for never consumed, 1 for sometimes, and 2 for frequently -- milk, cheese, ice cream, and butter -- and then these scores were added.

Those consuming dairy products more frequently are associated with higher levels of eight of the organochlorines (significantly so for five); those with more months of previous lactation with lower concentrations of all the organochlorines (significantly so for six) in agreement with (2), (7), and (8); those longer on the vegetarian diet with lower concentrations for seven of the organochlorines (but significantly so for only two); and those who are older with higher concentrations for eight organochlorines (significantly so for five), in agreement with Wasserman (13). As expected, there is some statistical interaction among the three temporally defined variables (the correlation between age and months previous lactation is 0.43; between age and years on diet 0.39; between months previous lactation and years on diet 0.60). Exclusion of AGE tends to increase the significance of MONTHS (months previous

lactation), to decrease the significance of YEARS (years on diet), but to have little effect on DAIRY or the regional variables. However, there also appears to be an interaction between age and direct exposure (correlation between AGE and EXP is 0.52). With AGE excluded EXP is associated with higher concentrations of eight organochlorines (significantly so for four). Of the regional variables only Southeast is significant for many of the organochlorines, but there are only three vegetarians from the Southeast, too few to draw conclusions. Regressions excluding the regional variables altogether did not notably change the results for the other variables. The coefficients of determination, adjusted for degrees of freedom ($\bar{R}^2$), are higher in the regressions on vegetarians alone, and a stepwise analysis suggests that DIARY, MONTH, and YEARS contribute more or less equally to this difference.

Egg consumption, mentioned by Hayes (5), as a possible source of DDT, was disaggregated from DAIRY and included as a separate variable but no association was apparent; nor for variables describing previous smoking history. Inclusion of these variables does not notably affect DIARY, YEARS, MONTHS, AGE, or EXP. Diet was analyzed in fairly aggregative terms because of the relatively small number of vegetarians investigated. With a larger number of vegetarians, diet could be further disaggregated; and some of the association reported negatively here could be resolved more completely.

Three other issues suggest themselves for further investigation, beyond the scope of this study. (i) The above statistical analysis suggests that agricultural pesticides may have different pathways from industrial organochlorines (PCB and HCB). Possibly the agricultural pesticides are transferred through the food chain more than the industrial

organochlorines (Table 4, last two rows), but the industrial organochlorines are transferred more through dairy products than other vegetarian foods (Table 5, last two rows). (ii) The regional variables had relatively little effect in the analysis but the effect of the Southeast is opposite to that found by Savage. A considerably larger number of observations and an analysis including the above identified factors simultaneously, along with data on the regional discharge of organochlorines into the environment could help clarify the role of region. (iii) With AGE excluded, the variable describing direct exposure (EXP) is significant for heptachlor epoxide, oxychlordane, and TNC, all derivatives of chlordane, used for termite control. AGE may be a proxy for accumulated previous exposure, and the nonsignificance of EXP for these three organochlorines may be a result of colinearity between AGE and EXP (correlation between AGE and EXP is 0.52). Further investigation with additional statistical data is required to resolve this question as well.

The basic findings of our study are these: vegetarians have lower levels of many organochlorines in their breast milk than those on normal diet. The presence of a food chain effect is suggested at two levels -- in the pooled regressions, the lower levels of organochlorines concentrations for abstainers from meat pointing to a food chain mechanism in meat fats; and in the regressions on the vegetarians the lower levels of organochlorine concentrations for those eating dairy products less frequently pointing to a food chain mechanism in dairy fats. Previous lactation was found to lower residue concentrations as did the length of time on a vegetarian diet.

All the organochlorines in this study are either carcinogens or

suspected carcinogens. While the extent of the risk to the infant is not known, it is worth noting that the above-trace average, for 1,436 mothers, (column 7, Table 2) for heptachlor epoxide slightly exceeds the World Health Organization's Acceptable Daily Intake (0.5 ug/kg/day); the above-trace average for dieldrin exceeds the WHO ADI (0.1 ug/kg/day) by ninefold; and the above-trace average for DDE exceeds the WHO ADI (5 ug/kg/day) by a factor of two (20). Moreover, it appears that the first child is most heavily dosed, with following children receiving decreasing burdens (21). This study indicates that a vegetarian diet, or a diet low in animal fats, offers some protection against the risk of organochlorine contamination of breast milk (22). And finally, this study suggests that with the identification of the food chain as a principal route of exposure, organochlorine contamination of breast milk (and probably other foods high in animal fats) will continue for many years after the organochlorines have been banned or restricted in use.

TABLE 1

FREQUENCY OF DETECTION OF ORGANOCHLORINES IN FOODS

(Percentages)

	Meat Fish Poultry	Dairy	Potatoes	Grains Cereals	Root Vegetables	Leafy Vegetables	Legumes	Oils & Fats	Fruits
DDE	100	90	23	0	27	17	0	13	0
DDT	33	93	27	10	0	0	0	13	0
Dieldrin	100	93	23	7	0	0	0	0	7
Hep. Epox.	97	90	27	0	0	10	0	10	7
BHC	90	97	0	7	0	0	0	0	13
HCB	13	10	7	0	10	0	10	23	0

Source: Compliance Progress Evaluation FY 1974, Total Diet Studies (7320.08), Food and Drug Administration, Washington, D.C.

TABLE 2

COMPARISON OF VEGETARIANS AND MATCHED PAIRS
TO THE GENERAL POPULATION

	Percent in Sample Greater than Zero			Percent in Sample Greater than Trace			Average Above Trace in ppb, Fat Adjusted		
	Total Sample	Matched Pairs	Vegetarian	Total Sample	Matched Pairs	Vegetarian	Total Sample	Matched Pairs	Vegetarian
	1	2	3	4	5	6	7	8	9
DDE	*	100	100	100	100	100	3521	3126	1948
p,p'-DDT	*	100	100	99	91	60	553	515	284
o,p'-DDT	*	36	4	*	0	0	*	†	†
Dieldrin	96	100	100	81	54	44	164	112	90
Heptachlor Epoxide	93	98	95	63	58	33	91	64	66
Oxychlordane	96	98	98	74	74	53	96	75	60
TNC	*	100	76	70	72	67	93	78	62
β-BHC	*	89	80	87	56	40	193	253	114
HCB ††	*	95	100	46	47	87	78	85	48
PCB	*	100	100	*	33	31	*	2395	2700

* Not reported.
† Not applicable.
†† Note that the average of above trace concentrations for HCB is lower for the vegetarians (48) than for the matched pairs (85); the reason HCB is found significantly higher for the vegetarians is because of the greater frequency of values reported trace for the vegetarians.

TABLE 3

NONPARAMETRIC SIGN TEST

Vegetarians significantly less than matched pairs	p Value
DDE	.003
p,p'-DDT	.0003
Dieldrin	.09
Heptachlor Epoxide	.006
Oxychlordane	.03
TNC	.05
β-BHC	.003
Vegetarians significantly more than matched pairs	
HCB	.07
No significant difference	
PCB	.67

TABLE 4

VEGETARIANS AND GENERAL POPULATION

	DIET	BRFD	AGE	Region				FAT	$\bar{R}^2$
				NE	SE	NW	SW		
DDE	-26.96**	-11.97	.48	-8.09	-22.4	-8.27	17.61	14.21***	.18
p,p'-DDT	-5.78***	-1.43	-.04	-2.64	-5.02	2.24	-.44	2.16***	.23
Dieldrin	-.40	-.24	-.19	.005	-.82	-.04	.32	.57***	.31
Heptachlor Epoxide	-.60***	-.05	.002	-.30	-.68*	-.38	-.57	.27***	.33
Oxychlordane	-.56*	-.28	.002	.27	-.43	-.14	.16	.47***	.33
TNC	-.20	-.16	.01	.39	.48	-.39	.54	.61***	.18
β-BHC	-1.96***	-.93**	.03	-.36	-1.86	1.74*	2.23**	1.02***	.38
HCB	.62	-.49**	.08**	.34	.00	1.73***	1.67**	.30***	.32
PCB	9.2	-10.62	-1.02	10.39	-19.07	-3.64	5.16	10.15***	.09
Total Pesticides	-30.58**	-13.46	.59	6.70	-12.61	7.98	39.17**	17.13***	.26
Total Organochlorines	-15.29	-29.9**	1.71	13.84	-59.72	7.09	26.28	26.07***	.24

* Significant at the 10% level.
** Significant at the 5% level.
*** Significant at the 1 % level.

TABLE 5

VEGETARIANS ONLY

	DAIRY	YEARS	MONTHS	AGE	EXP	Region				FAT	$\bar{R}^2$
						NE	SE	NW	SW		
DDE	6.05**	-3.82	-.87***	2.58**	7.72	9.52	-30.86	7.88	9.81	16.60***	.64
p,p'-DDT	.57	.18	-.06*	.09	-.1	4.11*	.16	1.90	-.48	1.16**	.44
Dieldrin	.06	.20	-.03***	.04	-.23	-.42	-2.25**	-.13	.14	.79***	.56
Heptachlor Epoxide	.10*	-.15**	-.01*	.06**	.05	-.25	-.83**	.22	-.14	.37***	.60
Oxychlordane	.13*	-.22**	-.02***	.07*	.38	-.14	-1.54**	.06	-.17	.58***	.70
TNC	-.13	-.27	-.03	.07	1.72**	-.37	-2.2	-1.43	-.49	.63**	.34
β-BHC	.48***	-.61***	-.028*	.22***	-.34	.55	-2.35*	.60	.95	1.21***	.68
HCB	.18*	-.11	-.013	.11**	-.33	.37	-.80	1.62***	.45	.48***	.50
PCB	15.93***	-6.25	-1.00**	-3.29	28.3*	-34.47	-75.6**	-23.84	25.96	26.22***	.49
Total Pesticides	7.25**	-4.69	-1.05***	3.13**	9.20	13.0	-39.87	8.66	9.35	21.32***	.68
Total Organochlorines	23.37***	-11.05	-2.06**	-.05	37.17*	-21.1	-116.27**	-13.55	35.76	48.02***	.65

* Significant at the 10% level.
** Significant at the 5% level.
*** Significant at the 1% level.

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6. Indirect evidence of a correlation is from cows for which the concentration of DDE in milkfat is about the same as the

6. concentration in body fat, and similarly for HCB and PCB: see G. F. Fries and G. S. Marrow, Hexachlorobenzene retention and excretion by dairy cows, J. Dairy Science 59 (March 1976), 475-80; G. F. Fries; G. S. Marrow, Jr.; and C. H. Gordon, Long-term studies of residue retention and excretion by cows fed a polychlorinated biphenyl (aroclor 1254), J. Ag. Fd. Chem. 21, 1 (1973), 117-21.

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(1974), 148-51.

11. For example, HCB is much more tightly held than DDE: for comparisons of organochlorine retention in cows see references in [6].

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16. For example, we found that an analysis of the 1038 PCB

levels, using a Tobit limited dependent variable model, suggested that the average value reported trace might be somewhat greater than half the level of sensitivity: see G. S. Maddala, "Econometrics," McGraw-Hill, New York, N. Y., 1977, 162-7.

17. E. I. Lehmann, "Nonparametrics: Statistical Methods Based on Ranks," Holden Day, San Francisco, 1975, 156-200.

18. The five regions are characterized by four dummy variables, the first being one if the mother is from the Northeast, zero otherwise; and similarly for the Southeast, Northwest, and Southwest. The constant term in the regression incorporates the effect of the Midwest, and the dummy variable coefficients estimate the difference in effect from this term.

19. Each of these factors was scored between zero and one and then added (occupational exposure of wife and husband being minimal, except through farm residence).

20. Cows' milk and formula milk are considerably lower in organochlorine concentration than breast milk, but of course without the benefits of breast-feeding.

21. Savage ([8], pp. 53-61) found that the organochlorine concentration declined linearly with the number of children previously breast-fed.

22. The importance of previous lactation in lowering the concentration of organochlorines in breast milk suggests that breast-feeding may transfer some cancer risk from the mother to the infant. However, B. MacMahon et al. did not find a difference in breast cancer between women with differing lactation (Bull. Wld. Hlth. Org. 42 (1970), 135). See also R. Ing; J. H. C. Ho; and N. L. Petrakis, who found among women who nursed only on one side, more cancer of the unnursed breast than the nursed breast (Lancet (July 16, 1977), 124).