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Organochlorine Insecticide Residues in Human Breast Milk: A Survey of Lactating Mothers from a Remote Area in Papua New Guinea

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Persistent halogenated hydrocarbon pesticides and fungicides such as the organochlorines and organophosphates have been used extensively in industry and agriculture throughout the world over the last few decades. Organochlorines have been detected in human breast milk in various countries in recent years, for instance Brazil (Matuo *et al.* 1980), Sweden (Hofvander *et al.* 1981), India (Saxena & Siddiqui 1982; Rama Krishnan *et al.* 1985), Iraq (Al Omar *et al.* 1985), and Australia (Stacey *et al.* 1985; Spicer 1987). The purpose of this study was to investigate the degree of contamination of nursing mothers in a very remote area in Papua New Guinea.

MATERIALS AND METHODS

The survey was undertaken in November 1990 in four remote villages in the Star Mountains region in the northern part of the Western Province of Papua New Guinea. The villages from which the breast milk samples were collected are situated in the rugged remote mountainous terrain at around 600 - 1500 metres above sea level, and half to two days walking from Tabubil, a mining town with an estimated population of 6000 people.

A survey team was in the area in November 1990 as part of the Ok Tedi Mining Medical Department malaria and microfilaria control programme. Milk was collected from lactating mothers who volunteered 50 - 100mls of their breast milk. Milk was only collected from mothers where it was considered that the child would not be jeopardised in any way ie suffering from an illness, too small or malnourished.

Milk was collected into glass containers which had been thoroughly cleaned and rinsed in acetone then N hexane then carefully dried. Milk was collected by the mother expressing the breast and collecting the milk in the previously cleaned bottle. A plastic cap was screwed on over aluminium foil.

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The bottles and contents were then placed immediately into a small mobile refrigerator at 4°C and transported within 12 hours to Tabubil where they were all deep frozen. The samples were later transported still frozen to the New South Wales Dairy Corporation laboratories in Sydney, Australia.

Organochlorine pesticide residues are fat soluble and, if present are found associated with the lipids of milk. The first step of the analysis was to extract the organochlorine residues together with the fat from the milk using organic solvents.

Isolation of the organochlorine residues from the co-extracted fat was done using Gel Permeation Chromatography (GPC). This method utilises molecular size exclusion with BioBeads SX-2 resin to separate compounds of interest with smaller molecular weights from interfering components with larger molecular weights. The instrument is calibrated experimentally to determine elution profiles and appropriate fractions relating to the pre-programmed dump/collect times were collected by a fraction collector. The samples were then concentrated down to suitable volumes for analysis.

Analysis mixtures were analysed on a Shimadzu Gas Chromatograph (GC 9AM) which utilises two Electron Capture Detectors (ECD) and two capillary columns: J & W DE 1701 for quantitation and SGE BP5 for simultaneous confirmation of residues. Hydrogen gas was used as the carrier and nitrogen as the make-up gas. Peak heights or areas were quantified, depending on the level found, against analysis standards. Spike controls were tested at regular intervals to determine recovery of the organochlorine pesticides. The recoveries were incorporated into the final calculations.

Samples were tested for organochlorines:

HCB	DDE
aBHC	DDD
bBHC	DDT
Lindane	Oxychlordane
Aldrin	alpha Chlordane
Heptachor	gamma Chlordane
Hept. Epoxide	Endrin
Dieldrin	Methoxychlor

Samples were screened for:

Pherate	Diazinon
Chlorpyrifos-ne	Pirimiphos-me
Fenchlorphos	Chlorpyrifos-D
Fenitrothion	Chlorfenvinphos-Z
Parathion	Ethion
Bromophos-me	Iodophenphos

Minimum level of reporting of pesticide residue is 0.01 mg/kg of sample.

Note: Samples were tested as received. As arranged fat samples were cleaned up using GPC technique by another NATA registered laboratory prior to GC analysis. Sample 14 -

analysis mixture had evaporated due to ill fitting lid. This was reworked and an estimated value has been reported. Sample 14 (Table 1) was excluded from any calculations because the value was estimated after reworking the analysis.

The ages of the mothers were generally estimated since the majority of them did not know when they were born and some only had a rough idea how long they had lived.

RESULTS AND DISCUSSION

In spite of the remoteness of the villages 100% of the mothers had DDT in their breast milk. Ten were above the allowable limit of 1.25 mg/kg fat for cow's milk (NSW regulation 6 of the Pure Food Act 1908 [as amended]). Six mothers had Heptachlor epoxide found in their breast milk. All were well below the maximum residual concentration of 0.15 mg/kg fat. Testing for other organochlorines did not reveal detectable amounts of any other compounds. Screening for the organophosphates also did not reveal detectable amounts present. In Papua New Guinea DDT was found to be present in all samples of human adipose tissue (Siyali *et al.* 1973). A correlation between organochlorines in serum and adipose tissue in man is now accepted. Siddiqui *et al.* (1981) showed that there is a correlation between maternal blood levels of organochlorines and their transfer through placenta and the excretions through breast milk. It is therefore not altogether surprising that all of the mothers from this very remote part of the world should have detectable amounts of DDT in their breast milk. Studies from other parts of the world have also shown this to be so (Spicer 1987; Al Omar *et al.* 1985). Our figures suggest however that the DDT levels are at least half to one third lower than that found in other studies.

There was a significant difference between the amount of total DDT in breast milk of mothers feeding their first babe compared to those feeding their second, third or more babes. First babe n=13, mean 1.3331; second or more n=27, mean=0.6019 ($t=3.8044$; $P<0.001$). The significant difference between the amount of DDT in the milk of primigravidas and multigravidas is therefore demonstrated. This supports the theory of shedding the total body load of organochlorines via the breast milk with each successive pregnancy (Slorach and Vaz 1983). However the literature does appear to be somewhat contradictory on this point. Krauthacker (1991) and Drijver *et al.* (1988) found no significant differences between primigravidas and multigravidas. Our figures although showing a significant difference between DDT in the milk of primigravidas and multigravidas did not show any real correlation between organochlorines in the breast milk and the number of pregnancies.

No direct correlation could be found between the amount of total DDT present in the breast milk and parity of the mothers ($r=0.05124$).

Table 1. Levels of DDT and its metabolites, heptochlor epoxide in breast milk samples according to age, number of previous pregnancies and age of infant being fed (mg/kg)

Sample No.	Age Mother	Vill-age	DDE	DDD	DDT	Total DDT	Hepto Epox	Vill-age	No. of Preg.	Infant Age
1	39	KWT	0.06			0.06		1	3	22
2	26	KWT	0.13		0.15	0.28		2	2	21
3	20	ATM	1.65	0.15	1.20	3.00		2	1	13
4	26	KWT	0.15		0.12	0.27		3	1	4
5	28	ATM	1.23	0.05	1.15	2.43		2	1	5
6	30	KWT	0.06		0.04	0.10		3	3	12
7	38	ATM	0.36		0.58	0.94		2	5	7
8	30	KWT	0.22		0.14	0.36		4	4	14
9	19	KWT	0.14		0.10	0.24		1	2	5
10	28	ATM	0.20		0.13	0.33		1	5	16
11	38	ATM	0.39	0.03	0.35	0.77		3	4	10
12	38	ATM	0.22	0.03	0.12	0.37		3	5	19
13	22	ATM	0.83	0.06	0.86	1.75	0.02	2	2	19
14	26	KAV	1.19	0.06	1.80	3.05	0.01	1	3	14
15	26	KAV	0.68	0.03	0.45	1.16	0.01	1	2	8
16	20	KAV	0.93	0.04	0.79	1.76		1	1	8
17	30	KAV	0.87	0.04	1.06	1.97		1	3	7
18	30	KAV	0.49	0.02	0.41	0.92		1	3	19
19	20	KAV	0.64	0.04	0.95	1.63	0.02	1	1	30
20	20	KAV	0.86	0.04	0.62	1.52		1	1	36
21	20	KOR	0.77	0.02	0.64	1.43	0.01	2	1	24
22	26	KOR	0.14		0.08	0.22		3	2	8
23	26	KOR	0.04		0.03	0.07		2	1	12
24	26	KOR	0.23		0.53	0.76		4	2	4
25	26	KOR	0.50	0.02	0.39	0.91		2	2	24
26	26	KOR	0.04		0.03	0.07		1	2	7
27	20	KOR	0.20	0.01	0.11	0.32		4	1	24
28	35	DER	0.36		0.50	0.86		1	7	9
29	26	DER	0.15		0.19	0.34		2	2	10
30	30	DER	0.13		0.24	0.37		2	5	24
31	30	DER	0.21		0.03	0.51		2	4	0.25
32	26	DER	0.39	0.04	0.47	0.90		1	2	3
33	20	DER	0.77	0.04	0.63	1.44		1	1	17
34	20	DER	0.18	0.01	0.16	0.35		1	1	0.25
35	30	KUM	0.27	0.03	0.13	0.43		3	4	4
36	20	KUM	0.65	0.05	0.36	1.06		3	1	8
37	20	KUM	1.25	0.04	0.76	2.05	0.02	2	1	21
38	26	KUM	0.16	0.02	0.08	0.26		0	3	15
39	35	KUM	0.32	0.03	0.21	0.56		3	9	5
40	26	KUM	0.17		0.11	0.28		2	5	3
41	35	KUM	0.36	0.04	0.13	0.53		1	7	1

There was mildly significant difference between the amount of total of DDT in those mothers more than 30 years of age n=14, mean=0.625 compared to those under 30 years old n=26, mean=0.9565 ($t=1.04992$; $0.5>P>0.1$). There was no correlation between the age of the mother and the amount of DDT secreted in the milk ($r=-0.3614$). Since the ages were only an estimation the figures are not reliable. Other studies have shown a correlation between age and amount of DDT (Spicer 1987; Siddiqui & Saxena 1985)

Heptachlor epoxide was found in six samples (including sample 14). Because of the low numbers no statistical analysis could be performed. It is noted however that all six had DDT levels above the mean and appeared to be in the younger age group. Parity for the six varied between one and six. They came from different villages.

The secretion of DDT, DDE and DDD in the breast milk is a fast mechanism of eliminating these lipotrophic compounds (Wolf 1983). Screening for the organophosphates, as expected, did not show any to be present. What is surprising is that ten were above 1.25 mg/kg fat level. There had been no spraying of DDT in or near any of the villages. All mothers except one (sample 38) had lived in different villages in the immediate area during their lives but only one (sample 31) had lived in Tabubil. Eleven of the samples have DDT higher than DDE, implying recent and/or direct exposure. There is no obvious explanation for these findings. Most of the mothers who donated their milk had lived majority of their lives in total isolation apart from the occasional visit or short stay in Tabubil. Their staple diet would be locally grown taro, ibeka, sweet potato, occassionally bird and, on special feastings, pig. Primitive agriculture of the "hunter gatherer" / "slash & burn" type is practised here. All would have, at some stage, visited Tabubil and eaten food from the old commissary during construction days of the township or more recently from the Tabubil tradestore, thus substituting their staple diet with rice, tinned beef and fish, all of which are imported from Australia and Japan. Vector control spaying of DDT was not performed in these villages because of their remoteness and until very recently, previously unknown existence. Vector control spraying of houses has been performed in the nearby less remote villages since 1983 and in Tabubil township from 1983 to 1987.

Almost all studies throughout the world have shown DDT to be present in the milk of 100% of lactating mothers. The literature however appears to be contradictory regarding correlation of age of the mother, number of pregnancies, type of diet and place of residence. The probable answer is that all the variables are responsible to a more or lesser degree.

Jenson (1983) highlights the potential hazards versus the benefits of breast feeding. Human breast milk has the correct constituents for human beings, is the right temperature and should be relatively free from pathogenic organisms. It also contains host resistant factors and

gives the infant an immunological protection. The emotional bonding between mother and child is also of considerable importance. Some of the hazards associated with breast feeding are the possible transfer of infectious organisms in the milk from infected or vaccinated mothers, the presence of some drug residues or contamination of the milk by chemicals such as the organochlorines. The existence of contaminants in breast milk is known but no resulting damage to breast fed infants has yet been proved. The benefits of breast feeding still outweigh the possible disadvantages (Doucette 1978). It must be remembered that breast feeding is all important in some developing countries such as Papua New Guinea where cost and prevention of gastroenteritis are important consideration. The levels found are small compared with other countries but nevertheless do show the ubiquitous nature of the organochlorines. It would be appropriate to continue monitoring breast milk samples for residual herbicides and pesticides to see if a downward trend is maintained.

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