1989

Dichlorodiphenyldichloroethene (DDE) in human milk from South African women

Audrey Zarba-Vary
Iowa State University

INFORMATION TO USERS

The most advanced technology has been used to photograph and reproduce this manuscript from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book. These are also available as one exposure on a standard 35mm slide or as a 17" x 23" black and white photographic print for an additional charge.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

U·M·I

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313/761-4700 800/521-0600

Order Number 8920202

Dichlorodiphenyldichloroethene (DDE) in human milk from South African women

Zarba-Vary, Audrey, Ph.D.

Iowa State University, 1989

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Dichlorodiphenyldichloroethene (DDE) in human milk from South African women

by

Audrey Zarba-Vary

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Food and Nutrition
Major: Toxicology
Nutrition

Approved:

Signature was redacted for privacy.
In Charge of Major Work

Signature was redacted for privacy.
In Charge of Major Work

Signature was redacted for privacy.
For the Major Department

Signature was redacted for privacy.
For the Major Department

Signature was redacted for privacy.
For the Graduate College

Iowa State University
Ames, Iowa

1989

TABLE OF CONTENTS

DEDICATION

Dedicated to my parents, Wanda Mary Abraham and William Philip Zarba; my sisters, Regina Ann Zarba and Mary Patricia Zarba; and my sons, William James Vary and Brian Edward Vary.

With gratitude for your love, nurturing, and acceptance and providing me with challenges and opportunities to grow physically, intellectually, emotionally, and spiritually.

GENERAL INTRODUCTION

Some 60,000 chemicals are available on the market throughout the world which will pass into the environment and can enter humans (WHO, 1978). Chemicals can enter the human body by several pathways: (1) through the intestine by ingestion of contaminated food, (2) through the lungs by inhalation of airborne chemicals in dust, vapor, and aerosols, and (3) by penetration through the skin (Loomis, 1978). Within the human body, the chemical is exposed to numerous pathways and metabolic processes. In the case of lipophilic organohalogenated pesticides such as DDT, heptachlor, dieldrin, and industrial contaminants, polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs), the parent compound and/or its metabolites are bioconcentrated and stored in the lipid portion of adipose tissue, milk, and liver (Pellizzari et al., 1982; Geyer et al., 1987; Slorach & Vaz, 1983).

Although the usage pattern of persistent pesticides has greatly changed in industrialized countries (Calabrese, 1982), these pesticides continue to be used in Third World countries because they are cheap, persistent, and effective. The toxicity of these environmental contaminants has been reviewed (Hayes, 1975; WHO, 1979). However, the long term effects of chronic low level exposure of these environmental contaminants is still unknown. Many countries have programs for the analysis of residues in crops (Pennington & Gunderson, 1987; Conacher, 1987; Smart, 1987) using one or more of three approaches based on food consumption data to estimate the daily

intake of contaminants: Total diet ("Market Basket" studies), selective studies on individual foodstuffs, and duplicate portion studies (GEMS, 1985).

Levels of environmental contaminants in blood or hair and urine are relatively low and in some cases below the level of detection with current methodology. Biological monitoring of these lipophilic contaminants often entails invasive technique using adipose tissue during surgery or autopsies. Biological monitoring may be undertaken to monitor the effectiveness of pesticide regulations, and to identify analytes of potential public health significance.

The collection of human milk is an alternative to the use of adipose tissue. In lactating women, milk is the major vehicle of excretion of lipophilic environment contaminants. There are several reasons for human milk studies: 1) as part of a series to survey prevalence of environmental contaminants in the general population, 2) to elucidate the mother's body burden of a specific or variety of environmental contaminants, 3) to estimate the body burden of the contaminant in a healthy growing infant. Only a limited amount of work has been undertaken in this last area. Laug et al. first reported the presence of pesticides in human milk in 1951. Interest in pesticide residues in human milk did not really develop until the 1960s (Calabrese, 1982). Since that time 1,1bis-(4-chlocophenyl)-2,2,2-trichloroethane (DDT) and other environmental contaminants have been found in human milk throughout the world (Jensen, 1983; Slorach & Vaz, 1983; WHO, 1985).

In the past 15 years, industrial contaminants such as PCBs, PBBs and more recently polychlorinated dibenzo-p-dioxins (PCDDs) have been detected in human milk in industrial countries. Levels of DDT and its metabolites are much higher in human milk in Third World countries when compared with industrial countries.

The mining industry in the Republic of South Africa enables the country to play an important role in world economy. Agriculture is also an important industry, since South Africa strives to grow food sufficient to meet demands of its growing population but also to export foods to other countries in southern Africa and to Europe. South African agriculture is at an intermediate stage of development (Van Dyk et al., 1982). It is not as intensive and highly mechanized as in industrial countries but, it is far from subsistence agriculture practiced in many other parts of Africa. The intermediate stage is reflected in its application of pesticides. A limited amount of work has been undertaken in South Africa and on the African continent to evaluate pesticide residues in human milk of various segments of the population (Jensen, 1983; WHO, 1985).

REVIEW OF LITERATURE

Republic of South Africa

Demographic information

Country Profile

Formal Name: Republic of South Africa

Common Name: South Africa

Three Capitols: Pretoria (Administration Capitol)

Cape Town (Legislative Capitol)

Bloemfontein (Judicial Capitol)

Geography Size: 1,229,260 square kilometers, southernmost country of continental Africa.

Topography Major features include a high interior plateau surrounded by a steep semi-circular escarpment and a narrow stretch of coastal lowlands in the east south and west. The terrain varies from temperate and semi-tropical farmlands, grassland plains, and deep valleys to rugged mountainous areas, semiarid scrubland, and a sparsely settled desert. No more than 47% of the land is arable and of this portion of the land only 10% is cultivated (Cubitt & Helfet, 1983).

Climate South Africa's weather and climate are determined by the subcontinent's position under the confluence of the three subtropical atmospheric high pressure cells. The stable subsiding air of these high pressure cells inhibit rain-forming processes resulting

in dry sunny days and clear cool nights. The coastal areas of the east have warmer and more humid conditions than the interior plateau, the western coastal marginal areas are arid and also cooler than equivalent latitudes on the east coast (Fuggle, 1983). There are eleven climatic regions in South Africa (Cubitt & Helfet, 1983). Sixty-five percent of the country has less than 500 mm precipitation annually which is usually regarded as a minimum for successful dry-land farming (Fuggle, 1983).

Boundaries The Atlantic Ocean makes up the western boundary and the Indian Ocean is found on the southern and eastern boundaries of South Africa. The following countries all form a part of the northern borders: Namibia, Botswana, Zimbabwe, Mozambique, and Swaziland.

Society

Population According to the 1980 census, the total South African population is 24,885,960, excluding the Republics of Transkei, Bophuthatswana, and Venda, and approximately twenty-eight million people if these independent states are included, growing at a rate of 2.2 percent per year. There are four officially recognized racial categories: Blacks, Whites, Coloreds, and Asians (mainly Indians). The composition of the population in 1980 was Blacks 68%, Whites 18.2%, Coloreds 10.5%, and Asians 3.3%. Fifty-three percent of the total population lives in urban areas: 90.6% of Asians live in urban areas, 88% of Whites, 76.6% of Coloreds, and 38% of Blacks live in

urban areas (Fuggle, 1983).

Ethnic groups Each of the four officially recognized racial categories which are Black, White, Colored, and Indian, are composed of several historically important ethnolinguistic groups varying in social and political significance. There are eight distinct Black groups: Xhosa; Zulu; Swazi; Tswana; Sepedi, Seshweshwe; Shangaan; Venda (Cubitt & Helfet, 1983). Over sixty percent of Whites are Afrikaners, and almost forty percent are English speakers. The Cape Colored are a major group among the Colored, while the Cape Malays are an important minority. Among the Asian or Indian population, there are important regional distinctions. The majority of Indians live in Natal within a 150 kilometer radius of Durban.

Languages English and Afrikaans are the official languages, both are spoken by most of the Whites. English is the primary language of a minority of Coloreds, the primary and secondary language of many Asians (Indians) a secondary language of educated urban Blacks. Afrikaans is the primary language of most Coloreds and secondary language of many Blacks who have worked for White Afrikaners. The following dialects are widely used and officially recognized for educational and broadcasting purposes: Xhosa, Zula, Swazi, North Sotho, South Sotho, Tswana, Tsonga, Venda. Hindi, Urdu, Tamil, and Telugu are the home languages of the Indian community (Kaplan & Nelson, 1981).

Religion Most Whites and Coloreds, and more than half of all Blacks are Protestant Christians. The Roman Catholic religion is an important minority among the three groups (de Gruchy, 1986). Indians are mostly Hindu, while about one-fifth are Muslim. Among the Afrikaners, the predominant religion is Nederduitse Gereformeerde Kerk (Dutch Reformed Church); while most of the English speaking Whites are Anglicans, Methodists, or Roman Catholics. Blacks practice the Methodist religion, Roman Catholicism and a variety of independent African denominations. About twenty-five percent of the Black population adhere to indigenous religious beliefs. More than one-quarter of the Coloreds are Dutch Reformed and the remainder, Anglicans or Roman Catholics. Cape Malays are Muslims (de Gruchy, 1986; Kaplan & Nelson, 1981).

Education Separate education exists for the four racial groups from primary through university level. Recently, racial integration for a few university students has started at the University of Witwatersrand. School attendance is compulsory for Whites and Coloreds from ages seven to sixteen; for Asians, between seven and fifteen; and for Blacks, between seven and fifteen.

Health Among low income groups, the morbidity and mortality rates of tuberculosis, gastroenterititis, venereal disease, kwashiorkor, pellagra, and other diet-associated illnesses are high (Heyns, 1986; FAO, 1982; Kaplan & Nelson, 1981). Esophageal cancer is endemic in the Black population (Wyndham, 1986). In 1982, the infant

mortality rate was reported to be 120 per 1000 live births, averaged over all racial groups (Grant, 1984). Infant mortality is higher among Blacks. Whites have a higher incidence of cardiovascular disease, stroke, and cancer, in general (Bradshaw & Harrington, 1985; Wyndham, 1985). Sophisticated medical treatment in government hospitals and many private hospitals is available to most urban populations. However, quality and availability decline in rural areas and among all low income groups everywhere (FAO, 1982; de Beer, 1984).

Economy Prominent features: there is a significant contrast between the industrialized economy of the White area (RSA) and the subsistence economies of the Black homelands (CIIR, 1986). The country has large diversified mineral resources, advanced stages of manufacturing development and great agricultural expansion capabilities; but the entrenched apartheid system and shortage of skilled labor keep the growth rate below its potential (Brewer, 1986).

Agriculture, livestock, forestry, and fisheries: about 90% of the total agricultural output is produced by 69,000 farms in White area, the remainder is produced by subsistence agriculture in Black homelands. The highly variable climate produces wide fluctuations in crop production, but use of modern technology results in adequate food supplies for the domestic market and surpluses for export in most years. In the Black homelands, agricultural produce does not meet minimum food needs for the population (Whitaker, 1981). The major crops grown include maize, sorghum, wheat, sugar cane, potatoes, citrus and deciduous fruits, cotton, tobacco, and ground nuts (FAO,

1985). Depending on the size of the harvest, all of these agricultural products are normally exported. More than 50% of the land area of South Africa is unsuitable for cultivation due to insufficient rainfall; however, these areas are favorable for livestock production. Wool (primarily Merino wool) from this production effort is an important export commodity. South Africa is the world's fourth largest wool producer. The area suited for natural forests is very limited in South Africa. Nevertheless, the country is self sufficient in timber output as a result of large scale production by private commercial and government forestry plantations.

The country has more than 5,000 kilometers of coastline along the Atlantic and Indian Oceans. The west coast of South Africa is well-known for its prolific marine life. The fishing industry is greatly aided by a 200 nautical mile fishing zone along South Africa's entire coastline. Cape hake and cape anchovy contribute 46% and 25%, respectively, of the total metric tons of fish caught (FAO, 1980).

Mining: nine and one-half percent of the economically active population are engaged in the mining industry which contributes 16.3% to the gross domestic product (GDP). It is the largest employer of Black males. The following minerals are of economic importance: gold, uranium, platinum, nickel, copper, coal, antimony, diamonds, vanadium, asbestos, iron ore, fluorspan, chromium, manganese, and limestone.

Manufacturing: in 1986, manufacturing contributed 21.5% to the GDP while employing 17% of the economically active population. There

are four major manufacturing areas, Pretoria-Witwatersrand-Vereenigning complex; Cape Town, Durban, and Port Elizabeth, which produce a large part of the requirement for consumer goods, intermediate products, and, increasingly, heavy machinery.

Foreign trade: South Africa is dependent on international trade. The major export items were listed above. The major importers of South African goods in 1985 include the United States, Japan, the United Kingdom, Netherlands, Switzerland, and the Federal Republic of Germany in decending order of amount imported.

Transportation Roads and road transport: there are approximately 650,000 kilometers of roads, 25% national, and the remaining, provincial and rural. Modern highway systems including some toll roads connect all principal cities. Railroads: The South African rail system is the most important mode of transportation in the 1980s. The system is state owned and operated. Approximately 23,000 route kilometers connect all principal cities, population and economic areas (Whitaker, 1981). Many lines are electrified. South African rail system also interconnects rail systems in Zaire, Zambia, Zimbabwe, and Mozambique.

Civil aviation: South African Airways (SAA), a part of the South Africa Railway and Harbor Administration (SARAH) provides major domestic, regional, and international service and is the largest national airline on the African continent. Since the mid-1980s, the number of foreign airlines serving South African has decreased due to international political pressure.

Ports and shipping: there are six major ports in South Africa incuding Durban, East London, Port Elizabeth, Cape Town, all built in colonial times, and Richards Bay and Saldanha Bay both built during the 1970s. All ports are operated by SARAH except for the stevedoring. There was nearly an 18% increase in total cargo handled between 1984 and 1985. Based on the total cargo handled, Durban was the largest port in Africa in 1986. Worldwide shipping services are provided by South African Marine Corporation Limited, which has a fleet of approximately 40 ships including container and cargo vessels; refrigerated vessels for exporting South African fresh fruit; bulk carriers for sugar, grain and ore, and super tankers.

Government and politics Government: South Africa has a parliamentary form of central government achieved May 31, 1910 when the Union of South Africa was created; it became a sovereign state of the British Empire in 1934; became a republic on May 31, 1961; left the Commonwealth of Nations in October, 1961. Under the 1961 Constitution, State President (Chief of State) and the Prime Minister provide the executive functions and head the Executive Council (cabinet). Parliament consists of three Houses: House of Assembly, 166 directly elected plus 12 indirectly placed seats, representing Whites; the House of Representatives, 80 directly elected plus 5 indirectly placed seats, representing the Coloreds; the House of Delegates 40 directly elected plus 5 indirectly placed, representing the Indians. Voting is restricted to only White, Colored, or Indian South African citizens who are eighteen years of age or older and

electing a member to their respective parliamentary House. Blacks within South Africa are represented by their homeland governments. There are ten of these local homeland governments representing major ethnic groups: Four of the Black homelands, Transkei, Bophuthatswana, Venda, and Ciskei were granted "independence" by South Africa in 1976, 1977, 1979 and 1980, respectively. Independence of these areas is not recognized by any country in the world except South Africa and other involved homelands. The other Black homelands are: Gazankulu (Shangaan-Tsonga); Na Ngwane (Swazi); Kwa Kdebele (Ndebele); Kwa Zulu (Zulu); Lebowa (Sepedi); Qwaqua (Seshoeshoe) (Tartter, 1981; MacGregor & Hutcheson, 1987).

Administrative: there are four provinces: Cape Province, Orange Free State, Natal, and Transvaal.

Judicial system: the Roman-Dutch law is the common law of the Republic of South Africa. The law of England is not recognized as authoritative, although English law principles have been introduced in relation to civil and criminal procedures, evidence and mercantile matters. However, the former law prevails in all other matters. The Supreme Court consists of an Appellate Division. The provinces are divided into districts and regions with Magistrates' Courts, whose criminal and civil jurisdiction is clearly defined, as well as Provisional and Local Divisions of the Supreme Court. Appeals originate from Magistrates' Courts, followed by Provisional and Local Divisions of Supreme Court, and finally to the Appellate Division (Thompon, 1982).

Politics: as of May, 1987, in the House of Assembly representing Whites, there were six parties, the National Party clearly being the most popular party with 133 seats of 178 seats available; followed by the Conservative Party with 23 of 178 seats, and the Progressive Federal Party with 20 of 178 seats. As of August, 1984, in the House of Representatives representing Coloreds, there were four parties, with the Labour Party representing the majority of voters, controlling 77 of the possible 85 seats. In the House of Delegates, representing Indians, as of August, 1984, there were three parties; both the Solidarity Party and the National People's Party represent over 80% of the Indian voters and occupy 17 and 18, respectively of 40 directly elected seats (Thompon, 1982).

Foreign relations: there are presently 103 embassies, legations, consulates, and special missions of the Republic of South Africa abroad, but only 24 countries and the Vatican currently maintain diplomatic facilities in South Africa. South Africa is a member of the United Nations and some of its specialized agencies, but the relations are strained.

Pesticide Usage in South Africa

Legislation: central legislative control of pesticides was mandated by the Fertilizers, Farm Foods, Seeds, and Pest Remedies Act 20 of 1907 soon after the Union of South Africa was formed. The act was later repealed and substituted by an Act of 1917 aimed at protecting the farmer from inferior farming remedies. This Act was amended in 1947 and again substantially, in 1977. The current Fertilizers, Farm

Feeds, Agricultural Remedies and Stock Remedies Act protects agriculture and the consumer by requiring that only approved pesticides are used on agricultural products.

Van Dyk et al. (1982) recently reviewed the regulatory aspects of pesticide management in South Africa, while the effects of pesticide usage on marine pollution were recently discussed by Brown (1987). There are several reasons why persistent organochlorine pesticides were applied in lesser quantities in South Africa than other western industrialized countries (1) uncertain climatic conditions in most of the country (Cubitt & Helfet, 1983), (2) greater availability of cheap labor for weed control, (3) strict enforcement of pesticide regulation originating in 1947. In addition, Van Dyk and Van Der Linde (1976) and Wiese and Basson (1966) report that soil and climatic conditions enhance degradation of persistent organochlorinated pesticides.

Until 1960, the Republic of South Africa had no systematic pesticide residue monitoring for any edible commodities. When West Germany, The Netherlands, and the Scandinavian countries lowered the maximum pesticide residue limit permitted on fruits and vegetables, including imports, South Africa began to monitor fruit for export.

Pesticide residues on food stuffs are regulated by three laws:

1. The Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act mandates the process of pesticide registration, the marketing, and manner of application of pesticides (Wiese & Bot, 1971).
2. The Foodstuffs, Cosmetics Disinfectant Act mandates the

maximum pesticide residue limits on all edible commodities for local consumption.

3. The Agricultural Produce Export Act mandates the maximum residue limits of pesticides on certain export fruit.

The last two laws are not identical. South Africa requires all pesticides including those which are internationally recognized to undergo field tests under South African climatic and soil conditions performed by the South African Bureau of Standards (Anon., 1980). All legislation pertaining to pesticides in South Africa is administered at the national level. The Department of Agriculture enforces the Agricultural Products Export Act and the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act while the Department of Health and Welfare enforces the Foodstuffs, Cosmetics, and Disinfectants Act (Rabie, 1983).

Monitoring

Although South Africa pesticide regulatory legislation is sophisticated (Van Dyk et al., 1982), enforcement of the legislation is difficult if there is insufficient monitoring of some of the following aspects: pesticide residue limits on local commodities; evaluation of effectiveness of educational programs to prevent accidental pesticide poisoning in various segments of South Africa population; the requirement of toxic substances to be administered by trained operators but an absence of formal higher education program in pesticide application; planned training programs to meet a critical shortage of residue chemists and technicians.

There seems to be conflicting information in the literature pertaining to monitoring pesticide residues in foods in South Africa. Fourie (1986) reports that pesticide residue determinations were being conducted on a regular basis by the Department of Health officials from local authorities, municipalities, and State departments. The samples obtained are for evaluation of the tolerance or maximum residue limit set for that commodity and are not representative of that food supply. Lück (1983) reported that there is an urgent need to investigate pesticide residues in milk and dairy products in South Africa.

Pesticide residues in South African foods

Since 1972, citrus fruit analyses have shown a significant steady decline in pesticide residues exceeding the maximum residue limit. Pesticide residues in deciduous fruit have followed a sporatic pattern. A level of 2-4% of fruit sampled during 1974-76 exceeded the maximum residue limit permitted. This was followed by a decline and then a sharp increase in the percent of fruit sampled which exceeded the maximum residue limits. The pesticides observed included parathion, malathion, methidathion, and dimethoate (Van Dyk et al., 1982). Organochlorinated pesticide residues continue to be found in many products (Van Dyk et al., 1982) (Table 1).

Fourie (1986) conducted the first South African total diet study for the purpose of estimating dietary intakes of contaminants in the White population and selective studies of individual foods for the purpose of estimating dietary intakes of contaminants in the Colored

Table 1. Occurrence of pesticide residues in food products in South Africa

Last year sampled	Product	Pesticide Residue
1979	Fresh milk	Dieldrin, Total DDT
1979	Dairy Products (milk powder, condensed milk, butter)	Dieldrin, Camphechlor
1979	Meat and Poultry (beef, chicken, mutton, processed meats)	Total BHC, Total DDT, Dieldrin, Endosulfan
1978	Separated Fish (cooking oil, soft and hard margerine)	Total BHC, Total DDT, Endosulfan
1978	Bread (white and whole wheat)	Total BHC, Total DDT, Dieldrin, Endosulfan
1979	Vegetables only carrots and not lettuce, cabbage, cauliflower, but not tomatoes or turnips	Total DDT
1979	Subtropical fruit only papaya and not melons, avocados, pineapples, guava	Dichlorvos
1980	Same as above	Dithiocarbamate
1978	Fish, marine and fresh water	Dieldrin, Total DDT Total BHC, Endosulfan

and Black segments of the South African population.

Four residues were found in 8 of the 11 composite food groups. No residues were found in soups, sugars, and beverage food groups. DDT, dieldrin and dichloran were present in seven groups, whereas the organophosphate, mercaptothion, was present in the cereal food group. It is interesting to note that dichloran was found in the vegetable food group, since it is permitted only for use on peaches (Van Dyk et al., 1982) and, that mercaptothion residues were present at a concentration of 0.05 mg/kg in ready-to-eat cereals (Fourie, 1986). Insofar as Whites do not consume nearly the amount of cereals and grains that the Black population do (Wiese, 1964), it may be that certain segments of the population are at a risk of chronic exposure due to the presence of agrochemical contaminants in their food.

Residue levels in human adipose and milk samples among South Africans

Residues of pesticides and their metabolites found in various tissue fluids collected from the general population indicate the total body burden of these pesticides and the individual's past and present exposure to them (Murphy et al., 1983). In the mid-sixties, Wasserman began to study the storage of DDT in general population groups in various parts of the world. In 1966, he published a world map of the distribution of levels of organochlorinated pesticides stored in humans (Wasserman et al., 1975). It is important to determine on a national scale the incidence and level of exposure to pesticides experienced by the general population and to identify trends in these factors when they occur.

There have been no extensive studies of pesticide residue levels in human adipose or human milk in South Africa (Van Dyk et al., 1987). In 1973, the Working Group on Pesticide Residues (WGPR) requested the Department of Health to analyze approximately 300 samples of human adipose samples per year. They used the following criteria:

--samples should be equally divided among the White, Colored, Indian and Black population group;

--samples should be equally divided between males and females;

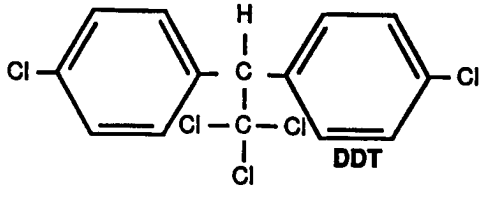
--sample age group should be 25-60 years.

Wasserman et al. (1970) reported that the mean total DDT level in adipose tissue in the South Africa general population (n = 114) was 6.38 ppm; the mean total DDT levels for the Black population (n = 73) was 5.94 ppm and for Whites (n = 41) it was 7.18 ppm. However, there was no information pertaining to the Indian or Colored population. Wiese (1976) reported concentrations of organochlorine pesticides in adipose tissues in the general population as in 1968/69, total DDT = 6.4 ppm, total BHC = 2.4 - 10.6 ppm; Dieldrin 0.04 ppm; 1971/73 total DDT = 5.9 ppm; total BHC = 3.5 ppm; Dieldrin, not detectable; in 1973/74, total DDT = 5.3 ppm; total BHC = 2.0 ppm; Dieldrin, not detectable. Van Dyk et al. (1982) reported levels for 1974/75, as total DDT 7.9 ppm; total BHC, 6.6 ppm; Dieldrin, not detectable. Van Dyk et al. (1987) described the results of an ad hoc project of monitoring adipose tissue and human milk. He reported medium values of human adipose in 1982 in three urban areas as, total DDT 2.04 ppm to 3.78 ppm; Dieldrin 0.03 ppm to 0.1 ppm; and total BHC, 0.012 ppm to 0.057 ppm. There was

no significant decrease in total DDT residues whereas there was for Dieldrin and total BHC. Sixty-two samples of human milk were collected and analyzed. Pesticide residues were found in 59 of these. Forty-seven samples contained p,p'-DDE ranging from 0.1 to 3.6 mg/kg fat and 22 contained p,p'-DDT ranging from 0.1 to 1.0 mg/kg fat. Unfortunately, there is little description of the sampling technique of the human milk nor a description of the sample population.

DDT

DDT is the acronym for dichlorodiphenyltrichloroethane which is the prototype of broad spectrum of action persistent insecticides (WHO, 1979). Table 2 lists compounds which occur in commercial DDT and the analogs that have some use as insecticides.



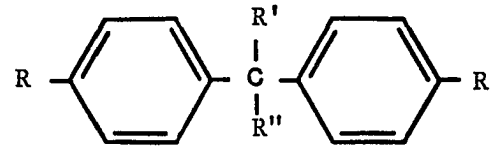
$C_{14}H_9Cl_5$ mol. wt. 354.5

1,1 bis-(4-chlorophenyl)-2,2,2-trichloroethane

DDT is a mixture of approximately 3 parts of 1,1 bis-(4-chlorophenyl)-2,2,2-trichloroethane(p,p'-DDT) to 1 part 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloro ethane (o,p'-DDT).

The p,p' isomer forms colorless crystals with melting point 108.5°C and the vapor pressure is 1.9×10^{-7} mm Hg at 20°C (Windholz, 1983). This isomer is practically insoluble in water, moderately soluble in hydroxylic acids, polar solvents, and petroleum oils. It

Table 2. Structure of p,p'-DDT and its analogues of the form:



(many of the compounds also exist as o,p'-isomers and other isomers)

Name DDT and its major metabolites	Chemical name	R	R'	R"
DDT	1,1'-(2,2,2-trichloro-ethylidene)bis[4-chloro-benzene]	-Cl	-H	$-CCl_3$
DDE[1]	1,1'-(2,2,dichloro-ethenylidene)bis[4-chloro-benzene]	-Cl	None	$=CCl_2$
TDE(DDD)[a,b]	1,1'-(2,2,dichloro-ethylidene)bis[4-chloro-benzene]	-Cl	-H	$-CHCl_2$
DDMU[a]	1,1'-(2-chloroethenyl-idene)bis[4-chlorobenzene]	-Cl	None	=CHCL
DDMS[a]	1,1'-(2-chloroethylidene)-bis[4-chlorobenzene]	-Cl	-H	$-CH_2Cl$
DDNU[a]	1,1'-bis(4-chlorophenyl)-ethylene	-Cl	None	$=CH_2$
DDOH[a]	2,2-bis(4-chlorophenyl)-ethanol	-Cl	-H	$-CH_2OH$
DDA[a]	2,2-bis(4-chlorophenyl)-acetic acid	-Cl	-H	-C(O)OH

[a]Recognized metabolite of DDT in the rat.

[b]As an insecticide, this compound has the International Organization for Standardization (ISO) approved name of TDE, and it has been sold under the name Rothane®; in metabolic studies the same compound has been referred to as DDD; as a drug, it is called mitotane.

Table 2. (continued)

Name DDT and its major metabolites	Chemical name	R	R'	R''
Some related insecticides				
Bulan®	2-nitro-1,1-bis-(4-chlorophenyl)butane	-Cl	-H	$-\overset{NO_2}{\overset{\vert}{C}}HC_2H_5$
Prolan®	2-nitro-1,1-bis (4-chlorophenylpropane)	-Cl	-H	$-\overset{NO_2}{\overset{\vert}{C}}HCH_2$
DMC	4-chloro-a-(4-chloro-phenyl)-a-(methyl)benzene-methanol	-Cl	-OH	$-CH_3$
dicocol (Kelthane®)	4-chloro-a-(4-chloro-phenyl)-a-(trichloro-methyl)benzenemethanol	-Cl	-OH	$-CCl_3$
chlorobenz-ilate[c]	ethyl 4-chloro-a-(4-chlorophenyl)-a-hydro-xybenzeneacetate	-Cl	-OH	$-C(O)OC_2H_5$
chloropropo-pylate[c]	1-methylethyl 4-chloro-a (4-chlorophenyl)-a-hydroxy-benzeneacetate	-CL	-OH	$-C(O)OCH(CH_3)_2$
methoxychlor[c]	1,1'(2,2,2-trichloro-ethylidene)-bis[4-metho-xybenzene]	$-OCH_3$	-H	$-CCl_3$
Perthane®	1,1'-(2,2-dichloroethy-lidene)-bis[4-ethylbenzene]	$-C_2H_5$	-H	$-CHCl_2$
DFDT	1,1'-(2,2,2-trichloroethy-lidene)-bis[4-fluoro-benzene]	-F	-H	$-CCl_3$

[c]Common name approved by the ISO.

is relatively soluble in most aromatic and chlorinated solvents. DDT is dechlorinated by alkalis or organic bases in solution. Otherwise, it is stable in the presence of acid and alkaline permanganate and aqueous acids and alkalis. DDT is resistant to complete enzymatic degradation by soil microorganisms and higher organisms (WHO, 1979).

DDT is a potent nonsystemic stomach and contact insecticide known for its extreme persistence on solid surfaces (Mercier, 1981). Recently, Coats (1982) reviewed steric and electronic parameters pertaining to toxicity, degradation, and other factors of DDT and its analogs. DDT was first synthesized in 1877 by Zeidler, but it wasn't until the late 1930s that Paul Müller discovered its insecticidal properties. It was used extensively during World War II to prevent vector borne disease and later in the United States and elsewhere it was used for agricultural purposes (Hayes, 1982). However, in 1970, Western countries began to resist the use of DDT due to its persistence in the environment and possible threat to human health. DDT has been responsible for some large-scale kills of birds, fish, and other non-target species, and other effects (e.g., egg shell thinning) have been produced in species near the top of the food chain (Hutson, 1981). DDT is still used exclusively for agriculture and vector control in some countries. It continues to be the choice insecticide for malarial control. Substituting malathion, an organophosphate insecticide, or propoxur, a phenyl carbamate insecticide, for DDT would increase the cost of malarial control 3.5-8.5 times, respectively (WHO, 1979). If DDT were not used, then there

would be a significant loss of manpower due to endemic and epidemic malaria.

Hayes and coworkers studied the fate of DDT ingested by human volunteers (Hayes et al. 1956, 1961, 1971). Several groups of male human volunteers were fed DDT in corn oil solution at doses representing about 1.20, and 200 times the ordinary dietary intake of DDT. The first study lasted 18 months and the other two studies, 21.5 months. Individuals were followed for as long as 5 years. During these studies no subject complained of any symptoms nor did any of their clinical tests demonstrate any sign of illness that could clearly be caused by exposure to DDT.

The conclusions reached from these studies were:

(1) Intakes of DDT 1250 times those normally present in the food were not demonstrably harmful even after 21.5 months of ingestion.

(2) DDT plus DDE was concentrated in adipose tissue and reached a plateau when the intake was equal to the amount metabolized and/or excreted.

(3) Stored DDT is progressively metabolized to DDE which is stored with residual DDT.

In a more recent study by Morgan and Roan (1971) the metabolic pathway proposed by Peterson and Robison (1964) was used as the background. Like most species, Homo sapiens converts DDT to 1,1,bis-(p-chlorophenyl)-2,2 dichloroethylene (DDE) by dehydrochlorination. DDE is stored even more tenaciously than the parent compound in

adipose tissue. DDT also undergoes a reductive dechlorination to form 2,2 bis-(p-chlorophenyl)-1-dichloroethane (TDE or DDD), an intermediate in the formation of the main excretory product 2,2-bis-(p-chlorophenyl)-acetic acid (DDA) (Roan et al., 1971). See Figure 1. Technical DDT is more readily excreted and less readily stored than p,p' DDT because it contains 15-20% o,p'-DDT.

Human Milk Studies

Rationale

Studies have attempted to evaluate the relationship between the levels of certain organochlorine contaminants in human milk fat and their corresponding adipose tissue levels (Acker & Schulte, 1970; Yakushiji, et al., 1978); between certain organochlorine contaminant levels in human milk fat and whole blood (Yakushiji et al., 1977; Mes et al., 1984; Masuda et al., 1978) or plasma (Polishuk et al., 1977a; Rogan et al., 1986; Krauthacker et al., 1980; Curley & Kimbrough, 1969; Dyment et al., 1971; Siddiqui et al., 1981). The relationship between certain organochlorine pesticide residue levels in human adipose tissue and blood (Polishuk et al., 1977b) or serum (Wyllie et al., 1972) aldo have been investigated. An equilibrium between some of the organochlorine pesticide residues in blood and other tissues was demonstrated by Radomski et al. (1971) and Brown (1972). Levels in human milk fat are generally better correlated to the levels in blood or blood serum lipids than to those in adipose tissues. Acker and Schulte (1970) found almost the same mean concentration of certain

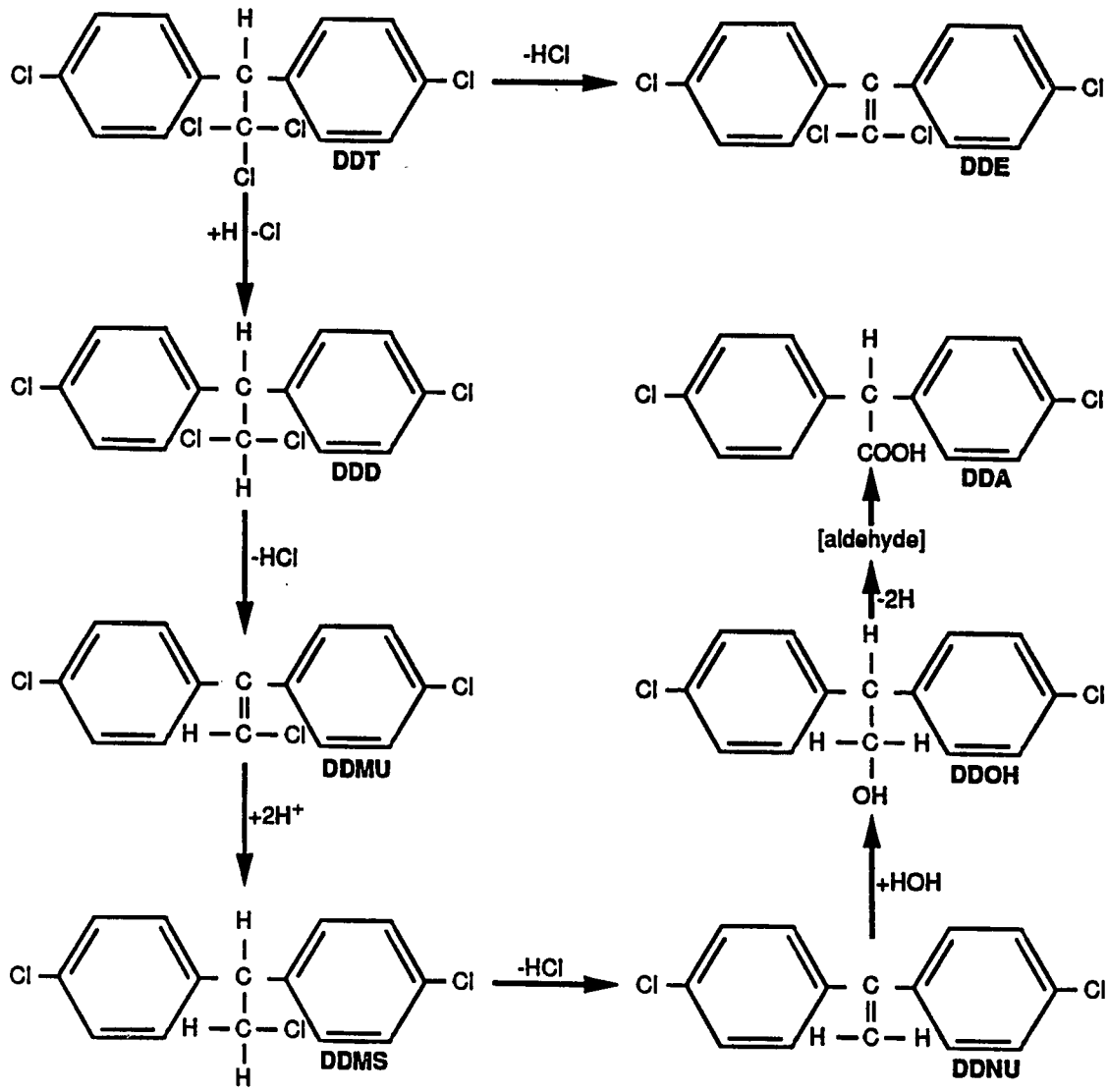


Figure 1. Biotransformation of DDT in mammals (Peterson & Robison, 1964)

organochlorine contaminants (including DDT, DDE, B-HCH and PCBs) in adipose tissue and human milk fat, while PCBs were approximately 50% higher in adipose tissue than human milk fat. Despite the many studies to evaluate the relationship between organochlorine contaminant levels in human milk fat and various tissues (adipose, blood, and plasma or serum), there is no clearly defined relationship. It is difficult to compare results from one study to another because several factors may influence levels of organochlorine contaminants in human milk. The organochlorine contaminants are found in the fat of mother's milk, and the fat content of human milk varies widely. Studies by Lucas et al. (1980) and Hall (1979), respectively, observed a two-fold and three-fold increase in the percent lipid during a feeding and confirmed the earlier findings of Hytten (1954a), Macy et al. (1931), and Gunther and Stainer (1949).

Methods of collection of human milk

Human milk may be collected in several ways: manual expression, expressed milk using a hand operated or an electric breast pump; or drip collected, that is milk which drips from one breast into a nipple shield while the infant is suckling at the other breast, Figure 2. Drip milk contains significantly less fat than milk collected either by hand or by using a pump (Davis and Carroll, 1982; Gibbs et al., 1977). Other investigators studied the lipid composition of milk by comparing manually expressed milk with milk expressed using various breast pumps on the market. Whereas Green et al. (1982) found the Egnell electric breast pump allowed the donor to express a greater volume of milk

than by using hand expression or than by using other hand operated pumps, there was no significant difference in the percent lipid between either method of collection. However, their method of lipid analysis may not have been sensitive enough to determine small differences due to the method of collection. Garza et al. (1982) also found the volume significantly greater using the Egnell electic breast pump as compared with manual expression. The percent lipid was 25% greater using the Egnell pump, but this was not statistically significant.

Factors affecting human milk lipid content

The content of lipids in human milk varies with the method of collection, within a feeding, diurnally, in relation to the donor's diet, over the lactation period, and by the type of weaning. In human lactation studies, investigators found the fat content of fore milk, the milk initially secreted during the first 2 minutes, to be significantly lower in fat than hind milk, the milk secreted at the end of nursing (Hytten, 1954a; Picciano, 1978; Neville et al., 1984). Neville et al. (1984) observed that a single sample taken 2 minutes after let down may not give an adequate estimate of fat content when the value for an individual is of importance; however, it would be an acceptable method for use during population studies. "Let down" refers to the contraction of the mammary myoepithelium and the release, or let down, of the milk.

Hall (1979) observed diurnal variations in the amount of total

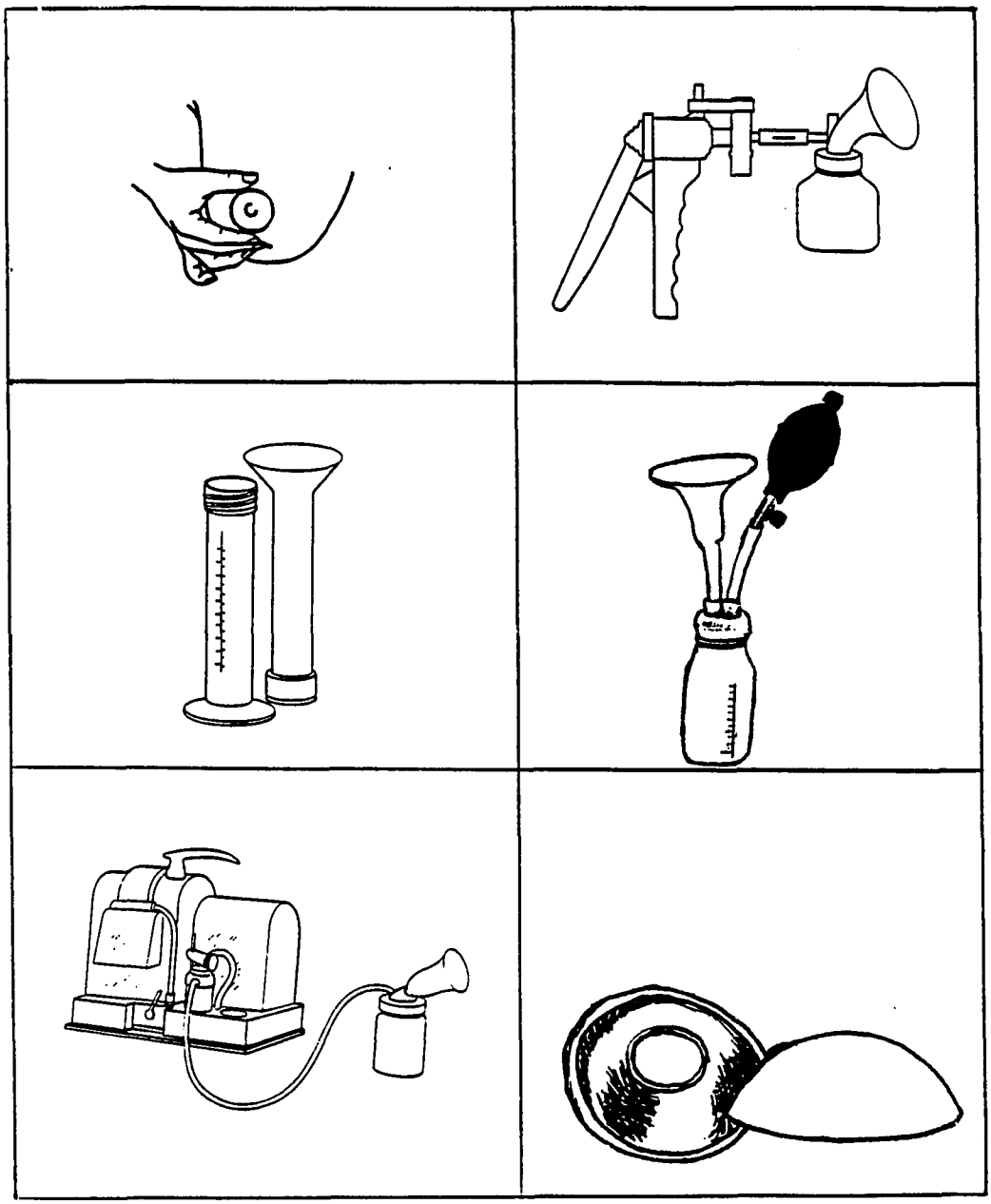


Figure 2. Methods of collection of human milk adapted from Lawrence (1985), pp. 465-468.

lipid as did Hytten (1954b), and Gunther and Stainer (1949). Total lipid increases from early morning, reaches a peak about midmorning, and then declines gradually throughout the day.

The fat content of human milk changes during lactation. The changes are most pronounced during early lactation and during weaning. The milk fat content is lowest in colostrum and increases to a higher level during the first few weeks postpartum. Thereafter, the fat content remains fairly constant during the lactation period (Hamosh et al., 1985). This antecedant appears to be true for women who deliver very premature infants (26 to 30 weeks gestation), premature infants (32 to 36 weeks), and full term infants.

Garza et al. (1983) observed the concentration of fat to increase during gradual weaning until the tenth week of weaning; the sample collected during the twelth week of weaning had a value of fat concentration similar to the period immediately preceding the weaning period. Hartmann and Kluski (1978) observed no significant change in the total lipid during the first eight days after abrupt termination of breast-feeding; there was a 2.5 fold increase in total lipid during the remaining 6 weeks during involution of the mammary gland.

It is difficult to evaluate the extensive data available pertaining to organochlorine pesticides levels in human milk and factors which affect these levels due to differences in methodologies. Among the difficulties are the lack of well-defined sampling methodology, the analytical determination of environmental contaminants, and a consistent manner for reporting of the results.

Sampling effects on organochlorine pesticides residue levels in human milk

Few studies have dealt with the sampling techniques and what constitutes a representative sample for determining organochlorine pesticide levels in human milk. Newton and Greene (1972) determined levels of pesticide residues in milk from one donor who collected samples of fore-milk, mid-milk, and hind milk during three different feedings in one day. These individual samples were composited by group to provide three samples for analyses. On a fat basis, the levels of total DDT and hexachlorobenzene were twice as high at the end of a feeding than they were in the middle. The results were even greater when calculated on a whole milk basis. Wilson et al. (1973) reported a significant difference between pesticide residue levels in 24 matched fore milk and hind milk samples (that is, obtained from the same donor at the same feeding). The total DDT was significantly higher in the hind milk ($p < .01$). Barnett et al. (1979) analyzed nine matched fore milk and hind milk samples. They reported the difference in total DDT and other pesticides of hind milk residues minus fore-milk residues on a fat basis were not significant, but the average difference in total DDT and other pesticides on a whole milk basis was significant ($p < 0.05$). Mes and Davies (1978) studied levels of organochlorine pesticide residues in human milk by collecting 20 ml and 10 ml fractions from 2 donors at three different times during one day. Milk was expressed from the right breast and the donors were asked not to feed the infant from this breast between

morning and evening feeding. This may also explain why the average percent fat was 2.40 and 1.62, respectively, since Hall (1979) reported a lower level of total fat occurs when there is a long lapse of time between feedings. Mes and Davies (1978) did not find a strict linear relationship between the amount of fat and the levels of organochlorine pesticide residues, although levels of contaminants increased with the increasing fat content of the milk. Stacey et al. (1985) reported similar results. Based on these findings, Mes (1981) recommended obtaining a random sample by collecting milk at different times during a particular feeding, at all feedings during the day, and alternating between the right and the left breast.

Norén (1983c) studied six women at different times during their lactation to determine the correlation between organochlorine pesticide residue levels and the fat content of their milk. Two donors supplied 50 ml fractions from one feeding. Another donor in early lactation collected samples during each feeding for a 24 h period, and, thereafter, one sample was collected weekly for four weeks. Three other donors collected one milk sample almost every week for a period of 4-6 months. Norén observed that the amount of contaminants excreted by the donors was constant when considering analytical error, when calculated on a fat basis regardless of difference in the volume of milk produced and expressed, and the amount of fat in the milk. He also observed the levels of organochlorine pesticide residues remained constant during the day when calculated on a fat basis, and the slight fluctuations observed

over a one month time period could be attributed to analytical error.

Methods for Human Milk Lipid Analysis

To study total lipids in human milk or to study lipophilic substances in human milk, it is necessary to use the best method(s) to isolate human milk fat. At least six extraction methods have been used to study lipids in human milk (Jensen et al., 1980; Jensen and Clark, 1984). Direct methods for isolation and determination of human milk fat include: creamatocrit (Lucas et al., 1978); an enzymatic technique utilized by Hibberd et al. (1982); a colorimetric method (Lönnerdal et al., 1984; Frings & Dunn, 1970); a turbidimetric method (Nakai & Le 1970). Lipids are also isolated by solvent extraction, and measured gravimetrically or densitometrically. The Roese-Gottlieb method involves treatment of milk with ammonium hydroxide and extraction with ethanol, diethyl ether and hexane. The method requires 1-10 ml of milk (Horowitz, 1975). The Mojonnier method is a modification of the previous method utilizing a special flask to hasten separation of aqueous and solvent phases (Atherton & Newlander, 1977). A modified Folch method extracts lipids with methylene chloride and methanol in a two to one volume ratio (Clark et al., 1982). This method can extract 0.1-10 ml of milk or larger volumes. Erickson and Dunkley (1964) in a modified Monjonnier method acidified milk by heating with hydrochloric acid and absolute ethanol. The volume of milk was 500 1. Dry column extraction involves mixing sodium sulfate and celite and extracting the lipids with methylene chloride and methanol using a 9 to 1 volume ratio. This method uses

1-10 ml of milk (Maxwell et al., 1980). Anderson et al. (1981) estimated the quantity of fat in milk with the procedure by Van der Kamer (1949) which measures the amount of fat in feces by saponification, acidification, extraction, and titration of the resulting free fatty acids. This method is very similar to the method used by Hudson et al. (1979), but is not recommended due to so many inherent errors (Jensen et al., 1985). Recently, Hundrieser et al. (1984) compared four methods for total lipid analysis of human milk: modified Folch, dry column extraction, a spectrophotometric micro method and creamatocrit method, to the AOAC standard method for bovine milk, Roese-Gottlieb. All four methods correlated highly with the Roese-Gottlieb method but the modified Folch was recommended for detailed total lipid analysis in human milk.

Methods for pesticide residue analysis in human milk

No study has compared various methods for organohalogen pesticide (OCP) analysis despite the numerous human milk studies conducted. A standard acceptable method of analysis as well as standardized collection technique for a representative sample of human milk for OCP analysis would allow investigators to evaluate factors contributing to the storage and excretion of these substances in the mother and determination of exposure of environmental contaminants to the infant.

A number of methods pertaining specifically to analysis of OCPs in human milk have been published: EPA (1980); Yakushiji et al. (1978); Brevik (1978); Tessari and Savage (1980); Bush et al. (1983); and

McKinney et al. (1984). With the exception of Bush who used a lengthy Soxhlet extraction, all the other methods are modifications of Mills (1961) and de Faubert Maunder et al. (1964). Mills added ethanol and potassium oxalate to the milk and the fat was extracted repetitively with diethyl ether-petroleum ether (1:1 v/v). Pesticides were extracted from the fat with acetonitrile and then partitioned back into petroleum ether by an aqueous dilution of the acetonitrile extraction. The petroleum ether extract is concentrated by evaporation and further purified by Florisil fractionation. De Faubert Maunder et al. (1964) isolated the fat from milk by a combination of physical separation (maceration and centrifugation) and solvent extraction (acetone-hexane 1:1, v/v). The pesticides were extracted repeatedly from the fat with dimethylformamide (DMF) saturated with hexane and then partitioned back into hexane after the DMF extracts were washed with 2% aqueous sodium sulphate solution. The hexane extract is concentrated by evaporation and further cleaned using activated alumina chromatography. The final extracts are identified and quantitated by electron-capture chromatography.

Brevik's method utilizes concentrated sulphuric acid and 10% sodium hydroxide in methanol instead of liquid-liquid partitioning and column chromatography for cleanup after a solvent extraction of the fat from milk. Brevik's method improves the recovery rate of hexachlorobenzene, but the acid destroys endrin if it is present (Langlois et al., 1963). Concentrated sodium hydroxide alters DDT and DDD to DDE (Lampbert, 1964).

Norén (1983b) compared the extraction efficiency of the methods of Mills (1963) and de Faubert Maunder and found the concentrations of organohalogen pesticides and PCBs were in similar close agreement, for each milk sample, but the percentage of fat was slightly higher by de Faubert Mauder's method. Krauthacker et al. (1980) compared two micro methods of extraction of p,p'-DDE, EPA (1980) and Curly and Kimbrough (1969), and found the latter method gave a slightly higher geometric mean 60.6 ug/l vs 52.6 ug/l and fewer signals due to impurities. Mes (1981) compared various aspects in analysis for OCPs, including 5 extraction methods, sample size (25 g vs. 2 g), clean-up procedures, and separation. Yakushiji et al. (1978) reported about 10% of fat was lost in the aqueous layer in the step involving washing the n-hexane-diethyl ether extracts with 2% sodium chloride solution in the Mills method. They recommended centrifuging the aqueous layer and combining the upper layer to the hexane-diethyl ether extracts resulting in consistent recovery rate as of 95% for human milk fat. Kuwabara et al. (1982) reported that when dry column chromatography was compared to acetonitrile partitioning in the modified Mills method regarding the recovery of pesticides in human milk, recovery rates were greater than 80% for hexachlorocyclohexane isomers, p,p'--DDT and metabolites, and dieldren in either method. However, the recovery rate of hexachlorobenzene increased from 66% to 83% using dry column chromatography.

The method reported by Tessari and Savage (1980) was used in the largest human milk epidemiological study (1976) to date. This method

used a triple extraction of a 7 g sample of milk and reported percent recovery for DDE to be 89.9% with a detection limit for DDE to be 2.5 ppb. McKinney et al. (1984) recently examined extraction and purification methods for another large human milk epidemiological study (Rogan et al., 1986). They reported detection limits for DDE using a 10 g sample to be 2 ppb.

Factors affecting levels of OCPs and DDE in particular in human milk

Because fat is the most variable nutrient in human milk, one can expect to find fluctuations and variations in lipophilic substances including levels of organochlorinated contaminants. This is particularly true when there is no standardized sampling or analytical methodology to evaluate various factors which may contribute to these levels. Nevertheless, many investigators have tried to relate the DDT level found in human milk to factors outlined here.

1. Parity—Since exposure to DDT and metabolites begins *in utero* through transplacental passage (Curley & Kimbrough, 1969; O'Leary et al., 1970), it seems reasonable to assume multiparous women would have lower levels of DDT and its metabolites. Many investigators have found higher levels of DDE in human milk of mothers who are breastfeeding for the first time (Kroger, 1972; Bradt & Herrenkohl, 1976; Matuo et al., 1980; Noren, 1983a, 1983b; Rogan et al., 1986). Some investigators have found no correlation between levels of total DDT and number of children (Dillon et al., 1981;

Takahaski et al., 1981; Vuori et al., 1977; Wickström et al., 1983; Weisenberg et al., 1985; Weisenberg et al., 1980). Barril et al. (1977), however, reported a significantly negative correlation between total DDT levels in human milk and number of children.

2. Maternal Age—Polishuk et al. (1977a), Wilson et al., (1973), and Al-Omar et al. (1985) all reported the levels of total DDT in milk were higher in younger women than older women. Yet, more investigators observed a trend of higher levels of total DDT in human milk as age of the mother increased (Knoll & Jayaraman, 1973a; Matuo et al., 1980; Schüpbach and Egli, 1979; Rogan et al., 1986); while Dillon et al. (1981) reported varying results in different hospitals in Quebec. Norén (1983b) observed a positive correlation of levels of p,p'-DDT and p,p'-DDE in human milk fat to the age of mother, but only for donors nursing their first infant. Still others reported observing no correlation between the age of the donor and levels of total DDT and/or DDE (Takahashi et al., 1981; Vuori et al. 1977; Wickström et al. 1983; Weisenberg et al., 1980; Barril et al. 1977; Krauthacker et al., 1980; Currie et al., 1970).
3. Cigarette smoking—When agricultural usage of DDT reached its peak in the late sixties and early seventies, DDT was applied to tobacco fields (Mussalo-Rauhamaa et al., 1986). Consequently, it is not surprising to find reports in the

contemporary literature of that period that women who smoked had higher levels of DDT and metabolities in their milk than those who didn't (Bradt & Herrenkohl, 1976; Miller & Fox, 1973; Savage, 1976; Vuori et al., 1977). However, more recently Dillon et al. (1981) observed a significant positive correlation between cigarette smoking and the concentration of DDE in human milk, while Rogan et al. (1986) reported smokers had 15% higher levels of DDE in their milk compared to non-smokers. Collins et al. (1982) observed no difference between the mean concentration of p,p'-DDE in human milk fat of cigarette smokers and non-smokers. A limited amount of data collected prevented Hofvander et al. (1981) from discussing any clear relationship between cigarette smoking habits and the levels of organochlorine contaminants, including DDE, in human milk.

4. Season--Wilson et al. (1973) observed a seasonal dependence of total DDT concentration in human milk with DDT concentrations ranging up to 60% higher in late summer than in late winter. Using pooled milk samples from various milk banks Westöö and Norén (1978) were unable to detect any relationship between total DDT and seasons over an 18-month period. Luguet et al. (1972) also were unable to detect any seasonal change in pooled milk samples from milk banks in France.

5. Occupation--Very high levels of p,p'-DDT and p,p'-DDE have

been found in human milk in areas where DDT had been applied to various crops, e.g., tobacco (Miller & Fox, 1973), cash crops (Kanja et al., 1986) and cotton (de Campos & Olszyna-Marzys, 1979) resulting in occupational exposure. Graca et al. (1974) reported levels of total DDT in a woman who cleaned pesticide glassware manually in the laboratory for six years. After 73 days, her milk contained 1.38 ppb total DDT. Takahashi et al. (1981) found no significant difference in any organochlorine pesticide residue levels in milk samples of women whose husbands were exposed regularly to pesticides and women whose husbands were not engaged in agricultural activities. Occupation was found to affect levels of DDE in a large study (Rogan et al., 1986). Women who identified themselves as professional, farmer or laborer had the highest level of DDE in their milk, while those whose main occupation was housewife, paraprofessional or student had lower levels.

6. Maternal Residence--In general, levels of total DDT and/or DDE in human milk are higher in urban women than in rural women as long as DDT had not been used commercially in that area (Newton & Greene, 1972; Graca et al., 1974; Dillon et al., 1981). Fytianos et al. (1985) observed no difference between rural and urban areas in northern Greece for DDT levels in human milk fat.

In areas where DDT has been aerially sprayed (Miller &

Fox, 1973; Karakaya et al., 1987; Matuo et al., 1980) levels of total DDT in milk are higher in rural mothers than urban mothers. Extremely high levels of total DDT have been reported in Central America where DDT is aerially sprayed for malaria control and/or agricultural purposes (Olszyna-Marzys et al., 1973; Winter et al., 1976; Olszyna-Marzys, 1978; de Campos & Olszyna-Marzys, 1979; Barnett et al., 1979).

Levels of total DDT are significantly higher in developing countries (Slorach & Vaz, 1983; Atuma & Vaz, 1986; Kanja et al., 1986) than in industrialized countries due to continued use of DDT. Kalra and Chawla (1981) reported mean levels of 0.5 ppm of total DDT in human milk representing an infant intake of 0.09 mg/kg/day, which is 18 times the acceptable daily intake (0.005 mg/kg/day) recommended by WHO (FAO/WHO, 1974). Kanja et al. (1986) reported the highest mean total DDT residue level in human milk fat found in Kenya to be 18.73 mg/kg. This antecedant is among the highest mean levels found in any country since 1974; but this antecedant is only 20% of total DDT residue levels found in Guatemalan human milk in 1970-71 which are the highest ever reported levels of DDT residue in human milk (de Campos & Olszyna-Marzys, 1979).

Other investigators have reported significant regional differences in DDT and DDE levels in human fat. In five geographic regions of the largest epidemiology milk study

undertaken, Savage (1976) reported the highest mean levels of DDT and DDE were in the Southwest and Southeast regions of the United States, 704 ppb, 606 ppb, and 5293 ppb, and 4277 ppb, respectively. The lowest mean values for DDT and DDE in human milk fat were found in the Northwest (396 ppb) and Northeast (468 ppb) areas of the United States, respectively. Earlier, studies in Norway (Brevik & Bjerk, 1978) reported regional differences in levels of total DDT in human milk while a later study by Skaare (1981) found no differences in mean levels of total DDT from different areas of Norway. Mes et al. (1986) found DDE levels were slightly lower in the central region of Canada than the other four regions. Ritchey et al. (1972) observed the lowest mean levels of total DDT in human milk were found the western provinces. In a later study by Musial et al. (1974), mean values for DDT and DDE in human milk collected from New Brunswick and Nova Scotia were approximately one-third the value reported by Ritchey. These values may be lower due to sampling methodology; mean percent lipid values were 1.44 and 1.08, respectively.

7. Home use of pesticides--There is some evidence that large use of house and garden pesticides may contribute to higher levels of total DDT in human milk. Wilson et al. (1973) reported lower concentrations of total DDT ($p = 0.05$) in milk of women employing commercial home exterminators as compared with women reporting self use of pesticides in their home and

garden. Bradt and Herrenkohl (1976) reported a statistically significant positive correlation between levels of total DDT in human milk and greater self use of non-persistent pesticides. Knoll and Jayaraman (1973a, 1973b) and Weisenberg et al. (1980) also reported similar findings, whereas, Stacey & Thomas (1975), Dillon et al. (1981), and Takahashi et al. (1981) found no correlation between total DDT levels in milk of women who were self users of pesticides around the home.

8. Different cultures--In the United States, there are studies of blood serum residues in which DDT levels are higher in blacks than whites (Davies et al., 1972) and a study of DDT in adipose tissue in which levels are higher in blacks than whites (Kutz et al., 1977). The reason for these differences in unknown. Woodward et al. (1976) observed significantly higher levels of total DDT in human milk of low income rural blacks compared to urban middleclass whites; 447 ppb and 75 ppb, respectively. However, other socioeconomic factors may have contributed to these differences. Savage (1976) reported 65% of black women had p,p'-DDT in human milk fat greater than 500 ppb as compared with only 25% of the white women sampled. All black women sampled had p,p'-DDE levels in human milk fat greater than 500 ppb compared to 96% of the white women. Rogan et al. (1986) found substantial racial differences in DDE levels of human milk fat. Almost half the

black donors had values over 6 ppm, while only 5 percent of the white donors reached this level. In Brazil, Matuo et al. (1980) found non-white women had total DDT levels in human milk two and one-half times higher than white women, 0.13 mg/kg and 0.05 mg/kg, respectively. However, other socioeconomic factors may have contributed to these differences. Other investigators have observed significant differences in levels of total DDT in human milk among immigrants compared to citizens (Ritchey et al., 1972; Luquet et al., 1972; Westöö & Norén, 1978; Schüpbach & Egli, 1979).

9. Maternal body weight—A number of investigators have addressed the effect of maternal weight on levels of organochlorine pesticide residues in human milk or human milk fat. Stacey and Thomas (1975) reported no apparent correlation between total DDT, DDE in human milk and the weight of the donor. However, by dividing the value reported for total DDT on a whole milk basis by the percent lipid reported for the individual donor, one can determine the total DDT on a lipid basis for the individual. A trend was seen when the arithmetic means are determined for donors whose weight was 50 kg or less; those who weigh between 50.5 kg and 60 kg; and those who weighed 60.5 kg or more. The results are: 0.045 mg/kg fat total DDT (n = 4); 0.068 mg/kg fat total DDT (n = 12); .075 mg/kg fat total DDT (n = 7),

respectively. Vuori et al. (1977) reported the mean weight of 49 donors to be 65 $\pm$ 11.9 kg. They did not observe any significant correlation between total DDT or DDT in human milk fat and the donor's weight. Polishuk et al. (1977a) observed that donors weighing more than 72 kg had lower mean total DDT in whole milk than those who weighed less than 63 kg; 67.8 ppb, 92.5 ppb, respectively; whereas the converse was true for total DDT in whole plasma, 72.9 ppb and 62.8 ppb, respectively. However, these differences were not statistically significant. Using a ratio of total DDT in whole milk to that in whole plasma, it seems that donors who weigh more than 72 kg are excreting less of this pesticide than donors who weigh less than 63 kg. Takahashi et al. (1981) reported the mean weight of 50 donors was 122 lbs (55.4 kg) with a range between 102 lbs (46.3 kg) and 175 lbs (79.5 kg). The weight of the donor did not appear to affect the excretion of pesticides in milk, including p,p'-DDE and p,p'DDT. Matuo et al. (1980) reported weights for all donors and most of the heights of donors. They observed that the mean levels of DDT were high (0.111 mg/kg) in milk from overweight donors, but decreased in samples obtained from normal and thin donors, 0.095 mg/kg and 0.083 mg/kg, respectively. In a recent large survey, Rogan et al. (1986) observed that levels of DDE in human milk fat were affected not significantly by the donor's weight. The conflicting

results may be explained by methodological differences in sample collection and chemical analyses, as well as failure to define ideal weight of the population group.

Some authors have suggested that weight reduction during lactation may lead to increasing concentrations of organochlorine pesticides and contaminants in human milk due to fat mobilization from fat depots (Atkinson, 1979; Rogan et al., 1980; Barthel & Nguyen Thi, 1983; Fooken & Butte, 1987). Eckenhausen et al. (1981) studied organochlorine pesticide concentrations in perinatal samples from donors and their infants. The subjects included those who were breast feeding and those who were bottle feeding. Within each of these groups, were those who were on a weight reduction program and those who were not. They reported that (1) there is no evidence that breast-feeding results in higher concentrations of organochlorine contaminants (including p,p'-DDT and p,p'-DDE) in infant's blood compared to those infants who were bottle-fed; (2) organochlorine pesticide concentrations in milk samples of non-slimming donors were not compared with those of slimming donors due to insufficient number of participants, (3) there were small, but not significant differences between concentrations in the blood of slimming and non-slimming donors and their infants. Vuori et al. (1977) did not find a significant correlation between DDT levels in human milk and weight loss of donors.

The mean weight lost during lactation was 2 ± 1.5 kg and a range of 0-7.5 kg. This antecedant was a cross-sectional study, i.e., one donor gave one sample during 1 time during her individual lactation. Fooken and Butte (1987) could not confirm an association between weight reduction and increasing concentration of p,p'-DDT and p,p'-DDE. Five donors were studied longitudinally from 5 to 9 months and four of the five donors lost 4 to 8 kg weight during lactation without any increase in levels of DDT and DDE in their milk.

10. Dietary habits--The major metabolite of p,p'-DDT is the more persistant metabolite of p,p'-DDE (Hayes, 1982). DDT is dechlorinated in the human body to TDE and then metabolized to the water soluble, and excretable, DDA, or reduced to DDE and stored in adipose tissue, although it is slowly metabolized and eliminated (Morgan & Roan, 1971). When DDT is banned or severly restricted, p,p'-DDT levels in foods of plant origin decrease rapidly. Because animals exposed to p,p'-DDT metabolize this substance much like humans, they store its metabolite, p,p'-DDE, and are the primary source of this metabolite through dietary sources. Since DDE is not effectively metabolized and eliminated in man and animals, the body burden of DDE increases in them.

High levels of total p,p'-DDT (i.e., DDT + DDE + DDD) and a large DDT/DDE ratio in adipose tissue in certain geographical areas would

probably indicate continued use of DDT in agriculture or for vector control. This antecedant is seen in some developing countries, but not industrialized countries.

It follows, then, that contamination of human milk fat by DDT indicates relatively recent exposure of the mother to DDT from food or some other direct exposure. Contamination of human milk fat by DDE would indicate an earlier exposure of the mother to DDT and/or consumption of foods of animal origin.

It is not surprising then, that a number of investigators have attempted to identify important exposure routes by correlating the total DDT, the DDE/DDT ratio, or individual level of pp'DDT and/or pp'DDE to food habits.

Szokolay et al. (1977) studied the dynamics of DDT and hexachlorocyclohexane (HCH) isomers residues in the food chain in Czechoslovakia and observed that the transfer of DDT differs from that of HCH isomers. The values of DDT and DDE found in soil are only found in foods of animal origin and he observed a strong accumulation of p,p'-DDE in milk fat. Wilson et al. (1973) observed no significant correlation between total DDT concentration in human milk and the number of days per week the donor consumed meat or fish. He did observe that donors who consumed margarine had higher levels of total DDT in their milk than donors who consumed butter ($p = < 0.04$). The findings may be related to the use of cottonseed oil in the manufacturing of margarine. Woodard et al. (1976) reported that the cotton market accounted for 70% of DDT use on farms in the United

States. In a more recent study, Matuo et al. (1980) observed no difference in total DDT concentration in human milk from donors who consumed butter, margarine, or neither, (0.091 mg/kg milk, 0.088 mg/kg milk, and 0.077 mg/kg milk, respectively). Bradt and Herrenkohl (1976) reported that higher levels of total DDT in human milk were statistically related to a diet high in calories as evaluated by a nutritionist; however, the source and distribution of calories were not delineated. Vuori et al. (1977) also inquired about the frequency of meat and fish consumption in 49 donors. Despite the fact that almost every donor consumed meat daily and the majority of the donors consumed fish or liver once or twice per week, they could not determine a significant correlation between concentration of total DDT in human milk and the donorss dietary habits. Other factors, such as, length of time from previous lactations, sample collection methodology, and method of reporting results may explain the lack of correlation. Takahashi et al. (1981) also inquired about the dietary habits of Hawaiian donors. Although no significant correlation was observed between intake of meat and dairy products which were the most important food in the diet of the donors, they reported that individuals who consumed meat more frequently tended to have higher levels of organochlorine contaminants (p,p'-DDT and p,p'-DDE) in their milk fat than those who ate meat less frequently. Watanabe et al. (1979) confirmed that approximately 70-90% of PCBs in the Japanese diet was derived from marine products and that the same trend was found to be true with p,p'-DDE. However, Yakushiji et al. (1979)

could not show a correlation between dietary habits and levels of p,p'-DDE in human milk fat due to insufficient number of donors' milk samples. Hofvander et al. (1981) reported that a limited amount of data pertaining to dietary habits of donors did not permit them to make a clear association between the levels of DDT and DDE in human milk fat and this factor. Norén (1983b) compared levels of p,p'-DDT and p,p'-DDE in human milk fat from donors consuming a lacto-vegetarian diet, a mixed diet, and a mixed diet in which fatty fish from the Baltic was consumed regularly. The lowest levels of p,p'-DDT and pp'-DDE were found in the milk fat from lacto- vegetarians, and the highest levels of these compounds from donors who regularly consumed Baltic fatty fish. Hergenrather et al. (1981) reported significantly lower values of p,p'-DDT and p,p'-DDE in human milk fat of vegetarians than nonvegetarians. However, other lifestyle aspects may influence their lower levels too. Rogan et al. (1986) reported 14% higher levels of p,p'-DDE in human milk fat from donors who regularly consumed sportsfish during pregnancy than those donors who did not consume sports fish. In a recent study, in Kenya, Kanja et al. (1986) showed significant differences of total DDT and the DDE/DDT in relation to dietary habits, e.g., fish consumers and strictly vegetarian consumers, as well as agricultural activities and pesticide use in various sampling areas.

Purpose of the Study

The South African population is diverse in food habits and DDT was used until 1976 for agricultural purposes and continues to be used

today for malarial control. Since a limited amount of work to evaluate chronic exposure of the South African population to persistent pesticide residue is available, a study was undertaken to determine levels of p,p'-DDE in human milk and to examine various factors that affect this organochlorine pesticide residue level in human milk fat.

Explanation of the Dissertation Format

This dissertation is composed of two papers. The first paper addresses the effects of age, parity, cigarette smoking, pesticides usage, previous breast-feeding, percent lipid in human milk, and location of their residence on levels of p,p;-DDE in human milk fat from South African women living in the Johannesburg area. The second paper addresses the effects of food habits, drug use, alcohol consumption, and body mass index on levels of p,p'-DDE in human milk fat from South African women living in the Johannesburg area.

PART I. THE INFLUENCE OF MATERNAL AND ENVIRONMENTAL FACTORS ON THE LEVELS OF DDE RESIDUES IN HUMAN MILK FROM SOUTH AFRICAN WOMEN

Abstract

Full expressions of breast milk were obtained from 184 healthy women in Johannesburg, South Africa. The mean collection day was 51 days after parturition. Human milk samples were analyzed for p,p'-DDE concentration by gas chromatography and human milk lipids were isolated by solvent extraction and determined gravimetrically. Mean concentrations of p,p'-DDE in human milk (mg/kg fat) of Black, Colored, White, and Indian donors were: 0.99 ± 0.12; 1.00 ± 0.16; 1.30 ± 0.17; 0.76 ± 0.45, respectively.

The Codex Alimentarius Commission maximum residue limit for total DDT was exceeded by 28% of the samples based only on concentration of DDE. Positive correlations were observed between DDE concentration and age class, among the Colored donors only, including primiparae. No correlation was found between parity and DDE concentration, however, significant decreasing differences of DDE concentration and parity classes were found among Black donors while the opposite was found in Colored donors. The total accumulated time of previous lactations was inversely correlated to DDE concentration. Thirty percent higher concentrations of DDE were observed in those smoking between 11-20 cigarettes per day than those smoking less than 10 cigarettes per day. Levels of DDE in human milk (mg/kg fat) are comparable to other Western countries and those in the Global Environmental Monitoring System.

Introduction

Ever since the discovery of DDT in human milk by Laug and co-workers in 1951, interest in the presence and levels of environmental contaminants in human milk has continued. Jensen (1983) extensively reviewed the extent of human milk contamination. While organochlorine pesticides are banned in industrialized countries, they continue to be used in many developing countries because, in general, they are effective and persistent, but less expensive and less toxic to the non-target organisms than the organophosphate insecticides.

The Republic of South Africa plays an important role in world economy, especially in relation to its natural resources. However, agriculture is also an important industry. South African agriculture is at an intermediate stage of development (Van Dyk et al., 1982). It is not as intensive and highly mechanized as in industrial countries of North America and Europe, but it is also far from a subsistence agriculture as practiced in other parts of Africa. A limited amount of work has been undertaken in South Africa to evaluate pesticide residue levels in adipose tissue of various segments of the diverse population (Wiese, 1976; Van Dyk et al., 1982; Van Dyk et al., 1987), while the usage pattern of organochlorine pesticides has changed greatly. The results of a survey undertaken in 1985 to evaluate factors contributing to and levels of 1,1'-(2,2-dichloroethenylidene)-bis 4-chlorobenzene (p,p'DDE) in human milk fat of South African women in the Johannesburg area are reported in this paper.

Collection of milk samples and interviews

Samples of human milk were collected with an electric breast pump from 184 individuals in Johannesburg between July and November, 1985. All women had delivered full term healthy singleton infants and were solely breast-feeding their infant at the time of the study. Women were enrolled in the study during their post-partum check-up. The entire contents of one breast were expressed with the Egnell mechanical breast pump (Egnell Inc., Cary, Illinois). Most samples were collected during the mother's post partum check-up at a hospital. Some samples were collected in individual homes. Samples collected at a hospital were kept on ice transferred to pesticide-free glass jars, and stored at -20°C within 4 hours. Samples collected in the individual homes were transferred to the hospital on ice and transferred to pesticide-free glass jars and stored at -20°C within 4 hours. The frozen samples were then transferred to a laboratory, placed in styrofoam shipping containers, and stored at -90°C until December 1985. The frozen samples were shipped air freight to Des Moines, Iowa, and transported to the Iowa State University Veterinary Diagnostic Laboratory where they were stored at -35°C for up to or all 18 mo. At the time aliquots were, samples were thawed overnight in a refrigerator then sonicated until homogeneous by using a sonicator water bath containing cold water. A 10 g milk sample for pesticide analysis was weighed into a 20 ml vial and sealed with a teflon lined screw cap. All aliquots were immediately refrozen at -35°C. At the time of preparation for pesticide residue analysis, the samples were

thawed in tepid water.

Donors were interviewed at the time of milk collection to obtain demographic information as well as information pertaining to parity, lactation history, use of medications, food consumption, pesticide usage, smoking habits and alcohol consumption, and assessment of any weight loss. The University of the Witwatersrand Committee for Research on Human Subjects reviewed this project and concluded that the rights and welfare of the human subjects were adequately protected, those risks were outweighed by the potential benefits and expected value of the knowledge sought, that confidentiality of data was assured and that informed consent was obtained by appropriate procedures.

Methods of analysis of the milk samples

Analyses were performed at the Iowa State University Veterinary Diagnostic Laboratory, Ames, Iowa. Deionized water was passed through a Millipore Q system; each gallon of millipore water was shaken with 250 ml petroleum ether after which the petroleum ether was discarded. Solvents were glass distilled and each 4-liter bottle was tested by gas chromatography after concentration from 250 ml to 0.5 ml. Anhydrous, granular sodium sulfate (Mallinkrodt 8024, Paris, KY) was treated at 500°C 12 hours and then maintained at 125°C. Florisil® (PR grade, 60-100 mesh, Pittsburgh, PA) was activated and stored at room temperature. Elution patterns of standards were determined with each new lot of Florisil and for every 2 weeks. All glassware was tested for impurities.

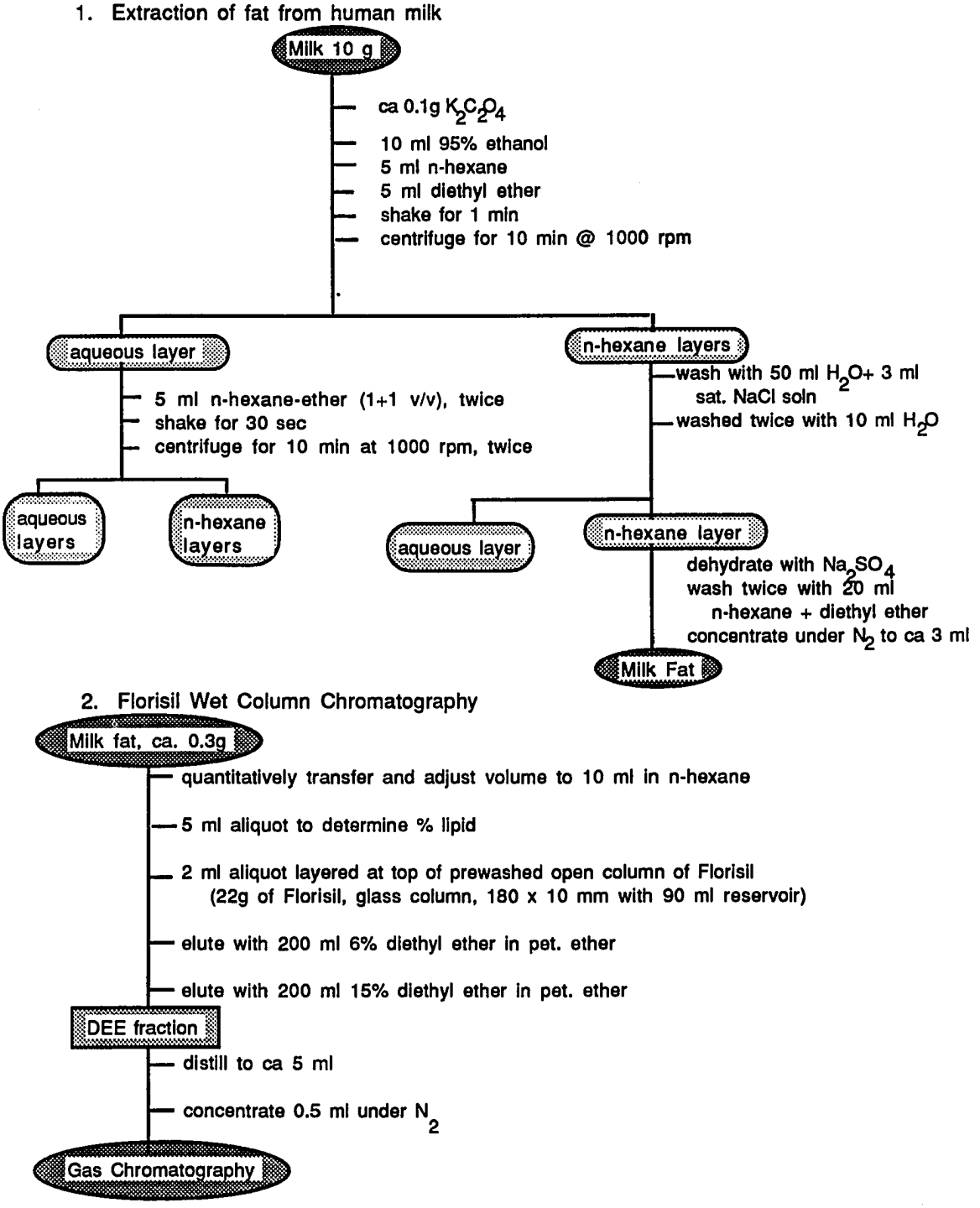


Figure 1. Flow chart, analysis of DDE in human milk

Extraction and cleaning

The triplicate extraction method of McKinney et al. (1984) utilizing ethanol-petroleum ether-diethyl ether was used to quantitatively extract the fat from human milk. Each 10 g milk sample was thawed and gently shaken to ensure homogeneity and then transferred to a 50 ml centrifuge tube containing approximately 0.1 g potassium oxalate. The sample was spiked with 100 ppb oxychlordane. The cleaning of the extract was based on the method of Stahr (1980) except 22 g of activated Florisil was used. Two milliliters of the lipid extract were applied to the Florisil column. Fraction one was eluted with 200 ml of 6% diethyl ether-petroleum ether; fraction two with 15% diethyl ether-petroleum ether. Both fractions were combined and concentrated to 5 ml on a steam bath and then concentrated to 1 ml under a stream of nitrogen over a water bath (30-35°C) (Figure 1).

Chromatography Primary identification and quantification of p,p'-DDE was accomplished by using a Packard Model 540 gas-liquid chromatograph equipped with a 63 Ni (10 mCi) electron-capture detector utilizing a constant-current pulse mode and a borosilicate glass packed column (1.8 m long, 6 mm, o.d., 4mm i.d.) contained 1.5% OV-17 and 1.95% OV-210 on Gas Chrom Q (100-120 mesh). The operating conditions of the gas-liquid chromatograph were as follows: Electron capture oven temperature, 240°C; column temperature, 225°C; injector temperature, 240°C; carrier-gas flow, 50 ml N_2/min and detector scavenger-gas flow, 20 ml N_2/min. The electron capture detector was adjusted to give a sensitivity of full scale on a 1 mv recorder for 1

ng pesticide.

Quantitative evaluation The peak heights of p,p'-DDE were compared with the peak height for a known quantity of pure standard of p,p'-DDE. The retention times were measured and found to agree with a standard mean.

Analysis of the Data

A data set consisting of the concentration p,p'-DDE (mg/kg fat) for each individual along with her demographic and other information was constructed. The mean differences in p,p'-DDE among various classifications of the data were tested using F-statistics in the context of the analysis of variance. The independence of the proportions of individuals in various classifications from other demographic variables were examined using chi-square statistics. Correlations among measures were also calculated.

Results and Discussion

The average age of the donors was 25.4 y and ranged from 15 to 42 y. Forty percent of the donors were breast-feeding their first child; 25% their second child; 22% their third child; the remaining 13% were breast-feeding their fourth or later child. The average number of children per donor was 2.24 $\pm$ 1.4 children (range from 1 to 10). The mean day of lactation was 52.2 $\pm$ 12.6 days (range from 33 to 184 days). Only 21 of 184 donors lived in a rural setting of the Johannesburg-Pretoria-Vereeniging complex. Thirty-five of 182 donors were currently smoking (two mothers did not respond to this question).

Fifty percent of the donors were Black; 24% were Colored; 23% were White, and 3% were Indian. The Colored are a racial group of mixed ancestry recognized in South Africa. More than one-half of donors identified themselves as homemakers (n = 100), but a few indicated they were professional women. Of those employed, only a small number were White donors while a larger number of Colored and Black donors worked outside the home. The average percent human milk fat of donors from 4 ethnic groups was similar. The values were: Black donors, 3.5% $\pm$ 0.1%; Colored donors, 3.0 $\pm$ 0.2%; White donors, 3.6 $\pm$ 0.2%; Indian donors, 3.3 $\pm$ 0.6%.

Some DDE was found in all samples, but two samples had only traces. The mean level of DDE was 1.1 $\pm$ 1.1 mg/kg fat (range:trace to 9.7 mg/kg fat). The two highest concentrations were found in Black women who were breast-feeding for the first time, 9.7 and 7.7 mg/kg fat, respectively.

Van Dyk et al. (1987) reported 47 of 62 samples of human milk collected in Johannesburg had concentrations of p,p'-DDE between 0.1 and 3.6 mg/kg, fat.

A total of 184 milk samples from individual donors were analyzed. The results are summarized and compared with other recent studies on the African continent in Table 1. The Codex Alimentarius Commission (Joint FAO/WHO Food Standards Program, 1974) has set a residue limit of 1.25 mg/kg fat for total DDT (that is sum of DDT + DDE + DDD). In this study, 28% of the samples exceeded the maximum residue limit for total DDT, based only on levels of DDE.

Age

The donors were grouped into five age groups, 15-19, 20-24, 25-29, 30-34, 35-42 yr, and the mean level of DDE reported for each class. The level of DDE increased from the first to the fourth class and then declined (Table 2). Overall, there was no correlation observed between the age of the individual donor and level of p,p'-DDE. Only the correlation between age group and p,p'-DDE level in the Colored group was statistically different from zero; $r = 0.423$ ($p = 0.003$). A similar correlation between age group and p,p'-DDE concentration was found also in primiparae (Table 3).

Although there is a weak trend of increasing amounts of DDE with increasing age classes, the trend is more pronounced when the following criteria are applied and compared to the original whole data set; primiparae nursing their child within the first 12 weeks (Table 2). Thus the amount of DDE stored in the body is clearly a function of age. It appears that younger women, in general, were exposed less to DDT and its major metabolite, DDE via the food chain, since agricultural use of DDT was severely restricted since 1970 (Van Dyk et al., 1982) and finally banned for agricultural use in 1976 (Van Dyk et al., 1987, 1982). In this study, donors who were older than 35 y might also be expected to have lower levels of DDE in human milk, because DDT was not commercially available in countries outside the USA until after 1954 (Hayes, 1982).

Table 1. DDT residues in human milk, African

Nation	Sampling Year	Residue	Median	Reference
Ghana n =	1972	DDT	29 ppb whole milk	Polishuk et al., 1977
Zaire n = 77	1981	DDE	29 ug/1 whole milk	WHO, 1985
		DDD	2 "	
		DDT	3.5 "	
Nigeria n=35	1981-1982	p,p'DDE	1.1 mg/kg milk fat (range: 0.28-1.9 mg/kg milk fat)	Atuma and Vaz, 1986
		p,p'-DDT	0.41 mg/kg milk fat (range: 0.12-1.0 mg/kg milk fat)	
Kenya			Mean	Kanja et al.,
Karatina n = 50	1983	p,p'DDE	1.72 mg/kg milk fat (range: 0.37-15.60 mg/kg milk fat	1986
		p,p'-DDT	1.59 mg/kg milk fat (range: 0.12-24.17 mg/kg milk fat)	
		DDT	3.5 mg/kg milk fat (range: 0.61-41.48 mg/kg milk fat)	
Turkana n = 68	1983-1984	p,p'-DDE	1.73 mg/kg milk fat (range: 0.04-7.80 mg/kg milk fat)	
		p,p'-DDT	5.15 mg/kg milk fat (range: 0.29-26.23 mg/kg milk fat)	
		DDT	7.02 mg/kg milk fat (range: 0.44-32.82 mg/kg milk fat)	
Loitokitok n = 13	1984	p,p'-DDE	0.43 mg/kg milk fat (range: 0.11-1.64) mg/kg milk fat	
		p,p'-DDT	0.47 mg/kg milk fat (range: 0.16-0.83 mg/kg milk fat)	

Table 1. (continued)

Nation	Sampling Year	Residue	Median	Reference
		DDT	1.69 mg/kg milk fat (range: 0.44-2.60 mg/kg milk fat)	
Nanyuki n = 42	1984-1985	p,p'-DDE	1.52 mg/kg milk fat (range: 0.21-5.48 mg/kg milk fat)	
		p,p'-DDT	2.47 mg/kg milk fat (range: 0.87-18.42 mg/kg milk fat)	
		DDT	4.32 mg/kg milk fat (range: 0.60-23.02 mg/kg milk fat)	
Rushing Island n = 25	1984-1985	p,p'-DDE	7.61 mg/kg milk fat (range: 1.38-21.83 mg/kg milk fat)	
		p,p'-DDT	9.60 mg/kg milk fat (range: 1.74-44.53 mg/kg milk fat)	
		DDT	18.73 mg/kg milk fat (range: 3.70-69.87 mg/kg milk fat)	
Embu n = 48	1985	p,p'-DDE	5.23 mg/kg milk fat (range: 0.83-32.89 mg/kg milk fat)	
		p,p'-DDT	3.63 mg/kg milk fat (range: 0.60-28.08 mg/kg milk fat)	
		DDT	9.76 mg/kg milk fat (range: 1.71-54.54 mg/kg milk fat)	
Homa Bay n = 12	1985	p,p'-DDE	3.48 mg/kg milk fat (range: 0.32-7.99 mg/kg milk fat)	
		p,p'-DDT	4.08 mg/kg milk fat (range: 0.64-9.12 mg/kg milk fat)	

Table 1. (continued)

Nation	Sampling Year	Residue	Median	Reference
		DDT	9.76 mg/kg milk fat) (range: 0.99-16.63 mg/kg milk fat)	
Meru n = 44	1985	p,p'-DDE	1.41 mg/kg milk fat) (range: 0.02-9.80 mg/kg milk fat)	
		p,p'-DDT	0.65 mg/kg milk fat) (range: 0.05-2.68 mg/kg milk fat)	
		DDT	2.20 mg/kg milk fat) (range: 0.02-12.95 mg/kg milk fat)	
South Africa Johannesburg	1985			This study
Blacks (n = 92)		p,p'-DDE	0.99 mg/kg milk fat (range: trace - 9.70 mg/kg milk fat)	
Coloreds (n = 45)		p,p'-DDE	1.00 mg/kg milk fat) (range: trace - 3.50 mg/kg milk fat)	
Asians (n = 5)		p,p'-DDE	0.76 mg/kg milk fat) (range: 0.43- 1.18 mg/kg milk fat)	
Whites (n = 42)		p,p'-DDE	1.3 mg/kg milk fat) (range: 0.96 - 5.11 mg/kg milk fat)	

Table 2. Mean DDE concentration (mg/kg fat) for age groups all donors and Primiparae

Age Class (all donors)	N	Mean p,p'-DDE mg/kg fat	S.E.
15-19	33	1.0	1.2
20-24	57	1.1	1.3
25-29	51	1.2	0.8
30-34	25	1.2	0.8
35-42	18	0.8	0.6
Age Class (primiparae)	N	Mean p,p'-DDE mg/kg fat	S.E.
15-19	31	0.97	0.88
20-24	27	1.24	0.94
25-29	10	1.50	1.54
30-34	4	1.78	2.45
35-42	1	0.60	4.90

Table 3. Correlation between age classes and concentrations (mg/kg fat) in human milk among ethnic groups.

	All r	Primiparae r
All donors	0.04 n = 184 avg. age = 25.4	0.12 n = 73 avg. age = 21.7
Black	-0.086 n = 92 avg. age = 26.2	-0.020 n = 34 avg. age = 21.2
Colored	0.429* n = 45 avg. age = 23.8	0.571** n = 17 avg. age = 19.8
White	0.173 n = 42 avg. age = 25.9	0.341 n = 20 avg. age = 24.4
Asian	0.706 n = 5 avg. age = 23.4	only 2 donors

*p = 0.003.

**p = 0.0017.

Parity

Lipophilic organochlorinated pesticides and contaminants are known to cross the placenta (Polishuk et al., 1977) and may be one reason why the concentration of these substances decreases in older women. However, there were no significant differences in the levels of DDE among parity classes for this study. There was no trend observed in lower levels of DDE in human milk with each subsequent pregnancy, in general, for all donors. However, significant differences were observed between mean concentration of DDE in human milk fat among the parity classes for Black donors and Colored donors (Table 4).

This observation may be related to the average length of lactation for these Black donors which was 15.5 months. White, Colored, and Asian donors average length of lactation periods were: 8.1 mos, 12.6 mos, 5.8 mos, respectively. Despite the similarity in average length of lactation with Colored donors, the opposite trend was observed, that is, with each parity an increase in the mean DDE level was observed (Table 5).

It is difficult to explain the opposite trends observed in Black and Colored donors. It seems that other factors may be affecting this trend, however, it was not possible to identify these factors within the scope of the present study. A larger sample in the Johannesburg area as well as other major urban centers could validate these observed trends. Many investigators have found higher levels of DDE in human milk of donors who are breast-feeding for the first time

Table 4. Mean DDE concentration (mg/kg fat) in relation to parity in Black donors

Parity	N	Mean p,p'-DDE mg/kg fat	S.E.
1	34	1.33	0.19
2	21	0.96	0.23
3	20	0.92	0.24
4	7	1.25	0.40
5	3	0.60	0.62
6	5	0.63	0.48

Table 5. Mean DDE concentrations (mg/kg fat) in relation to parity in Colored donors

Parity	N	Mean p,p'-DDE mg/kg fat	S.E.
1	17	0.84	0.26
2	14	0.94	0.29
3a	11	1.17	0.32
6	2	2.06	0.76
7	1	0.55	1.07

[a]No Colored mothers had borne 4 or 5 children.

(Kroger, 1972; Bradt & Herrenkohl, 1976; Matuo et al., 1980; Norén, 1983a, 1983b; Rogan et al., 1986), while some investigators have found no correlation between total DDT (DDT + DDE + DDD) and number of children (Vuori et al., 1977; Dillon et al., 1981; Takahashi et al., 1981; Weisenberg et al., 1980; Weisenberg et al., 1985). Barril et al. (1977) and Wickström et al., 1983; reported a significant negative correlation between total DDT levels in human milk and parity.

Cigarette smoking

Only 35 donors reported they were currently smoking cigarettes at the time of the study. No difference in mean DDE levels in human milk fat between cigarette smokers and non-smokers of cigarettes were observed. Sixty-seven percent of the donors who were smoking currently reported smoking less than 10 cigarettes per day. A 32% higher mean concentration of DDE in milk fat was observed in donors who were smoking 11 to 20 cigarettes per day (Table 6). No significant differences were observed between mean DDE levels in human milk fat and the and the past use of cigarette smoking and those currently smoking cigarettes. No differences were observed in Black donors. Colored donors who were smoking at the time of the survey, had 25% higher mean levels of DDE in milk fat than those Colored donors who were not currently smoking cigarettes, while White donors had 13% lower mean DDE concentrations in human milk fat compared with White non-smokers of cigarettes (Table 7). When agricultural usage of DDT reached its peak in the late sixties and early seventies, DDT was applied to tobacco fields (Mussalo-Rauhamaa et al., 1986).

Table 6. Comparison of mean DDE concentration (mg/kg fat) levels among smokers

Quantity	N	Mean DDE mg/kg fat	S.E.
< 10 cigarettes per day	23	0.95	0.22
10 to 20 cigarettes per day	9	1.39	0.35

Table 7. Mean DDE concentration (mg/kg fat) and current smoking habits

Group	N	Smoking at Time of Survey	Mean p,p'-DDE mg/kg fat	S.E.
Black	6	Y	1.06	0.44
	86	N	1.06	0.12
White	10	Y	1.17	0.34
	32	N	1.34	0.19
Colored	18	Y	1.13	0.25
	25	N	0.85	0.21

Consequently, it is not surprising to find reports in the earlier literature that women who smoked cigarettes had higher levels of DDT and its metabolites in their milk than those who did not smoke (Bradt & Herrenkohl, 1976; Miller & Fox, 1973; Savage, 1976; Vuori et al., 1977). Even more recently, Dillon et al. (1981) observed a significant positive correlation between cigarette smoking and the concentration of DDE in human milk fat, while Rogan et al. (1986) reported cigarette smokers had 15% higher levels of DDE in their milk fat as compared with non-smokers. Collins et al. (1982) observed no difference between the mean concentration of p,p'-DDE in human milk fat of smokers and non-smokers. A limited amount of data collected by Hofvander et al. (1981) prevented them from discussing any clear relationship between cigarette smoking habits and levels of organochlorine contaminants, including DDE, in human milk. Matuo et al. (1980) observed differences amongst three racial groups in Brazil. However, smoking habits were not studied. A larger sample group of donors in Johannesburg and/or other areas of South Africa is necessary to substantiate if there are significant differences between cigarette smokers and non-smokers and among ethnic groups.

Pesticide usage

Using aerosol insecticides in the home may lead to dermal or respiratory exposure. In this study, 77% of the participants indicated a variety of pesticides were used in their household. The four most common pesticides used were non-persistent aerosol products.

Sixty-four percent of the donors indicated they applied the

pesticides in the household. There was no indication that household use of pesticides or the pesticide use by the donor contributed to higher levels of DDE in human milk fat amongst the donors. However, among Black donors who reported household use of pesticides, the mean DDE levels in human milk fat were lower ($p = 0.05$) than Black non-users of household pesticides.

Total duration of lactation

Lactation is one of the pathways of elimination of persistent lipophilic organohalogen contaminants, including DDT and its major metabolite, DDE (Hayes, 1975). Despite the large number of human milk studies in the past and more recently, few investigators have examined the effect of the donor's total length of lactation (i.e., the total accumulative number of weeks or months the subject has previously lactated) on levels of persistent lipophilic contaminants in human milk. Hahne (1985) observed lower levels of total DDT concentrations in human milk fat with both parity and the total time of previous lactations. Parity, the number of children born to a woman, often is correlated to levels of these contaminants. The total lactation time also should not be confused with studies of changes in organohalogen residue levels in human milk during an individual's period of lactation. Here, there are conflicting results (Norén, 1983c; Krauthacker et al., 1980; De Bellini et al., 1977; Mes et al., 1984).

Our results indicate a correlation between a diminishing DDE concentration with the number of months of previous lactation ($r = -0.30$, $p = 0.002$) for all donors. Differences were also observed within

ethnic groups (Table 12). A larger number of Indian donors would be necessary to verify the observed positive correlation in this ethnic group. Since the mean day of lactation was similar for all donors and within Black, Colored, and White ethnic groups, 52 ± 12, 51 ± 8, 54 ± 20, 54 ± 10 days, respectively, the effect of an individual's total time of previous lactations on the concentration of DDE in human milk fat was evaluated. The data for three ethnic groups and all donors are shown in Figure 1. The concentration of DDE decreased significantly in Black and White donors (regression equations, DDE = 1.1-7.4(months), (DDE) = 1.7-27.9(months), respectively). The weak correlation between DDE concentration and number of months of previous lactation in the Colored donors may be related to one high value and fewer donors breast-feeding for an extended period. This deserves further investigation, however.

Other environmental factors

Only 18 of 166 donors in this study lived in a rural area, the remainder lived in the urban area of Johannesburg. When mean concentration of DDE in human milk and in human milk fat were determined geographic differences were found. There was a trend ($p = 0.10$) of higher concentrations of DDE in human milk fat among rural donors than among urban donors. However, a statistically significantly higher ($p = 0.05$) concentration was found in human milk among the rural donors as compared with urban donors. The rural area surrounding Johannesburg is used both for farming and grazing. It would appear that at one time these rural donors were more exposed to

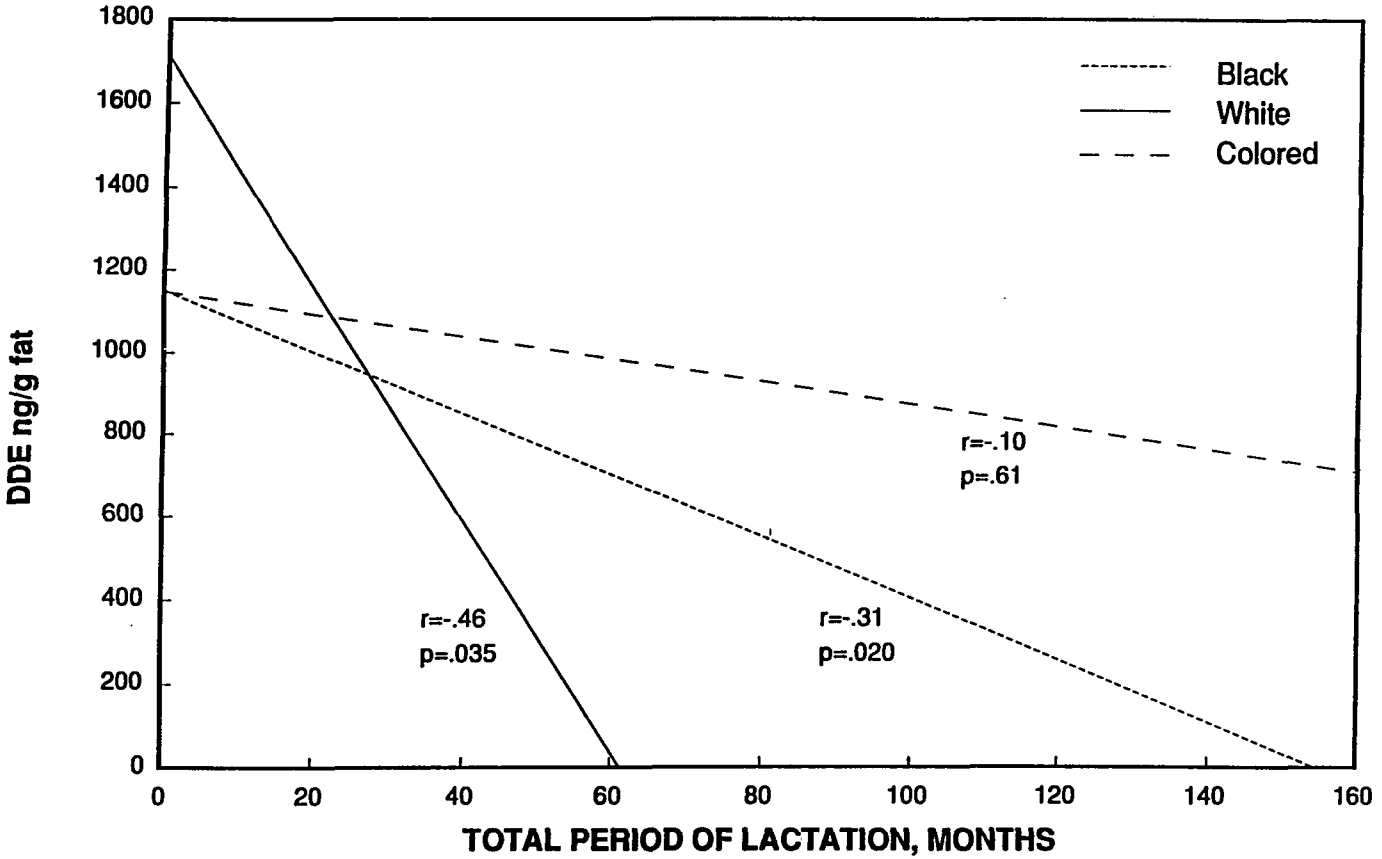


Figure 1. Regression of DDE on total months of previous lactation by ethnic groups

more DDT than urban donors. They continue to be exposed to more pesticides presently; whether or not this is contributing to higher levels is unknown. This was also the case when DDE was analyzed on a whole milk basis. These results also point out some of the problems with sampling human milk and reporting the results (Mes & Davies, 1978; Mes, 1981; Picciano, 1984; Ferris and Jensen, 1984). Human milk samples taken prior to a feeding or within 2 minutes after the infant starts suckling tend to have a lower level of fat and lower levels of DDE and other lipophilic contaminants when reported on a whole milk basis. Human milk samples taken at the end of a feeding tend to have a higher level of fat and higher levels of DDE and other lipophilic chemical contaminants when data are reported on whole milk. Norén (1983c) reported that DDE and other lipophilic organochlorine contaminants were constant within analytical error for each donor if reported on a fat basis.

Other factors, which may contribute significantly to higher levels of DDE in human milk and human milk fat, are: country of origin, frequency of moves within a country; as well as, visiting countries where pesticide regulatory aspects on a national level are not well monitored. No significant differences were observed in mean concentrations of DDE in human milk (mg/kg fat) between native borne South Africans and immigrants. A greater number of White donors moved once, or more in the past 5 years, than Black or Colored donors. However, no differences in DDE concentration in human milk fat were observed between donors who moved frequently and those who did not.

Table 8. Mean DDE concentration (mg/kg fat) in relation to household pesticide usage. (Parentheses indicates DDE mg/kg whole human milk x 10^2)

Household pesticide use	Mean p,p-DDE mg/kg fat	SE	LSD p = 0.05
Yes	1.40	0.09	
(n = 141)	(3.35)	(0.28)	0.36 (0.66)
No	1.27	0.16	
(n = 43)	(3.84)	(0.50)	

Table 9. Mean DDE concentration (mg/kg fat) in relation to donor self-use of pesticides. (Parentheses indicates DDE mg/kg whole human milk x 10^2)

Self Use	Mean p,p'-DDE mg/kg fat	SE	LSD (p = 0.05)
Yes	1.00	0.10	
(n = 118)	(3.26)	(0.30)	0.32 (1.00)
No	1.24	0.13	
(n = 66)	(3.84)	(0.40)	

Table 10. Mean DDE concentration (mg/kg fat) in relation to pesticide usage for each ethnic group

Ethnic Group	Household Pesticide Usage	Mean p,p'-DE mg/kg fat	Mean p,p'-DDE mg/kg Whole Milk x 10^2
Asian (n=3)	No	0.84	2.32
Asian (n=2)	Yes	0.63	2.40
Black (n=12)	No	1.70	5.10
Black (n=80)	Yes	0.97	3.25
Colored (n=20)	No	0.97	2.57
Colored (n=25)	Yes	1.03	2.76
White (n=8)	No	1.61	5.87
White (n=34)	Yes	1.23	4.06

Table 11. Mean DDE concentration (mg/kg fat) in relation to self-use of pesticides (parentheses indicates DDE in human whole milk, mg/kg c 10^2)

	Yes			No			Y vs N
Ethnic Group	p,p-DDE Mean	SE	N	p,p'-DDE Mean	SE	N	LSD
All donors	1.01 (3.26)	0.98 (0.30)	118	1.25 (3.84)	0.13 (0.40)	66	0.32 (1.00)
Blacks	0.98 (3.25)	0.12 (0.37)	80	1.67 (5.09)	0.31 (0.95)	12	0.65* (2.04)
Whites	1.24 (3.93)	0.23 (0.70)	22	1.38 (3.92)	0.24 (0.74)	20	0.65 (2.09)
Coloreds	0.89 (2.40)	0.28 (0.85)	15	1.05 (2.80)	0.20 (0.60)	30	0.66 (2.09)
Asians	0.58 (1.78)	0.29 (3.29)	1	0.80 (2.50)	0.15 (1.65)	4	0.91 (11.78)

*$p \leq .05$.

Table 12. Correlation between total previous lactation periods in months and DDE concentration (mg/kg fat) in human milk (number of observations in parentheses)

	r	p
All donors (n = 104)	-0.30	0.002
Black (n=55)	-0.31	0.020
Colored (n=25)	-0.10	0.61
White (n=21)	-0.46	0.036
Asian (n=3)	0.60	0.60

Conclusions

Twenty-eight percent of the samples exceeded the Codex Alimentarius Commission maximum residue limit for total DDT based on the p,p'-DDE content alone. Our results are similar to those reported for p,p'-DDE in an earlier South African study that Van Dyk et al., 1987 had conducted in the same geographical area. One major difference was noted, the range of values was greater (tr to 9.7 mg/kg fat;0.1-3.6 mg/kg fat, respectively). We also observed a positive correlation between age class and DDE content in human milk fat among the Colored donors ($r = 0.43$, $p < .05$); a negative correlation between the total accumulation time a mother has lactated and levels of DDE in human milk fat among donors in general as well as the Black and White donors.

Among those who were smoking cigarettes higher levels of DDE were found in those smoking between 11-20 cigarettes than those smoking less than 10 cigarettes per day. Other factors may be contributing to the lower level of DDE among Blacks who used aerosol pesticides in their households and those who did not.

When the results were analyzed on a whole milk basis, different results were obtained. Since our sample collection consisted of one full expression of breast milk, we prefer to analyze and report our results on a fat basis. However, we believe more research is necessary in determining what constitutes a representative sample for estimating the amount of environmental contaminants, individual or groups of contaminants, present in human milk. Until such time, we

prefer to recommend the benefits of breast-feeding.

References

Atuma, S. S., Vaz, R. (1986). A pilot study on levels of organochlorine compounds in human milk in Nigeria. Intern. J. Environ. Anal. Chem. 26, 187-192.

Barril, C. R., Reyes, N. A., Consolacion E. C., Rosellon, L. B. (1977). DDT residue levels in human milk in Laguna, Philippines. Philipp. Entomologist. 3, 321-338.

Bradt, P. T., Herrenkohl, R. C. (1976). DDT in human milk. What determines the levels? Sci. Total Environ. 6, 161-163.

Collins, G. B., Holmes, D. C., Hoodless, R. A. (1982). Organochlorine pesticide residues in human milk in Great Britain, 1979-80. Human Toxicol. 1, 425-431.

De Bellini, Y., Cressely, J. Deluzarche, A., Hazemann, A. (1977). Pesticides organochlores dans le lait de femme. Ann. Fals. Exp. Chim. 70, 567-572.

Dillon, J. C., Martin, G. B., O'Brien, H. T. (1981). Pesticide residues in human milk. Food Cosmet. Toxicol. 19, 437-442.

FAO/WHO Food Standards Programme. (1974). Recommended International Maximum Limits for Pesticide Residues: Fourth Series CAC/RS 65-1974 Food and Agricultural Organization, Rome.

Ferris, A. M., Jensen, R. G. (1984). Lipids in human milk: A review. 1. Sampling, determination, and content. J. Pediatr. Gastroenterol. Nutr. 3, 109-122.

Hahne, K. H. (1985). Untersuchungen über Vorkommen und Eintragswege persistenter chlorierter Kohlen wasserstoffe sowie von Blei und Cadmium in Fraunmilch. pp. 72-73. Dissertation, Universität Kiel.

Hayes, W. J. (1975). Toxicology of Pesticides. William and Wilkins, Baltimore.

Hayes, W. J. (1982. Pesticides Studied in Man. William and Wilkins, Baltimore.

Hofvander, Y., Hagman, U., Linder C. E., Vaz, R., Slorach, S. A. (1981). WHO collaborative breast feeding study. I. Organochlorine contaminants in individual samples of Swedish human milk, 1978-79. Acta Paediatr. Scand. 70, 3-8.

Jensen, A. A. (1983). Chemical contaminants in human milk. Residue Rev. 89, 1-128.

Kanja, L., Skåre, J. U., Nafstad, I., Maitai, C. K., Løkken, P. (1986). Organochlorine pesticides in human milk from different areas of Kenya 1983-1985. J. Toxicol. Environ. Health. 19, 449-464.

Krauthacker, B., Alebic-Kolbah, T., Buntic, A., Tkalcevic, B., Reiner, E. (1980). DDT residues in samples of human milk and in mothers and cord blood serum in a continental town in Croatia (Yugoslavia). Int. Arch. Occup. Environ. Health. 46, 267-273.

Kroger, M. (1972). Insecticide residues in human milk. J. Pediatr. 80, 401-405.

Lang, E. P. Kunze, F. .M., Prickett, C. S. (1951). Occurrence of DDT in human fat and milk. Arch. Ind. Hyg. 3, 245-246.

Matuo, Y. K., Lopes, J. N. C., Lopes, J. L. C. (1980). DDT levels in human milk from Ribeirað Preto. (Brazil). Rev. Brasil. Biol. 40, 293-296.

McKinney, J. D., Moore, L., Prokopeta, A., Walters, D. B. (1984). Validated extraction and cleanup procedures for polychlorinated biphenyls and DDE in human body fluids and infant formula. J. Assoc. Off. Anal. Chem. 67, 122-129.

Mes, J., Davies, D. J. (1978). Variation in the polychlorinated biphenyl and organochlorine pesticide residues during human breast feeding and its diurnal pattern. Chemosphere 9, 699-706.

Mes, J., Doyle, J. A., Adams, B. R., Davies, D. J., Turton, D. (1984). Polychlorinated biphenyls and organochlorine pesticides in milk and blood of Canadian women during lactation. Arch. Environm. Contam. Toxicol. 13, 217-223.

Miller, G. J., Fox, J. A. (1973). Chlorinated hydrocarbon pesticide residues in Queensland human milk. Med. J. Aust. 2, 261-264.

Mussalo-Rauhamaa, H., Salmela, S. S., Leppänen, A., Pyysalo, H. (1986). Cigarettes as a source of some trace and heavy metals and pesticides in man. Arch. Environ. Health 41, 49-55.

Norén, K. (1983a). Organochlorine contaminants in Swedish human milk from the Stockholm region. Acta Peditatr. Scand. 72, 259-264.

Norén, K. (1983b) Levels of organochlorine contaminants in human milk in relation to the dietary habits of the mothers. Acta Paediatr. Scand. 72, 811-816.

Norén, K. (1983c). Some aspects of the determination of organochlorine contaminants in human milk. Arch. Environm. Contam. Toxicol. 12, 277-283.

Picciano, M. F. (1984). What constitutes a representative human milk sample? J. Pediatr. Gastroenterol. Nutr. 3, 280-283.

Polishuk, Z. W., Ron, W., Wasserman, M., Cucos, S., Wasserman, D., Lemesch, C. (1977). Organochlorine compounds in human blood plasma and milk. Pest. Monit. J. 10, 121-129.

Rogan, W. J., Gladen, B. C., McKinney, J. D., Carreras, N., Hardy, P., Thullen, J. Tingelstad, J., Tully, M. (1986). Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: Effects of maternal factors and previous lactation. Am. J. Public Health. 76, 172-177.

Savage, E. P. (1976). National study to determine levels of chlorinated hydrocarbon insecticides in milk. Washington, Environmental Protection Agency (EPA 540/9-781005).

Stahr, H. M. (1980). Supplement to Analytical Toxicology Methods Manual. Iowa State University Press, Ames, Iowa.

Takahashi, W., Saidin, D., Takei, G., Wong, L. (1981). Organochlorine pesticide residues in human milk in Hawaii, 1979-80. Bull. Environm. Contam. Toxicol. 27, 506-511.

Van Dyk, L. P., Lötter, L. H., Mullen, J. E. C., de Kock, A. (1987). Organschlorine insecticide residues in human fat in South Africa. Chemosphere 16, 705-711.

Van Dyk, L. P., Wiese, I. H., Mullen, J. E. C. (1982). Management and determination of pesticide-residues in South Africa. Residue Rev. 82, 38-124.

Vuori, E., Tyllinen, P., Kuitunen, P., Paganus, A. (1977). The occurrence and origin of DDT in human milk. Acta Paediatr. Scand. 66, 761-765.

Weisenberg, E., Ackerman, E., Freier, S., Reshef, A., Sahm, Z., Shoenberg, J. (1980). Pesticides in the milk of mothers in Jerusalem. In Human Milk. Its Biological and Social Value. Edited by S. Freier and A. I. Eidelman. pp. 147-152. Excerpta Medica, Princeton.

Weisenberg, E., Arad, I., Grauer, F., Sahm, Z. (1985). Polychlorinated biphenyls and organochlorine insecticides in human milk in Israel. Arch. Environm. Contam. Toxicol. 14, 517-521.

WHO. (1985). The Quantity and Quality of Breast Milk. World Health Organization, Geneva.

Wickström, K., Pyysalo, H., Siimes, M. A. (1983). Levels of chlordane, hexachlorobenzene, PCB, and DDT compounds in Finnish human milk in 1982. Bull. Environm. Contam. Toxicol. 31, 251-256.

Wiese, I. H. (1976). Pesticides and the South African population. SAMJ 50, 1801-1805.

PART II. INFLUENCE OF DIET AND OTHER MATERNAL FACTORS ON LEVELS OF DDE RESIDUES IN HUMAN MILK FROM SOUTH AFRICAN WOMEN

Abstract

Full expressions of breast milk were obtained from 184 healthy lactating women in Johannesburg, South Africa. The mean collection day was 51 days after parturition. Human milk samples were analyzed for dichlorodiphenyl dichloroethene (DDE) extraction, concentration, purification, and gas chromatography and milk lipids were isolated by solvent extraction and determined gravimetrically. Three racial groups were identified and body mass index (BMI), food frequency consumption, and use of steroidal contraceptives, and the fat content of the donor's milk were assessed.

The mean concentration of DDE in human milk for White, Colored, Black and Indian donors was: 1.30 mg/kg fat; 1.00 mg/kg fat; 0.90 mg/kg fat; and 0.76 mg/kg fat, respectively. A significant positive correlation between body mass index (BMI) or weight/height^2, and the concentration of DDE mg/kg fat was observed among Colored donors. A significant inverse relationship between DDE concentration (mg/kg fat) and the fat content of milk among Black and Colored donors was seen while a nearly significant ($p = 0.055$) similar relationship was observed in White donors. Significant differences ($p = 0.05$) in the concentration of DDE mg/kg fat were found among ethnic groups when 2 high values from Black donors were removed. Black donors consumed fewer foods ($p = 0.05$) than Colored donors who consumed fewer foods ($p = 0.05$) than White donors. Consumption of breads and cereals, and

vegetables was associated with a lower concentration of DDE in human milk, whereas, consumption of fat containing foods was not significantly correlated with DDE concentration. The use of oral contraceptives concurrent with lactation was not associated with a significantly higher concentration of DDE in human milk (mg/kg fat).

Introduction

Persistent lipophilic organohalogen compounds including agrochemical and environmental contaminants, are known to accumulate in adipose tissue and are excreted in human milk. The study of these contaminants in human milk is a useful way to monitor the exposure of the mother-infant dyad as well as to detect trends in levels of exposure in the general population (Slorach & Vaz, 1983). In addition to determining levels of environmental contaminants in human milk in population groups, factors which influence these levels are important. Studying these factors allows comparisons between studies and may enable prediction of population groups at an increased risk of morbidity and mortality from chronic low dose exposure from contaminants.

Several investigators (Jensen, 1983; Slorach & Vaz, 1983; Seifert & Caprioli, 1986) have reviewed the literature and outlined at least ten factors influencing the concentration of various lipophilic environmental contaminants in human milk. A number of investigators have evaluated the relationship of pesticide residue concentration in human milk with milk fat concentration (Stacey & Thomas, 1975; Barril et al., 1977; Vuori et al., 1977) and with the donor's weight

(Polishuk et al., 1977; Takahashi et al., 1981; Rogan et al., 1986).

The fat content of mature human milk is influenced very little by the nutritional status or dietary intake of the mother (WHO, 1985b). However, nutrients and other dietary components regulate xenobiotic metabolizing enzyme systems (Williams, 1978). Organohalogenated pesticides are substrates for the Mixed Function Oxidase (MFO) system (Hathcock, 1985). Therefore, diets which are low or marginal in certain nutrients or high in others may have an effect on the concentration of certain pesticides in body tissues and fluids even though the fat content of human milk may not be affected.

Since the first report of pesticides in human milk by Lang et al. (1951), oral contraceptives have been widely used in Western countries and in Third World countries. Oral contraceptives have been reported to decrease the fat content of human milk (Drury et al., 1985; Toddywalla et al., 1977). If the fat content of milk was decreased, this might contribute to lower concentrations of pesticides in a population group study.

Our objectives in this study were to examine DDE concentrations in human milk from South African women in relation to anthropometric indices; food frequency and consumption patterns; alcohol consumption; medication usage, including steroidal contraceptives, and the fat content of the milk.

Materials and Methods

Samples of human milk were collected with an electric breast pump from 184 individuals in Johannesburg between July and November 1985.

All women had delivered full term healthy singleton infants and were solely breast-feeding their infant at the time of the study. Donors were enrolled in the study during their post-partum check-up. All aspects of this study protocol were approved by the University of Witwatersrand, Johannesburg, South Africa, Committee for Research on Human Subjects. Information about the donors, and handling, storage, and analysis of the milk samples are described elsewhere (Zarba-Vary et al. (1989). Height and weight measurements were made by nurses during the post-partum check-up. The height was taken with subjects standing erect in bare feet, heels together and head in the Frankfort horizontal plane position. Height was recorded to the nearest centimeter. Weight was also taken in bare feet and light clothing (hospital examining gown), using a balance beam scale and recorded in kilograms. Weight was recorded to the nearest 0.1 kg.

Food consumption habits were determined by using a food frequency questionnaire. The food frequency questionnaire was modified from the one used by the California Department of Health (1975). Three different food frequency questionnaires were used for three different population groups the questionnaire for Blacks contained 53 items, for Whites 57 items, and 49 items for Colored Indian donors. Non-English speaking donors were interviewed by a trained researcher who spoke Africaans and 5 dialects. The questionnaire and food frequency schedule were checked for accuracy and acceptability by dietitians, nurses, and physicians working with the various population groups. The demographic questionnaire and food frequency questionnaire were

pretested to determine whether they were understood, whether they elicited the information desired, and the length of time to complete. Minimal changes were necessary. The approximate time to complete both forms was 45 minutes.

Statistical analysis

A data set consisting of p,p'-DDE for each individual along with the demographic and other information was constructed. The mean differences in p,p'-DDE among various classifications of the data were tested using F-statistics in the context of the analysis of variance. The independence of the proportions of individuals in various classifications from other demographic variables were examined using chi-square statistics. Correlations among measures were also calculated.

Results

Anthropometric indices

The means, standard deviations, and ranges for anthropometric measurements are given in Tables 1 and 2. These are calculated for 165 lactating women as a group and within each ethnic group.

Height

The mean height for this population was 158 cm. Black and Colored donors were 2 cm shorter, while White donors were 6 cm taller than the mean. A positive correlation was observed between age and height (Table 3). This correlation was significant for the entire group ($r = 0.29$; $p = 0.0002$) and among Black and White donors ($r =$

Table 1. Anthropometric measurements and indices for entire group of South African lactating women (n=165)

Variable	Mean	SD	Range
Height (cm)	158.0	0.08	140.0 - 187.0
Weight (kg)	60.4	12.7	39.0 - 105.5
Body mass index (wt/ht^2)	24.2	4.7	16.0 - 40.2

Table 2. Anthropometric measurements and indices for South African lactating women by ethnic group. Means ± SD, and (range) (n=165)

Ethnic Group	Height (cm)	Weight (kg)	Body Mass Index (kg/m^2)
Black	155.8 ± 0.06	61.0 ± 11.5	25.1 ± 4.3
	(144.0 - 176.0)	(41.8 - 105.5)	(18.1 - 40.1)
Colored	156.6 ± 0.05	55.2 ± 12.5	22.4 ± 4.5
	(144.0 - 168.0)	(39.0 - 91.2)	(16.0 - 35.2)
White	164.5 ± 0.08	65.0 ± 14.0	23.9 ± 5.2
	(152.0 - 187.0)	(43.2 - 94.1)	(17.2 - 31.2)

0.35; p = 0.0008 and r = 0.43; p = 0.005, respectively). Older donors among Black and White ethnic groups tended to be taller, while Colored donors were more likely to be similar in height.

Weight

The mean weight for the entire group and Black donors was similiar, 60.4 kg and 61.0 kg, respectively; whereas White donors weighed approximately 5 kg more on the average and Colored donors weighed 5 kg less on the average than the group as a whole. Only among Black donors was there a correlation between age and weight (Table 3). Younger Black donors were shorter and weighed less than older Black donors. A correlation between weight and age was observed for the entire group and among Black donors only (r = 0.30; p = 0.0001 and r = 0.37; p = 0.0004, respectively). The correlation between weight and height was also significant among Black donors, suggesting that the BMI, which adjusts weight for height, may better distinguish the relationship between age and weight in this ethnic group. There was a low correlation between weight and age for White and Colored donors, indicating that within each ethnic group, younger and older donors had similar weights. Younger donors tended to be shorter than older donors in the Black and White ethnic groups.

Body mass index

When the individual donor's BMI was compared with the concentration of DDE in the donor's milk (mg/kg fat) no significant correlations were found in the entire group or by ethnic group, except

among Colored donors ($r = 0.45$; $p = 0.007$). The concentration of DDE significantly increased in Colored donors (regression equation, DDE = -0.535 + 70.78(BMI)).

No significant differences were found among the various BMI classes and mean DDE concentration in human milk (mg/kg fat) (Table 4). Although not statistically significant, donors with BMI > 16 $\leq$ 20 had a mean concentration of DDE in milk (mg/kg fat) 35% lower than those with a BMI > 20 $\leq$ 25, and 49% lower than those donors with a BMI > 35. In a WHO report (1985a), the desirable average for BMI in women is suggested to be 20.8 with a range from 18.7 - 23.8. Women with a BMI > 28.6 are considered obese.

Diet

Food frequency analysis

Significant differences ($p = 0.0001$) among ethnic groups were present pertaining to the frequency of foods consumed per week. Black donors consumed fewer foods than Colored donors; Colored donors fewer than White donors. Among Indian donors the variability was too great to infer anything. Cereal grain products and fruits were consumed by Black, Colored, and White donors with similar frequency on a weekly basis. Foods which contributed to sources of fat in the diet, vegetable fats, and animal muscle and legumes were consumed with considerable differences in frequency per week. Black and Colored donors consumed dairy products 43% and 50%, respectively, as frequently as White donors. Black donors consumed sources of fat half as frequently as either White or Colored donors (Table 5).

Table 3. Correlations among anthropometric indices and DDE concentrations (mg/kg fat) and milk composition

Variable	Age	BMI	Weight	Lipid %
Height (cm)	0.29*	-0.09	0.37*	
	0.35*	-0.04	0.39*	
	0.14	0.10	0.41*	
	0.43*	-0.23	0.24	
Weight (kg)	0.30*	0.89*		
	0.37*	0.90*		
	0.12	0.95*		
	0.22	0.89*		
BMI	0.20*			
	0.24*			
	0.18			
	0.01			
DDE (mg/kg fat)		0.48		-0.29*
		-0.03		-0.25*
		0.45*		-0.39*
		0.02		-0.30

1st line = entire group
2nd line = Black donors
3rd line = Colored donors
4th line = White donors

*$p < 0.05$.

Table 4. Mean DDE concentration (mg/kg fat) in relation to body mass index

BMI Class	N	Mean p,p'-DDE mg/kg fat	SE
$\geq$ 16, $\leq$ 20	21	0.83	0.23
> 20, $\leq$ 25	86	1.27	0.12
> 25, $\leq$ 35	53	0.94	0.15
> 35	5	1.61	0.48

Table 5. Frequency of servings of food from major food groups per week consumed by lactating women

Food Group	Black Donors	White Donors	Colored Donors
Cereal & bread	28.0	23.9	28.1
Fruits	16.1	16.6	19.0
Vegetables	13.6	18.2	16.3
Sub-total A	57.7	58.7	63.4
Dairy products	14.0	31.7	15.8
Fats	11.3	23.9	20.5
Meat	16.7	16.0	12.9
Subtotal B	42.6	71.6	49.2
Total A + B	99.7	130.3	112.6

Analysis of variance examination of the effect of food consumption frequency in six major food groups per week on DDE concentration in human milk (mg/kg fat) showed no significant differences between the frequency of foods consumed in the meat and legume group, dairy products group, or fats group. Significant differences ($p = 0.05$) were found between the frequency of foods consumed in the bread and cereal grain group, and the vegetable group. A trend toward significance ($p = 0.06$) was found between the frequency of fruit consumption per week and DDE concentration in human milk (mg/kg fat). DDE concentration was found to be less by 14, 21, and 16 mg/kg fat for each time of consumption of such foods. This finding suggests a protective effect of plant based foods, perhaps in relation to absorption of DDE.

Alcohol consumption

One hundred eighty of 184 donors reported whether they consumed the equivalent of 1 oz spirits, 4 oz wine, or 12 oz beer and how frequently. Seven donors reported daily consumption of an alcoholic beverage, while 13 others reported weekly consumption of alcohol, ranging from 2-5 drinks/wk. No significant differences in mean DDE concentration in human milk (mg/kg fat) were found among the three general groups of consumers, i.e., non-consumers of alcohol, daily, or weekly consumers of alcohol. However, those who consumed alcohol on a weekly basis has a 32% higher mean DDE concentration in milk (mg/kg fat) than those who did not consume alcohol, and a 15% higher mean DDE concentration in milk (mg/kg fat) than daily consumers of alcohol.

Other maternal factors

Medications Of 184 donors, 28 reported taking prescribed medications at the time of this study. The types of medications and frequency of use are as follows: antibiotics, 3; cardiovascular agents, 3; anticonvulsants, 1; antiparasitic agents, 1; vaccination, 1; hormones, 16; bronchodilators, 3. Of those taking hormones, 15 donors reported taking these for contraceptive purposes. Thirteen donors regularly took oral contraceptives, and 2 donors received Depo-Provera. Donors using oral contraceptives had a significantly higher ($p = 0.05$) mean concentration of DDE (mg/kg fat) than those who were not using oral contraceptives (Table 6).

Fat content of milk

The average concentrations of milk fat in the samples among all of the groups were similar (Table 7). Significant correlations were found between the concentrations of DDE (mg/kg fat), and mg/kg whole milk and the percent lipid of the sample (Table 3). Although not statistically significant, similar trends in correlations were found in White donors for DDE mg/kg fat and mg/kg whole milk ($p = 0.06$, $p = 0.11$, respectively). Since all samples were randomized before analysis, differences may not be attributed to improved technique by repetition.

Discussion and Conclusion

The mean anthropometric indices among this group of South African lactating women were similar to those found in a WHO study (1985b).

Table 6. Mean DDE concentration (mg/kg fat) in relation to contraceptive use

Type of Contraceptive	N	Mean p,p'-DDE mg/kg fat	SE
Depo-Provera	2	1.28	0.75
Micronovum	13	1.73	0.30
No steroidal contraceptives	169	1.04	0.08

Table 7. Means and standard errors by ethnic group for 5 variables

Ethnic Group	Ave yrs.	Servings/ week	Lipid %	DDE mg/kg fat	Whole DDE mg/kg milk x 10^2
Black	26.3	99.7	3.50	0.895	2.96
(n=90) s.e.	(0.61)	(3.4)	(0.14)	(0.10)	(0.275)
Colored	23.8	112.2	3.04	1.00	2.67
(n=45) s.e.	(0.87)	(4.8)	(0.20)	(0.14)	(0.388)
White	25.9	130.5	3.59	1.30	4.40
(n=42) s.e.	(0.90)	(5.0)	(0.20)	(0.14)	(0.442)
Indian	23.4	142.6	3.31	0.76	2.35
(n=5) s.e.	(2.60)	(14.4)	(0.59)	(0.42)	(1.16)

In that study, Zaire urban donors were approximately 5 kg lighter in weight than donors from Soweto. Their BMI was also correspondingly smaller than donors from Soweto. Colored donors' anthropometric measurements and indices were similar to those of Zaire lactating women. Indian donors (4) were slightly shorter than low income Punjabi women, but weighed more and had a higher mean BMI than corresponding high income lactating Punjabi women (Hira et al., 1988).

Several investigators have evaluated the relationship between the donor's weight and the concentration of DDE and other persistent lipophilic contaminants in human milk (mg/kg fat) and found no relationship (Vuori et al., 1977; Takahaski et al., 1981; Rogan et al., 1986). Other investigators (Stacey & Thomas, 1975; Polishuk et al., 1977; Matuo et al., 1980) found no relationship between DDE concentration in whole human milk and the donor's weight. We also found no relationship between DDE concentration in human milk or human milk fat and the donor's weight.

Height and weight are by far the two more used and most useful body measurements but there is considerable lack of agreement on how best they should be expressed in relation to each other and to specific references values. The body mass index is useful in epidemiological studies of diverse population groups as an index of relative weight (Thomas et al., 1976). BMI is considered the most valid single clinical estimator of total body fat in females (Roche et al., 1981; Cronk & Roche, 1982). Our findings indicate that among White and Colored donors there was no correlation between age and

weight suggesting that within each ethnic group weights were similar, but a significant positive correlation between age and height was found among Black and White donor's. This suggests that older donors were taller. BMI was not correlated with height, but was strongly correlated with weight. Only among the Black donors is there a significant correlation between age and BMI. This suggests that BMI may better distinguish between age and weight in this group. DDE concentration (mg/kg fat) and BMI were positively correlated but only among the Colored donors. The inconsistencies between the various anthropometric correlations among ethnic groups is difficult to explain. A larger group of White and Colored donors may show significant correlations. While the lack of correlation between BMI and DDE concentration in White and Black donors may be masked by other factors such as parity, previous breast-feeding experience, diet, and energy balance.

Body mass index is a weight corrected for height index and should have little correlation with height of the population(s) to which it is applied but significant correlation with weight (Lee et al., 1981). It is a useful tool when there is an interest in body fitness or leanness and data can be compared with percentile from a nationally representative sample (Roche et al., 1981). A BMI of < 16 indicates very little total body fat; a BMI of > 35 indicates excessive total body fat, and a BMI of $> 20, \leq 25$ suggests appropriate total body fat for the individual (Bray, 1985).

The frequency of food consumed in general was significantly

different among the various ethnic groups of donors. Blacks consumed foods least frequently of all groups. This finding may be attributed to a distinct eating pattern (Langenhoven et al., 1988) and/or socioeconomic aspects. Since the dietary intake of these donors is unknown, we can only qualitatively evaluate the diet. Black donors consumed foods which contributed to total fat in the diet much less frequently than Colored or White donors. The differences in frequency of consumption of foods contributing to fat in the diet (i.e., meat group, fat group, and dairy products) was not as noticeably different among Black, Colored, and White donors. White donors consumed dairy products 2-2 1/2 times more frequently than either Colored or Black donors, respectively. Lück (1983) and Fourie (1986) indicate dairy products are important sources of DDE in South African foods. The greater frequency of consumption of dairy products may explain the significant differences in DDE concentration in human milk (mg/kg fat) among the ethnic groups. Maternal diet is known to influence the fatty acid composition of milk without changing milk volume or milk fat output by changes in the dietary fat (Mellies et al., 1979; Vuori et al., 1982); by changes in dietary energy (Insull et al., 1959); and by changes in dietary carbohydrate levels (Read et al., 1965a, 1965b; Vuori et al., 1982).

In our study, only 7 donors reported daily usage of alcohol. However, the differences among women in DDE concentration were too great to see a significant effect. Rogan et al. (1986) reported no effect of alcohol consumption (at least one drink per week) on DDE

concentrations in human milk.

Several investigators evaluated the correlation between the concentration of DDT and other lipophilic pesticides and the percent fat in human milk. De Campos and Olszyna-Marzys (1979) and Stacey and Thomas (1975) found no correlation between the amount of fat and the concentration of DDT reported on a whole milk basis. Stacey and Thomas obtained 40 ml samples from donors, which may be less than a full expression of breast milk. This may contribute to a lower amount of fat, since this may include fore and mid milk, but probably not hind milk. Hind milk is considerably higher in fat content. Also the time of collection during the day may be a factor. Wickström et al. (1983) reported a positive but not significant correlation between the concentration of total DDT in whole human milk and fat content of the donor's sample. Barril et al. (1977) reported a significant positive correlation ($r = 0.424$, $p = 0.025$) between total DDT in whole human milk and the fat content of the donor's sample. Our results were consistent with the previous two investigators ($r = 0.32$; $p = 0.0001$; $r = 0.17$; $p = 0.01$) with and without three high values removed, respectively. However, the observation of a negative correlation ($r = -0.29$; $p = 0.0001$) between DDE concentration (mg/kg fat) and the fat content of the donor's sample is interesting. Since milk samples were collected at only one time during the day and the diurnal pattern of fat concentration among women is known to vary, the significance of this association is unclear. It may be that other maternal factors are contributing to this finding.

Oral contraceptives are known to have a variety of effects on lipid metabolism (Fotherby, 1985). Drury et al. (1985) and Toddyvalla et al. (1977) reported that the use of depo-medroxyprogesterone acetate (DMPA) decreases the fat content of milk over a period of 4-6 months. Sas et al. (1986) reported a significantly higher concentration of fat in human milk among well-nourished mothers whose diets contributed 38-42% energy from fat then. In our study, 15 donors reported the use of steroidal contraceptives. Thirteen of the 15 donors reported the use of oral contraceptives (OC). Among the thirteen reporting OC use, 11 were White donors. OC users had significantly higher concentrations of DDE in human milk than donors who reported no use of steroidal contraceptives. Among White donors there was no significant inverse relationship between fat content and DDE concentration (Table 3). Recently, Rogan et al. (1987) reported that higher levels of DDE were associated with shorter lactation time. These women were not taking any OCs. He hypothesized that shorter duration of lactation may be due to an inhibitory effect of DDE. The o,p-DDT and its metabolite are weak estrogens which are known to depress lactation. Zarba-Vary et al. (1989) reported elsewhere that among White donors in this study, the average length of lactation was considerably shorter than Black or Colored donors. White donors also had significantly higher concentrations of DDE than Black or Colored donors (Table 7). Whether or not this is a chance occurrence among OC users occurs pursuing.

Human milk is the ideal and recommended nourishment for infants

until 6 months. In many parts of the world, the infant continues to receive a significant portion of its nourishment from its mother's milk for an extended period of time. The amount of DDE consumed in human milk can be estimated (Table 8).

The Codex Alimentaries Commission (FAO/WHO, 1974) has established a maximum residue limit (mrl) of 1.25 mg/kg fat for total DDT in raw agricultural commodities, while the South African mrl for total DDT is set at 1.00 mg/kg fat. The World Health Organization has set an Acceptable Daily Intake (ADI) for total DDT at .005 mg/kg body weight. In Table 11, the average intake of DDE for a 3-month infant consuming the average DDE in human milk from this study would be less than the ADI. The infant intake was reported by Hofvander et al. (1981) and Stastny et al. (1984), while the mean weight for a 3-month infant is also from the literature. In this study, 24% of the donors had concentrations of DDE in human milk which would exceed the ADI. DDE is less toxic than DDT and to date no case reports of illness due to environmental chemicals through human milk have been reported (Rogan, 1980, 1987). But there are regions of world where DDT continues to be used extensively whose populations are already at an increased risk of morbidity and mortality in which continued frequent monitoring of DDT/DDE in human milk is encouraged.

Table 8. Estimated intake of DDE from human milk, South Africa

	3 mos infant
Avg. quantity human milk consumed	780 g/day
Avg. body wt. infant, kg	6.3
Mean conc. DDE whole human milk, mcg/kg	34 (tr-306)
Mean conc. DDE human milk fat, mg/kg	1.1 (tr-9.7)
Mean fat content, %	3.4 (0.80-8.7)
Daily intake DDE from human milk, mcg/kg body wt.	4.2 (tr-38)
ADI for DDT mcg/kg	5

References

Barril, C. R., Reyes, N. A., Consolacion, E. C., Rosellon, L. B. (1977). DDT residue levels in human milk in Laguna, Philippines. Philipp. Entomologist 3, 321-338.

Bray, G. A. (1985). Obesity: Definition, diagnoses, and disadvantages. The Medical Journal of Australia 142, 52-58.

California Department of Health (1975). Nutrition during pregnancy and lactation. Sacramento, Calif.

Cronk, C. E. Roche, A. F. (1982). Race-and sex-specific reference data for triceps and subscapular skinfolds and weight/stature^2. Am. J. Clin. Nutr. 35, 347-354.

de Campos, M., Olszyna-Marzys, A. F. (1979). Contamination of human milk with chlorinated pesticides in Guatemala and in El Salvador. Arch. Environm. Contam. Toxicol. 8, 43-58.

Drury, P. J., Crawford, M. A., Ayeni, O., Pinol, A. (1985). Comparative studies on human milk lipids in Hungary and Thailand and the effect of steroidal contraceptives. In J. Schaub (Ed.), Composition and Physiological Properties of Human Milk, (pp. 189-200). Elsevier Science Publishers, New York.

FAO/WHO Food Standards Programme. (1974). Recommended International Maximum Limits for Pesticide Residues: Fourth Series CAC/RS 65-1974 Food and Agricultural Organization, Rome.

Fotherby, K. (1985). Oral contraceptives, lipics, and cardiovascular disease. Contraception 31, 367-394.

Fourie, H. O. (1986). Toxicological consequences of pesticide uses in the Republic of South Africa. Ph.D. dissertation, University of Cape Town.

Hathcock, J. N. (1985). Nutrient and non-nutrient effects on drug metabolism. Drug and Nutr. Interact. 4, 217-234.

Hira, C. K., Murgai, V., Palhak, V. (1988). Nutritional intake of lactating Punjabi women. Ecology of Food and Nutr. 21, 159-166.

Hofvander, Y., Hagman, U., Linder, C. E., Vaz, R., Slorach, S. A. (1981). WHO collaboration breast feeding study. I. Organochlorine contaminants in individual samples of Swedish human milk, 1978-79. Acta Paediatr. Scand. 70, 3-8.

Insull, W., Jr., Hirsch, J., James, T., Ahrens, E. H., Jr. (1959). The fatty acids of human milk. II. Alteration produced by manipulation of caloric balance and exchange of dietary fats. J. Clin. Invest. 38, 443-450.

Jensen, A. A. (1983). Chemical contaminants in human milk. Residue Rev. 89, 1-128.

Langenhoven, M. L., Steyn, K., Van Eck, M. (1988). The food and meal pattern in the Cape Penisular Colored population. Ecology Food and Nutr. 22, 107-116.

Lang, E. P., Kunei, F. M., Prickett, C. S. (1951). Occurrence of DDT in human fat and milk. Arch. Ind. Hyg. 3, 245-246.

Lee, J. Kolonel, L. N., Hinds, M. W. (1981). Relative merits of the weight-corrected for height indices. Am. J. Clin. Nutr. 34, 2521-2529.

Lück, H. (1983). Pesticides in milk and dairy products. S. Afr. J. Dairy Tech. 15, 75-79.

Matuo, Y. K., Lopes, J. N. C., Lopes, J. L. C. (1980). DDT levels in human milk from Ribeiraõ Preto (Brasil). Rev. Brasil. Biol. 40, 293-296.

Mellies, M. J., Ishikawa, T. T., Gartside, P. S., Burton, K., MacGee, J., Allen, K., Steiner, P. M., Brady, D., Glueck, C. J. (1979). Effects of varying maternal dietary fatty acids in lactating women and their infants. Am. J. Clin. Nutr. 32, 229-302.

Polishuk, Z. W., Ron, W., Wasserman, M., Cucos, S., Wasserman, D., Lemesch, C. (1977). Organochlorine compounds in human blood plasma and milk. Pest. Monit. J. 10, 121-129.

Read, W. W. C., Lutz, P. G., Tashjian, A. (1965a). Human milk lipids. II. The influence of dietary carbohydrates and fat on the fatty acids of mother's milk, a study of four ethnic groups. Am. J. Clin. Nutr. 17, 180-183.

Read, W. W. C., Lutz, P. G., Tashjian, A. (1965b). Human milk lipids. III. Short term effects of dietary carbohydrate and fat. Am. J. Clin. Nutr. 17, 184-187.

Roche, A. F., Siervogel, R. M., Chumlea, W. C., Webb, P. (1981). Grading body fatness from limited anthropometric data. Am. J. Clin. Nutr. 34, 2831-2838.

Rogan, W. J., Bagniewska, A., Damstra, T. (1980). Pollutants in breast milk. N. Engl. J. Med. 302, 1450-1453.

Rogan, W. J., Gladen, B. C., McKinney, J. D., Carreras, N., Hardy, P., Thullen, J., Tingelstad, J., Tully, M. (1987). Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: Effects on growth, morbidity, and duration of lactation. Am. J. Public Health 77, 1294-1297.

Rogan, W. J., Gladen, B. C., McKinney, J. D., Carreras, N., Hardy, P., Thullen, J., Tingelstad, J., Tully, M. (1986). Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: Effects of maternal factors and previous lactation. Am. J. Public Health 76, 172-177.

Sas, M., Gellen, J. J., Dusitsin, N., Tunkeyoon, M., Chalapati, S., Crawford, M. A., Drury, P. J., Lenihan, T., Ayeni, O., Pinol, A. (1986). An investigation on the influence of steroidal contraceptives on milk lipid and fatty acids in Hungary and Thailand. Contraception 33, 159-178.

Seifert, W. E. Jr. Caprioli, R. M. (1986). Chemical contaminants in human milk. In R. R. Howell, F. R. Moriss, Jr., L. K. Pickering (Eds.), Human Milk in Infant Nutrition and Health. (pp. 279-300). Chas. C. Thomas Publisher, Springfield.

Slorach, S. A. Vaz, R. (1983). Assessment of Human Exposure to Selected Organochlorine Compounds through Biological Monitoring. Swedish National Food Administration, Uppsala.

Stacey, C. I. Thomas, B. W. (1975). Organochlorine pesticide residues in human milk, Western Australia--1970-71. Pest. Monit. J. 9, 64-66.

Stastny, D., Vogel, R. S., Picciano, M. F. (1984). Manganese intake and serum manganese concentration of human milk-fed and formula-fed infants. A. J. Clin. Nutr. 39, 872-879.

Takahashi, W., Saidin, D., Takei, G., Wong, L. (1981). Organo-chlorine pesticide residues in human milk in Hawaii, 1979-80. Bull. Environm. Contam. Toxicol. 27, 506-511.

Thomas, A. E., McKay, D. A., Cutlip, M. B. (1976). A monograph method for assessing body weight. Am. J. Clin. Nutr. 29, 302-304.

Toddywalla, V. S., Joshi, L., Virkar, K. (1977). Effect of contraceptive steroids on human lactation. Am. J. Obstet. Gynecol. 127, 245-249.

Vuori, E., Kiueu, K., Makinen, S. M., Vayrynen, P., Kara, R., Kuitunen, P. (1982). Maternal diet and fatty acid pattern of breast milk. Acta Paediatr. Scand. 71, 959-963.

Vuori, E., Tyllinen, P., Kuitunen, P., Pagarus, A. (1977). The occurrence and origin of DDT in human milk. Acta. Paediatr. Scanda. 66, 761-765.

WHO. (1985a). Energy and protein requirements. WHO Technical Report Series No. 724, Report of Joint FAO/WHO/UNU Expert Consultation, World Health Organization, Geneva.

WHO. (1985b). The Quantity and Quality of Breast Milk. pp. 61-63. World Health Organization, Geneva.

Wickström, K., Pyysalo, H., Siimes, M. A. (1983). Levels of chlordane, hexachlorobenzene, PCB, and DDT compounds in Finnish human milk in 1982. Bull. Environ. Contam. Toxicol. 31, 251-256.

Williams, R. T. (1978). Nutrients in drug detoxication reactions. In J. N. Hathcock J. Coon (Eds.), Nutrient and Drug Interrelations. (pp. 303-318). Academic Press, New York.

Zarba-Vary, A., Stahr, H. M., Pettifor, J. J. M., Sefuba, M., Hathcock, J. N. (1989). The influence of maternal and environmental factors on the concentration of DDE residues in human milk from South African women. Food and Chem. Toxicol. in preparation.

GENERAL SUMMARY AND CONCLUSIONS

Limited research is available on the levels of DDE in human milk in various African populations, and also among South African women. A research study was conducted to determine baseline levels of p,p'-DDE in human milk and to examine selected factors that affect the concentration of DDE in human milk (mg/kg fat). The city of Johannesburg was chosen as the research site because of its large urban population, temperate climate, and representative food supply from around South Africa.

On hundred and eighty-four lactating women, 15 to 42 yrs were selected to represent healthy lactating women who were solely breast-feeding singleton infants.

Data on demographic information, previous breast-feeding experience, medication usage, smoking habits, pesticide usage, and dietary information were obtained through a personal interview. Participants were categorized by ethnic group, Black, White, Colored, and Indian. A full expression of breast milk was collected between 8 am and 12 noon typically at the second feeding of the day, either at the post-partum clinic or at the donor's home. Milk was analyzed for fat'content and the concentration of p,p'-DDE using a modification of the 'FDA' method.

The mean concentration of DDE in human milk (mg/kg fat) was not significantly affected by ethnic group, age, or age class, parity, and total previous lactation or the ethnic group by age or age class, parity, or total previous lactation time interaction. Some

investigators have shown various effects on DDE concentration and others have not. This antecedent was reviewed recently by Jensen (1983) and Seifert and Caprioli (1986).

Cigarette smoking habits, current and in the past, pesticide usage, occupation, and location of residence, i.e., urban and rural or native-born and emigrant did not significantly affect the mean concentration of DDE in human milk (mg/kg fat) among donors.

Within ethnic groups, a significant positive correlation between the concentration of DDE (mg/kg fat) and age class were seen among Colored donors, while a significant inverse relationship between total previous lactation time and the concentration of DDE (mg/kg fat) were seen in Black and White donors. If indeed the correlations seen among Black and White donors are different than factors contributing to the pattern of elimination of DDE in human milk (mg/kg fat) should be pursued.

Based on the Global Environmental Monitoring System (GEMS), the mean concentration of DDE in human milk (mg/kg fat) is comparable to values reported for Sweden and Federal Republic of Germany (Table 1). It is important to be cautious in interpreting these values as this may reflect baseline levels for only region of a large and diverse country. Values may be higher in coastal areas where temperature, soil conditions, and pesticide usage patterns may contribute to higher levels.

In Part 2 of this dissertation, the effect of other maternal and dietary factors on the concentration of DDE in human milk (mg/kg fat)

Table 1. Levels of p,p'-DDE in the fat of human milk. Medians and ranges are shown and for p,p'-DDE levels the 90th percentiles. Data for all the mothers studied

Country/Area	Samples Collected		Mother's age, years	No. of samples		Fat (%)	p,p'-DDE, mg/kg fat median/90th perc. (range)
	Year(s)	Wks. post-partum		Total	Postive		
Belgium	1982	3	26	47	47	2.7	0.94/3.0
Brussels		(1-37)	(17-30)			(1.1-6.0)	(0.15-5.2)
China	1982	5	27	100	100	2.5	4.4/7.2
Beijing		(1-17)	(22-31)			(0.19-6.3)	(0.66-26)
F R Germany	1981	1	25	81	81	3.1	1.2/2.4
Hanau		(1)	(15-38)			(0.8-6.2)	(0.12-5.1)
India	1982	5	24	50	50	4.8	4.8/13
Ahmedabad		(1-34)	(18-30)			(1.8-9.2)	(0.39-17)
Israel	1981/82	4	26	52	52	3.4	2.2/6.5
Jerusalem		(1-16)	(19-30)			(0.90-7.9)	(0.50-8.1)
Japan	1980/81	12	27	107	107	3.1	1.5/3.1
Osaka		(6-22)	(19-35)			(1.3-8.0)	(0.39-5.4)
Mexico	1981	12	21	48	48	3.1	3.7/7.0
Morelia		(3-32)	(16-37)			(0.43-6.6)	(1.2-30)
Sweden	1981	13	27	58	58	2.8	0.81/1.5
Uppsala		(12-15)	(21-31)			(0.61-5.9)	(0.36-2.2)
USA	1979	7	28	50	50	2.6	1.6/2.6
22 states		(1-75)	(19-38)			(0.3-6.3)	(0.40-17)
Yugoslavia	1981/82	4	26	50	50	3.7	1.9/3.9
Zagreb		(1-22)	(18-31)			(1.5-7.4)	(0.20-4.8)
RSA	1985	7	25	184	184	3.4	0.83/1.8
Johannesburg		(4-25)	(15-42)			(0.80-8.7)	(tr-9.7)

are presented and discussed.

There have been few research studies which evaluated the effects of maternal anthropometry, alcohol consumption, fat content, and hormonal use on the concentration of DDE or other persistent environmental contaminants.

In our study, maternal anthropometry and alcohol consumption did not significantly affect the concentration of DDE in human milk (mg/kg fat). The significant positive correlation between BMI and DDE concentration among Colored donors is interesting and requires further investigation to rule out chance occurrence.

A number of research studies have attempted to explain the contribution of DDE in human milk by consumption of certain foods. In our study, dietary patterns pertaining to the frequency of food consumption and food composition were significantly different among ethnic groups. Although White donors consumed more frequently sources of food more like to contribute to DDE in the diet, a different effect was seen. Frequencies of foods consumed in the bread and cereal grain group and the vegetable group had a significant inverse effect on DDE concentration in human milk. There are at least several possible explanations for this observation. Dietary fiber increases fecal bulk which can reduce the concentration of DDE in the gut. Increased fecal bulk also decreases the transit time of substance in the gut and thereby reduces intestional absorption of the substances. Certain types of fibers are known to be in xenobiotics which may also reduce absorption of DDE.

Fat content and hormonal usage did significantly affect the mean concentration of DDE in human milk (mg/kg fat). The significant inverse relationship between fat content and DDE concentration is interesting and is difficult to interpret at this time. Maternal age and parity are known to affect the fat content of human milk.

The variation of DDE concentration in human milk might be expected but to the individual's absorption and excretion of DDE, nutritional and other socio-economic factors. The variation of DDE in human milk is probably greater between women than within a woman's day to day milk supply despite the factors affecting the lipid component of milk which transports DDE and other environmental contaminants. Fat content varies within a feeding, between breast, diurnally, and during lactation. Before the various factors which effect DDE concentration can be elicited, what constitutes a representative sample of human milk for environmental contaminants either specific ones and/or collectively must first be determined.

GENERAL BIBLIOGRAPHY

Acker, L. & Schulte, E. (1970). Über das Vorkommen von chlorierten Biphenylen und Hexachlorbenzol neben chlorièrten Insektiziden in Humn milch und menschlichem Fettgewebe. Naturwissenschaften 57, 497.

Al-Omar, M. A., Tawfig, S. J., & Al-Ogasly, N. (1985). Organochlorine residue levels in human milk from Baghdad. Bull. Environ. Contam. Toxicol. 35, 65-67.

Anderson, G. H., Atkinson, S. A., & Bryan, M. H. (1981). Energy and micronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. Am. J. Clin. Nutr. 34, 258-265.

Anon. (1980). Control of pests. Community Health in South Africa. August, pp. 2-15.

Atherton, H. V. & Newlander, J. A. (1977). Chemistry and Testing of Dairy Products. 4th ed. Avi Publishing Company, Westport, Conn.

Atkinson, S. A. (1979). Chemical contamination of human milk: A review of current knowledge. J. Can. Dietet. Assoc. 40, 223-226.

Atuma, S. S. & Vaz, R. (1986). A pilot study on levels of organochlorine compounds in human milk in Nigeria. Int. J. Environ. Anal. Chem. 26, 187-192.

Bakken, A. F. & Seip, M. (1976). Insecticides in human breast milk. Acta Paediatr. Scand. 65, 535-539.

Barnett, R. W., D'Ercole, A. J., Cain, J. D., & Arthur R. D. (1979). Organochlorine pesticide residues in human milk samples from women living in northeast and northwest Mississippi, 1973-75. Pestic. Monit. J. 13, 47-51.

Barril, C. R., Reyes, N. A., Consolacion, E. C., & Rosellon, L. B. (1977). DDT residue levels in human milk in Laguna, Philippines. Philipp. Entomologist 3, 321-338.

Barthel, R., Nguyen, Thi, H. (1983). Stillen ohne Angst. Kriterienvergleichende Untersuchungen auf Schadstoffe in der Muttermich. Die Medizinische Welt. 34, 967-73.

Bradshaw, E. & Harington, J. S. (1985). The changing pattern of cancer mortality in South Africa, 1949-1979. SAMJ 68, 455-465.

Bradt, P. T. & Herrenkohl, R. C. (1976). DDT in human milk. What determines the levels? Sci. Total Environ. 6, 161-163.

Brevik, E. M. (1978). Gas chromatographic method for the determination of organochlorine pestides in human milk. Bull. Environ. Contamin. Toxicol. 19, 281-286.

Brevik, E. M. & Bjerk, J. E. (1978). Organochlorine compounds in Norwegian human fat and milk. Acta Pharmacol. et Toxicol. 43, 59-63.

Brewer, J. (1986). After Soweto, an Unfinished Journey. Clarendon Press, New York.

Brown, A. C. (1987). Marine pollution and health in South Africa. SAMJ 71, 244-248.

Brown, J. R. (1972). Concentration of chlorinated hydro-carbon pesticides in human blood and their relation to the concentration in depot fat. Toxicol. Appl. Pharmacol. 22, 327-328.

Bush, B., Snow, J. T., & Connor S. (1983). High resolution gas chromatographic analysis of nonpolar chlorinated hydrocarbons in human milk. J. Assoc. Off. Anal. Chem. 66, 248-255.

Calabrese, E. J. (1982). Human breast milk contamination in the United States and Canada by chlorinated hydrocarbon insecticides and industrial pollutants: Current status. J. Amer. Coll. Toxicol. 1, 91-98.

CIIR, Catholic Institute for International Relations. (1986). South Africa in the 1980s-State of Emergency. pp. 15-17. Russell Press Ltd., Nottingham.

Clark, R. M., Ferris, A. M. Fey, M., Brown, P. B., Hundriesser, K. E., Jensen, R. G. (1982). Changes in the lipids of human milk from 2 to 16 weeks postpartum. J. Pediatr. Gastroenterol. Nutr. 1, 311-315.

Coats, J. R. (1982). Structure-activity relationships in DDT analogs. In J. R. Coats (Ed.), Insecticide Mode of Actin. (pp. 29-43). Academic Press, New York.

Collins, G. B., Holmes, D. C., & Hoodless, R. A. (1982). Organochlorine pesticide residues in human milk in Great Britain, 1979-80. Human Toxicol. 1, 425-431.

Conacher, H. B. S. (1987). Importance of quality assurance standards in Canadian pesticide analysis. J. Assoc. Off. Anal. Chem. 70, 941-943.

Cubitt, G. & Helfet, A. (1983). South Africa. C. Struik (Pty) Ltd., Cape Town.

Curley, A. & Kimbrough, R. (1969). Chlorinated hydrocarbon insecticides in plasma and milk of pregnant and lactataing women. Arch. Environ. Health 18, 156-164.

Currie, R. A., Kadis, V. W., Breitkreitz, W. E., Cunningham, & G. B., Bruns, G. W. (1970). Pesticides residue in human milk, Alberta, Canada--1966-1970, 1977-78. Pestic. Monit. J. 13, 52-55.

Davies, J. E., Edmundson, W. F., Raffonelli, A., Cassady, J. C., & Morgade, C. (1972). The role of social class in human pesticide pollution. Am. J. Epidemiol. 96, 334-341.

Davis P. D. & Carroll, L. (1982). Donor breast milk and growth of preterm babies. In Human milk banking (pp. 171-8). International Institute of Refrigeration, Paris.

de Beer, C. (1984). The South African Disease: Apartheid Health and Health Service. South African Research Service, Johannesburg.

de Campos, M. & Olszyna-Marzys, A. F. (1979). Contamination of human milk with chlorinated pesticides in Guatemala and in El Salvador. Arch. Environ. Contam. Toxicol. 8, 43-58.

de Faubert Maunder, J. F., Egan, H., Godly, E. W., Hammond, E. W., Roburn, J., & Thomson, J. (1964). Clean-up of animal fats and dairy products for analysis of chlorinated pesticide residues. Analyst 89, 168-174.

de Gruchy, J. W. (1986). The Church Struggle in South Africa. Wm. B. Eerdmans Publishing Co., Grand Rapids.

Dillon, J. C., Martin, G. B., & O'Brien, H. T. (1981). Pesticide residues in human milk. Food Cosmet. Toxicol. 19, 437-442.

Dyment, P. O., Hebertson, L. M., & Decker, W. J. (1971). Relationship between levels of chlorinated hydrocarbon insecticides in human milk and serum. Bull. Environ. Contam. Toxicol. 6, 449-452.

Eckenhausen, F. W., Bennett, D., Beynon, K. I., & Elgar, K. E. (1981). Organochlorine pesticide concentration in perinatal samples from mothers and babies. Arch. Environ. Health 36, 81-92.

EPA. (1980). Manual of analytical methods for the analysis of pesticides in human and environmental samples. U. S. Environmental Protection Agency EPA-600/8-80-038. U. S. Government Printing Office, Washington, DC.

Erickson, D. R. & Dunkley, W. L. (1964). Spectrophotometric determination of tocopherol in milk and milk lipids. Anal. Chem. 36, 1055-1058.

FAO. (1980). Yearbook of Fishery Statistics, Food and Agricultural Organization, Rome.

FAO. (1982). Apartheid, poverty, and malnutrition. Food and Agriculture Organization, Rome. FAO Economic and Social Development Paper 24.

FAO/WHO Food Standards Programme. (1974). Recommended International Maximum Limits for Pesticide Residues: Fourth Series CAC/RS 65-1974 Food and Agricultural Organization, Rome.

Ferris, A. M. & Jensen, R. G. (1984). Lipids in human milk: A review. I. Sampling, determination, and content. J. Pediatr. Gastroenterol. Nutr. 3, 108-122.

Fooken, C. & Butte, W. (1987). Organochlorine pesticides and polychlorinated biphenyls in human milk during lactation. Chemosphere 6, 1301-1309.

Fourie, H. O. (1986). Toxicological consequences of pesticide uses in the Republic of South Africa. Ph.D. dissertation, University of Cape Town.

Frings, C. S. & Dunn, R. T. (1970). A colorimetric method for determination of total serum lipids based on the sulfophospho-vanillin reaction. Am. J. Clin. Pathol. 63, 89-91.

Fuggle, R. F. (1983). Elements of the natural environment. In R. F. Fuggle and M. A. Rabie (Eds.), Environmental Concerns in South Africa. (pp. 9-29). Juta and Co., Ltd., Cape Town.

Fytianos, K., Vasilikiotis, G., Weil, L., Kavlendis, E., & Laskaridis, N. (1985). Preliminary study of organochlorine compounds in milk products, human milk, and vegetables. Bull. Environ. Contam. Toxicol. 34, 504-508.

Garza, C., Johnson, C. A., O'Brian Smith, E., & Nichols, B. L. (1983). Changes in the nutrient composition of human milk during gradual weaning. Am. J. Clin. Nutr. 37, 61-65.

Garza, C., Johnson, C. A., Harrist, R., & Nichols, B. L. (1982). Effects of methods of collection and storage on nutrients in human milk. Early Hum. Dev. 6, 295-303.

Geyer, H. J., Scheunert, I., Korte, F. (1987). Correlation between the bioconcentration' potential of organic environmental chemicals in humans and their n-octanol/water partition coefficients. Chemosphere 16, 239-252.

Gibbs, J. H., Fisher, C., Bhattacharya, S., Goddard, P., & Baum, J. D. (1977). Drip breast milk: Its composition, collection, and pasteurization. Early Hum. Dev. 1/3, 227-245.

Global Environmental Monitoring System. (1985). Guidelines for the Study of Dietary Intakes of Chemical Contaminants. World Health Organization, Geneva.

Graca, I., Fernandes, A. M. S. S., & Mourão, H. C. (1974). Organochlorine insecticide residues in human milk in Portugal. Pestic. Monit. J. 8, 148-156.

Grant, J. P. (1984). The State of the World's Children 1985. Oxford University Press, New York.

Green, D., Moye, L., Schreiner, R. L., & Lemons, J. A. (1982). The relative efficacy of four methods of human milk expression. Early Hum. Dev. 6, 158-159.

Gunther, M. & Stainer, J. E. (1949). Diurnal variation in the fat content of breast milk. Lancet 2, 235-242.

Gunther, S. A. (1980). Interpreting pesticide residue data at the analytical level. Residue Rev. 76, 155-171.

Hall, B. (1979). Uniformity of human milk. Am. J. Clin. Nutr. 32, 304-312.

Hamosh, M., Bitman, J., Fink, C. S., Freed, L. M., York, C. M., Wood D. L., Mehta, N. R., & Hamosh, P. (1985). Lipid composition of preterm human milk and its digestion by the infant. In J. Schaub (pp. 153-162). Composition and Physiological Properties of Human Milk. Elsevier Science Publishers, New York.

Hartmann, P. E. & Kluski, J. K. (1978). Changes in the composition of the mammary secretion of women after abrupt termination of breast feeding. J. Physiol. 275, 1-11.

Hayes, W. J. (1975). Toxicology of Pesticides. Williams and Wilkins, Baltimore.

Hayes, W. J. (1982). Pesticides Studied in Man. William and Wilkins, Baltimore.

Hayes, W. J., Jr., Dale, W. E., & Pirkle, C. I. (1971). Evidence of safety of longterm, high, oral doses of DDT for man. Arch. Environ. Health 22, 119-135.

Hayes, W. J., Jr., Dale, W. E., & Pirkle, C. I. (19). Effect of known repeated oral doses of DDT, Working Paper 6.12. Expert Committee on Insecticides (Toxic Hazards to Man). World Health Organization, Geneva.

Hayes, W. J., Jr., Durham, W. F., & Cueto, C., Jr. (1956). The effect of known repeated oral doses of chlorophenothane (DDR) in man. JAMA 162, 890-897.

Hergenrather, J., Hlady, G., Wallace, B., & Savage, E. (1981). Pollutants in breast milk of vegetarians. N. Eng. J. Med. 304, 302.

Heyns, R. (1986). South Africa 1986 Official Yearbook of the Republic of South Africa. CTP Book Printers, Cape Town.

Hibberd, C. M., Brooke, O. G., Carter, N. D., Haug, M., & Harzer, G. (1982). Variation in the composition of breast milk during the first 5 weeks of lactation. Implications for the feeding of premature infants. Arch. Dis. Child. 57, 658-662.

Hofvander, Y., Hagman, U., Linder C. E., Vaz, R., & Slorach, S. A. (1981). WHO collaborative breast feeding study. I. Organochlorine contaminants in individual samples of Swedish human milk, 1978-79. Acta Paediatr. Scand. 70, 3-8.

Horowitz, W. (1975). Official Methods of Analysis. 13th ed. Association of Official Analytical Chemists, Washington, D.C.

Hudson, G. J., Gerber, H., & John, P. M. W. (1979). Creamatocrit procedure versus triacylglyerol analysis. A comparison of methods for the determination of human milk fat in epidemiological studies. J. Hum. Nutr. 33, 283-287.

Hundrieser, K. E., Clark, R. M., Jensen, R. G., & Ferris, A. M. (1984). A comparison of methods for determination of total lipids in human milk. Nutr. Res. 4, 21-26.

Hutson, D. H. (1981). The metabolism of insecticides in man. Progress in Pesticide Biochemistry 1, 287-333.

Hytten, F. E. (1954a). Clinical and chemical studies in human lactation. II. Variations in major constituents during a feeding. Br. Med. J. i, 176-179.

Hytten, F. E. (1954b). Clinical and chemical studies in human lactation. III. Diurnal variations in major constituents during a feed. Br. Med. J. i, 179-82.

Jensen, A. A. (1983). Chemical contaminants in human milk. Residue Rev. 89, 1-128.

Jensen, R. C. & Clark, R. (1984). Methods of lipid analysis. J. Pediatr. Gastroenterol. Nutr. 3, 296-299.

Jensen, R. G., Bigman, J., Wood, L., Hamosh, M., Clandinin, M. T., & Clark, R. M. (1985). In R. G. Jensen and M. C. Neville (Eds.), Human Lactation, Milk Components, and Methodologies. (pp. 97-112). Plenum Press, New York.

Jensen, R. G., Clark, R. M., & Ferris, A. M. (1980). Composition of the lipids in human milk: A review. Lipids 15, 345-355.

Kalra, R. L. & Chawla, R. P. (1981). Occurrence of DDT and BHC residues in human milk in India. Experientia 37, 404-405.

Kanja, L., Skåre, J. U., Nafstad, I., Maitai, C. K., & Løkken, P. (1986). Organochlorine pesticides in human milk from different areas of Kenya 1983-1985. J. Toxicol. Environ. Health 19, 449-464.

Kaplan, I. & Nelson, H. D. (1981). The society and its environment. In H. D. Nelson (Ed.), South Africa, a country study. (pp. 63-158). U. S. Government Print Office, Washington, D.C.

Karakaya, A. E., Burgaz, S., & Kanzik, I. (1987). Organochlorine pesticide contaminants in human milk from different regions of Turkey. Bull. Environ. Contamin. Toxicol. 39, 506-510.

Knoll, W. & Jayaraman, S. (1973a). Zur kontamination von humanmilch mit chlorierten kohlenwasserstoffen. Nahrung 17, 599-615.

Knoll, W. & Jayaraman, S. (1973b). Chlorierte kohlenwasser stoffe in human milch. Z. Ges. Hyg. 19, 43-45.

Krauthacker, B., Alebic-Kolbah, T., Buntic, A., Tkalcevic, B., & Reiner, E. (1980). DDT residues in samples of human milk and in mothers and cord blood serum in a continental town in Croatia (Yugoslavia). Int. Arch. Occup. Environ. Health 46, 267-273.

Kroger, M. (1972). Insecticide residues in human milk. J. Pediatr. 80, 401-405.

Kutz, F. W., Yobs, A. R., Strassman, S. C., & Viar, J. F. (1977). Effects of reducing DDT usage on total DDT storage in humans. Pestic. Monit. J. 11, 61-63.

Kuwabara, K., Maeda, K., Murakami, Y., & Kashimoto, T. (1982). Comparative studies on the recovery of organochlorine pesticides between dry column chromatography and acetonitrile partitioning. Bull. Environ. Contam. Toxicol. 29, 347-353.

Lampbert, L. M. (1964). Rapid separation of fat for pesticide residue analysis of milk products. J. Dairy Sci. 47, 1013-1014.

Langlois, B. E., Stemp, A. R., & Liska, B. J. (1963). Milk fat from the Babcock Test for insecticide residue analysis in dairy products. J. Dairy Sci. 46, 854-855.

Laug, E. P., Kunai, F. M., & Prickett, C. S. (1951). Occurrence of DDT in human fat and milk. Arch. Ind. Hyg. 3, 245-246.

Lawrence, R. A. (1985). Breastfeeding: A Guide for the Medical Profession. 2nd ed. The C. V. Mosby Company, St. Louis.

Lönnerdal, B., Smith, C., & Keen, C. L. (1984). Analysis of breast milk. Current methodologies and future needs. J. Pediatr. Gastroenterol. Nutr. 3, 290-295.

Loomis, T. A. (1978). Essentials of Toxicology. 3rd ed. Lea and Febiger, Philidelphia.

Lucas, A., Gibbs, J. A. H., Lyster, R. L. J., & Baum, J. D. (1978). Creamatocrit; simple clinical technique for estimating fat concentration and energy value of human milk. Br. Med. J. 1, 1018-1020.

Lucas, A., Lucas, P. J., & Baum, J. D. (1980). The nipple-shield sampling system: A device for measuring the dietary intake of breast-fed infants. Early Hum. Dev. 4, 365-72.

Lück, H. (1983). Pesticides in milk and dairy products. S. Afr. J. Dairy Tech. 15, 75-79.

Luquet, F. M., Gorsaud, J., & Gaudier, B. (1972). Étude de la pollution des laits humains par les résidues de pesticides. Pathol. Biol. 20, 137-143.

MacGregor Hutcheson, A. (1987). South Africa. In Africa South of the Sahara 1988 (pp. 888-941). Europa Publications Limited, London.

Macy, I. G., Nims, B., Brown, M., & Hunscher, H. A. (1931). Human milk studies. VII. Chemical analysis of milk representative of the entire first and last halves of the nursing period. Am. J. Dis. Child 42, 569-589.

Masuda, Y., Kagawa, R., Kuroki, H., Kuratsune, M., Yoshimura, T., Taki, I., Kusuda, M. Yamashita, F., & Hayashi, M. (1978). Transfer of polychlorinated biphenyls from mothers to foetuses and infants. Food. Cosmet. Toxicol. 16, 545-546.

Matuo, Y. K., Lopes, J. N. C., & Lopes, J. L. C. (1980). DDT levels in human milk from Ribeirað Preto. (Brazil). Rev. Brasil. Biol. 40, 293-296.

Maxwell, R. J., Marmer, W. N., Zubillage, M. P., Dalikas, & G. A. (1980). Determination of total fat in meat and meat products by a rapid, dry column method. J. Assoc. Off. Anal. Chem. 63, 600-603.

McKinney, J. D., Moore, L., Prokopetz, A., & Walters, D. B. (1984). Validated extraction and cleanup procedures for polychlorinated biphenyls and DDE in human body fluids and infant formula. J. Assoc. Off. Anal. Chem. 67, 122-129.

Mercier, M. (1981). Criteria (Dose/Effect Relationships) for Organochlorine Pesticides. Pergamon Press, Oxford.

Mes, J. (1981). Experiences in humn milk analysis for halogenated hydrocarbon residues. Int. J. Environ. Anal. Chem. 9, 283-299.

Mes, J. & Davies, D. J. (1978). Variation in the polychlorinated biphenyl and organochlorine pesticide residues during human breast feeding and its diurnal pattern. Chemosphere 9, 699-706.

Mes, J., Davies, D. J., Turton, D., & Sun, W. F. (1986). Levels and trends of chlorinated hydrocarbon contaminants in the breast milk of Canadian women. Food Addit. Contam. 3, 313-322.

Mes, J., Doyle, J. A., Adams, B. R., Davies, D. J., & Turton, D. (1984). Polychlorinated biphenyls and organochlorine pesticides in milk and blood of Canadian women during lactation. Arch. Environ. Contam. Toxicol. 13, 217-223.

Miller, G. J. & Fox, J. A. (1973). Chlorinated hydrocarbon pesticide residues in Queensland human milk. Med. J. Aust. 2, 261-264.

Mills, P. A. (1961). Collaborative study of certain chloriniated organic pesticides in dairy products. J. Assoc. Off. Anal. Chem. 44, 171-177.

Morgan, D. P. & Roan, C. C. (1971). Absorption, storage, and metabolic conversion of ingested DDT and DDT metabolites in man. Arch. Environ. Health 22, 301-308.

Murphy, R. S., Kurtz, F. W., & Strassman, S. C. (1983). Selected pesticide residues or metabolites in blood and urine specimens from a general population survey. Environ. Health Perspect. 48, 81-86.

Musial, C. J., Hutzinger, O., Zitko, V., & Crocker, J. (1974). Presence of PCB, DDE, and DDT in human milk in the provinces of New Brunswick and Nova Scotia, Canada. Bull. Environ. Contam. Toxicol. 12, 258-267.

Mussalo-Rauhamaa, H., Salmela, S. S., Leppänen, A., & Pyysalo, H. (1986). Cigarettes as a source of some trace and heavy metals and pesticides in man. Arch. Environ. Health 41, 49-55.

Nakai, S. & Le, A. C. (1970). Spectrophotometric determination of protein and fat in milk simultaneously. J. Dairy Sci. 54, 276-278.

Neville, M. C., Keller, R. P., Seacat, J., Casey, C. E., Allen, J. A., & Archer, P. (1984). Studies on human lactation. I. Within feed and between breast variation in selected components of human milk. Am. J. Clin. Nut. 40, 635-646.

Newton, K. G. & Greene, N. C. (1972). Organochlorine pesticide residue levels in human milk-Victoria, Australia, 1970. Pestic. Monit. J. 6, 4-8.

Norén, K. (1983a). Organochlorine contaminants in Swedish human milk from the Stockholm region. Acta Peditatr. Scand. 72, 259-264.

Norén, K. (1983b). Levels of organochlorine contaminants in human milk in relation to the dietary habits of the mothers. Acta Paediatr. Scand. 72, 811-816.

Norén, K. (1983c). Some aspects of the determination of organochlorine contaminants in human milk. Arch. Environ. Contam. Toxicol. 12, 277-283.

O'Leary, J. A., Davies, J. E., Edmundson, W. F., & Reich. G. A. (1970). Transplacental passage of pesticides. Am. J. Obstet. Gynecol. 107, 65-68.

Olszyna-Marzys, A. E., De Campos, M., Farvar, M. T., & Thoman, M. (1973). Residous de plaguicidas clorados en la leche humana en Guatemala. Bol. Of. Sanit. Panama 764, 93-107.

Olszyna-Marzys, A. E. (1978). Contaminants in human milk. Acta Paediatr. Scand. 67, 571-576.

Pellizzari, E. D., Hartwell, T. D., Harris, S. H., Waddell, R. D., Whitaker, D. A., & Erickson, M. D. (1982). Purgeable organic compounds in mother's milk. Bull. Environ. Contam. Toxicol. 28, 322-328.

Pennington, J. A. T., & Gunderson, E. (1987). History of the food and drug administration's total diet study--1961-1987. J. Assoc. Off. Anal. Chem. 70, 772-782.

Peterson, J. E. & Robinson, W. H. (1964). Metabolic products of p,p;-DDT in the rat. Toxicol. Appl. Pharmacol. 6, 321-327.

Picciano, M. F. (1978). Mineral content of human milk during a single nursing. Nutr. Rep. Int. 18, 5-11.

Polishuk, Z. W., Ron, W., Wasserman, M., Cucos, S., Wasserman, D., & Lemesch, C. (1977a). Organochlorine compounds in human blood plasma and milk. Pestic. Monit. J. 10, 121-129.

Polishuk, Z. W., Wasserman, D., Wasserman, M., Cucos, S., & Ron, M. (1977b). Organochlorine compounds in mother and fetus during labor. Environ. Res. 13, 278-284.

Rabie, M. A. (1983). Biocides. In R. F. Fuggle & M. A. Rabie (Eds.), Environmental Concerns in South Africa. Technical and Legal Perspectives. Juta & Co. Ltd., Cape Town.

Radomski, J. L., Deichmann, W. B., Rey, A. A., & Merkin, T. C. (1971). Human pesticide blood levels as a measure of human body burden and pesticide exposure. Toxicol. Appl. Pharmacol. 20, 175-185.

Ritchey, W. R., Savary, G., & McCully, K. A. (1972). Organochlorine insecticide residues in human milk, evaporated milk and some milk substitutes in Canada. Can. J. Public. Health 63, 125-132.

Roan, C., Morgan, D., & Paschal, E. H. (1971). Urinary excretion of DDA following ingestion of DDT and DDT metabolites in man. Arch. Environ. Health 22, 309-315.

Rogan, W. J., Bagniewska, A., & Damstra, T. (1980). Pollutants in breast milk. N. Eng. J. Med. 302, 1450-1453.

Rogan, W. J., Gladen, B. C., McKinney, J. D., Carreras, N., Hardy, P., Thullen, J. Tingelstad, J., Tully, M. (1986). Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: Effects of maternal factors and previous lactation. Am. J. Public Health. 76, 172-177.

Savage, E. P. (1976). National study to determine levels of chlorinated hydrocarbon insecticides in milk. Environmental Protection Agency (EPA 540/9-781005).

Schüpbach, M. R. & Egli, H. (1979). Organochlorpestizide und polychlorierte Biphenyle in Muttermilch. Mitt. Gebiete Lebensm. Hyg. 70, 451-463.

Siddiqui, M. K. J., Sakena, M. C., Bhargava, A. K., Seth, T. D., Krishna, Murti, C. R., & Kutty, D. (1981). Agrochemicals in the maternal blood, milk and cord blood: A source of toxicants for prenates and neonates. Environ. Res. 24, 24-32.

Skaare, J. U. (1981). Persistant organchlorinated compounds in Norwegian human milk in 1979. Acta Pharmacol. et Toxicol. 49, 384-389.

Slorach, S. A. & Vaz, R. (1983). Assessment of Human Exposure to Selected Organochlorine Compounds Through Biological Monitoring. Swedish National Food Administration, Uppsala.

Smart, N. A. (1987). Organophosphorus pesticide residues in fruits and vegetables in the United Kingdom and some other countries of the European Community since 1976. Rev. Environ. Contam. Toxicol. 98, 99-160.

Stacey, C. I., Perriman, W. S., & Whitney, S. (1985). Organochlorine pesticide residue levels in human milk: Western Australia, 1979-80. Arch. Environ. Health 40, 102-108.

Stacey, C. I. & Thomas, B. W. (1975). Organochlorine pesticide residues in human milk, Western Australia--1970-71. Pestic. Monit. J. 9, 64-66.

Stahr, H. M. (1980). Supplement to Analytical Toxicology Methods Manual. Iowa State University Press, Ames, Iowa.

Storck, W. J. (1987). Pesticides growth slows. Chem. Engrg. News __, 35-42.

Szokolay, A. L., Rosival, J., Uhnak, J., & Medaric, A. (1977). Dynamics of benzene hexachloride (BHC) isomers and other chlorinated pesticides in the food chain and in human fat. Ecotoxicol. Environ. Safety 1, 349-359.

Takahashi, W., Saidin, D., Takei, G., & Wong, L. (1981). Organochlorine pesticide residues in human milk in Hawaii, 1979-80. Bull. Environm. Contam. Toxicol. 27, 506-511.

Tartter, J. R. (1981). Government and politics. In H. D. Nelson (Ed.), South Africa, a country study. (pp. 219-292). U.S. Government Printing Office, Washington, D.C.

Tessari, J. D. & Savage, E. P. (1980). Gas-liquid chromatographic determination of organochlorine pesticides and polychlorinated biphenyls in human milk. J. Assoc. Off. Anal. Chem. 63, 736-741.

Thompon, L. & Prior, A. (1982). South African Politics. Yale University Press, New Haven.

Van der Kamer, J. H. (1949). Rapid method for determination of fat in feces. J. Biol. Chem. 177, 347-355.

Van Dyk, L. P. & Van Der Linde, A. (1976). Persistence of endosulfan in soils of the Loskop dam irrigation area. Agrochemphysica 8, 31-34.

Van Dyk, L. P., Wiese, I. H., & Mullen, J. E. C. (1982). Management and determination of pesticide-residues in South Africa. Residue Rev. 82, 38-124.

Van Dyk, L. P., Lötter, L. H., Muller, J. E. C., & de Kock, A. (1987). Organochlorine insecticide residues in human fat in South Africa. Chemosphere 16, 705-711.

Vuori, E., Tyllinen, P., Kuitunen, P., & Paganus, A. (1977). The occurrence and origin of DDT in human milk. Acta Paediatr. Scand. 66, 761-765.

Wasserman, M. L., Tomatis, L., & Wasserman, D. (1975). Organochlorine compounds in the general population of the seventies and some of their biological effects (in man and animals). Pure Appl. Chem. 42, 189-208.

Wasserman, W., Wasserman, D., Lazarovici, S., & Coetzee, A. M. (1970). Present state of the storage of the organochlorine insecticides in the general population of South Africa. S. Afr. Med. J. 44, 646-648.

Watanabe, I., Yakushiji, T., Kuwabara, K., Yoshida, S., Maeda, K., Kashimoto, T., Koyama, K., & Kunita, N. (1979). Survelliance of the daily PCB intake from diet of Japanese women from 1972 through 1976. Arch. Environ. Contam. Toxicol. 8, 67-75.

Weisenberg, E., Ackerman, E., Freier, S., Reshef, A., Sahm, Z., & Shoenberg, J. (1980). Pesticides in the milk of mothers in Jerusalem. In S. Freier & A. I. Eidelman (Eds.), Human Milk. Its Biological and Social Value (pp. 147-152). Excerpta Medica, Princeton.

Weisenberg, E., Arad, I., Grauer, F., & Sahm, Z. (1985). Polychlorinated biphenyls and organochlorine insecticides in human milk in Israel. Arch. Environ. Contam. Toxicol. 14, 517-521.

Westöö, G. & Norén, K. (1978). Organochlorine contaminants in human milk, Stockholm, 1967-1977. Ambio 7, 62-64.

Whitaker, D. P. (1981). The economy. In H. D. Nelson (Ed.), South Africa, a country study. (pp. 159-218). U.S. Government Printing Office, Washington, DC.

Wickström, K., Pyysalo, H., & Siimes, M. A. (1983). Levels of chlordane, hexachlorobenzene, PCB, and DDT compounds in Finnish human milk in 1982. Bull. Environ. Contam. Toxicol. 31, 251-256.

Wiese, I. H. (1976). Pesticides and the South African population. S. Afr. Med. J. 50, 1801-1805.

Wiese, I. H. & Basson, N. C. J. (1966). The degradation of some persistent chlorinated hydrocarbon insecticides applied to different soil types. S. Afr. J. Agr. Sci. 9, 945-970.

Wiese, I. H. & Bot, J. (1971). Pesticide regulation in South Africa. Residue Rev. 35, 49-63.

Wilson, D. J., Locker, D. J., Ritzen, C. A., Watson, J. T., & Schaffner, W. (1973). DDT concentrations in human milk. Am. J. Dis. Child. 125, 814-817.

Windholz, M. (1983). The Merck Index. 10th ed. Merck & Co., Inc., Rohway, NJ.

Winter, M., Thomas, M., Wernick, S., Levin, S., & Farvar, M. T. (1976). Analysis of pesticide residues in 290 samples of Guatemalan mother's milk. Bull. Environ. Contam. Toxicol. 16, 652-657.

WHO. (1976). Environmental Health Criteria 2, Polychlorinated biphenyls and terphenyls. World Health Organization, Geneva.

WHO. (1978). Environmental Health Criteria 6, Principles and methods for evaluating the toxicity of chemicals. Part I. World Health Organization, Geneva.

WHO. (1979). Environmental Health Criteria 9, DDT and its derivatives. World Health Organization, Geneva.

WHO. (1983). Environmental Health Criteria 27, Guidelines on studies in environmental epidemiology. World Health Organization, Geneva.

WHO (1985). The Quantity and Quality of Breast Milk. World Health Organization, Geneva.

Woodard, B. T., Ferguson, B. B., & Wilson, D. J. (1976). DDT levels in milk of rural indigent blacks. Am. J. Dis. Child. 130, 400-403.

Wyllie, J., Gabica, J., & Benson, W. W. (1972). Comparative organochlorine pesticide residues in serum and biopsied lipoid tissue: A survey of 200 persons in southern Idaho. Pestic. Monit. J. 6, 84-88.

Wyndham, C. H. (1985). A comparison of mortality rates from cancer in white, Indian, and coloured adults in 1970 and 1980. S. Afr. Med. J. 67, 709-711.

Wyndham, C. H. (1986). Comparison of mortality rates for cancer in black adults--1970 vs. 1980. S. Afr. Med. J. 70, 469-471.

Yakushiji, T., Tanaka, R., & Kunita, N. (1977). PCB and organschlorine pesticides in mothers milk, blood, and foods. Pediatrician 6, 28-33.

Yakushiji, T., Watanabe, I., Kuwabara, K., Yoshida, S., Hori, S., Fukushima, S., Kashimoto, K., Koyama, K., & Kunita, N. (1979). Levels of organochlorine pesticides and polychlorinated biphenyls (PCBs) in mothers' milk collected in Osaka Prefecture from 1969 to 1976. Arch. Environ. Contam. Toxicol. 8, 59-66.

Yakuushiji, T., Watanabe, I., Kuwabara, K., & Yoshida, S. (1978). Identification of low chlorinated biphenyls in human milk by gas chromatography-mass spectrometry. J. Chromatogr. 154, 203-210.

ACKNOWLEDGMENTS

I would like to especially thank Dr. Henry Michael Stahr, my major professor, for his enthusiasm and support of my research study; Dr. John M. Pettifor, Director of the Pediatric Mineral Metabolic Unit at Barazwanath Hospital, Soweto, for his interest and assistance with the field work aspects of this research study; the World Food Institute, Iowa State University, for their financial support; the Egnell Company, Cory, Illinois, for their donation of two electric breast pumps; my very special friends, Marlaine Domoto, Julie Kinker, Margaret Manatt, Diana McFarland, Bonnie N., Robin Orr, Bonnie Theisen, and Suzanne Root.

I gratefully acknowledge the help Sister Muriel Sefuba provided with the collection of data in South Africa; Dr. David F. Cox and Mary Sayler for their assistance with the statistical analysis; Martin Kahm for the long hours of help in the laboratory; and Rebecca Shivvers, who was always so helpful and cheerful in the preparation of this manuscript. I sincerely wish to thank the women who volunteered to participate in this research project. Without their interest and willingness, this research study would not have been possible.

APPENDIX

CLEARANCE CERTIFICATE DOCUMENT A/3/33

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
OFFICE OF THE DEPUTY REGISTRAR (RESEARCH) 144 PROTOCOL NO: 3/6/85

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS

PROJECT: Pesticide and Aflatoxin concentrations in Breast milk of three population groups in Johannesburg.

INVESTIGATOR/S: Professor J M Pettifor

DEPARTMENT: Paediatrics and Paediatric Mineral Metabolism Research Unit

DATE CONSIDERED: 21 June 1985

RECOMMENDATION OF COMMITTEE

NOT APPROVED: ☐

APPROVED: [X] subject to the following conditions:-
Approved, on condition that any information obtained be made available to the mothers, if desired.
Informed consent.

DATE: 85/07/09

CHAIRMAN: [signature]

"INFORMED CONSENT" FORMS ATTACHED
FURTHER "I/C" FORMS AVAILABLE AT FACULTY OFFICE

DECLARATION BY INVESTIGATOR/S

To be completed in duplicate and one copy returned to the OFFICE OF THE DEPUTY REGISTRAR (RESEARCH), ROOM 10025, SENATE HOUSE, UNIVERSITY.

I/We fully understand the conditions under which I am/we are authorised to carry out the abovementioned research and I/we guarantee to ensure compliance with those conditions.

Should any departure be contemplated from the research procedure as approved I/we undertake to resubmit the Protocol to the Committee.

DATE: 16/7/85 SIGNED: [signature]
JM PETTIFOR

Today's date:

Sample No. _____

Appendix B Figure 1

FOOD AND NUTRITION DEPARTMENT
IOWA STATE UNIVERSITY
AMES, IOWA

HUMAN MILK STUDY PARTICIPANT'S INTERVIEW

HOSPITAL: _____ DATE OF BABY'S BIRTH : _____ SAMPLE NO.:____
MOTHER'S NAME: _____ ADDRESS: _____
_____ TELEPHONE NO: _____
RESIDENCE SITE: _____ URBAN: _____ RURAL: _____

When was milk sample collected: Before feeding _____
After feeding _____ During feeding _____

INTERVIEWER: Circle the number(s) for each question, or fill in the blank. Circle or write in as many answers as apply. If more specific information concerning a question is needed, write in the blank space to the right of the question, or at the bottom of the page.

Background Information on Mother

What was your age in years at last birthday?_____

A. Mother's Birth Date: _____
Month Day Year

B. Ethnic group: 1 - Black 3 - Colored
2 - White 4 - Asian

C. How many pregnancies have you had? _____
1. How many of these pregnancies resulted in live births? _____
2. Were any of these twins or triplets? Yes _____ No _____
3. How many children have you given birth to altogether? _____

D. How many children were breast fed? _____
Beginning with your first child, the oldest, please tell me how long were they breast fed:

	Days	Weeks	Months	Years
1st child				
2nd child				
3rd child				
4th child				
5th child				
6th child				
7th child				
8th child				
9th child				

Sample No. _____

E. Beginning with the first child you breastfed, would you please tell me why you decided to wean your infant.

1. This is first breastfeeding experience
2. Another pregnancy
3. Return to work
4. Baby not satisfied
5. Not enough milk
6. Baby old enough
7. Mother ill
8. Baby ill
9. Other (please specify)

1st child _____
2nd child _____
3rd child _____
4th child _____
5th child _____
6th child _____
7th child _____
8th child _____
9th child _____

F. What nutritional supplement(s) including any herbal preparations have you taken during the past week? (brand name/amt./day in parenthesis):

1. None
2. Multivitamin (_____)
3. Multivitamin/mineral (_____)
4. Vitamin A (_____)
5. Vitamin D (_____)
6. Vitamin E (_____)
7. Vitamin C (_____)
8. Thiamin (_____)
9. Riboflavin (_____)
10. Niacin (_____)
11. B6 (_____)
12. B12 (_____)
13. Calcium (_____)
14. Iron (_____)
15. Potassium (_____)
16. Trace minerals (_____)
17. Others (_____)

G. Why are nutritional supplement(s) or herbal preparations taken? (specifics in parenthesis):

1. Not applicable; supplements not taken.
2. Prescribed by physician (_____)
3. Recommended by traditional healer (_____)
4. Self desire to improve general health (_____)
5. Self desire to improve health-related symptom (_____)
6. Health maintenance or illness prevention (_____)
7. Other (_____)

H. What medications or drugs, including non-prescription items are you taking presently? (name and amt./day in parenthesis):
1. None
2. Medications (_____)
3. Non-prescription item(s) (_____)

I. Has there been anytime in your life when you can remember losing alot of weight, enough weight so that your clothes felt too big?
Yes_____ No _____
1. If yes, when did you lose the weight?_____
_____
2. Did weight loss occur with:
1. loss of close relative
2. family stress
3. marital stress
4. illness
5. other (specify)_____

J. Mother's Ht:_____ Mother's Wt:_____

GENERAL INFORMATION

A. What kind of job does head of household now have? _____
What kind of job do you have now?_____

B. How long has household head held present job ?/ How long have you held present job?

1. Less than 1 year	1. Less than 1 year
2. 1-2 years	2. 1-2 years
3. 3-5 years	3. 3-5 years
4. 6-10 years	4. 6-10 years
5. Over 10 years	5. Over 10 years

C. What did household head do prior to this job? _____
What did you do prior to this job?_____

D. Where were you born? City _____
Province _____
Nearest large town _____
Country _____

E. List all your residences for the past 10 years beginning with the present:
1. City _____ Province _____
Months _____ Years _____
2. City _____ Province _____
Months _____ Years _____
3. City _____ Province _____
Months _____ Years _____
4. City _____ Province _____
Months _____ Years _____
5. City _____ Province _____
Months _____ Years _____

E(a) What was the highest level of education completed by head of household/and yourself?
_____head of household; _____self.

Sample No. _____

F. Do you or any members of your household use pesticides, for example, bug sprays for fruit, vegetable, or flower gardens or farms or animals, including house pets? Yes No
If yes, what are the name(s) of the product(s) used?_____

G. Which members in your household use pesticides?
1. No one
2. Self
3. Spouse
4. Son
5. Daughter
6. Father
7. Other

H. Are any protective or precautionary measures observed during application of pesticides (for example, protective clothing, use of a mask, restriction of humans or animals in treated area)? Yes____ No____

I. Where are the areas of your pesticide usage?
1. Household
   a. Inside
   b. Yard
   c. Trees
   d. Garden
   e. Pets
2. Farm
   a. Crops
   b. Pasture
   c. Building, pens
   d. Livestock
   e. Fence rows
   f. Seed treating
3. No pesticides used

J. Do you eat vegetables or fruits out of your garden? Yes ____ No ____
If yes, how many months out of the year? _____

K. Has a commercial applicator ever treated your premises for pests?
Yes ____ No ____

If so, when, for what reason, and what type of brand of chemical was used?
1. Date_____
2. Chemical_____
3. Why_____

Sample No. _____

L. From where does your drinking water come?
1. Municipal (Tapwater)
2. Private (Borehole)
3. Water supply points provided by authority
4. River, stream, dam, and other untreated water sources

If water is from untreated sources, do you treat the water yourself?
Yes_____ No_____

M. Please indicate which best describes your smoking habits now:
1. Do you regularly smoke now? _____Yes _____No
If yes, do you smoke:
a. less than 1/2 packet of cigarettes per day
b. 1/2 to 1 packet of cigarettes per day
c. more than a packet of cigarettes per day
d. a pipe

How many cigarettes are there in your packet?
a. 10
b. 20
c. 30
d. make my own

2. Have you ever smoked? _____Yes _____No
If yes, how long did you smoke? (specify cigarettes, pipe, or other) _____Months _____Years

How much did you smoke?
a. less than 1/2 packet of cigarettes per day
b. 1/2 to 1 packet of cigarettes per day
c. more than a packet of cigarettes per day

3. Dose anyone else smoke in your household? Yes:_____ No:_____ Don't know
If yes, check all that apply: _____cigarettes:_____cigars_____pipe:_____

N. How often do you drink alcoholic beverages:
1. Never
2. _____ x daily
3. _____ x weekly
4. _____ x monthly
5. less than monthly

Other(specify)

If 2-5 were answered, then ask the following:

What type of beverage and amount are taken during one period:
1. regular beer or ale; _____ oz.
2. homemade beer; _____ oz.
3. wine; _____ oz.
4. liquor: gin, scotch, vodka, whiskey; alone or with a mixer
5. brandy, cordial, cognac _____ oz.

Sample No. _____

O. What food is raised in home garden, orchard, nut trees:
1. None
2. Starchy vegetables
3. Dark green/yellow vegetables
4. Other vegetables
5. Nuts
6. Fruit

P. What other foods are produced at home:
1. None
2. Meat
3. Eggs
4. Milk
5. Butter
6. Other

Q. What foods are preserved by any of the following methods: (type of food in parenthesis):
1. Food not preserved
2. Canning (_____)
3. Freezing (_____)
4. Jelly, preserves (_____)
5. Pickling (_____)
6. Salting (_____)
7. Smoking (_____)
8. Other (_____)

R. What items are in your kitchen:
1. No kitchen in home
2. Stove, electric or gas
3. Stove, wood
4. Refrigerator
5. Hot plate
6. Dry storage area
7. A place to wash dishes

S. Where is most of food for home bought:
1. Food not bought for home
2. Large supermarket
3. Small grocery store
4. "Quick" market
5. Produce market
6. Other _____

T. How many times a week is each cooking method used for preparing foods for yourself:
1. Boiling
2. Stewing
3. Steaming
4. Baking
5. Frying
6. Grilling
7. Roasting
8. Smoking
9. Other (_____)

Food frequency form, Black donors 151	Food	Don't Eat	Do Eat		
			Times/ Day	Times/ Week	Times/ Month
I.	Chicken				
	Beef				
	Sausage				
	Pork/Ham				
	Bacon				
	Lamb				
	Offal				
	Liver,kidney,chitterlings				
	Insects (mopanie worms)				
	Eggs				
	Nuts,seeds (groundnuts)				
	Beans				
	Peanut butter				
	Wild animal				
II.	Fresh milk				
	Tinned milk				
	Buttermilk				
	Yogurt				
	Sourmilk				
	Cottage cheese				
	Cheese				
III.	Cremora + similar products				
	Butter				
	Margarine				
	Lard				
	Fishoil				
IV.	Mealie, green				
	Mealie, dry				
	Samp				
	Mealie rice				
	Mealiepop				
	Rice				
	Cereals (Matabella)				
	Brown bread				
	White bread				
	Noodles, macaroni				
V.	Tomato,tomato sauce, juice				
	Orange or orange juice				
	Tangerine				
	Grapefruit or juice				
	Papaya or mango				

	Food	Don't Eat	Do Eat Times/ Day	Times/ Week	Times/ Month
	Lemonade				
	White potato				
	Turnip				
	Peppers (green,red,chili)				
	Strawberries,cantaloupe				
VI.	Carrots				
	Sweet potato or yam				
	Summer squash				
	Winter squash				
	Apples,peaches,pears				
	Banana				
	Pineapple				
VII.	Other foods not listed which you regularly eat				

Food frequency form, White donors Food		Don't Eat	Do Eat		
			Times/ Day	Times/ Week	Times/ Month
I.	Chicken				
	Beef				
	Sausage				
	Pork/Ham				
	Bacon				
	Lamb				
	Liver of Kidney				
	Fish				
	Game, such as biltong				
	Eggs				
	Beans, lentils				
	Nuts, seeds (groundnuts)				
II.	Milk, full cream				
	Milk, low fat				
	Tinned milk				
	Yogurt				
	Buttermilk				
	Cottage cheese				
	Cheese				
	Ice cream				
	Pudding and custard				
III.	Cremora or other brands				
	Cream, including whipped				
	Butter				
	Margarine				
	Lard				
	Cooking oil:				
	Corn				
	Peanut				
	Soybean				
	Blend				
IV.	White bread				
	Brown bread				
	Mealiepop				
	Rolls,biscuits,muffins				
	Pastry				
	Cereals				
	Noodles,macaroni				
	Rice				
	Mealierice				
	Samp				

Food	Don't Eat	Do Eat Times/ Day	Do Eat Times/ Week	Do Eat Times/ Month
V. Tomatoes, tomato sauce, juice				
Orange or orange juice				
Tangerine				
Grapefruit or juice				
Papaya, mango				
Lemonade				
White potato				
Turnip				
Peppers (green, red, chili)				
Strawberries, cantaloupe				
VI. Carrots				
Sweet potato or yam				
Summer squash				
Winter squash				
Apples, peaches, pears				
Banana				
Pineapple				
VII. Other foods not listed				
which you regularly eat				

Food frequency form, Colored and Indian donors 155		Don't Eat	Do Eat		
	Food		Times/ Day	Times/ Week	Times/ Month
I.	Chicken				
	Veal				
	Beef				
	Lamb				
	Fish				
	Eggs				
	Beans and Dal (all types)				
	Lentils				
	Nuts, seeds (cashews)				
	Peanut butter				
II.	Buttermilk				
	Yogurt				
	Milk, full cream				
	Milk, low fat				
	Tinned milk				
	Cheese				
III.	Panir				
	Ghee				
	Butter				
	Margarine				
	Cooking oil:				
	Corn				
	Peanut				
	Soybean				
	Blend				
IV.	White bread				
	Brown bread				
	Pastry				
	Cereals				
	Rice				
	Noodles,macaroni				
	Pivus				
	Chapati				
	Roti				
V.	Tomato,tomato sauce,juice				
	Orange or orange juice				
	Tangerine				
	Grapefruit or juice				
	Papaya, mango				
	White potato				
	Turnip				

	Food	Don't Eat	Do Eat Times/ Day	Times/ Week	Times/ Month
	Peppers (green,red,chili)				
	Strawberries,cantaloupe				
VI.	Carrots				
	Sweet potato or yam				
	Summer squash				
	Winter squash				
	Apples,peaches,pears				
	Banana				
	Pineapple				
VII.	Other foods not listed which you regularly eat				