"Organochlorine pesticide residues in Mothers' milk; evaluation of possible Drug interaction in humans"

A THESIS PREPARED

BY

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DEDICATION

To my dearest wife Perpetua and children, Tinoashira, Sakhile and Rujeko; special thanks for their enduring patience.

In memory of my father, and plenty of gratitude to my loving mother for moral and spiritual support.

ABSTRACT

The aim of the study was to investigate exposure levels of organochlorine pesticides (OCPs) in mother's milk and in selected staple foods. The study also aimed to evaluated effects of 1,1,1-trochloro-2-bis-(4-chlorophenyl)ethane (DDT) on paracetamol half-life in highly exposed and least exposed breast-feeding mothers as a way of investigating possible drug interaction.

This was an experimental study where milk and food samples from Esigodini, Harare, Kadoma Kariba Nyanga and Mudzi were collected and analysed for OCPs levels using GC-ECD. Evaluation of induction of the hepatic cytochrome P450 enzymes by DDT was carried out by pre-treatment of female rats with a single intraperitoneal dose of DDT (0.3 mg/g) body weight. Cytochrome P450 enzyme was quantified by potassium phosphate buffering the microsomal fraction followed by spectral determination of the reduction of cytochrome P450. Blood samples from selected mothers in areas showing results of higher exposure levels to DDT and selected mothers with low exposure levels to DDT (controls) were evaluated for paracetamol drug interaction. Concentration levels of paracetamol in rats were measured using UV-Visible spectrophotometry. Levels of paracetamol in human mothers were measured using the Immunochemistry System (ICS) based on immunochemical precipitation of paracetamol and nephelometric detection of the precipitate.

The highest mean levels of DDT in mother's milk were found in Mudzi with levels were16838.90 ng/g and the lowest was found in Esigodini with 934.12 ng/g. Results obtained from the analysis of hexachlorohexane (HCH) levels were comparably lower than DDT levels in the selected areas. There was a statistical significant correlation ($r=0.8857$, $p= 0.0188$) between DDT levels in staple food and mother's milk. However, there was no statistical significant correlation ($r=0.6571$, $p= 0.1562$) between HCH levels in staple food and mother's milk.

Maximum induction of cytochrome P450 enzyme for test animals was at its highest (8.51 n mol /mg) on day 14 when compared to control animals, which had 0.55 n mol/mg at day 14. Hepatomegally was directly related to higher levels of cytochrome P450 in test animals while no liver enlargement was observed in control animals.

DDT induced female rats and DDT exposed breast-feeding mothers showed interference with the pharmacokinetics of paracetamol at therapeutic dose level. The half-life of paracetamol in DDT pre-treated rats was 144 minutes when compared to control rats with 380 minutes a difference of 236 minutes. A similar trend was observed in the sampled breast-feeding mothers; analysis of the pharmacokinetic (half-life) data for highly DDT exposed mothers (212 minutes) and 13 least exposed (318 minutes), showed a significant difference (106 minutes) of paracetamol half-life.

The conclusion from the study is that Kariba and Mudzi areas are highly exposed to DDT while Harare and Esigodini areas are least exposed. Exposure levels in breast milk are correlated to levels analysed in staple foods. Continued use of DDT for vector control is welcomed, but it should be done with intense monitoring to prevent long term effects of POPs on exposed individuals. More research is recommended so that scientists could assess the effects of the induction properties of OCPs on the efficacy, toxicity and the therapeutic index on dosage of many drugs used by animals and humans.

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DDT	Dichlorodiphenyltrichloroethane
DDE	Dichlorodiphenyldichloroethylene
α-HCH	Alpha- Hexachlorohexane
β-HCH	Beta-Hexachlorohexane
γ-HCH	Gamma- Hexachlorohexane
HCB	Hexachlorobenzene
PCB	Polychlorinated Biphenyl
GLC	Gas Liquid Chromatography
OCS	Organochlorine Compounds
OCPs	Organochlorine Pesticides
DDD	Dichlorodiphenyldichloroethane
ECD	Electron Capture Detector
ADI	Acceptable Daily Intake
EDI	Estimated Daily Intake
FAO	Food and Agricultural Organization
WHO	World Health Organization
WP	Wettable Powder
DDA	Dichlorodiphenyl-acetic acid
LD50	Lethal dose fifty
ITCZ	Inter Tropical Convergence Zones
UNEP	United Nations Environmental Programme
NSAIDs	Non Steroid Anti-Inflammatory Drugs

AUC	Area Under the Curve
Vd	Volume of Distribution
T1/2	Half-life
NAPQI	N-acetyl- para- benzoquinane imine
op	oral Administration
ip	intraperitoneal
iv	intravenous
Im	intramuscular
POP	Persistent Organic Pollutant
CPM	Chlorinated Pesticides Mixture

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CHAPTER 1

1.0 Introduction

Organochlorine pesticides (OCPs) are persistent in the environment and have significant bio concentration and bio magnification properties in the food chain. Dichlorodiphenyltrichloroethane (DDT) is not the only compound magnified in the global food chains, but polychlorinated biphenyls (PCBs) that are widely used in the developed countries as industrial chemicals, are also very persistent. The PCBs have not so far been a big environmental threat to most developing countries including Zimbabwe (Slorach and Vaz, 1983; Kanja, *et al*., 1986; Chikuni. *et al*., 1997b; Barkatina, *et al*., 1999).

Organochlorine pesticides have been used through out the world, with the aim of eliminating pests that threaten human health and insects that devour crops. The insecticidal properties of DDT were discovered in 1942. It was during this period that it was first medically used for the suppression of typhus epidemic in Naples. It was also applied directly to the human body to control lice. However, by the early 1980s, use of OCPs especially DDT was banned in the developed world after discovering their persistence in the environment. The large predominance of 1,1-dichloro-2-bis (4-chlorophenyl) ethyl (pp-DDE), which is a more persistent metabolite of DDT in almost all samples analysed, suggests a general decline of DDT in the environment in general (Brooks, 1974; Curtis and Lines; 2000, Hernandez, *et al*., 1993).

In the different regions of North America, levels of organochlorine residues in human tissues have declined from 0.55 mg/kg to 0.050 mg/kg, during the last 20-30 years. In southern Canada, a ten-fold decline in DDT in human tissue has been observed over the last 20 years. This decline was due to the decrease of the dietary intake of DDT. Other organochlorine pesticides such as aldrin, dieldrin, heptachlor and heptachlor epoxide in milk samples from Canadian women have also exhibited decreasing trends between 1967 and 1982 (Conacher and Mes, 1993; Mes, 1994).

In Africa DDT has been in use since 1946 for control of body pests and vector control programmes. DDT has been extensively used in Africa primarily in the prevention of malaria, typhus, yellow fever and sleeping sickness, but also in agriculture as an insecticide for cotton plants. DDT is being used in some Africa countries such as in South Africa's KwaZulu Natal area, for malaria control in the homes ((Phelps, *et al*., 1986; Bouwman, *et al.,* 1990).

In Zimbabwe, agriculture is the most important economical activity with about 80% of the population depending on it. The country started using DDT in the early 70s. Information on the accumulation of organochlorine pesticides in humans, animals and in the environment is meager. At the beginning of 1982, restrictions on the use of DDT were imposed and DDT was only to be used in tsetse and mosquito control programmes as a 3% aerosol spray formulation. (Mpofu, 1987; Nhachi, *et al.,* 1995; Chikuni, *et al*., 1997a).

Methoxychlor that is structurally related to DDT is now increasingly being used as a replacement for DDT in Zimbabwe because it is less toxic to mammals. It does not persist in the body as long as DDT. It is stored in the adipose tissue to about 0.2 % of the extent of DDT and its half-life in rats is only two weeks, while that of DDT is about 6 months (Murphy and Harvey, 1985). A shorter half-life is the result of a more rapid metabolism by o-demethylation, after which it is conjugated and excreted in the urine (Kapoor*, et al*., 1970); Li, *et al*., 1995b; Kinyamu, *et al*., 1998). However, the concern with methoxychlor usage is that it is metabolized to estrogenic products. It has been established that methoxychlor can act as a hormone by blocking hormone receptors and as such, it has endocrine-disrupting effects (Bulger, *et al.,* 1983; Colborn*, et al*., 1993; Eriksson 1997).

Another OCP aldrin has been used in Zimbabwe in preference to dieldrin (Plant Protection Research, 1977). Dieldrin is a metabolite of aldrin, and is more persistent than aldrin. In the human body aldrin is quickly metabolised to dieldrin therefore, the presence of dieldrin residues in mother's milk reflects exposure to aldrin (Ackerman, 1980; Casey and Vale, 1994).

Lindane is another OCP that Zimbabwe is still using but it was restricted for use on edible crops by the early 80s. It is used clinically as an ectoparasiticide and also as a pediculicide shampoo formulation for head lice. Exposure to lindane produces signs of poisoning that resemble that of DDT (Forget*, et al*., 1993; Mhlanga and Madziva, 1990). Chronic exposure to mice of this pesticide causes

an increase in hepatocellular tumors. The ∝- and γ- isomers of hexachlorohexane are CNS stimulants. CNS stimulation appears to be due to the blocked effects of γ-aminobutyric acid (GABA) (IARC 1974; Matsumura and Ghiasuddin 1979). Lindane also induces hepatic microsomal enzymes (Gonzalez and Korzekwa, 1995). Compared to DDT, lindane has a relatively low persistence in the environment (Drummond, *et al*., 1988; London and Myers, 1995).

Chlorinated cyclodienes stimulate the CNS; most signs and symptoms of poisoning resemble those of DDT. Unlike DDT, these compounds tend to produce convulsions before other less serious signs of illness have appeared (Hayes, 1982; Attaran and Roberts, 2000). Studies have shown that aldrin produces dose-related hepatomas in mice and has a greater carcinogenic potential compared to DDT (Saxen, and Siddiqui, 1983; Dewailly, *et al*., 1996).

Studies of DDT exposure in Zimbabwe have given an indication that some areas in this country are exposed. Previous studies of DDT residues in human breast milk have indicated that areas such as Kariba, Mudzi and Nyanga have shown high levels of 25.3 mg/kg, 23.4 mg/kg and 10.1 mg/kg (fat weight) respectively (Chikuni, *et al.,* 1991; Chikuni, *et al.,* 1997a).

In Zimbabwe, vector control programmes are the possible major cause of environmental exposure in the Kariba area. DDT has been used to control the prevalence of mosquitoes (malaria) and tsetse flies (sleeping sickness) in the surrounding game parks and areas within the Zambezi Valley. In 1983, after the world ban on DDT Zimbabwe was restricted to about 127 tonnes of DDT and these were used for tsetse control in Binga, Gokwe and Hurungwe areas of the

Zambezi operational area (Zaranyika and Makhubalo, 1996). The washout from the main rivers assists in distributing the DDT, which eventually finds its way into Lake Kariba which is the largest water reservoir in the country (Zaranyika, *et al*., 1994). The lake is the major source of water to the nearby town, townships and rural dwellings and it is the main source of fish to the local population and even to the country's hinter land. People in this area also eat vegetables from small back-yard gardens, which are all possible sources of DDT exposure.

1.1 Presence of OCPs in Zimbabwe Foods

Developed countries set programmes that constantly monitor pesticide residues in foods and this has led to the setting up of tolerance levels for organochlorine compounds in foods and beverages and a reduction or ban in the usage of some OCPs. In order to show that Zimbabwe was also committed towards a better environment for all, parliament recommended creating a Ministry of Environment and Tourism. The recommendation to establish this ministry was adopted and passed by parliament in 1990. Zimbabwe has in the past permitted usage of an array of pesticides for agro-industry and domestic food crops; amongst the pesticides used are the organochlorine pesticides of which DDT was extensively used (Mukahanana, 1990).

Increased use of pesticides in crop protection in Zimbabwe has increased the possibility of food contamination. Some of the consumables, which are exposed to pesticides, are fish, drinking water, vegetables, meat, fruits, grains (maize and wheat), cooking oil, peanut butter, milk and milk products. For in instance

people in Kariba area consume vegetables from small back yard gardens that are watered using the DDT contaminated water, and often consume fish, from the same water source (Lake). A research project has since documented high residues of DDT in mothers milk as 25 259 ng/g (fat weight)) as compared to acceptable levels of 35 ng/g (fat weight) which may implicate diet source of high exposure residues in humans (Chikuni, *et al*., 1997b; FAO/WHO, 1993). The spraying of the crops using pesticides is done at all the stages of crop growth. Pesticide residues remain in the structures such as roots, stem, leaves or fruits of the crop until humans or animals consume them. The effects on humans can be acute or chronic (Nhari, 1996). Pesticide residues of 976 ng/g (fat weight) were also detected in fresh water sardines (Kapenta) from the Kariba Lake (Mhlanga, *et al.,* 1986; Zaranyika, *et al.,* 1994).

The Global Environmental Monitoring System (GEMS), established in 1983, collects data on pesticide residues in food and the environment throughout the world (Dogheim *et al,* 1996). Zimbabwe's participation in GEMS has been poor, but within the past five years the situation has improved. The preferred method for the detection of the organochlorine residues in the various consumables is the capillary gas chromatograph (GC) method. In this method the capillary column uses a special deactivation technique and the column shows maximum inertness, resulting in a linear column response. The method includes extraction with a solvent mixture and then quantitation of the different OCPs on the GC (Brevik, 1978; Skaare, *et al*., 1988).

1.2 Chemistry of Organochlorine Pesticides

The organochlorine pesticides belong to three chemical classes and these include the dichlorodiphenylethanes, the chlorinated cyclodienes, and the chlorinated benzenes and cyclohexane-related structures. They are persistent and are biotransformed at a slow rate due, in part to the complex aromatic ring structures and the extent of chlorination. These ring substitutes are exceedingly difficult to remove by enzymatic processes available in body tissues. The molecular formula of DDT is C_{14}–H_9–Cl_{l5} and the formula shows the extent of chlorination. This pesticide has very low solubility in water (12 µg/kg) and a high solubility in fat (10 g/kg). This is why the pesticide is lipophilic and easily absorbed in fat. Once absorbed in the body tissues of a living organism, DDT is concentrated in the adipose tissue/fat depots (Leighty, *et al.,* 1980). The pesticide is a potent non-systemic and contact pesticide and storage in the fat tissue is important because it decreases the amount of the pesticide at the site of toxic action (Sharma, 2001).

1.2.1 Toxicokinetics of Organochlorine Pesticides

The lipoprotein, the chlorine bonds and the stabile covalent bonds of the OCPS described in the proceeding paragraph are the precursors that lead to their persistence and toxicity. The toxicity is evidenced in the different stages of the time course of OCPs disposition (Toxicokinetics) that include their absorption, distribution, metabolism and excretion in the whole living animal (Medinsky and Klaassen, 1996).

Absorption

The absorption of OCPs involves the direct transportation to the liver via the hepatic- portal vascular system of the body. DDT is lipid-soluble, it is readily absorbed by the skin and mucous membranes of the gastrointestinal tract (Riviere, 1994; Roberts, *et al*., 1997). Other lipophilic compounds such as polychlorinated biphenyls (PCB) can also get into the systemic circulation by passive transportation of lipids via the micelles and subsequent biological processes related to lipid metabolism (Klaassen and Rozman, 1996). The human skin comes into contact with many toxic agents; fortunately the skin is not very permeable and therefore is a relatively good barrier. When the skin absorbs these chemicals in sufficient quantities they can elicit systemic effects. DDT is much less toxic to mammals than to insects when it is applied to the skin. DDT passes more readily through the chitinous exoskeleton of insects than through the skin of mammals (Albert, 1965, Hayes, 1965).

Distribution

After administration DDT is transported into the blood stream by absorption. For lipid soluble toxicants, the major determinant of the rate of movement across capillaries is the partition coefficient. DDT accumulates in certain parts of the body as a result of protein binding, active transport and its high solubility in fat. The alpha and beta lipoproteins are very important in the transport and distribution of lipophilic xenobiotics such as DDT with the interactions being non-covalent (Wilkinson 1983; Oliyai and Stella, 1993). DDT binds to proteins that have hydrophobic regions such as lipoproteins and albumin. Chlordane,

DDT and PCB, are highly lipophilic, and become localised in adipose tissue, where they seem to exert little effect until mobilized by starvation of the animal.

Distribution of the residues in tissues may be a protective mechanism if the tissue is not the target site for toxicity, and it also reduces toxic effects in other organs. Although sequestration into adipose tissue reduces exposure of other tissues, mobilization of the fat in the adipose tissue may cause a sudden release of the organochlorine into the bloodstream with a dramatic rise in concentration and toxic consequences (Timbrell, 1991a). Sequestration into tissues occurs due to the action of an endogenous substance such as the protein glutathione transferase 1,2,3 (GST 1, GST 2 GST 3). This protein binds and transports both endogenous and exogenous compounds into the liver. These toxicants accumulate in fat by dissolution in neutral fats, which constitute about 50 % and 20 % of the body weight of obese individuals and lean athletic individuals respectively. Storage lowers the concentration of the toxicants in the target organ. (Ecobichon, 1996; Rivero, *et al.*, 1997). A study of the perch (Perca fluviatilis) showed that the body burden of OCPs' residues was lowered by storage in adipose tissue (Olson, *et al.,* 1999).

Metabolism

The metabolism of DDT and other organochlorine compounds converts these lipophilic pesticides into more polar substances and hence into more excretable metabolites (Redetzke and Applegate, 1993). Sometimes transformed substances (metabolites) may be more toxic than the parent compound and this is

the case with DDT, where the metabolite pp-DDE is more persistent and toxic than the parent DDT (Stevens, *et al*., 1993,Timbrell, 1991b). Xenobiotic metabolism is divided into two main phases, which are phases I and phase II (Nebert, 1994). These sometimes, but not always, occur sequentially as in an experiment by (Pratap, *et al*., 2000) in which he observed that the reaction of an ethanolic extraction of the leaves of Adhatoda vesica (Acanthaceae) sequentially influenced phases I and II enzyme systems in Albino Swiss mice. However, xenobiotic metabolism is now well recognised as three phases where the elimination from the cell is regarded as the phase III (Bernard, *et al*., 2003). Phase 1 reactions commonly involve hydrolysis, reduction or oxidation (Otto, *et al*., 1999). The reactions expose the original compound so that the functional group (-OH, - NH2, -SH, or -COOH) can be added and then conjugated in phase II reactions (Williams, 1983, Nichols, *et al*., 1994). Phase II stage, involves conjugation reactions; these include glucoronidation, sulphonation, acetylation, methylation, conjugation with glutathione, or conjugation with amino acids.

Enzymes catalyse metabolism of xenobiotics, and most of these enzymes are very specific in metabolising particular compounds. Studies have been carried out to show catalytic activity of P-450 enzymes as expressed in bacterial, yeast or mammalian cells (Gonzalez and Korzekwa, 1995). These enzymes are localised in the sub-cellular environments such as the smooth endoplasmic reticulum and in the cytosol and a few are found in such organelles as the mitochondria. Most oxidation reactions are catalysed by one enzyme system, which is the cytochrome P-450 mono-oxygenase or the mixed function oxidases (Farrell, 1987; Krishna and Klotz, 1994). Cytochrome P-450 system ranks first

in terms of catalytic versatility in the numbers of xenobiotics it detoxifies or activates. (Guengerich, 1994; Werck-Reichhart and Feyereisen, 2000). Studies have been done in-vitro using human cells on the use of antipeptide antibodies directed against defined regions of human cytochrome P-450 enzymes to show the function of the enzyme on compounds (Raunio, *et al*., 1995; Boobis, *et al.,* 1996).

All cytochrome P-450 enzymes are haeme-containing proteins. The haeme iron in the cytochrome is usually in the ferric (Fe^{3+}) state. When reduced to the ferrous (Fe^{2+}) state, cytochrome P-450 can bind ligands such as oxygen (O^2) and carbon monoxide (CO). The complex between ferrous cytochrome P-450 and CO absorbs light maximally at 450 nm, from which cytochrome P-450 derives its name (Guengerich, 1987). The preferred method for the analysis of the enzyme cytochrome P-450 is the microsomal fractioning method, followed by the colour complexing of the enzyme, which is calorimetrically determined on a scanning spectrophotometer. Quantitation of the enzyme is based on determination against microsomal proteins (Wilson, 1995). Human liver microsomes contain 15 or more different P-450 enzymes (isoenzymes) such as CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, 3A7, 4A9, and 4A11 (Guengerich, 1994; Yoshitomi, *et al*., 2001).

The P450 enzymatic system is known to exist in a multiplicity of forms (isoenzymes) with overlapping substrate specificities. Recent studies have shown that the definition of families and subfamilies has shifted toward lower cut-offs for inclusion. A more realistic view today is that a cut-off window is

better than an absolute value like the original 40% identity or higher for family inclusion (Estabrook, 1999). More problematic is the merging or blurring of subfamilies that used to be distinct, and the CYP4 family is a prime example. It is now so full of subfamilies that they are hard to keep from overlapping (Daly, *et al*., 1996). Cytochrome P450 research is a field that has benefited by having a systematic standardized nomenclature (Nelson, *et al*., 1996).

The CYP induction response occurs mainly in the liver, depending on the nature of the xenobiotic, but also in lungs, kidney and placenta. The induction is characterized by increased expression of the CYP genes and associated catalytic activities for one or more isoforms (Okey, 1990). Exposures to environmental pesticides such as DDT, leads to induction of CYP 2B1, CYP 2B2 isoforms and to the CYP 3A4 subfamily (Lubet, *et al.*, 1990; Kocarek, *et al.,* 1991). These soluble cytochrome P-450 enzyme systems catalyze the pesticide DDT by dehalogenation. The overall reaction is a dehydrogenation and the formation of the metabolite pp'-DDE (Nims, *et al*., 1998).

Studies have shown that DDT and its major metabolites pp-DDE and pp-DDD, as well as many other organochlorine pesticides, are phenobarbital (PB)-type cytochrome P450 inducers in the rat (Li, *et al*., 1995a; Nims and Lubet, 1995). Thus, like administration of PB, exposure to such organochlorine pesticides results in the induction in rat liver or cultured rat hepatocytes of CYP2B-selective catalytic activities. In addition, DDT and other organochlorine pesticides elicit in the rat many of the other manifestations of the hepatic pleiotropic response caused in rat by PB including hepatomegaly and induction

of the CYP3A subfamily, NADPH cytochrome P450 oxidoreductase, and certain phase II activities and genes such as microsomal epoxide hydrolyase (Campbell, *et al*., 1983; Yashioka, *et al*., 1984; Kocarek, *et al*., 1991; Lubet, *et al*., 1992).

Excretion

Toxicants are eliminated from the body through several routes. The kidney is one of the most important organs for the excretion of polar/water soluble xenobiotics as more chemicals are eliminated from the body by this route than by any other. Removal of a toxic compound may help to reduce the extent of damage to the organs and body tissue. The rate of elimination of foreign compounds is reflected by the parameters such as plasma half-life (t1/2), elimination rate constant (Ke1) and total body clearance of the area under the curve (AUC). The half-life of some diphenyl aliphatics such as DDT and the cyclodienes such as aldrin may range from days to weeks or months. Residual elimination of organochlorine pesticides may be described by a two-compartment model, where the first phase is relatively rapid, and the second phase is prolonged (Osweiler, 1996). Many xenobiotics have to be biotransformed to water-soluble products before they can be excreted in urine. However, most body secretions appear to have the excreted chemicals thus some toxicants have been found in sweat, saliva, tears and milk (Drummond, *et al*., 1988; Veberk, *et al*., 1990). The biliary route of elimination is probably the most important route of elimination in as far as the chlorinated ethane derivatives such as DDE are concerned The major excretory route is from bile to the digestive tract where some enterohepatic recycling may take place. The liver removes the

toxic agents from the blood after absorption from the gastrointestinal tract. Some of the metabolites that are formed are excreted directly into the bile (Rozman and Klaassen, 1996). The metabolites are slowly released from lipid storage depots until equilibrium with blood is achieved. DDT is metabolised to 1,1-dichloro-2-bis-(4-chlorophenyl) ethane (pp-DDD) and pp-DDE; then pp-DDD is further metabolised to bis-(4-chlorophenyl) acetic acid (pp-DDA), which is water-soluble and is excreted in the urine (Fraklin, *et al*., 1986; He, 1999).

However, there is no efficient elimination mechanism for non-volatile highly lipophilic chemicals such as polyhalogenated biphenyls and chlorinated hydrocarbons insecticides such as DDT, more so if the pesticides are not easily biotransformed (Okonkwo, *et al*., 1999). These chemicals are excreted very slowly and tend to accumulate in the body upon chronic exposure (Gladen and Rogan, 1995; Czaja, *et al*., 1997). However, there are a few rather slow processes for elimination and excretion of these lipophilic pesticides, such as excretion by the mammary gland after the pesticides have been dissolved in milk lipids (Bouwman, *et al.,* 1992; Barkatina, *et al*., 1998).

The secretion of organochlorine pesticides such as DDT and HCH through the breast milk is a form of excretion or a form of elimination processes (Hernandez, *et al*., 1993; Hooper and Petreas, 1997). Research has demonstrated that for suckling infants, milk is their major source of the body burden of OCPs (Lederman, 1993; Franchi and Farcardis, 1993). The health importance of secretion of DDT and other organochlorine pesticides in human breast milk depends entirely on the dosage received by suckling babies (Skaare*, et al*., 1988;

Kanja*, et al.,* 1992). Studies have demonstrated that milk as a route of excretion can be used as a biomarker for measuring and monitoring environmental exposure levels of DDT and HCH (Nair and Pillai, 1992; Johansen, *et al*., 1994).

1.3 Toxicity of Organochlorine Pesticides

The major toxic action of DDT is on the central nervous system (CNS), and the impact of the pesticide is its action on the membranes in the nervous system especially axonal membranes. Studies have shown that the nervous system both central and peripheral and the cells that make up these systems are particularly susceptible to damage by these compounds (Handerson, *et al*., 1989; Silbergeld, 1993; (Mutti, 1999; Aitio, 2002).

Endocrine disruption has been shown in animals exposed to DDT. Studies in mice have suggested that the oocytes in the reproduction system of the female mice can be damaged or destroyed by the metabolic action of the organochlorine pesticides (Carson, *et al*., 1989; Savitz and Harlow, 1991). Other studies have shown that DDT can cause an estrogenic response in rats, leading to the thickening of the endometrium and an increase in the uterus weight. This is due to competitive inhibition of DDT to binding of estradiol to receptor sites in the uterus cells (Alisan and Morgan, 1987; Carson, *et al*., 1989; Safe, 2000).

1.3.1 Mechanism of Toxicity of DDT

DDT alters the porous channels through which sodium ions pass by interfering with the active transport of sodium out of the nerve axon during repolarization.

Studies have demonstrated that at the level of the neuronal membrane, DDT affects the permeability to K^+, reducing K^+ across the membrane. It was also observed that, DDT inhibits neuronal adenosine triphosphatase (ATPase), particularly the Na^+, K^+, -ATPase and Ca^{2+} -ATPase that play a vital role in neuronal repolarization. It was observed that they is the inhibition of (ATPase) enzyme by exposure to DDT when a preparation of this enzyme from a nerve-ending fraction of the rat or rabbit brain was exposed to DDT concentration of 0.00001mol/) in vitro (Matsumura, 1985; Guillette, *et al.,* 1999). Finally it was also observed that the pesticide inhibits the ability of calmodulin, a calcium mediator in nerves, to transport calcium ions essential for neurotransmitters (Ecobichon, 1996). Heptachlor and lindane may inhibit the binding of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Elderfrawi, *et al.,* 1985; Fonseca*, et al.,* 1993).

Acute and sub-acute toxicity of OCPs in humans and animals is rare, from an environmental situation. Chronic toxicity of OCPs is the main, since it is from an environmental exposure set-up. The present DDT exposure situation in Zimbabwe is predominantly of a chronic nature (Mpofu, 1987; Chikuni, *et al*., 1997a). Zimbabwe's DDT exposure is caused by chronic usage of DDT for environmental vector control programmes in the past (Matthiessen, 1985; Chikuni, *et al*., 1997b). Chronic low-level exposure is difficult to confirm. It is by measuring residual levels of OCPs that scientists can obtain a true picture of physiological and behavioral changes of species living within the exposed ecosystem (Furst, *et al*., 1994).

Most chronic low-level exposures have been associated with reproductive dysfunction in wild birds, especially in fish eagles, as was shown in a study on wild birds exposed to DDT. Eggshell thinning in this species is a result of DDT blocking the oestrogen receptors that mediate the deposition of Ca^{2+} into the eggshell, thereby resulting in reduced shell strength or increased shell brittleness. Studies were done in birds that were chronically exposed to aldrin (Matthiessen, 1985; Okoniewski, 1993; Holmes, *et al*., 1994). A study done on the shores of Lake Kariba showed that DDT residues were present in eggs and in fat depots of crocodiles (Niloticus laurentis) (Kubus and Berg, 1991; Zaranyika, *et al*., 1994).

Exposure at these low levels has also been associated with potential carcinogenic effects in animals (Falck, *et al*., 1992; Wolf, *et al*., 1993). Exposure to the low levels is also associated with neurological diseases in human. Behavioural changes were correlated with chronic pesticide exposure when studied in rats (Becking, *et al*., 1993; Joy, 1994).

1.3.2 Drug Interaction

Drugs may interact with each other in a number of ways. It could be by addition, synergism, antagonism, and potentiation or through drug metabolism. It was observed that there was an increase in half-life of 22% in 6 patients who were given paracetamol, at a dose of 100 mg intravenously 2 hours after intravenously receiving a dose of 1-gram chloramphenicol, suggesting interference with the metabolism of the antibiotic. Studies have shown that, the rate of metabolism sets a limit to the rate at which the drug can be administered, and if this rate

exceeds the therapeutic dose level, the amount of the drug in the body will in principle increase. Thus, variations in the rate of metabolism through enzyme induction will also produce disproportionately large changes in the plasma concentration of the drug (Safe, 1994; Nims, *et al*., 1998).

Paracetamol is metabolised by several routes, and induction of the cytochrome P-450 enzyme system may alter the balance of these routes. The drug is a substrate for the cytochrome P-450 1A2 (CYP1A2), CYP2E1 and CYP3A4. The isoenzyme CYP 1A2 is present in both humans and rats and this similarity makes rats the choice animal for comparative studies (Rang, *et al.,* 1999). CYP3A4 is capable of metabolising paracetamol to a reactive quinoneimine (Werck-Reichhart and Feyereisen, 2000). Studies have demonstrated that DDT or methoxychlor is associated with induction of CYP3A4 in rats or in humans (Li, *et al*., 1995a; Wienker, 2001). These studies show that DDT induces CYP3A4 and the isoenzyme is also associated with metabolism of paracetamol. The hepatotoxicity of paracetamol in rats and humans is increased by induction with DDT due to an increase in the cytochrome P-450 isoenzymes such as CYP3A4 that activates the drug. Thus, changes in the rate of metabolism of paracetamol due to the induced cytochrome P-450 mono-oxygenase enzyme system (CYP3A4) should have an effect on the pharmacokinetics of paracetamol. These effects should be a decrease in half- life, a decrease in plasma concentration, probable change in the proportions of metabolites, and a probable change in the toxic effects of the drug (Rang, *et al*., 1999).

1.4 Pharmacokinetics of Paracetamol

Paracetamol is rapidly and well absorbed from the gastrointestinal tract after oral administration, which is the commonly used route of administration (Welch, 1993). Peak plasma concentration is reached in 30 to 60 minutes in normal situations that is, in patients with normal hepatic and renal functions. A variable proportion is bound to plasma, and the drug is inactivated in the liver by a series of metabolic pathways. Metabolism of paracetamol is mainly in the liver and can be conjugated to give the glucuronide and the sulphate metabolites (Malfertheiner, *et al*., 1994; Brown, *et al*., 1999; Wilson, *et al*., 2000). It is then excreted within 24 hours in urine and small amounts of hydroxylated metabolites are also excreted (Clissold, 1986; Brown, *et al*., 1992). At high doses of paracetamol, one of these metabolites formed is N-acetyl- p-benzoquinone, which is the metabolite responsible for the toxicity of the drug. At the therapeutic dose level, the plasma half- life of paracetamol is 2 hours up to 4 hours but at the toxic dose level it may be extended from 4 hours up to 8 hours (Ahrens, 1996; Rang, *et al.,* 1999).

Excretion of mercapturic acid from paracetamol metabolism was found to be dose-dependent, and it was found to decrease in experimental animals (mouse) in over-dose situations (Myers, *et al*., 1995; Yoshitomi, *et al*., 2001). An approximately 20% depletion of hepatic glutathione was apparently correlated to covalently bound protein and glutathione. In humans, the increased metabolism of paracetamol due to the induction of CYP1A2, CYP2E1 and CYP3A4 is

responsible for producing the N-acetyl-p-benzoquinone imine (Morrison, 1995; Halmes, *et al.*, 1998; Otto, *et al*., 1999).

1.5 The Public Health and Environmental Concern

Organochlorine pesticides' persistence in soils and sediments cause biomagnification in the food chain. The extent of upward biomagnification from the concentration in lake sediments is up to 500 times the concentration in plankton which is up to 5 000 times the concentration in oligocheates. Concentration of oligocheates is up to 50 000 times the concentration in kapenta fish, which is up to 100 000 times the concentration in tiger fish and other larger fish, and this is finally magnified up to 200 000 times in humans. Exposure to these persistence organochlorine pesticides is a public health concern (Szokolay, *et al*., 1977; Chikuni, *et al.*, 1997c; Asplund, *et al.,* 1994; Zaranyika, *et al*., 1994).

The half-life of DDT in the soil varies from 2 to 15 years depending on biodegradation to pp-DDE. Organisms may accumulate DDE through the food chain (IARC, 1991). In humans acute DDT poisoning is associated with effects on the central nervous system (CNS). However, pathological changes relating to the effects of exposure can be demonstrated in animal tissues (Anger, 1990; Joy, 1994). Studies suggest that exposure of the developing foetus or newborn to DDT during the critical stages in nervous system development may cause developmental toxicity manifested later on in life. This was demonstrated by a researcher who found behavioural changes in mice stemming from doses of

DDT (approximately 0.5 mg/kg) of the same order as doses received by exposed human neonates during lactation (Eriksson, 1993).

An association between DDE levels in mother's blood and the likelihood of pre-term birth has been suggested (Lave and Ennever, 1990; Longnecker, 2001). Major pathological changes are observed in the liver and reproductive organs. Morphological changes in mammalian liver include hypertrophy of hepatocytes and sub-cellular organelles such as mitochondrion, proliferation of smooth endoplasmic reticulum and formulation of inclusion bodies (Lavy and Mattice, 1986; Battershill, 1994; Anwar, 1997). Centro lobular necrosis and an increase in the incidence of hepatic tumours have been observed following exposure to high concentrations of DDT (IARC, 1974).

Studies evaluating the evidence of DDT carcinogenicity in experimental animals show evidence of a dose-related increase in liver tumors following subcutaneous injection and oral administration in mice and rats. (Austin, *et al*., 1989; IARC, 1991). However, there has been no epidemiological evidence linking DDT to carcinogenicity in humans (Hayes, 1982). Epidemiological data on cancer risk associated with exposure to DDT are suggestive, but limitations in the exposure assessment and the finding of small and inconsistent excesses complicate the evaluation. Most recent studies of breast cancer risk associated with increased serum levels of pp- DDE have yielded conclusive results, and there has been the high occurrence of breast cancer in mothers with high concentration of DDT and DDE (Amoateng-Adjepong, *et al*., 1995; Lopez- Carrillo, *et al*., 1997; Hunter, *et al*., 1997).

The op-isomer of DDT has been shown to compete with estradiol for binding the oestrogen receptors in rat uterine cytosol. Studies have found that estrogenic effects could be caused in mice at levels of DDT (0.018mg/kg and above). It was shown that these effects can also be established in DDT exposed humans (Kupfer and Bulger, 1976; Safe, 1994). Recent studies have shown that pp-DDE is a potent inhibior of the androgen receptor. This has raised further concern about a possible link between environmental contamination from DDT derivatives and a supposed decline in human fertility (Keice, *et al*., 1995; Ulrich, *et al.,* 2000). Previous studies have established that the Zimbabwe environment is exposed to organochlorine pesticides. High residues were reported in and around Lake Kariba (Mhlanga, *et al*., 1986; Zaranyika, *et al*., 1994; Nhachi and Kasilo, 1990; Chikuni, 1997c).

However, in recommending restrictions or withdrawal of organochlorine pesticides, as a gesture of public concern, considerations had to be given to the availability of safer and more effective alternatives or compounds with less undesirable effects, which are also economically sustainable. Zimbabwe is now faced with escalating prices of the safer alternatives, which are environmentally acceptable, but expensive. Because DDT is not expensive and has long lasting residual effect the government of Zimbabwe has resorted to the use of DDT for vector control programmes.

1.6 Objectives

The main aims and objectives of this study were: -

1.6.1 To establish whether there is a correlation between the levels of DDT and HCH in breast milk of breast feeding mothers and DDT and HCH in staple foods of mothers from different exposure backgrounds.

1.6.2 To demonstrate the induction of the hepatic cytochrome P-450 enzyme by DDT in rats.

1.6.3 To investigate the effect of DDT pretreatment on the pharmacokinetics of paracetamol in rats.

1.6.4 To investigate the effect of DDT on the pharmacokinetics of paracetamol in humans, as an example of drug interaction.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Glassware

The glassware that was used in the method was as listed in Table 2.1 below.

TABLE 2.1 List of Glassware

Item	Description	Manufacturers
Centrifuge tubes	5, 10, 20, 50 ml volumes	Cole-Palmer International, USA
Pipettes	Fixed volumes – 1,2,5 µl	Kebo Laboratories, Norway
Vials with screw-on top	2, 5ml volume	Chromopack, Norway
Polycarbonate centrifuge tubes	2, 5ml volume	Cole-Palmer International, USA
Vials	2ml with Teflon tops	Chromopack, Norway
Eppendorf tubes	1, 2, 5ml volume	Fisher Bioblock Scientific, France
Volumetric dispensers	2,4,5ml volume	Kebo Laboratories, Norway
Cuvettes	Glass quartz 1ml volume	Cole-Palmer International, USA
Graduated pipettes	Straight, up to 10,25 ml	Technical Scientific Enterprises UK
Test tubes with glass top	2, 5 ml volume	Technical Scientific Enterprises UK
Flat-bottomed bottle	2, 5 ml volume	Chromopack, Norway
Zymark, tubes	2ml end-point mark	Zymark Company, USA

All the glassware used was ultra clean and fat free. The clean up of the glassware was done as follows: -

Residual extracts from centrifuge tubes (for example) were poured out into a disposal container and then the tubes were rinsed with clean water. The tubes were left immersed for an hour in 96% ethanol. The ethanol was then poured out and the tubes and the rest of the glassware were rinsed with water and then immersed overnight in a basin with 2% deconex solution. The glassware was then removed from the 2% deconex solution and thoroughly cleaned in a 1% deconex solution. The glassware was then rinsed with ethanol and finally rinsed out with distilled water and left to dry in a drying oven (Ahlborg, *et al.,* 1994)

The chemicals and reagents used in the methods were of analytical quality as listed in the Table 2.2 below.

TABLE 2.2 List of Chemicals and Reagents

Chemical & Reagent	**Description**	**Manufactures**
Cyclohexane	HPLC, AR grade	Rathburn Chemicals, Scotland
Acetone	HPLC AR grade	Rathburn Chemicals, Scotland
Sulphuric acid	96% AR grade	Chemscan Ab. Elverum, Norway
Hydrochloric acid	36% AR grade	May and Baker, Zimbabwe
Ethanol	96%, AR grade	Protea Medical Services, Zimbabwe
Sodium dithionate	Crystals AR grade	Merck, Germany
Paracetamol	500 mg tablets	Varichem Pharmaceuticals, Zimbabwe
Paracetamol	Powder 100% pure	Varichem Pharmaceuticals, Zimbabwe
Sodium chloride	Crystals AR grade	May and Baker P/L, Zimbabwe
Glycerol	Oil 100% pure	Geddes P/L. Zimbabwe
Deconex Liquid soap	Glassware wash-up.	Heigar & Co. A/S, Norway

Key for abbreviation: - HPLC = High Pressure Liquid Chromatograph.
AR = Analytical Reagent

The gas chromatograph equipment used was constantly standardized and calibrated. The GC and other equipment used in the method were as listed in the Table 2.3 below.

TABLE 2.3 List of Equipment

Item	Equipment description	Manufactures
Ultrasonic homogenizer	4710 series	Cole-Palmer International, USA
Zymark Turbovap II	Evaporation unit	Beckman Instruments Inc. Germany
Auto-sampler AS 800	For GC, equipment	Fisons, USA
HRGC 5300 Mega Series GC	High Resolution Gas Chromatograph equipment	Carlo Erba, Germany
Homogenizer Systems	With a cutting blade & and a build-in rotor-stator generator, reduces particles to 2 μm size	Cole-Palmer International USA
Cobas Mira analyzer	Auto-analyzer – proteins	Beckman Instruments
Hermle ZK 40	Cool spin centrifuge, refrigerated	Hermle P/L, Germany
Spectrophotometer	Scanning with Graphic display monitor	Shimadzu, Japan UV-160 Model
Centrifuge	High speed refrigerated with Ti50 fixed angles	Beckman Instruments Inc. SA
Balance	Analytical, with 4-decimal point	Sartorius, Germany
Computer-GC 820	Maxima Chromatograph work-station	Millipore Waters USA

2.2 Method Validation

2.2.1 GC Method Validation

Consistency and reliability of the equipment and the method was confirmed by inclusion of internal standards such as PCB 112 with a concentration of 10ng/ml. It was also confirmed by running in standard calibration curves of individual OCP standards such as pp-DDE and β-hexachlorohexane and running in a series of standard CPM for recoveries. A volume of 100 µl of an internal standard of PCB 112 isomer with a concentration of 10 ng/ml was added to each and every sample to be analysed.

A series of standard dilutions were prepared by diluting working standards with cyclohexane from a 40-ng/ml stock standard of pp-DDE and β-HCH, and these concentrations were 10, 20, 30 and 40 ng/ml. A volume of 1ml of the prepared series of standard pp-DDE was pipetted into vials. Then 1µL of the standard was then injected in to the GC and analysed with every batch of samples. The height ratio was noted and a calibration curve was drawn against the standard concentrations. The same procedure was done for the standard series of β-HCH and a calibration was also drawn as mentioned above.

The standard CPM 10 with a concentration of 10 ng/ml was used as a recovery standard for the method. The Chlorinated Pesticide Mixture 10 (CPM 10) is a mixture of pesticides standards. The actual composition of this CPM 10 standard was, HCH 2.5 ng/ml, γ-HCH 2.5 ng/ml, β-HCH 10.0 ng/ml, heptachlor 2.5 ng/ml, aldrin 5.0 ng/ml, heptaepoxide 8.0 ng/ml, dieldrin 12.0 ng/ml, pp-DDE 10.0 ng/ml, op-DDD 20.0 ng/ml, endrin 20.0 ng/ml, op-DDT 22.5 ng/ml, pp-DDD 19.0 ng/ml and pp-DDT 26.0 ng/ml.

The concentration of standard CPM 10 was based on the concentration of pp-DDE, which was 10.0 ng/ml. Homogenised commercial fresh milk; this was milk that was extracted, as in section 2.4, and was analysed as in section 2.4.2 for OCPs. There were no detectable OCPs residues in this milk and this was the milk that was used as the pooled control and test milk through out the study. The standard was spiked with 100 µl of a CPM 10 standard and extracted and cleaned-up as the test samples. A volume of 1ml was pipetted into four vials and these were randomly loaded on the auto sampler and analysed on the GC with every batch of the test samples. The results were noted and the coefficient of variance was calculated.

2.2.2 Validation of Paracetamol Method

A calibration curve was used to check the validity of the paracetamol method (Nagaraja, *et al.,* 1998; Parajcic, *et al.,* 2003)

The sample results were compared with an internal standard from the paracetamol programme card. For the paracetamol standard, the stock paracetamol standard solution was made by accurately weighing 0.4 grams of paracetamol and dissolving it in 10 ml of dehydrated ethanol and topped up to 100 ml with distilled water thus making a stock solution of 4g/L. Bovine plasma was used to dilute the stock to working standards containing paracetamol at the following concentrations: 0.50, 100, 200, and 400 mg/l in bovine plasma. The working standards were prepared weekly and stored at -20°C until ready for use. The paracetamol concentration was observed and noted after each colorimetric analysis.

2.3 Determination of DDT and HCH in breast milk and in foods

2.3.1 Sampling

Breast milk samples were collected from mothers living in one of the six selected areas. These areas were selected on the basis of agricultural activities (Kadoma, Nyanga and Esigodini) industrial activities (Harare) and vector control programmes (Kariba and Mudzi). To select breast feeding mothers to participate in the study an inclusion criteria system was employed and it was as follows: -

- The mothers had to be between 16-35 years old.
- They had to be nursing their first, second or third child at the time of sampling.
- Mothers had to have lived in the sampled area for at least five years.
- Both the mother and the infant had to be healthy at the time of sampling.
- The mother's milk was collected between 1 to 12 months post-partum.

The mothers who satisfied the above criteria completed a questionnaire (Appendix 2).

Collection of milk from Mothers

Milk samples were collected between 10:00 and 12:00 at clinics or hospitals during the times mothers came for post-natal visits. The samples were collected into 20 ml clean vials with Teflon lined caps. Each vial was clearly marked with the sample number and the date of collection. Before manually expressing the milk sample, the mothers were advised to clean their hands with clean water and also to clean the breast with a wet clean cotton pad, so as to remove any oily material or dirt that may be on the breast.

Each mother was required to give only one sample of at least 10 mls of breast milk. For preservation of the samples, 50 µl of 30% formalin was added to every 10 mls of the milk. The samples were then stored in a freezer at minus 20^0 C until ready for the GC analysis.

Collection of staple foods

Staple food samples were collected from each of the six selected areas. These were collected from local back-yard gardens, village gardens, local farms, and milk from domestic animals and fish from local rivers or lakes. The food samples collected were representative of the local diet of mothers living in the selected areas and these included covo vegetables or kale *(Brassica oleracea),* mealies, and maize meal for sadza, meat, tomatoes, peanuts, cooking oil, sour milk, fresh milk, and fish such as Kapenta (*Limnothrissa miodon),* bream (*Talapia andersonic and Tilapia mossambica)* and trout (*Salmo gairdneri*). The samples were collected in 20 ml vials. The tubes were then stored in the freezer at minus 20^0 C until ready for GC analysis.

2.4 Extraction of Milk Samples

The milk samples were removed from the freezer and left at room temperature for one hour to thaw. The bottles were then placed in an incubator at 37^oC for 1 hour. The tubes were then mixed well on a whirl-mixer. Ten mls of each mother's milk was weighed in a 100 ml centrifuge tube. Pooled control fresh commercial milk was included in each batch of samples to be analyzed as a pooled sample blank. 100 µl of an internal standard with concentration of 10 µg/ml was added to all the standards and sample

tubes. The internal standard of each batch was polychlorinated biphenyls (PCBs) of isomer 112 (Polder, *et al.,* 1998).

The samples were extracted twice with a solvent mixture. The first extraction was with 20 mls of cyclohexane and 15 mls of acetone and this was homogenized for 2minutes with a Cole-Palmer ultrasonic homogenizer. The homogenate was then centrifuged for 10 minutes in a refrigerated centrifuge at 3000 rpm. The cyclohexane layer was carefully pipetted into a Zymark evaporation tube. The homogenate was again extracted for the second time for 1 minute with the ultrasonic homogenizer after addition of 15mls of cyclohexane and 10 mls of acetone. The homogenate was again centrifuged for 10 minutes at 3000 rpm. The cyclohexane layer was again removed and added to the Zymark evaporation tube. The cyclohexane layer was then evaporated to about 2ml under a gentile stream of Nitrogen air with a Zymark Turbovap II evaporation unit. The remaining fat extract was quantitatively transferred to a 5 ml volumetric flask and adjusted to the mark with cyclohexane. After adjustment of the volume, 1ml of the fat extract was used to determine the fat percentage and another 1ml was used for the clean up procedure and was then ready for GC analysis Polder, *et al.,* 1998; Vaz, *et al.,* 1993).

The clean up was done by pipetting 1ml of the cyclohexane-fat extract into a 10 ml centrifuge tube. The extract was then cleaned up with 4 ml of ultra pure Sulphuric acid (H_2S0_4) and gently mixed. After mixing, the tubes were left in a dark cupboard for an hour, after which they were centrifuged. Then 1µl of the cyclohexane layer was pipetted into a screw cap 1 ml vial with a conical insert, for the GC analysis.

2.4.1 Extraction of OCPs from Staple Foods

Homogenisation of foods

Staple food samples were first homogenised in n-hexane solvent by a Cole Palmer homogeniser to a smooth paste. The homogenate was then transferred into a 100 ml centrifuge tube and the weight of each homogenate used was recorded as; fresh milk 9.67g, sour milk 10.82g, covo vegetables 9.21g, cabbage 10.55g, maize meal 11.57g, peanut butter 2.26g, fish 8.78g and river water 9.13g (American Society of Testing and Materials, 1999). An internal standard of 100 µl PCB 112 isomer with a concentration of 50 ng/ml was added into each of the centrifuge tube with the sample. Recovery of the internal standard in the Mudzi area was used to determine extraction efficiency and validation of food results (Dogheim, *et al.,* 1996; Polder, *et al.,* 1998).

In extraction of foods the methodology is similar to that of milk, but the times of sonication and centrifugation are longer as described below. A solvent mixture of 20 mls cyclohexane and 15 mls acetone was added to the homogenate and the mixture was sonicated for 4 minutes with an ultra-sonicator. The mixture was centrifuged in a refrigerated centrifuge at 3000 rpm for 15 minutes. The cyclohexane layer was pipetted into the Zymark evaporation tube. Then 15 mls of cyclohexane and 10 mls of acetone solvent mixture were added to the homogenate and this was again homogenized for 3 minutes with an ultra-sonicator. The homogenate was again centrifuged in a refrigerated centrifuge at 3000 rpm for 10 minutes. The cyclohexane layer was again added to the evaporation tube. The cyclohexane layer was evaporated as in the breast milk method mentioned above. The clean-up and GC analysis was done as previously mentioned for the breast milk samples. The quantitation of the OCP residues in food was calculated using the standards mentioned in human milk analysis.

2.4.2 GC-Analysis

Analysis of OCPs was done according to the GC–ECD method by Brevik (Bjerk and Brevik, 1980). The OCP extracts were automatically injected by an auto-sampler Fisons model AS 800 into a Carlo Erba, high-resolution gas chromatograph (HRGC) model 5300 Mega Series equipped with an electron-capture ^{63}Ni detector and a Maxima model 820 chromatography work station. The Carlo Erba gas chromatograph was equipped with a split / split-less injector. The split ratio for milk samples was set to a ration of 1:20 of 1µl of the cleaned samples, and the split time was set to 180 seconds. The septum flash was set at 1.25 ml / minute, and the injection temperature was 270^0 C. An electron capture ^{63}Ni detector detected the components. Hydrogen was used as the carrier gas at a linear velocity of 0.1 cm^3 / second. Nitrogen was used as make-up gas with a flow rate of 30 ml / minute.

The separation was carried out in a SPB- 5 capillary column 60 m long, 0.25 mm ID, 0.25µm film thickness. The initial oven temperature was set at 90^0 C and held at this temperature for 2 minutes, then raised by 25^0 C / minute to 180^0 C and then held at this temperature level for 2 minutes, and then raised again by 1.5^0 C / minute to 220^0 C and held at this temperature for 2 minutes, and raised again by 30^0 C / minute to the final temperature of 270^0 C, and finally held at this level for 15 minutes. Individual peaks from different pesticide components in the samples were quantified by comparison with individual peaks from the external standard of chlorinated pesticide mixtures (CPM). Serial dilutions of single external standards (pp-DDE, β-HCH and HCB) were used for quality assurance. Table 2.4 below, is a list of the standards used in this study.

Analytical Standards

Table 2.4 List of Analytical Standards

Standard	Description	Manufacturer
β-hexachlorohexane (β-HCH)	5ml of 40 ng/ml in n-hexane a quant-kit of 99.5% Chemically pure.	Chromopack International B.V. Middleburgh, The Netherlands.
pp-DDE	5ml of 40 ng/ml in n-hexane a quant-kit of 99.5% Chemically pure	Chromopack International B.V. Middleburg, The Netherlands
Chlorinated Pesticide Mixture (CPM/ 40)	10ml of pesticide mixture 10 ng/ml in n-hexane a quant-kit of 99.5% Chemically pure.	Chromopack International B.V. Middleburg, The Netherlands.
PCB 112 isomer	5ml of 10 ng/ml in n-hexane a qual-kit of 99.5% Chemically pure.	Chromopack International B.V. Middleburg, The Netherlands.

2.5 Demonstration of Cytochrome P-450 Induction by DDT

To demonstrate induction by DDT the following procedure was done: -

Female Sprague-Dawley rats of weight 295 – 305 grams, from the animal house at the University of Zimbabwe's Faculty of Medicine were used to investigate the induction of the hepatic cytochrome P-450 enzyme by DDT. The rats were transported in Perspex

cages to the department of Clinical Pharmacology's holding room fitted with a temperature-control unit. Both the test animals and the control animals were kept in animals-holding room for 7 days so that they stabilize and acclimatize with the room conditions and a room temperature set at 25^0 C (Landi, *et al.,* 1982; Tuli, *et al.,* 1995). The cages were cleaned, saw dust was changed, and the animals were given clean water and pellets on a daily basis. The rats were also exposed to the natural day and night cycle of the environment they were kept. The rats for the cytochrome P450 induction studies were kept in two cages each with 26 animals for the test rats and 26 animals for the control rats.

2.5.1 Test rats (TR): -

After acclimatization of the test and control rats, the following procedure was done: -

On day one of the induction period, 26 of the rats were marked as test rats (TR). The animals were marked on the tail with a black marker for identification, and then weighed and the weights recorded. They were then injected intra-peritoneally (i.p) with DDT in corn oil at a dose of 20 mg/kg body weight. The DDT dose that was used for induction of the animals was based on the exposure levels detected in milk samples of the highly exposed mothers. Two rats were used for each step of the experiment and the animals were kept for three days for the next procedure.

Control Rats (CR): -

After acclimatization to conditions in the department of Clinical Pharmacology holding-room, 26 female rats were marked and labeled on the tail with an edilable marker. They were then weighed and their weights were recorded. The rats received the same regime volume of corn oil intra-peritoneally as was with the test animals. This procedure was

also done in duplicate and the animals were kept in a separate cage under the same conditions as the test animals until day three.

Sample Preparation and Collection

On day four between 9:30 and 10:30, 2 animals from the control group and 2 from the test group were sacrificed by carbon dioxide asphyxiation. The thoracic cavity of the animal was opened, and the liver was thoroughly perfused with 25 mls of ice-cold 0.25 M sucrose solution. The whole liver from each rat was quickly removed, trimmed free of extraneous tissue and blotted dry. The liver was weighed on a Satorius balance.

A portion of the central median lobe of the liver was quickly excised, weighed, minced with a sharp scissors and homogenized. Then 16 mls of phosphate buffer pH 7.4 containing 1.15 % potassium chloride was added to the homogenate. The volume was finally and accurately made up to 20 mls with the phosphate buffer pH 7.4 and this was capped and stored at minus 70° C prior to enzyme assay. This procedure was done for all the other rats. The last day of asphyxiation was day 16. All the liver homogenates were numbered and stored at minus 70°C, until ready for the enzyme assay.

2.5.2 Preparation of Hepatic Microsomal Fraction by Ultra Centrifugation

Ultra-centrifugation was done according to the method used by Estabrook and Werringloer and slightly modified by others (Estabrook and Werringloer, 1978; Yun, *et al.,* 1991).

The stored homogenates tubes were thawed and then gently mixed by gentle inversion of the tube. Four mls of the homogenate were accurately aliquoted into capped poly-carbonated centrifuge tubes. The tubes were pre-cooled to 4°C and then centrifuged at 10 000 rpm for 20 minutes in a high spin refrigerated centrifuge. The supernatant was carefully removed after discarding the fatty layer on top. Three mls of the supernatant was pipetted into ice cooled clean polycarbonated tubes and ultra centrifuged at 40 000 rpm for 1 hour in a Beckman L5-40 ultra centrifuge refrigerated with a Ti_{50} fixed angles rotor.

The cytosol fraction, which was the supernatant layer, was removed with a Pasteur pipette, and any lipid layer left on the microsomal pellet was discarded. The microsomal pellet was rinsed twice with 0.25 M sucrose solution and resuspended with 2 mls ice-cold sucrose of 0.25 M solution. It was centrifuged at 40 000 rpm for 1 hour. After centrifugation, the pellet formed was resuspended in a phosphate buffer solution pH 7.4 to a concentration of 0.25 g of fresh liver tissue per 1 ml of phosphate buffer. Then 3 ml of buffer solution and 1 ml of 20% glycerol were added in order to stabilize the microsomes. Finally 2 ml of the microsomal fraction were added to make up a total volume of 6 ml in each tube. One ml of the microsomal fraction was taken for protein assay. The protein assay was done with the Cobas Mira analyzer.

Measurement of Cytochrome P-450

The method that was used for measuring cytochrome P-450 was the one used by Omura and Sato and slightly modified by Estabrook and others (Omura and Sato, 1964; Werringloer and Estabrook, 1975; Basselink, *et al.,* 1997).

Microsomal preparation of 1 mg/ml protein concentration in 0.1 M phosphate buffer pH 7.4 was placed in a cuvette of 1-cm optical path. The base-line spectrum was read using a Shimadzu scanning spectrophotometer between a wave length of 400 nm and 500 nm. A few grains of sodium dithionate were added to the microsomal solution into the other cuvette. The test cuvettes were then gently bubbled with carbon monoxide for 30 seconds, at the rate of 1 bubble/second. The spectrum was then rescanned from 400 nm to 500 nm, and the difference in absorbance between 450 and 490 nm was measured and recorded. All spectrophotometer readings were done at room temperature, which was read at 25° C.

Determination of Microsomal Protein using the Pyrogall Red Method

The method for determining microsomal protein was based on the fact that Pyrogall red complexes with protein in an acidic solution containing molybdate ions. The resulting blue coloured complex absorbs maximally at 600 nm. A Shimadzu scanning spectrophotometer was used to measure the absorbance. The maximum absorbance at 600 nm was directly proportional to the protein concentration in the liver samples. The method was attenuated to detect quantities as low as 10 µg/ml proteins. The Biuret method could not be used in this project because it was less sensitive, being unsuitable for the assay of proteins at concentrations less than 10 µg/ml (Doumas, 1975; Chromy, *et al*., 1980). The Biuret method is affected by gross haemolysis and lipoemia, hence it was not used in this projected.

Calculation of Cytochrome P-450 levels

The difference in absorbance between the absorption maximum at 450 nm and the base line at 490 nm was calculated from the spectrophotometer chart. The extinction coefficient for reduced cytochrome P450 binding with CO was determined. Hence cytochrome P450 concentration expressed in (n mol/ml) of the sample was calculated. The concentration of cytochrome P450 was expressed in n mol/mg by dividing the added volumes of the microsomal sample and that of the phosphate buffer by the calculated microsomal protein. The difference in the cytochrome P-450 enzyme concentration between the TR and CR rats was the indication of induction of the enzyme by DDT.

2.5.3 Effect of DDT on the Pharmacokinetics of Paracetamol in Rats

DDT was used as the cytochrome P-450 enzyme inducer and the pharmacokinetics of paracetamol were observed in female Sprague-Dawley rats.

Preparation of animals for Paracetamol study

A total of 48 rats were used for the pharmacokinetics experiment. The animals were divided into two groups, i.e. 24 rats for the Test Group and 24 rats for the Control Group. The schematic diagram figure 2.1 represents how the rats for the study were treated.

The Test Group

Twenty-four Sprague Dawley rats for induction with DDT were accurately weighed and the weights were recorded. All the 24 animals were clearly marked on their tails with

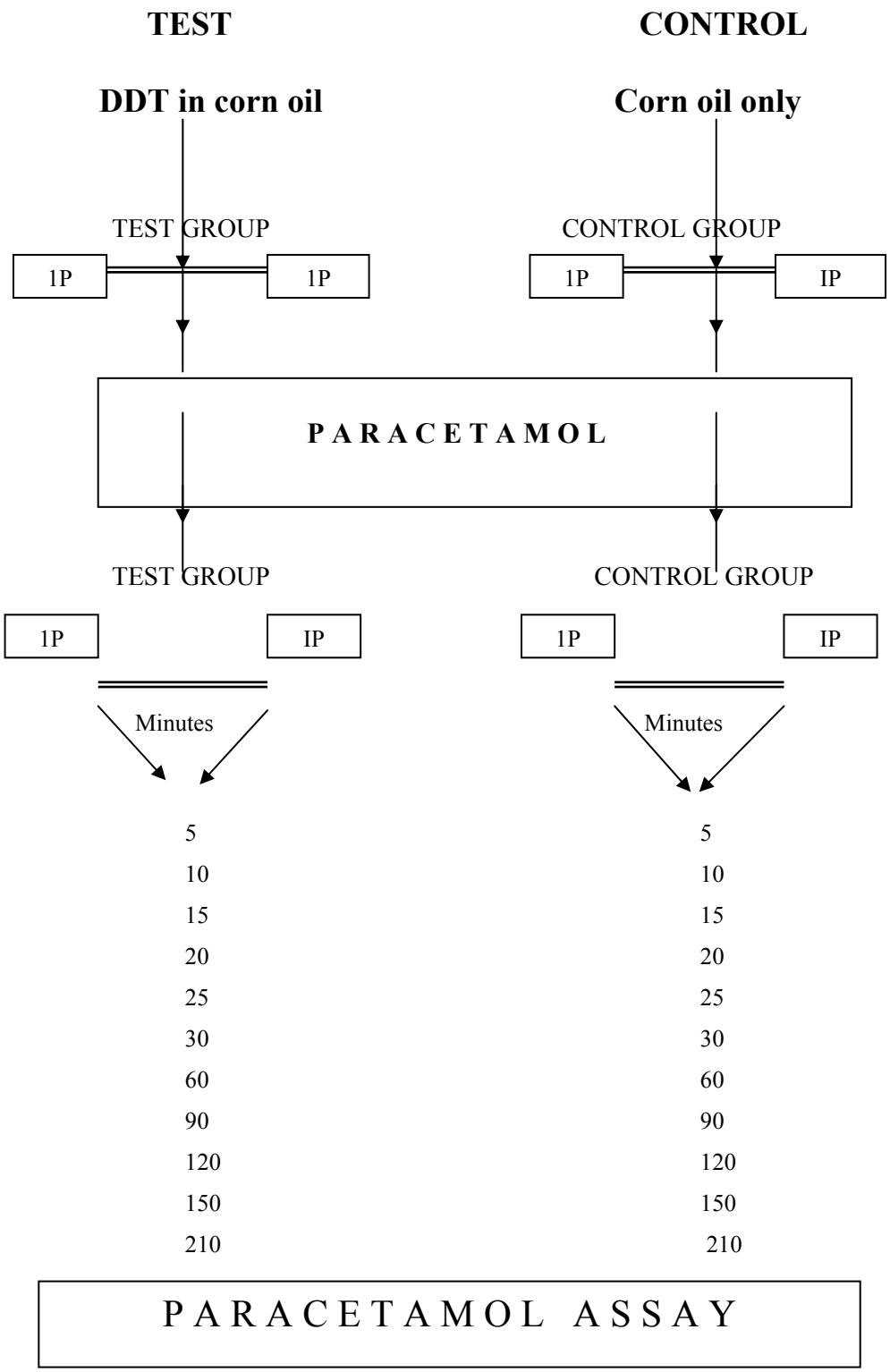
indelible ink and kept in a cage labelled 'Test Group'. These animals were intra-peritoneally (i.p) injected with a stat dose of 300 mg DDT/ kg body weight. The pesticide was dissolved in corn oil. After administration with DDT, the test group animals were kept for 14 days in order to allow maximum exposure to DDT before administration of paracetamol. The rats were kept under normal day/night circle. The cages were cleaned frequently and food and clean water replenished daily.

The Control Group

Twenty-four rats were used as the control group. They were given only corn oil (i.p). Corn oil was injected intra-peritoneal (i.p) to each rat, at a dose of 2 µl corn oil/g body weight. The animals were also clearly marked on their tails, accurately weighed and kept in a separate cage labelled "Control Group". The animals were also kept for 14 days in the department of Clinical Pharmacology's holding room before the administration of the paracetamol drug. They were also kept under similar conditions as the test group, and these were, a normal day/night circle and clean cages with food and water replenished daily.

Administration of Paracetamol

On day 14 all the 48 rats, were administered with paracetamol as shown in Figure 2.1 below.



IP = intraperitoneally

Figure 2.1 Schematic Presentation of Treatment and Grouping of Rats

Test Group

All the rats were injected intra-peritoneally (i.p) with paracetamol. The paracetamol dose was based on the normal dose for humans that are 1000 mg paracetamol/70 kg body weight. The paracetamol was suspended in a volume of isotonic solution (0.9% NaCl) and injected to each rat i.p as a paracetamol based on average body weight of each rat.

Control Group

All the control animals were also injected intra-peritoneally (i.p) with a similar dose of paracetamol suspension as was done to the test group.

Time Schedule for Administration of Paracetamol

In both the groups, the time schedule for injection of the animals with the paracetamol drug was as illustrated in table 2.5. In each of the two groups 12 rats were treated in duplicates making a total of 24 rats for each of the two groups stated. The rats at zero minutes were used as the blank samples, thus the rats were not given the paracetamol drug. The rats from schedule 2-12 from each of the two groups were given a dose of paracetamol as indicated in the diagram. The time-scale was from 5 minutes up to a time-scale 210 minutes.

Collection of samples for Paracetamol assay

At the end of the each time scale as per table 1, the animals were sacrificed by asphyxiation with ether. Each animal was quickly dissected and 5 ml of blood was collected from the superior vena cava of each animal. The blood was placed in a labeled centrifuge tube and was centrifuged. The separated serum was then frozen at minus 20^0

C ready for the paracetamol assay. The whole liver was excised after irrigation with ice cold 0.25 M/L sucrose solution, blotted dry with filter a paper, weighed and stored at minus 70^0 C. This whole procedure collection of blood and of the liver was duplicated as shown in Table 2.5 below.

TABLE 2.5 Time Scale for Administration of Paracetamol in Rats

SCHEDULE	TEST RATS	CONTROL RATS	TIME SCALE (MINUTES)	PARACETAMOL DOSE (per rat)
1	2	2	0	Nil (blank)
2	2	2	5	4.3 mg
3	2	2	10	4.3 mg
4	2	2	15	4.3 mg
5	2	2	20	4.3 mg
6	2	2	25	4.3 mg
7	2	2	30	4.3 mg
8	2	2	60	4.3 mg
9	2	2	90	4.3 mg
10	2	2	120	4.3 mg
11	2	2	150	4.3 mg
12	2	2	210	4.3 mg

TG = Test group expose to DDT: CG = Control group not exposed to DDT.

Two rats were used for each time scale in each group.

Paracetamol assay in Animal serum

The principle of the method is based on the fact that paracetamol is metabolized by conjugation with glucuronic acid and sulphate prior to urinary excretion. Hydrolysis of the glucuronate and sulphate conjugates with concentrated hydrochloric acid gives p-aminophenol. P-aminophenol is conjugated with o-cresol to form a coloured dye. The intensity of the colour formed by this reaction is proportional to paracetamol concentration and this is measured by a spectrophotometer (Nagaraja, *et al.,* 1998; Parajcicic, *et al*., 2003)

Precipitation of protein with trichloroacetic acid is followed by treatment with nitrous acid that converts the formed complex to a nitroso compound that is yellow in colour. The absorbance of the solution is measured on a spectrophotometrical at wavelength of 450 nm. For the serial paracetamol standards, a stock standard solution of paracetamol was made by accurately weighing 0.4 grams of pure paracetamol drug. The drug was then dissolved in 10 ml of dehydrated ethanol and topped up to 100 ml with distilled water. The stock solution was 4 g/l. Bovine serum was used to dilute the stock to working standards that contained paracetamol at concentrations of 0, 50, 100, 200, 400 mg/l. The paracetamol working standards were prepared daily and used for the calibration curve. The linear calibration curve confirmed to the Beer and Lambert's Law; which states that- under suitable conditions the amount of light absorbed by a solution when illuminated with light of a suitable wavelength is directly proportional to the concentration of the coloured solution and the length of the light path through the solution. Its signified by the formula: -

A= e b c; where, A = absorbance, e = molar absorbivity (M), b = path of light (cm),

and c = concentration of paracetamol.

Determination of Paracetamol Concentration

The assay was done in duplicate for each sample of serum collected from each rat. To 1 ml of the serum or standard, 2 ml of trichloacetic acid was added, mixed and centrifuged for 5 minutes. In a separate tube 1 ml of hydrochloric acid was added to 2 ml of sodium nitrite solution and mixed.

To the mixture from step 2, 20 ml of the supernatant from step 1 was added, mixed and allowed to stand at room temperature (25°C) for 2 – 3 minutes. Then 2 ml of ammonium sulfamate was carefully added drop by drop to insure that excess nitrous acid was removed. To this mixture 2 ml of sodium hydroxide solution was added, vortex-mixed to remove any gas bubbles that may interfere with the readings. The absorbance of each sample was measured using a Shimadzu spectrophotometer at 450 nm wavelength against plasma blank. The serum paracetamol concentration of each sample was extrapolated form the standard curve.

2.6 Effects of DDT on the Pharmacokinetics of paracetamol

The blood sample for the half-life study was collected from the studied breast-feeding mothers from Harare and Kariba. A questionnaire was also used to find out if paracetamol was a commonly used drug as analgesic and as antipyretic amongst the breast-feeding mothers. It was therefore accepted as the most commonly used drug in these areas.

2.6.1 Paracetamol Administration

Paracetamol was presented as white tablets, manufactured by Varichem a Zimbabwean company. The strength of each tablet was 500 mg paracetamol. The dose administered to each participating mother was 2 x 500 mg paracetamol tablets. This is the recommended dose for an adult human being (EDLIZ, 2000). This dose was given orally (o.p) to each mother and washed down with a glass of water. Each participating mother should not have used the paracetamol drug for the past 24 hours. The plasma half-life of paracetamol at therapeutic dose is 2 – 4 hours for a normal adult.

2.6.2 Blood Sample Collection

Blood samples were collected from mothers for OCPs analysis. A tourniquet was used on the arm to enhance blood flow from the vein. The skin on the area to be punctured was cleaned with methylated spirits using clean cotton wool. Blood samples were collected with a 5 ml disposable plastic syringe from the basilic or cephalic superficial vein, into a plain 10 ml (EDTA) blood sample tube. The tubes were gently turned to mix blood with anticoagulant. Blood was then centrifuged for 10 minutes at 2 000 g and plasma was collected into properly labelled tubes. The tubes were stored in a freezer at minus 20°C.

2.6.3 Blood Collecting Time Scale

Seven blood samples were collected from each mother. Sample number 0 was collected from each mother before giving the participant paracetamol tablets. This was the blank or control sample for each mother. Sample 1 was collected 30 minutes post dose, then after that every thirty minutes, three times and then after 60 minutes, two times. Total

collection time for each mother was 240 minutes (4 hours). The following Table 2.6 illustrates the time scale for collection of blood samples post dose. The exact time point when each blood sample was drawn after the dose intake was accurately recorded.

TABLE 2.6 Time Interval for Human Blood Collection

TUBE NUMBER	TIME INTERVAL (MINUTES)	ACCUMULATIVE TIME (MINUTES)	ADMINISTRATION OF DRUG
0	0	0	No Paracetamol
1	30	30	Post dose
2	30	60	Post dose
3	30	90	Post dose
4	30	120	Post dose
5	60	180	Post dose
6	60	240	Post dose

A total of fourteen mothers were sampled for the paracetamol pharmacokinetics, and these were participating mothers with high DDT levels and fourteen from mothers with low DDT levels. A total of 28 mothers were sampled for this paracetamol study 14 for the test group and 14 for the control group. A total of 196 blood samples were collected from all the 28 mothers. The samples were centrifuged and the plasma stored in a freezer at -20°C in clearly labeled sample bottles until ready for the paracetamol assay.

2.6.4 Determination of Paracetamol concentration in Human Blood

Paracetamol concentration in the human plasma samples was measured by the automated immunochemistry system (auto ICS). The model used for this assay was a (FPIA, TDX) from Abbot. The (Auto ICS) is a modular microcomputer-controlled system, used for therapeutic drugs monitoring (measuring) in human biological fluid. The system consisted of a nephelometer that measured the rate of light scatter-formation that resulted from an immunoprecipitation reaction. The equipment has a data processor that controls the activities and prints out the results. The reagents for the test kit were, phosphate buffered saline, with 0.1% sodium azide and a borate buffer in 0.9% sodium chloride. A paracetamol programme-card was used to set up the electronic parameters of paracetamol. A volume of 1µl was injected into the reaction flow cell for calibration. After the calibration, 42 µl of the human blood sample was pipetted into the test cell were it was diluted with 8µl of phosphate buffer solution and gently mixed. From this buffered sample solution, 1µl was pipetted and injected into the reaction cell for analysis. The paracetamol concentration results were read from a data processor.

The method used an antigen – antibody reaction in which paracetamol was the antigen. The formation of light-scattering complexes was dependent on the presence of antigen and antibody molecules in optimal proportions. For a constant amount of antibody, the degree of complexation increased with the amount of antigen/paracetamol present. By maintaining a constant level of antibody, and measuring the peak rate signal generated by increasing concentrations of antigen, it was possible to form a peak rate, which was a function of antigen concentration. The peak rate which was a function of changes in scattered light was detected and converted to peak rate units which were a measure of

paracetamol concentration, and was read on the display unit (Marrack and Richards, 1971).

CHAPTER THREE

3.0 RESULTS

3.1 Validation GC Methodology

3.1.1 Validation of calibration standards.

The results of validation of the GC method obtained are demonstrated by the standard curves of pp-DDE, β-HCH and HCB standards, as shown in Figures 3.1, 3.2 and 3.3 whose values are derived from Tables 3.1, 3.2 and 3.3.

Table 3.1 Calibration Standard pp-DDE

Tube Number	Concentration in ng/ml	Height Ratio
1	5.0	0.60
2	10.0	1.30
3	15.0	2.00
4	20.0	2.75
5	25.0	3.50
6	30.0	4.25
7	35.0	5.00
8	40.0	5.75

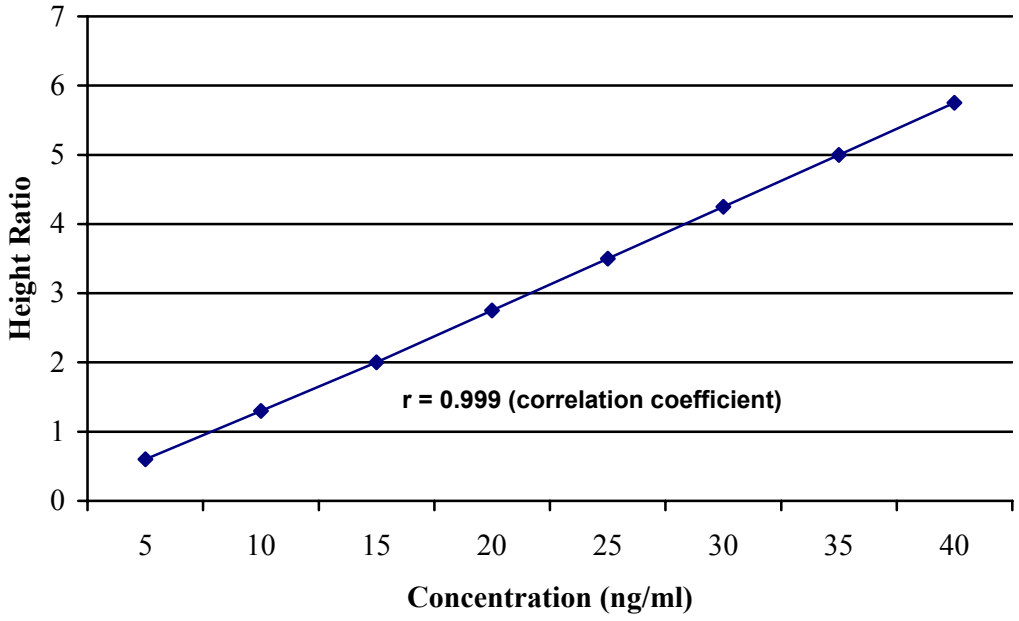


Figure 3.1 Calibration Standard Curve pp-DDE in (ng/ml)

Table 3.2 Calibration Standard β-HCH

Tube	Concentration in ng/ml	Height Ratio
1	5.0	0.6
2	10	1.1
3	15	1.6
4	20	2.1
5	25	2.6
6	30	3.1
7	35	3.6
8	40	4.1

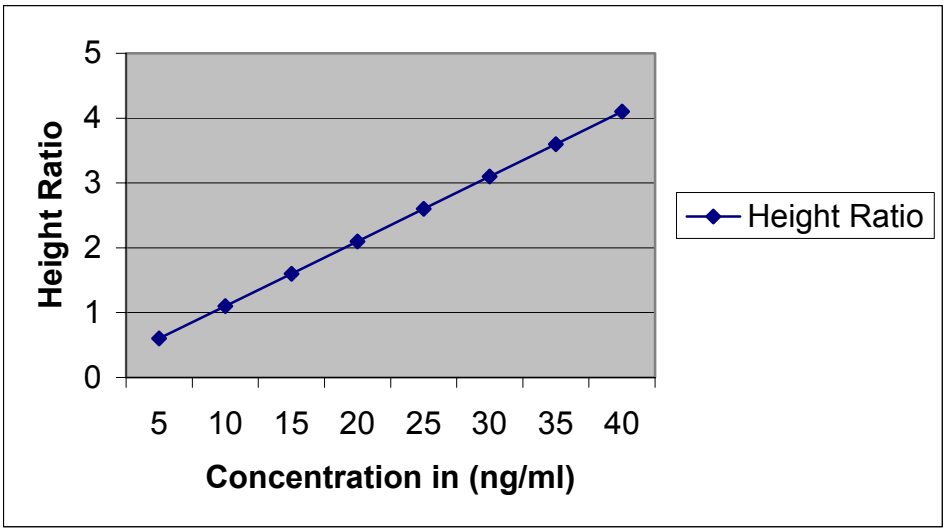


Figure 3.2 Calibration Standard Curve β-HCH in (ng/ml)

r = Correlation coefficient

Table 3.3 Calibration Standard HCB

Tube Number	Concentration In ng/ml	Height Ratio
1	0.5	0.50
2	1.50	1.50
3	3.00	3.35
4	6.00	7.00

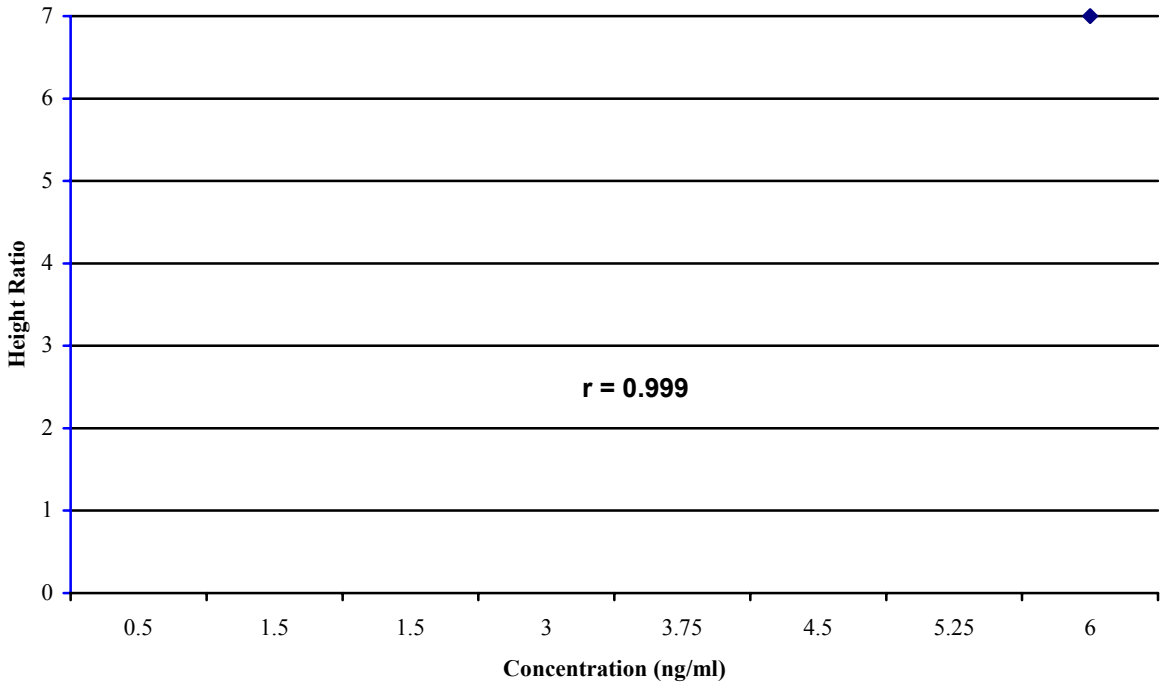


Figure 3.3 Calibration Standard Curve of HCB in (ng/ml)

r =Correlation Coefficient

A linear relationship is observed in the above standard calibration curves i.e. Figure 3.1, 3.2 and 3.3.

3.1.2 Validation of Metabolites Standards Method

Reproducibility of the method for analysis of different organochlorine pesticides i.e. HCB, HCH α, β, and γ isomer standards and chlorinated pesticides mixture standards are shown in Table 3.4 below.

Table 3.4 Reproducibility of HCB, HCH and CPM standards

Standards used / Conc. In ng/ml	Inject. A	Inject. B	Inject. C	Mean of 3 inject.	% Standard Deviation
HCB 1.50	1.49	1.50	1.50	1.50	0.4
Oxy-Klordan 3.75	3.74	3.75	3.75	3.75	0.2
Trans-Klordan 2.50	2.50	2.50	2.49	2.50	0.2
Cis-Klordan 2.50	2.49	2.50	2.49	2.49	0.2
Trans-Nonaklor 3.75	3.75	3.75	3.75	3.75	0.0
Cis-Nonaklor 2.50	2.51	2.50	2.50	2.50	0.2
Mirex 12.50	12.50	12.49	12.50	12.50	0.1
α-HCH 2.50	2.49	2.50	2.50	2.50	0.2
β-HCH 10.00	9.85	10.00	10.00	10.00	0.9
γ-HCH 2.50	2.50	2.50	2.50	2.50	0.0
pp-DDE 10.00	10.00	9.95	10.00	9.98	0.3
op-DDD 20.00	20.0	20.05	20.0	20.02	0.1
pp-DDD 19.00	18.95	19.00	19.00	18.98	0.2
op-DDT 22.50	22.50	22.50	22.50	22.50	0.0
pp-DDT 26.00	26.00	25.98	26.00	25.99	0.1

Key: -
HCB = Hexachlorobenzene.
HCH = Hexachlorohexane.
CPM= Chlorinated Pesticide Mixture.
Conc.= Concentration in ng/ ml.
A = 1st injection.
B = 2nd injection.
C = 3rd injection.

The above results show reproducibility of the method with % standard deviation or coefficient of variance ranges of 0.2 – 3.3.

Results of method reliability are demonstrated by recovery runs of HCH isomers and DDT metabolites standards, as in Table 3.5 below.

Table3.5 Recoveries of α, β and γ HCH, and DDT metabolites standards

Standards used / Conc. in ng/ml		Recov. A	Recov. B	Recov. C	Recov. D	Mean	SD%
α-HCH	5	4.96	4.98	5.00	5.00	4.99	0.38
β-HCH	20	19.98	19.95	20.00	20.00	19.98	0.13
γ-HCH	5	4.96	5.00	5.00	4.98	4.99	0. 38
pp-DDE	20	19.96	19.98	20.00	20.00	19.99	0.10
op-DDD	40	39.98	40.00	39.95	40.00	39.98	0.06
pp-DDD	38	38.01	37.95	37.95	38.00	37.98	0.08
Op-DDT	45	44.95	45.00	44.98	45.00	44.98	0.05
pp-DDT	52	51.98	51.96	52.00	52.00	51.99	0.04

Key: -
Mean. = Average of 4 runs. % SD= %Standard deviation.
Conc. = Concentration.
Recov.= The matrix used for the recoveries was, pooled commercial cows milk.

α-HCH, β-HCH and γ-HCH = Isomers of Hexachlorohexane.
op- DDD, pp- DDE, pp- DDD, op- DDT and pp- DDT = Metabolites of DDT.

In the above Table 3.5 results show method reliability as demonstrated by recovery runs and % standard deviation or Coefficient of variation with ranges of 0.04 – 0.38 %.

3.1.3 Validation of Staple Foods Results

The following Table 3.6 is validation of staple foods results, using internal standard recoveries in staple foods from Mudzi area.

Table 3.6 Recoveries of Internal Standards in Foods- Mudzi

Staple Foods	Internal Std. Conc. ng/g	Recoveries Conc. Ng/g	Individual Difference
River Water	5.0	4.81	- 0.19
Fresh Milk	5.0	4.87	- 0.13
Sour Milk	5.0	4.96	- 0.04
Vege. (Covo)	5.0	4.88	- 0.12
Cabbage	5.0	4.84	- 0.16
Maize Meal	5.0	4.85	- 0.15
Peanut Butter	5.0	4.87	- 0.13
Fish	5.0	4.93	- 0.07
Total	**40.0**	**39.43**	**- 0.99**
Mean N=8	**5.0**	**4.92875**	**- 0.12375**

Key: -

N= Number of sampled foods from Mudzi. Internal Standard = PCB 112 isomer

Conc. = Concentration of standard in ng/ml

The above Table 3.6 showed good recoveries (ranges 4.81 – 4.93) from the food matrices (8) with a % standard deviation (Coefficient of variation) of 1.77%.

3.1.4 Validation of Paracetamol Method

Figure 3.4 below shows results whose values are derived from Table 3.7.

Table 3.7 Paracetamol Standards

Standard Number	Concentration in mg/ml	Absorbance
1	0.00	0.00
2	50.00	0.10
3	100.00	0.20
4	200.00	0.30
5	400.00	0.40

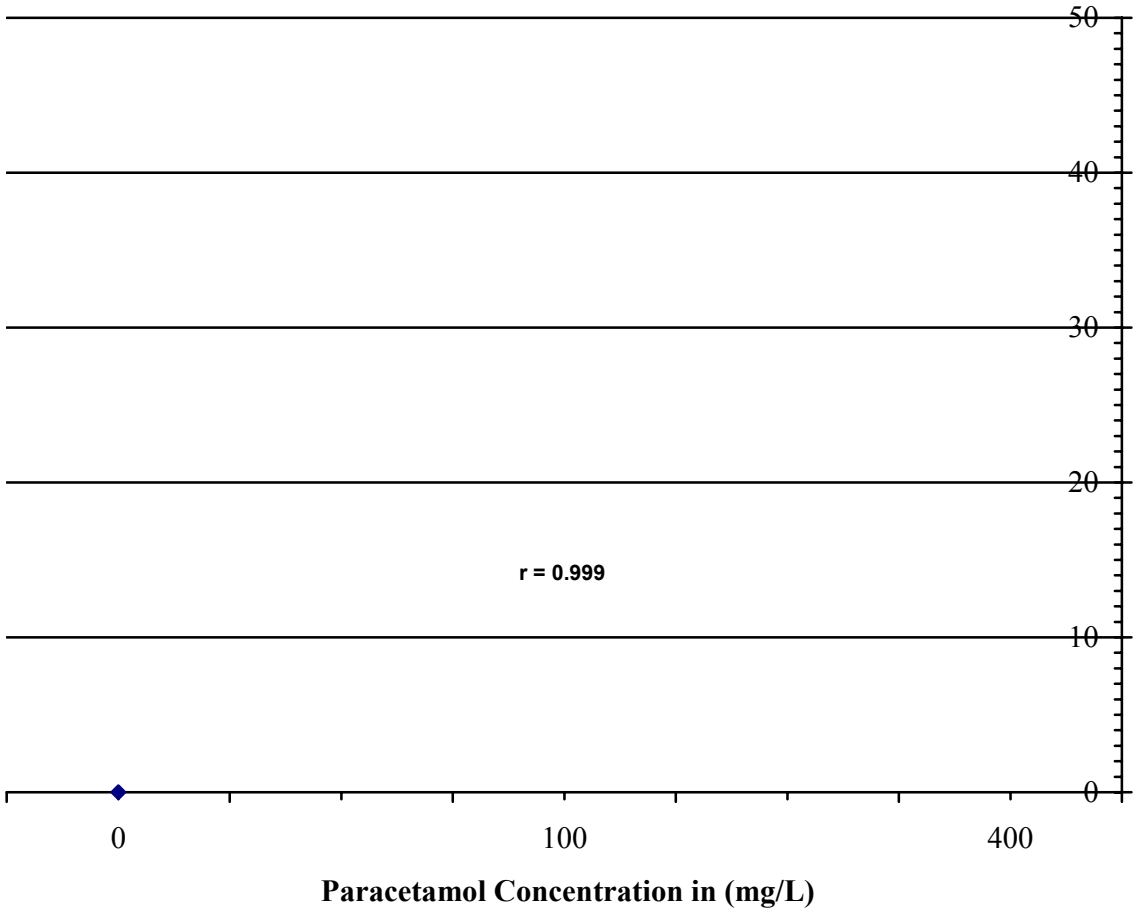


Figure 3.4 Paracetamol Standard Curve

r = Correlation coefficient.

The paracetamol curve above demonstrates linearity.

3.2 DDT and HCH residues in Selected Staple foods.

The results obtained on DDT and HCH residues in selected staple foods in six sampled areas of Zimbabwe are shown in Table 3.8.

Table 3.8 DDT and HCH Residues in Selected Staple Foods from 6 Areas of Zimbabwe.

Staple Foods / Areas	Nyanga		Mudzi		Kariba		Kadoma		Esigodini		Harare	
	HCH	DDT	HCH	DDT	HCH	DDT	HCH	DDT	HCH	DDT	HCH	DDT
Water (local river)	5 ±0.9	26 ±1.0	nd	27 ±0.9	1 ±0.9	33 ±0.8	Nd	nd	Nd	Nd	nd	5 ±0.8
Milk fresh (Local Cow)	119 ±0.9	79 ±1.0	36 ±1.0	75 ±0.7	Nd	609 ±0.8	3 ±0.7	43 ±0.8	10 ±1.0	32 ±0.9	10 ±0.9	13 ±0.6
Milk Sour (local cow)	116 ±0.1	78 ±1.0	32 ±1.0	79 ±0.7	Nd	603 ±0.8	3 ±0.9	46 ±0.9	9 ±0.9	29 ±1.0	9 ±1.2	12 ±0.8
Vegetable (covo)	35 ±0.0	421 ±1.0	26 ±1.0	695 ±0.6	6 ±1.3	547 ±0.8	66 ±1.0	466 ±0.6	3 ±0.8	9 ±0.9	3 ±0.8	36 ±0.8
Cabbage (local)	33 ±0.8	522 ±1.0	26 ±1.0	591 ±0.8	7 ±0.9	148 ±0.6	53 ±0.6	446 ±0.9	2 ±0.9	20 ±0.9	4 ±0.8	39 ±1.3
Maize meal (upfu)	18 ±0.2	662 ±1.0	663 ±0.9	874 ±0.8	3 ±0.9	27 ±0.5	nd	nd	1 ±0.6	7 ±0.7	Nd	27 ±0.9
Peanut butter (dovi)	16 ±1.0	298 ±0.9	121 ±0.8	372 ±0.8	nd	90 ±0.7	nd	nd	nd	nd	4 ±1.3	37 ±0.7
Fish (local river)	76 ±0.9	124 ±0.9	71 ±0.9	103 ±0.6	3 ±0.8	956 ±0.2	10 ±0.9	16 ±0.9	nd	nd	4 ±0.7	320 ±0.9
Total	418	2210	975	2816	20	3013	135	1017	25	97	34	489
Mean	52	276	122	352	3	377	17	127	3	12	4	61

Key: -
nd = Residues not detectable. Total = Sum of the residues analysed in 8 selected foods in each area.
n = Number of types of foods sampled in that area. Mean = average of 8 selected foods from each. Concentration of samples in ng/g

Figures represent means ± standard deviations

The highest DDT level of 956.0 ng/g was observed in the Kariba area from bream fish (*Tilapia andersonic and Tilapia mossambica*) from Kariba Lake. Mudzi area had the highest HCH level that was analysed in maize meal and this was 85.5 ng/g. Kariba area had the highest mean residue levels of DDT i.e. 376.6 ng/g while Esigodini a rural area had the lowest of 12.1 ng/g.

3.3 Residues of Organochlorine Pesticides in Mothers Milk

A total of 111 milk samples were collected from six selected areas. Table 3.9A shows the characteristics of the mothers sampled in different areas.

Table 3.9A Characteristics of Participating Mothers

Areas Sampled	**Esigodini**	**Harare**	**Kadoma**	**Kariba**	**Nyanga**	**Mudzi**
Number (N)	9	10	12	37	27	16
Av. Mother age (Yrs.) Range	23 19-33	24 19-35	22 18-32	23 17-29	23 16-30	23 17-36
Av. Infant age (Mths.) Range	5 2-18	9 4-14	7 2-22	5 3-12	6 3-18	8 4-18
Av. Parity Range	1 1-2	2 1-3	1 1-2	1 1-2	1 1-2	1 1-2

Key: -

N= Number of milk sampled. Av. = Average. Yrs. = years. Mths. = months.

Table 3.9B below shows different levels of HCH isomers in six areas.

Table 3.9B Mean Levels of HCH isomers in Mother's Milk- Conc. (ng/g fat)

Areas Sampled N	**Esigodini 9**	**Harare 10**	**Kadoma 12**	**Kariba 37**	**Nyanga 27**	**Mudzi 16**
α-HCH	**10.5** ±3.5	**7.2** ±2.6	**6.8** ±2.1	**4.9** ±1.4	**121.1** ±19.7	**27.8** ±6.8
β-HCH	**31.3** ±5.9	**59.3** ±7.4	**62.2** ±8.9	**25.7** ±5.3	**552.1** ±28.4	**124.8** ±19.7
γ-HCH	**6.6** ±3.1	**6.8** ±2.8	**5.9** ±1.3	**5.5** ±1.6	**214.1** ±22.2	**45.9** ±4.5
Total HCH	**48.4 ±6.3**	**73.3 ±6.4**	**74.9 ±6.2**	**36.1 ±4.2**	**887.3 ±35.2**	**198.5 ±15.5**

Key: -

Conc. = Concentration. N= Number of milk samples. ± = Associated error estimates.

Table 3.9B above shows that β- HCH isomer has the highest mean level of residues. Nyanga area, a forestry and fruit growing area had the highest mean levels of HCH (887.20 ng/g) compared to all the other areas analysed. While Kariba a national park area with vector control programmes had the lowest mean level of HCH (36.11 ng/g).

Levels of metabolites of DDT in six areas are shown in Table 3.9C below.

Table 3.9C Mean Levels of DDT Metabolites in Mother's Milk- Conc. (ng/g)

Area Sampled N	**Esigodini 9**	**Harare 10**	**Kadoma 12**	**Kariba 37**	**Nyanga 27**	**Mudzi 16**
pp-DDE	**581.2** ±13.5	**1735.6** ±20.9	**1314.4** ±19.8	**5782.7** ±21.6	**5958.9** ±24.3	**13784.4** ±38.7
op-DDD	**18.6** ±7.8	**19.2** ±4.1	**8.6** ±2.5	**15.2** ±2.0	**71.3** ±5.9	**23.5** ±6.4
pp-DDD	**14.5** ±6.3	**3.1** ±0.6	**2.4** ±0.2	**71.7** ±6.7	**52.4** ±4.6	**156.9** ±7.7
op-DDT	**51.5** ±8.1	**18.7** ±4.7	**17.4** ±3.6	**109.9** ±7.8	**846.5** ±11.9	**238.1** ±8.9
pp-DDT	**268.4** ±9.2	**536.3** ±7.9	**33.2** ±4.7	**2006.5** ±15.2	**816.1** ±10.2	**2636.1** ±13.7
Total DDT	**934.2 ±10.8**	**2312.9 ±11.9**	**1376.0 ±10.3**	**7986.0 ±14.5**	**7745.2 ±13.2**	**16838.9 ±17.9**

Key: -

N= Number of milk samples. Conc. = Concentration. ± = Associated error estimates.

Mudzi had the highest mean residues of DDT (16838.90 ng/g) and Esigodini showed the lowest i.e. 934.12 ng/g.

3.3.1 Relationship between pp-DDT/pp-DDE of Mothers Milk in Six Areas

Table 3.10 below shows the relationship of pp-DDT/pp-DDE.

Table 3.10 Ratio of pp-DDT/pp-DDE of Breast Milk in Six areas

Sampled Areas	pp-DDT	pp-DDE	Ratio pp-DDT/pp-DDE
Esigodini N = 9	268	581	0.46
Harare N = 10	536	1736	0.31
Kadoma N = 12	33	1314	0.03
Kariba N = 37	2 007	5783	0.35
Nyanga N = 27	816	5 959	0.14
Mudzi N = 16	2 636	13 784	0.19

Key: -
N= Number of milk samples from each area. Concentrations in ng/g fat

pp- DDT and pp-DDE = Metabolites of DDT

From Table 3.10 above the highest ratio was observed in Esigodini and the lowest was in observed in Kadoma.

3.3.2 Relationship of DDT and HCH mean residues in staple Food and in Mother's milk

The above relationship is shown in Table 3.11 below.

Table 3.11 Relationship of mean levels of DDT and HCH in Staple Food and Mothers Milk.

Source / Area	Esigodini.	Harare	Kadoma	Kariba	Nyanga	Mudzi
Staple Food (DDT)	12.1	61.0	127.2	376.7	276.1	352.0
Mother's Milk (DDT)	934.1	2312.9	1375.8	7985.7	7745.2	16838.9
Staple Food (HCH)	3.1	4.2	16.9	2.3	38.2	22.5
Mother's Milk (HCH)	48.5	73.3	74.9	36.1	887.2	37.6

Key: -

Values = Concentration in ng/g. Mean values = Mothers milk and Staple foods

From the Table 3.11 above the highest mean DDT levels in food is Kariba, and the highest in mother's milk is Mudzi. The highest in mean HCH in food is Nyanga and the mean highest in mother's milk is also Nyanga. The lowest mean HCH level in both food and mother's milk is in Kariba area and the lowest mean DDT levels both in food and mother's milk is in Esigodini area.

Correlation analysis revealed a significant correlation (r = 0.8857, p = 0.0188) between DDT levels in staple food and mother's milk. A correlation was observed between HCH levels in staple food and mother's milk; however, this correlation was not statistically significant (r = 0.6571, p= 0.1562).

3.4 Induction of Hepatic Cytochrome P-450 Enzyme by DDT in Rats

3.4.1 Cytochrome P 450 Enzyme against Time in Rats

Figure 3.5 below shows induction of cytochrome P-450 by DDT in rats. The variables are derived from Table 3.12 below.

Table 3.12 Cytochrome P 450 Concentration against Time in Rats

Sample Number	Time Days	Cytochrome P 450 (n mol/ mg)	Sample Number	Time Days	Cytochrome P 450 (n mol/mg)
TR 1	4	3.15 ± 3.22	CR 1	4	0.51 ± 0.54
TR 2	5	3.46 ± 3.50	CR 2	5	0.53 ± 0.55
TR 3	6	3.47 ± 3.42	CR 3	6	0.55 ± 0.58
TR 4	7	4.13 ± 4.20	CR 4	7	0.54 ± 0.55
TR 5	8	4.39 ± 4.42	CR 5	8	0.56 ± 0.57
TR 6	9	4.75 ± 4.64	CR 6	9	0.55 ± 0.54
TR 7	10	5.03 ± 5.00	CR 7	10	0.55 ± 0.55
TR 8	11	5.26 ± 5.32	CR 8	11	0.56 ± 0.54
TR 9	12	5.27 ± 5.34	CR 9	12	0.55 ± 0.56
TR 10	13	7.35 ± 6.89	CR 10	13	0.56 ± 0.55
TR 11	14	8.51 ± 8.48	CR 11	14	0.55 ± 0.52
TR 12	15	5.00 ± 4.86	CR 12	15	0.56 ± 0.58
TR 13	16	4.42 ± 4.45	CR 13	16	0.55 ± 0.53

Key: -
TR= Test rats DDT induced. CR= Control rats not induced.
Concentration = Cytochrome P 450 concentration in n mol/mg. ± = Results of duplicate animal.

Figure 3.5 Cytochrome P450 Conc. against Time after Treatment with DDT

Maximum induction of cytochrome P450 for test animals was on day 14 at 8.51 n mol/mg while the control rats displayed no change in cytochrome P450 levels during the course of the experiment. The range of cytochrome P 450 concentration for the control animals was (0.51 – 0.56 n mol/mg) with a mean value of 0.55 and a standard deviation of 0.019.

3.4.2 Comparison of Liver and Body Weight Ratio in Rats

Figure 3.6 below displays liver and body weight ratio in rats and the figures are derived from Table 3.13 below.

Table 3.13 Liver and Body weight in Rats

Sample Number	Body Wt. (g)	Liver Wt. (g)	Liver Wt./ Body Wt. %	Sample Number	Body Wt. (g)	Liver Wt. (g)	Liver Wt./ Body Wt.%	Time Days
TR 1	156.7	7.0	4.5	CR 1	159.1	4.3	4.5	4
TR 2	158.9	7.2	4.5	CR 2	157.9	4.3	4.6	5
TR 3	159.4	7.2	4.5	CR 3	159.6	4.3	4.6	6
TR 4	138.7	6.8	4.9	CR 4	143.1	3.9	4.6	7
TR 5	170.9	9.1	5.3	CR 5	165.9	4.2	4.6	8
TR 6	159.5	9.1	5.7	CR 6	162.2	4.2	4.6	9
TR 7	326.9	18.6	5.7	CR 7	328.0	4.1	4.6	10
TR 8	153.8	8.8	5.7	CR 8	165.3	3.9	4.6	11
TR 9	293.1	17.0	5.8	CR 9	290.8	4.2	4.6	12
TR 10	285.7	16.7	5.9	CR 10	279.9	4.3	4.6	13
TR 11	329.5	19.4	5.9	CR 11	331.1	4.2	4.6	14
TR 12	304.6	17.9	5.9	CR 12	329.9	3.9	4.6	15
TR 13	177.6	10.3	5.8	CR 13	180.3	4.3	4.6	16

Key: -

TR = Test rats DDT induced. CR = Control rats. Wt. = Weight. g = gram weight.

Time = Post dose time in days

Figure 3.6 below displays liver weight/body weight expressed as a percentage against days after treatment with DDT in the two groups. The graph shows maximum liver enlargement (hepatomegaly) on day 14 for the test animals. The control animals showed no significant difference in liver/body weight ratio.

Figure 3.6 Relationship between Liver and Body Weight after Treatment with DDT.

3.5 Effects of DDT on the Pharmacokinetics of Paracetamol in Rats

3.5.1 Effects of Cytochrome P450 induction on Paracetamol bioavailability

Figure 3.7 below displays the effect of induction of cytochrome P450 on the in vivo metabolism of paracetamol in the female rats and the variables were derived from Table 3.14 below.

Table 3.14 Time against drug Concentration in Rats

Sample Number	Time Minutes	Drug Conc. in (mg/L)	Sample Number	Time Minutes	Drug Conc. in (mg/L)
IPT 1	5	10.1 ± 9.8	IPC 1	4	2.2 ± 2.4
IPT 2	10	19.5 ± 20.0	IPC 2	12	5.0 ± 4.9
IPT 3	20	33.4 ± 34.0	IPC 3	23	11.4 ± 11.5
IPT 4	25	43.5 ± 44.2	IPC 4	30	15.6 ± 15.4
IPT 5	30	50.2 ± 49.6	IPC 5	40	21.5 ± 21.8
IPT 6	60	33.6 ± 34.0	IPC 6	60	31.8 ± 32.0
IPT 7	90	24.2 ± 24.5	IPC 7	90	27.4 ± 26.8
IPT 8	120	14.8 ± 15.0	IPC 8	120	24.2 ± 24.6
IPT 9	154	11.6 ± 12.2	IPC 9	156	20.5 ± 21.0
IPT 10	210	5.8 ± 4.9	IPC 10	210	16.2 ± 16.9

Key: -

IPT= Test rats DDT induced. IPC = Control rats not induced. Drug = Paracetamol

Conc. = Concentration of drug. ± ± = Results of duplicate animal.

Time = Post dose time in minutes

Figure 3.7 Effect of Cytochrome P450 induction on the Bioavailability of Paracetamol

From the Figure 3.7 above maximum drug concentration was 50.1 mg/L at 30.5 minutes for the test animals while the maximum drug concentration for the control animals was 31.6 mg/L at 60.0 minutes.

3.5.2 Effects of Cytochrome P450 induction on paracetamol half-life in rats

Effects of enzyme induction on drug half-life after Figure 3.8 below demonstrates pre-treatment with DDT and the values were derived from Table 3.15 below.

Table 3.15 Post dose Time and drug concentration on log scale in rats

Sample Number	Time Minutes	Drug Conc. Log (mg/L)	Sample Number	Time Minutes	Drug Conc. Log (mg/L)
IPT 1	5	1.1 ± 1.0	IPC 1	4	0. 31 ± 0.29
IPT 2	10	1.1 ± 1.1	IPC 2	12	0.68 ± 0.70
IPT 3	20	1.3 ± 1.2	IPC 3	23	0.98 ± 0.94
IPT 4	25	1.5 ± 1.5	IPC 4	30	1.19 ± 1.16
IPT 5	30	1.7 ± 1.7	IPC 5	40	1.33 ± 1.30
IPT 6	60	1.8 ± 1.7	IPC 6	60	1.50 ± 1.48
IPT 7	90	1.4 ± 1.4	IPC 7	90	1.44 ± 1.45
IPT 8	120	1.2 ± 1.2	IPC 8	120	1.39 ± 1.42
IPT 9	154	1.1 ± 1.1	IPC 9	156	1.31 ± 1.28
IPT 10	210	0.8 ± 0.7	IPC 10	210	1.21 ± 1.20

Key: -

IPT = Rats pre-treated with DDT. IPC = Control rats not treated.

L = logarithm. Conc. = Concentration of drug in mg/L.

± ± = Duplicate results from 2nd animal used in each time scale.

Figure 3.8 Serum concentration/ Time profile of paracetamol in Female rats

From the Figure 3.8 above half-life of paracetamol in DDT pre-treated rats was 144.0 minutes while the half-life of paracetamol in the non-treated animals was 380 minutes. The difference in t½ of paracetamol between the two groups (induced and non-induced) is 236 minutes.

Area under the curve (AUC), volume of distribution (Vd) and total body clearance (TBC) were deduced from the graph as shown in Table 3.16 below.

Table 3.16 Pharmacokinetic values of Paracetamol in rats

	Vd (L/kg)	AUC- (sq cm)	TBC- (ml/minute)
Pre-treated rats	**0.06**	**4953.5**	**2.62**
Non- treated rats	**0.09**	**9138.3**	**1.42**

Key: -

Vd = Volume of distribution.

AUC = Area under the curve.

TBC = Total body clearance.

Pre-treated rats = DDT induced rats.

Non-induced rats = Not induced rats.

3.6 Effects of DDT on the Pharmacokinetics of Paracetamol in Humans

3.6.1 Choice of Pain Relief Drugs

The Table 3.17 below show pain relief drugs used during the period 1995 to 1999.

Table 3.17 Different drugs used for pain relief by interviewed mothers

DRUG	NUMBER/MOTHERS USING THE DRUG	% Mothers Using The Drug
Aspirin	87	21.5
Ibuprofen	5	1.2
Paracetamol	242	59.9
Stopayne	38	9.4
Indomethacin	12	3.0
"No Drug	20	5.0
Total	404	100

Paracetamol had the highest usage rate of 59.9%; followed by aspirin with a 21.5% usage rate, and the least was ibuprofen with a 1.2% usage rate.

3.6.2 Effects of DDT on the Pharmacokinetics of Paracetamol

Table 3.18 represents data of group mothers highly exposed to DDT and the plasma concentration of paracetamol after an oral dose of the drug. The Table 3.18 also shows log concentration of paracetamol.

Table 3.18 Plasma Concentration of Paracetamol Following an Oral Dose in Highly Exposed Mothers

Sample Gp.	DDT ng/g	Tm	Dc	Tm	Dc	Tm	Dc	Tm	Dc	Tm	Dc	Tm	Dc	Tm	Dc
1	1835	0	3	31	98	63	141	94	112	153	66	216	15	241	0
2	4115	0	0	30	103	62	92	87	81	153	27	215	14	240	0
3	2725	0	0	32	124	74	111	92	99	158	64	219	16	240	0
4	4688	0	0	31	120	62	90	91	78	152	32	215	17	243	0
5	4371	0	0	36	104	63	102	91	87	150	78	214	18	240	0
6	3654	0	0	31	127	67	101	93	81	150	44	214	19	243	0
7	1897	0	0	30	119	60	86	105	62	165	30	216	16	225	10
8	4908	0	0	30	98	60	85	125	63	160	32	215	14	253	0
9	2307	0	0	30	96	70	93	108	67	170	40	217	10	244	8
10	1897	0	1	40	113	80	97	120	64	167	27	216	12	250	6
11	3019	0	3	33	103	70	86	98	83	160	26	218	15	246	9
12	2292	0	0	29	105	61	75	91	84	159	37	216	16	227	0
13	3988	0	0	27	125	62	103	92	98	152	38	212	16	239	0
Mean N =13	**3207**	**0**	**1**	**32**	**110**	**65**	**97**	**99**	**81**	**157**	**41**	**216**	**15**	**241**	**3**
Log_{10} Conc.	**-**	**-**	**0**	**-**	**2.1**	**-**	**2.0**	**-**	**1.9**	**-**	**1.6**	**-**	**1.2**	**-**	**0.5**

Key: -

Thirteen mothers exposed to 1500 – 5000 ng/g DDT**.**
Conc. = Log concentration of paracetamol. Tm = Post dose time in minutes.

Dc = Paracetamol concentration in mg/L. Mean = Average of thirteen mothers.
Gp = Highly DDT Exposed Mothers N = Number of mothers

Table 3.19 shows 13 mothers least exposed to DDT. The table also shows log concentration of paracetamol against time.

Table 3.19 Plasma Concentration of Paracetamol Following an Oral dose in low Exposed mothers

Sample Gp.	DDT ng/g	Tm	Dc	Tm	Dc	Tm	Dc	Tm	Dc	Tm	Dc	Tm	Dc	Tm	Dc
1	523	0	0	29	16	62	24	92	89	152	60	209	22	252	0
2	567	0	0	32	22	66	28	101	72	157	47	218	21	250	0
3	570	0	0	31	14	63	21	93	55	159	42	211	24	251	0
4	1254	0	0	34	12	65	23	96	53	159	64	218	25	250	0
5	932	0	0	29	11	59	21	96	51	156	66	213	22	252	1
6	923	0	0	33	14	61	22	91	54	158	58	217	20	250	0
7	1448	0	0	34	18	63	25	96	76	157	47	217	21	249	0
8	647	0	0	30	10	67	25	97	56	162	63	218	26	251	0
9	1347	0	0	30	18	60	27	99	75	161	61	212	20	225	2
10	916	0	1	33	19	65	29	101	73	160	43	215	23	253	0
11	1347	0	0	30	10	63	26	98	76	164	59	214	25	251	0
12	854	0	0	31	25	64	27	94	63	163	64	225	23	250	1
13	546	0	2	34	10	64	25	94	73	156	69	223	25	251	0
Mean N=13	**913**	**0**	**0**	**32**	**15**	**63**	**25**	**96**	**67**	**159**	**57**	**216**	**23**	**250**	**0**
Log_{10} Conc.	**-**	**-**	**0**	**-**	**1.2**	**-**	**1.4**	**-**	**1.8**	**-**	**1.7**	**-**	**1.4**	**-**	**0**

Key: -
Thirteen mothers exposed to 500 – 1500 ng/g DDT.
Dc = Concentration of paracetamol in mg/L. Tm = Post dose time in minutes.
Conc. = Log concentration of drug. Mean = Average of the thirteen mothers.
Gp = Low DDT Exposed Mothers N = Number of the mothers.

3.6.3 Relationship in Low and Highly DDT exposed Breast-feeding Mothers

Figure 3.9 below shows comparison of the pharmacokinetics of paracetamol in mothers highly exposed to DDT and mothers with low exposure to DDT. The values of Figure 3.9 are derived from Table 3.20 (derived from Tables 3.18 and Table 3.19) below.

Table 3.20 Paracetamol Conc. in least and highly DDT exposed mothers

LEAST EXPOSED		HIGHLY EXPOSED	
Time-Minutes	**Drug Conc. (mg/L)**	**Time-Minutes**	**Drug Conc. (mg/L)**
0	0	0	1
32	15	32	110
63	25	65	97
96	67	99	81
159	57	157	41
216	23	216	15
250	0	241	3

Key: -

Conc. = paracetamol concentration mg/L

Figure 3.9 Relationship of Pharmacokinetics of Paracetamol in Low and Highly Exposed Mothers

Figure 3.9 above show a graph of mothers highly exposed to DDT with paracetamol maximum concentration (Cmax) of 111 mg/L at Tmax 37 minutes. The least DDT exposed mothers show a paracetamol Cmax of 68 mg/L at Tmax of 107 minutes. The difference in the Tmax in the least and high exposed mothers is 70 minutes a 65.4% difference.

3.6.4 Paracetamol Half-Life (t½)

Figure 3.10 below is the graphical interpretation of the paracetamol half-life of two groups of mothers one with low and one with high DDT exposure levels. The values are derived from Table 3.21 below.

Table 3.21 Paracetamol Concentration Log Scale in Low and Highly exposed Mothers

LEAST	**EXPOSED**	**HIGHLY**	**EXPOSED**
Time- Minutes	**Drug Conc. Log**	**Time- Minutes**	**Drug Conc. Log**
0	0	0	0
32	1.24	32	2.04
63	1.46	65	1.99
96	1.84	99	1.91
159	1.75	157	1.61
216	1.44	216	1.17
250	1.25	241	1.10

Key: -
Drug Conc. = Drug concentration in mg/L. Log = Concentration in log.
Least Exposed = Mothers least exposed to DDT.
Highly Exposed= Mothers highly exposed to DDT.

Figure 3.10 Relationship of Half–life in Low and Highly Exposed Mothers

The results of Figure 3.10 above show that t½ of the mothers with low DDT exposure levels is 318 minutes at 1.04 mg/L log concentration while that of the mothers with high DDT exposure levels is 212 minutes at 1.26 mg/L log concentration. Half-life difference in the two group is 106 minutes a 33.3 % difference. Table 3.22 below shows the results of the pharmacokinetics of the two groups.

Table 3.22 Pharmacokinetics Values of Paracetamol in Human Mothers.

	Vd (L/kg)	AUC (sq cm)	TBC (ml/minute)
Least DDT Exposed	**0.21**	**11607.06**	**86.21**
Highly DDT Exposed	**0.14**	**14080.11**	**71.05**

Key: -

Vd = Volume of distribution.

AUC = Area under the curve.

TBC = Total body clearance.

CHAPTER 4

4.0 DISCUSSION

4.1 Validation of Methodology

Standard curves of individual standards of pp-DDE, β-HCH and hexachlorobenzene (HCB) were performed as shown by Figures 3.1, 3.2 and 3.3. The standards showed a linear relationship within the given concentration of each standard analysed, the standards also displayed a correlation coefficient of 0.999 indicating that the method was valid for analysis of organochlorine pesticides (OCPs) within the given concentration ranges as shown in Tables 3.1; 3.2 and 3.3. Reproducibility and consistency of the method were confirmed as indicated by Tables 3.4 and 3.5 whose % standard deviation or coefficient of variance had ranges of 0.2 - 3.3 % and 0.04 - 0.38 % respectively. Paracetamol curve Figure 3.4 displayed linearity within the given concentration ranges of 0-400 mg/ml standards. The paracetamol curve demonstrates that Beer and Lambert's law (previously described) was applicable and that these standard results can be used to calculate paracetamol concentration of the control and test samples. A coefficient correlation of 0.999 was also an assurance of results.

4.2 DDT and HCH

Studies previously done in this country to assess organochlorine pesticides exposures have shown that the country is exposed to organochlorine pesticides; especially the pesticide DDT [1,1,1,trichloro-2.2-bis (4-chlorophenyl) ethane] (Zaranyika and Makhubalo, 1996; Chikuni *et al.,* 1997a). The results in this study and others have shown that the intensity of residue levels of organochlorine pesticides (OCPs) in mother's milk vary from area to area (Nhari 1996, Nhachi and Kasilo 1990). The exposure levels for DDT (Table3.8C) shows that Mudzi, Kariba

and Nyanga (16 838.90; 7 985.97 and 7 745.17 ng/g respectively) are areas that registered the highest exposure levels of DDT in the selected areas. The main reason for the very high levels in these areas was a result of exposure from vector control programmes (Mudimu *et al.,* 1995). In 1982 restriction on usage of DDT was imposed in the country. It was only to be used for tsetse and mosquito control programmes (Muchena, 1991). The last recorded DDT ground spraying programme for vector control in Mudzi area was last done in 1994 (Mudimu, *et al.,* 1995). For the Kariba area, which also recorded high levels of DDT, usage of this pesticide for vector control was last recorded in 1989 (Mukahanana, 1990). The results of the high DDT exposure levels in the country are a direct indication of usage of the pesticide for vector control programmes.

In comparison to the other six areas sampled, Esigodini had the lowest levels of DDT (934.12 ng/g). It is a rural area, with semi-arid climatic conditions and with a low annual rainfall. Thorny bushes and cactus like plants which grow in hot and dry regions, are abundant in this area, suggesting an area that is not conducive to intense agricultural activities and therefore minimum use of pesticides. Tsetse flies and mosquitoes are not pandemic in this area suggesting minimum use of organochlorine pesticides for vector control.

Nyanga area had the highest levels of HCH (887.20 ng/g) and Kariba area had the lowest (36.11 ng/g) as in figure 3.8B. The high HCH residues detected in Nyanga area are an indication of high usage of this pesticide. The area has the biggest forest industry and fruit- farming industry in the country, and HCH is the major pesticide that is used. The low residues in Kariba area are an indication of low usage of HCH since DDT is the major pesticide used for mosquitoes and tsetse flies control (Mathiessen, 1985; Chikuni *et al*., 1997b).

This study has shown that high residues of DDT and its metabolites detected in breast milk (16 838.90 ng/g) of mothers living in Mudzi area are a result of consumption of DDT contaminated staple foods (352.0 ng/g). When DDT was banned or severely restricted, the levels of DDT in foods of plant and animal origin fell rapidly. However, exposure to its metabolites especially pp-DDE is still evident. The ratio of pp-DDT / pp-DDE is an expression of the period of adherence to the restriction or ban imposed on the usage of DDT pesticide for domestic or for agricultural purposes (Torres-Arreola *et al*., 1998). A higher ratio indicates high levels of the pp-DDT metabolite that is not as persistent as the pp-DDE metabolite and therefore indicating recent usage of the parent DDT pesticide. The ratio of pp-DDT / pp-DDE recorded in the various areas of the country indicates that DDT use has indeed been reduced. The comparison of the ratio reveals that some areas have a more recent use of DDT than others. Areas like Esigodini (0.46) and Kariba (0.35) recorded a higher pp-DDT / pp-DDE ratio than other areas such as Kadoma (0.03). In the case of Kariba, the higher ratio of recent DDT usage was expected as confirmed by vector-control programmes still going on this area (Mudimu, *et al*., 1995; Chikuni, *et al*., 1997a). However, in the case of Esigodini, a rural area, with comparably low usage of DDT, the results (934.12 ng/g) shown are an anomaly that would suggest a recent usage of this pesticide. This anomaly could be explained by human mobility of a mother from an area that was probably exposed to DDT through vector control programmes, and would have came to live in Esigodini at the time of sampling. Implying inclusion in the Esigodini sampled population a mother who probable could have migrated from an area that had recent exposure to DDT who gave false information concerning her previous residential status.

Other studies have reported similar results in food and water. Studies previously done in this country to assess organochlorine pesticide residues in water and fish have shown presence of these OCPs especially DDT (Mhlanga *et al*., 1986, Zaranyika and Makhubalo, 1996). Another

study that evaluated OCP exposure using breast milk also detected presence of these residues in human breast milk (Chikuni *et al.,* 1997b). This study has gone beyond the evaluation of presence of these residues in water, food and human milk (Conacher and Mes, 1993), but has proved an association between the level of exposure and the presence of these OCP residues in human milk.

Of the 8 foods analysed, maize meal (*Upfu*) (which is the main component in the diet of most Zimbabweans); had the highest levels of DDT compared to other staple foods. Maize meal is eaten as porridge in the morning and *sadza* for lunch and supper. In one area (i.e. Mudzi) that recorded high levels of DDT in breast milk (16 838.90 ng/g), maize meal (874.21 ng/g) contributed the largest proportion into the sum DDT in staple food (2 815.73 ng/g) analysed. This area, which is a rural area, has a history of DDT use for vector control (Mudimu *et al*., 1995).

The other area that recorded high DDT levels in breast milk (i.e. Kariba) had the highest DDT amounts in fish (956.00 ng/g). Fish is the main source of protein in this area as established through a questionnaire. It was also noted that vegetables in Mudzi, i.e. *covo* (695.30 ng/g) and cabbage (591.31 ng/g) that are served commonly in the Zimbabwean dishes had high amounts of DDT levels. The occurrence of pesticides residues in food samples was associated with the usage of similar pesticides in the sampled areas. The results also confirmed that food of animal origin as exemplified by fish (956.00 ng/g) in Kariba contained higher levels of DDT compared to other foods for instance cabbage (591.31 ng/g) (Barkatina *et al*., 1999).

This study suggests that the main source of exposure in the population besides occupational exposure, is dietary related. The study, therefore underscores the significance of diet in

environmental exposure as reported in earlier studies conducted in Zimbabwe (Nhachi*, et al*., 1995; Chikuni, *et al.,* 1997a).

4.3 Relationship between levels of DDT and HCH in staple food and mother's milk

The results of this study are based on mothers who were environmentally exposed to DDT and HCH as determined by a questionnaire. The study observed a statistically significant Correlation (r=0.8857, p=0.0188) between DDT levels in staple food and mother's milk. This association was also observed between HCH levels in staple food and mother's milk, even though it was not statistically significant (r=0.6571, p=0.1562). These results suggest that diet is a main source of exposure. Areas with high DDT levels in food also had high levels of these residues in mother's milk, as reflected by the results from Mudzi (352.04 ng/g in food and 16 838.90 ng/g in mother's milk) Kariba (376.65 ng/g in food and 7985.70 ng/g in mother's milk). Conversely, areas that recorded low DDT in food also had low levels in breast milk as observed in Esigodini (12.13 ng/g in food and 933.82 ng/g in mother's milk) and Harare (60.95 in food and 2312.85 in mother's milk). The same pattern was also observed for lowest HCH in Kariba (2.28 ng/g in food and 36.11ng/g in mother's milk; and for highest HCH in Nyanga (38.22ng/g in food and 887.20 ng/g in mother's milk.

4.4 Hepatic Induction of Microsomal Enzyme

Enzymes involved in the metabolism of foreign compounds (xenobiotics) are involved in the catalytic rate of metabolism of these chemicals. These enzymes include cytochrome P-450, NADPH cytochrome P-450 reductase, cytochrome b5, glucoronosyl transferases, epoxide hydrolases, epoxide hydrolases and glutathione transferase. They are all involved in catalytic

activation of the metabolic rate of xenobiotics. (Raunio, 1995; Estabrook, 1999). This study only focused on Cytochrome P-450 enzymes.

Microsomal enzymes induction has been demonstrated in many animal species including humans. In this study female Sprague Dawley rats were used to demonstrate enzyme induction. Usually, induction results from repeated or chronic exposure, but in this study a chronic intraperitoneal dose of DDT a CYP-450 enzyme inducer was given (Stresser and Kupfer, 1998).

Induction results in an increase in the amount of enzymes and enzyme activity and in this study, the increase of cytochrome P-450. Maximum induction day was used in all the studies there after, as other researchers have observed (Nims *et al.,* 1998). The study demonstrated induction of cytochrome P450 by DDT as reported elsewhere (Lubet *et al,* 1992; Li *et al*., 1995). In addition maximum concentration of cytochrome-P450 coincided with maximum liver/body weight percentage when highest induction was achieved as shown by graphs (Figure 3.5 and 3.6)

4. 5 Effect of DDT on Pharmacokinetics of Paracetamol

Liver microsomal enzyme induction in rats can be demonstrated by a reduction in half-life of paracetamol, maximum drug plasma concentration (Cmax), and an increase in liver weight. The enzyme cytochrome P-450 induction by compounds such as polycyclic aromatic hydrocarbons (DDT) can increase the rate of metabolism or increase drug toxicity after an initial induction with DDT. Paracetamol was the drug that was selected for this project.

The choice of the drug (paracetamol) for this study was a result of the response from 404 breast-feeding mothers. Out of the five drugs mentioned in the questionnaire i.e. aspirin, ibuprofen,

paracetamol, stopayne and indomethacin, 59.9% of the mothers responded that paracetamol was their drug of choice for pain.

This study has demonstrated pharmacokinetic interactions between DDT and paracetamol as indicated by a reduction in half-life which was the time taken to reach peak plasma concentration and time taken for clearance of paracetamol, in both rats and humans. As already mentioned above, the reduction in the half-life of paracetamol is due to enzyme induction caused by DDT residues. The reduction in the half-life of paracetamol in exposed mothers has important clinical implications. First, it is a commonly used analgesic whose therapeutic effects can be affected by reduction in half-life and secondly, whose toxicity can be enhanced by high doses which could be a result of an individual patient over dosing in an effort to achieve the required therapeutic index or amelioration of pain. Individuals with high levels of DDT in the body appear to be at greater risk of drug interactions in relation to paracetamol and other drugs that are metabolized by the DDT induced enzymes.

DDT or methoxychlor has been observed to induce CYP3A4 in rats and humans (Li *et al*., 1995; Wienkers, 2001). Paracetamol is known to be a substrate of CYP1A2 and CYP3A4 enzymes. The hepatoxicity of paracetamol in rats and humans is therefore increased by induction of the enzymes by DDT, due to an increase in the catalytic rate of reaction of the enzyme CYP3A4. The reaction will reduce the amelioration of pain period and hence the need for more medication. Absorption of DDT in the gastrointestinal tract will potentiate the volume and viscosity of the intraluminal content and thereby making the intestinal mucosal cells more permeable to drugs such as paracetamol (Chabra and Eastin, 1984).

This study also observed a reduction in the time taken to reach maximum plasma concentration in both rats (30.5 minutes) and humans (37.0 minutes). In addition, peak plasma concentration in the exposed rats (50.1 mg/L) and in humans (111.0 mg/L) was higher than in the control rats (31.6 mg/L) and in humans (68.0 mg/L). The pesticide DDT is highly lipophilic and is sequestrated into fat tissues including the tissues lining gastrointestinal and intraperitoneal cavity (Smyth and Hottendorf, 1980; Kupfer *et al.,* 1990). Studies have shown that DDT is covalently bound to plasma lipoproteins, and because of this bonding it has difficulty in actively permeating cell membranes. It cannot leave intestinal capillaries by diffusion and hence its difficulties in dissociating from plasma proteins. Because of the strong bonds to plasma proteins there is a delay and prolongation in the absorption of DDT into intestinal mucosa and hence its sequestration into these tissues. Sequestration of DDT into the intestinal tissue lining, will enhance rate of absorption of paracetamol and hence its increase in peak plasma concentration (Acra and Ghishan, 1991). Some studies have also shown that glucuronide of paracetamol can be hydrolyzed β-glucuronidase and can be reabsorbed in the intestinal lumen and therefore enhancing paracetamol absorption (Gregus and Klassen, 1986).

Other indicators of the mothers' ability to metabolize paracetamol drug and therefore the elimination of the drug were obtained from the calculation of total body clearance (Kalant *et al*., 1985). In the study, the control mothers had a higher clearance of 86.2 ml/min than the test mothers with a clearance of 71 ml/min. Thus, paracetamol in the test mothers had a faster rate of elimination due to higher concentration of metabolizing enzymes. Another indication of the dynamics of metabolism used in the study was volume of distribution (Vd). A compound with a high Vd will tend to be excreted more slowly than a compound with a low Vd. The result of control mothers had a higher Vd of 0.2 L/kg compared to the test mothers with a Vd of 0.14 L/kg a significant difference of 0.06L/kg. The difference is a result of the higher rate of

paracetamol elimination in the test mothers due to higher metabolism in enzyme induction, thus Vd in the test mothers was lower than in the control mothers.

Calculated results show that the test mothers (DDT-induced) had a low AUC (11.66 mg/hr/l) in comparison to control mothers (non-induced) who had a higher AUC (14.7 mg/hr/l). The AUC is another parameter of importance that provides information to estimate bioequivalence of the drug after induction by xenobiotics. The results however, showed that the control mothers (low DDT residues) were subjected to slightly more paracetamol per unit time than the test mothers (higher DDT residues).

The decrease in half-life coupled with an increase in clearance means that a standard dose of paracetamol usually taken by non- exposed individuals for the desired effects, if taken by the exposed will have a decreased therapeutic effect. Exposed individuals may need to take higher doses of paracetamol or take this drug more frequently than usual to achieve the same analgesic effect.

CHAPTER 5

5.0 CONCLUSIONS

The study has shown that human beings are mainly exposed to organochlorine pesticides through consumables of contaminated foods. The study has established that there is indeed a correlation between the levels of DDT and HCH in breast milk of breast-feeding mothers and presence of these residues in staple diet of mothers living in the exposed areas. The results confirmed the close relationships between OCPs exposure and dietary habits; as exemplified by the high residues of DDT and it's metabolites in breast milk (16 838.9 ng/g) of mothers living in Mudzi; and the associated high levels (352.0 ng/g) in consumed foods from the same area.

Induction by DDT resulted in increased levels of the metabolizing enzymes. Exposure of the animals to DDT induced the cytochrome P-450 enzymes, predominantly CYP2B (CYP2B1 and CYP2B2) and to a lesser extent CYP3A (CYP3A1 and CYP3A4). Hepatic mitochrodianic proliferation of cytochrome P-450 enzymes that was a result of induction by DDT was demonstrated by liver enlargement (hepatomegaly) in rats. This demonstrates how the human body is able to metabolize and eliminate from the body, most xenobiotics using the liver as the major metabolizing organ. Proliferation of the cytochrome P-450 enzymes in most animals is a result of metabolic activation of the detoxifying mechanism of the living organisms.

The study confirmed that DDT affects the pharmacokinetics of paracetamol in both animals and humans as indicated by a reduction in the half-life of exposed mothers compared to mothers who are not exposed. At a normal dose, paracetamol is conjugated to give the glucuronide and the sulphate conjugate by the phase 1 reaction and the end product is mercarpuric acid. At a higher dose paracetamol is metabolized to n-acetyl-p-benzoquinane imine (NAPQI) by P-450

mixed function oxidase. If this metabolite is not inactivated by conjugation with glutathione, it will react with liver cell proteins, resulting in cell damage (liver necrosis), and hence the resultant hepatotoxicity.

Other pharmacokinetic parameters such as maximum concentration (Cmax), area under the curve (AUC); volume of distribution (Vd) and clearance of paracetamol, confirmed that DDT influence the metabolism of paracetamol in both humans and animal tests. This was demonstrated by the pesticide DDT's properties to induce Cytochrome P 450 enzymes in vitro.

The continued use of DDT in malaria control is welcomed, but it should be done with intense environmental monitoring to prevent the long-term (chronic) effects of these pesticides on exposed individuals. More research is recommended so that scientists could assess the influence of the induction properties of organochlorine pesticides on the efficacy, toxicity, and the therapeutic index on dosage of many drugs used by animals and humans.

REFERENCES

Ackerman LB. Humans: Overview of Human exposure to Dieldrin residues in the Environmental current trends in tissue. *Pest Monitor J*. 1980; 14: 64 – 69.

Acra SA and Ghishan FK. Methods of investigation intestinal transport. *Jpn J Parenter Enteral Nutr.* 1991; 15: 93s – 98s.

Ahrens FA. The National Vet Medicine Series. Williams and Wilkins 1996: 174 – 175.

Aitio A. Zero exposure – a goal for environmental and occupational health. *Toxicol Lett.* 2002; 134: 3-8.

Albert A. Selective Toxicity 3rd (ed). London: Methuen 1965.

Alison RH and Morgan KT. Ovarian neoplasma in F344 rat and B6C3FI mice. *Environ Health Perspect.* 1987; 73: 91-106.

American Soceity of Testing and Materials. Standard guide for combating terrestial plant toxicity tests. In: *Annual Book of ASTM Standards.* E1963-98. West Canshohocken, PA.1995; 29 – 138.

Amoateng-Adjepong Y, Sathiakumar N, Delzell E, Cole P. Mortality among workers at a pesticide manufacturing plant. *J Occup Environ Med*. 1995; 37: 471 – 478.

Anger WK. Worksite behavioral research: results, sensitive methods, test batteries and the transition from laboratory data to human health. *Neurotoxicol*. 1990; 11: 629-720.

Anwar WA, Biomarkers of Human exposure to pesticides. *Environ Health Perspect.* 1997; 105 (4): 801 – 806.

Asplund L, Svensson BG, Nilsson A, Eriksson U, Jansson B, Jensen S, Widequist U, and Skerfving S. Polychlorinated Biphenyls, pp-DDT and pp-DDE in human plasma related to fish consumption. *Arch.Environ Hlth.* 1994; 49: 477-486.

Attaran A and Roberts D. "Balancing Risks on the backs of the poor", Nature Med. 2000; 6: 729 – 731.

Austin H, Keil J E, Cole P. A prospective follow –up study of cancer mortality in relation to serum DDT. *Am J Public Health*. 1989; 79: 43 – 46.

Barkatina EN, Pertsovsky AL, Murokh VI, Kolomiets ND, Shulyakovskaya OV, Navarich ON and Makarevich VI. Organochlorine pesticide residue in breast milk in the Republic of Belarus. *Bull Environ. Contam Toxicol*. 1998; 60: 231-237.

Barkatina EN, Pertsovsky AL, Murokn VI Kolomites ND, Shulyakovskaya OV, Venger on and Makarevich VI Organochlorine Residues in basic food products and diets in the Republic of Belarus. *Bull Environ Contam Toxcol* 1999; 63: 235 – 242.

Basselink HT, Van Sante E Varstman W, Vethaok AD, Koeman JH and Browver A. High induction of CYP P-450 1A activity without changes in Retinoid and Thyroid Hormone levels in flounder ((Platichthys flesus Exposed to 2,3,7,8 – tetrachlorodibenze-p-Dioxin. Environ Toxco and Chem. 1997; 16 (4): 816 – 823.

Battershill JM. Review of the safety assessment of Polychlorinated Biphenyls (PCBs) with particular reference to reproductive toxicity. *Human and Exper Toxicol.* 1994; 13 (58): 581-587.

Becking G C, Boyes W K, Darmastra T, Macphail R C. Assessing the neurotoxic potential of chemicals- a multidisplinary approach. *Environ Res*. 1993; 61: 164 – 175.

Bernard SF, Thil MA and Groscolas. Lipolytic and metabolic response to glucagon in fasting king penguins: phase II vs. phase III. *Am J Physiol Regulatory Integrative Comp Physiol*. 2003; 284(2): R444 – 454.

Bjerk JE and Brevik. EM. Organochlorine Cmpds in aquatic environments. *Archives of Environ Contamin and* Toxico. 1980; 9(6):743-750.

Boobis AR, Edward RJ, Adams DA and Davies DS. Dissecting the Function of P- 450 *Br. J Clin Pharmacol* 1996; 42: 81-89.

Boobis AR, Fawtrhrop DJ, Davies DS. Mechanism of cell Death Trends. *Pharmacolo*. 1989; 10: 275 – 280.

Bouwman H, Becker P J, Cooppan R M, Reinceke A J. Transfer of DDT used in malaria control to infants via breast milk. *Bulletin of the, World Health Organisation.* 1999.

Bouwman H, Becke PJ, Coopan RM, Reinecke AJ. Transfer of DDT used in malaria control to infants via breast milk. *Bull. World Hlth Org*. 1992; 70: 241-250.

Bouwman H, Reinecke AJ, Coopan RM, Becker PJ. Factors Affecting Levels of DDT and Metabolites in human breast Milk from KwaZulu. *J Toxicol Environ Health* 1990; 31: 93- 115.

Brevik EM. Gas Chromatographic Method for the Determination of Organochlorine Pesticides in human Milk. *Bull Environ Contam. Toxicol* 1978; 19: 281- 286.

Brooks GT, Chlorinated Insecticides. Technology and application, Cleveland Ohio, *CRC*; 1974; 1: 12 –13.

Brown RD, Wilson JT, Kearns GL Eichler VE, Johnson VA and Bertrand KM. Single dose pharmacokinetics of ibuprofen and acetaminophen in febrile children. *J Clin. Pharmaco.* 1992; 32: 231 -241.

Brown RD, Kearns GL, Wilson JT. Integrated pharmacokinetics/pharmacodynamic model for acetaminophen, ibuprofen and placebo antipyresis children. *J Pharmakin Biopharm*. 1999; 26: 555-579.

Bulger WH and Kupfer D. Estrogenic action of DDT analogs. Am. J Ind. Med. 1983; 4: 163-173.

Campbell MA, Gyorkos J, Leece B Hamonko K, Safe S. The effects of twenty-two organochlorine pesticides as inducers of the hepatic drug-metabolising enzymes. *Gen Pharmacol*. 1983; 14: 445 – 454.

Carson RS, Zhang Z, Hutchson LA. Growth factors in ovarian function. *J Reprod Fert*. 1989; 85: 735-746.

Casey PB and Vale JA. Death from Pesticide Poisoning England and Wales, 1945 – 1989. *Human and Exp Toxicol*. 1994; 13: 95 – 101.

Chhabra RS, and Eastin WC Jr. Intestinal absorption and metabolism of xenobiotics in laboratory animals . In: *Intestinal toxicology*, (ed) by Schiller CM. 1984; 145 – 160.

Chikuni O, Nhachi CFB, Nyazema NZ, Polder A, Nafstad I and Skaare JU. Assessment of Environmental pollution by PCBs, DDT and its Metabolites Using Human Milk of Mothers in Zimbabwe. *The Sci of the Total Environ*. 1997a; 199: 183 – 190.

Chikuni O, Nhachi CFB, Polder A, and Skaare JU. An evaluation of DDT and DDT residues in human Breast Milk in the Kariba Valley of Zimbabwe. *Bull Environ Contam Toxicol*. 1997b; 58 5: 776.

Chikuni O, Skaare JU, Nyazema NZ, Polder A. Residues of Organochlorine Pesticides in Human Milk from mothers living in the Greater Harare Area of Zimbabwe. *Central African J Med*. 1991; 37: 136-141.

Chikuni O. DDT and Oganochlorine Pesticides Residues an Assessment of their presence in Human Breast Milk of mothers in Zimbabwe. Thesis 1997c: 1 – 2.

Chromy BA, Fischer J, Vaznicek J. Assay of total serum protein by the Beirut method, reaction rate and lipernia. *J Biochem*. 1980; 21 (6); 333 – 3337.

Colborn T, vom Saal FS and Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect.* 1993;101:379-384.

Conacher H and Mes J. Assessment of Human Exposureto chemicalin food. Food Addit. Contam. 1993; 10: 5-15.

Curtis C F and Lines J D. Should DDT be banned by International Treaty? *Parasitology Today*. 2000; 16: 3.

Czajak, Ludwicki JK, Goralczyk K and Strucinski P. Effect of age and number of deliveries on mean concentration of organochlorine compounds in human breast milk in Poland. *Bull Environ Contam Toxicol* 1997; 59: 407-413.

Daly AK, Brockmöller J,Broly F, Eichelbaum M, Evans WE, Gonzelz FJ, Huang JD, Idle JR, Ingleman-Sundberg M, Ishizaki T, Jacqz-Aigrain E Meyer UA, Nebert Dw, SteenVM, Wolf CR, Zanger UM. Nomenclature of human CYP2D6 alleles. *Pharmacogenetics*. 1996; 6: 193 – 201.

Dekant W, Vamuakas S Amnders MW: Bioactivation of nephrotoxic haloalkenes by glutathione conjugation: Formation of toxic and mutagenic intermediates by cystine conjugate β-lyase. *Drug Metb Rev*. 1989; 20: 43 –83.

Dewailly E, Ayotte P, Lalibertte C, Weber JP, Gingras S and Nantel AJ. Polychlorinated Biphenyls (PCBs) dichlorodiphenyl dichloroethylene (DDE) concentrations in the breast milk of women in Quebec. *Am J Public Health.* 1996; 86: 1241 – 1246.

Dogheim SM, Mohamed EZ Gad-Alla SA, El- Saired S and Mohsen AM. Monitoring of Pesticides Residues in human Milk, Soil Water and Food samples Collected from Kafi El-Zayat Governarate. *Jnr of AOAC Int*. 1996; 79 (1): 111 – 116.

Doumas BT. Estimation of serum total protein and serum Albumin- Beirut, *Bochem Methods* 1975; 21: 1195-1166.

Drummond L, Gillanders EM, Wilson HK. Plasma gama-hexachlorocyclohexane concentrations in forestry workers exposed to Lindane. *Br. J Ind Med.* 1988; 45: 493-497.

Ecobichon DJ. Toxic effects of pesticides. In Casarett and Doull's Toxicology: The basic science of poisons, (ed) Klaassen CD, editors emirit Amadur MO and Doull J. 5th edition. McGraw-Hill.1996; 643 – 683.

Eldefrawi MES, Sherby SM, Abalis IM, Eldefrawi AT. Interactions of pyrethroid and cyclodiene insecticides with nicotinic acetylcholine and GABA receptors. *Neurotoxicology.* 1985; 6: 47 – 62.

Eriksson P. Neonatal Exposure to DDT induces increases susceptibility to pyrethroid (Bioallenthrin) exposure at adult age- changes in cholonergic muscarinic receptorand behavioural variables. *Toxicol.* 1993; 77: 21 - 30.

Estabrook RW and Werringloer J. The measurement of different spectra: application to the cytochrome of microsomes. *Methods. Enzymol.* 1978; 52: 212 – 220.

Estabrook R. An introduction to the cytochrome P450s. In human cytochrome P450s. (ed) Hasler JA. *Molecular Aspects Med*. 1999; 20 (1/2): 5 – 12.

Falck F Jr, Ricci A Jr, Wool FM, Godbold J and Deckers P. Pesticides and polychlorinated biphenyls residues in human breast lipids and their relation to breast cancer. *Arch Environ Hlth.* 1992; 47:143-146.

FAO/ WHO Pesticides Residues in Food. Codex Alimentaries Comisssion Vol II 1993.

Farrell GC Drug Metabolism in Extrahepatic Diseases. *Pharmacol Ther.* 1987; 35: 375- 404.

Fonseca RG, Resende LAL, Silva MD, Camargo A. Chronic motor neuron disease possibly related to intoxication with organochlorine insecticides. *Acta Neurol Scand.* 1993; 88: 56 – 58.

Fordtran JS, Rector FCJ, Euton MF, Soter N and Kinney T. Permeability of human small intestine. *J Clin Invest.* 1965; 44: 1935 – 1944.

Forget G, Goodman T and de Villiers A. Impact of pesticide use on the Health in developing countries. Ottawa Canada: *International Development Research Centre* (IRDC): 1993.

Fraklin CA, Muir NJ, Moody RP. The use of biological monitoring in the estimation of exposure during the application of pesticides. *Toxicol Lett.* 1986; 33:127-136.

Franchi E, and Facardis S. Polychlorinated congeners, hexachlorocyclohexane and DDTs in human milk from central Italy*, Sci. Total Environ. 1991*; 102: 223-228.
from Mexico. *Cancer Res*. 1997; 57: 3728 – 3732.

Furst P, Furst C, Wilmers K. Human milk as a bioindication for body burdern of PCDDs, PCDFs, organochlorine pesticides and PCBs. *Environ. Health Perspect,* 102 Suppl. 1994; 1:187-193.

Glanden BC and Rogan WJ. DDE and shortened duration of lactation in a Northern Mexican town. *AM J. Public Health.* 1995; 85:504-508.

Gonzalez FJ and Korzekwa KR. Cytochromes P-450 Expression Systems. *Ann Rev Pharmacol Toxicol* 1995; 35: 369 - 390.

Guengerich FP. Catalytic selectivity of human Cytochrome P-450 enzymes: Relevance to drug metabolism and toxicity. *Toxicol Letters.* 1994; 70:133-138.

Guengerich FP, Hosea NA, Johnson WW, Gillam EMJ, Shimada T.Catalytic properties of cytochrome P450 3A enzymes. In: Microsomes and drug oxdations, Proceedings of the 12th International Symposium. 1998: 27: 345- 349.

Guillette L J Jr, Rooney A A, Crain D A, Orlando E F. Steroid hormones as biomarkers of endocrine disruption in wildlife. In: Hensel D (eds) Environmental toxicology and risk assessment. *ASTM, STP1364*. 1999; 8-9.

Gregus Z, Klassen CD. Enterohepatic circulation of toxicants, in Kozman K Hanninien O (eds): *Gastrointestinal Toxicology*. Amsterdam: Elsevier/ North Holland, 1986; 57 –118.

Halmes NC, Samakyszyn VN,Hinton TW, Hinson JA and Pumford NR. The Acetaminophen Regioisome 3-hydroxyacetamine inhibits and covalently binds to cytochrome P-450 2E1. *Toxicolo Letters*. 1998; 94: 65-71.

Hayes WJ Jr. Clinical Handbook on Economic Publications. Public Health Service publication No. 476 US. Government Printing Office, Washington DC 1963- *Pesticides Studies in Man*. The Williams and Wilkins CO. Baltimore. 1982.

Hayes WJ Jr. Pesticides Studies in Man; William and Wilkins, Baltimore, MD 1982, 172- 283.

He F. Biological monitoring of exposure to pesticides: current issues. *Toxicol Lett.* 1999;108: 277-283.

Handerson RF, Bechtalid WE, Bond JA and Sun JD. The use of biological markers in Toxicology. *Crit Rev Toxicol.* 1989; 20: 65-82.

Hernandez LM, Fernandez MA, Hoyas E, Gonzalez MJ and Garcia JF. Organochlorine insecticides and polychlorinated biphenyls residues in human breast milk in Madrid (Spain). *Bull Environ Contam Toxicol*. 1993; 50: 308-315.

Holmes SB, Thompson DG, Wainio-Keizer KL, Copell SS and Staznik B. Effects of lethal and sublethal concentrations of the herbicide, triclopyrobutoxyethyl ester, in the diet of Zebra flinches. *J Wild Diseases.* 1994; 30: 319-327.

Hooper K, Petreas MX and She J. Analysis of Breast milk to asses exposure to chlorinated contaminants in Katzakstan: PCBs andorganochlorine pesticides in Southern Katzakstan. *Environ Health Perspect.* 1997; 105: 43 – 58.

Hunter D J, Hankinsson S E, Laden F, Colditz G A, Manson J E, Willet W C, Speizer FE and Wolf M S. Plasma organochlorine levels and the risk of breast cancer. *N England J Med.* 1997; 337: 1253 – 1258.

IARC. Occupational Exposures in In: IARC Monograms on the Evaluation of Carcinogic risks to Humans, volume 53. Ryan: *International Agency for Research on Cancer*, 1991; 179- 234.

IARC: Monograms on the Evaluation of Carcinogenic risks of chemicals to man. Some organochlorine pesticides. Lyons, *France International Agency for research on Cancer*, 1974; 5.

Johansen HR, Becker G, Polder A and Skaare JU. Congener- Specific Determination of Polychlorinated biphenyls and Organochlorines Pesticides in human Milk from Norwegian Mothers living in Oslo. *J Toxicol Environ Hlth*. 1994; 42: 157- 171.

Joy RM. Chlorinated Hydrocarbons in Insecticides in Ecobichon Chou DJ, Joy RM: Pesticides and Neurological Diseases 2nd (ed) Boca Raton, *CRC* 1994; 81- 170.

Kalant H, Roschlau WHE and Sellers EM. Principles of Medical Pharmacology, Oxford University Press, INC NY 4th ED, 1985: 864.

Kanja L, Skaare JU, Nafstud I, Maitai CK and Lokken O. Organochlorine Pesticides in Human Milk from different areas in Kenya (1983- 1985). *J Toxicol Environ Hlth*. 1986; 19: 449- 464.

Kanja WL, Skaare JU, Ojiwang SBO, and Maitai CK. A comparison of organochlorine pesticides residues in maternal adipose tissue, maternal blood, cord blood and human milk form infant pairs. *Arch. Environ Contam Toxicol.* 1992; 22: 21-24.

Kapoor IP, Metclaf RL, Nystrom RF and Sangha GH. Comparative Metabolism of Methoxychlor, Methiohlor and DDT in mouse, insects and in a model ecosystem. *J Agric Food Chem* 1970; 18: 1145- 1152.

Keice W R, Stone C R, Laws S C, Gray E L, Kemppainenja J A and Wilson E M. Persistent DDT metabolite pp-DDE is a potent androgen receptor antagonist. *Nature.* 1995; 375: 581 – 585.

Kinyamu JK, Kanja LW, Skaare JU and Maiho TE. Levels of Organochlorine Pesticides Residues in milk of Urban mothers in Kenya. *Bull Environ Contam Toxicol*. 1998; 60: 732- 738.

Klaassen CD, and Rozman K. Absorption, distribution and excretion of toxicants. In Casarett and Doull's *Toxicology, eds*. M.O. Amdur, J Doull and Klaassen, New York: Mcgraw-Hill, 1996: 50-87.

Kocarek TA, Schuets RG and Guzelian PS. Selective induction of Cytochrome P-450 by Kepone (chlordecone) in Primary Cultures of Adult rat Hepatocytes. *Mol Pharmacol*. 1991; 40: 203- 210.

Krishna DR and Klotz U. Extrahepatic Metabolism of Drugs in humans. *Clin Pharmacokinetic*. 1994, 26: 144- 160.

Kubus M and Berg H. Pesticides in Lake Kariba: A report prepared for the department of Systems Ecology, University of Stockholm 5-10691, Stockholm, Sweden. 1991.

Kupfer D and Bulger WH, and Theoharides AD. Metabolism of methoxychlor by heptic P 450 monoxygenases in rat and human, characterization of a novel catechol metabolite. *Chem Res Toxicol*. 1990; 3: 8 – 16

Landi MS, Kreider JN, Lang CM and Bullock LP. Effects of shipping on the immune function in the mice. *Am J Vet Res.* 1982; 43: 1654 – 1657.

Lave L and Ennever FK, Toxic substance control in the 1990s: Are we poisoning ourselves with low-level exposure? *Ann Rev of Pub Health.* 1990; 11: 69 - 87.

Lavy TL and Mattice JD. Progress in Pesticides Exposure studies and Future concerns. *Toxcol Letter.* 1986; 33: 61- 71.

Lederman SA. Environmental contaminants and their significance for breast feeding in the Central Asia Republics. *Wellstart International*. 1993; 21:245-321.

Leighty EG, Fentiman, AF Jr, and Thomas RM. Conjugation of fatty acids to DDT in the rat: Possible mechanism for retention. *Toxicol.* 1980; 15: 77-82.

Li HC, Dehal SS and Kupfer D. Induction of the Hepatic CYP2B, and CYP3A Enzymes by the proestrogenic Pesiticides Methoxychlor and DDT in the rat: Effects on Methoxychlor Metabolism. *J Biochem Toxicol*. 1995a; 10: 51- 61.

Li X, Weber LWD and Razman KK. Toxicokinetics of 2,3, 78- tetrachlorodibenzo-p-dioxin (TCDD) in Female Sprgue-Dawley rats including placenta and lactation transfer to foetuses and neonates. *Fundam Appl Toxicol*. 1995b; 27: 70- 76.

London L and Myers J E. Critical issues in agrochemical safety in South Africa. *Am J Ind Med.* 1995; 27: 1 – 14.

Longecker M. Association between maternal serum once triation of the DDT metabolite DDE and preterm and small for gestational age babies at birth. *The lancet*. 2001; 358: 110 – 114.

Lopez-Carrillo L, Lapez-Cervantes M, Cebrian M, Rieda M, Reyes R, Mohar A and Bravo J. Dichlodiphenyl trichloethane serum levels and breast cancer risk: a case-control study from Mexico. *Cancer Res.* 1997; 57: 3728- 3732.

Lowry OH, Rosebrough NJ, Farr RJ and Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193: 265 – 275.

Lubert RA, Dragnev KH, Chauhan DP, Nims KW, Diwan BA, Ward JM, Jones CR, Rice JM and Miller MS. A pleiotropic response to Phenobarbital- type enzyme inducers in the F 344/ NCR rat: Effects of chemicals of varied structure. *Biochem. Pharmacol.*1992; 45: 1067-1078.

Lubet RA, Guengerich FP, and Nims RW. The induction of Alkaloxyresorufin Metabolism: A Potential Indicator of Environmental Contamination. *Arch Environ Contam Toxicol*. 1990; 19: 157-163.

Malfertheiner P, Daminguez-Munaoz JE and Buchler MW. Chronic pancreatitis: management of pain. *Digestion*. 1994; 55 (1): 29 – 34.

Marrack JR, and Richards CB. Light Scattering Studies of the formation of aggregates in mixtures of antigen and antibody. *Immunology*. 1971; 20: 1019 – 1020.

Mathiesssen P. Contamination of Wildlife with DDT Insecticide Residues in Relation to Tsetse Fly Control Operations in Zimbabwe. *Environ. Pollut Serv Bull* 1985; 10:189 – 211.

Matsiumura F, and Ghiasuddin SM. DDT – Sensitive Ca^{2+} Adipose- in the Oxomic Membrane. In: Neurotoxicology of Insecticides and Pheromones. (Narahashi T (ed) Plenum Press, New York, 1979; 245 – 257.

Matsiumura F. Toxicology of Insecticides. New York: Plenum, 1985: 122-128.

Medinsky MA and Klaassen CD. Toxicokinetics. In Casarett and Doull's Toxicology; The basic Science of poison, (ed) Klaassen CD, editors emirit Amadur MO and Doull J. 5th edition. McGraw-Hill. 1996: 187 – 197.

Mes J. Temporal changes in some chlorinated hydrocarbons residual levels of Canadian breast milk and infants exposure. *Environ. Pollut*. 1994: 84: 261-268.

Mhlanga AT and Madziva JJ. Pesticides Residues in Lake McLlwaine. Ambio, 1990; 19: 31 – 36.

Mhlanga AT, Taylor RD and Phelps RJ. HCH and DDT residues in the freshwater sardines (Kapenta) at the Ume River mouth Kariba. *Zimbabwe Science News* 1986; 32:285 – 289.

Morrison HG, Oleksiak MF, Cornell NW, Sogin ML and Stegeman JJ. Identification of Cytochrome P-450 1A (CYP 1A) genes from two toleost fish toad fish (Opsanus tau) and Scuys (Stenotomus Chrysops) and Pheylogenetic Analysis of CYP1A genes. *Biochem J*. 1995; 308: 97 – 104.

Mpofu SM. DDT and its Use in Zimbabwe. *Zim Sci. News*. 1987; 21:31 – 36.

Muchena P. "Zimbabwe Country Report on Pesticide Management" FAO/SADC Sub-regionalWorkshop on Pesticide Management, Harare, Zimbabwe, 20-27; 1991.

Mudimu GD, Chigume S and Chikanda M. Pesticides Use and Policies in Zimbabwe, Pesticide Policy Project Publications Series. 1995; 2.

Mukahanana M, Strategy for Sustainability. Zimbabwe's Case Study *Govnt Publish.* 1990.

Murphy R and Harvey C. Residues and metabolites of selected persistent halogenated hydrocarbons in blood specimens from a general survey. *Environ Health Perspect*. 1985; 60:115-120.

Mutti A. *Bio*logical monitoring in occupational and environmental toxicology. *Toxicol Letters*. 1999; 108: 77-89.

Myers TG, Dietz EC, Anderson NL, Khairallah EA Cohen SD and Nelson SD, A Comparative Study of Mouse Liver Protein Arylated by Reactive metabolites of Acetaminophen and its Neuro-hepatotoxic regioisomers, 3-hydroacetanilide. *Chem Res Toxicol* 1995; 8: 403 – 413.

Nagaraja P, Srinivasa KC and Rangappa KS. Spectrophotometric method for the determination of paracetamol and phenacetin. *J Pharmacet Biomed Analys.* 1998; 17 (3): 501 – 506.

Nair, A and Pillai MKK. Trends in Abient Levels of DDT and HCH Residues in Humans and the Environment in Delhi, *India Sci Total Environ*. 1992; 121:145 – 57.

Nebert DW, Drug- Metabolism enzymes in ligand- adulated trasnscription. *Biochem.* 1994: 4: 53 – 65.

Nelson DK, Koymans L, Kamataki T. P450 superfamily: update on new sequences, gene mapping accession numbers and nomenclature. *Pharmacogenetics.* 1994; 6: 1 – 42.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Eastabrook RW, Gunsalus IC, Nebert DW. P450 superfamily: Update on new sequences, gene mapping accession numbers and nomenclature. *Pharmacogenetics*. 1996; 6: 1- 42.

Nhachi CFB and Kasilo OMJ. Occupational exposure to DDT among mosquito control sprayers. *Bull Environ Contamn Toxicol*. 1990; 45: 189 – 192.

Nhachi CFB, Urombo R, Murambiwa W and Kasilo OMJ. The use and status of DDT in Zimbabwe-A review. *East Africa J Hlth Sci.* 1995; 2: 331-332.

Nhari DB. Acute Pesticide Poisoning: Government Analyst Laboratory Records. In CFB Nhachi and OMJ Kasilo (eds) Pesticides in Zimbabwe Toxicity and Health Implications. The UZ Publications, Harare. 1996; 38 – 49.

Nichols J, Rheingans P, Lothenback D. Three- dimensional visualization of physiologically based kinetic model outputs. *Environ Health Persepect*. 1994; 102 (11): 952 – 956.

Nims RW and Lubet RA. Induction of Cytochrome {-450 in Norway Rat, Rathus Norwegious, following exposure to potential environmental contaminants Jr. *Toxicol, Environ Hlth*. 1995; 46: 271 – 292.

Nims RW, Lubet RA, Fox SD, Jones CR, Thomas PE, Reddy AB, Kocarek TA. Comparative pharmacodynamics of CYP2B induction by DDT, DDE and DDD in male rat liver and cultured rat hepatocytes. *J Toxicol Environ Health*. 1998; 53 (6): 455 – 477.

Okey AB. Enzyme Induction in the Cytochrome P-450 System. *Pharmacol, Ther*. 1990; 45: 241 – 298.

Okoniewski JC and Novesky E. Bird poisonings with cyclodiens in surburbia: links to historical use on turf. *J Wild Manag.* 1993; 57: 630-639.

Okonkwo JO, Kampira L and Chingakule DDK. Organochlorine Insecticides Residues in Human Milk: A Study of Lactating Mothers in Siphofaneni Swaziland . *Bull Environ Contam Toxicol.* 1999; 63: 243 – 247.

Oliyai R, and Stella V J. Prodrugs of peptides and proteins for improved formulation and delivery. *Ann Rev Pharmacol Toxicol.* 1993; 33: 521 – 544.

Olson A, Vitinsh M, Plikshs M and Bergman A. Halogenated environmental contaminants in Perch (Perca fluviatilis) from Latvian costal areas. *Sci Total Environ* 1999; 239: (1-3), 19-30.

Omura T and Sato R. The carbon monoxide-binding pigment of liver microsomes. Evidence of its hemo-protein nature. *J Biol Chem* 1964; 239: 2370 – 2379.

Osweiler GD. Toxicology (The National Veterinary Medical Series for Independent Study) Williams and Wilkins. The Science of Review 1996; 238 – 304.

Otto DME, Sen CK, Casley WL and Moon TW. Regulation of Cytochrome P-450 1A metabolism by Glutathione. *Pharm Toxicol* 1999; 84; 201 – 210.

Parajcic J, Karljikovic-Rajc K, Duric Z, Jovanovic M and Ibric S. Development of the second-order derivative UV spectrophotometer method for biopharmacology. *Drug Dispo*. 2003; 24 (7): 309 – 314.

Phelps RJ, Facordi S, Fossie C, Leonzio C, and Renzoni A. Chlorinated Hydrocarbons and heavy metals in Crocodile Eggs from Zimbabwe. *Trans Zimbabwe Scient. Ass*. 1986; 63: 8 – 15.

Plant Protection Research Institute. The Policy to be adopted Towards the Use of Oranochlorine Pesticides in Rhodesia. *Draft* 1977.

Polder A, Becher G, Savinova JN, Skaare JU. Dioxins, PCBs and some chlorinated pesticides in Human Milk from the Kola Pensila, Russia. *Arch Environ Contamin* 1998; (9-12): 1795 – 1806.

Pratap SR, Bandhuvula P and Ramesha RA. Modulatory influence of Adhatoda vesica (Justicia adhatoda) leaf extract on the enzymes of xenobiotic metabolism, antioxidant status and lipid peroxidztion im mice. 2000; 213(1-2): 99 – 109.

Rang HP, Dale MM and Ritter JM. Pharmacology 4th (ed). Churchill Livingstone: Edinburgh, London, New York, 1999; 235 – 236.

Raunio H, Pasanen M, Maenpaa J, Hakkola J Pelkman O.ExpressioN of extrahepatic cytochrome P 450 in human. In: Pacific G M, Francchia G M. (eds) Advance in drug metabolism in man. *European Commission, Luxembourg.* 1995; 233-287.

Redetzke KA and Applegate HG. Organochlorine pesticides in adipose tissue of persons from El Pa, Texas. *J Environ Health*. 1993; 56(3): 25 –27.

Rivero L, Borja V, Santos C and Walszensky S. Exposure assessiment for works applying DDT to control malaria in Veracruz, Mexico. *Environ Health Persepest*. 1997; 105: 98 – 101.

Riviere JE, Absorption and distribution. *I*n introduction to biochemical toxicology eds. Rodriguez LA, Jick H. Risk of gastrointestinal bleeding and perforation associated with non-steroidal anti-inflammatory drugs. *Lancet.* 1994; 343: 769 – 772.

Roberts DS, Laughlin LL, Hsheih P and Legters LJ. DDT, global strategies, and a Malaria Control Crisis in South America. Emerging Infectious Diseases. 1997; 3(3): 295- 302.

Rozman KK and Klaassen CD. Absorption, Distribution and Excretion of Toxicants. In Casarett and Doull's Toxicology: The Basic Science of Poisons, 5th (ed).Klaassen CD, Amdur MO and Doull J. Mcgraw- Hill Companies. 1996; 101 – 109.

Safe SH. Endocrine disruptors and human health- is there a problem.? up date. *Environ Health Perspect*. 2000, 108: 487-493.

Safe SH. Dietary and environmental estrogens and antiestrogens and their possible role in Human Diseases. *Environ Sci Pollut Res*. 1994; 1 (1): 29 – 33.

Savitz DA and Harlow SD. Selection of reproductive health and points for environmental risk assessments. *Environ Health Perspect.* 1991. 8: 56- 60.

Saxen MC and Siddiqui MKJ. A comparison of Organochlorine insecticide Contact in specimens of maternal blood placenta and umbilical-cord blood from still born and live- born Cases. *J Toxicol Environ Health.* 1983; 11: 71 – 79.

Sharma V P, Health hazards of mosquito repellents and safety alternatives. *Current Science.* 2001; 80: 3.

Silbergeld EK. Neurochemical approaches to developing biochemical markers of neurotoxicity: review of current status and evaluation of future prospects. *Environ Res.* 1993; 63: 274-286.

Skaare JU, Tuveng JM and Sande HA. Organochlorine Pesticides and Polychlorinated Biphenyls in Maternal Adipose Tissue, Blood Milk and Cord Blood from Mothers and their infants living in Norway. *Arch Environ contamin Toxicol* 1988; 17: 55 – 63.

Slorach, A.S. Vaz R "Assessment of Human Exposure to Selected Organochlorine Compounds Through Biological Monitoring' Global Environmental Monitoring System (GEMS). UNEP/WHO. Prepared by the Swedish National Food Administration, Uppsala, Sweden, 1983.

Stevens M F, Ebell G F, Psaila-Savon P. Organochlorine pesticides in Western Australia nursing mothers. *Med J Aust.* 1993; 158: 238 – 241.

Smyth RD, Hottedorf GH. Effect of First Pass Metabolism; Toxical Appl. Pharmc. 1980; 53: 179.

Stresser DM and Kupfer D. Human Cytochrome P450- Catalyzed Conversion of the Proestrogenic Pesticide Methoxychlor into an Estrogen role of CYP2C19 and CYP1A2 in O-Demythylation. 1998; 26-9: 868-874.

Szokolay A, Rosival L, Uhnak J and Madaric A. Dynamics of benzene hexachloride (BHC) isomers and other chlorinated pesticides in the food chain and in human fat. *Ecotoxicol.Environ.Safety*.1977; 1: 349-350.

Timbrell JA. Principles of Biochemical Toxicology 2nd (ed). Factors affecting toxic responses: disposition. *Taylor and Francis London*; 1991a; 47 – 53.

Timbrell JA. Principles of Biochemical Toxicology 2nd (ed). Factors affecting metabolism and disposition. *Taylor and Francis, London* 1991b; 172 – 184.

Tuli JS, Smith JA and Morton DB. Stress measurements in mice after transportation. *Lab Anim.* 1995; 29: 132 – 138.

Ulrich E. Environmentally relevant xenoestrogen tissue concentration correlated to biological responses in mice. *Environ Helth Perspect.* 2000; 108: 973 – 977.

Vaz R, Slorach SA and Hofuander Y. Organochlorine contaminants, in Swedish human milk: Studies conducted at the National Food Administration. 1981–1990. *Food Additives and Contaminats*. 1993; 10: 407 – 418.

Verbek MM, Brouwer DH and Brouwer EJ. Health effects of pesticides in the flower-bulb culture in Holland. *Med. Lav.* 1990; 81: 530-541.

Welch KMA. Drug therapy of migraine. *N Engld J Med.* 1993; 329 (20): 1476 – 1483.

Werck-Reichhart D and Feyereisen R. Cytochromes P450: A Success Story. Genomebiology Com. 2000; 1(6): 3003.

Werringloer J and Estabrook RW. Heterogeneity of liver microsomal Cytochrome P 450 Spectral characterization of reactants with reduced cytochrome P–450. *Archives Biochem and Biophys.* 1975; 167(1): 270 – 286.

Wienkers LC. Problems associated with in vitro assessment of drug inhibition/induction of CYP3A4 and other P-450 enzymes and its impact on drug discovery. *J Pharmacol and Toxicol Methods*. 2001; 45(1): 79 – 84.

Wilkinson GR. Plasma and Tissue Binding considerations in Drug Disposition. *Drug Metab. Rev*. 1983; 14: 427 – 465.

Williams RT. Detoxification Mechanism, 2nd (ed). New York: Wiley, 1971. Wilson JT. Determinants and Consequences of Drug excretion in Breast Milk. *Drug Metab. Rev* 1983; 14: 619 – 652.

Wilson JT, Helms R, Pickering BD, Danahue and Brown R. Acetaminophen controlled- release sprinkles versus acetaminophen intermediated-release Elixir in fibrile- children. *J Clin Pharmacol.* 2000; 40: 360 – 369.

Wolf M S, Toniolo P G, Lee E W, Rivera Mand Dublin N. Blood levels of organochlorine residues and risk of breast cancer. J *Natal Cancer Inst.* 1993; 85: 648 – 652.

Yoshioka H, Miyata T and Amura T, Induction of Phenobarbital induced form of cytochrome P-450 in rat liver microsomes by 1,1 di(p-chloropheny) – 2,2-dichloroethylene. *J. Biochem* 1984; 95: 937 – 947.

Yoshitomi S, Ikemoto K, Takahashi J, Miki H, Namba M and Asahi S. Establishment of the transformants expressing human cytochrome P-450 subtypes in HepG2, and their applications on drug metabolism and toxicology. Toxicol in Vitro. 2001; 15 (3): 245 – 256.

Yun CH, Shimada J and Guengerich FP. Purification and Characterisation of Human liver Microsomal cytochrome P-450 2A6, Molecular Pharmaco. 1991; 40 (5): 679 – 685.

Zaranyika MF and Makhubalo JM. Organochlorine Pesticide Residues in Inland Water in Zimbabwe. In: Nhachi CFB and Kasilo OMJ (eds) Pesticides in Zimbabwe, Toxicity and Health Implications. *The University of Zimbabwe Publications*. Harare. 1996; 89 – 106.

Zaranyika MF, Mambo E and Makhubalo JM, Organochlorine Pesticides in the Sediments of Selected River Bays in Lake Kariba, *Zimbabwe the Science of the Total Environ* 1994; 142: 221 – 6.

APPENDIX 1

APPENDIX 2