MALARIA VECTOR CONTROL AND OCCURRENCE, LEVELS AND DYNAMICS OF DDT IN BREAST MILK IN CERTAIN RURAL AREAS OF KWAZULU

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OPSOMMING

MALARIAVEKTORBEHEER EN DIE VOORKOMS, VLAKKE EN DINAMIEK VAN DDT IN BORSMELK IN SEKERE GEBIEDE VAN KWAZULU

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Die binnenshuise aanwending van DDT vir die voorkoming van transmissie van die malaria parasiet deur muskiete, word deur die Wêreld Gesondheidsorganisasie aanbeveel. Hierdie gebruik word as veilig vir mens en omgewing beskou. Daar is egter weinig navorsing oor die moontlike gesondheidsgevare vir moeders en suigelinge, wat die binnenshuise gebruik van DDT en sosiale gebruike in ag neem, gedoen. Die doel van die studie was:

1. Die bepaling van die vlakke van DDT en sy metaboliete (DDD en DDE) in die borsmelk van 'n toets en kontrole bevolking, asook die bepaling van verandering in hierdie vlakke met binnenshuise toediening van DDT.

2. Die bepaling van die opname van DDT en sy metaboliete deur die suigeling via borsmelk, en die ontwerp van 'n statistiese model wat die dinamiek beskryf.

3. Die bepaling van die gesondheidsrisiko vir die moeder en die suigeling wat aan die blootstelling aan DDT en sy metaboliete toegeskryf kan word.

Die eksperimentele ontwerp en metodes is deur die Etiese Komitee van die Navorsingsinstituut vir Siektes in 'n Tropiese Omgewing van die Mediese Navorsings Raad goed gekeur. Lakterende moeders wat die babaklinieke by Mseleni (toetsgroep) en Murchison Hospitale (kontrole groep) besoek het, is gedurende vier geleenthede in 1986 en 1987 gevra om melk te skenk. Bloed is ook van 23 babas van die toetsgroep geneem. Ektraksieprosedures wat self ontwikkel is, is gebruik en analise is m.b.v. gaschromatografie met 'n elektronvangs-detektor gedoen. DDT is in al die melkmonsters van die toetsgroep, en in meeste van die monsters van die kontrolegroep gevind. Die gemiddelde vlak van ΣDDT (totale DDT) in die borsmelk van die toetsgroep was 15,83 mg l^{-1} ΣDDT (melkvet), statisties betekenisvol hoër ($p < 0,05$) as die 0,69 mg l^{-1} ΣDDT (melkvet) vir die kontrole groep. Dieselfde was ook die geval vir DDE, DDD en persentasie DDT.

Hierdie vlakke, wat die liggaamsbelading van die moeder weerspieël, word nie as 'n gesondheidsrisiko vir die moeder beskou nie. Die ΣDDT konsentrasie in die borsmelk van die toetsgroep oorskry die berekende Aanvaarbare Daaglikse Inname vir suigelinge (0,02 mg kg^{-1} ΣDDT dag^{-1}). Die konsentrasie van DDT in die bloed van die suigelinge is suksesvol gemodelleer. Pariteit van die moeder en ouderdom van die suigeling was twee van die belangrikste faktore in die model. Die persentasie DDT het ook statisties betekenisvol ($p < 0,05$) met pariteit toegeneem.

Inligting in die literatuur dui daarop dat blootstelling aan die hoë vlakke van DDT moontlik 'n gesondheidsrisiko vir die baba inhou. Immunologiese en neurologiese sisteme is van die sisteme wat geaffekteer kan word. Die bepaling van hierdie risiko is as 'n navorsingsprioriteit geïdentifiseer.

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III. LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
ANOVA	Analysis of Variance
AR	Analytical Reagent
BGT	Bender visuomotor Gestalt Test
BHC	Benzene-hexachloride
BMDP	Biomedical Computer Programs, P-series
BNBAS	Brazelton Neonatal Behaviourial Assessment Scale
°C	Degrees Centigrade
CC	Correlation Coefficient
CD	Coefficient of Determination
cm	centimetre
CNS	Central Nervous System
CSF	Cerebral Spinal Fluid
%CV	Percentage Coefficient of Variation
DB	Liquid phase identification
DC	District of Columbia
DDA	2,2-bis(2,2 dichloroethylidene)bis(4-chlorobenzene)
DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene
DDOH-PA	DDT conjugated with palmitic acid
DDR	German Democratic Republic
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
Dept	Department
ECD	Electron capture detector
ED_{50}	Dose which elicits a certain effect in 50% of a test population
EEG	Electro-encephalogram
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization
FID	Flame Ionization Detector
Fig	Figure
Figs	Figures

GC	Gas-chromatograph
GIT	Gastro Intestinal Tract
IA	Infant Age
IB	Institute for Biostatistics
IBM PC	International Business Machine - Personal Computer
ICPS	International Programme on Chemical Safety
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQ	Intelligence coefficient
JUN/Jun	June
J&W	Company name (capillary columns)
l	litre
LD_{50}	Dose which is lethal to 50% of a test population
Ln	Natural logarithm
kg	kilogram
μg	microgram
μl	microlitre
m	meter
MA	Maternal Age
MAR/Mar	March
Max	Maximum
mCi	milli Curie
mg	milligram
min	minute
Min	Minimum
mm	millimetre
MRC	Medical Research Council
n or N	Number of samples
N2	National road 2
NC	Not Calculable
ng	nanogram
NLM	National Library of Medicine

NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOV/Nov	November
p	Probability value
PAR	Parity
PBB	Poly-Brominated-Biphenyls
PCB	Poly-Chlorinated-Biphenyls
pg	picogram
pH	Logarithm of the reciprocal of the hydrogen ion concentration
PTZ	pentylenetetrazol
PU for CHE	Potchefstroom University for Christian Higher Education
R	Rand
RD	Raw Data
RIDTE	Research Institute for Diseases in a Tropical Environment
RIED	Research institute for Environmental Diseases
RSA	Republic of South Africa
ΣDDT	sum of DDT, DDE and DDD
SD	Standard Deviation
SE	Standard Error
sec	second
sIgA	Secretory immunoglobulin A
USA	United States of America
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Although the existing knowledge on the health effects of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) is extensive, large gaps still exist. This is especially true for DDT in the context of malaria control. As malaria control is almost exclusively practised in Third World situations, the scientific effort to determine, not only the occurrence and levels of DDT, but also its dynamics in such a context, is fragmentary. Also, in South Africa, no studies on DDT-levels in humans in this regard have been done.

The largely negative public perception of the continued use of DDT, together with an increasing concern for the environment, has resulted in mounting pressure for a total ban on DDT. The printed press has sketched a picture of an almost total disregard for human safety by the health authorities. One report entitled "THE MILK OF DEATH" (Ferreira, Sunday Tribune, 27 Jan. 1985), actually stated that DDT was one of the most dangerous chemicals used, and that breast-feeding mothers were poisoning their babies with DDT as a consequence of the malaria control program.

Environmentalists have also actively discouraged the continued use of DDT. They state that, no matter the purpose, method and control of application, DDT will always be harmful to the environment. They no doubt mean well, but the absence of any locally derived data supporting the contrary, results in counter arguments that can only be based on studies from abroad, which, although applicable in a general sense, do not carry any convincing weight. Conservation bodies (such as the KwaZulu Bureau of Natural Resources) have also actively lobbied for the use of alternative agents. In their opinion DDT should not be allowed under any circumstances.

Further evidence for the need of a more controlled study in South Africa has come from a local study by Van Dyk *et al.* (1987), who reported some data on

contaminated breast milk and human fat. They stated "The PCB and DDT levels certainly require further attention and monitoring should continue in this area."

1.1 AIMS OF THE STUDY

The three main aims of this study were:

A. To determine the levels of DDT and its metabolites in the breast milk of lactating mothers in relation to malaria control activities, and to develop statistical models describing the dynamics of DDT in this compartment.

B. To determine the uptake of DDT and its metabolites from breast milk by the infant[1] exposed to DDT in breast milk, and to develop a statistical model describing the relationship between different factors that determine the levels in the infant.

C. To determine the risk to infants and lactating mothers, posed by the exposure to, and uptake of DDT.

The objectives set to achieve these aims were:

1. To develop appropriate analysis techniques.

2. To define the study and control populations and to design an epidemiologically sound sampling method.

3. To conduct an exposure and risk assessment of DDT in lactating mothers, and to characterize the associated uncertainties.

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[1]For the remainder of this thesis, children younger than two years of age will be referred to as infants.

4. To conduct an exposure and risk assessment of DDT in breast feeding infants, and to characterize the associated uncertainties.

These aims will aid in defining areas that need further research as well as testing the safety of the current malaria control policy in the RSA.

The following guidelines (Environmental Protection Agency, 1986) have been taken into consideration for the collection and evaluation of the sources of information used in the present study.

1. The availability of relevant information on every appropriate aspect of this study.

2. The reliability and applicability of this information to the aims of the study.

3. The qualitative and quantitative nature of this information.

4. The limitations on the ability to assess exposure (and risk) from available information.

Relevant information has been readily available on most aspects of the present study, except in relation to malaria control (as a source of DDT in humans) and the significance of DDT exposure to the infant. As previous studies were not designed to determine the risk of DDT in breast milk to infants, information from less pertinent investigations, such as animal exposure studies, had to be used.

The majority of the available information seemed to be reliable, corroborated each other in most aspects and appeared in refereed journals. Some articles were, however, of questionable quality (doubtful reporting of methods, data and data analysis) and this was taken into account by limiting its evidential value. Differences of opinion were frequent in respect of the actual advisability of the

continued use of DDT, as well as the significance of sub-lethal effects such as cancer. Many articles reported on levels of DDT and its metabolites in breast milk, serum, whole-blood and body fat, but, in general, represented low-level background or occupational exposure. Particular problems were experienced with the omission of advanced data analysis. There were cases where obvious disparities or relationships were not mentioned or discussed. Problems with ill defined populations, age groups and method reporting (such as epidemiological and chemical analysis methods) were also encountered.

1.2 MALARIA, DDT AND VECTOR CONTROL

1.2.1 MALARIA

> *"For centuries, malaria has outranked warfare as a source of human suffering. Over the past generation it has killed millions of human beings and sapped the strength of hundreds of millions more. It continues to be a heavy drag on man's efforts to advance his agriculture and industry"*
>
> John F Kennedy, 1962 (Schmidt and Roberts, 1985)

Despite all the advances made since 1962, these words are still true. Man is continuing to battle perhaps the most important disease in the world. Recognisable descriptions of malaria were deciphered from Egyptian papyri in which it was linked with the annual flooding of the Nile (Schmidt and Roberts, 1985). Hippocrates described the fevers and splenomegaly associated with malaria. Greek cities built in low lying areas were made nearly uninhabitable by this disease. Even then, the link between the disease and marshes (bad air or malaria) was suggested. The discovery that malaria was transmitted by *Anopheles* mosquitoes was made in 1897 by Ronald Ross in India. The full life cycle of the parasite was only described in 1948 (Schmidt and Roberts, 1985).

Perhaps the first concerted campaign to control malaria was made by William C. Gorgas during the building of the Panama canal (Schmidt and Roberts, 1985). This operation was a huge success, as the hospital admissions for malaria dropped from 1263 per 1000 (of the population) to only 76 per 1000. An estimated 71000 lives were saved. This illustrates the tremendous influence that malaria, and its control, can have on the economies of entire continents (Schmidt and Roberts, 1985).

To date, the most effective measure to limit transmission of malaria remains the control of the vector with chemicals. Vaccines still seem to be a long way off. Insecticides such as DDT are at present the most important control measure.

Our knowledge regarding the safety of this compound needs to be continually reevaluated in the light of new discoveries or insights.

1.2.2 A SUMMARY OF THE USE OF DDT AS AN INSECTICIDE IN AFRICA

DDT has been used in Africa since 1946 for the control of malaria (Brown, Haworth and Zahar, 1976). As far as can be established, the first anti-malarial control operations in Africa took place in Transvaal, using DDT as a larvicide and as a house spray, with very good results. Other programs started in Natal and Swaziland, with apparently no other national projects anywhere else in Africa by 1950. By 1957, the disease was eradicated in large areas of Natal and Transvaal and the programs were almost completed by 1961. Programs in Zanzibar and Pemba were started in the same year. Only the lowlands in the north of Zimbabwe were actively controlled, while other areas were kept under surveillance. Successful pilot projects with DDT in Central African states, like Cameroon and Uganda, led to the implementation of eradication schemes in 13 tropical African countries between 1962 and 1964. These schemes were discontinued due to lack of finances and infra structure (Brown *et al.*, 1976).

By 1982, more than 359 million people, out of a total of 397,4 million, remained unprotected against malaria in the original malaria endemic areas of Africa south of the Sahara (World Health Organization, 1984). Only 9,22 million people were considered to be living in areas freed of malaria by that time, which was due mainly to the use of DDT. Because of a lack of information from most African countries, further information, such as number of cases or areas under attack were not available. Seventy-one million clinical cases of malaria were estimated for this region for 1981, with only about two million being reported. Control operations, mainly using DDT (and sometimes dieldrin), were reported for Nigeria, Cameroon, Liberia, Uganda, Tanzania, Zimbabwe, Botswana, Ethiopia, Somalia, Sudan and Swaziland, with activities in other countries limited to selected urban and suburban areas and some economically important areas (World Health Organization, 1984).

In the Sudan, DDT (which was replaced by BHC - 1,2,3,4,5,6-hexachloro-cyclohexane - because of vector resistance) was effective for only four years, when resistance again set in. The following years saw a malaria epidemic which only abated after malathion was introduced in 1975 (El Gaddal *et al.* 1985). By 1985, the situation in Africa for an estimated population of 400 million seemed worse, with an estimated 80 million clinical cases (World Health Organization, 1987).

DDT is not only used against malaria vectors; tsetse fly is also extensively controlled by the use of this agent. In Zimbabwe, DDT has been used for this purpose from at least 1968, and in Mozambique since 1969. Before then dieldrin was used. During the 1969 campaign, more than 36,5 tons of DDT (corrected for active ingredient) were applied to water bodies, trees and other likely hiding and breeding places of the fly, using very intense application (Robertson *et al.* 1972). Since 1973, the use of DDT has been restricted in Zimbabwe for agricultural use on maize and cotton only. DDT replaced BHC in 1973 as malaria vector control agent in Zimbabwe (Tannock, Howells and Phelps, 1983). The use of DDT against *Simulium* in streams in Uganda has also been reported (Hynes and Williams, 1962). Koeman and Pennings (1970) reported the use of DDT and dieldrin for tsetse fly control in Nigeria and Kenya.

Elsewhere in Africa, agricultural application of DDT was started in 1954, and its use, together with other chlorinated pesticides, has increased to very high levels (Deelstra, Power and Kenner, 1976). In Nigeria DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene) was still present in soil of cocoa farms more than ten years after the farms had been abandoned (Adedeji, 1983). Other reports also implicate agriculture as a source of DDT in the environment. El-Zorgani (1976) found DDT in fish and birds in Sudan. Atuma and Okor (1987) mentioned extensive and indiscriminate use of pesticides as possible reason for the presence of DDT in human breast milk. Koeman *et al.* (1972), Ulfstrand and Södergren (1972), Peakall and Kemp (1976), Frank *et al.* (1977) and Phelps *et al.* (1986) reported DDT residues in biological material from Kenya, Tanzania, Transvaal, Kenya and Zimbabwe, respectively. They all postulated agricultural

use of DDT as the main source. Lincer *et al.* (1981) and Matthiessen (1985) implicated malaria and tsetse fly control as also contributing to the environmental load.

Levels of DDT and metabolites in humans from Africa somehow attracted less interest, with mostly reports on levels in milk being available. Atuma and Vaz (1986) reported levels of DDE between 0,28 to 1,9 mg kg^{-1} (milk fat[2]) and 0,12 to 0,41 mg kg^{-1} (milk fat) for DDT in 35 samples from Nigeria. Mean levels of 2,9 mg kg^{-1} ΣDDT[3] (2,9 in males and 1,2 mg kg^{-1} in females) were detected in 75 fat samples (obtained at autopsy) from Ugandans (Wassermann *et al.*, 1974). In the same article, mean levels of 6,5 and 4,5 mg kg^{-1} in human fat samples from Nigeria and Kenya, respectively, were reported. The living conditions of these people, as well as the source of DDT were not described. Atuma and Okor (1987) reported mean levels of 2,37 and 3,83 mg kg^{-1} ΣDDT (fat basis) in colostrum (n=34) and mature milk (n=38). Kanja *et al.* (1986) analyzed 302 samples from eight different areas in Kenya. Mean levels ranged from 1,1 to 18,7 mg kg^{-1} ΣDDT (fat basis) from the different areas. El-Zorgani and Musa (1976) measured blood levels of DDT in exposed personnel from a research farm in Gezira (Sudan). A mean (calculated from their data) of 140 $\mu g\ l^{-1}$ ΣDDT in whole-blood[4] was found.

Mpofu (1986) analyzed 34 serum samples from people living in DDT sprayed dwellings in Zimbabwe. He found no DDT, DDE or DDD. People involved in cotton, maize, wheat and vegetable farming (subsistence and commercial) for which DDT had been used for pest control did, however, have detectable levels,

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[2]Concentrations of contaminants in milk are expressed in one of two ways: on the basis of its concentration in milk fat or on a whole milk basis. To obtain an approximate milk fat value, the whole milk value can be divided by 30.

[3]ΣDDT refers to the sum of the concentrations of DDT, DDE and DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane) isomers. This notation will be used throughout this thesis.

[4]Levels of contaminants in blood can be based on whole-blood or serum, depending on how it is collected and stored.

which makes his data suspect. The highest mean serum level (29 $\mu g\ l^{-1}$ ΣDDT, n = 69) was for a community involved in maize and cotton farming. A mean serum level of 2220 $\mu g\ l^{-1}$ ΣDDT was reported for nine malaria control applicators.

1.2.3 DDT IN SOUTH AFRICA

For South Africa, only a few reports on levels of DDT in humans, animals and the environment were noted. Some of them were based on small numbers or samples of convenience. The review article by van Dyk, Wiese and Mullen (1982) summarized their findings on DDT-levels as declining overall, with localized high contamination. The registration of DDT for agricultural use in South Africa was withdrawn in 1976 (van Dyk *et al.*, 1982). Wassermann *et al.* (1970) conducted the only prospective study published on the levels of DDT in adipose tissues from South Africans. They analyzed 123 fat samples from blacks and whites and found a mean concentration of 6,38 mg kg^{-1} ΣDDT. Levels were slightly higher in whites than in blacks, possibly due to a higher intake of meat by the more affluent whites (Wiese, 1976). This is corroborated by Deichmann (1972), who included in his article "*The debate on DDT*", information obtained as a personal communication from Dr J. Robinson of Shell. Mean body fat DDT concentrations for white males, white females, black males and black females from South Africa were 2,6; 2,21; 2,72 and 1,87 mg kg^{-1}, respectively. For DDE the respective values were 7,79; 7,43; 6,27 and 4,31 mg kg^{-1}. The higher levels in whites were attributed to domestic use of pesticides.

Van Dyk *et al.* (1987) reported results from *ad hoc* monitoring programs. No effect of age on levels was found, but organochlorines were detected in 95% of the fat samples. ΣDDT levels ranged from a low of 2,04 to a high of 7,3 mg kg^{-1} from different sites at different times. DDT was also detected in breast milk from Johannesburg (sampled during 1983). The levels ranged from 0,1 to 3,6 mg kg^{-1} (fat basis) for DDE and from 0,1 to 1,0 mg kg^{-1} (fat basis) for DDT. The levels of DDT were only slightly less than when DDT was still in general use.

The main finding was that DDT was still present in human fat and milk, seven years after restriction of its use in agriculture.

Although it seems that some work has been done in Africa, well controlled studies on humans are rare. In most studies, only a few factors have been considered. The lack of consistent sample collection methods and basis of calculation is more confusing than helpful in determining the extent of human and environmental contamination by DDT, and the risk that it poses. The data indicate that contamination is widespread, due to a wide variety of applications. People may thus be exposed to DDT (from agricultural and vector control as sources) by more than one route. Contaminated fish and food will add to the body burden, while the exposure is increased by house spraying. Comparisons with countries like Kenya (Kanja *et al.*, 1986), India (Jani *et al.*, 1988) and Guatamala and El Salvador (de Campos and Olszyna-Marzys, 1979) where comparable malaria control operations are carried out, will be covered.

1.2.4 SCOPE AND SUCCESS OF VECTOR CONTROL IN KWAZULU

The present endemic malaria areas of KwaZulu in Natal (South Africa) are situated between the Lebombo Mountains to the west, the Indian Ocean to the east, Lake St Lucia to the south and the Mozambique border in the north. Localized outbreaks have been recorded as far south as Durban in 1905 and Umzinto in 1930. During 1928-29, more than 2750 out of an estimated total population of 241000 people, died from malaria (Sharp *et al.*, 1988).

Anti-larval measures were introduced in 1932, and from 1934 pyrethrum was used for indoor control of adult mosquitos. This was replaced by DDT in 1946 for both larviciding and indoor control. In 1953 the measures were extended to include the districts of Ubombo, Hlabisa and Ingwavuma (Sharp *et al.*, 1988).

The control measures and strategies used in combatting malaria in the above mentioned areas are described by Sharp *et al.* (1988). The success of this program is phenomenal, as cases of malaria are recorded as infections, not

mortality which hardly occurs. The mean yearly mortality for the period 1976 to 1985 was 4,3 (Sharp *et al.*, 1988).

Each homestead covered by the control program is numbered, and has a control card with records of DDT-treatment, entomological investigations and malaria surveillance visits. This allows the mapping of malaria outbreaks, and is also very helpful during health related surveys such as the present investigation. Movement of people from Mozambique to these areas account for a major part of the malaria cases. This migration was also a factor in the planning of the present study, as no malaria control is presently being practised in Mozambique. These people have therefore been exposed to DDT for only a short while after having entered South Africa.

1.3 CHEMICAL AND PHYSICAL PROPERTIES OF DDT, DDE AND DDD

As DDT is an agent that has been shown to adversely affect non-target organisms, the characteristics of this compound and its two major metabolites must be known in order to explain the findings of environmental, biological and medical research. The information was obtained from an on-line database, Toxnet, of the National Library of Medicine (NLM) in 1988.

1.3.1 PROPERTIES OF DDT

p-p'DDT[5] will be referred to as DDT throughout the rest of this thesis, unless specified otherwise (such as o-p'DDT[6]). The following are synonyms of p-p'DDT (National Library of Medicine, 1988c).

1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane
4,4'-DDT
4,4'-dichlorodiphenyltrichloroethane
ethane, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)
ethane, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)
p,p'-DDT
p,p'-dichlorodiphenyltrichloroethane
alpha,alpha-bis(p-chlorophenyl)-beta,beta,beta-trichloroethane
diphenyltrichloroethane
trichlorobis(4-chlorophenyl)ethane
1,1,1-trichloro-2,2-di(4-chlorophenyl)-ethane

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[5]para-para'DDT, referring to the chlorine substitutions on the para positions of the phenyl rings.

[6]ortho-para'DDT, referring to the chlorine substitution on the ortho position of one of the phenyl rings

NCI-C00464
ENT-1506
Dicophane
Chlorophenothane
1,1'-(2,2,2-trichloroethylidene)bis(4-chlorobenzene)

The molecular formula of DDT is $C_{14}H_9Cl_5$. DDT is formed by the condensation of chlorobenzene and trichloro-acetaldehyde. It is formulated as aerosols, dusts, solutions, wettable powders, emulsifiable concentrates or granules. Some 76 different commercial formulations are listed in National Library of Medicine (1988c) under such descriptive names as End-O-Pest, Miller's Fly-Ro-Cide, Mysterious Roach Killer Outfit, Fly-Ded, Sapho Insect Bombs, Ded-Tox, Dee-Dex, Didimac, Lethalaire, Pest-B-Gon Spray, Sani-Deth, Will-Kill and Water Bug Death.

Crystalline DDT has a boiling point of 260°C, a melting point of 108,5°C, a molecular weight of 354,5 and a density of 0,98 to 0,99. It starts to decompose at 110°C. The vapour pressure is $1,5 \times 10^{-1}$ mm Hg at 20°C. The log of the octanol \ water partition coefficient is 3,98. It has a very low solubility in water (12 $\mu g\ l^{-1}$) and a high solubility in fat (10 $g\ kg^{-1}$). Solubility in some solvents are: 58 g 100 ml^{-1} in acetone, 78 g 100 ml^{-1} in benzene, 2 g 100 ml^{-1} in alcohol, 28 g 100 ml^{-1} in ethyl ether and 116 g 100 ml^{-1} in cyclohexanone. It is not soluble in diluted acids and alkalies. DDT is odourless and tasteless. DDT does not occur as a natural product (National Library of Medicine, 1988c).

1.3.2 PROPERTIES OF DDE

As the synonyms used for DDT are almost the same for DDE, only two will be listed. p-p'DDE will be referred to as DDE throughout the thesis.

p,p'-dichlorodiphenyl dichloroethylene
1,1-dichloro-2,2-di(p-chlorophenyl)ethylene

The molecular formula of DDE is $C_{14}H_8Cl_4$. DDE has a melting point of 88,4°C and a molecular weight of 318,0. Its solubility in water is 120 μg l^{-1} at 25°C and has a vapour pressure of 6,5 x 10^{-1} mm Hg at 20°C. Solubilities of DDE in solvents are comparable with DDT. DDE does not occur as a natural product. A log octanol \ water partition coefficient of 4,74 has been calculated. DDE is not formulated or sold commercially. It is odourless and tasteless (National Library of Medicine, 1988b).

1.3.3 PROPERTIES OF DDD

Synonyms of p-p'DDD are given below. p-p'DDD will be referred to as DDD throughout this thesis.

1,1-bis(4-chlorophenyl)-2,2,-dichloroethane
1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
1,1-dichloro-2,2-bis(parachlorophenyl)ethane
2,2-bis((4-chlorophenyl)-1,1-dichloroethane
4,4'-DDD
dichlorodiphenyl dichloroethane
p,p'-DDD
p,p'-TDE
TDE
tetrachlorodiphenylethane

DDD is formed by condensing dichloro-acetaldehyde with chlorobenzene. It is formulated as an insecticide (Rothane). It is used for tomato horn worm, against which it is more effective than DDT, and mosquito larvae.

The molecular formula of DDD is $C_{14}H_{10}Cl_4$ with a molecular weight of 320,05 and a density of 1,476 at 20°C. DDD crystals are colourless. It has a boiling point of 193°C and a melting point of 109°C. The log octanol / water partition coefficient is 5,99. The maximum solubility in water is 160 μg l^{-1} at 24°C. It is odourless and tasteless. DDD is not a natural product (National Library of Medicine, 1988a).

1.4 ASPECTS OF BREAST MILK AND BREAST-FEEDING

A thorough understanding of the synthesis and composition of milk is not only needed to understand the significance of breast-feeding, but also to understand how and where DDT is incorporated in the milk. The next two sections will give a summary of the bio-synthesis and composition of human breast milk. Breast-feeding patterns differ between societies and the special significance of this practice in Africa will be discussed.

1.4.1 BIO-SYNTHESIS AND SECRETION OF BREAST MILK

Lactation is an integral part of reproduction in all mammals. The bio-synthesis and secretion of milk takes place in the mammary gland in the breast, which is one of the most complex endocrine organs in the body. The mammary gland is also unique in that the production of milk demands a tremendous contribution from the maternal system, without any direct physiological advantage to the maternal organism. The onset of lactation alters the metabolism of the mother. The higher metabolic rate requires a redistribution of blood supply and an increased nutrient demand, and milk may be produced at the metabolic expense of other organs. These changes are suckling dependant. Suckling releases the anterior pituitary hormones under neuronal control via tactile sensors located in the nipple. These hormones stimulate milk bio-synthesis, as well as the milk-ejection hormones that are necessary for milk production (Lawrence, 1980; White, Handler and Smith, 1973).

The bio-synthesis of milk occurs in the epithelial cells of the gland. They are differentiated into stem cells and secretory cells. The secretory cells change from a cuboidal to a cylindrical shape after stimulation by prolactin just before secretion, with an increased cellular water uptake. The cell enlarges and the club-like tip pinches off, enclosing or encapsulating fat. The droplet thus contains lipid surrounded by the secreted cell wall which also contains proteins.

Secretion of milk is a combination of apocrine (lipid and protein) and merocrine (lactose) processes.

The nucleus of the secretory cell contains the DNA necessary for transcription of RNA for protein and lipid synthesizing enzymes. The nucleus in the resting cell migrates from the base of the cell to the apex just prior to secretion. The mitochondria in the secretory cell increase at the onset of lactation to supply the increased demand for oxidative capacity and expanded mitochondrial function. The citrate from the mitochondria is the precursor for fatty acid synthesis and the mitochondria also supply carbon for non-essential amino acid synthesis. Lipid, protein and carbohydrate synthesis involve the Golgi apparatus, endoplasmic reticulum and cell membranes of the secretory cell. The protein and most of the lipid and carbohydrates are thus synthesized *de novo* in the cell and not imported from the blood. The water and ions are derived from the plasma store (Lawrence, 1980; White *et al.*, 1973).

Lactose is derived from blood glucose in a reaction requiring α-lactalbumin, a whey protein, as catalyst. The bio-synthesis of α-lactalbumin is increased with increased prolactin levels after the removal of the placenta, with a concomitant reduction in progesterone and oestrogen levels which are rate limiting for α-lactalbumin synthesis. It is interesting that lactose synthesis requires glucose and not the phosphorylated derivative.

Short chain fatty acids, derived from acetate are produced and esterified with glycerol from plasma in the endoplasmic reticulum. This process is under the control of prolactin and insulin. The synthesized glycerides, as well as some derived from plasma, collect in small droplets on the base of the cell and move to the apex, coalescing with other droplets. These are engulfed by the apical membrane and discharged. The long chain fatty acids are derived from plasma (Lawrence, 1980; White *et al.*, 1973).

Most of the protein is formed from amino acids in the secretory cell, under the hormonal control of prolactin, cortisol and insulin. The three major proteins are casein (40%) and the two whey proteins α-lactalbumin and ß-lactalbumin. They

are produced on the ribosomes from where they move to the apex of the cell. They are predominantly discharged apocrinically, but some merocrine secretion also takes place. A number of enzymes are also present in milk, of which lipase is the most significant as it splits triglycerides.

The major buffer system of milk is citrate, formed as described above. The secretory mechanism is not known, but is probably secreted with lactose merocrinically. Inorganic phosphate is also present as a buffer (Lawrence, 1980; White *et al.*, 1973).

1.4.2 COMPOSITION OF BREAST MILK

An understanding of the nature and composition of human breast milk is necessary for a proper evaluation of the many possible options available for extraction and clean-up of milk for the determination of DDT. Transport and preferential association of DDT with certain components of milk will also influence the approach. The composition of breast milk is, of course, also of relevance to the development of the infant.

Human milk consists of 3,8% fat, 0,4% casein, 0,6% whey protein, 0,2% ash and 7% lactose. Water (88%) makes up the volume. The white appearance of milk is due to the emulsified fat and the calcium salt of casein. The pH of fresh milk is between 6,6 and 6,8. Pigments (carotene and xanthophyll) sometimes add a yellow colour. Nutrients such as copper, iron and vitamins C and D are present only in very low concentrations. The infant can develop anaemia due to insufficient intake of iron and copper if milk is the sole source of food. Calcium, phosphorous, potassium, sodium, magnesium and chlorine are present (Lawrence, 1980; White *et al.*, 1973).

Lactose is a disaccharide of galactose and glucose and occurs only in milk. Glucose and galactose are also present in small amounts in milk. The digestive enzyme lactase is only present in mammals and gradually disappears in the infant and at age five, almost none is present. Lactose enhances calcium

absorption in the intestinal tract. Galactose, derived from lactose, is essential to the production of galactolipids such as cerebroside which is essential for central nervous system (CNS) development.

The lipids in milk provide the necessary energy for growth. The lipid droplets are kept in suspension by the adsorbed membrane layer that is made up of phospholipid. Other classes of lipids represented in milk are triglycerides, diglycerides, monoglycerides, free fatty acids, glycolipids, sterols, and sterol esters. Lipid soluble vitamins such as A, D and K are also associated with the lipid fraction. The fatty acid pattern of human milk shows that even numbered fatty acids from C12 (lauric acid) to C18 (stearic acid), and unsaturated derivatives are the major constituents with oleic acid (18:1) and palmitic acid making up the bulk (39,5% and 22,5% respectively). Altogether 167 fatty acids have been identified in human milk. Bovine milk has 437 fatty acids. The longer chained fatty acids are apparently necessary for brain development in the infant. Grey matter contains a high proportion of un-myelinated neurons, and white matter consists mainly of myelinated nerve fibres. Characteristic fatty acids of grey matter are C20:4 and C22:6. Fatty acids that play a role in the quality of myelin are C18:2-6 (linoleic acid), and C18:3-3 (linolenic acid) which are obtained from milk. They are the precursors of arachidonic (C20:4) and docosahexaenoic (C22:6) acids (Lawrence, 1980; White *et al.*, 1973). The possible significance of these fatty acids in relation to DDT will be addressed in the sections on extraction and exposure.

Apart from casein and α and ß-lactoglobulins, other proteins are also present in milk. Serum albumin, lactoferin, immunoglobulins, enzymes and glycoproteins make up the rest. These proteins supply the essential amino acids and are derived from plasma. Other non-essential amino acids are synthesized in the secretory cells. Human milk contains three sulphur-containing amino acids, methionine, cysteine and taurine (absent from cows milk), which cannot be synthesized by the infant. Taurine may play a role as a neurotransmitter or neuromodulator in the brain and retina. Lactoferin is an iron binding protein that inhibits growth of iron dependant bacteria. It could play a role in the prevention of certain gastrointestinal infections (Lawrence, 1980).

The major immunoglobulin in human breast milk is IgA, with IgG second, the reverse of the serum profile. Both are derived from serum and bio-synthesis in the mammary gland. IgA occurs in the milk as secretory IgA (sIgA), synthesized from two IgA molecules linked by disulfide bonds from sulphur containing amino acids. It confers protection against viruses and bacteria to the infant. The immunoglobulin is stable at low pH and is resistant to proteolytic attack (Lawrence, 1980; White *et al.*, 1973).

Lipases present in milk act on fat globules to present more surface for digestion. This action supplies free fatty acids and monoglycerides for absorption. Several types of lipases are present, some stimulated and others inhibited by bile salts. The bile salt stimulated lipases are stable in the duodenum and hydrolyse triglycerides, whilst being protected by bile salts from enzymatic action. Even in a deep-freeze, lipolytic action continues in the absence of bile salts (Lawrence, 1980; White *et al.*, 1973).

A host of other compounds occur in breast milk (Lawrence, 1980; White *et al.*, 1973 have summarized). Carriers of DDT (Charman and Stella, 1986; Eriksson and Nordberg, 1986) will be addressed in the discussion. The positive aspects of breast-feeding as compared to bottle feeding will not be discussed here. Those aspects will be covered in the discussion on breast-feeding with contaminated milk and the associated risks.

During the course of a single feeding, and over the entire period of lactation, changes in the composition of milk occur, that must be taken into account for sampling. The first few millilitres that are secreted during a single feeding is called "fore milk" and the last "hind milk". It is the change in lipid content that is a major consideration for this study. The lipid content changes from 2,42% to 7,48% from the fore milk to the hind milk, respectively (Hall, 1979). Other changes in composition are from 1,00% to 1,36% and 8,29% to 7,95% for

protein and lactose, respectively[7]. Diurnal changes also occur. Lipid composition changes from less than 4% to 7% from 06:00 hours to 18:00 hours (combined fore and hind milk of a feed). A plateau is reached at around midday, with the greatest change occurring at around 10:00 hours. Protein composition does not show any changes (Lawrence, 1980).

1.4.3 BREAST-FEEDING AS PRACTISED BY BLACKS

Breast-feeding is practised as a norm in most Third World situations. This is partly due to the convenience, and represents the cheapest way of feeding infants. Breast-feeding under these circumstances also constitutes the major form of defence against infant diarrhoea. Under conditions where clean water is not available, prevalence of this disease will increase if lactation is terminated earlier. In Chile it has been shown that a doubling in the infant mortality was associated with a decrease in the prevalence of breast-feeding (Gerrard, 1974).

Breast-feeding is also used as a form of contraception (lactational amenorrhoea), as continued breast-feeding prevents ovulation (World Health Organization, 1983). The average length of pregnancy interval differs for lactating and non-lactating mothers. For Nigeria, Bangladesh and Senegal (under conditions of food shortage) the intervals were 26,5, 25,0 and 24,0 months for lactating mothers. For non-lactating mothers from the same countries, the intervals were 8,0, 10,0 and 11,0 months respectively (Jelliffe and Jelliffe, 1985). This represents a better "couple-years protection" in Third World situations than technical methods. Therefore, changes in breast-feeding practices, especially reduction in length and frequency, will alter this protection if alternative prophylaxis is not concurrently introduced (Olukoya, 1986). A recent study from South Africa (Wagstaff, Yach and Kkhasibe, 1989) reported that breast-feeding in Soweto was continued for more than twelve months by 60 percent of the mothers.

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[7]Various methods of extraction of these compounds are in use, which explains the variation of reported values

1.5 DDT IN THE ENVIRONMENT

DDT, as an ubiquitous contaminant, can be described in the context of the compartments that make up an environment. No attempt will be made to give an exhaustive review of all data, and many of the processes will only be referred to if deemed relevant to this study. Some of the compartments will receive less attention than others. This must be seen as establishing priority and pertinent concepts for motivation and consideration relevant to the aims of this study. The compartments that will be considered are: air, soil, water and the biota, each with a different contribution towards exposure to humans.

1.5.1 DDT IN THE AIR

Air is usually the medium of application of DDT in agriculture. The same is also true for application on the walls of the dwellings in KwaZulu. Some of this application will not reach the wall and will circulate in the enclosed space until adsorption on a surface (such as the floor or respiratory tract) or dust particles. Levels in air have been measured during indoor spraying operations at a maximum of 7,1 mg m^{-3} in the breathing space of the applicator (Wolfe *et al.*, 1959). Because DDT has a finite vapour pressure, DDT applied on the walls and other surfaces will go into the gaseous phase and will be under influence of factors such as temperature and humidity. Because DDT is not applied outside the dwellings, little DDT will find its way outside. The contribution of this to the air compartment outside the hut can thus be ignored. The relatively short half-life of 1,76 days that was calculated for DDT, 4,63 hours for DDE and 1,71 days for DDD in the atmosphere by photochemical reaction, contributes toward reducing the levels in air (National Library of Medicine, 1988a,b,c). The DDT in vapour phase and that associated with suspended particles inside the dwellings are not exposed to photochemical action. Measuring DDT inside the dwellings for this study was attempted, but was not successful.

Agricultural application of DDT has resulted in elevated levels in the air six kilometres away (Bevenue, 1976). Levels ranged from 2200 ng m^{-3} (Orlando, USA) to 282 ng m^{-3} (Alabama, USA). Urban areas had lower levels, ranging from 25 ng m^{-3} (Maryland, USA) to 8 ng m^{-3} (Iowa City, USA) (Bevenue, 1976). Measuring levels of pesticides in air is difficult because of the presence of both vapour and particulate matter (Edwards, 1970).

1.5.2 DDT IN THE SOIL

Soil is a complex and variable composite of minerals, organic material, water and air. Most pesticides are either directly applied to, or reach the soil in large amounts. This is true for agriculture, as well as for indoor application, as most Third World type dwellings are constructed from mud and clay. The various compartments that make up the soil contribute towards parameters such as half life of the pesticide, biological availability and movement. Some of the determining factors of the half life of pesticides in soil are pH, water content, particle size, humic acid content, temperature, microbial activity and exchange capacity. The contribution of these factors towards pesticide parameters varies between soils, climate and season.

DDT levels were measured before and after aerial application to plantations in Pennsylvania (Cole, Barry and Frear, 1967). Background levels of DDE and DDT were determined before application. A year later (to allow leaf litter to reach the forest floor) some DDT-levels increased more than 50-fold. The levels remained relatively constant, in contrast to sediments and water in which a relatively rapid decline was noticed. The soil retained much of the DDT with little or no run-off. In New Brunswick (Canada) DDT residues were present in only the top layer of soils nine years after aerial application, with little evidence of transport or breakdown (Yule, 1970). This was ascribed to the adsorption of DDT onto organic material under low pH conditions. The type of forest litter apparently also influenced adsorption, as balsa, fir and spruce (softwoods) bound more than beech, birch and maple (hardwoods). This was established by measuring the bio-availability using contact-toxicity tests with fruit flies. Surface

dwelling insects and spiders contained less DDT than the litter layer. Thus the ecological significance of contamination can differ from that suggested by direct analyses of the pesticide content of the inorganic component at the exchange boundary only.

A sequential series of soil samples from an apple orchard in England suggested a half life of 11,7 years for DDT and 3,3 years for DDD (Cooke and Stringer, 1982). The DDE content increased as a result of the breakdown of DDT, and the estimated time for doubling the DDE concentration was 9,1 years. The ΣDDT-levels did not decline appreciably over an 11 year period, with an estimated half life of 57,54 years. Some factors mentioned by Cooke and Stringer (1982) as possibly contributing towards these long half lives included decreased microbial activity due to herbicide application, negligible volatilization, no leaching and strong adsorption.

In a very different environment in Arizona, half lives were estimated in soil treated 11 years earlier (Buck, Estesen and Ware, 1983). At first, the half life for cultivated fields was estimated at 7 years and 2,5 years in desert soil. The increase in percentage DDE (such as found by Cooke and Stringer, 1982) changed this to 12 and 7 years, respectively. A possible confounding influence was the deposition of contaminated dust from nearby treated fields on the desert surface.

The adsorption of DDT to tropical soils and the concomitant loss of insecticidal activity has been studied in some detail. Higher humidity increased the insecticidal activity of DDT sprayed on mud blocks (Barlow and Hadaway, 1956). However, prolonged high humidity increased the migration of dieldrin deeper into Ugandan mud blocks, thereby decreasing bio-availability. At a relative humidity of 90%, 96% of the dieldrin was recovered after 1 hour and only 6% after 13 days. The respective values at 10% relative humidity were 92% and 52%. This has been explained (partially) by competition with water molecules replacing pesticides from active sites in the soil and thereby increasing bio-availability (Hadaway and Barlow, 1951).

1.5.3 DDT IN WATER

Surface water, from an environmental point of view, can be considered as the most important compartment. The amounts of contaminants present may be relatively small, but their effects are enhanced if they are highly toxic, persistent, mobile and readily accumulated in aquatic organisms and those that feed on the latter.

The unidirectional flow of most water bodies transports, disperses and mostly dilutes pollutants from their origins. Degradation (and adsorption on sediment) takes place, thereby reducing the concentration in the water (Hellawell, 1988). The reduction in DDT concentrations in water has been measured and some of those reports have been taken up in Toxnet (National Library of Medicine, 1988a,b,c). DDT was recovered unchanged after eight weeks in river water at 27 °C and a pH of between three and five under sunlight and fluorescent light (National Library of Medicine, 1988c). Natural substances present in water may contribute to indirect photolysis, for half lives of between a couple of hours to a few days (National Library of Medicine, 1988c). No change in DDT was observed after seven days in distilled water exposed to sunlight (National Library of Medicine, 1988c). The same variation in results were reported for DDE and DDD (National Library of Medicine, 1988a,b). The local conditions seem to be the major determinants of persistence in aqueous media.

1.5.4 DDT IN THE BIOTA

The process underlying the phenomenon of bio-amplification (higher concentrations of persistent compounds in higher trophic levels) observed in many systems (Niethammer *et al.*, 1984; Baumann and Whittle, 1988), has not been fully resolved. It was generally accepted that a transfer from lower orders to higher ones was the principal factor for the observed phenomenon in water (Edwards, 1970). Recently however, direct uptake from water via gills has also received considerable attention (Hamelink and Spacie, 1977; Harding, Vass and Drinkwater, 1981; Gobas and Mackay, 1987). It does seem, however, that both

mechanisms are present and that the principle one will depend on the local conditions, the biological systems under consideration and the concentration in water (Bevenue, 1976; Moriarty, 1988).

Persistence of DDT in aquatic organisms indicates that the sediment and drainage area act as sinks, and concentrations can temporarily increase (depending on the season) years after application has ceased, because of run-off from agricultural land (Johnson, Norton and Yake, 1988). Agricultural use of DDT in the USA was banned in 1972, and residues in water fell below the detection limit (0,01 $\mu g\ l^{-1}$) in the same year. Levels in whole fish started to decline only after 1974 and still persisted at levels half of that of the maximum 1974 level in 1978 (Johnson *et al.*, 1988). Direct and indirect effects of DDT on fish and fish populations remained largely localized due to spillage or heavy contamination.

Accumulation of contaminants, especially DDT and DDE, in piscivorous organisms, probably poses the greatest environmental danger. Animals such as fish eagles, pelicans, king fishers, otters and crocodiles feed extensively on fish. Elevated levels have been attributed to bio-accumulation (the food being the primary source of body burdens). A whole range of effects, such as reduced reproduction and induced microsomal enzymes have been found in piscivorous birds and ducks. DDT can thus be transferred within a food chain from the lower trophic levels, where no ill effect is observed, to higher levels where the bio-accumulated (or bio-amplified) DDT can elicit reaction or damage.

Cows' milk may be contaminated with DDT and contribute towards body burden. Kapoor, Chawla and Kalra (1980) showed that the presence of DDT and BHC in the milk of the water buffalo corresponded remarkably well with the pattern of DDT use. Mean levels of ΣDDT (whole milk) from three different areas ranged closely around 360 $\mu g\ kg^{-1}$. Agricultural and veterinary sources of co-contamination could, however, not be ruled out.

All roofed structures in controlled areas of India are sprayed, including cattle sheds (Kapoor *et al.*, 1980). No such structures can be found in KwaZulu and

only living quarters were treated. If DDT is found in cows' milk, it stands to reason that it will also be present in its meat and meat products. The level of contamination in tissues will depend mainly on the fat content of the tissue and the octanol-water partition coefficient of DDT and metabolites (Travis and Arms, 1988).

1.6 ADULT EXPOSURE

1.6.1 APPROACH TO EXPOSURE ASSESSMENT

The ADI (Acceptable Daily Intake) is based on a review of data from all relevant disciplines such as biochemistry and toxicology, from animal and human studies (World Health Organization, 1988). The most sensitive marker is used to establish a No-Observed-Adverse-Effect Level (NOAEL). Consideration of the type of expected effect, the severity or irreversibility of the effect and inter-species extrapolation uncertainties, is applied to the NOAEL to arrive at an ADI (World Health Organization, 1988). The ADI of a chemical is defined by the WHO (1988) as "*the daily intake which, during an entire lifetime, appears to be without appreciable risk, on the basis of all the facts known at the time. It is expressed in milligrams of the chemical per kilogram body weight*".

The ADI for humans determined by the World Health Organization (1979) is 0,005 mg kg^{-1} ΣDDT day^{-1}. A new ADI of 0,02 mg kg^{-1} ΣDDT has been established (Coulston, 1985b).

There are a number of sources and routes for exposure and uptake of xenobiotics, with various factors governing the amounts present and / or available in any given environment (sub-section 1.5). Exposure to these substances is defined as the contact with a chemical (or physical) agent (Environmental Protection Agency, 1986). The magnitude of the exposure is determined by measuring, for a defined time, the amount of the agent available at the exchange boundaries. The boundaries include lungs, skin or intestinal mucosa. The qualitative or quantitative determination or estimation of the magnitude, frequency, duration and route of exposure is called "exposure assessment". Past, present and future exposures can be considered using appropriate techniques. Measurement of accumulation will relate to past exposure. Measurement of levels present in the different compartments at the exchange boundaries will relate to present exposure. Modelling will consider

future exposure. This data is usually combined with existing data on environmental and health aspects of the agent under consideration. The amount absorbed from the amount present at the exchange boundaries constitutes uptake or the absorbed dose. The presence of an agent in tissue or fluid indicates exposure and, in certain cases, allows a quantitative assessment of exposure (Environmental Protection Agency, 1986). The relationship between body burden of DDT in humans and dose has already been determined (World Health Organization, 1979).

In accordance with the Environmental Protection Agencies' "Guidelines for Exposure Assessment" (Environmental Protection Agency, 1986), the amounts of DDT present at the exchange boundary of that part of the population assumed to be most exposed and susceptible (in this case the breast feeding infant), and the associated uptake have been measured and the results presented in this thesis. This measurement also allowed the determination of serial change in DDT-levels in breast milk, and the estimation of maternal exposure.

1.6.2 ADULT EXPOSURE AND ROUTES OF UPTAKE

The compartments mentioned in sub-section 1.5 should all be considered as routes of exposure. DDT in the air is a likely candidate as an indoor route, when considered in the context of malaria control. The doors of the dwellings are kept closed during the day, allowing saturation levels of gaseous DDT to be achieved, as little exchange of air can be expected. In other circumstances, DDT in the gas phase is considered as negligible in contributing to body burden (Geyer, Scheunert and Korte, 1986). DDT adsorbed on dust particles or disturbed from the rafters could also be inhaled.

DDT adsorbed on the walls of the dwellings is another possible route as a contact surface. DDT is not readily absorbed by the skin (Klaassen, Amdur and Doull, 1986), especially in powder form. However, there is growing evidence of effective insecticidal action of residual DDT more than a year after application

(Sharp, 1988, personal communication), indicating residual bio-availability. Another conceivable route is bedding, which is not removed during spraying. DDT in water might only be a problem during spraying when water containers are kept in or near the sprayed dwellings. Otherwise the intake from contaminated surface water would only be a factor if it is heavily contaminated, due to a large spill. Data from a related study (Bouwman, Coetzee and Schutte, 1990) show that the fish were not heavily contaminated (Table 1.6.1), indicating low water levels.

The most likely route of uptake, and also the one best supported by literature, is food. For rural blacks, this route includes food such as staple crops, vegetables, cows' milk, meat and fish. Meat is used by all the families that participated in the serum study. The intake frequency depends on availability, as no refrigeration is available. Therefore, meat is only available after slaughtering, and is usually shared or sold to neighbouring homesteads shortly after slaughter. No attempt was made to obtain further information on this aspect.

Two relevant studies from Africa, concerning contaminated food, were available. Atuma (1985) extracted DDT from a wide range of vegetables and meat products from Nigeria. The levels were reported on the basis of extractable lipids, so that the values for vegetables would be very low. For cows' liver and meat the levels were 0,08 mg kg^{-1} ΣDDT and 0,08 mg kg^{-1} ΣDDT, respectively. No other meat products contained any measurable amount of DDT. Fish was not included in this survey. Kahunyo, Froslie and Maitai (1988) analyzed chicken eggs from Kenya and found that 18 of the 105 samples contained levels higher than the "practical residue limit" of 0,5 mg kg^{-1} ΣDDT. The highest value was 9,2 mg kg^{-1} ΣDDT. They argued that the route of contamination by DDT was from contaminated chicken feed and from the environment.

The most obvious route of exposure, considering the present study would be contaminated fish. This was determined separately, but concurrently with the present study for the Pongolo Flood Plain system and the results are summarized in Table 1.6.1.

Table 1.6.1. Mean levels of DDT and metabolites in fish collected from pans in the Pongolo Flood Plain system. Concentration is given as $\mu g\ kg^{-1}$ wet weight (Bouwman *et al.*, 1990).

SPECIES	DDE	DDD	DDT	ΣDDT
Hydrocynus vittatus	47,0	19,6	12,9	79,5
Oreochromus mossambicus	9,4	12,9	6,0	28,3
Eutropius depressirostris	11,6	7,0	4,2	22,8

These levels indicate widespread contamination of all fish. Assuming a mean body weight of 60 kg and a daily fish consumption of 200 g, the intake calculated for a mean of 93,6 $\mu g\ kg^{-1}$ ΣDDT (wet weight) through fish intake (mean level for all fish from the most highly contaminated pan) is 0,312 $\mu g\ kg^{-1}$ ΣDDT day^{-1}, much less than the ADI. Further reductions of DDT due to cooking of the fish has not been taken into account. Intake of DDT via this route therefore does not constitute a health hazard (Bouwman *et al.*, 1990). Other sources may also contribute to body burdens, e.g. alcoholic beverages and wild nuts and fruit, but these should be negligible.

1.7 DDT: EFFECTS ON THE HEALTH OF ADULTS

Review articles, research articles and letters will be used to determine what has already been done, or can be inferred from animal studies, on the pathology of DDT to human adults. Review articles tend to take a more balanced look at results and present issues more clearly. The review articles adequately reflected the content of those articles that were consulted, but differed in the interpretation. Letters are more personal and are included, as it will give a better understanding of the diverse opinions maintained by informed people that do not always surface when consulting research articles.

1.7.1 EFFECTS OF DDT EXPOSURE EXCLUDING CANCER

The 90-day oral LD_{50} of DDT to rats was 46 mg^{-1} day^{-1} (World Health Organization, 1979). The acute LD_{50} for male rats of DDE was 880 mg kg^{-1} and for DDT it was 113 mg kg^{-1}. The NOEL (No Observed Effect Level) of DDT to rats was 0,05 mg kg^{-1} day^{-1} (Klaassen *et al.*, 1986). For dogs and monkeys it was 8 and 2,2-5,54 mg kg^{-1} day^{-1}, respectively. The acute dose for human adults is not known, but is higher than 285 mg kg^{-1} (World Health Organization, 1979).

Attempts have been made to detect any adverse effects of DDT in female dogs. Deichmann *et al.* (1971) conducted a study on the adverse effects of DDT in beagle dogs, dosed at 12 mg kg^{-1} DDT for 5 days a week. He found significant adverse effects in the female dogs of the exposed group, as measured by delayed oestrus, increased number of stillbirths, lack of mammary development, reduced milk production and high pup mortality. This study was repeated by Ottoboni, Bissell and Hexter (1977) with 198 mature dogs, which produced 650 pups. The dosage rates (DDT) were 1, 5 and 10 mg kg^{-1} day^{-1}. They did not find any statistically significant differences, other than oestrus cycles that were two to three months earlier in the exposed group.

In the study by Hayes, Dale and Pirkle (1971), 24 incarcerated male volunteers ingested up to 35 mg DDT day^{-1} for 21,5 months. Some were observed for a further five years. The fat of those receiving dosages of 3,5 and 35 mg man^{-1} day^{-1} contained 39,8 and 105,6 mg kg^{-1} ΣDDT, respectively. No definite clinical or sub-clinical evidence of adverse effects was determined. They concluded that DDT had a high degree of safety. In the WHO document "DDT and its derivatives" (1979), several studies, relating the daily dosages of pesticide workers with health effects, were used to stress the absence of any adverse effect. The hemopoietic, immune, nervous, renal, cardiovascular, reproductive and gastrointestinal systems and endocrine organs, after review of the literature, were not damaged by apparently any dose less than acute. Some evidence as to the mutagenicity of serum levels of DDT higher than 200 μg l^{-1} was presented, but no further comments about this aspect were made.

1.7.2 CANCER

The detection of possible carcinogenic action of DDT in humans was the focus of many articles. Deichmann (1972) compared the levels of DDT in body fat of a normal population to the levels in people who died from liver and nervous system diseases. Patients who died from portal cirrhosis (n=33), toxic hepatitis (n=2), primary liver cancer (n=4) and metastatic liver cancer (n=30) had 2 to 2,5 times more ΣDDT than the normal body fat concentration of 9,7 mg kg^{-1} ΣDDT. Of the neurological diseases, 24 cases of encephalomalacia had a mean level of 18,3 mg kg^{-1} ΣDDT. Analysis of these results did not present evidence that these elevated levels were either caused by the disease, or resulted in the disease. A valid point, about the studies by Hayes and other people on pesticide workers, was that only males were considered (Deichmann, 1972). Deichmann (1972) also warned about sub-clinical effects that are difficult to detect, such as hormonal changes, oestrus, milk production, hepatic microsomal activities and cancer (both causative as well as preventative, according to Laws, 1971). Laws *et al.* (1973) conducted a study on 31 men occupationally exposed, with an equivalent oral intake of 3,6 to 18 mg DDT daily for an mean of 21 years. Based on extensive medical examinations, liver function tests and serum level

measurements (mean ΣDDT-level of 1373 $\mu g\ l^{-1}$), no liver function abnormalities or evidence of hepatic diseases could be detected.

Deichmann and MacDonald (1977) reviewed and compared liver cancer data before and after the introduction of organochlorines in the USA. They found a significant decrease in the total (primary and secondary) liver cancer death rate from 8,8 per 100000 in 1930 to 5,6 per 100000 in 1972. This failed to support the assumption that DDT is carcinogenic in the general population. Unger, Olsen and Clausen (1982) conducted a case control study on people with and without cancer, and found a significant association between levels of DDT and cancer. Confounding influences such as weight, occupation and residence were taken into account. Wong *et al.* (1984) conducted a historical prospective mortality study on 3579 white male workers, some of them occupationally exposed to DDT. "*No significant overall or cause-specific mortality excess was detected among employees potentially exposed to either TRIS or DDT*". Austin, Keil and Cole (1989) published a retrospective follow-up study of cancer mortality, in relation to serum DDT levels, in 919 subjects. They found no relationship between either overall mortality or cancer mortality and increasing DDT-levels. However, they did find a weak association between respiratory cancer and levels of DDT in serum. These findings, they concluded, did not support the assertion that DDT is a carcinogen in humans.

Some recent studies on cancer rates in animals found proof of the carcinogenic potential of DDT. Cabral *et al.* (1982b) observed significant increases of liver-cell tumours in female rats fed for life with food containing DDT at levels of 125, 250 and 500 mg kg^{-1} DDT. No metastases were found. This was not observed in male rats. The lack of carcinogenicity of DDT (at 500 and 1000 mg kg^{-1}) to hamsters was described in two further reports from the same group (Cabral *et al.*, 1982a and Rossi *et al.*, 1983). They did, however, observe that DDE produced liver-cell tumours in hamsters at dosage rates ranging between 40 and 80 mg kg^{-1} day^{-1}. Apparently no metastases were observed. Hayes (1982) concluded that carcinogenic potential had been shown at high doses in laboratory animals, and that this evidence should not be discarded, but "*man*

does not appear to be susceptible to the tumorigenic action of the chlorinated hydrocarbon insecticides" (Hayes, 1982).

On "The dilemma of DDT", Coulston (1985a) said "*But the real lesson to be learned from DDT as a scientist is that we have been led down a thorny trail by a few wilful scientists who are willing to call any chemical that changed even a single cell a potential carcinogen.*" He also said that it is no use to study DDT in the dog as it does not metabolise DDT as humans do. In the review article by Coulston (1985b) published in the same issue of Regulatory Toxicology and Pharmacology, he advocated the use of monkeys as experimental animals, since they are closely related and have enzyme and hormonal systems much like humans. Monkeys, however, do not metabolize DDT to DDE (World Health Organization, 1979).

Jukes (1974) found that pesticide handlers had 50 times the residues of DDT in body fat compared to the general population. No interference with the health of these workers, despite the long term contact with this agent, was shown. Jukes (1974) also stated that the absence of cancer in 39 pesticide workers, who had between 9 and 19 years occupational contact with a daily dose 400 times that of the general population, "*makes it unlikely that DDT is a carcinogenic risk to the general population*".

In a reply to the article by Jukes (1974), Wurster (1975) asserted that *"DDT is a carcinogen."..."It may be the most widespread of all man-made carcinogens"*. As cancer in man can develop years after exposure, human cancer rates cannot be used to evaluate carcinogenic activity. Even if DDT caused an additional 100000 cancers in the USA, it could be missed. The example of the cancer risk attributed to smoking, which took decades to establish, was mentioned (Wurster, 1975). The reference by Jukes (1974), to the 39 pesticide workers, was considered as having little chance of detecting cancer, as the study was too small, too short and without any clinical observations. Regarding malaria control with DDT, Wurster (1975) stated *"its benefits to society, if any, were greatly exceeded by its costs"*.

In the rebuttal by Jukes (1975) in the same issue of Nature, numerous further examples of excessive exposure to DDT without any harmful effect were given. The observation that 130000 malaria control sprayers did not show any observed symptoms was noted. He concluded with a quote from World Health Organization (1971a) *"there is at present no sound reason to believe that the millions of people protected against vector-borne diseases are at tangible risk from their small exposure to DDT....the withdrawal of DDT would indeed be a major tragedy in the chapter of human health".*

The above exposition of the literature, regarding the inferred or known health effects of DDT to adults, gives an idea of the considerable controversy that exists. After 50 years of research on DDT, people, both informed and naive, can still get quite emotive about the issues that mainly revolve around extrapolation between species, and its implications.

1.8 INFANT EXPOSURE

A special case of exposure to and uptake of DDT, is the infant deriving nutrition mainly from breast milk. The breast-feeding infant could be considered as belonging to the highest trophic state, as it derives most of its nutrients maternally. Bio-accumulated DDT in the maternal system will largely determine the exposure of the infant. DDT was first reported in breast milk by Laug, Kunze and Prickett, (1951). They analyzed 32 milk samples from Negro mothers resident in Washington DC and found (using the colorimetric Shechter method) a mean concentration of 130 $\mu g\ l^{-1}$ (whole-milk), with a maximum of 770 $\mu g\ l^{-1}$. Further evidence was obtained from a more elaborate study by Quinby, Armstrong and Durham (1965), and at least 200 more studies since then. The various factors, such as pharmacokinetics and maternal factors, that determine the levels of DDT and its metabolites in breast milk will be discussed below.

1.8.1 PHARMACOKINETICS OF DRUG EXCRETION VIA BREAST MILK

Blood flow to the mammary tissue (30-40 litre blood $hour^{-1}$) exceeds milk excretion 400-500 times. This makes a very high milk concentration, relative to blood concentration, possible for lipid soluble chemicals such as DDT, which has a high milk to plasma ratio as explained in the scheme below (Fig. 1.8.1), adapted from Wolff (1983).

Milk-plasma ratios for various chemicals can be derived from the literature. Caffeine has a milk-plasma ratio of 0,5, lead a ratio of $\leq$ 1 and DDT 6 to 7. Therefore the clearance time for DDT is much faster than for lower ratio chemicals. This fact allows the DDT concentration of the fat portion of the milk to approximate the concentration of the body fat, as predicted from normal diffusion. In terms of the above scheme, the milk-plasma ratio is the same as the adipose blood ratio for DDT and DDT-like compounds. This means that, given a constant fatty acid composition and an equilibrium concentration of the fat compartments of the maternal body and milk, the concentration in the milk fat will be

independent of the volume of milk excreted. This assumption seems to be valid in light of the stability and lipid solubility characteristics of DDT. Most articles report both whole milk and milk fat based concentrations, but the ratio of concentrations in milk fat and whole-milk of larger samples remains relatively constant (subject to percentage fat determined, thereby making direct comparisons difficult) at between 25 and 40.

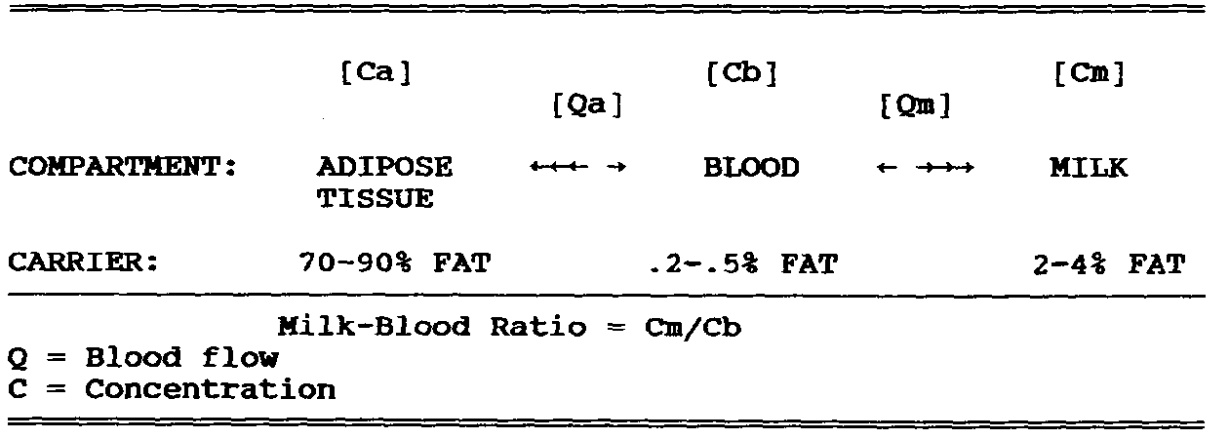

Fig. 1.8.1. Schematic representation of the partition processes between different compartments in the body: Arrows indicate transfer and the number of arrows the magnitude for a lipid soluble chemical (Adapted from Wolff, 1983).

1.8.2 LEVELS OF DDT AND METABOLITES IN HUMAN BREAST MILK

A selection of the analyses of DDT in human breast milk, reported by other workers, will be listed. The main criteria for inclusion were its possible relevancy to the present study, i.e. studies done in less developed countries or those studies reporting high values. Values based on whole-milk were divided by 30 to arrive at an approximate level based on milk fat (Spindler, 1983). The results will be presented chronologically, in table form (Table 1.8.1). This sub-section is not meant to be an exhaustive review. Jensen (1983) included more than 200 reports worldwide, and more have appeared since then.

Table 1.8.1. Concentration of DDE, DDD, DDT and ΣDDT in breast milk from different parts of the world. Concentration is mg kg^{-1} milk fat.

AREA	N	DDE	DDD	DDT	ΣDDT	REFERENCE (shortened)
Munster	43	2,7	-	1,1	3,8	Acker & Schulte, 1970
Ghana	18				0,97	Gejvall *et al.*, 1972 *
New Guinea						
Goroka	7	0,96	0,15	2,28	3,39	Hornabrook *et al.*, 1972
Okapa	10	0,22	-	0,26	0,48	"
Kar Kar	16	0,06	-	0,06	0,12	"
Saidor	6	0,07	-	0,05	0,12	"
DDR	96	7,0	-	3,0	10,7	Knoll & Jayaraman, 1973
Canada						
N Brunswick	6	1,51	-	0,77	2,28	Musial *et al.*, 1974
Nova Scotia	9	1,80	-	0,61	2,41	

* Original could not be traced by a literature search. Referred to by Polishuk *et al.* (1977) as an article in preliminary draft.

Table 1.8.1. (Continued) Concentration of DDE, DDD, DDT and ΣDDT in breast milk from different parts of the world. Concentration is mg kg^{-1} milk fat.

AREA	N	DDE	DDD	DDT	ΣDDT	REFERENCE (shortened)
France						
Lyon	?				3,92	Luquet *et al.*, 1975
Nantes	?				4,58	"
St-Etienne	?				3,40	"
Austria						
Vienna	22	3,38	-	1,06	4,44	Pesendorfer, 1975
Mistelbach	9	3,92	-	1,76	5,68	"
Guatemala						
Livingston	30				28,8	Winter *et al.*, 1976
La Bomba	31				19,6	"
Asuncion	31				16,3	"
Guatemala	78				7,8	"
Nebaj	28				1,16	"
Oslo	50	2,17	-	0,60	2,72	Bakken & Seip, 1976
Nashville						
Blacks	38				14,9	Woodard *et al.*, 1976
Whites	14				2,5	"
Iran	131	0,37	0,17	0,9	0,80	Hashemy-T *et al.*, 1977
Israel	29	1,81	0,48	0,97	5,77	Polishuk *et al.*, 1977

Table 1.8.1. (Continued) Concentration of DDE, DDD, DDT and ΣDDT in breast milk from different parts of the world. Concentration is mg kg^{-1} milk fat.

AREA	N	DDE	DDD	DDT	ΣDDT	REFERENCE (shortened)
Guatemala						
La Bomba	10				37	Olszyna-Marzys, 1978
El Rosario	27				61	"
Escuintla	10				118	"
Guatemala	15				16	"
El salvador	40				23	"
Canada						
East	10	0,97	-	0,17	1,14	Mes & Davies, 1979
Quebec	25	1,13	-	0,23	1,36	"
West	20	1,97	-	0,27	2,24	"
India	25	2,39	0,43	1,47	4,24	Siddiqui *et al.*, 1981
India	75	8,33	-	8,66	17,0	Kalra & Chawla, 1981
Hawaii	38	2,0	-	0,17	2,17	Takahashi *et al.*, 1981
Madrid	20	5,67	0,1	2,77	8,54	Baluja *et al.*, 1982
Helsinki	50	0,85	0,009	0,036	0,89	Wickström *et al.*, 1983
Stockholm						
1978	745	1,27	-	0,27	1,54	Norén, 1983a
1979	805	1,13	-	0,21	1,34	"
1980	973	1,05	-	0,17	1,22	"

Table 1.8.1. (Continued) Concentration of DDE, DDD, DDT and ΣDDT in breast milk from different parts of the world. Concentration is mg kg^{-1} milk fat.

AREA	N	DDE	DDD	DDT	ΣDDT	REFERENCE (shortened)
Australia						
Urban	45	1	-	0,4	1,53	Stacey *et al.*, 1985
Rural	140	0,9	-	0,47	1,37	"
Baghdad	50	2,27	0,76	0,73	3,76	Al-Omar *et al.*, 1985
N Carolina	733	2,43	-	-		Rogan *et al.*, 1986a
Nancy	39	0,11	-	-		Klein *et al.*, 1986
Nigeria	35	1,1	-	0,41		Atuma & Vaz, 1986
Kenya						
Rusinga	25	7,61	-	9,60	18,73	Kanja *et al.*, 1986
Embu	48	5,23	-	3,63	9,76	"
Karatina	50	1,72	-	1,59	3,51	"
Loitokitok	13	0,43	-	0,47	1,69	"
Canada						
Inuit	18	0,76	0,02	0,06	0,83	Davies & Mes, 1987
Whites	?	0,91	0,02	0,08	1,01	"
Turkey						
Ankara	61	2,71	-	0,42	3,66	Karakaya *et al.*, 1987
Adana	52	8,55	-	1,17	10,57	"
India	50	4,78	0,07	1,10	6,7	Jani *et al.*, 1988

1.8.3 EFFECTS OF PARITY ON LEVELS OF DDT IN BREAST MILK

Bradt and Herrenkohl (1976) analyzed 55 breast milk samples, and found that higher levels of ΣDDT were statistically related to the fewer number of children nursed. Multiple correlation analysis of the variables revealed that 54% of the variance was accounted for by the influence of parity (21%), number of cigarettes smoked (15%), the use of non-persistent pesticides (9%) and diet in calories (9%).

Norén (1983a) followed the levels of DDT longitudinally in the breast milk of three mothers. One was followed over three deliveries. The ΣDDT-level in milk at delivery of the third child was 2,5 times less than for the first child. The other two mothers were not sampled on the same schedule, but a definite decrease was apparent. In another study, Norén (1983b) mentioned parity as decreasing the levels, especially during copious milk production.

Wickström, Pyysalo and Siimes (1983) presented a mathematical model which related maternal age, parity and percentage milk fat with the ΣDDT-levels in milk of 50 milk samples from a milk bank in Helsinki, but the dependence on parity was not significant. This model will be discussed later. Unfortunately, they did not present specific levels associated with parity.

Stacey, Perriman and Whitney (1985) showed that the percentage of donors with milk levels of dieldrin above 9 $\mu g\ l^{-1}$ (whole-milk) decreased from 35,4% for primiparous mothers to 19,1% for mothers breast-feeding their second child. Confounding factors such as age of residential suburb, maternal age and white ant (presumably termite) treatments were also significant.

Rogan *et al.* (1986a) reported that levels of DDE were 17% lower in mothers with more than one child, compared to primiparous mothers ($n > 700$). Other factors looked at were smoking, race, maternal age and alcohol consumption. They were also able to do follow ups on sequential parity of 45 mothers, and reported a 23% drop in the mean DDE-level following the next birth.

Kroger (1972) on the other hand, reported that mothers with three or more babies had ΣDDT-levels well below the mean of the whole group (2,40 mg l^{-1} ΣDDT). The four highest values were for milk from primiparous mothers. Knoll and Jayaraman (1973) found that the mean level in milk of primiparous mothers

was 0,05 mg l^{-1} ΣDDT (milk fat) higher than the mean for multiparous mothers (0,38 mg l^{-1} ΣDDT in milk fat). They suggested that a larger exposed group, with multiple sampling of each mother, should be employed to test this observation. Mussalo-Rauhamaa, Pyysalo and Antervo (1988) found a significant increase in levels (*correlation coefficient* = 0,22 and $p < 0,01$) with parity. They ascribed this to the age of the mothers, which was better correlated with concentrations of DDT than parity.

Weisenberg *et al.* (1985) reported no significant differences in levels of ΣDDT in breast milk between mothers with their first or second child. They analyzed 100 samples and the mean ΣDDT was about 2,73 mg l^{-1} (milk fat). Takahashi *et al.* (1981) reported on organochlorine levels of milk samples from Hawaii. They did not find a linear relationship with parity, and no further analysis or explanation was given. It must be noted that most of the literature consulted did not report on this aspect. Vukavic *et al.* (1986) for instance, did not present analysis based on parity, but claimed to have included this information on the questionnaire which was completed for each participant. The same was true for Dommarco *et al.* (1987), who analyzed 65 samples from Rome.

Parity can affect the levels of DDT and its metabolites in the breast milk of the mother and therefore also the exposure to the infant.

1.8.4 EFFECTS OF INFANT AGE ON LEVELS OF DDT IN BREAST MILK

In as early as 1969, Curley and Kimbrough reported perhaps the first longitudinal study done on five lactating women. The first sample was taken 3-6 days after delivery and had a mean ΣDDT-level of 63,2 μg l^{-1} (whole-milk). Samples taken 37-60 days after delivery had a mean level of 69,9 μg l^{-1} ΣDDT (whole-milk) and those taken 90-96 days after delivery had a mean level of 78,4 μg l^{-1} ΣDDT (whole-milk).

Knoll and Jayaraman (1973) determined the longitudinal changes in levels of DDT in breast milk of a single woman over 140 days. The ΣDDT-level fifteen days after delivery (9 mg l^{-1}; milk fat) was more than twice as high as the level just after delivery (4 mg l^{-1}; milk fat). For the rest of the lactation period, the concentration did not vary much from the 6 mg l^{-1} ΣDDT (milk fat) level.

Hagyard *et al.* (1973) sampled breast milk of six lactating mothers for 120 days. Excretion increased from 93 $\mu g\ l^{-1}$ ΣDDT (whole-milk) after delivery to 575 $\mu g\ l^{-1}$ ΣDDT (whole milk) between 46 and 60 days later. The increase, strangely enough, was not significant ($p > 0,05$).

Woodard, Fergusen and Wilson (1976) tried to relate infant age with ΣDDT-levels, but failed because questionnaires were not properly completed. Bakken and Seip (1976) determined DDT-levels in the breast milk of three women for some days, but no explanation, other than variable pesticide intake via the diet, could be given for contradictory results and further work was suggested. Eckenhausen *et al.* (1981) sampled breast milk of 14 women over 11 days. The mean ΣDDT-values for every second day were 24,2 ; 29,0 ; 25,7 ; 40,8 and 34,2 $\mu g\ l^{-1}$ (whole-milk). They stated "*Trends of concentrations in the milk samples with time of sampling post-partum were not observed*". Klein *et al.* (1986) followed a similar procedure with 39 women from Nancy (France). DDE-levels were 112 $\mu g\ l^{-1}$ (milk fat) two days after birth and declined rapidly to 38 $\mu g\ l^{-1}$ on the third day, after which it decreased very slowly to 16 $\mu g\ l^{-1}$ on day 10.

Perhaps the best study relating to this aspect was that of Rogan *et al.* (1986a), who included 880 women in their study. Levels (median) of DDE at birth were 2,43 mg l^{-1} (milk fat), 2,03 mg l^{-1} at three months, 1,85 mg l^{-1} at six months and 1,39 mg l^{-1} after nine months.

Infant age, as a time factor, must therefore be included in the analysis of results, to determine how this affects exposure.

1.8.5 EFFECTS OF MATERNAL AGE ON LEVELS OF DDT IN BREAST MILK

It was difficult to find unanimity in the literature about the influence of maternal age on levels of DDT and its metabolites in milk. Jensen (1983) stated in his review that no clear consensus has been reached on this aspect, probably due to the confounding influence of parity.

Knoll and Jayaraman (1973) found indications of increased levels of ΣDDT with an increase in maternal age. The mean level at 17 years of age was around 6,5 mg l^{-1} ΣDDT (milk fat). At 33 years of age it was 15,5 mg l^{-1} ΣDDT (milk fat). No

further statistical analysis was given, but it would seem, from the graph presented, that there was a trend.

A significant correlation between ΣDDT concentration in milk and maternal age of lacto-vegetarians nursing their first infant was the only significant age / concentration relationship found by Norén (1983b). This increase was attributed to a longer contact of the older mothers with a more polluted environment than was the case with younger mothers.

Wickström *et al.* (1983) presented a statistical model in which maternal age featured. The maternal age factor was included as positive. This indicated that an increase in maternal age was associated with an increase in ΣDDT-levels in milk of 50 milk bank samples from Helsinki. Stacey *et al.* (1985) also modeled maternal age and found a positive covariate regression coefficient.

Hashemy-Tonkabony and Fateminassab (1977) analyzed 131 samples from Iran. They selected three age intervals. Lactating mothers between 14-20 years of age had a mean ΣDDT-level of 35 $\mu g\ l^{-1}$ (whole-milk); mothers between ages 21 and 30 had a mean ΣDDT-level of 38 $\mu g\ l^{-1}$, while the age interval 31-42 had a mean level of 57 $\mu g\ l^{-1}$ ΣDDT. No further statistical analysis was given, but it was stated that a large variation in levels was found.

Rogan *et al.* (1986a) reported DDE-levels in milk fat of 1,90; 1,94; 2,41; 2,78 and 2,31 mg l^{-1} (milk fat) for maternal age intervals of 16-19, 20-24, 25-29, 30-34 and 35-41 years, respectively. Increases between the second and third, and third and fourth intervals were statistically significant. The increase was attributed to "*life-time accumulation*".

Mussalo-Rauhamaa *et al.* (1988) found a significant positive association (correlation coefficient = 0,30, $p < 0,01$) between maternal age and levels in milk. The ΣDDT-level was 0,52 mg kg^{-1} (milk fat) for the mothers aged between 19 and 24. For mothers between 35 and 39 years of age, the mean ΣDDT-level was 1,54 mg kg^{-1} (milk fat).

Polishuk *et al.* (1977) reported a mean ΣDDT-level of 73,5 $\mu g\ l^{-1}$ (whole-milk) for maternal ages between 20 and 29 and a level of 64,0 $\mu g\ l^{-1}$ ΣDDT for mothers aged between 30 and 39 years. No indication of significance was given, but they maintained that this finding contradicted that of Knoll and Jayaraman (1973).

Al-Omar, Tawfiq and Al-Ogaily (1985) found a mean ΣDDT-level (approximated from the means of four metabolites from their data) of 145 $\mu g\ l^{-1}$ ΣDDT (whole-milk) and also reported data for other pesticides. They reported "*remarkable differences*" in residue levels with maternal age, with women younger than 30 years having levels higher than older women. No specific values for any of the compounds relating to age intervals were given.

Woodard *et al.* (1976) found no correlation of ΣDDT-levels in milk of indigent rural mothers with age.

All three possible associations (increase, decrease and no change) with maternal age could therefore be expected. Obvious reasons for this might be differences in exposure or a changes in exposure and breast-feeding patterns.

1.9 DDT: EFFECTS ON THE HEALTH OF INFANTS

The adverse effects of DDT on infants is not well known and this issue is still contentious. An important dialogue that illustrates the differences of opinion concerning the possible sub-lethal toxic effects of DDT in infants, as well as the emotive issues involved, is described below.

Shapiro, Eron and Beckwith (1969) made the following comment in a letter to Nature. "*Breast feeding is becoming a serious health hazard because of the high concentrations of DDT and other pesticides in human milk*". Jukes (1970a) replied a month later on this specific quote by saying that they "*have selected a singularly infelicitous example to give substance to their fear that the world faces immediate doom*". Jukes (1970a) also made the following important comment. "*It would undoubtedly be possible to show a linear positive correlation between infant health and DDT content of breast milk in tropical countries where,....malaria is the great killer of babies*".

Jukes (1970a) also referred to an unnamed scientist who delivered the apparently devastating news of the presence of DDT in milk. The unnamed scientist in question was G. Löfroth, who published his rebuttal (Löfroth, 1970). He called the review by Jukes (1970a) "*meagre*", and claimed to have been misquoted. Although, he asserted, the presence of DDT in breast milk was first reported in 1951, the general public was not told until he, and others, pointed it out in 1968 and 1969. The review, he claimed, was biased, in that it only referred to acute effects and DDT should therefore be banned totally. To which Jukes (1970b) replied: "*This is not so. Publication in the open scientific literature should be equivalent to a public disclosure...*". He also said that, because of the lack of an acceptable replacement agent, a ban on DDT would result in "*millions of deaths*".

Although this dialogue happened 20 years ago, the thrust of the arguments and the lack of knowledge concerning sub-lethal effects has remained much the same

until today. This dialogue also illustrates an important moment in the ongoing discussion on the health effects of DDT. Biased or subjective interpretation of facts can be seen as a necessary part of scientific endeavour, from which a more or less objective consensus will be reached. The consensus regarding the pathology of DDT in humans and infants at that stage, seemed to have given DDT a clean bill of health. This is important to keep in mind, since the formulation of the WHO position *vis a vis* DDT was reached shortly afterwards (World Health Organization, 1971a and b). However, a number of articles have since appeared concerning possible harmful effects to the infant exposed to DDT, which would suggest a reappraisal of the WHO position regarding DDT. Also, more knowledge concerning the uptake, metabolism and excretion of drugs by the infant has become available. These articles will be discussed in this and the following sub-sections.

1.9.1 EFFECTS OF DDT ON LACTATION

An inverse relationship between DDE concentration in human milk and the duration of breast-feeding was discovered by Rogan and Gladden (1982). A study on 865 breast-feeding children revealed that the 5% of mothers with the highest DDE levels, had a median lactation period of nine weeks. The lowest 6% had a median lactation period of 35 weeks. Reasons for termination were given as a lack of milk or difficulty with feeding. They proposed that the DDE affected either the mother or the child. Higher levels of DDE in milk might not afford the child with a full measure of immunologic protection or nutrition. Measuring these effects in exposed infants, if present, would be difficult.

Deichmann, MacDonald and Cubit (1975) exposed seven generations of mice to low levels of dietary DDE, but no effect on the growth of the young was detected. Ottoboni *et al.* (1977) exposed beagle dogs to DDT over three generations, also with no effect on any measured parameter. On the other hand, Del Pup *et al.* (1978) noted a significant decrease in neonatal survival in stable populations of mice continuously exposed to DDT. Impairment of lactation or a toxic effect on the young could have been possible causes.

Partly in reaction to the findings of Rogan and Gladden (1982), Kornbrust *et al.* (1986) exposed female rats to 10 mg kg^{-1} DDE in the diet five weeks before mating, and continued with this exposure throughout gestation and lactation. Parameters such as neo-natal growth, milk production, milk composition and mammary-gland weight were measured. No negative effects were found.

It is possible that the shorter lactation period found in the study by Rogan and Gladden (1982), will not be paralleled in animal models[8]. The effect could also be ascribed to the more toxic (in humans) effect of the DDT component. The possibility of mothers consciously or subconsciously terminating breast-feeding if aware of the contaminated status of the primary food source of their infant, cannot be ruled out. Rogan and Gladden (1982) partially ruled out this possibility by not informing the mothers, but some were already aware of their levels from their participation in an earlier study.

1.9.2 ANALYSIS OF RISK TO THE INFANT FROM LITERATURE REPORTING ON DDT IN HUMAN MILK

Already in 1965, Egan *et al.* (1965) predicted the presence of DDT in infants as derived from breast milk. Quinby *et al.* (1965) calculated a dosage of 0,0112 mg kg^{-1} ΣDDT day^{-1} which was 2,24 times the ADI at that stage. The safety factor, they maintained, was less for infants than for adults, and they stressed the need to determine undetected toxic effects due to long-term low-level exposure.

Engst, Knoll and Nickel (1970) suggested that, because of under developed enzyme systems, the ability of the child to excrete or metabolize DDT is less than that of the adult, and DDT may thus cause more harm.

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[8]The rat is not a good comparative model, as DDT is metabolized and retained differently.

In a report in Russian, which could not be traced in South Africa, Nikitina (1974) determined a level of 0,12 mg kg^{-1} in the breast milk of vineyard workers. They were also exposed to many other pesticides. There was apparently a relationship between the high levels and increased miscarriages and low birth weight neo-nates, but it could have been caused by any or all of the contaminants. This is the only known study done on women occupationally exposed to DDT (Coulston, 1985b)

Knoll and Jayaraman (1973) found that a daily intake of 500 ml of breast milk that was contaminated with DDT was enough to exceed the ADI by five times. They gave a number of reasons why infants could be more susceptible to pesticides than adults. These included immature kidney and liver function and the protective barriers of the Central Nervous System (CNS) that are not yet effective. Based on some animal studies, they came to the conclusion that the physical development of the breast-fed infants could be slower than those who are not exposed.

Bakken and Seip (1976) excluded the possibility of acute intoxication if the ADI is exceeded by a factor of 10, but warned about other possible health effects, such as the induction of the p_{450} enzyme system and cancer. The rapid growth and development of the infant might make it more susceptible (Bakken and Seip, 1976).

Hofvander *et al.* (1981) determined that the mean intake of ΣDDT by infants decreased from a mean of 0,008 mg kg^{-1} day^{-1} at three months to 0,005 mg kg^{-1} after six months, and concluded that these levels were not harmful. The mean volume of milk ingested was 780 ml and 595 ml at three and six months, respectively.

Siddiqui *et al.* (1981) mentioned the immunosuppressive properties of DDT in certain animals as potentially making the infant more vulnerable to infections. Klein *et al.* (1986) also mentioned immunosuppression in the infant as a possible effect.

Skaare, Tuveng and Sande (1988) determined a mean intake of 0,005 mg kg^{-1} ΣDDT day^{-1} for babies of Norwegian mothers, and four to five times more for infants of immigrant mothers. They mentioned that, because of the lack of any well-founded, long-term, epidemiological studies, the possibility of any adverse or injurious long-term effects could not be excluded. They advocated regular surveillance for changes in environmental pollution.

From the above it can be concluded that DDT was not associated with any adverse effects in infants, although none of the above mentioned studies reporting on levels of DDT in breast milk, as well as numerous others consulted, conducted any clinical examinations. The difficulties associated with eco-toxicological studies (see sub-section 4.6.3), must, however, be taken into account. Most authors, who did elaborate on the possible effects, advised either continued monitoring or further studies on the possible effects of DDT. Essentially the same was expressed after review of the literature by Deichmann *et al.* (1971), Kendrick (1980), Rogan, Bagniewska and Damstra (1980), Jensen (1983), Spindler (1983), Rogan and Gladen (1985), Coulston, (1985b) and Shou-zhen (1987).

1.9.3 PREMATURITY AND ABORTION ASSOCIATED WITH DDT

There have been a number of papers dealing with the possible role of chlorinated hydrocarbons in prematurity and stillbirths. Curley, Copeland and Kimbrough (1969) analyzed DDT and a number of other pesticides in necropsied adipose tissue and other material from eight stillborn infants and two who died soon after birth. The range of concentrations determined corresponded with that of the general adult population of the USA at that time, but was very wide, precluding any correlations.

O'Leary *et al.* (1970a) reported on levels of DDE in fetal whole-blood. The mean level for term babies (white) was 4,9 $\mu g\ l^{-1}$ DDE; for pre-term (white) babies it was 22,1 $\mu g\ l^{-1}$ DDE. For negro babies the values were 6,1 and 19,0 $\mu g\ l^{-1}$ DDE, respectively. Significance was not reported. O'Leary, Davies and Feldman

(1970b) reported on the association between DDE-levels in maternal blood and abortion. Normal pregnancy was associated with maternal blood levels of 11 and 9 $\mu g\ l^{-1}$ DDE in white and negro mothers, respectively. The levels for abortion cases were 9 and 12 $\mu g\ l^{-1}$ DDE (whole-blood), respectively. No significant association was found.

No relationship was also found by Ron *et al.* (1988), at levels of 24,12 $\mu g\ l^{-1}$ ΣDDT in maternal serum, and by Leoni *et al*, (1989) at 5,76 $\mu g\ l^{-1}$ ΣDDT in maternal whole-blood.

Saxena *et al.* (1983) compared organochlorine levels in samples from mother and infant from both stillborn and normal infants in India. ΣDDT in maternal blood from control mothers was 26,2 $\mu g\ l^{-1}$ (whole-blood; 35% DDT). It was 96,8 $\mu g\ l^{-1}$ ΣDDT (65% DDT) for the group of mothers who had stillbirths . The difference was significant for ΣDDT and DDT ($p < 0,01$). Placental levels for the two groups were 38,8 and 60,8 $\mu g\ kg^{-1}$ ΣDDT, respectively, and significantly different ($p < 0,01$). The levels of ΣDDT in cord blood were 30,9 and 33,6 $\mu g\ l^{-1}$, respectively, and not significantly different. Aldrin was similarly related, so that any conclusions in this respect could not be ascribed to the effect of DDT alone. They suggested a possible mutagenic or metabolic effect that may lead to congenital abnormalities and interaction with other unknown factors.

Treinen and Kulkarni (1986) found that DDT, DDE, DDD and DDOH (1,1-bis(p-chlorophenyl)-2,2-dichloroethanol) significantly inhibited the placental Ca^{+2}-ATPase from human term placentas. They postulated that this effect could be one of many such biochemical effects that may influence outcome of the pregnancies.

1.9.4 ANALYSIS OF RISK ASSESSMENT TO THE INFANT FROM THE LITERATURE ON ANIMAL STUDIES

Despite the extensive literature on the effects of DDT on laboratory animals, only a few relate to the effect of DDT on the newborn and pre-weanling animals.

Some studies only looked at the distribution of the pesticide in various organs, but made no comment regarding any possible effects. This sub-section will not examine neurological investigations using animals. These will be covered in depth in sub-section 1.9.7.

It is perhaps best to first determine at which dose lethality can be expected in relation to age. Two studies investigated the age dependant lethal dose of DDT in rats. Lu, Jessup and Lavallée (1965) determined an LD_{50} of 4000 mg kg^{-1} DDT for one-day old rats and 438 mg kg^{-1} DDT for single doses for rats between 14 and 16 days old. The LD_{50} for repeated doses are almost the same for adults and pre-weaned rats, at 286 and 279 mg kg^{-1} DDT, respectively. Harbison (1975) used pups aged between two and five days, and adult rats. DDT in corn oil was administered intraperitoneally for seven days. The LD_{50} value for adult males was 225 mg kg^{-1} DDT and for newborns it was 2356 mg kg^{-1} DDT.

There were also studies that looked at the toxicity of DDT transferred via milk to weanlings. Hayes (1976), acting on some reports suggesting that infant rats may be more susceptible than adults to DDT, determined dose / response effects of DDT administered to the young via milk using a tube. He found that, at dosages as high as 200 mg kg^{-1} DDT to the dams, the pups were as resistant as the dams, perhaps even more so. The pups, he found, were in no danger from DDT in milk, except perhaps at levels that were also toxic to the dams.

Fahim, Bennet and Hall (1970) published some findings that, strangely, perhaps because of the dramatic results, have not often been referred to in the literature. They dosed female rats intraperitoneally at rates of 1, 5 and 25 mg kg^{-1} DDT in sesame oil, starting 24 hours after delivery for 21 days. The neo-natal mortality rate was 3,8; 13,2; 32,4 and 100% for 0; 1; 5 and 25 mg kg^{-1} DDT dosages, respectively. Growth of survivors was also depressed. World Health Organization (1979) did not list this article, neither did Harbison (1975), Hayes (1982), Spindler (1983), Coulston (1985b) and Kornbrust *et al.* (1986), indicating that the results of Fahim *et al.* (1970) should be attributed less evidential weight.

Five studies dealing with the sub-lethal effects of DDT on reproduction in laboratory animals, over at least one generation, where DDT was transferred via milk, were located. Ottoboni (1969) started weanling rats on dosages of 0, 20 and 200 mg kg^{-1} DDT in food. The experiment was terminated after weaning of the third litter of the F_1 generation. All litters were checked for daily survival, and were examined at birth and after 21 days for number, weight, sex and health. There were no adverse effects, other than a significant increase in ringtail, a disease where a part of the tail is spontaneously amputated. Fertility data also suggested that DDT might prolong the reproductive life-span of the rat. In a study to determine this, Ottoboni (1972) used an exposed group of rats maintained on 20 mg kg^{-1} DDT (a dosage of approximately 1 mg kg^{-1} DDT day^{-1}) and a control group. The DDT-treated rats had a significantly increased reproductive life-span (14,6 months) over that of the control group (8,9 months). No simple explanation for this could be found. These two studies did, however, indicate that the transfer of DDT to the young, via milk, apparently had no adverse effect.

Clement and Okey (1974) found a reduction in the fertility of rats only at a very high dosage of 500 mg kg^{-1} DDT in diets. Del Pup *et al.* (1978) fed mice a dosage of 100 mg kg^{-1} DDT in food. They found a significant decrease in neo-natal survival between 20 and 30 weeks after exposure. This also happened in subsequent generations. Lactation was also affected. No tumours or any other pathological changes were detected.

Kornbrust *et al.* (1986) dosed female rats with DDE at 10 mg kg^{-1} five days a week, starting five weeks before mating and continuing through lactation. Neo-natal growth, milk production, milk composition, mammary gland weight, histopathology and organ weights were measured. The dose was not toxic to the dams, and did not have a "*pronounced effect*" on neo-natal mortality. There were no significant differences between the exposed and control groups for any of the lactation parameters. Litter weight gains for the two groups were almost identical. The mean level of DDE in the milk was 24,4 mg kg^{-1}.

1.9.5 UPTAKE, PHARMACOKINETICS AND EXCRETION

The uptake of DDT from the GIT seems to be more efficient when given in animal fat or vegetable oil, than when given in a petroleum fraction which may act as a laxative (Hayes, 1982). DDT in milk is associated with the lipid globules (Hugunin and Bradley, 1971), and will be absorbed by pinocytic action in the small intestine of the newborn. This also allows the uptake of intact immunoglobulins that are associated with the fat (Hoffmann, 1982). The blood flow to the GIT is normally higher in the neo-nate than in the adult, and drugs are absorbed two to three times more efficiently by ten day old rats than by 30 day old rats. This is also the case for dogs and pigs (Hoffmann, 1982).

Age dependent absorption of drugs by the lung was also reviewed by Hoffmann (1982). This process is mainly determined by diffusion, and will therefore be determined by the properties of the lipid membrane such as thickness, porosity and surface area. Lipid soluble drugs, such as procaine-amide-hydrochloride and sulfisoxalone, were absorbed by newborn rats at rates similar to those for adult rats (Hoffmann, 1982).

Age dependent absorption of drugs by the skin has not been well defined (Hoffmann, 1982). DDT is poorly absorbed by the skin (Hayes, 1982), but no age related studies could be traced. Site of application and agent used could play a major role (Hoffmann, 1982). From accidental exposure of infants to chemicals it would seem that the rate of absorption through skin is reduced with an increase in age, but this aspect needs further investigation.

Morselli (1976), in an overview of clinical pharmacodynamics in neo-nates, stated that the newborn infant cannot be considered as a small adult. The neo-nate is adapting rapidly to a new environment, through a continuous sequence of physiological and anatomical changes. The immaturity of various organs, absorption, protein binding, excretion, metabolism and distribution depends on birth weight and gestational age, and these functions develop with age. He also mentioned that newborns have less adipose tissue than older children and adults. Absorption from the GIT is regulated by pH-dependant diffusion and

gastric emptying time. After parturition, the pH in the stomach drops from around seven to between one and three within 24 hours. After that, for about nine days, there is no acid secretion. Gastric acidity reaches adult values after three years (Morselli, 1976). The effect of this on the uptake of DDT from the GIT is not known.

The gastric emptying time for the neo-nate is much longer (6-8 hours) and only reaches adult values after about seven months (Morselli, 1976). Biliary function is also not yet fully developed. Plasma protein binding of drugs depends on the affinity constant and the amount of protein available (Morselli, 1976). A different albumin in the plasma of the newborn has a lower avidity for drugs. There is also a higher concentration of bilirubin and free fatty acids in plasma, other endogenous compounds that compete as substrates, and a lower blood pH. This low binding, coupled with the long half life of the lipid soluble drugs such as tri-cyclic anti-depressants, may result in high concentrations of such drugs in the heart and the brain of the newborns who are exposed to them.

The reduced plasma protein binding and the increased "apparent volume of distribution" (quotient between the amount of chemical in the body and plasma concentration; Klaassen *et al.*, 1986) could have an important influence on the toxic effect of certain drugs (Morselli, 1976). The plasma levels of the infant represent a higher level in the tissues, than for the older children and adults. Whether this is also the case for DDT is not known. If binding proteins are present at lower levels, the higher levels of free fatty acids, if they are associated or conjugated with DDT, could play a role. Conjugation was proposed as a mechanism explaining the retention of DDT in the rat (Leighty, Fentiman and Thompson, 1980). The (presumably less toxic) conjugates formed by the rat liver can be hydrolysed to release the DDT again. This detoxification reaction (conjugation) may, however, not be fully developed in the human new-born (Klinger, 1982).

The effect of lipid class on intestinal uptake of DDT by rats was studied by Charman and Stella (1986). They administered DDT in peanut oil, oleic acid and a 2:1 mixture of oleic-acid and mono-olein, which represented the lumenal

digestion products of triglycerides. Oleic-acid or the mixture was a significantly better class of lipid from which DDT was taken up. The uptake was independent of dosage and volume administered. Oleic acid was also identified as a constituent of breast milk, and comprised between 35 and 46% of the fatty acid and lipids (Lawrence, 1980).

Renal function, comparable to adults, is only reached between six and twelve months after birth (Morselli, 1976). Therefore, compounds that are not readily metabolized and that depend on renal performance for their excretion are eliminated at a slow rate. This results in a long plasma half life and increases the risk of toxic effects. Morselli (1976) concluded that the infant is a "*unique drug recipient*" and, because of the lack of present knowledge, it is difficult to determine safe dosages. He stressed the need for clinical trials and the monitoring of any risk situations, so that relevant information could be obtained.

Klinger (1982) reviewed the bio-transformation of drugs and xenobiotics during post-natal development. He stated that "*...lipid soluble foreign compounds which are not metabolized....would stay in the organism for weeks or months by continuous reabsorption in the gut and the kidneys, if they were not metabolized to more hydrophobic metabolites..*". He also mentioned that the enzymes responsible for the metabolism of xenobiotics have low substrate specificity, due to the high structural variability characterizing these foreign compounds.

Most of these detoxification activities are localized in the liver. Hepatic excretion (including bile acids and neutral compounds) develops post-natally in rats (Klinger, 1982). The uptake rate is ten times that of the excretion rate in rats. The immature hepatic uptake of the newborn rat leads to an accumulation of some drugs in the plasma, with increased lethality. PCBs have been shown to stimulate hepatic uptake of quabain in 15 day old rats, but not in adult rats. Quabain is 40 times more toxic to newborn than to adult rats. Stimulation of the microsomal enzymes reduced the toxicity, due to increased hepatic excretion of the compound (Klaassen *et al.*, 1986). Enzyme induction has also been shown

for kepone, dieldrin, DDT, lindane and dioxin in neo-natal rats and mice (Klaassen *et al.*, 1986).

Klinger (1982) reviewed many enzyme systems, the majority of them developing after birth. Hydroxylation of aromatic compounds, for instance, is present in rat fetal-liver, but the main activity develops mainly post-natally (Klinger, 1982). De-hydrochlorination activity, which transforms DDT to DDE, was unfortunately not discussed.

1.9.6 IMMUNOLOGICAL STUDIES

The immune system has been shown to be perturbed by some pesticides at low levels (Moon *et al.*, 1986; Olsen *et al.*, 1987). Other pesticides, such as malathion, were not found to be immunosuppressive (Rodgers *et al.*, 1986). There are, however, several papers dealing with the effect of DDT, which will be discussed in this sub-section.

Wassermann *et al.* (1969) found that the immune responses of rats receiving 200 mg l^{-1} in water was moderated. Anti-ovalbumin antibodies fell by 30%. The serum of treated rats had reduced globulin fractions and more albumin than normal. Adrenals of control rats subjected to trauma (such as gonadectomy or hymithhyriodectomy) increased in weight, but this increase was almost completely inhibited in DDT-treated rats. Wassermann *et al.* (1973) reported a significant trend of increased levels of IgG and IgM in serum of rabbits receiving DDT or Dieldrin, at dosage rates between 50 and 200 mg l^{-1} in water. The IgG and IgM levels decreased with PCBs. They considered this as indicative of "*moderation*" of the immune system.

Lower dosages were used by Gabliks, Askari and Yolen, (1973). They injected guinea pigs with 10 to 20 mg kg^{-1} DDT before and after immunisation with diphtheria toxin, and determined serum antibody levels. These were not altered. What was found, however, was that the anaphylactic shock was significantly less severe when the DDT-treated animals were challenged with the toxin. A

possibility of induced histaminase activity was suggested. The same group (Askari and Gabliks, 1973) subsequently found that the levels of histamine in the lungs as well as the numbers of mast cells were reduced in DDT-treated animals.

Street and Sharma (1975) endeavoured to determine a dose-response relationship, using several response tests for the rabbit. The dosages used were: 0,00; 0,18; 0,92; 2,10 and 6,54 mg kg^{-1} DDT day^{-1} presented in food (respective DDT-concentrations in food: 0; 4; 20; 45 and 150 mg kg^{-1}). They found a decreased count of plasma cells in popliteal lymph nodes, reduction of germinal centres in the spleen, increasing athropy of the thymus cortex, depressed antigen induced increase in serum gamma-globulin, higher pre-antigen gamma-globulin values and decreased sensitivity to tuberculin (skin test) at the higher dosage rates. They concluded that the effect of environmental chemicals on, especially the cell mediated immune system, is an important aspect of toxicology.

Banerjee, Ramachandran and Hussain (1986) and Banerjee (1987a and 1987b) looked at the longer term effects of DDT on the immune system of mice and rats. A depression of primary and secondary humoral immune responses (immunisation with sheep red blood cells) was related to time and dosage (concentrations in food were: 0; 20; 50 and 100 mg kg^{-1}; compare these with the daily dosage given by Street and Sharma (1975), above). The suppressive effect was more pronounced in the secondary response, and was more dependent on time than on the dose (Banerjee *et al.*, 1986). To obtain more information on the type of cells involved (i.e. T or B cells), the humoral immune response to inoculated *Escherichia coli* lipo-polysaccharide, a thymus independent antigen in mice, was tested (Banerjee, 1987a). A significant time dependent suppression in the IgM response was found, at levels of 50 and 100 mg kg^{-1} DDT in food after three to 12 weeks of exposure. This suggested a direct influence of the DDT on the B-lymphocytes. This could have happened at either the germinal centres in the spleen, by cyto-toxicity, or by interference with steroid metabolism. They suggested that the observed reduction in response would place the host at a disadvantage when dealing with infections.

The effect of DDT on various immunological parameters, using rats inoculated with tetanus toxin, was determined (Banerjee, 1987b). Treatment at 100 mg kg^{-1} DDT (in food) for 18 and 22 weeks and at 50 mg kg^{-1} DDT (in food) for 22 weeks showed a significant effect on spleen weight, but not on thymus weight. Serum antibody titre to tetanus toxin was significantly decreased in rats exposed to 100 mg kg^{-1} DDT for 18 and 242 weeks. Globulin levels (using albumin to globulin ratios) were significantly depressed in the groups exposed to 50 and 100 mg kg^{-1} for 18 and 22 weeks, respectively. Again, the increase in IgG levels (following tetanus toxin stimulation) was significantly less in the groups exposed to 50 and 100 mg kg^{-1} DDT for 18 and 22 weeks respectively. This, they suggested, indicates an important change in the immunity of the host after exposure to DDT. The rats of the same two groups exposed to 50 and 100 mg kg^{-1} DDT and immunized with the toxin, also showed a significant decrease in the ability of the antigen-sensitised cells to release the factors that prevent macrophage and leucocyte migration. This effect was also time dependent. Banerjee (1987a and 1987b) suggested that this was an indication of a threshold susceptibility to exposure, as the responses were more related to time, than to dosage.

Two articles relating to the susceptibility of organochlorine-treated animals to virus infections were traced. Friend and Trainer (1970) exposed 10 day old ducklings to 25, 50 and 100 mg kg^{-1} PCB (Arachlor 1254). Five days later these ducklings were challenged with duck hepatitis virus. Ducks exposed to 25, 50 and 100 mg kg^{-1} PCB suffered 35, 65 and 44% mortality, respectively, after 80 hours. The hepatitis exposed control group (no PCBs) had 14,3% mortality and the control group (no PCBs and no virus) none.

In the second article, Fournier *et al.* (1988) determined the interaction of two immunosuppressive factors, the inhibition of lymphocyte mitogenesis, and sub-chronic exposure to dieldrin and aminocarb. DDT was not included in the initial trial. Immunosuppression was observed for the lymphocytes exposed to dieldrin, but not for aminocarb. The *in vitro* cultures were then exposed to mouse hepatitis virus 3, but synergism was not observed for either dieldrin or aminocarb. They concluded that dieldrin induced immunosuppression of the murine cellular immunity was the primary factor, and was potentially responsible

for the impairment of the cellular response. They suggested that this system could be a potential target for pesticide exposure.

None of the above articles looked at age specific effects of xenobiotics on immune competence. Andersson *et al.* (1981) presented their own results (using cell cultures) and reviewed cellular and humoral immunity in humans from birth to two years of age. The B cell function (antibody production) was fully mature at birth, with regard to IgM formation, but not for IgG or IgA. This might explain the high susceptibility of newborns to infections. Full IgG (1 and 3) production was reached within 24 months, while IgA production took longer. T cell helper function for Ig synthesis was absent in 90% of the newborns, probably because of cellular immaturity. By six months of age, this function was fully developed.

Other immune system characteristics, such as complement synthesis, T-cell population in the spleen, neutrophil count and function approach that of the adult (Soothill, Hayward and Wood, 1983). There were, however, indications that mononuclear phagocytes are still immature at birth and that this could limit both antibody and cell mediated immunity of new born animals, but apparently not in human newborns (Soothill *et al.*, 1983).

1.9.7 NEUROLOGICAL STUDIES ON ANIMALS

Neurological involvement characterizes the action of many insecticides with acute as well as chronic exposure. The central, as well as the peripheral nervous systems may be involved. To affect the central nervous system (CNS), DDT must be able to cross the blood-brain barrier. Morrison (1971) showed that DDT is able to penetrate this barrier and that it was also present in the cerebral spinal fluid (CSF) of rats after a single oral dose in peanut oil. According to them, the level of DDT in blood after an acute dose does not reflect the level in the brain (also Hayes and Dale, 1964). A good relationship was, however, found with chronic adminstration.

The principal symptoms of acute DDT poisoning are related to the effects on the CNS (Hrdina, Singhal and Ling, 1975). Following oral administration (200-300 mg kg^{-1} DDT to rats) there was a latent period of about two hours, followed by hyper-excitability to tactile and sound stimuli. Tremors increased in intensity and convulsions could follow. Death usually followed respiratory failure during extreme exhaustion. There were few pathological changes in the CNS.

The effect of chronic exposure on organisms can be measured using microscopic, neuro-physiological or behaviourial measurements. Chronic exposure of dogs to DDT resulted in a number of swollen, hyper-chromatic Purkinje cells with pyknotic nuclei. Higher dosages resulted in more severe damage to the cerebellum. The cerebrum, brainstem and peripheral nerves were, on microscopic examination, undamaged. The tremors and ataxia observed were similar to the symptoms observed in cerebellar dysfunction (Hrdina *et al.*, 1975).

Albrecht (1987) developed an assay, based on pentylenetetrazol (PTZ), a known CNS stimulant, to determine neuro-physiological changes. This allowed the comparison of CNS stimulation induced by pesticides in mice to controls, using a PTZ response curve. DDT, BHC and Arachlor 1254 (a PCB mixture) did not affect the PTZ ED_{50} in female mice. Kindling, the eventual appearance of a convulsive response from repeated non-convulsive stimuli, was observed. Dieldrin was much more neurotoxic than the other pesticides. A daily dose of 20 mg kg^{-1} DDT to rats resulted in marked changes in EEG (increased frequency and amplitude) after four weeks, and slight ataxia after five weeks (Hrdina *et al.*, 1975). This suggested that the cerebellum might be sensitive to DDT. The damage, tremors and ataxia determined after DDT treatment suggested that the cerebellum and cerebral motor cortex could be the most important target areas of DDT in the CNS (Hrdina *et al.*, 1975).

Behaviour can also be affected (Hrdina *et al.*, 1975). Dosages of 0,1 and 1 $\mu g\ l^{-1}$ DDT in the drinking water of pregnant mice and their offspring decreased the isolated aggression of the young male. Other behaviourial responses were also

reported, but not much attention had been given to this aspect up to 1975 (Hrdina *et al.*, 1975).

The effect of DDT (and other organochlorines) on avoidance response and seizure activity of rats was studied by Tilson, Shaw and McLamb (1987). They performed a systematic comparative study to determine the dose dependent effects of DDT, administered as a single large dose to rats aged between 10 and 12 weeks. They looked at the acquisition of a two-way shuttle-box avoidance response, and the acquisition and retention of a passive avoidance response, using electric foot-shock. The dosages used in the two-way shuttle-box response experiment were 25, 50 or 100 mg kg^{-1} DDT in corn oil, given by gavage, prior to testing. 30% Mortality was experienced at the highest dose. DDT did not affect the number of shuttle-box avoidance responses during a 60-trial period, but responses were reduced in the inter trial period. Irritability and tremors were observed. In the first experiment on passive avoidance, rats were dosed three hours prior to acquisition of the passive avoidance task. No effect was observed with DDT. In the second experiment they were dosed immediately after the task was completed and tested for retention. DDT still had no effect after seven days (Tilson *et al.*, 1987). Unfortunately, the experiments were not repeated with chronically dosed rats.

The distribution of DDT in the different areas of the brain has also been studied (Tilson *et al.*, 1987). DDT was apparently more concentrated in the grey matter than in the white matter. This might be related to the differences in lipid content and composition. Lipids in the areas of the brain that consist mainly of grey matter (cerebellum and neocortex) accumulated DDT faster and attained a higher final concentration (lipid weight), than did white matter (brain stem and spinal cord, with a high myelin content). The levels were similar, if the concentrations were expressed on a wet weight basis, rather than lipid weight, six and twelve hours after administration (Tilson *et al.*, 1987).

Eriksson and Darnerud (1985) studied the distribution and retention of DDT in the mouse brain during the pre-weaning period. The DDT was dissolved in a fat emulsion and administered to the rats via a tube at ages 3, 10 and 20 days. The

mice were sacrificed after one and seven days, and auto-radiographed. The retention of DDT was very pronounced when administered on the tenth day of life. The levels in the mouse brain seven days after treatment (2% radioactivity) were almost as high as those at 24 hours. DDT was evenly distributed throughout the brain, and not specifically associated with myelin. DDT was, however, slightly concentrated in the cerebellum and corpus callosum of the cerebrum 24 hours after treatment of the 20 day old mice. More DDT (20% radioactivity) was found in the liver than in the brain. Most of the DDT was associated with subcutaneous fat, but there was also some in the kidneys, adrenals and nasal cavity.

An important observation was that DDT does not seem to be very soluble in myelin. DDT is, of course, soluble in neutral fat. The pronounced retention in the brain of the ten day old mouse was attributed to lipid associated with the synthesis of new myelin. They concluded that, based on the findings that DDT may interfere with lipids and the synthesis thereof, DDT may interfere with maturation of the brain. The (single) dosage received by the young rats was 0,5 mg kg^{-1} (body weight) DDT.

A year later, Eriksson and Nordberg (1986) presented their findings on the effect of DDT and DDT conjugated with palmitic acid (DDOH-PA) on the muscarine receptors in the brains of ten day old mice. A significant increase in the density of muscarine receptors after seven days was found for both DDT and DDOH-PA. The percentage high-affinity binding sites decreased, while the low-affinity binding sites increased. The pre-synaptic sodium dependent uptake of choline was not affected by either agent. They also found that the conjugated DDT (DDOH-PA) was as potent as DDT in altering the muscarine receptors. Such an effect had not been shown before, and is potentially of great importance, as this is one of the forms in which DDT is transported in milk. These findings, they proposed, were indicative of a higher sensitivity to persistent xenobiotics over a long period for pre-weanling mice.

1.9.8 NEUROLOGICAL STUDIES ON ADULTS

The World Health Organization (1979) reported the findings of two studies conducted on malaria control personnel (India and Brazil). In both studies there were differences between exposed and control groups (stronger knee reflexes, slight tremor and a reduced performance of a timed Romberg test, referred to as ataxia), but these disappeared after re-examination a couple of months later. Hayes (1982) listed a number of studies on experimentally exposed adults. The results of these studies are obviously not comparable, but no negative effects on CNS function was found.

Misra, Nag and Krishna Murti (1984) studied cognitive functions in 29 DDT sprayers. They were subjected to clinical evaluation, Bhatia IQ tests, Wechsler memory tests, Bender visuomotor Gestalt Test (BGT; visuomotor function) and EEG tests. The sprayers had serum DDT-levels (401 $\mu g\ l^{-1}$ ΣDDT) more than eight times that of the control group. Headache (38%), ocular symptoms (20,7%) and rawness in the throat (17,2%) was noted for the sprayers (comparative values for the controls were not given). "*Soft neurological signs*" were determined in 24% of the workers. These included tremors, loss of ankle jerks, fasciculations and hypo-reflexia (4 subjects). The IQ and memory tests did not show a difference between the two groups. The BGT test revealed significantly higher scores in the oldest workers, with the same tendency in younger workers. This suggested involvement of the dominant frontoparietal lobe, and was moderately associated with DDT-levels (correlation coefficient = 0,46). Those sprayers with higher BGT scores were subjected to EEG examination. The changes in the encephalographs were diffuse and not lateralised to any one side. This also suggested the selective involvement of the frontoparietal lobe, together with diffuse involvement. Individual susceptibility seemed to have played a major role. The authors suggested that the potential role of DDT in the etiology of mental retardation, minimal brain dysfunction and presenile dementia needed to be studied (Misra *et al.*, 1984).

1.9.9 NEUROLOGICAL EFFECTS OF DDE IN INFANTS

The only study on the neurological effects of DDT on infants that could be located (using Medline, National Library of Medicine, 1989) was that of Rogan *et al.* (1986b). Unfortunately, the presence of PCBs, together with DDE in breast milk was a confounding influence that was difficult to control for. As this article is the most relevant regarding risk assessment of DDT in milk to the breast-feeding infant, it will be extensively discussed.

During the North Carolina Breast Milk and Formula Project, a birth cohort of 930 infants was subjected to the Brazelton Neonatal Behaviourial Assessment Scale (BNBAS). This scale assesses the reflexive and motor behaviour of the infant, and also monitors the state of the infant during the examination. The BNBAS scores 27 behaviourial and 20 reflex parameters. These are categorized in several clusters and the means of these clusters are used during statistical evaluation. The reflex cluster score was the number of abnormal reflexes such as: not elicited, low, high or asymmetric. Although the BNBAS should be administered in the first three days after birth, the majority were performed in the first month. Breast milk was also taken at the same time. A mean of 2,43 mg kg^{-1} and 1,77 mg kg^{-1} (milk fat) for DDE and PCBs, respectively, was found. Multiple regression was performed on physical information, such as birth weight, race, sex, mother's education, mother's occupation, mother's alcohol consumption, parity, etc.

There was no association between the PCB or DDE-levels in the milk with birth weight, hyper-bilirubinemia or head circumference. The only clusters that were significantly affected by either PCB, DDE, or both, were the tonicity and reflex scores. The tonicity score was affected by PCBs. Of the four scores that made up the tonicity cluster, the higher levels of PCBs were associated with less muscle tone and activity.

The reflex cluster score was affected by both PCBs and DDE. Both PCBs and DDE were associated with hypo-reflexia, but not with hyper-reflexia. The effect of PCBs was only noticed at high levels, while the effect of DDE was more

gradual and the effect increased with concentration. The regulation of states cluster (average of the self-quieting activity and hand to mouth facility scales), was also associated with higher levels of DDE ($p = 0,06$). Addition of other variables (mainly maternal diseases) that were associated with PCB or DDE levels ($p < 0,01$), were also included in the model, but little change resulted. Caesarean section, forceps delivery or suction resulted in more abnormal reflexes, but this did not influence the significance of DDE or PCB.

The dose response for abnormal reflexes is presented in Table 4.6.1. The daily intake for DDE, based on 6 mg kg^{-1} (milk fat) was calculated at 0,037 mg kg^{-1} day^{-1}. The correlation coefficient between PCBs and DDE was 0,23. They concluded that PCBs was associated with hypotonicity and hypo-reflexia and DDE with hypo-reflexia.

Table 1.9.1. Association of PCBs and DDE with percentage abnormal reflexes (out of 20 tests; Rogan *et al.*, 1986b).

	N	ABNORMAL REFLEXES	LOW REFLEXES
PCB-levels[a]			
0,0-0,09	49	12,2	8,2
1,0-1,49	241	10,4	6,2
1,5-1,99	276	14,1	8,3
2,0-2,49	151	14,6	9,3
2,5-2,99	66	13,6	9,1
3,0-3,49	34	14,7	8,8
3,5-3,99	20	25,0	25,0
4,0+	29	27,6	17,2
DDE-levels[a]			
0-0,9	59	6,8	3,4
1-1,9	235	11,5	8,1
2-2,9	252	13,5	8,7
3-3,9	163	12,9	5,5
4-4,9	60	23,3	16,7
5-5,9	34	17,6	11,8
6+	64	20,3	14,1

a - mg kg^{-1} (milk fat)

1.9.10 PSYCHOLOGICAL INVOLVEMENT

An important factor complicating risk assessment of DDT to infants is the potential psychological involvement of the mother with respect to breast-feeding. Not much work has been done on this aspect, and only one article could be traced after a Medline search (National Library of Medicine, 1989). Hatcher (1982) determined the psychological responses of mothers, after learning of the presence of toxic substances (poly-brominated-biphenyls, PBBs) in their milk. The attitude and emotions of the mother towards breast-feeding is closely related to the experience of breast-feeding. A conscious (attitudinal), as well as subconscious (defensive), conflict could arise when a mother is informed about the contamination of her milk. Mothers could show denial of the problem or behaviourial adaptation. Mothers in lactation could also show more denial than those that knew about the contamination before. More than 60% of the mothers that participated could not remember the levels of PBB in their milk, even though they were informed by letter. A third of the mothers thought that PBB was not excreted at all. More than half of the mothers thought that PBB did not affect their babies. Only 8% reported that they felt their relationship with the baby was affected by the situation. Fifteen percent of the mothers switched to bottle feeding upon hearing the news.

The results were interpreted as follows. Mothers who were informed that they had "*higher levels of PBB*" were the most likely to use denial. They either forgot the level, or forgot that it is transmitted via milk to the infant. They also exhibited less knowledge about PBB. These mothers were, however, not ambivalent towards their nursing, suggesting conscious and unconscious conflict. Mastery (changing breast-feeding patterns, or locating elsewhere) was negatively correlated with denial. These mothers had more knowledge of the situation, and were more conscious of what they wanted to change.

Hatcher (1982) concluded "*A mother's psychological reaction to a chemical contamination of her breast milk could have lasting developmental effects on her child. If in addition to the initial psychological issues that the mother brings to the nursing relationship,*

there are added conflictual feelings because of fears of contaminated breast milk, the psychological burden of guilt and stress may indeed be far-reaching"

CHAPTER 2

MATERIALS AND METHODS

2.1 STUDY POPULATIONS AND AREAS

The description of populations from which a sample is drawn must be accurate enough to relate findings to aspects such as culture, habits, living conditions and diet. This will also allow comparisons with other studies if the same information is available. Some of the characteristics of the population (lactating mothers attending baby clinics) have been quantified and will be presented in sub-section 3.1 and discussed in sub-section 4.1.

2.1.1 EXPOSED POPULATION

There are four hospitals in the northern part of KwaZulu, but the location of the Mseleni hospital offered some unique opportunities (Fig 2.1.1). This area, where the participating mothers were drawn from, is situated on the shore of Lake Sibaya. It is removed from any of the major routes of migration for people travelling from Mozambique southward in search of employment. It is also a stable community and only one of the mothers that participated in this study originally came from outside a sprayed area. All the other hospitals are situated on or close to major north-south migration and transport routes.

In a pilot study, performed the year prior to the present study and close to the Mozambique border, a number of the participating serum donors were of dubious origin. Mozambicans frequently give false information to hide their nationality, fearing possible repatriation. The serum levels obtained in the pilot study covered a very wide range. Some samples had no detectable residues at all, indicating no exposure to DDT, which is consistent with the absence of malaria control in Mozambique. A study, concurrent with, but separate from, the present study, was done to determine the longitudinal change in the levels of DDT in

twelve families living near the Pongolo river (Bouwman, 1990). Although the Mseleni area is not situated near the Pongolo river (37 km away), breast milk analyzed from participants from both areas (in a pilot study) had the same mean values of DDT.

The Mseleni hospital is situated 17 km inland, on the western shore of Lake Sibaya. It is served by a dirt road linking it with Manguzi (near Kosi Bay) in the north and Mbazwane (close to Sodwana Bay) in the south. The group that participated from this area will be referred to as the exposed or Mseleni group. The homesteads are widely spaced, with subsistence farming (maize is a staple crop) being practised on poor sandy soil patches or on more fertile soil closer to the water bodies. Huts are usually square, with walls made of branches as support and plastered with mud. Sometimes only the inner walls are plastered. Some poor families had thatch dwellings only. Cement and corrugated iron are being used more and more as building materials. The homesteads consist of one to seven (normally around three) living dwellings with, usually, a communal tree close by. A kitchen hut and a store hut are usually also present. From four to 22 people (mean 7,7) resided in these homesteads. The culture is still very traditional. The ubiquitous presence of the radio provides access to otherwise unavailable information. Because the people in the flood plain are not strictly speaking Zulu, the tribal and chieftain system is not as strictly applied as in the Zulu tradition (Torres, 1980).

A major source of income is migrant labour. The men usually go to the mines on the Witwatersrand (with an income of between R200.00 to R300.00 a month), or to the Natal sugar cane fields as cutters (R100.00 a month). The Mjindi farm (cotton) close to Jozini is also a source of income to some. Some make a living by selling fish (typical income of R6.00 a day for a good catch) or collecting and selling fire wood (R20.00 a month). Because of migrant labour, the economically productive males are absent from home for most of the year. The population structure, as measured in permanent residents, is therefore mainly females and children. There is no electricity or piped water. Water is collected from pans, rivers, lakes and sometimes boreholes. Crops, at the time of this study, were not dusted or treated with DDT or any other pesticides.

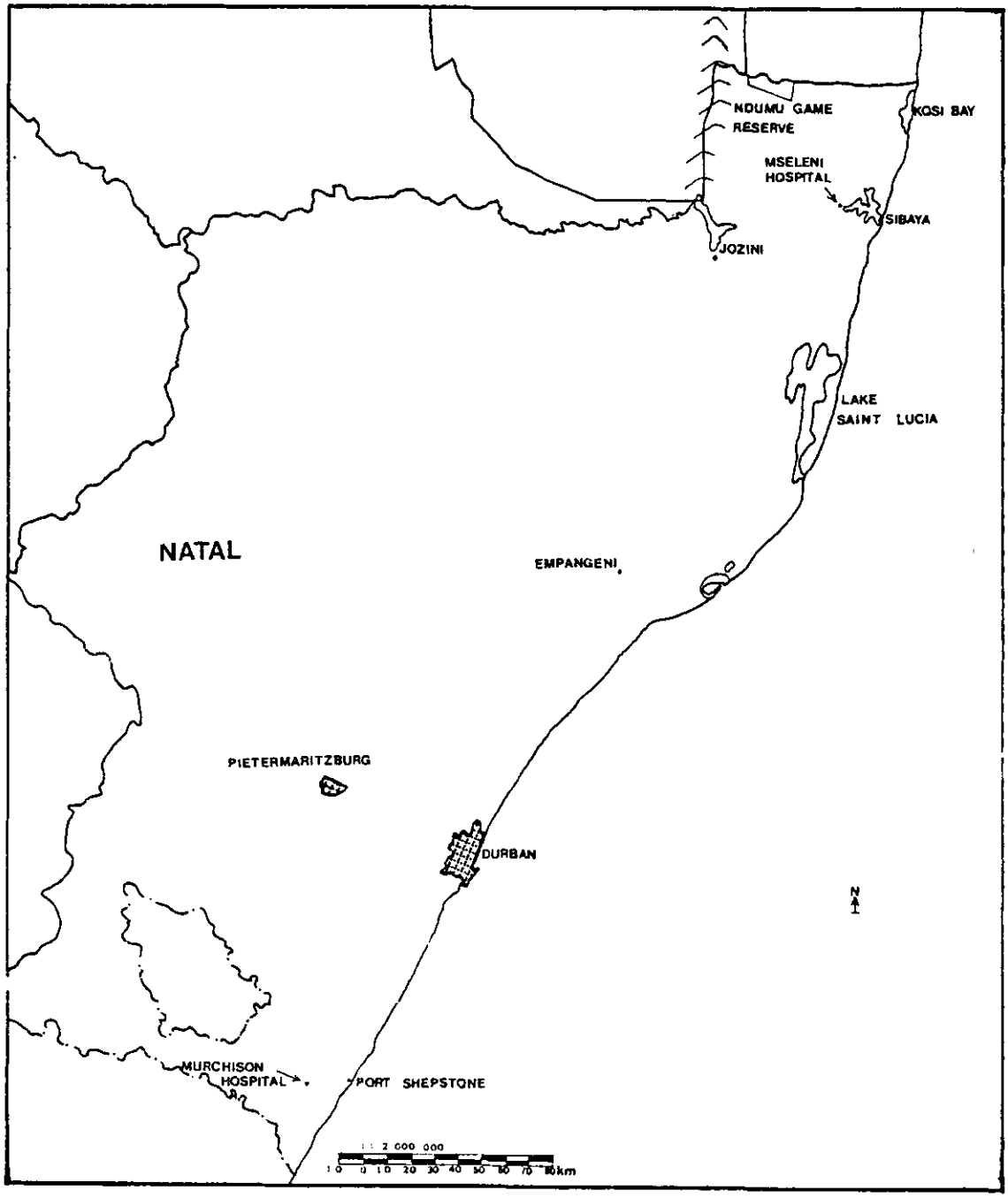

Fig 2.1.1. Map of Natal, showing the locations of major centra and the two hospitals.

The diet consists of maize meal, beans, vegetables, rice, beef and goat's meat, chicken, eggs and cows' milk. Fruit, nuts and roots are collected in the wild when available. Half the families own cattle, most own goats and everyone has chickens. The nutritional status was generally good, but malnutrition or stunted growth was seen in children of very poor families. The generally good nutritional status can be attributed to the diet, and the use of fish as a protein source. The use of alcohol is wide spread. Home brewing is practised and is also a source of income for some. Although the brews like marula beer and palm wine are not very potent, they are consumed in large quantities by both men and women. Palm wine (ubusulu) is an important source of vitamins and, in certain areas, an important part of the diet (Torres, 1980). Cases of child neglect were observed, because some parents spend their meagre income on drink.

2.1.2 CONTROL POPULATION

The ideal control population should, in terms of population structure, diet and geographical location, be indistinguishable from the exposed population, but with no known exposure to DDT. There are two major aspects that must be considered in the selection of such a group; location in relation to malaria control and geography.

Areas close to a sprayed area can only serve as a control group with diminished exposure. An attempt was made (in a pilot study conducted prior to this project) to obtain serum samples from people residing in Ngwavuma as a control for the group from the Flood Plain population. Ngwavuma lies on the Lebombo mountain range (Fig. 2.1.1) and because of the high altitude, malaria is not endemic. The two areas are adjacent, with daily bus and taxi services. The people are also related, and frequent visits take place. The Pongolo Flood Plain, particularly fish from the pans, serve as a source of food. These fish have been shown to be contaminated by DDT (Bouwman *et al.*, 1990) and will contribute to body burdens of DDT. This was reflected in the wide variation in serum levels obtained from this population, while the expected pattern of increased DDT-levels with age was not apparent.

The second aspect that was considered was that an area, geographically and climatologically comparable with the area around Mseleni, would also be subject to malaria. There are many areas to the south of St Lucia which, at the time of the study, were not sprayed, but which were previously malaria endemic. The use of DDT has eradicated malaria from this area, but sporadic cases of malaria, due to imported carriers, still occur (Sharp *et al.*, 1988). Such areas were thus excluded from consideration as a control area. The only alternative was to select a rural area to the south of Durban where no DDT has ever been used for malaria control.

KwaZulu consists of large tracts of land, traditionally and historically associated with the Zulu nation. These regions can be found all over Natal and a small enclave in the Transvaal. As the test area lies in the Ubombo district, another such KwaZulu district should be considered as a control area. The opinion of the Department of Health and Welfare of the KwaZulu Government Services was asked. Dr. R.M. Short, head of Communicable Diseases Section (and also the liaison person of that Department for this study), was approached. He suggested the Murchison Hospital (Fig 2.1.1) and the inhabitants of the surrounding areas as a suitable control group (R.M. Short, personal communication, 1986). Dr. P. Garde, of the same department considered the two hospitals as comparing favourably in most aspects, particularly in terms of population numbers served, and support to and referral of cases from surrounding clinics (P. Garde, personal communication, 1987).

The Murchison hospital also houses a mission station and is situated 12 km inland from Port Shepstone on the N2-National road. The area around the hospital is called "Muchasini" (after the name of the hospital) by the local population. The area will be referred to as the Murchison or control area and the participating mothers will be referred to as the Murchison or control group.

The Murchison area differs from the flat terrain of the Pongolo Flood Plain and the area surrounding Mseleni. Rivers and streams run through gorges lined with lush forests. The homesteads are more densely packed on flat areas and gentle slopes and more intensive farming (usually maize) is practised on the fertile soil.

Traditional type huts are giving way to more modern dwellings made from mud and cement. Homesteads usually consist of two to four dwellings. Culturally, the population is much more westernized than their northern counterparts. The working population (mostly men) commutes daily to and from the industrial areas at the coast. Occupations are usually manual labour in plumbing, carpentry, transport, painting and domestic service. The women farm the small, but productive patches, work as domestic servants or shop assistants or produce curios for the tourist market. Monthly incomes of this group range from small pensions to R300, which were comparable with the exposed group. Electricity and running water is not supplied. Two streams run through the area and a system applies in which one stream is used only for washing and the other for drinking. Some boreholes are also used.

The diet is more varied, when compared to the exposed group, due to the proximity of large retail outlets on the outskirts of Port Shepstone. The diet consists of maize meal, samp, mealie-rice, rice, bread, beans, vegetables, meat, chicken, eggs and tinned fish. Everybody owns chickens. Only a few families own cows or goats. The nutritional status is generally good. No child neglect was noticed.

Some form of agricultural pest control was noticed. "Blue death", a BHC formulation, is used as a dust by some of the farmers on a small scale. It is known by the users as DDT, creating some confusion. This came to light as one of the questions on the standard questionnaire, which was completed for all participants, relates to possible exposure to DDT in an occupational context.

Most of the information was obtained from talks with people from the areas and notes taken during visits by the author. A questionnaire was completed for some 30 families by a nurse that understood and knew the residents well. Specific information on living conditions in both areas was drawn from own observations and might not reflect conditions elsewhere, or be consistent with descriptions from other sources.

2.2 ETHICAL CONSIDERATIONS

The determination of DDT in a selected group of a population is in fact a study of the distribution and determinants of a health-related situation and its health implications. This is also a definition of epidemiology. In its dealings with individuals and populations, such studies are subject to ethical constraints in every aspect of its activity. There can be many different types of activities, depending on the aim of the study, ranging from controlled clinical trails to community based surveys. Ethical considerations will vary with the activity, according to the needs and scope of the study (Hessel and Fourie, 1987).

The overriding principle governing such studies is that the prime motivator should be the health and welfare of the individual and population (Hessel and Fourie, 1987). For this study it can be stated that, although a known and well researched compound is applied to the dwellings of a certain population in a controlled manner, the actual exposure, resultant body burdens and risk to health is not known. The implications of such (control) actions can, at the moment, only be determined from extrapolation from other studies that may not take the local conditions and customs of the people into consideration. The findings and recommendations, based on objective results of a non-biased protocol, should, in theory, be accepted by the people involved in malaria control and other interested parties (the press, environmentalists and conservation authorities).

The study design and the techniques were approved by the Institute Ethics Committee of the Research Institute of Diseases in a Tropical Environment of the Medical Research Council as well as the respective medical superintendents at both hospitals.

2.2.1 COLLECTION OF BREAST MILK

The ethical issues involved in the case of breast milk sampling are not as penetrating as those that pertain to the sampling of infant blood. No invasive techniques were used for breast milk sampling. Nor was the mother or baby in any way inconvenienced, other than some lost time. There are also no known risks involved. The mothers of the exposed group were informed of the aim of the study in clear, easily understood terms, in their own language, by a trained and experienced nurse with good communications skills. The subjects knew about DDT and malaria control and were generally positive about the control program. The mothers were told that the study aimed to determine "how good the milk is" in relation to DDT application and the implications it might have for the infant. The confidentiality of the information that was needed was stressed.

The ethical considerations involved with control subjects are more involved. There were no known benefits of this study for this group, except to quantify the expected low body burdens of DDT. The Institute Ethical Committee (of the RIDTE) accepted the need to know the levels in breast milk of a control group, considering the very safe nature of the sampling technique and the lack of any real inconvenience to the mother or baby.

The participating mothers of the control group were informed of the aim of the study, usually in group settings by the nurse in charge of the clinic. She explained to the mothers that they were involved as controls for other Zulu mothers living under malaria conditions. A brief vernacular explanation, on DDT as an insecticide was given, and that we were looking into possible health implications of this compound in breast milk.

2.2.2 SAMPLING OF INFANT BLOOD

Ethical considerations in the case of black minors should also take custom and tradition into account. Generally, black woman are considered minors and

permission must be obtained from either their husbands, or their tribal elders. KwaZulu law, on the other hand, considers a black woman as having acquired majority at the age of 21 and her status is not determined by any guardian. Normally, in such a case, the parents are the legal guardians, with the father having the final say (South African Medical Research Council, 1987).

The taking of finger-prick blood samples for active malaria screening is done on a continual basis by malaria control personnel. The aim and procedure is well known to every one in the malaria endemic areas of KwaZulu. This procedure also involves little risk to the subject. A procedure was developed for the present study, to collect as little blood as possible from the infant using a toe-prick, rather then a finger-prick. Collecting blood from a toe-prick was preferred, as the infant could get highly agitated during the finger prick procedure, that can last from 30 seconds to more than a minute.

It cannot be argued that such an experience would not be without any inconvenience to either the child or mother. However, prior experience with the finger-prick technique will enhance its acceptability. Collection was done at the baby clinic of the Mseleni Hospital, with the doctors on duty being informed of the procedure prior to commencement. Informed consent of the mother was obtained by explaining the possible transfer of DDT in breast milk to their infant and the need to know how much was transferred.

2.2.3 DISSEMINATION OF THE RESULTS OF THIS STUDY

The participants of the present study have the right of access to the information obtained by the study. This communication of information is also subject to proper review of the results and evaluation of the possible consequences. Dissemination may take different forms, depending on the results and type of study. This study was undertaken to determine exposure of the infant and to determine possible health effects. Information can be relayed to the participants individually by letter, to the superintendent at the hospital, to the relevant KwaZulu health authorities or by way of publication.

It was decided not to inform participants individually, as this was a survey and not a case study. The information was relayed to the superintendents and the KwaZulu and South African health authorities by the author as soon as the results became known. A technical report has been accepted by the MRC (Bouwman, 1990). The results will also be published as articles in appropriate journals. This document will serve as an extensive summary of the findings and recommendations, and could prove useful to health authorities involved in application of residual insecticides.

The personal records that were obtained are safely stored and will be destroyed after five years (Hessel and Fourie, 1987).

2.3 SAMPLING AND SCHEDULES

2.3.1 SAMPLING OF BREAST MILK

The possibility of doing a longitudinal study on the dynamics of DDT in breast milk was investigated. Some 30 primiparous mothers who had just started breast-feeding were needed. In the exposed group of 70 people from 12 families from which serum samples were taken (Bouwman, 1990), only three women were breast-feeding. During the course of the year one stopped breast-feeding and one was temporary unavailable because of a work engagement. The area that would need to be covered for a meaningful group would have been very large. It was decided to do a serial study, with cross-sectional surveys four times a year at the two baby clinics. All the mothers present at the clinics during visits were to be approached for participation. No known factor that might introduce a bias, other than multiple participation by individual mothers during subsequent visits, was envisaged. This was noted during questioning and the prevalence of multiple participation (2%) was as can be expected, considering the study design.

The areas and serving hospitals were as described previously. The spraying regime practised by the malaria control teams (January to March each year; Sharp *et al.* 1988) was taken into account. As stated in the aim of this study, the influence of spraying on levels of DDT in breast milk was to be examined. Sampling had to be scheduled to be able to determine minimum and maximum levels. Application of DDT to the huts will temporarily increase exposure to an unknown extent. It can, however, be compared to multiple dosages of a slow-acting drug once a year. Presumably the lowest concentration will be experienced just before the next application, as metabolism would have reduced the levels. Determination of this "trough" level will give the lowest levels of exposure and the one directly after application presumably the highest. The schedule is presented in Table 2.3.1.

The variation in fat composition of breast milk during a single feed and during the day has been discussed (sub-section 1.4.2). It was therefore decided to take

the first 10 ml of the fore milk. This minimised the inconvenience to the mothers. The sampling started at 10:00 hours. It must be noted that the women in KwaZulu normally rise very early. (Fetching of water by women has been observed at 03:00 hours, when dawn broke.) Before they arrive at the clinic, most would have done some work on the fields as well. The plateau for lipid composition will probably be reached earlier in the day than in the study of western women by Hall (1979). The collection procedures are given below.

PROCEDURE

COLLECTION OF BREAST MILK: With the prior permission from the superintendent at each hospital, a team consisting of a qualified nurse, an assistant and the author arrived early on Wednesday mornings. Necessary tables were set up and arrangements made for an adequate water supply. The matron of the hospital and also the sister in charge of the clinic was informed of the procedures. As far as possible, we attempted not to intrude on their clinic schedule.

Mothers were approached by the nurse as they entered or left. The mothers were informed of the aim of the study individually or in groups by the nurse or sister. They were then given a 100 ml beaker (washed before with chromic acid and rinsed with water and acetone) marked at the 10 ml level. They were instructed to clean the nipples with water and express breast milk manually up to the mark. Some mothers were unable to express the full amount, but all samples were collected. Mothers in the delivery ward were also approached and milk obtained in the same way. A questionnaire was completed for every mother and a number assigned to the sample and mother. The milk was immediately transferred to clean 10 ml test tubes and stoppered. Extra milk was pooled and stored in the same manner. Samples of less than 4 ml were also added to the pool and the number reassigned. This did not happen very often. The

tubes were immediately placed on ice. Each donor, whether successful in expressing the required amount or not, received, as appreciation, 1 kg maize meal and 250 g beans. Lollies were given to the babies and accompanying children. The milk was frozen the same day upon arrival at Jozini or Durban. It was kept frozen until analysis.

Only a few mothers refused participation on the grounds of inability to breast feed or painful breasts. The others donated successfully. The sampling schedule is presented in Table 2.3.1.

Table 2.3.1. Sampling schedule for breast milk at baby clinics at the Mseleni and Murchison hospitals.

	MSELENI	MURCHISON	NOTATION*
NOV'86	X	X	NOV'86
DEC'86			
JAN'87			
FEB'87			
MAR'87	X	X	MAR'87
APR'87			
MAY'87			
JUN'87	X	X	JUN'87
JUL'87			
AUG'87			
SEP'87			
OCT'87			
NOV'87	X	⁂	NOV'87

* This notation will be used throughout this thesis to indicate the specific survey.
⁂ Survey cancelled; see sub-section 4.2.1.

2.3.2 SAMPLING OF INFANT BLOOD

The need to determine the levels of DDT and its metabolites in infant blood became apparent when the results of the first analysis of serum (Bouwman, 1990) and breast milk were analyzed. It indicated very high levels in babies on

extrapolation of the serum levels of DDT. The levels determined for breast milk were also very high.

PROCEDURE

SAMPLING OF INFANT BLOOD: During the NOV'87 collection at Mseleni, with the permission of the superintendent, all the mothers that had been approached for milk, including those in delivery wards, were also asked permission for blood to be taken from their infants. The reason and the method of collection were explained carefully by the nurse to obtain informed consent. Infants, carried on the backs of their mothers and sleeping, were left in this position. Infants that were awake were placed on the laps of their mothers.

The big toe of the infant was cleaned with disinfectant and left to dry (about 30 seconds). A toe-prick was made with a sterile lancet and blood droplets collected in a clean 44,7 μl capillary glass tube (Coulter Pipets) used in haematological studies. Most babies hardly reacted and some did not even wake up during the whole process. Sometimes blood flow stopped half way through and a second prick was necessary, for which permission was asked from the mother. Excess blood on the tip of the capillary tube was wiped off with a tissue. The tubes were placed immediately in 3 ml test tubes containing 2 ml of distilled water. The stoppered tube was immediately shaken to dislodge the blood from the capillary tube and to lyse the cells. The tubes were then put on ice and frozen the same day. They were kept frozen until analysis.

This method was successful in accurately obtaining small volumes of whole-blood without too much commotion. Only four babies reacted cacophonously and proceedings were terminated. Only one five-day old baby from the delivery ward was sampled. Only four mothers objected to sampling of their babies, three of them from the delivery ward.

2.4 EXTRACTIONS AND DETERMINATIONS

The determination of past exposure, by measuring the level of accumulated DDT and its metabolites in breast milk and infant blood, was the main activity of this study, on which all conclusions and deductions were based. It stands to reason that the precision, accuracy and sensitivity of these determinations are therefore very important. Analysis of organochlorine residues in biological matrixes is often difficult and time consuming, due to the presence of fat and oil in which the DDT residues are dissolved. It therefore requires a rigorous sampling and extraction protocol to avoid cross contamination and loss of sample integrity. Thorough research is therefore needed to establish methods for sample purification and concentration from bulk samples. Co-extractives need to be removed to prevent interference during measurement and to facilitate qualitative and quantitative determinations of the compounds of interest. In general, fortification (also called spiking) of the sample with the compounds of interest is used to establish recoveries. Alternatively, given the availability of relevant equipment, radio-labelled compounds can be supplied endogenously to a biological system. In this instance, using radio-labelled compounds in humans was neither required, nor was it ethical.

2.4.1 EXTRACTION AND CLEAN-UP OF BREAST MILK

The Shechter-Haller colorimetric method was the first to be generally employed for the determination of DDT in biological samples. Quinby *et al.* (1965) used it to determine DDT in human milk. The lower limit of detection was 5 μg, but cross reaction with PCB was possible. This method was superseded by gas-chromatography using electron capture detection (ECD), which was first used in 1961 (Spindler, 1983). At that time, concentration of the solute while excluding interfering substances was still in its infancy. Curley and Kimbrough (1969) used a simple triple hexane extraction with no clean-up, which must have resulted in extremely

dirty extracts. However, they only extracted 0,2 ml of human milk and used packed chromatographic columns for separation. The advent of capillary chromatography made high efficiency separations possible, as well as lowering the limit of detection. This necessitated very thorough sample clean-up to get rid of co-extractives that could degrade column performance.

The stability of DDT type compounds in sulphuric acid makes it a very useful clean-up procedure which is still in general use (Veierov and Aharonson, 1980; Kapoor, Chawla and Kalra, 1981; Weisenberg *et al.* 1985; Kanja *et al.* 1986; Jani *et al.* 1988). Sulphuric acid is also used for the clean-up of extracts from fat samples (Murphy, 1972). However, the possible presence of other compounds such as dieldrin, which is also of considerable medical importance, did not permit use of this method.

Another method for clean-up is "liquid / liquid partitioning" of an extract. The compounds of interest (as well as the fat) is extracted from the liquid matrix into hexane and partitioned with acetonitrile (or any other suitable solvent) and sometimes partitioned back into hexane by increasing the polarity of the acetonitrile phase with a sodium sulphate solution (Gabica, Watson and Benson, 1974; Woodard *et al.*, 1976; Takei, Kauahikaua and Leong, 1983; Klein *et al.*, 1986). Adsorbents have also been used extensively in clean-up procedures. Aluminum oxide, silica gel and Florisil have been used to retain fat in extracts, while allowing the quantitative recovery of the compounds of interest by elution with appropriate solvents (Miller and Fox, 1973; Mes *et al.* 1986; Dommarco *et al.* 1987). This seems to be a widely used method. Combinations of clean-up procedures are common, usually with column clean-up as the last step. Freeze dried milk has been used to obtain milk powder for further extraction in a Soxhlet apparatus (Bush, Snow and Connor, 1983).

It is necessary to determine the efficacy of any extraction method in the laboratory, even if the method is taken directly from literature. Inconsistencies invariably creep in, and if recovery is not established, the data will be of little use. A problem facing all analysts is to

determine whether the exogenously introduced compounds (through spiking) are bound and extracted in the same way and over the full range of expected concentrations as the corresponding endogenous compounds are. It is not enough to determine recovery for one concentration only, as there seems to be a relationship between recovery and standard deviation (McKinney *et al.*, 1984). On the other hand, to spike orders of magnitude beyond the endogenous levels present in the sample could also contribute towards uncertainty, as its introduction and high concentration might alter the nature of the sample. There is no certain way of establishing recovery. Even introduction of radio labelled compounds endogenously is problematic, as radioactivity is counted, but the metabolism of the compound in the biological system also needs to be determined.

Recovery therefore, can only be an approximation of the efficacy. Depending on how equilibration is reached after the spike compound is introduced, it is usually a workable and acceptable indication of recovery. Veierov and Aharonson (1980), McKinney *et al.* (1984) and Kapoor *et al.* (1981) were among the few authors who adequately described spiking conditions, and their work will be referred to later.

An extensive effort was made to adapt an extraction method ("supported liquid / liquid" extraction) that was successfully developed for serum analysis (Bouwman, Sydenham and Schutte, 1989b), to extract DDT from breast milk. A column consisting of an equal mixture of diatomaceous earth (celite) and silica gel as adsorbent was used. This was found to be porous enough for serum, but serious clogging with milk required unacceptably high pressures for elution (the pipettes, used as columns, tended to split at the pressure required). Success with this method would have meant a major reduction in sample preparation time, as extraction and clean-up could have been combined in a single step. This was unsuccessful. Seymour *et al.* (1987) got around this problem by using a porous mixture of cellulose and Florisil to adsorb 10 ml of milk. This was then air-dried and extracted with a Soxhlet apparatus. This method though, would be time consuming, due to the mixing and the subsequent transfer of the mixture to a column followed by air-drying.

Barcarolo, Tealdo and Tutta (1988) passed 10 ml of milk through a C-18 Bond-Elut cartridge ("solid / liquid" extraction). The reversed phase C-18 silica adsorbent retained the compounds of interest, which were eluted with hexane after drying of the column. The fat and more polar compounds, such as triazine, were removed from the cartridge with a subsequent methanol wash. They did not describe spiking conditions, although they homogenized the milk and added a little toluene before adsorption. Consideration could be given to this method in future.

From the above it follows that the nature of the sample should receive careful consideration before method development or adaptation of published methods can begin. As DDT is highly lipophilic, it will mainly occur in the hydrophobic compartments of a given matrix. The composition and character of the fat of breast milk is therefore important. Lipid in breast milk consists of small droplets (Lawrence, 1980). These globules are mixed homogeneously in freshly secreted milk, but soon after secretion separation of phases takes place, and a layer of cream, that also contains most of the casein, is formed (White *et al.*, 1973).

The proteins present in milk whey could be a significant compartment for solubilizing DDT. The distribution of pesticide residues has been shown to depend on the fat content of milk. Dieldrin and DDT were found on the surface of the membrane (lipoprotein) and in the fat contained by the membrane (triglycerides) (Hugunin and Bradley, 1971). The pesticides were, on a fat basis, preferentially associated with the membrane fraction. The higher volume of the fat encapsulated by the membrane however, masked this effect. DDT and dieldrin were also associated with the soluble proteins (whey proteins), but to a lesser extent. ß-Lactoglobulin was the whey protein with which the DDT was best associated. The percentage contribution of this compartment was not high, being around 0,14% (Hugunin and Bradley, 1971). Cumulative intestinal transport of DDT was greatest when administered with oleic acid or oleic acid derivatives in comparison with the corresponding triglyceride (Charman and Stella, 1986). The preferential association of

DDT within the lipid class is important, and the extraction method must be able to present the pesticide to solvent action. This can be accomplished by ensuring a very small droplet size, disassociated from the cellular membrane, for effective solubilization for solvent action.

Spiking of samples usually consists of adding the compounds of interest, dissolved in a suitable solvent carrier. McKinney *et al.* (1984) used dimethyl sulfoxide as a solvent to introduce the solute in to the milk. Kapoor *et al.* (1981) added the solutes dissolved in acetone (1 ml acetone to 100 ml of milk). Veierov and Aharonson (1980) added 0,5 ml of standard solutions (solvent not identified) per 25 g of milk and stirred for 5 minutes. The presence of solvents, however, can change the composition and influence the characteristics (especially those pertaining to extraction) of the milk considerably. A matrix, different from the original, will be presented for extraction. Addition of the solutes, with immediate evaporation of a non-polar solvent, was used in the method developed. This removed most of the solvent from the sample, while introducing the solutes to the cream layer.

Lipases present in breast milk are apparently activated by freezing and thawing (Jensen, Clark and Ferris, 1980). This places a limit on the time that can be allowed for equilibration, as the nature of the sample will be affected. The following procedure for spiking was therefore adopted (Bouwman, Cooppan and Reinecke, 1989a).

PROCEDURE

SPIKING BREAST MILK: Fresh raw cows' milk was obtained from a dairy. A sub-sample was analyzed on the same day on GC-ECD (gas-chromatography with an electron capture detector) for organochlorine contaminants, using "liquid / liquid" extraction. A quantity of 500 ml was mixed and sub-samples of 2 ml were transferred to a 3 ml test tube. These tubes were capped and the

sub-samples kept frozen for a week. The milk sub-samples were then thawed and a small volume of hexane (Burdick and Jackson, Non Spectro 217) containing appropriate amounts of p-p'DDT, p-p'DDE and p-p'DDD (obtained from the EPA) was added carefully to each sample. The solvent, which formed a layer on the top of the sample, was evaporated using a gentle stream of nitrogen. The sample was then vortex mixed for ten seconds, allowed to equilibrate for four hours and then refrozen for at least three weeks. Breast milk from Murchison and Mseleni were pooled separately and similarly treated, to determine recovery for two levels of endogenously incurred residues.

It was established that the recovery immediately after equilibration (prior to freezing) was almost complete. Freezing reduced recovery. This reduction in recovery increased with time, so that at the end of two weeks recovery was reduced by 20%, compared with extracting prior to freezing. Metabolism of DDT, DDE and DDD was a possible reason, but this rate was too fast. The DDT compounds, with time, were bound in an unknown way to one or more components in the milk, and could not be recovered in the usual manner. With the addition of acetone or methanol, protein is precipitated and the bound fraction might be lost. A new approach was therefore necessary to develop a method to extract the bound fraction. Keeping the denatured protein solubilized, as described below, was therefore done.

Proteins derive their tertiary structure in part from disulphide bonds (White *et al.*, 1973; Stenlake, 1979). Breaking these bonds will expose the hydrophobic interior to the solvent. Mercapto-ethanol is an agent that will reduce these bonds (Stenlake, 1973) and prevent the formation of polymers (White *et al.*, 1973). Cholic acid is a bile acid and a strong anionic surface-active agent that consists of a large hydrocarbon fragment (non-polar) with polar groups at one end (Stenlake, 1973). It will emulsify and solubilize lipids. A finer state of distribution of the lipids in the aqueous phase is achieved with agitation of the mixture (White *et al.*, 1973) and a larger surface area is presented to the solvent.

Cholic acid will also solubilize proteins from membranes, thereby disrupting the cell wall coating of the lipid and protein droplets, exposing the interior (Calbiochem, 1987). The extraction procedure is given below (Bouwman *et al.*, 1989a).

PROCEDURE

EXTRACTION AND CLEAN-UP PROCEDURE FOR BREAST MILK: Solvents used were hexane (Burdick and Jackson, Non Spectro 217), ethyl ether (Saarchem AR 10094) and toluene (Merck 8325). These solvents gave clean chromatograms at a 100-fold concentration. Silica gel 60 (Merck 9385, 230-400 mesh) was heat activated at 250°C for 12 hours, deactivated with 5% double distilled water and stored in an airtight container. The columns used were 5 ml glass graduated pipettes (360 mm x 8 mm i.d.). They were prepared just before clean-up by inserting a glass wool plug, followed by 1 g of silica gel (dry packed). The packing was compacted with tapping. The column was cleaned and equilibrated with 6 ml ethyl ether followed with 6 ml of hexane, using pressure from a syringe, without letting the column packing run dry. A solution of 1% mercapto-ethanol (Merck 805740) and 2% sodium deoxycholate (Merck 43035) in distilled water was prepared every second day and stored in a refrigerator.

Samples were thawed under running tap water just before extraction and vortex mixed. The spiked samples were transferred to 11 ml test tubes. Two ml of the individual samples were transferred quantitatively from the thawed bulk sample (circa 10 ml) using a calibrated disposable-tip pipette to 11 ml test tubes. The spiked sample container was rinsed with 2 x 1 ml washings of the mercapto-ethanol / deoxycholate solution and the washings added to the test tube. Each sample tube was vortex mixed for 10 seconds and allowed to stand at

room temperature for 15 minutes with intermittent shaking of the test tube rack.

Four ml of hexane was added (in the case of the spiked samples the 3 ml containers were rinsed with the hexane) and vortex mixed for 30 seconds at high speed. A stable emulsion was formed in most cases, which was effectively broken by freezing at -70°C. The mixtures were thawed by running tap water over the side of the test tubes, in the rack. The samples were then centrifuged at 3000 rpm for 5 minutes. The hexane phase was drawn off, using a disposable pipette, into a tared 11 ml test tube. The extraction was repeated and the hexane extracts combined. The hexane was evaporated in a water bath at 40°C under a gentle stream of nitrogen. The tubes were then quickly heated to 50°C in an oven (2-3 minutes) to get rid of residual hexane, and allowed to cool down. The tubes were then weighed and the fat content determined.

The fat was taken up in 1 ml of hexane and transferred to the silica gel column. The tube was rinsed with 2 x 0,5 ml hexane washings that were also transferred to the column with a disposable pipette. The first 2 ml of the column eluent (hexane remaining from the equilibration step) was not collected, as this contained none of the organochlorines. Nine ml of hexane was passed through the column, using slight pressure from a syringe. The rate of elution was kept at about 0,5 ml min^{-1}. The eluent was collected and evaporated at 40°C, as before. The extract was taken up in 500 μl of toluene for the spiked and Murchison samples, and in 1000 μl of toluene (because of the high level of contamination) for samples from Mseleni. The toluene contained aldrin (50 pg μl^{-1}, supplied by the EPA) as an internal standard. The tubes were immediately capped and the contents swirled in the water bath so that the solvent covered the inner surface. The extract was analyzed according to sub-section 2.4.3.

Table 2.4.1. Determination of recovery of DDT and metabolites from raw cows' milk and pooled breast milk, using the spiking and extraction procedures given in the text. Units are given as ng per 500 µl final extract. Results were calculated from four replicates for the raw cows' milk, and five for the pooled sample. A DDE background level of 31,68 was subtracted for the Murchison samples (Bouwman *et al.*, 1989a).

	DDE	DDD	DDT	ΣDDT
RAW COWS' MILK				
ADDED	10	10	10	30
RECOVERED	9,8	7,5	8,3	25,6
%RECOVERY	98,4	74,7	83,2	85,4
STANDARD DEVIATION	12,5	5,0	6,9	7,8
STANDARD ERROR	6,2	2,5	3,4	3,9
%CV*	12,7	6,7	8,3	9,1
ADDED	50	50	50	150
RECOVERED	50,5	50,5	53,7	154,7
%RECOVERY	101,0	101,0	107,4	103,1
STANDARD DEVIATION	3,2	3,9	3,3	2,9
STANDARD ERROR	1,6	1,9	1,6	1,5
%CV	1,6	3,9	3,1	2,8
ADDED	100	100	100	300
RECOVERED	94,5	93,7	99,1	287,2
%RECOVERY	94,5	93,7	99,1	95,7
STANDARD DEVIATION	0,8	1,5	2,5	1,4
STANDARD ERROR	0,5	0,8	1,5	0,8
%CV	0,9	1,6	2,5	1,5
MURCHISON POOLED BREAST MILK				
ADDED	10	10	10	30
RECOVERED	10,3	10,1	10,2	30,6
%RECOVERY	102,5	101,5	102,4	102,1
STANDARD DEVIATION	8,5	9,0	8,3	7,7
STANDARD ERROR	3,8	4,0	3,7	3,4
%CV	8,3	8,9	8,1	7,5
ADDED	50	50	50	150
RECOVERED	45,7	52,8	50,0	148,5
%RECOVERY	91,4	105,6	100,0	99,0
STANDARD DEVIATION	1,8	2,5	2,4	0,3
STANDARD ERROR	0,8	1,1	1,0	0,1
%CV	2,0	2,3	2,4	0,3
ADDED	100	100	100	300
RECOVERED	86,5	98,5	98,9	284,0
%RECOVERY	86,5	98,5	98,9	94,7
STANDARD DEVIATION	2,0	2,6	2,7	1,8
STANDARD ERROR	0,9	1,1	1,2	0,8
%CV	2,3	2,6	2,7	1,9

* Percentage coefficient of variation

Toluene was used, as it has a much lower vapour pressure then hexane. Therefore changes in volume of the extract, due to evaporation prior to analysis, can be ignored. The addition of toluene was usually done five to ten minutes before injection. The detailed results of the recovery determination using the above clean-up procedures are given in Table 2.4.1. The overall results are summarized in Table 2.4.2.

A very good percentage recovery with this method was obtained for all compounds at all the levels tested (Tables 2.4.1 and 2.4.2). The high level of contamination for the Mseleni milk did not allow for proper calculations, as four fold dilutions of the extracts had to be made to obtain a detector response within the linear range. The use of dilutions were especially problematic for the two lower spiked amounts, as a difference of 2,5 and 12,5 pg after dilution had to be determined against a background of more than a hundred. These dilutions were made to allow for the response of the higher spiked compounds to fall within the linear detector range.

Table 2.4.2. Summary statistics for the determination of recovery of DDT and metabolites from raw cows' milk and pooled breast milk from Murchison, using the procedures described in the text. Four replicates were used for the raw cows' milk and five for the breast milk.

	DDE	DDD	DDT	ΣDDT
RAW COWS' MILK				
%RECOVERY	98,2	89,4	96,3	94,7
STANDARD DEVIATION	7,6	12,6	11,8	9,1
STANDARD ERROR	2,3	3,8	3,6	2,8
%CV	7,7	14,0	12,3	9,6
POOLED MURCHISON BREAST MILK				
%RECOVERY	93,5	101,9	100,4	98,6
STANDARD DEVIATION	8,4	6,0	5,1	5,3
STANDARD ERROR	2,1	1,5	1,3	1,4
%CV	9,0	5,9	5,1	5,4

Comparisons of the recovery results with the results of others will indicate whether this is a viable extraction and clean-up method. It must be noted, however, that very few studies reported the percentage recovery and other statistics, probably relying on routine laboratory methods. Kanja *et al.* (1986) reported average recoveries between 80 and 115% with a standard error of about 10%. The highest standard error for the present study was 5,25% of the percentage recovery. Woodard *et al.* (1976) had a mean recovery of 72% for the same compounds with a low of 28% for p-p' DDD, which was retained on the clean-up column. Woodard *et al.* (1976) also reported a percentage coefficient of variation (%CV) of 7; 15 and 6 for DDE, DDD and DDT, respectively. They also spiked with amounts of 16 ng and higher. They did not describe their method of spiking or equilibration. Steinwandter (1982) observed complete recoveries of DDT, DDD and DDE from milk and dairy products using a silica gel column and dichloromethane in petroleum ether (20:80), without removing the spike solvent. Dommarco *et al.* (1987) only reported a percentage recovery of higher than 80% for all

compounds. Bush *et al.* (1983) reported extensively on recoveries of PCBs, but not on DDT and metabolites. Seymour *et al.* (1987) reported percentage recoveries of 100,1 and 97,8 for DDE and DDT with a %CV of 2,8 and 6,0, respectively, for the Soxhlet method using cellulose and Florisil. However, they spiked the adsorbent (with 50 ng compound) and then added lipid and distilled water. This does not represent actual conditions and their results are probably biased. Barcarolo *et al.* (1988) reported percentage recoveries of 99,7 and 102,0 for DDE and DDT, with a %CV of 3,1 and 2,4, respectively. As remarked earlier, spiking and equilibration procedures were not reported.

Perhaps the most relevant paper is that of McKinney *et al.* (1984). Recoveries ranged from 74% (50 $\mu g\ l^{-1}$ DDE, %CV = 11,2) to 94,2% (1000 $\mu g\ l^{-1}$, %CV = 3,7). An apparent increase in recovery with higher concentrations seemed to be the norm in most studies that reported these values. The %CV's reported by McKinney *et al.* (1984) were all below 12%. The highest %CV in this study (12,7%) was for DDE at the lowest spiking level in raw cows' milk. The method of Kapoor *et al.* (1981) recovered 89,9% (%CV = 9,23) of DDE, 95,4% (%CV = 12,7) of DDD and 86,9% (%CV = 10,9) of DDT, with acetone as the spiking solvent.

It can be concluded, after comparison with other studies, that the present extraction method, based on a more rigorous spiking procedure and using chemical denaturation of proteins and solubilization of lipids, is comparable with, if not better than, other reports in literature. The relatively high percentage recovery obtained by this method is indicative of the efficacy. As percentage recovery is not constant over the entire range of expected concentrations, the results obtained will not be corrected for recovery. The trend in almost all studies was to report only obtained values and not corrected ones. It is obvious that the higher and more reliable the percentage recovery can be made, the more confidence can be attributed to the conclusions and deductions made from the data.

2.4.2 EXTRACTION AND CLEAN-UP OF INFANT BLOOD

Methods currently in use for extracting organochlorines in blood and serum involve multiple "liquid / liquid" extractions with solvents (Dale, Curley and Cueto, 1966; Peterson, Stahl and Meeker, 1976; Mckinney *et al.*, 1984) or multiple solvent extraction after acidification of the sample (Gupta *et al.*, 1978; Franken and Luyten, 1976). A major problem associated with most of these methods is the frequent formation of stable emulsions which are difficult to break, with the concomitant possibility of variable recoveries. A method was developed to extract DDT and metabolites from serum (Bouwman *et al.*, 1989b). This method involves a single extraction on a silica gel / celite column ("supported liquid / liquid" extraction), resulting in a clean extract that can be chromatographed. This method was validated against the triple hexane extraction method (adapted from Dale *et al.*, 1966). The latter technique was modified by increasing the time of extraction and using a more rigorous mixing method.

The method by Bouwman *et al.* (1989b) was used for the extraction of infant blood with one small adaptation. The peripheral blood from a toe-prick was collected with a capillary tube as described earlier (sub-section 2.2.2). The tube with the blood was then placed in two ml of distilled water and frozen. This increase in volume (44,5 μl to 2000 μl) was the only additional step necessary for extraction as compared to the serum extraction technique. Determination of recovery from this type of sample was not considered necessary. It would have entailed taking more blood from the baby for spiking, which would not be ethical. Spiking, because of the small volume and the fact that a such a small volume of whole-blood clots so fast (therefore preventing mixing), will be impossible to control. Spiking the two ml water and blood mixture would also not be acceptable, as this does not represent the original matrix (whole-blood). It was decided to accept the recovery results from serum as valid for the determination of DDT and metabolites in whole-blood, collected from peripheral capillary blood vessels. The method, as used for serum (Bouwman *et al.*, 1989b) and infant blood is given below.

PROCEDURE

EXTRACTION AND CLEAN-UP OF INFANT BLOOD:

Standards, solvents and spiking procedure for serum was the same as for the extraction of breast milk. Celite "545" (Saarchem 156200) was treated as follows. Concentrated hydrochloric acid was added to cover a batch of celite and stirred; it was left overnight and the supernatant poured of. Double-distilled water was added, the mixture stirred, allowed to settle and the supernatant poured of. This was repeated twice, after which the celite was transferred to a Buchner funnel and rinsed with double-distilled water until the effluent was neutral. The celite was then dried at 250° C for 24 hours. The silica gel (silica gel 60, 230-400 mesh, Merck 9385) was also dried at 250° C for 24 hours.

The column packing material was prepared by thoroughly mixing equal amounts of celite and silica gel and storing the mixture in an airtight container. The columns used were 10 ml graduated glass pipettes (240 mm x 8,24 mm i.d.), prepared by inserting a small plug of glass wool, followed by 3 g of packing material (dry-packed), tightly compacted to give a volume of 6,3 ml and topped with a thin layer of glass wool. The elution rate was controlled by using an adapted 20 ml syringe to deliver pressure onto the column. Holes were drilled through the top of the syringe wall and centre column of the plunger, through which a short metal rod immobilized the plunger.

The dry-packed column was preconditioned with 8 ml ether / hexane (40:60), using pressure from the syringe. When the solvent level reached the upper glass wool layer, the serum or diluted whole-blood was added with a disposable glass pipette. The sample container was rinsed with 3 x 0,5 ml ether / hexane (20:80), and the rinsings was added to the column. The capillary tube was also placed on the column, using a pair of

hexane rinsed forceps. The serum or diluted blood was forced into the packing material and the remaining solvent eluted at a rate of 0,3 ml min^{-1}, using the syringe, and the eluate discarded. When the solvent level reached the upper glass wool layer, a further 3,5 ml of ether / hexane (20:80) was added. The solvent was again eluted at the same rate, using pressure, followed by 6 ml ether / hexane (33:66) and both fractions were collected. The column was allowed to run dry and the combined eluates were evaporated to dryness under a stream of dry nitrogen in a water bath maintained at 50°C. The extract was taken up in 50 μl toluene (500 μl for serum), containing 50 pg μl^{-1} aldrin.

This method was compared with the triple hexane procedure (liquid / liquid extraction), adapted from Dale *et al.* (1966), using the same batches of spiked serum. Two ml of serum was extracted with 3 x 5 ml hexane by vortex mixing for two minutes each; this was centrifuged each time at 3000 rpm for 5 minutes and the hexane removed. Emulsions that formed were frozen at -70°C, thawed and centrifuged. The extracts were pooled, evaporated and taken up in toluene, and analyzed (see sub-section 2.4.3). The results of the two methods are given in Table 2.4.3.

Table 2.4.3. Summary results of the comparison of the two extraction procedures for serum using gas chromatography. Percentage recovery is given, with calculations based on 18 extractions at three different spiking levels for two different pooled sera samples (Bouwman *et al.*, 1989b).

	LIQUID/LIQUID	SUPPORTED LIQUID/LIQUID
DDE	80,3	90,7
DDD	67,5	80,9
DDT	79,0	90,8

The results in Table 2.4.3 indicate adequate recoveries using the supported liquid / liquid method. What is not apparent is that, because of inadequate recovery of endogenously incurred residues by the liquid / liquid method, overall extraction by the supported liquid / liquid method is actually 10% higher than indicated. This shows that bound residues are not always dislodged by conventional methods, and previous results could well be under estimates. Dale *et al.* (1966) reported recoveries of 42% for DDT and 67% for DDE, while Gupta *et al.* (1978) observed recoveries of 41% for DDE and 45% for DDT, also using the triple hexane method according to Dale *et al.* (1966). McKinney *et al.* (1984), using a triple hexane / ethyl ether (1:1) solvent phase, recovered 77% at low spiking levels and between 90 to 100% for the higher levels. Peterson *et al.* (1976), using 20% acetone in iso-octane as solvent, after adding sodium sulphate to the serum, reported recoveries of between 65% and 95,7% for DDE from spiked mallard serum.

Using ether / hexane (40:60) rather than ether / hexane (20:80) as column conditioning solvent, markedly improved overall recovery. The two different solvent polarities also improved the recovery of DDD and DDT, with DDT and DDE eluting mainly in the first fraction (ether / hexane 20:80) and DDD eluting mainly in the first two ml of the second fraction

(ether / hexane 33:66). Analysis of the solvent remaining on the column (1-2 ml, removed with water) revealed no trace of any of the compounds under investigation. As was the case with the milk samples, reduced extraction after freezing was observed. The dual solvent system adequately restored recovery to the reported levels.

The ease of this method can be attributed to the supporting phase of silica gel and celite. It completely retained all the serum components, including the water, whilst allowing the almost complete recovery of organochlorines. The combination of celite and silica gel made the packing porous enough to allow the flow of serum or diluted blood without clogging of the column. The diluted blood was completely retained and no haemoglobin or water was eluted. The whole-blood extract, because of its concentrated nature (50 μl final extract volume), did not chromatograph cleanly. Peaks that normally did not interfere were attenuated and made the determination of DDD impossible. Clean-up was, because of the very low levels (a mean level of 114 pg ΣDDT per injection), impossible without incurring unacceptable losses.

The method described here was found to be rapid and, except for DDD, efficient. Eight simultaneous extractions per hour could be performed. No loss of column performance as measured in constant separation and retention (chromatographic properties) was observed.

2.4.3 CHROMATOGRAPHIC CONDITIONS

The selection of a method for separating complex mixtures is the result of the consideration of many factors. Instrument availability, number of samples, accuracy, sensitivity and precision required, as well as cost (or time), plays a role. Most methods for the analysis of environmental samples require the use of GC in the final step. Gas-chromatographic detectors are more sensitive and selective than those used in liquid chromatography. GC is usually also cheaper (Grob, 1985). This separation

technique is by far the method of choice for a large group of researchers that have determined the environmental and health aspects of DDT.

A wide variety of GC instruments are available to the analyst, but cost is the one overriding factor governing the acquisition of an instrument and the required options. The first decision depends on the nature of the sample, as it will dictate the detector(s) that need to be used. Secondly, the type of column and inlet configuration necessary for optimal (i.e time saving) operation needs to be chosen. Lastly, data acquisition and manipulation need to be compatible with the sensitivity and accuracy required.

The choice of the ^{63}Ni Electron Capture Detector (ECD) was obvious. This detector is much more sensitive (and selective) than the ubiquitous Flame Ionization Detector (FID) (Poole, 1982). A high response on the ECD is shown by halogenated compounds, of which DDT and metabolites are members.

Choosing a separating column can be quite bewildering, given the wide choice in type of column, support, length and stationary phase. The choice is largely determined by the application, as well as the preference of the analyst. Capillary columns are becoming the laboratory standard because of their separating power and stability. However, they are limited to the sample amount that can be put onto the column. It is sometimes necessary to put most of the extract onto the column, because of the extremely low levels of xenobiotics in the extract. Speed of separation is also a factor, and the more samples that can be chromatographed adequately within the constraints of chromatographic practice, the better.

The choice of column type fell on a Megabore column. This is a large diameter, open tubular, wall coated, fused silica column. It can be classified, based on separating power, as falling between conventional packed columns and advanced narrow bore capillary columns. A discussion of the advantages and disadvantages of these column types falls outside the scope of this thesis. Suffice it to say that these columns can handle

larger sample amounts than normal capillaries, chromatograph them faster than packed columns, with an intermediate separation power (measured in plate height). The injection system is determined by the column, and a megabore adaptor kit was used to convert a conventional packed inlet. The instrument, chromatographic conditions, standard practice of operation and data capturing are described below.

PROCEDURE

CHROMATOGRAPHIC CONDITIONS: A Varian model 3300 gas chromatograph was used. It was equipped with a ^{63}Ni ECD with an activity of 8 mCi. A 15 m x 0,53 mm J&W fused silica megabore column with a DB-210+ liquid phase and a film thickness of 1 μm was used. Nitrogen (@ 38,5 cm sec^{-1}) was used as carrier gas. Detector make up gas was supplied at 30 ml min^{-1}. The injector temperature was 250°C and the detector was held at 300°C. The column oven was programmed as follows: the initial temperature was 125°C, rising to 190°C at 13°C min^{-1}. The oven was then maintained at this temperature for 1 minute, increased to 200°C at a rate of 27°C min^{-1}, the temperature held constant for 4,3 minutes and finally raised to 220°C and held for 2 minutes, so as to get rid of possible late eluting peaks.

Data capturing and integration was done with a Spectra Physics Integrator 4290, programmed to automatically calculate and present results on a mass basis, independent of sample amount injected and dilution. A non-linear calibration, using appropriate standards, based on aldrin as reference and internal standard, was used. Further calculations were done with a programmable calculator. Dilutions were done after the initial run was completed, and the required dilution (with toluene and internal standard) was estimated.

These chromatographic conditions gave good separation of all the compounds, with a minimum quantifiable limit of 0,5 pg / component. Injection volume was a constant 1 μl. Toluene was used as solvent for both standards and extracts, to eliminate possible discrepancies in response and retention time as a result of differences in the boiling points of solvents.

2.5 STATISTICS

Statistical methods are used to establish a systematic representation of the relationships between measurements. There are various ways of representing these relationships. Graphics can be used to visually display quantitative information. This allows the study of the relationship by presenting the whole picture and also allows scrutiny of smaller detail. Symbolic representations, such as mathematical expressions, allows quantitative predictions to be made, while tables can be used to summarize important information not directly readable from graphs or formulas. In the rest of this thesis, extensive use will be made of all three methods of presentation.

2.5.1 MODELS

There are various ways of describing the dynamics of pesticides in the environment. Models are used in many cases to describe (or predict) the dynamics of compounds between the various compartments of the environment. These models are based on a defined problem and will differ in complexity, depending on the extent of clarification and accuracy required. No model is, however, complete and it can only be based on our present knowledge of ecology, biology, chemistry, climatology and a host of other related disciplines. Design of these models must take into account all the knowledge available and make predictions for a defined set of parameters. Often various processes are not known and empirical treatment is required (Groth, 1974). The calculation of the various pathways, sinks and sources can only be based on measurements, or calculated from relevant data such as solubility and octanol / water partition coefficients (Gobas and Mackay, 1987). The applicability of such a model, checked against a real system, is limited by the assumptions made for a particular set of variables based on actual data (Groth, 1974). Greenhouse, Kass and Tsay (1987) states that "*statistical models are imperfect representations of the structure underlying the generation of data...*"

and "*one must assess critically the fit of a model before one uses it for inference*".

The role of models therefore, can only be of limited predictive and descriptive value. A set of circumstances can be mathematically modeled to fit the measured effect (such as storage), but a change in any of these might invoke a whole new set of determinants (such as induced metabolism by fungi or the reduced ecologic contribution caused by sub-lethal effects in soil invertebrates) not included in, or predicted by the model. Therefore, measurements of the real situation can never be replaced by modelling. Modelling will increase in sophistication, complexity and predictive ability, but its adaptive ability to a changing environment is usually lacking in adequacy or confirmability (Groth, 1974). Hamelink and Spacie (1977) have commented on the inclusion of all available parameters (in a model for aquatic pollution), such as changing kinetic rates and relative importance of body weights as different fish species goes through different stages, mathematically interacted with a concomitant interaction with seasonality, food selection and habitat selection. They estimated that 100 man-years was required to compile all the data needed for a growth model of bluegills in a small lake. An all encompassing model that includes all fishes in a system interacting with a pollutant, would require a monumental effort and is probably not necessary.

The limitations of modelling in any form must, however, not be seen as discrediting the concept of modelling. It is a valuable and necessary part of science to offer, in the first instance, a proposed explanation of the measured observations and the testing of theoretical concepts (Groth, 1974). Secondly, the gaps in knowledge become more apparent, even in very crude descriptive models. The EPA, with regard to exposure assessment, has stated the following; "*In the absence of sufficient reliable data and the time to obtain appropriate measurements, exposure assessments may be based on validated mathematical models. Whenever possible, exposure assessments based on modelling should be complemented by reliable measurements.*" (Environmental Protection Agency, 1986).

Modelling therefore, offers the scientist a base for deciding on further work to elucidate certain aspects for refining a model. A good example of such a model is presented by Smith (1987). He presented formulas for calculating the infant daily dose of dioxin from breast milk, based on the maternal daily intake of dioxins from incineration emissions. His work suggested major sources of dioxins, other than solid waste incineration, which need to be identified. For the present study, to determine exposure assessment, an understanding of the dynamics of DDT and metabolites is needed, to explain the findings in terms of source, uptake, storage and elimination of contaminants. During this study, various statistical models will be used and some new ones proposed, but they must be seen within the restrictions mentioned above.

2.5.1 STATISTICAL PROCEDURES USED

Most of the statistical analysis was conducted by myself in consultation with Dr P.J. Becker of the Institute for Bio-statistics of the Medical Research Council. Most of the analysis done in section 3.2 was done using the BMDP (Biomedical Computer Programs, P-series) statistical package (BMDP, 1985).

BMDP procedures used were:

BMDP1D - simple data description
BMDP3D - one sample and two sample t-tests
BMDP5R - polynomial regression
BMDP6D - bivariate scatter plots
BMDP7D - description of groups (strata) with histograms and analysis of variance

These are standard programs and the results of BMDP procedures are widely used and accepted. The rest of the analyses were done using Statgraphics version 2.6, a product for the IBM PC from Statistical Graphics Corporation (Statgraphics, 1987). Statistical procedures used from this package include:

Summary statistics
Frequency tabulation
Frequency histogram
Codebook procedure
One-sample analysis (t-test)
Two-sample analysis (t-test)
Comparison of Poisson rates
Distribution fitting (chi-square)
Fisher's exact test
One-way analysis of variance
Multifactor analysis of variance
Simple regression
Stepwise variable selection (regression)
Non-linear regression

Data input procedures were as outlined in the manual (Statgraphics, 1987). Remarks about the selection of some procedures are given in the results sections, where necessary. Regression plots are presented, together with the 95 percent confidence and prediction limits. Confidence limits are the limits within which the mean will fall for a given population, while prediction limits are limits of confidence for predicting a single value from such a population. Natural logarithms were used throughout and usually indicated with "Ln".

Graphs from regression analysis also include relevant data, such as the type of regression (linear, $Y = aX + b$; or multiplicative, $Y = aX^b$), correlation coefficient (CC), the coefficient of determination (CD) and the regression. Not all the possible graphs were included, as this would not have contributed any more meaningful information. Plots of DDD, for example, have been excluded, as none of the p-values were significant, indicating no significant slope. Non-linear regression analysis was done for both the data points (RD) and the means of the intervals. Probability plots were included to visually judge the distribution of residuals. This also allowed the determination of how and where the data

deviated, and whether there was any auto-correlation not detected by the Durbin-Watson statistic, which was also included.

The stepwise variable selection for multiple regression allowed various variables to be introduced or removed from the model, while determining their contribution to the regression. Once again, the expression for the regression as well as the coefficient of determination (CD) was included in the graph. Other graphical presentations were prepared using Harvard Presentation Graphics (Software Publishing Corporation, 1987), with data obtained from both BMDP and Statgraphics.

The possibility that raw data used for the two programs (same raw data sheets were used) might have been entered wrongly, was checked by comparing the summary statistics generated by both packages. No deviations, except with the rounding of numbers, was encountered.

CHAPTER 3

RESULTS

3.1 COMPARISON OF THE EXPOSED AND CONTROL GROUP PARAMETERS

The two groups under investigation must be comparable in order to draw valid conclusions. The most important aspects that must be looked at are: the mean rate of sampling, maternal age, parity, infant age and milk fat. Results of comparisons, both between and within groups, will be presented in tabular form. Profiles were used to visually compare the different parameters.

Table 3.1.1. Comparative summary of some characteristics of mothers and infants attending baby clinics. Standard deviations are given in parenthesis. Parameters marked * do not differ significantly between groups ($p > 0,05$).

	EXPOSED	CONTROL
NUMBER OF SURVEYS	4	3
NUMBER OF SAMPLES	132	88
MATERNAL AGE (YEARS)*	25,5 (5,6)	25,5 (7,1)
PARITY*	2,7 (1,8)	3,0 (2,1)
INFANT AGE (MONTHS)*	8,6 (5,4)	7,7 (5,9)
% BREAST-FEEDING	89	71
% BOTTLE-FEEDING[a]	1,5	4,6
% BREAST + BOTTLE-FEEDING	8,3	24,1
% MILK FAT*	3,95 (2,1)	3,76 (2,3)

[a] Those mothers that practised bottle-feeding were not included in the total, nor subsequent analysis.

Table 3.1.2. Summary table of maternal and infant parameters of the exposed group, as per survey. None of the parameters differ significantly between surveys ($p > 0,05$).

	MEAN	STANDARD DEVIATION	STANDARD ERROR
NOV'86			
n	39		
MATERNAL AGE[a]	26,5	6,17	0,99
PARITY	3,13	2,03	0,32
INFANT AGE[b]	8,46	5,76	0,38
% MILK FAT	4,01	2,09	0,33
MAR'87			
n	35		
MATERNAL AGE[a]	24,7	4,94	0,84
PARITY	2,51	1,36	0,23
INFANT AGE[b]	9,24	4,34	0,73
% MILK FAT	4,50	2,06	0,34
JUN'87			
n	28		
MATERNAL AGE[a]	24,5	5,72	1,08
PARITY	2,57	1,93	0,37
INFANT AGE[b]	7,24	6,11	1,15
% MILK FAT	3,54	2,11	0,40
NOV'87			
n	30		
MATERNAL AGE[a]	25,6	5,37	0,98
PARITY	3,10	1,81	0,33
INFANT AGE[b]	9,16	5,27	0,98
% MILK FAT	3,60	2,06	0,38

[a] Years.

[b] Months.

Table 3.1.3. Summary table of maternal and infant parameters of the control group, as per survey. None of the parameters differ significantly between surveys ($p > 0,05$)

	MEAN	STANDARD DEVIATION	STANDARD ERROR
NOV'86			
n	25		
MATERNAL AGE[a]	23,7	6,31	1,26
PARITY	2,64	1,85	0,37
INFANT AGE[b]	6,36	6,13	1,25
% MILK FAT	4,01	2,41	0,48
MAR'87			
n	29		
MATERNAL AGE[a]	27,0	7,30	1,36
PARITY	3,31	2,44	0,45
INFANT AGE[b]	6,78	4,99	0,93
% MILK FAT	3,34	2,54	0,47
JUN'87			
n	33		
MATERNAL AGE[a]	25,58	7,27	1,27
PARITY	2,94	2,09	0,36
INFANT AGE[b]	9,50	6,06	1,05
% MILK FAT	3,94	2,04	0,35

[a] Years.

[b] Months.

Table 3.1.4. Comparison of Poisson rates to compare the mean rate of samples per survey between the test and control groups.

	EXPOSED	CONTROL
EVENT COUNT	132	88
INTERVAL LENGTH	4	3
RATE ESTIMATE	33	29,3
RATE RATIO	1,125	
P-VALUE	0,00374	

Table 3.1.1 gives an overview of the two groups by combining the respective surveys. No significant differences between groups were found for maternal age, parity, infant age and % milk fat. The mean maternal age for both groups was the same (25,5). More mothers from the control group tended to bottle-feed than the test group and a quarter of them practised both feeding methods. Comparisons with each group between surveys revealed the same lack of statistical differences in mean values for all parameters (Tables 3.1.2 and 3.1.3). This was determined using two sided t-tests. The highest mean maternal age was 27,0 years for the Mar'87 control survey. The lowest mean maternal age was 23,7 years for the Nov'86 control survey.

The Poisson rates for the surveys revealed no difference of the survey rates between the two groups (Table 3.1.4). The low p-value of 0,00374 is highly significant.

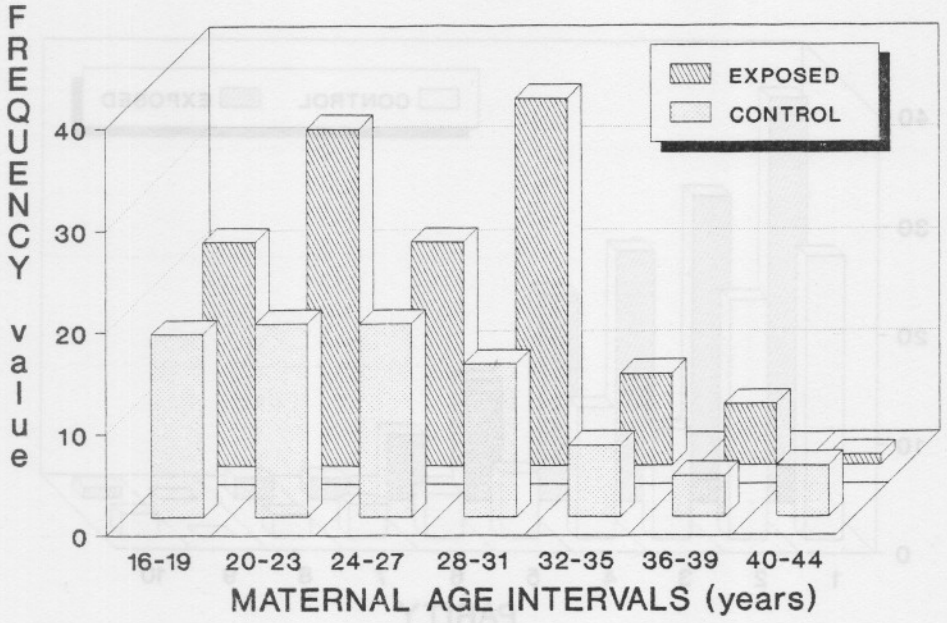

Figure 3.1.1. Frequency profile of maternal age intervals for the exposed and control groups.

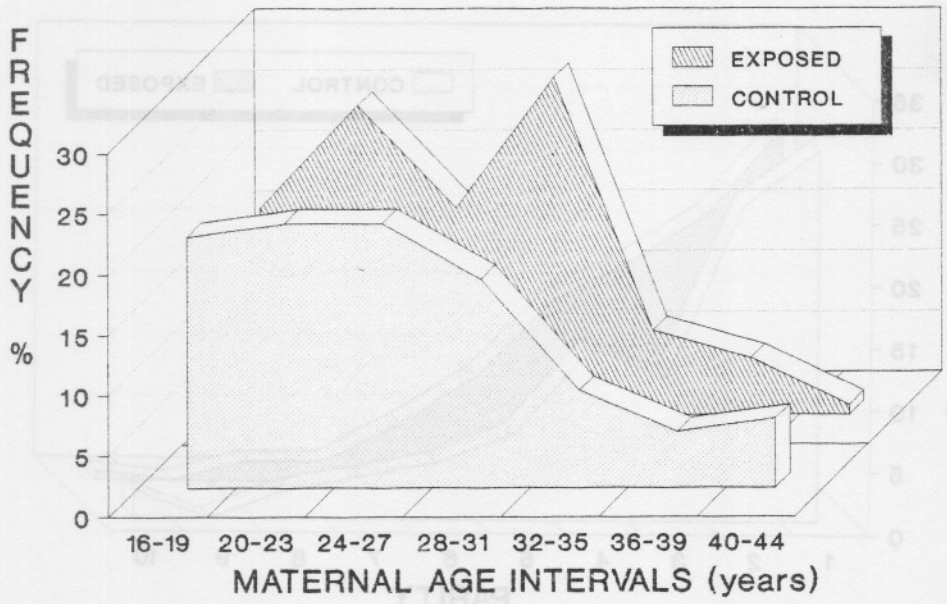

Figure 3.1.2. Percentage contribution of maternal age intervals for the exposed and control groups.

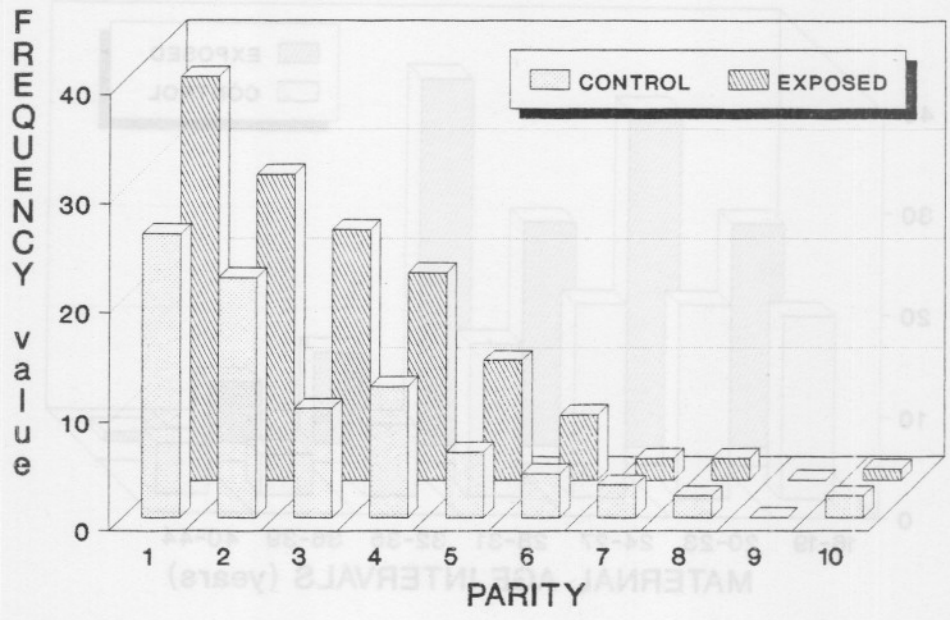

Figure 3.1.3. Frequency profile of parity for the exposed and control groups.

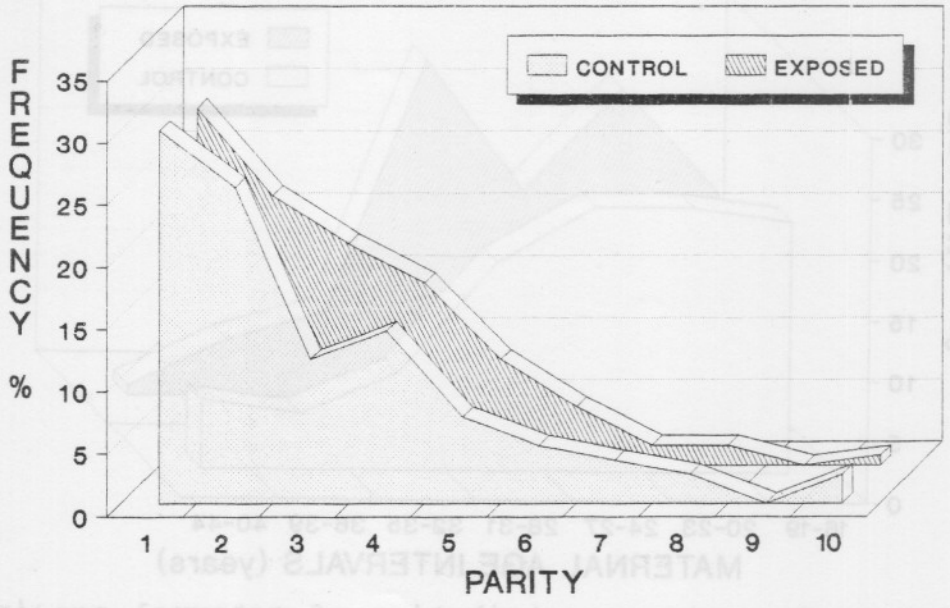

Figure 3.1.4. Percentage contribution profile of parity for the exposed and control groups.

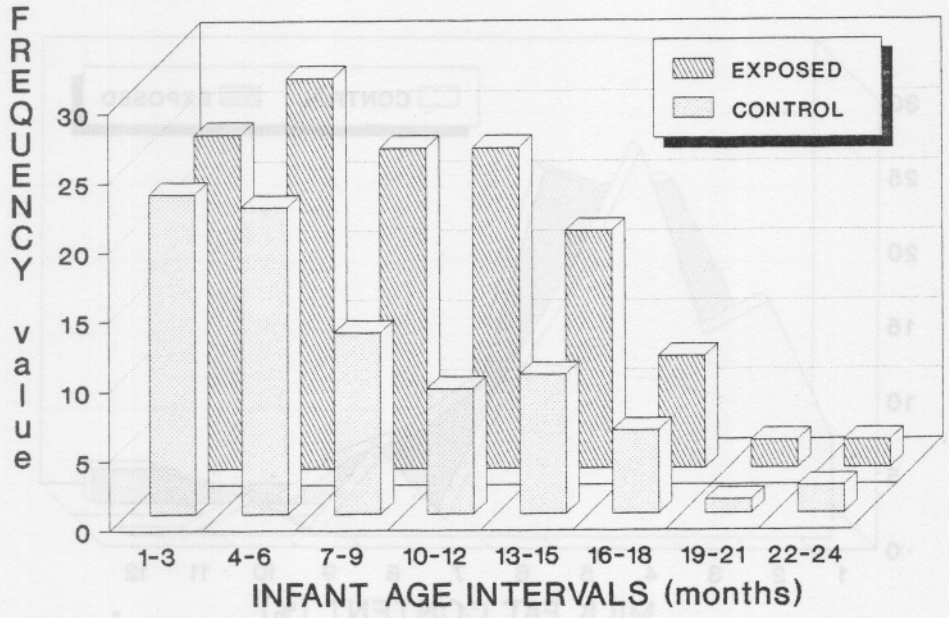

Figure 3.1.5. Frequency profile of infant age intervals for the exposed and control groups.

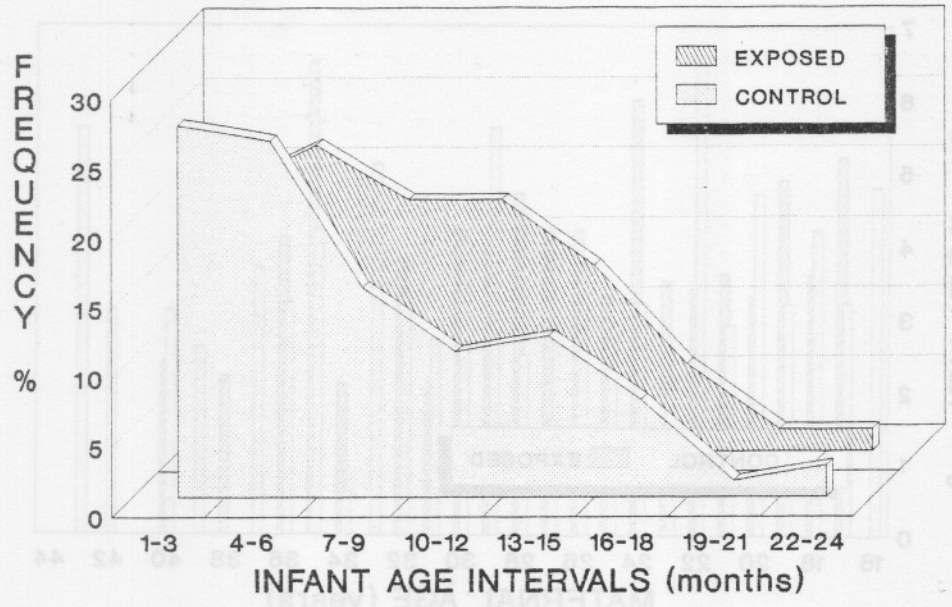

Figure 3.1.6. Percentage contribution profile of infant age intervals for the exposed and control groups.

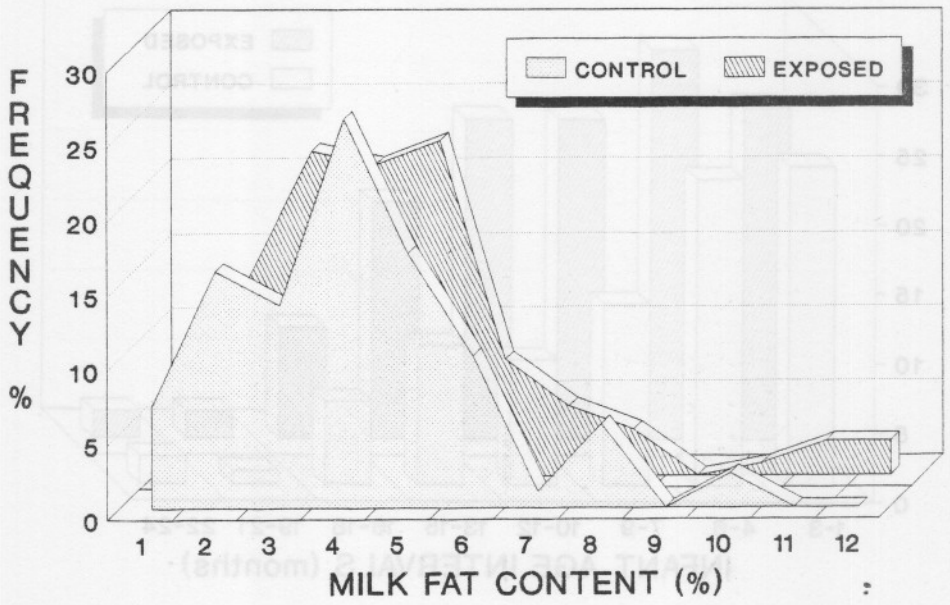

Figure 3.1.7. Frequency profile of milk fat content of breast milk for the exposed and control groups

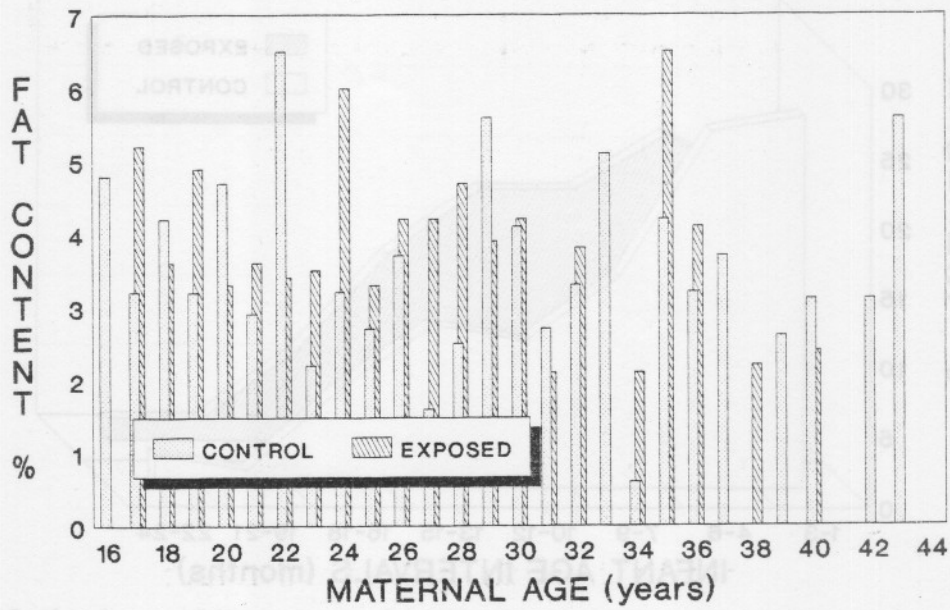

Figure 3.1.8. Percentage contribution of milk fat content of breast milk against maternal age for the exposed and control groups.

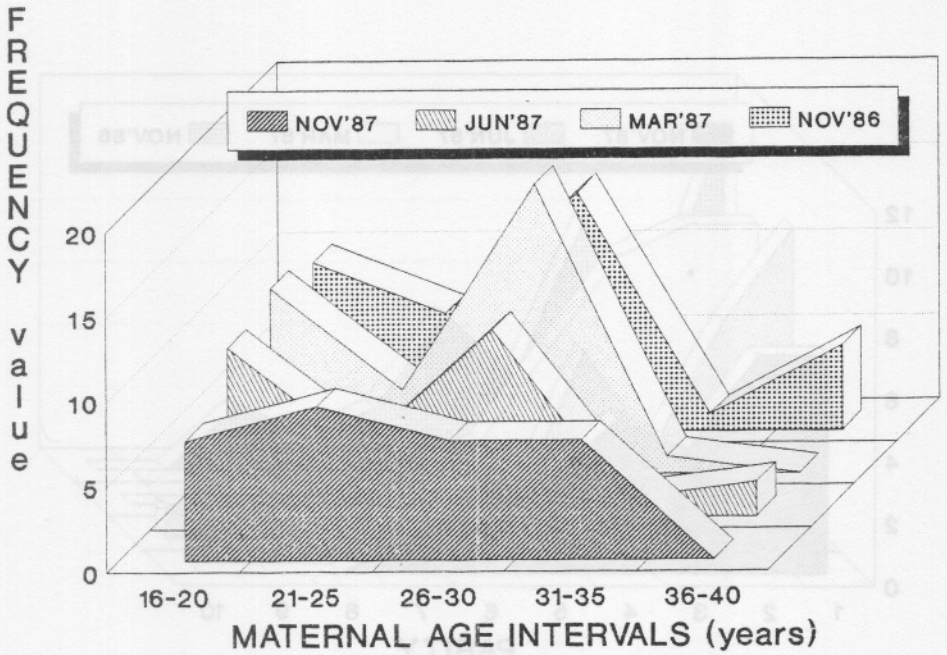

Figure 3.1.9. Frequency profile of maternal age intervals for the different surveys of the exposed group.

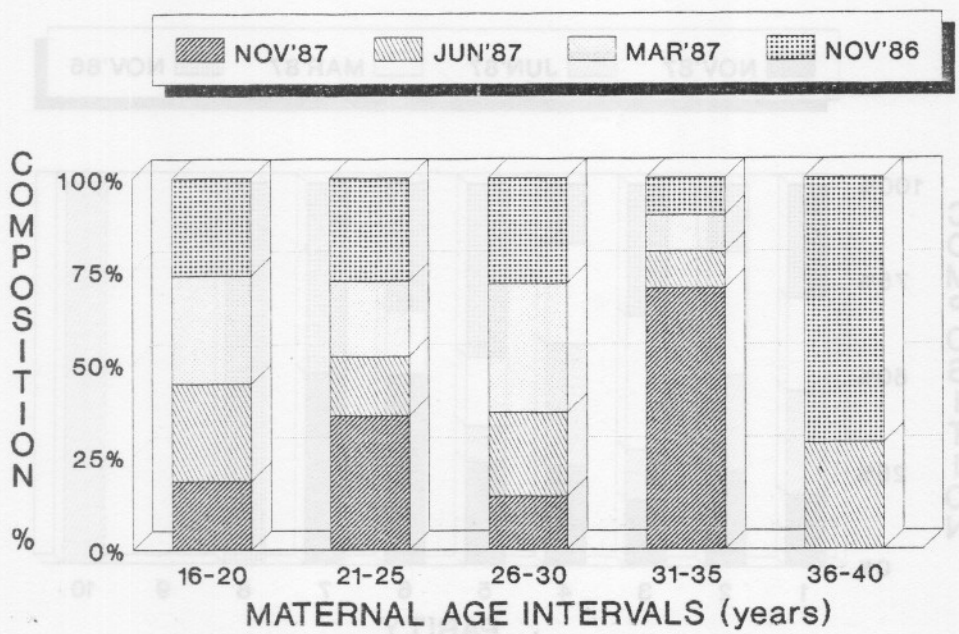

Figure 3.1.10. Percentage contribution of maternal age intervals for the different surveys of the exposed group.

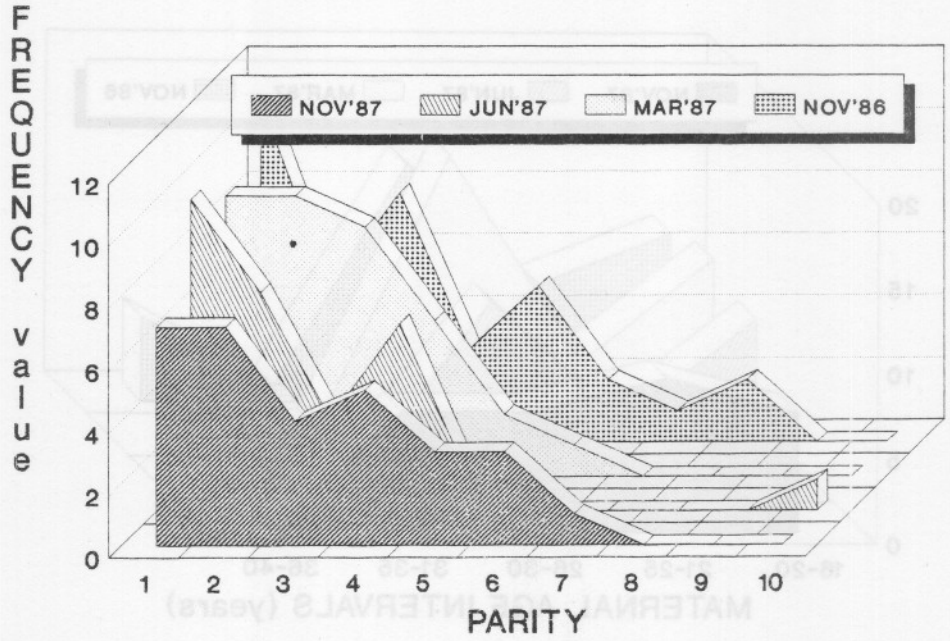

Figure 3.1.11. Frequency profile of parity for the different surveys of the exposed group.

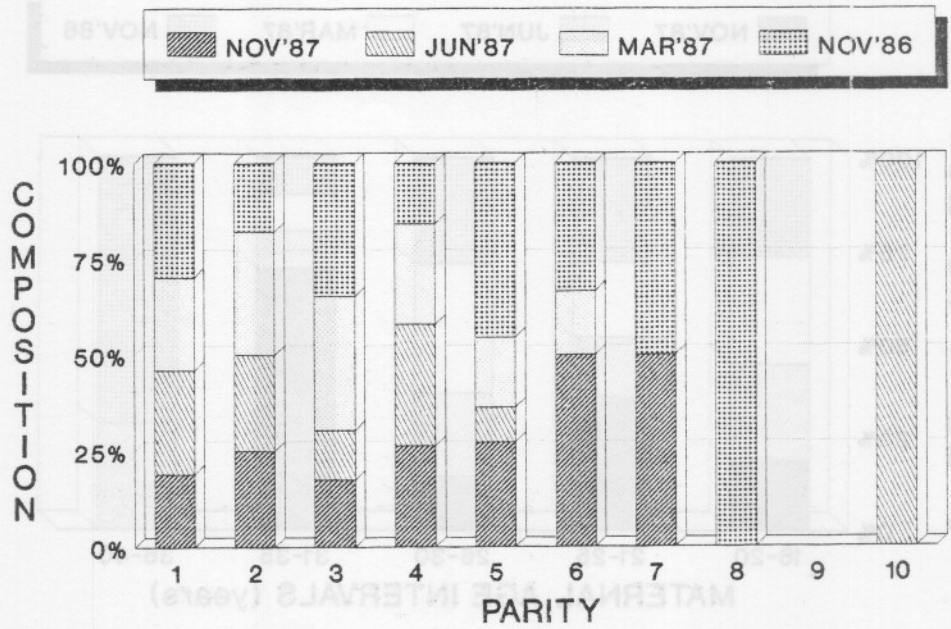

Figure 3.1.12. Percentage contribution of parity for the different surveys of the exposed group.

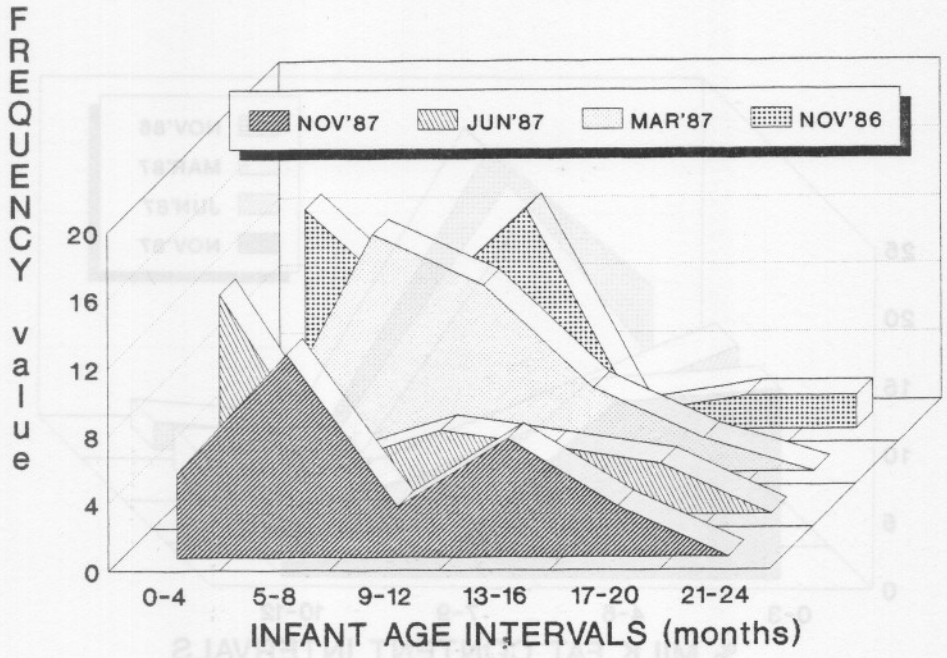

Figure 3.1.13. Frequency profile of infant age intervals for the different surveys of the exposed group.

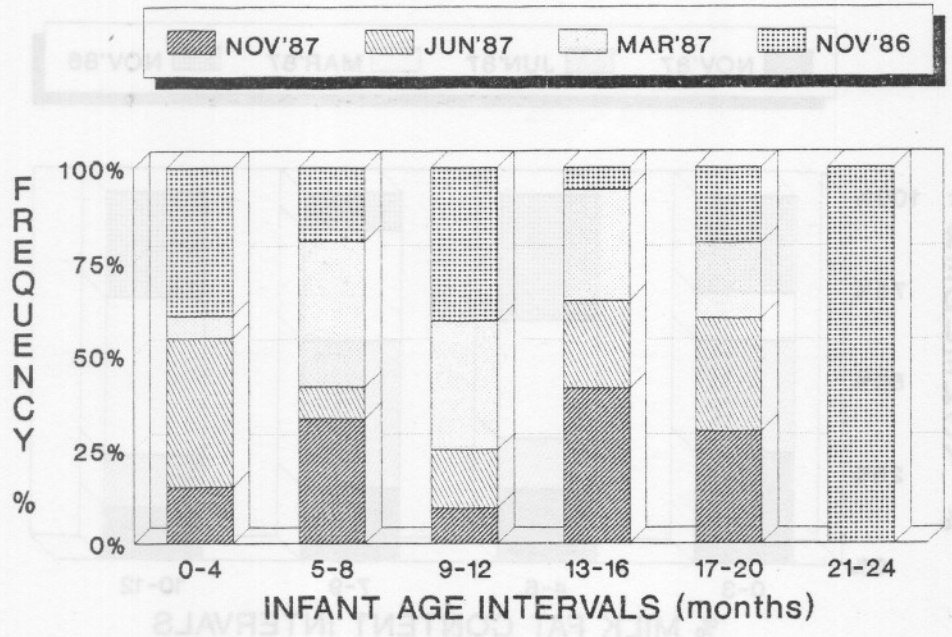

Figure 3.1.14. Percentage contribution of infant age intervals for the different surveys of the exposed group.

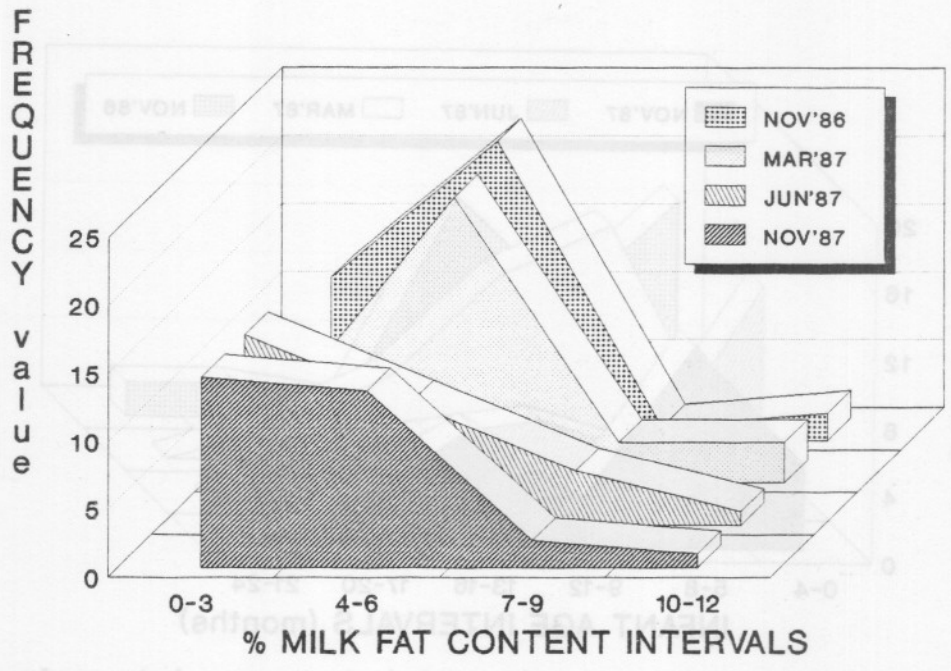

Figure 3.1.15. Frequency profile of milk fat content intervals for the different surveys of the exposed group.

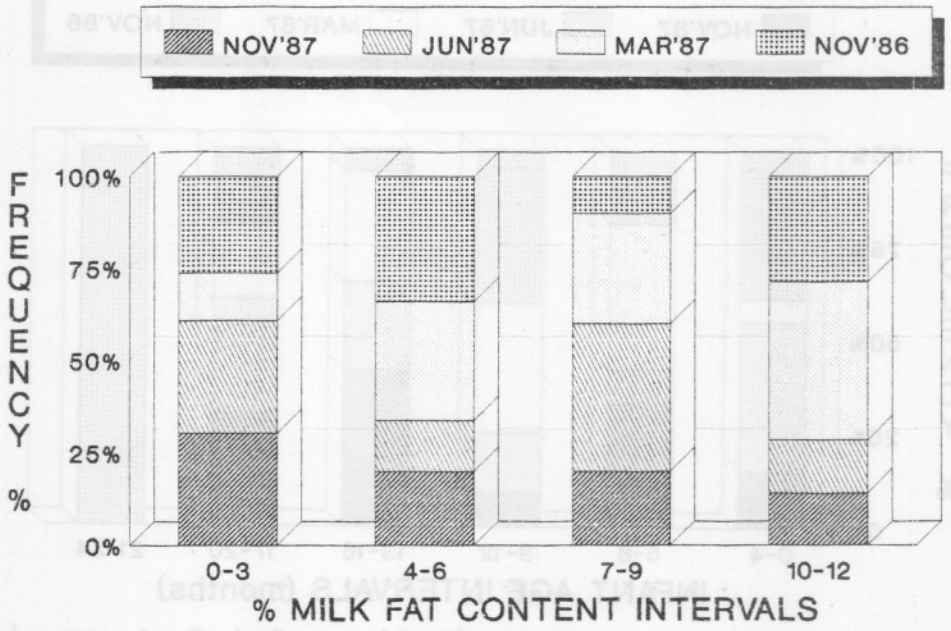

Figure 3.1.16. Percentage contribution of milk fat content intervals for the different surveys of the exposed group.

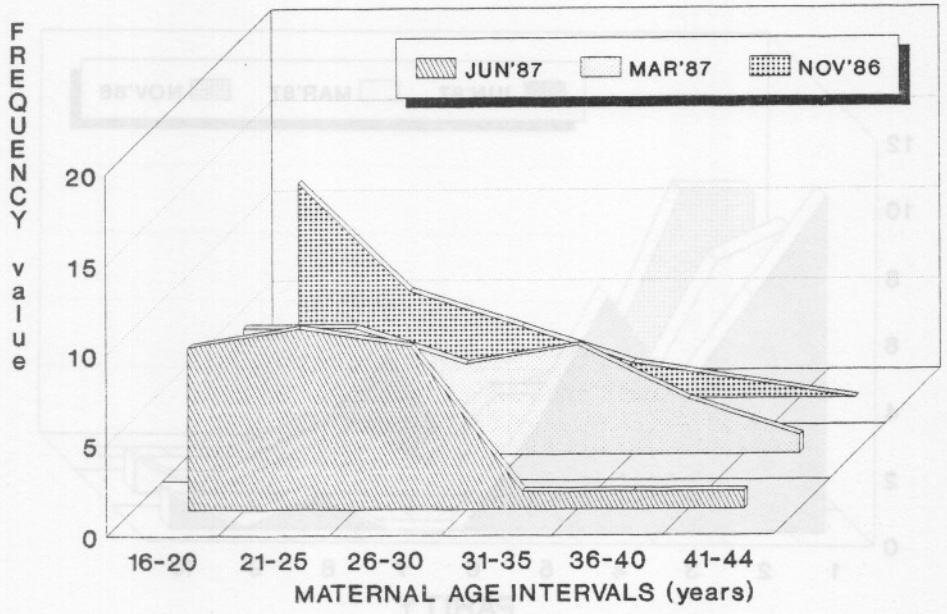

Figure 3.1.17. Frequency profile of maternal age intervals for the different surveys of the control group.

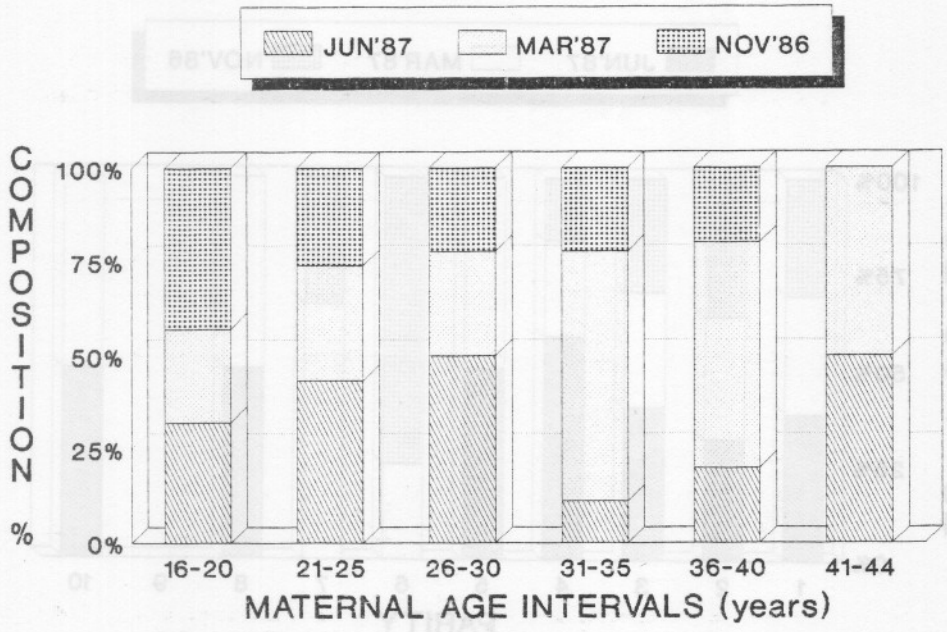

Figure 3.1.18. Percentage contribution of maternal age intervals for the different surveys of the control group.

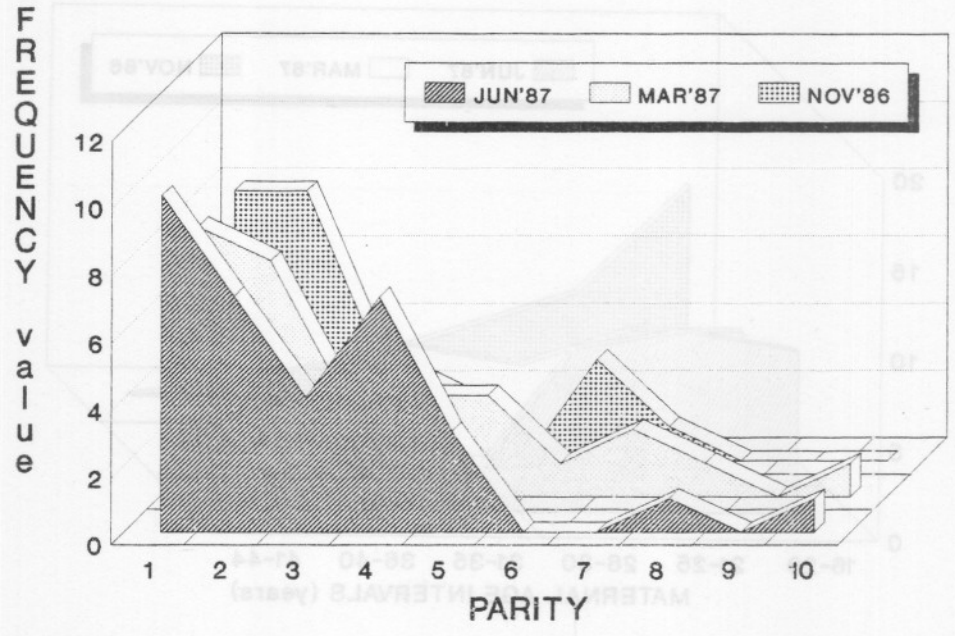

Figure 3.1.19. Frequency profile of parity for the different surveys of the control group.

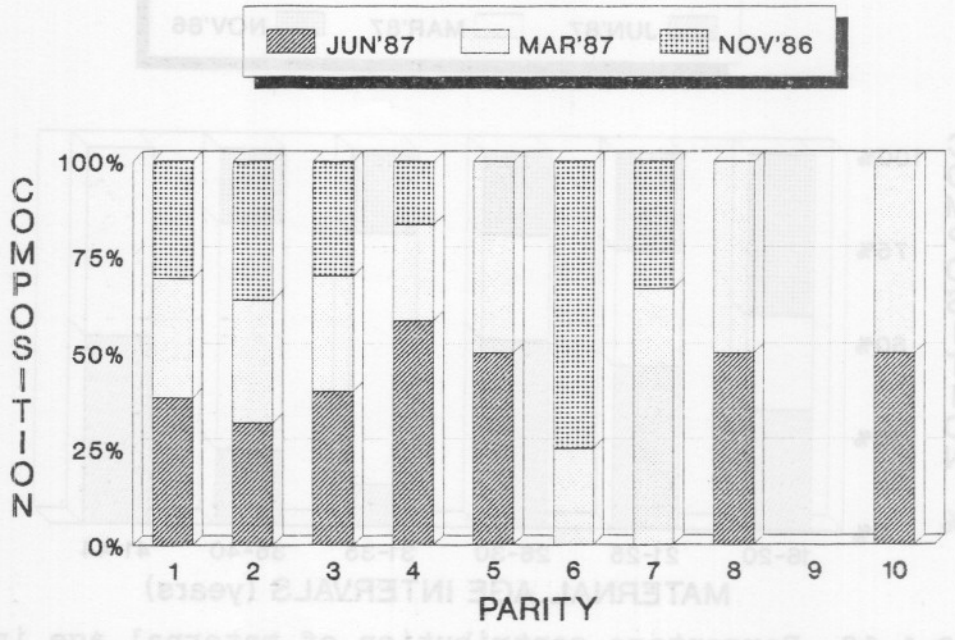

Figure 3.1.20. Percentage contribution of parity for the different surveys of the control group.

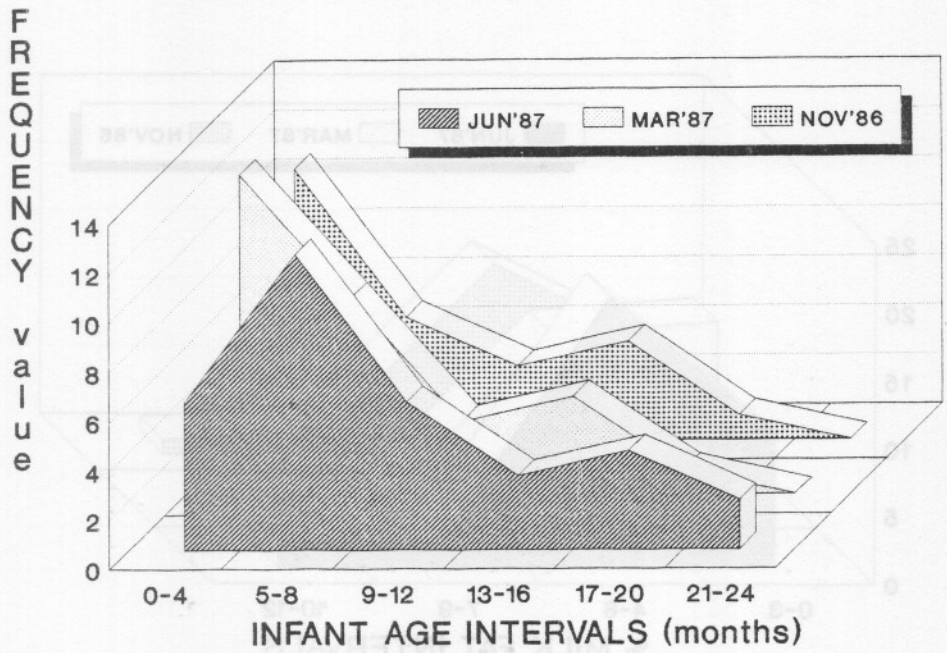

Figure 3.1.21. Frequency profile of infant age intervals for the different surveys of the control group.

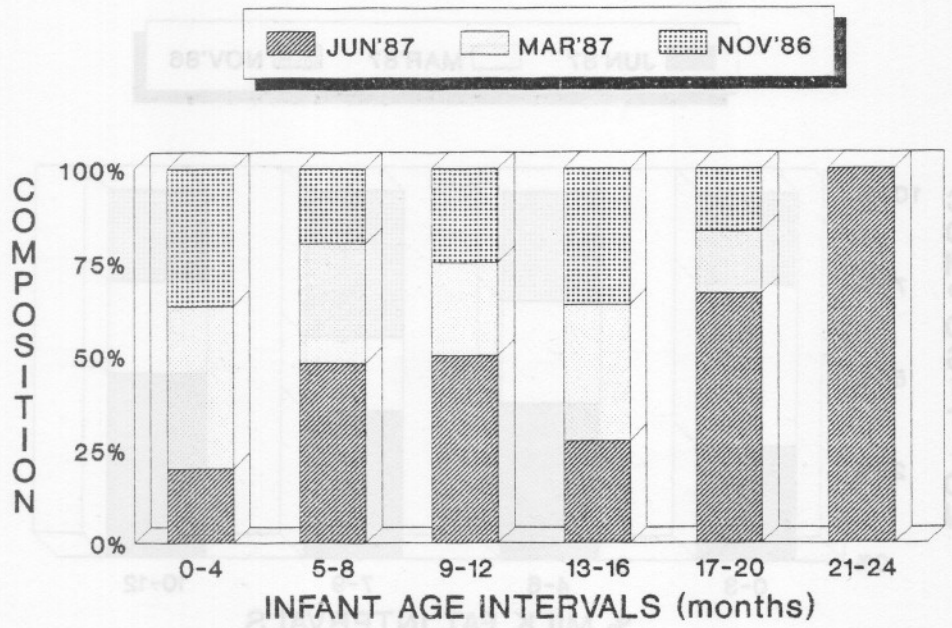

Figure 3.1.22. Percentage contribution of infant age intervals for the different surveys of the control group.

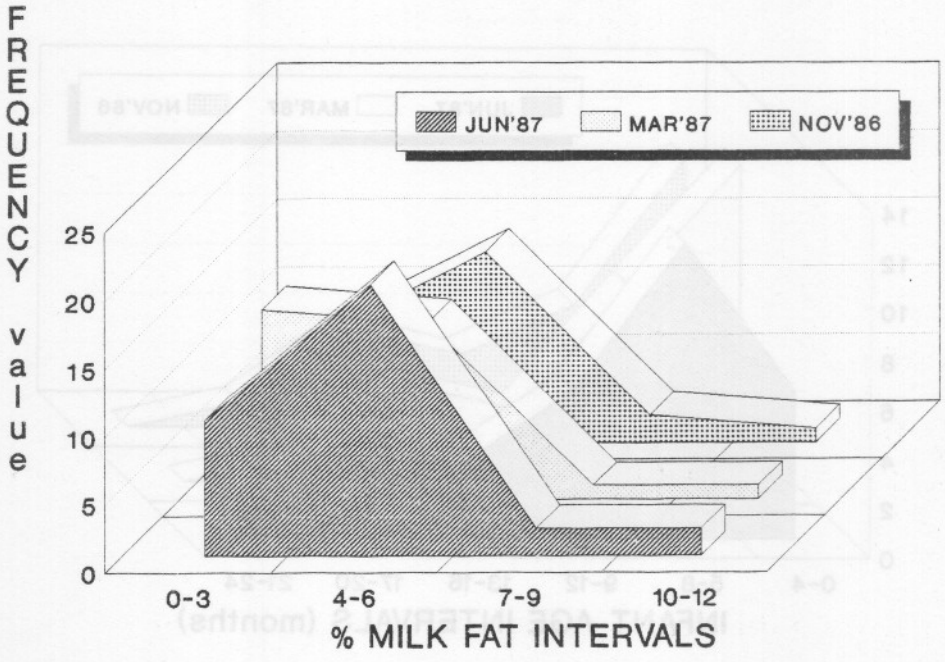

Figure 3.1.23. Frequency profile of milk fat content intervals for the different surveys of the control group.

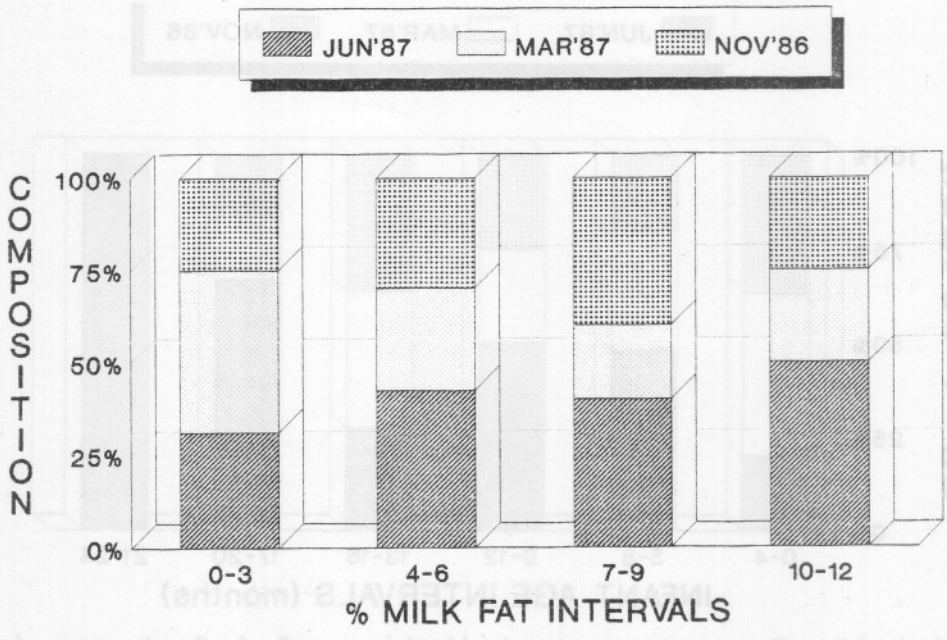

Figure 3.1.24. Percentage contribution of milk fat content intervals for the different surveys of the control group.

The maternal age profiles of the two groups were compared (Figs. 3.1.1 and Fig. 3.1.2). An obvious characteristic of the exposed group's maternal age profile (Fig. 3.1.1) was the two peaks at intervals 20-23 and 28-31 years, containing more than half of that group. This was due to the uncertainty of dates of birth of the exposed mothers, as no records were kept. The ages in many cases were estimated, and approximations to 20 and 30 years were common. Ages obtained for the control group were more accurate, because this was a more westernized society.

Figs. 3.1.3 and Fig. 3.1.4 compare the parity profile of the two groups. The frequency occurrence of parity of the control group was less than for the exposed group, corresponding to one less survey. On the percentage chart (Fig. 3.1.4) however, the profile compared very well except for an unexplained dip for mothers with three children for the control group. The combined proportion of mothers with five or more children was less than 22% for both groups.

Age profiles of breast-feeding infants of the two groups compared fairly well (Fig. 3.1.5), with some minor variations. The age interval 7-15 months was better represented in the exposed than the control group (Fig. 3.1.5). This was somewhat less obvious, but was still prominent, in the percentage composition chart (Fig. 3.1.6).

The frequency of the occurrence of percentage milk fat for both groups fell mainly between 1 and 6 percent, with almost 90% of both groups falling in this range (Fig. 3.1.7). The maxima corresponded closely to the stated means given in Table 3.1.1. There was no obvious pattern of difference in the percentage milk fat in breast milk between the two groups (Fig. 3.1.8).

A wider age interval (as compared to Figs. 3.1.1 and 3.1.2) was chosen for the maternal age profile of the exposed group (Figs. 3.1.9 and 3.1.10), as the this group was divided into four surveys with more data. The two peaks at 20 and 30 years were very obvious at the first three surveys. More care was taken at the last survey, and a better distribution, comparing well with the age profile of the control group (Fig. 3.1.2), was obtained. Fig. 3.1.10 showed the composition of the different age groups as percentages of the surveys. The relative contribution in the 16-30 years age interval was more or less evenly distributed

through the four surveys. The older mothers showed a less even distribution, but they contributed only a small percentage of the total.

The parity profile (Fig. 3.1.11) showed a varied distribution, with only the Mar'87 survey showing the expected distribution (Fig. 3.1.11). A more or less even percentage contribution to the total sample was found, up to a parity of four (Fig.3.1.12).

The infant age profiles for the four surveys of the exposed group varied, with maxima at the 0-4 months (Nov'86 and Jun'87), 5-8 months (Mar'87 and Nov'87) and 9-12 months intervals (Nov'86: Fig. 3.1.13). The same tendency of mothers to supply an approximate age was noted, which caused age intervals containing 3, 6 and 12 month old infants to be over represented. Relative contribution to the total sample was therefore uneven, with, for example, Mar'87 and Jun'87 showing reversed contribution in the 0-4 and 5-8 months age intervals (Fig. 3.1.14).

Milk fat content did not differ much between surveys, with most samples falling in the 0-6% intervals (Fig. 3.1.15). Relative contribution showed some variation (Fig. 3.1.16), but the t-test showed no significant differences ($p > 0,05$).

The maternal age information for the control group was much more accurately collected. Only one obvious peak (16-20 years for Nov'86: Fig. 3.1.17) was noted. The 31-35 years age intervals was well represented in the Mar'87 survey, which was reflected in the relative contribution chart (Fig. 3.1.18). These variations were not statistically significant ($p > 0,05$).

The parity chart (Fig. 3.1.19) showed that mothers with four children were well represented in the Jun'87 survey. This was also reflected in the relative contribution chart (Fig. 3.1.20). Contribution was comparable for mothers with one to three children, with no significant difference of the means between the surveys.

The infant age profiles showed a peak in the 5-8 months interval for the Jun'87 survey (Fig. 3.1.21). The other two surveys showed a decline, with a small increase in the 13-16 months interval. The relative contribution chart (Fig. 3.1.22) showed a large contribution of the Jun'87 survey in the 5-8, 9-12 and

17+ months age intervals, but without significant differences between surveys ($p > 0,05$).

The milk fat profiles of the control group followed very much the same pattern (Fig. 3.1.23) as that of the exposed group (Fig. 3.1.15), with the majority in the 0-6% range. The relative contribution chart (Fig. 3.1.24) showed some, but not significant ($p > 0,05$), variation.

3.2 SERIAL CHANGE OF DDT-LEVELS IN BREAST MILK

The DDT-levels in breast milk must be compared to further elucidate differences and similarities between surveys. The influence of the time factor (before and after spraying) on levels of DDT will be analyzed; this will be based either on a whole-milk basis or on a milk fat basis, and will be tabled or graphed where appropriate.

Table 3.2.1. Summary statistics of the levels of DDT and its two metabolites in the whole-milk of the exposed group. Concentration is expressed as $\mu g\ l^{-1}$.

	n	MEAN	MEDIAN	SD[a]	SE[b]	MAX	MIN
NOV'86							
DDE	39	255,4	189,9	201,8	32,3	854,3	41,7
DDD		16,9	11,7	17,7	2,8	86,0	0,0
DDT		183,1	166,0	124,2	19,9	706,5	20,1
ΣDDT		455,4	397,3	330,0	52,8	1646,9	76,4
MAR'87							
DDE	36	299,6	247,9	255,8	42,6	1275,4	12,5
DDD		5,2	4,9	4,4	0,7	17,3	0,7
DDT		273,1	268,3	131,9	22,0	561,4	25,1
ΣDDT		578,6	525,0	366,0	61,0	1831,2	37,7
JUN'87							
DDE	28	385,2	209,3	413,6	78,2	1636,1	18,5
DDD		15,8	16,4	10,6	2,0	38,6	0,0
DDT		275,8	208,4	222,9	42,1	934,2	46,0
ΣDDT		681,4	428,2	628,6	118,8	2392,3	67,6
NOV'87							
DDE	30	316,9	208,0	316,2	57,7	1758,8	67,1
DDD		14,8	12,7	9,8	1,8	43,7	1,1
DDT		230,4	204,1	170,1	31,1	967,9	35,6
ΣDDT		573,9	447,7	481,6	87,9	2743,4	144,1

[a]SD=Standard deviation [b]SE=Standard error

Table 3.2.2. Summary statistics of the levels of DDT and its two metabolites in the whole-milk of the control group. Concentration is expressed as $\mu g\ l^{-1}$.

	n	MEAN	MEDIAN	SD[a]	SE[b]	MAX	MIN
NOV'86							
DDE	25	20,98	20,70	11,83	2,36	39,90	1,50
DDD		0,04	0,00	0,19	0,04	0,93	0,0
DDT		1,74	0,90	2,47	0,49	12,50	0,0
ΣDDT		22,78	22,30	13,20	2,64	52,10	1,50
MAR'87							
DDE	29	23,81	17,60	18,91	3,51	72,70	0,10
DDD		0,05	0,00	0,17	0,03	0,69	0,0
DDT		1,34	0,70	2,43	0,45	13,10	0,0
ΣDDT		25,18	18,60	20,26	3,76	74,60	0,10
JUN'87							
DDE	34	17,94	12,25	16,01	2,75	70,20	0,60
DDD		0,0024	0,00	0,014	0,002	0,08	0,0
DDT		1,45	0,60	2,89	0,49	16,10	0,0
ΣDDT		18,95	12,90	16,70	2,86	72,80	0,60

[a]SD=Standard deviation [b]SE=Standard error

Table 3.2.3. Summary statistics on levels of DDT and its two metabolites in the milk fat of the exposed group. Concentration is expressed as mg kg^{-1} (milk fat).

	n	MEAN	MEDIAN	SD[a]	SE[b]	MAX	MIN
NOV'86							
DDE	39	6,86	5,90	4,95	0,79	18,50	0,60
DDD		0,43	0,43	0,36	0,06	1,78	0,0
DDT		4,89	4,70	2,47	0,40	11,00	0,42
ΣDDT		12,21	12,63	7,38	1,18	30,00	1,05
%DDT		42,57	42,54	8,37	1,34	67,20	25,00
MAR'87							
DDE	36	7,06	5,15	5,95	0,99	30,80	0,30
DDD		0,13	0,11	0,12	0,02	0,55	0,0
DDT		6,59	5,30	3,57	0,60	18,50	0,70
ΣDDT		13,79	14,11	9,08	1,51	44,20	1,00
%DDT		50,87	50,30	8,60	1,43	66,60	27,60
JUN'87							
DDE	28	10,95	5,50	10,96	2,07	46,90	0,50
DDD		0,58	0,46	0,53	0,10	2,14	0,0
DDT		7,93	6,40	5,80	1,10	28,80	1,40
ΣDDT		19,45	19,45	16,10	3,04	59,30	2,00
%DDT		45,85	45,79	9,84	1,86	72,60	19,20
NOV'87							
DDE	30	9,98	7,70	7,99	1,46	36,90	1,30
DDD		0,49	0,38	0,36	0,07	2,14	0,0
DDT		7,85	6,50	4,80	0,88	20,30	1,22
ΣDDT		18,34	18,34	12,60	2,30	57,60	2,70
%DDT		43,27	44,35	10,04	1,83	57,50	3,90

[a]SD=Standard deviation [b]SE=Standard error

Table 3.2.4. Summary statistics of the levels of DDT and its metabolites in the milk fat of the control group. Concentration is expressed as mg kg^{-1} (milk fat).

	n	MEAN	MEDIAN	SD[a]	SE[b]	MAX	MIN
NOV'86							
DDE	25	0,608	0,540	0,375	0,075	1,56	0,10
DDD		0,001	0,000	0,006	0,001	0,03	0,0
DDT		0,048	0,040	0,074	0,015	0,36	0,0
ΣDDT		0,658	0,550	0,411	0,082	1,56	0,12
%DDT		7,464	7,273	6,860	1,372	24,00	0,0
MAR'87							
DDE	29	0,922	0,590	0,954	0,177	4,73	0,0
DDD		0,0	0,000	0,0	0,0	0,0	0,0
DDT		0,037	0,020	0,048	0,009	0,23	0,0
ΣDDT		0,957	0,600	0,969	0,180	4,79	0,0
%DDT		4,244	2,857	5,246	0,974	4,79	0,0
JUN'87							
DDE	34	0,497	0,375	0,458	0,079	2,06	0,03
DDD		0,0	0,000	0,0	0,0	0,0	0,0
DDT		0,025	0,020	0,027	0,005	0,23	0,0
ΣDDT		0,522	0,405	0,471	0,081	2,10	0,03
%DDT		5,297	4,074	5,593	0,959	21,70	0,0

[a]SD=Standard deviation [b]SE=Standard error

DDE and DDT were detected in all samples of the exposed group. DDD was not detected in three samples of the exposed group. The control group was 100% positive for DDE, five samples were positive for DDD and 19 had no detectable DDT residues. Table 3.2.1 summarizes the DDT-levels in whole-milk of the exposed group. Standard deviations were relatively large, sometimes as large as the mean value, indicating a wide distribution. This was shown by the minimum and maximum values, as indicated. The largest range, 2599,3 $\mu g\ l^{-1}$ ΣDDT, was for the Nov'87 survey. The data were skewed, as indicated by the difference between the mean and median.

The DDT-levels of whole-milk of the control group (Table 3.2.2) were much lower, but the standard deviations were of the same order as that of the respective mean values. The largest range, 74,5 $\mu g\ l^{-1}$ ΣDDT, was for the Mar'87 survey. DDD was seldom present, with only some of the samples in the top range having detectable residues. Data were slightly skewed, as indicated by the difference between the mean and median.

The standard deviations of the means, expressed on a milk fat basis (mg kg^{-1}), for the exposed group (Table 3.2.3) did not improve much from that of the whole-milk. The highest value, 59,3 mg kg^{-1} ΣDDT, was sampled during Jun'87. Percentage DDT was also included in this table. It was only necessary to include percentage DDT in either the whole-milk or milk fat tables as percentage DDT is independent of basis of calculation. DDT comprised between 42 and 46% of the ΣDDT, except for the Mar'87 survey, when it increased to over 50%. Data were not skewed as much (considering the mean and median) as for the whole-milk (Table 3.2.1).

The values calculated for milk fat for the control group are presented in Table 3.2.4. The standard deviations, as in the case for the exposed group, did not improve from that of the whole-milk. Because of the dilution introduced by recalculating to milk fat and the change to a higher unit of reporting (μg to mg), the minimum values for DDD and DDT were 0,0 mg kg^{-1}. Skewness (considering mean and median) was also less for the data calculated on milk fat basis, as compared to whole-milk (Table 3.2.2).

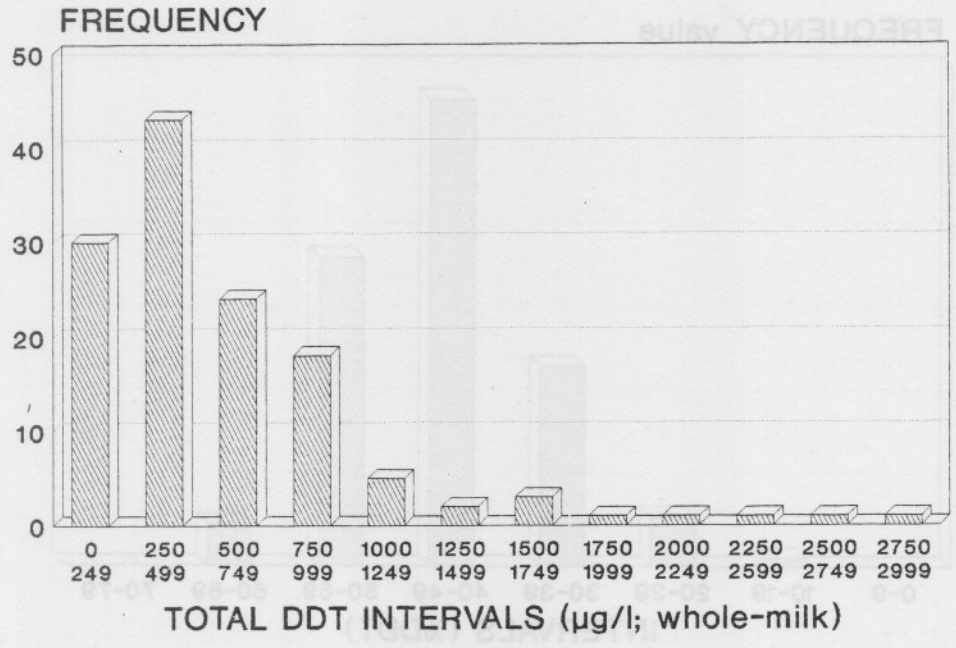

Figure 3.2.1. Frequency profile of the ΣDDT in whole-milk of the exposed group.

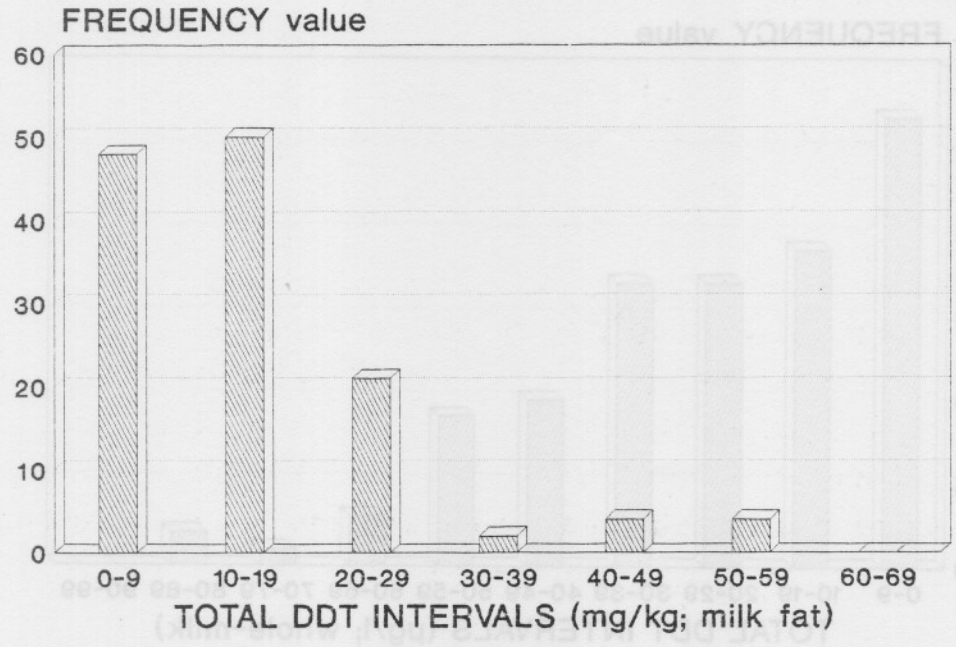

Figure 3.2.2. Frequency profile of the ΣDDT in milk fat of breast milk of the control group.

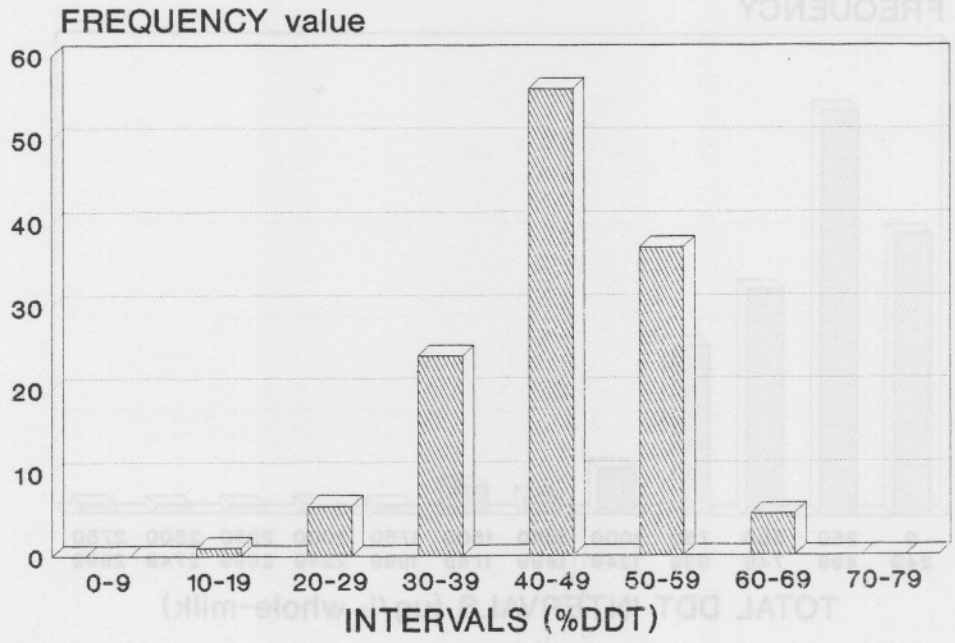

Figure 3.2.3. Frequency profile of the percentage DDT in breast milk of the exposed group.

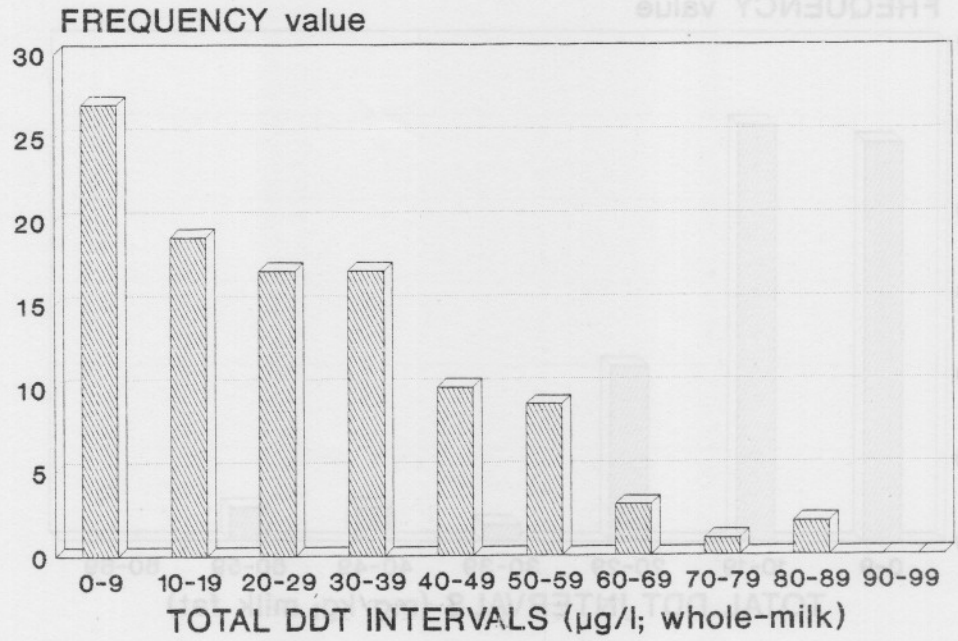

Figure 3.2.4. Frequency profile of the ΣDDT in whole-milk of breast milk of the control group.

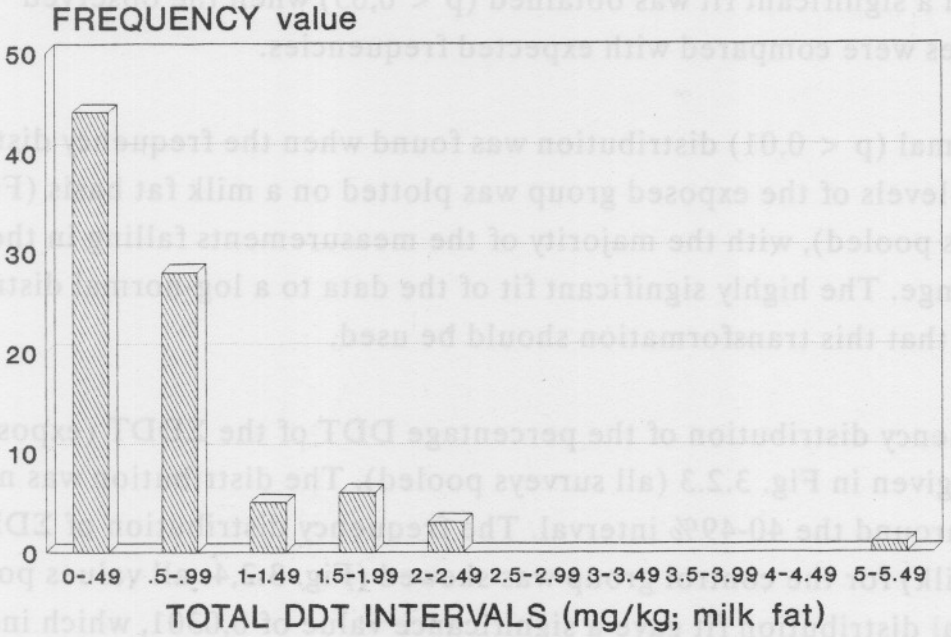

Figure 3.2.5. Frequency profile of the ΣDDT in milk fat of breast milk of the control group.

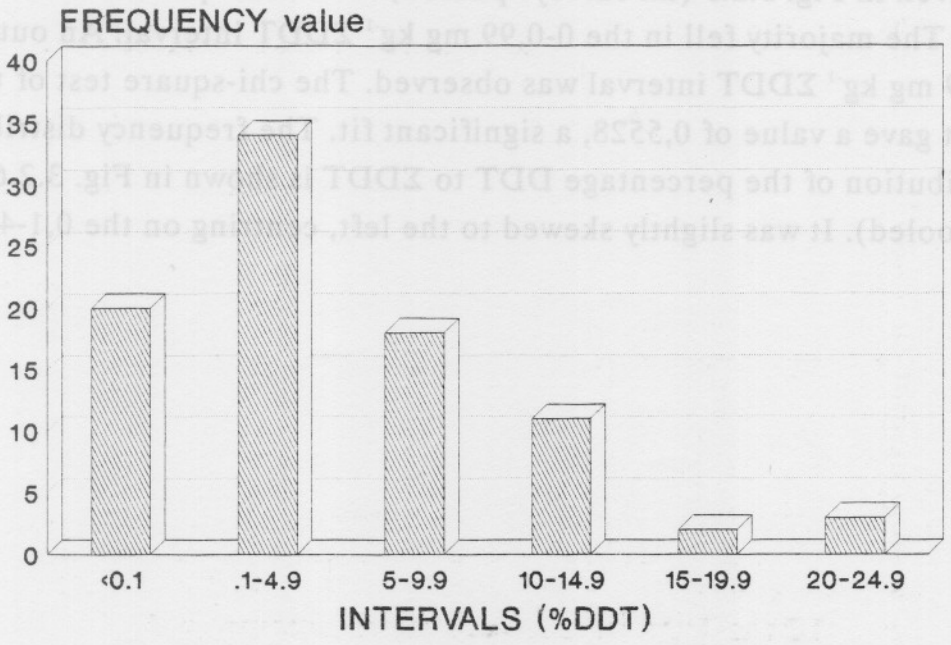

Figure 3.2.6. Frequency profile of the percentage DDT in breast milk of the control group.

Fig. 3.2.1 depicts the frequency distribution of the ΣDDT-levels in the whole-milk of the exposed group (all surveys pooled). There was a definite skewing, with the maximum frequency in the 250-499 $\mu g\ l^{-1}$ interval. The majority of measurements fell in the 0-999 $\mu g\ l^{-1}$ range. A log-normal distribution was fitted, and a significant fit was obtained ($p < 0,05$) when the observed frequencies were compared with expected frequencies.

A log-normal ($p < 0,01$) distribution was found when the frequency distribution of ΣDDT-levels of the exposed group was plotted on a milk fat basis (Fig. 3.2.2: all surveys pooled), with the majority of the measurements falling in the 0-29 $mg\ kg^{-1}$ range. The highly significant fit of the data to a log-normal distribution, indicated that this transformation should be used.

The frequency distribution of the percentage DDT of the ΣDDT (exposed group) is given in Fig. 3.2.3 (all surveys pooled). The distribution was normal, centring around the 40-49% interval. The frequency distribution of ΣDDT (whole-milk) for the control group was skewed (Fig. 3.2.4: all values pooled). A log-normal distribution fit gave a significance value of 0,0301, which indicates lack of fit. A normal distribution fit resulted in an even smaller value.

The frequency distribution plot of the ΣDDT-values calculated on a milk fat basis is given in Fig. 3.2.5 (all surveys pooled). The same picture as in Fig. 3.2.4 emerged. The majority fell in the 0-0,99 $mg\ kg^{-1}$ ΣDDT interval. An outlier in the 5-5,49 $mg\ kg^{-1}$ ΣDDT interval was observed. The chi-square test of the log-normal fit gave a value of 0,5528, a significant fit. The frequency distribution of the contribution of the percentage DDT to ΣDDT is shown in Fig. 3.2.6 (all surveys pooled). It was slightly skewed to the left, centring on the 0,1-4,9% interval.

Table 3.2.5. Comparison of log transformed single variables between the exposed and control group using t-tests. Percentage DDT was not transformed. Only positive cases were included.

VARIABLE	LEVENE	P-VALUE
NOV'86		
DDE[a]	0,7949	0,0000
DDD[a]	1,0000	0,0126
DDT[a]	0,2485	0,0000
ΣDDT[a]	0,7114	0,0000
DDE[b]	0,2865	0,0000
DDD[b]	1,0000	0,0261
DDT[b]	0,0664	0,0000
ΣDDT[b]	0,7421	0,0000
%DDT[b]	0,5989	0,0000
MAR,87		
DDE[a]	0,0543	0,0000
DDD[a]	0,2927	0,0002
DDT[a]	0,0559	0,0000
ΣDDT[a]	0,0155	0,0000
DDE[b]	0,1897	0,0000
DDD[b]	NC	NC
DDT[b]	0,0081	0,0000
ΣDDT[b]	0,0334	0,0000
%DDT[b]	0,0232	0,0000
JUN'87		
DDE[a]	0,3941	0,0001
DDD[a]	1,0000	0,0000
DDT[a]	0,2364	0,0000
ΣDDT[a]	0,8473	0,0000
DDE[b]	0,5848	0,0000
DDD[b]	NC	NC
DDT[b]	0,4604	0,0000
ΣDDT[b]	0,7939	0,0000
%DDT[b]	0,0634	0,0000

a=whole-milk basis
b=milk fat basis

NC = not calculable

Table 3.2.6. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for Ln DDE ($\mu g\ l^{-1}$: whole-milk) for the exposed group.

LEVENE	0,0244				
WELCH	0,5730				
T-TEST		NOV'86	VS		
				MAR'87	0,3591
				JUN'87	0,4110
				NOV'87	0,2273
BONFERRONI					
SIGNIFICANCE		MAR'87	VS		
NOTATION				JUN'87	0,9734
***	0,001			NOV'87	0,7410
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,7317

Table 3.2.7. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for Ln DDD ($\mu g\ l^{-1}$: whole-milk) for the exposed group.

LEVENE	0,5050				
WELCH	0,0000				
T-TEST		NOV'86	VS		
				MAR'87	0,0000 **
				JUN'87	0,7927
				NOV'87	0,9869
BONFERRONI					
SIGNIFICANCE		MAR'87	VS		
NOTATION				JUN'87	0,0000 ***
***	0,001			NOV'87	0,0000 ***
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,8148

Table 3.2.8. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for Ln DDT ($\mu g\ l^{-1}$: whole-milk) for the exposed group.

LEVENE	0,1245				
WELCH	0,0297				
T-TEST		NOV'86	VS		
				MAR'87	0,0044 *
				JUN'87	0,0866
				NOV'87	0,1516
BONFERRONI					
SIGNIFICANCE		MAR'87	VS		
NOTATION				JUN'87	0,3377
***	0,001			NOV'87	0,1979
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,7687

Table 3.2.9. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for Ln ΣDDT ($\mu g\ l^{-1}$: whole-milk) for the exposed group.

LEVENE	0,0246				
WELCH	0,3008				
T-TEST		NOV'86	VS		
				MAR'87	0,1053
				JUN'87	0,2234
				NOV'87	0,1461
BONFERRONI					
SIGNIFICANCE		MAR'87	VS		
NOTATION				JUN'87	0,7695
***	0,001			NOV'87	0,9295
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,8433

Table 3.2.10. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for Ln DDE (mg kg^{-1}: milk fat) for the exposed group.

LEVENE	0,1374				
WELCH	0,1364				
T-TEST		NOV'86	VS		
				MAR'87	0,8127
				JUN'87	0,1456
				NOV'87	0,0534
BONFERRONI SIGNIFICANCE NOTATION		MAR'87	VS		
				JUN'87	0,2241
***	0,001			NOV'87	0,0928
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,6739

Table 3.2.11. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for Ln DDD (mg kg^{-1}: milk fat) for the exposed group.

LEVENE	0,8645				
WELCH	0,0000				
T-TEST		NOV'86	VS		
				MAR'87	0,0000 ***
				JUN'87	0,1812
				NOV'87	0,4802
BONFERRONI SIGNIFICANCE NOTATION		MAR'87	VS		
				JUN'87	0,0000 ***
***	0,001			NOV'87	0,0000 ***
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,5137

Table 3.2.12. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for Ln DDT (mg kg^{-1}: milk fat) for the exposed group.

LEVENE	0,5957				
WELCH	0,0280				
T-TEST		NOV'86	VS		
				MAR'87	0,0263
				JUN'87	0,0168
				NOV'87	0,0077 *
BONFERRONI					
SIGNIFICANCE		MAR'87	VS		
NOTATION				JUN'87	0,4644
***	0,001			NOV'87	0,4306
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,8775

Table 3.2.13. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for Ln ΣDDT (mg kg^{-1}: milk fat) for the exposed group.

LEVENE	0,2685				
WELCH	0,0955				
T-TEST		NOV'86	VS		
				MAR'87	0,3793
				JUN'87	0,0492
				NOV'87	0,0237
BONFERRONI					
SIGNIFICANCE		MAR'87	VS		
NOTATION				JUN'87	0,2554
***	0,001			NOV'87	0,1569
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,8075

Table 3.2.14. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for percentage DDT of the ΣDDT for the exposed group.

LEVENE	0,000				
WELCH	0,0006				
T-TEST		NOV'86	VS		
				MAR'87	0,0001 ***
				JUN'87	0,1586
				NOV'87	0,7585
BONFERRONI SIGNIFICANCE NOTATION		MAR'87	VS		
				JUN'87	0,0371
***	0,001			NOV'87	0,0019 *
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,3280

Table 3.2.15. P-values associated with the Levene's test for equal variance, the Welch test to compare the means for the four surveys and pair-wise t-tests (separate variance) for percentage DDD of the ΣDDT for the exposed group.

LEVENE	0,0000				
WELCH	0,0000				
T-TEST		NOV'86	VS		
				MAR'87	0,0000 ***
				JUN'87	0,1899
				NOV'87	0,6527
BONFERRONI SIGNIFICANCE NOTATION		MAR'87	VS		
				JUN'87	0,0001 ***
***	0,001			NOV'87	0,0005 **
**	0,01				
*	0,05	JUN'87	VS		
				NOV'87	0,5345

Table 3.2.5 shows the significance of the differences of all the DDT and metabolite parameters between the two groups. All p-values, except for DDD of the Nov'86 survey, were less than 0,01, indicating the highly significant differences between the two groups.

Tables 3.2.6 to 3.2.15 examine the serial changes in the log transformed levels of DDE, DDD, DDT and percentage DDT between the surveys for the exposed group. The Levene's test was employed to test whether the variances of the surveys were significantly different. If the variances were not different, then the means were compared with one-way analysis of variance; otherwise the test of Welch was used. Specific differences between surveys were tested for, by making use of an approximate t-test, at the Bonferroni adjusted level of significance. A p-value of less than 0,00833 proves significance at the 5% level, less than 0,00166 proves significance at the 1% level and less then 0,000166 prove significance at the 0,1% level. A p-value of less than 0,05, 0,01 or 0,001 only indicates, but does not prove, significant difference.

No significant serial changes ($p > 0,05$) in the levels of DDE (whole-milk) between the surveys were found (Table 3.2.6). The high standard deviation for the Jun'87 survey (%CV = 107,0) was the reason that the difference in mean values between Nov'86 and Jun'87 (a difference of 129,8 $\mu g\ l^{-1}$ DDE or 33,7% of the Jun'87 level) was not indicated as significant ($p > 0,05$).

Significant serial changes ($p < 0,00166$) in the levels of DDD (whole-milk) were found (Table 3.2.7). They occurred between the Mar'87 survey and the Nov'86, Jun'87 and Nov'87 surveys. It is obvious from the means table (Table 3.2.1) that the mean for DDD of the Mar'87 survey was about a third of the other surveys. The means for the surveys before and after the Mar'87 survey, which was done directly after spraying, were significantly higher ($p < 0,00166$: Table 3.2.7).

One significant serial change ($p < 0,00833$) was found for DDT (whole-milk: Table 3.2.8). That was between the Nov'86 and the Mar'87 surveys. However, these were not the two surveys with the largest difference. The mean for Jun'87 was slightly more (275,8 $\mu g\ l^{-1}$ DDT) than that for Mar'87 (273,1 $\mu g\ l^{-1}$ DDT:

Table 3.2.1). A p-value for the difference of the means between Nov'86 and Jun'87 of 0,0866 also lacked significance ($p > 0,05$). The standard deviations were again the reason for the lack of significance, with a %CV for Mar'87 of 48,3% and for Jun'87 of 80,8%.

No significant serial changes ($p > 0,05$) were indicated for ΣDDT (whole-milk: Table 3.2.9). The lowest p-value was 0,1053, between Nov'86 and Mar'87, did not indicate significance. The large %CV for the Mar'87 survey (92,3%) probably prevented a more significant result.

As was the case for whole-milk (Table 3.2.6), DDE (milk fat: Table 3.2.10) showed no significant serial changes ($p > 0,05$). Compared with Nov'86 and Mar'87, Jun'87 had p-values of 0,1456 and 0,2241, not indicating significance.

The same combination of significant serial changes for DDD (whole-milk: Table 3.2.7) was found for the DDD (milk fat: Table 3.2.11).

As opposed to the significant serial changes found in means for DDT between Nov'86 and Mar'87 surveys (whole-milk: Table 3.2.8), this change was only indicated ($p < 0,05$) for the comparisons based on milk fat (Table 3.2.12). There was a significant change between Nov'86 and Nov'87 ($p = 0,0077$). The mean for Nov'87 (37,6%) was higher than the year before. There was a possible significant difference between the Nov'86 and the Nov'87 and Jun'87 surveys ($p < 0,05$).

The increase in DDT (milk fat) was not enough to make the serial changes between the same two surveys for ΣDDT significant (Table 3.2.13). The mean for the Nov'87 survey (18,34 mg kg^{-1} ΣDDT) was 33,3% higher than the mean for Nov'86 (12,21 mg kg^{-1} ΣDDT: Table 3.2.3). The changes in mean ΣDDT between Nov'86 and Jun'87 and Nov'86 and Nov'87 were possibly significant ($p < 0,05$).

The serial changes in percentage DDT between the surveys must be noted (Table 3.2.14). The mean for Nov'86 (42,57%) differed significantly ($p = 0,0001$) from the mean (50,87%) for the survey just after spraying (Mar'87). The same survey (Mar'87) also differed significantly from the mean of Nov'87 (mean =

43,27: p = 0,0019) but not from the survey after Mar'87 which was the Jun'87 survey (mean = 45,85: p = 0,0371).

The percentage DDD for Mar'87 was, as can be expected from the results from Table 3.2.7, also significantly different from the other three surveys. The low percentage for Mar'87 (2,01%) was more than three times less than any of the other surveys (Nov'86 = 9,96%: Jun'87 = 7,67%: Nov'87 = 8,99%).

The serial change in levels and percentages is illustrated in Figures 3.2.7 to 3.2.12. This, in conjunction with the relevant tables, will clearly illustrate the changes that occurred with time.

No statistics for the control group were included, since no significant serial changes were found for all the variables included in the above series between the Nov'86, Mar'87 and Jun'87 surveys.

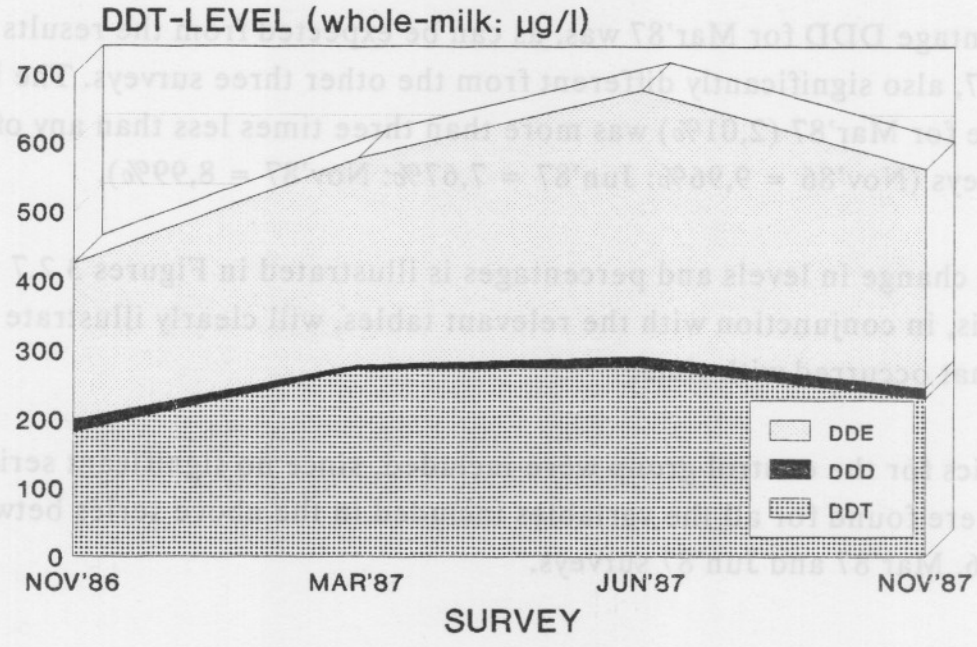

Figure 3.2.7. Serial change in the mean levels of DDT, DDE and DDD in whole-milk of the exposed group.

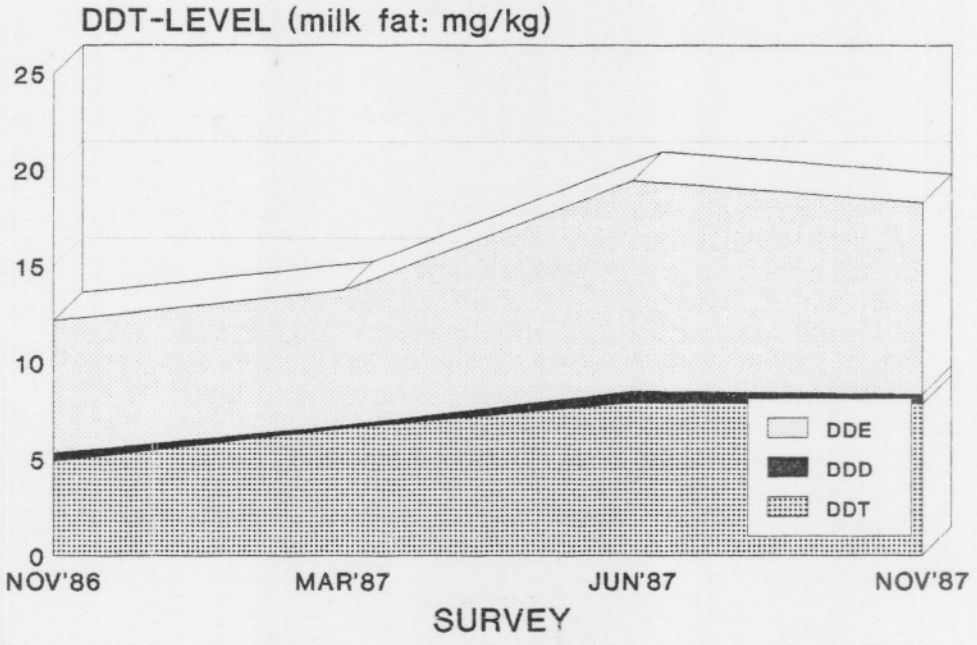

Figure 3.2.8. Serial change in the mean levels of DDT, DDE and DDD in milk fat of the exposed group.

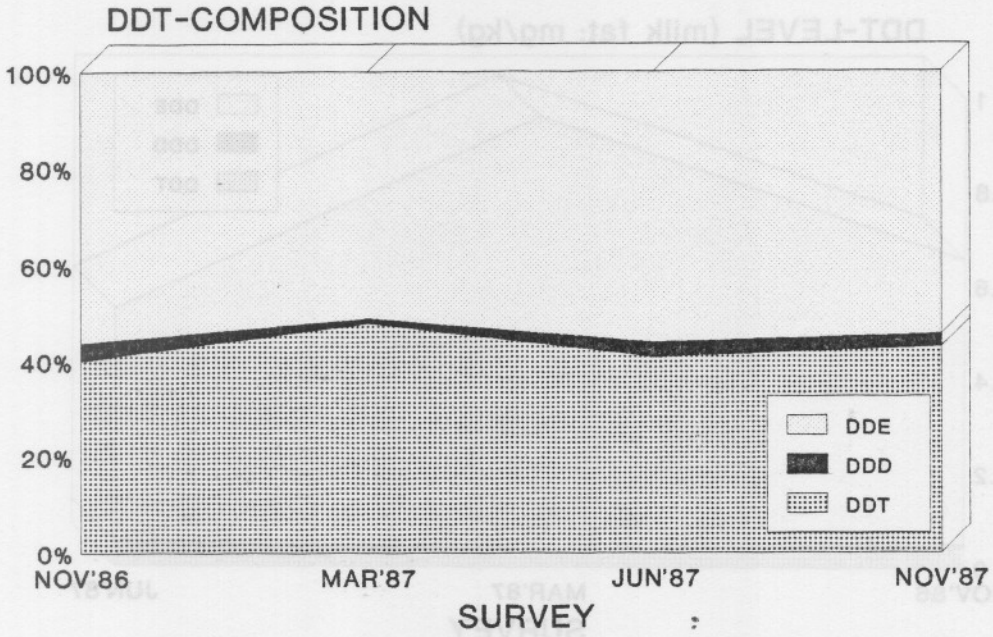

Figure 3.2.9. Serial change in mean percentage contribution of the various isomers to the ΣDDT-levels in milk of the exposed group.

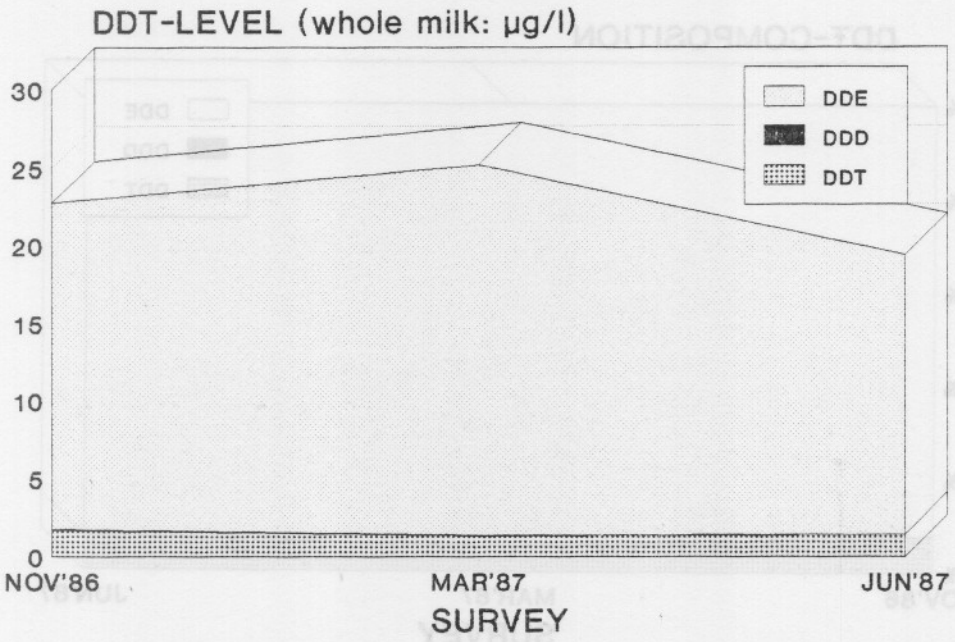

Figure 3.2.10. Serial change in the mean levels of DDT, DDE in whole-milk of the control group.

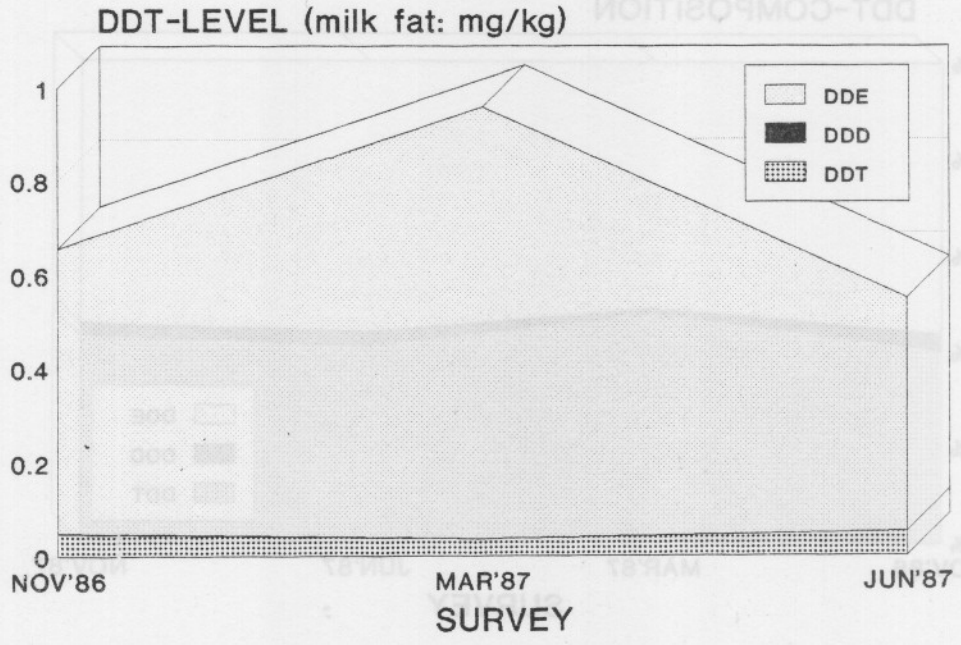

Figure 3.2.11. Serial change in the mean levels of DDT, DDE and DDD in milk fat of the control group.

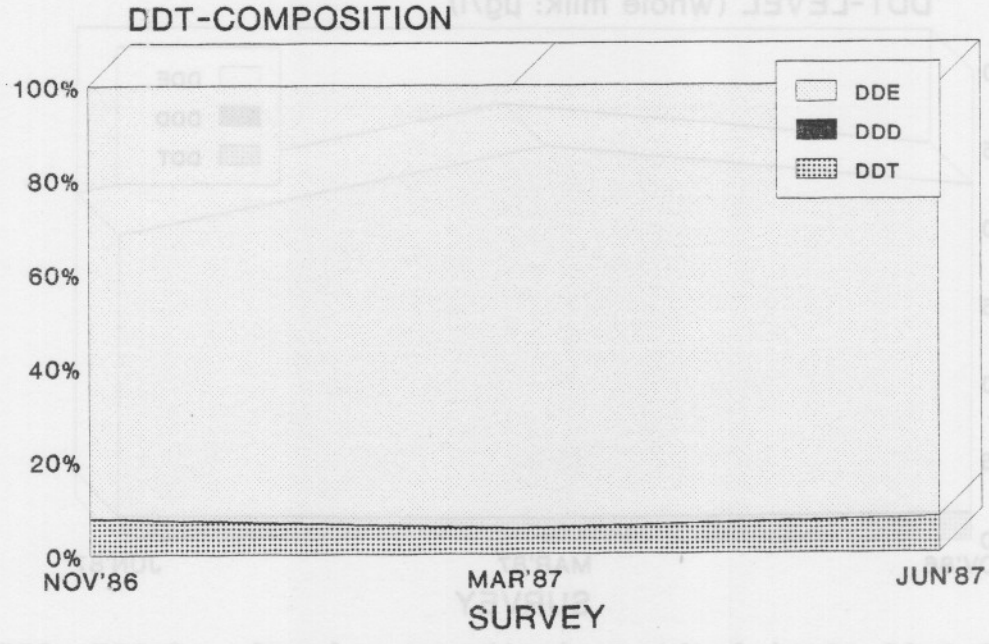

Figure 3.2.12. Serial change in the mean percentage contribution of the various isomers to the ΣDDT-levels in milk of the control group.

Fig. 3.2.7 shows that the mean levels of DDE and DDT (whole-milk) increased from Nov'86 to Jun'87 and then decreased slightly towards the end of the year (Nov'87). DDT-levels reached almost a plateau between Mar'87 and Jun'87. DDD-levels did not show any appreciable changes during the twelve months in question. The same general trend was found when the milk fat values were plotted (Fig. 3.2.8). The only obvious difference was that the DDT-level did not show a plateau between Mar'87 and Jun'87, but later, between Jun'87 and Nov'87.

The serial change in percentage composition of the various components of ΣDDT is graphically presented in Fig. 3.2.9. It shows the increase in percentage DDT for Mar'87, the survey period just after spraying operations ceased. Percentage DDD did not show any marked changes. Percentage DDE decreased at Mar'87 as a function of the increased percentage DDT, but returned to, it seemed, a more stable level of slightly less than 60%.

The serial chart based on whole-milk for the control group is presented in Fig. 3.2.10. No DDD was apparent because of the very low absolute values. No change in DDT levels was found. The picture based on milk fat (Fig. 3.2.11) was almost identical to that found with whole-milk (Fig. 3.2.10). Again, no DDD was apparent. The percentage contribution of the different components, as could be expected from the previous two figures, did not change serially.

3.3 FACTORS INFLUENCING DDT-LEVELS IN BREAST MILK

Table 3.2.12 indicated that, except for one instance where the DDT-levels (milk fat) of the exposed group were significantly higher than for the other sampling periods, no such differences were reflected in the ΣDDT (Table 3.2.13). The mean DDT-level of Nov'87 (exposed group) did not differ significantly from the mean of the pooled data of the exposed group (t-test, $p = 0{,}2278$). It was also shown that the relative contribution of DDD to the ΣDDT was very small (see sub-section 3.2). Therefore it was possible to pool all data to determine which factors influenced the levels of the DDE, DDT, ΣDDT and percentage DDT. DDD was not included because of the very low levels, and will only be referred to when necessary.

Chi-square analysis of two by two tables for the frequency of samples with detectable amounts of DDT, DDE and DDD were performed. Both the frequencies for detectable amounts of DDT and DDE were significantly different between the two groups ($p < 0{,}001$). DDE was found in all samples of the exposed and control groups. Only data calculated on a milk fat basis was used. This was done to facilitate comparison with other papers on this subject, as levels calculated on milk fat seemed to be the preferred reference base. Only ΣDDT and percentage DDT were examined for the control group, as the individual components were present in low levels or were, in the case of DDD and DDT, mostly below detection limits.

Regression analysis and analysis of variation (anova) results are presented together for ΣDDT, percentage DDT, maternal age and infant age variables. The anova presentation gives a clearer picture of the relationship between the means of intervals or sub-groups such as parity. The analysis of variation for ΣDDT of the control group was included to determine whether the same influence as for the exposed group was present, i.e. whether the same relationships were present. The coefficient of determination (to be indicated by "CD") and correlation coefficient (to be indicated by"CC") are displayed within the graphs where appropriate.

Non-linear regression was also performed on the parity and maternal age variables, as such a relationship seemed implied from the anova. The estimated coefficients were derived with the BMDP5R polynomial regression analysis program for the interval means (designated as "means" in the graphs) only. The second order polynomial regression was chosen, and the derived values entered as estimates. The Statgraphics non-linear regression program, through iteration, estimated the coefficients for the raw data (designated as "RD" in the graphs) in order to minimize the residual sum of squares (Statistical Graphics Corporation, 1987). Non-linear regression was not done for infant age, as only four intervals were selected and the BMDP5R program needed five values. Splitting the last interval in two would have weighted the fifth interval too much (mean calculated on four values) to be of any help.

The factors influencing DDT-levels, such as maternal and infant age and parity, seemed, at first glance, to be categorical. Anova can only be done on numeric variables. Parity and age measures are numeric and ratio scale variables, not non-metric variables, therefore the relevance of anova. Ages have been categorized to facilitate meaningful anova. Infant age was categorized quarterly, the first quarter presumably covering the time of highest breast-feeding frequency. Maternal age was categorized for five year intervals, the first category covering the most likely age of primiparous mothers.

Table 3.3.1. Summary statistics for pooled data of the exposed group.

	DDE[a]	DDD[a]	DDT[a]
SAMPLE SIZE	129	129	129
MEAN	315,5	15,98	242,21
MEDIAN	209,9	10,38	203,7
STANDARD DEVIATION	298,6	25,42	164,6
STANDARD ERROR	26,29	2,24	14,49
MINIMUM	18,5	0	20,1
MAXIMUM	1758,8	226,72	967,9
RANGE	1740,3	226,72	947,8

	ΣDDT[a]	DDE[b]	DDD[b]
SAMPLE SIZE	129	129	129
MEAN	574,89	8,65	0,400
MEDIAN	427,5	6,2	0,3
STANDARD DEVIATION	454,16	7,67	0,398
STANDARD ERROR	39,99	0,67	0,035
MINIMUM	67,6	0,5	0
MAXIMUM	2743,4	46,9	2,14
RANGE	2675,8	46,4	2,14

	DDT[b]	ΣDDT[b]	%DDT[b]
SAMPLE SIZE	129	129	129
MEAN	6,77	15,83	45,71
MEDIAN	5,8	12,8	45,83
STANDARD DEVIATION	4,31	11,62	8,77
STANDARD ERROR	0,38	1,02	0,77
MINIMUM	0,42	1,05	19,11
MAXIMUM	28,8	59,3	70
RANGE	28,38	58,25	50,89

[a] Whole-milk: $\mu g\ l^{-1}$

[b] Milk fat: $mg\ kg^{-1}$

Table 3.3.2. Summary statistics for pooled data of the control group.

	DDE[a]	DDD[a]	DDT[a]
SAMPLE SIZE	88	88	88
MEAN	20,74	0,03	1,30
MEDIAN	18	0	0,8
STANDARD DEVIATION	16,03	0,14	2,07
STANDARD ERROR	1,71	0,02	0,22
MINIMUM	0,1	0	0
MAXIMUM	72,7	0,93	13,1
RANGE	72,6	0,93	13,1

	ΣDDT[a]	DDE[b]	DDD[b]
SAMPLE SIZE	88	88	88
MEAN	22,07	0,65	0
MEDIAN	19,15	0,47	0
STANDARD DEVIATION	17,08	0,665	0
STANDARD ERROR	1,82	0,071	0
MINIMUM	0,1	0	0
MAXIMUM	74,6	4,73	0,03
RANGE	74,5	4,73	0,03

	DDT[b]	ΣDDT[b]	%DDT[b]
SAMPLE SIZE	88	88	88
MEAN	0,04	0,69	5,59
MEDIAN	0,02	0,49	3,96
STANDARD DEVIATION	0,05	0,68	5,94
STANDARD ERROR	0,0005	0,07	0,63
MINIMUM	0	0	0
MAXIMUM	0,36	4,8	24
RANGE	0,36	4,8	24

[a] Whole-milk: $\mu g\ l^{-1}$

[b] Milk fat: $mg\ kg^{-1}$

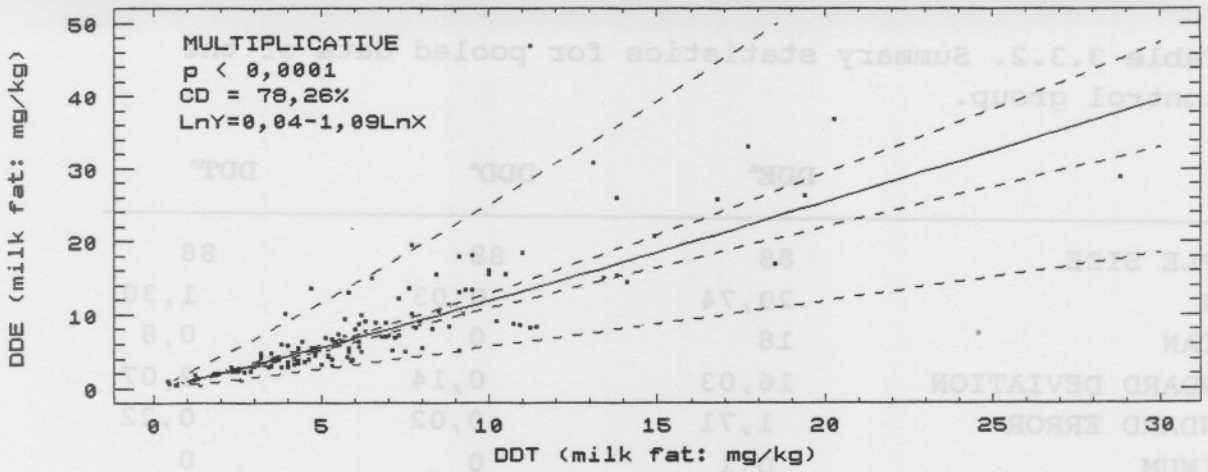

Figure 3.3.1. Relationship between the levels of DDT and DDE in milk fat of breast milk of the exposed group. 95% confidence and prediction limits are shown.

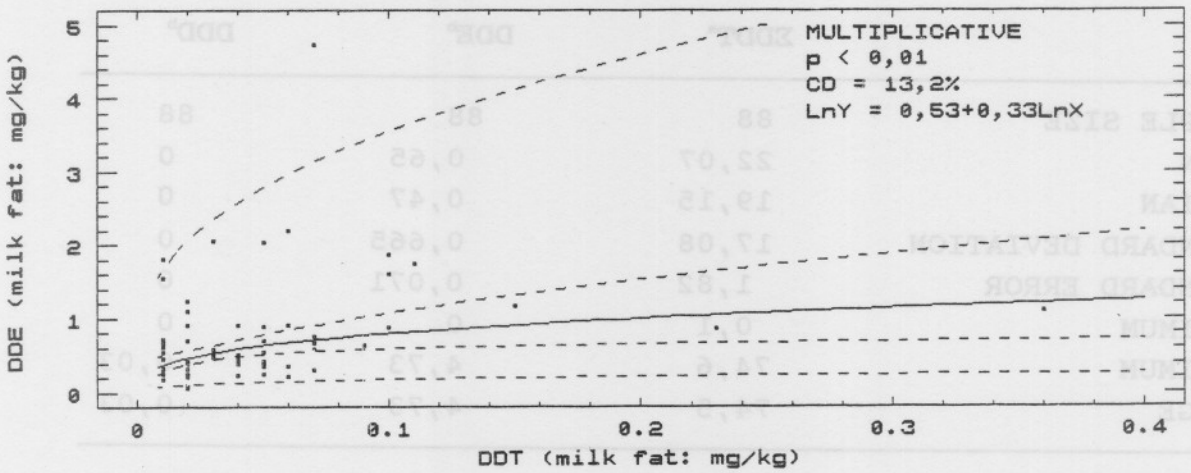

Figure 3.3.2. Multiplicative relationship between the levels of DDT and DDE in milk fat of breast milk of the control group. 95% confidence and prediction limits are shown.

Fig. 3.3.1 shows a very good positive relationship between the levels of DDT and DDE in breast milk of the exposed group. More than 78% of the DDE concentration is explained by the DDT concentration. For the control group (Fig. 3.3.2), this relationship, although also positive, was not so apparent. The fit of the raw data to the regression (indicated by the low coefficient of determination; CD) indicated that the DDE-levels were not well associated with the DDT-levels. The slope of the regressions summed up the relationships, in that the slope of the exposed group was much steeper (three times) than that of the control group.

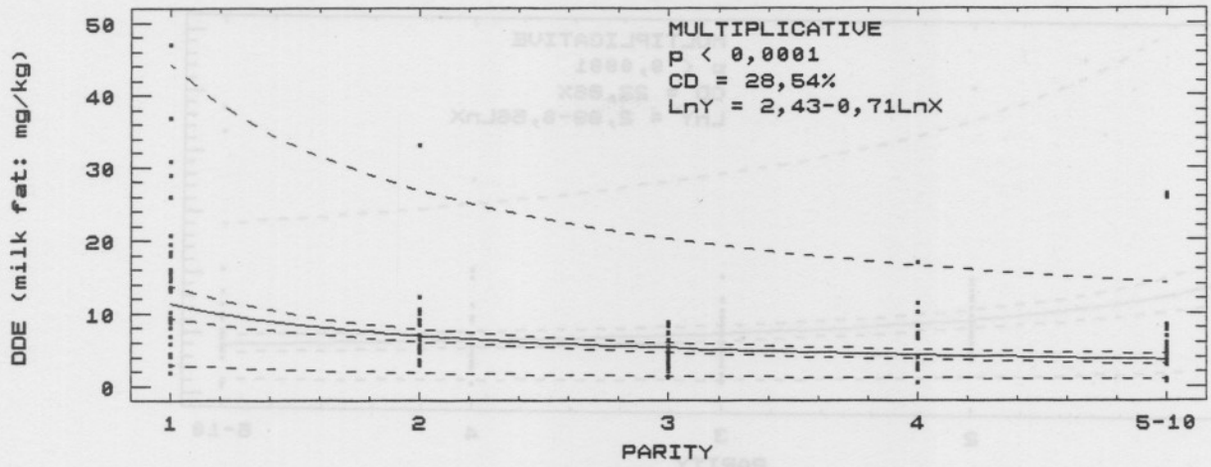

Figure 3.3.3. Multiplicative regression of the DDE in breast milk from the exposed group on parity. 95% confidence and prediction limits are shown.

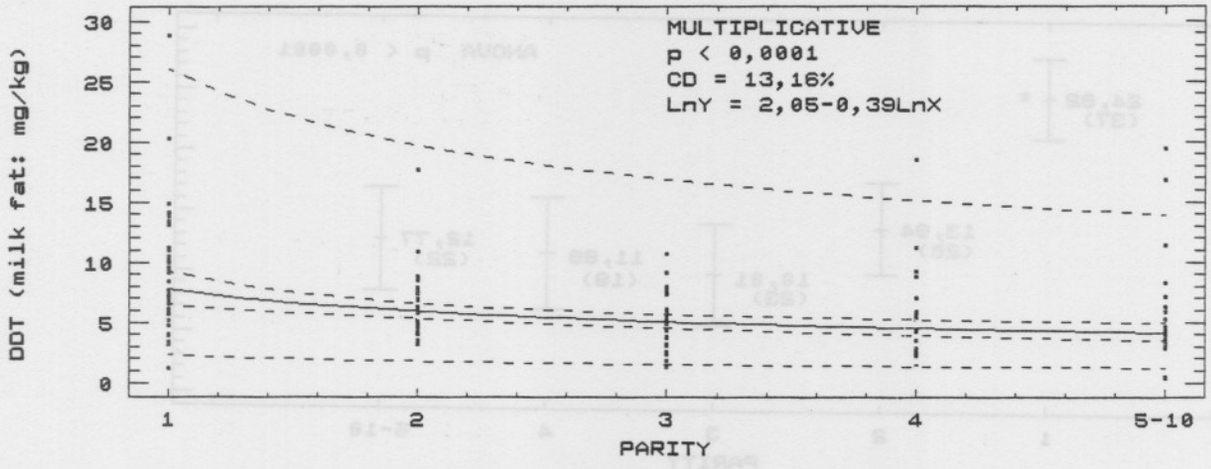

Figure 3.3.4. Multiplicative regression of the DDT in breast milk from the exposed group on parity. 95% confidence and prediction limits are shown.

Figs. 3.3.3 and 3.3.4 show the negative (and significant) regressions between DDE and DDT-levels with parity of the mothers of the exposed group. The coefficients of determination (CD) were not good. The slope of the DDE regression (standard error = 0,0992) was almost twice that of the DDT regression (standard error = 0,0884). The slope of the DDD regression (graph not shown) was -0,1985 (standard error = 0,1530).

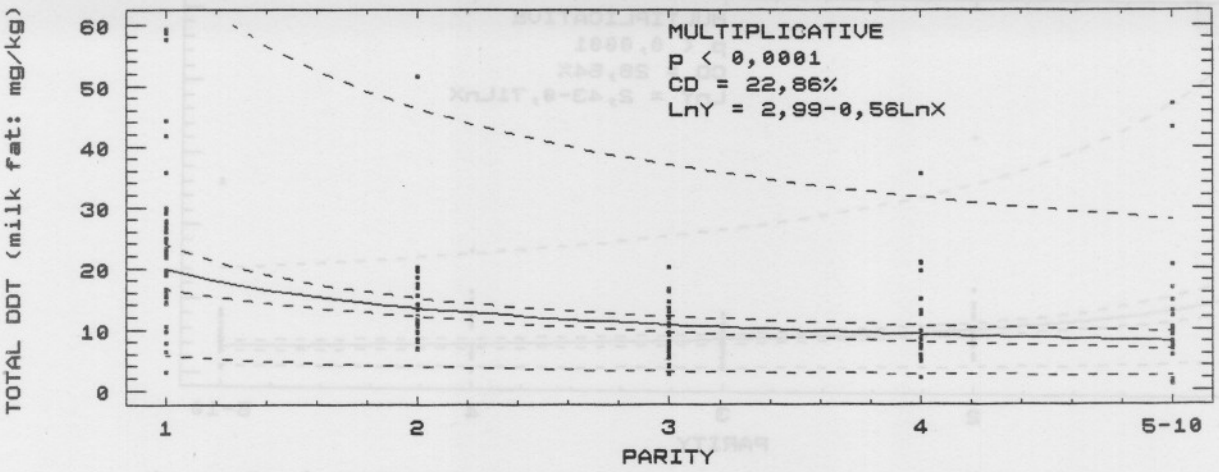

Figure 3.3.5. Multiplicative regression of the ΣDDT in breast milk of the exposed group on parity. 95% confidence and prediction limits are shown.

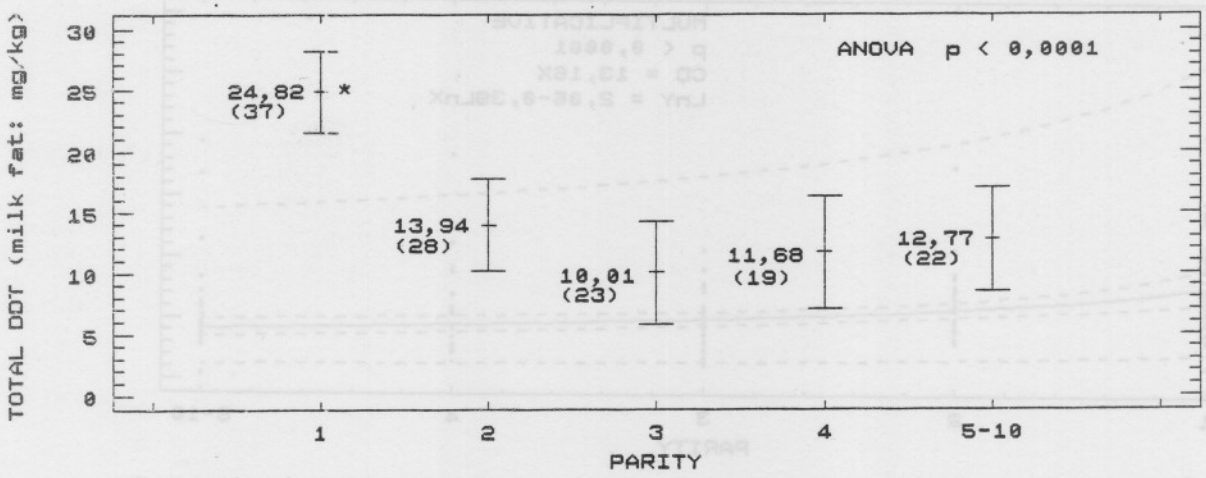

Figure 3.3.6. Means plot of the analysis of variance of the ΣDDT in breast milk of the exposed group by parity. 95% confidence intervals, means and sample size () are shown. Parities, significantly different from each other ($p < 0,05$), are indicated by *.

Fig. 3.3.5 shows the regression of the ΣDDT-levels of the exposed group on parity and Fig. 3.3.6 the analysis of variance of the same. The slope of the regression (standard error = 0,0906) fell between the slope of the DDE and DDT regressions (Figs. 3.3.3 and 3.3.4), of which ΣDDT is a function. The analysis of variance showed that the levels of ΣDDT for the primiparous mothers was significantly higher ($p < 0,005$) than for mothers with two or more children.

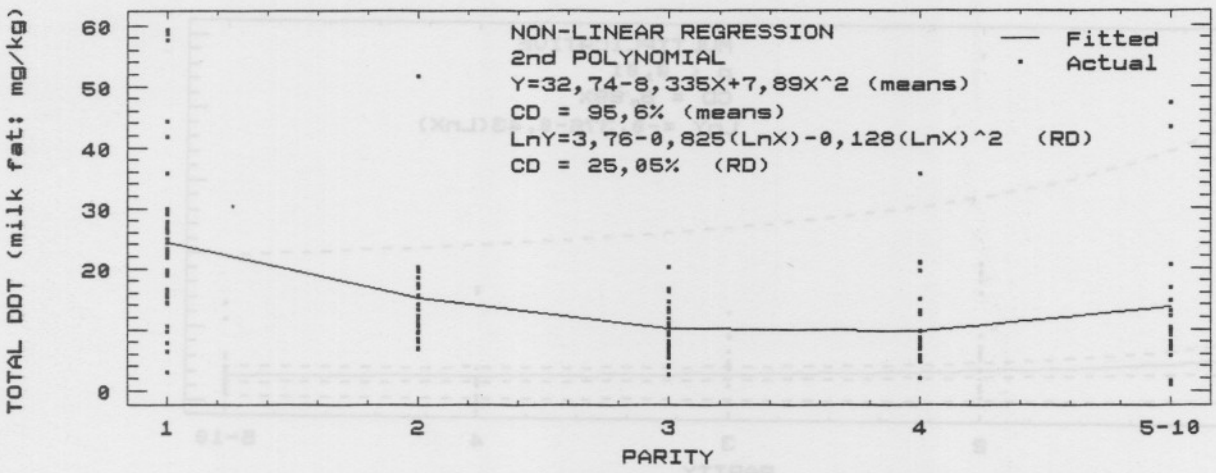

Figure 3.3.7. Non-linear regression of the ΣDDT in breast milk of the exposed group on parity. Information for both means (calculated separately) and raw data are given.

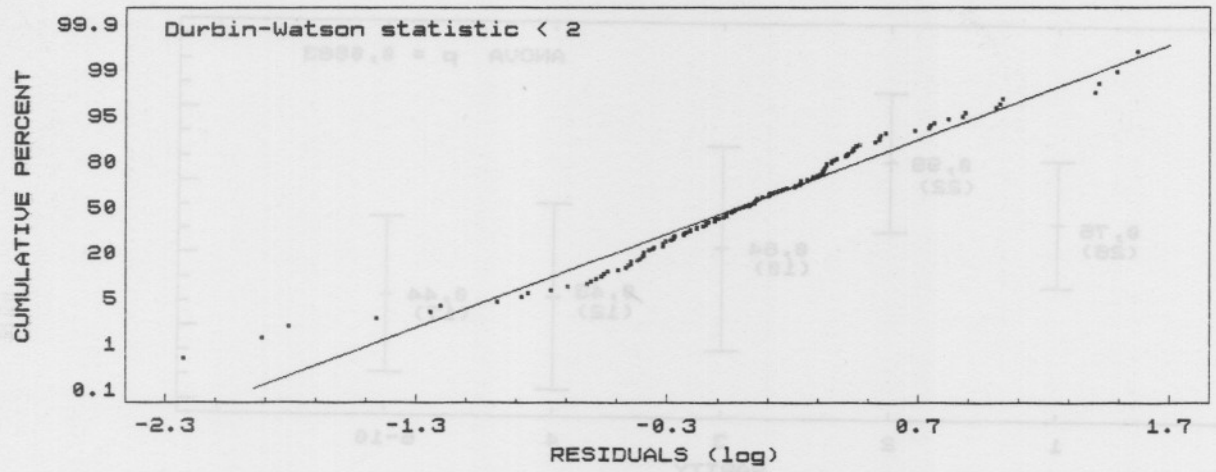

Figure 3.3.8. Probability plot of the residuals of the non-linear regression of the ΣDDT in breast milk of the exposed group on parity.

A very good fit of the non-linear regression for the ΣDDT means for different parities was obtained (Fig. 3.3.7). The coefficient of determination for the raw data (RD), due to the numerous outliers, was much lower than for the means. The Durbin-Watson statistic (Fig. 3.3.8) indicated no auto-correlation, although a cyclic pattern can be seen.

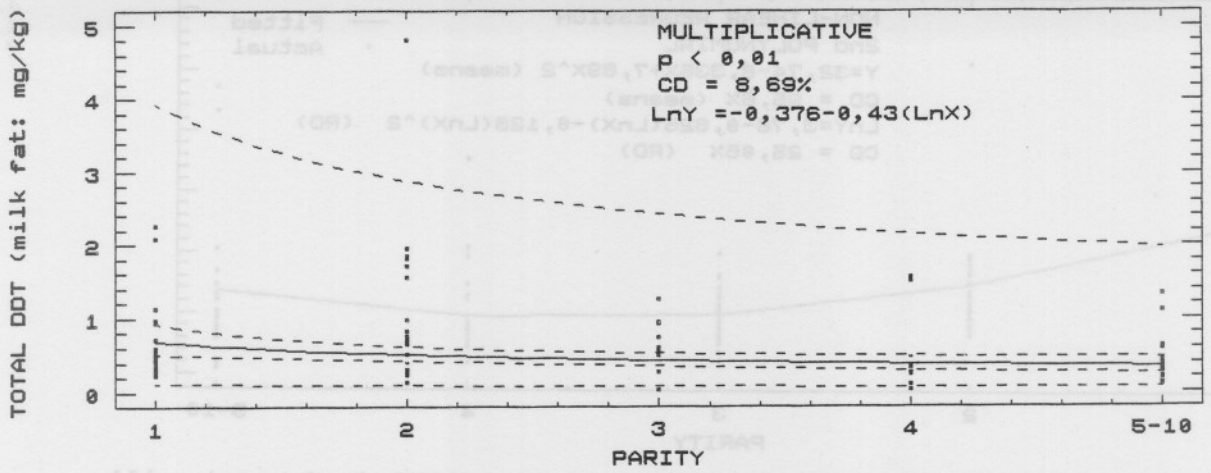

Figure 3.3.9. Multiplicative regression of the ΣDDT in breast milk of the control group on parity. 95% confidence and prediction limits are shown.

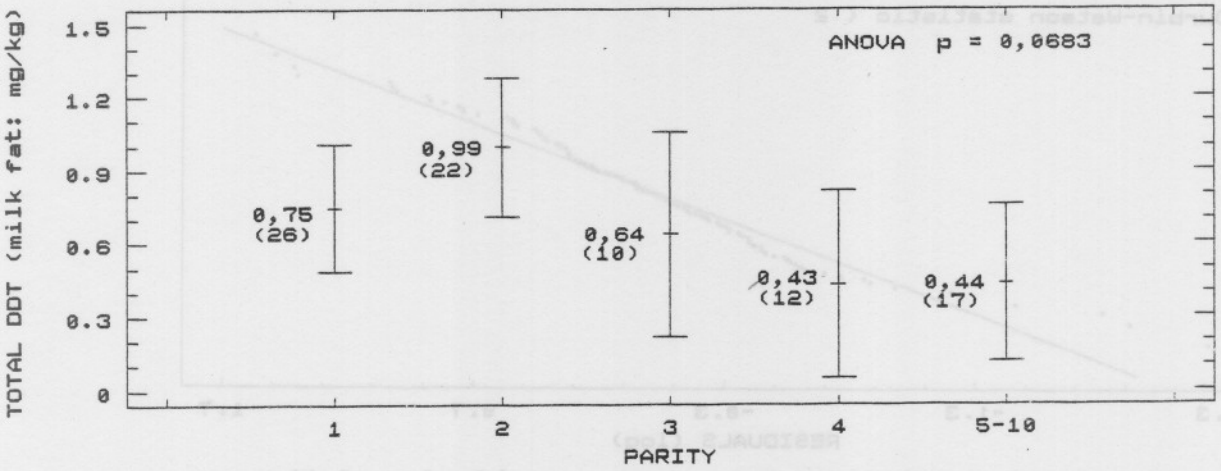

Figure 3.3.10. Means plot of the analysis of variance of the ΣDDT in breast milk of the control group by parity. 95% confidence intervals, means and sample size () are shown.

The regression of the ΣDDT-levels in breast milk of the control group on parity (Fig. 3.3.9) was significant, and had a slope similar to that of the exposed group, although the standard error (0,1510) was larger. The coefficient of determination was also small. The analysis of variance (Fig. 3.3.10) showed no significant differences between parities.

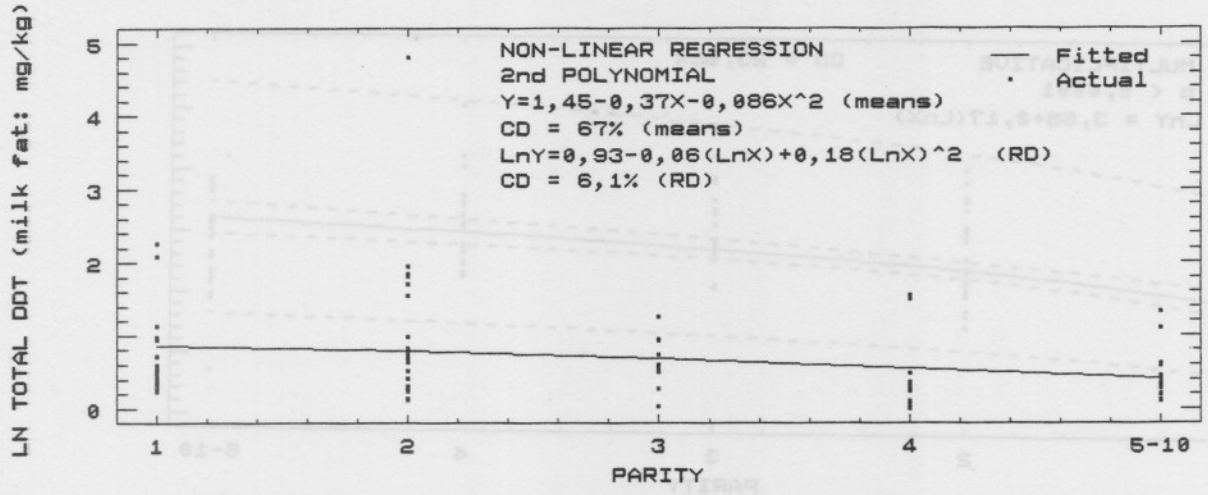

Figure 3.3.11. Non-linear regression of the ΣDDT in breast milk of the control group on parity. Information for both the means (calculated separately) and raw data are given.

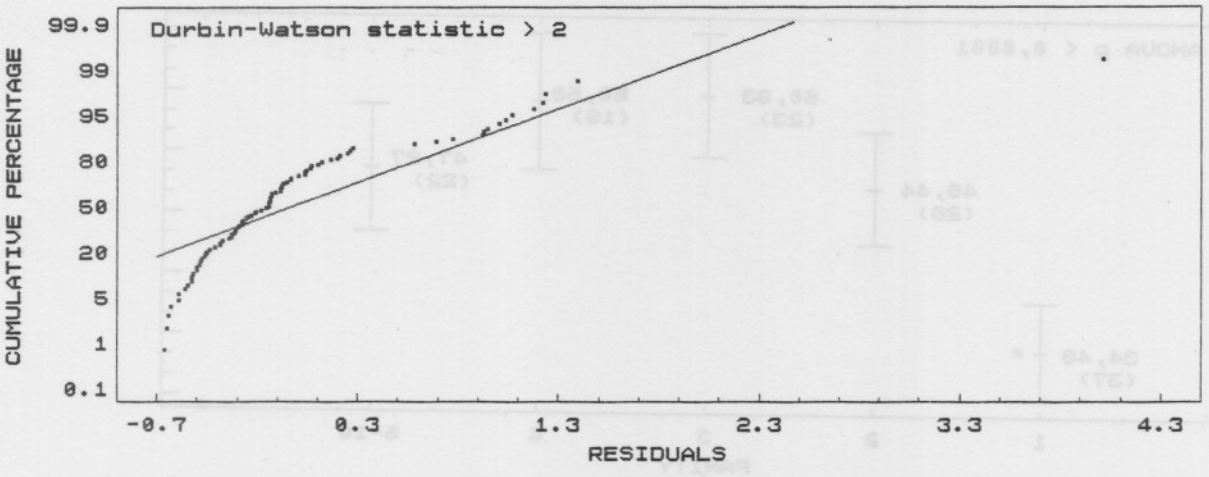

Figure 3.3.12. Probability plot of the residuals of the non-linear regression of the ΣDDT in breast milk of the control group on parity.

The non-linear regression (Fig.3.3.11) was a reasonable explanation for the variation of the means of the ΣDDT in breast milk of the control group, but for the log transformed data it was not very good. The Durbin-Watson statistic and the probability plot (Fig. 3.3.12) indicated that a systemic error was present in this model.

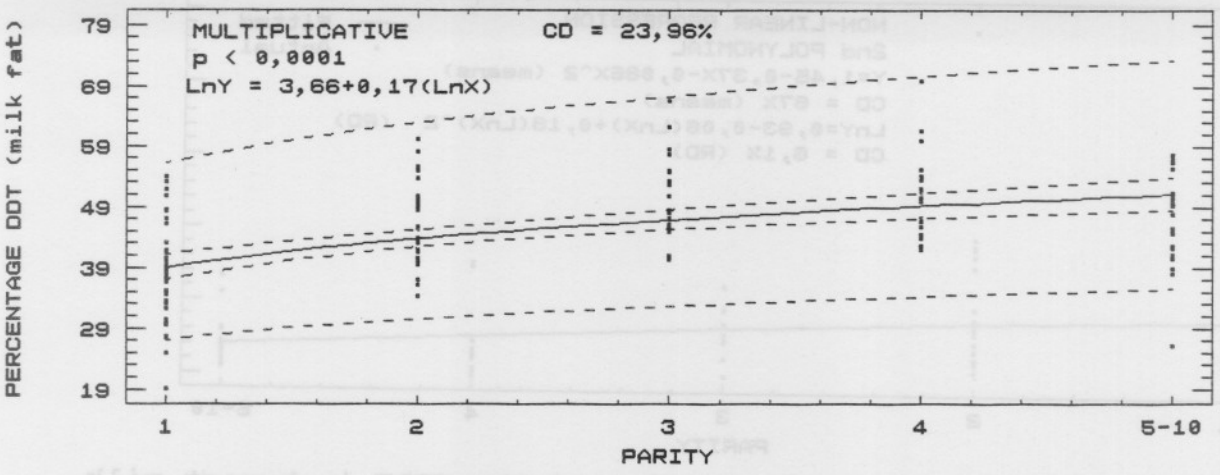

Figure 3.3.13. Multiplicative regression of the percentage DDT in breast milk of the exposed group on parity. 95% confidence and prediction limits are shown.

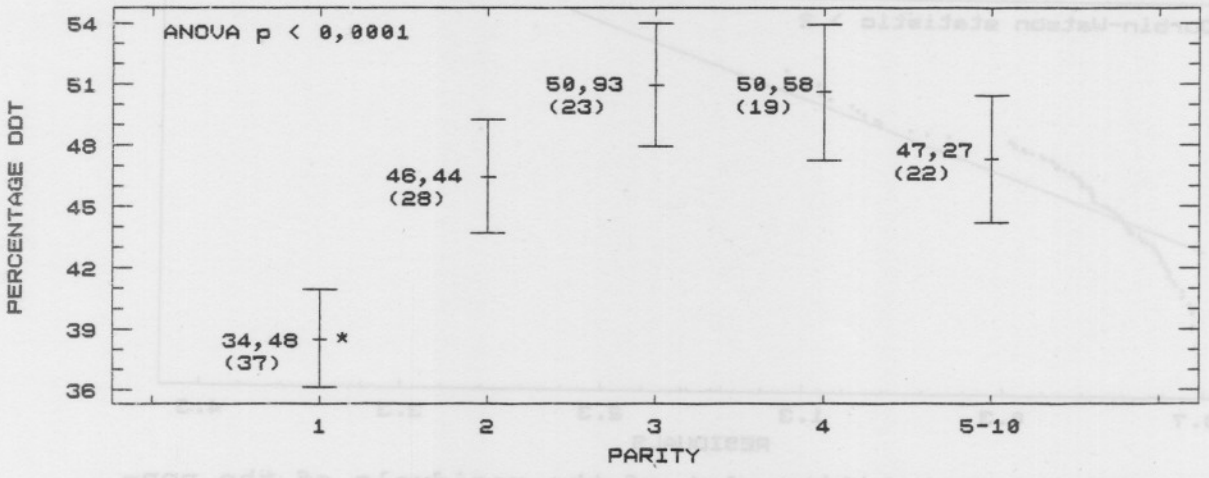

Figure 3.3.14. Means plot for the analysis of variance of the percentage DDT in breast milk of the exposed group by parity. 95% confidence limits, means and sample size () are shown. Parities, significantly different from each other ($p < 0,05$), are indicated by *.

Percentage DDT in breast milk of the exposed group increased significantly with an increase in parity (Fig. 3.3.13). The coefficient of determination indicated that parity explained 24% of the percentage DDT. The fit of the regression was highly significant. Fig. 3.3.14 shows that the mean percentage DDT of the primiparous mothers was significantly lower than for the mothers with two or more children. A decrease in percentage DDT can be noticed for mothers with four or more children.

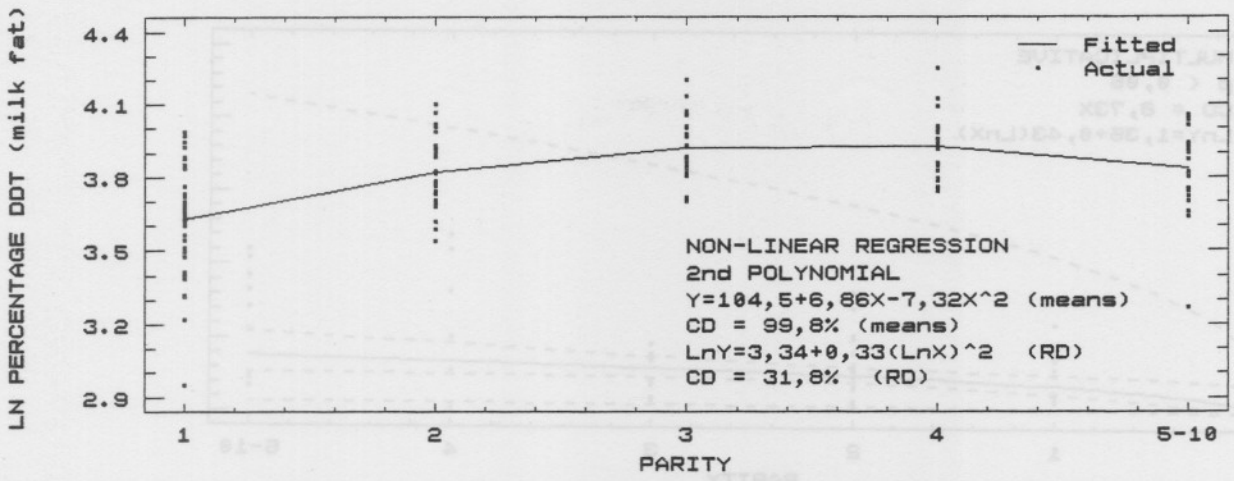

Figure 3.3.15. Non-linear regression of the percentage DDT in breast milk of the exposed group on parity. Information for both the means (calculated separately) and raw data are given.

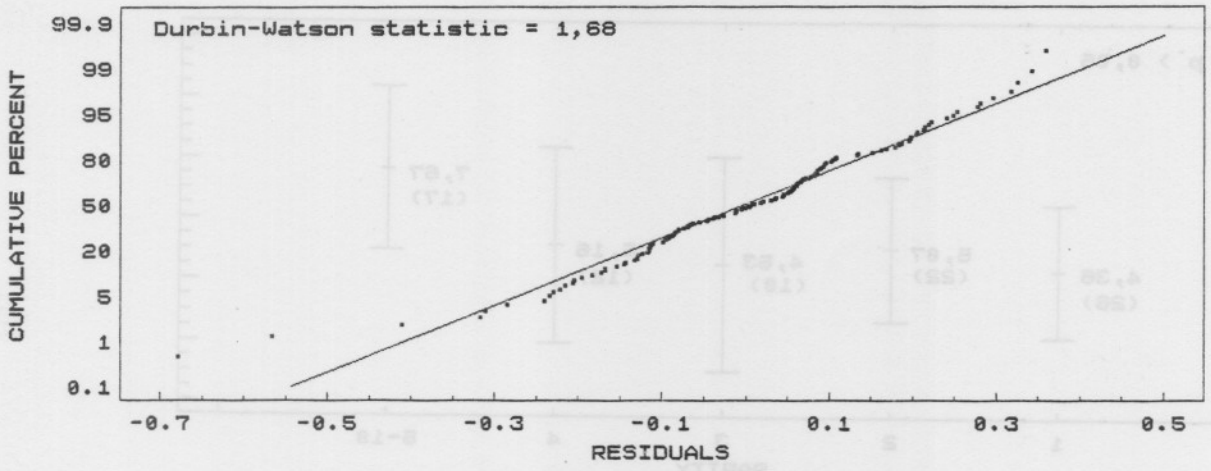

Figure 3.3.16. Probability plot of the residuals of the non-linear regression of the percentage DDT in breast on parity milk of the control group.

The non-linear regression, fitted through the means of the percentage DDT of the exposed group (Fig. 3.3.15), had a very good coefficient of determination. The same value for the regression fitted through the log transformed raw data dropped to 31,8% which was still better than for the multiplicative model (Fig. 3.3.14). The Durbin-Watson statistic showed no auto-correlation between residuals, but the probability plot (Fig. 3.3.16) indicates that some deviations at the lower and higher limits were present.

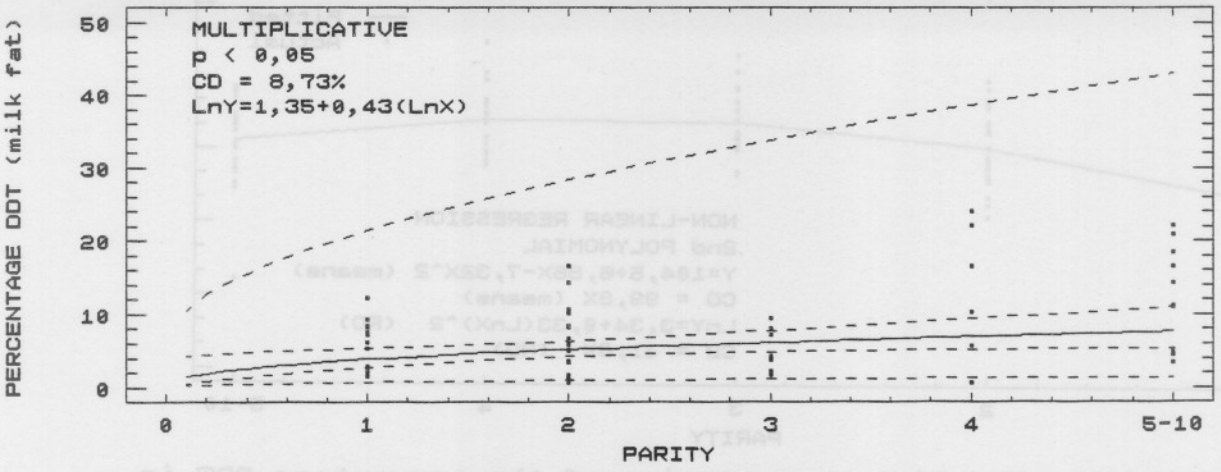

Figure 3.3.17. Multiplicative regression of the percentage DDT in breast milk of the control group on parity. 95% confidence and prediction limits are shown.

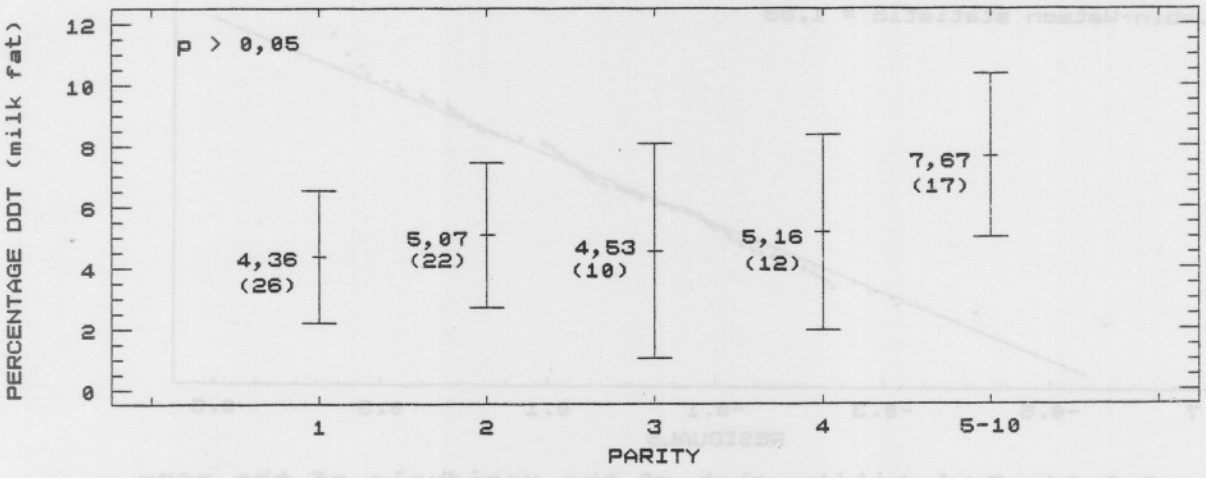

Figure 3.3.18. Means plot for the analysis of variance of the percentage DDT in breast milk of the control group by parity. 95% confidence limits, means and sample size () are shown.

The mean percentage DDT for the control group increased slightly, but significantly, as indicated by the significant p-value (Fig. 3.3.17). The fit of the raw data was not good. The anova (Fig. 3.3.18) indicated no significant differences (p > 0,05) between parities.

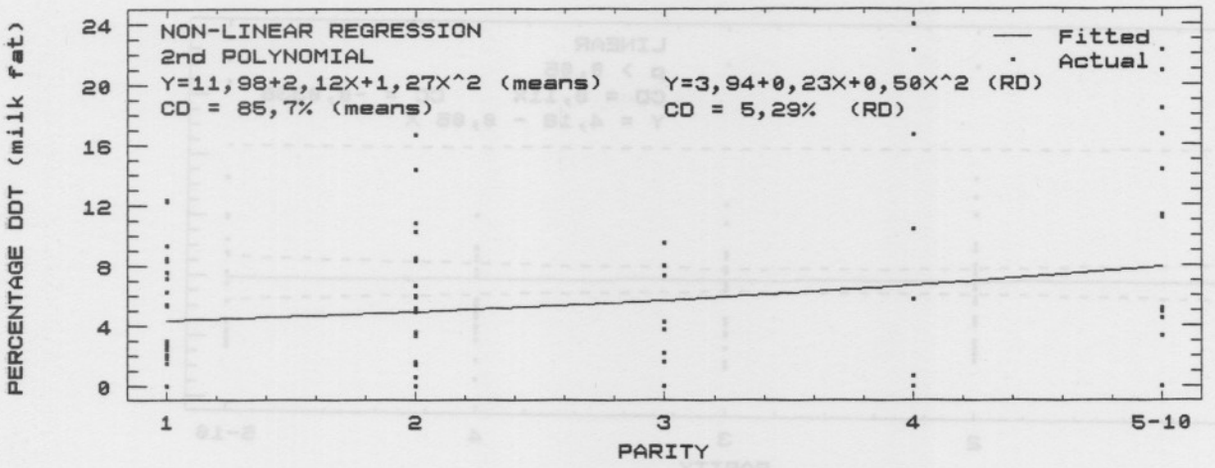

Figure 3.3.19. Non-linear regression of the percentage DDT in breast milk of the control group on parity. Information for both the means (calculated separately) and raw data are given.

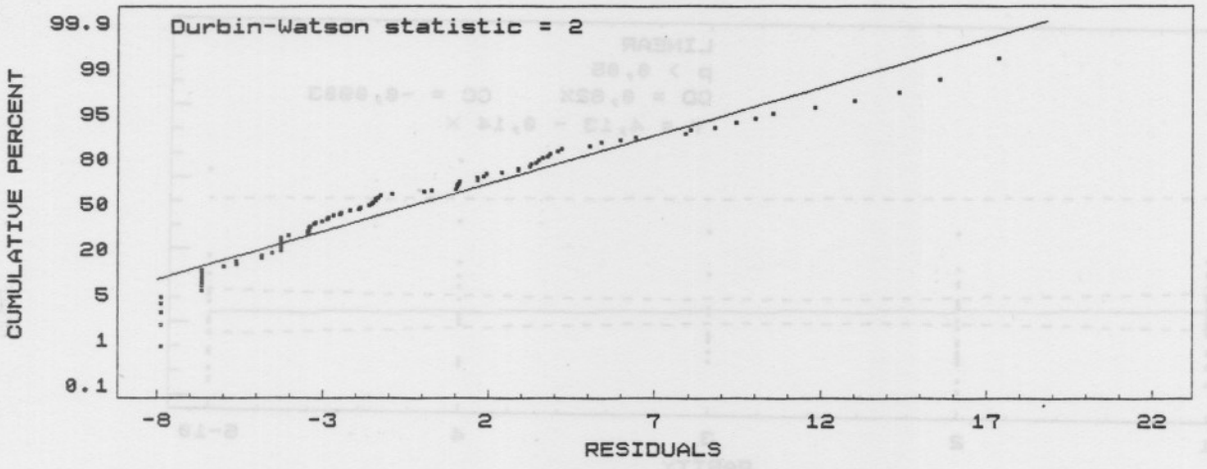

Figure 3.3.20. Probability plot of the residuals of the non-linear regression of the percentage DDT in breast milk of the control group on parity.

A good coefficient of determination for the non-linear regression for the means of the percentage DDT on parity (Fig. 3.3.19), was negated by the correspondingly low value for the log transformed raw data. This indicated a bad fit of the data. This was confirmed by the Durbin-Watson statistic, and the strong deviation from the mean of the residuals at the lower end (Fig. 3.3.20).

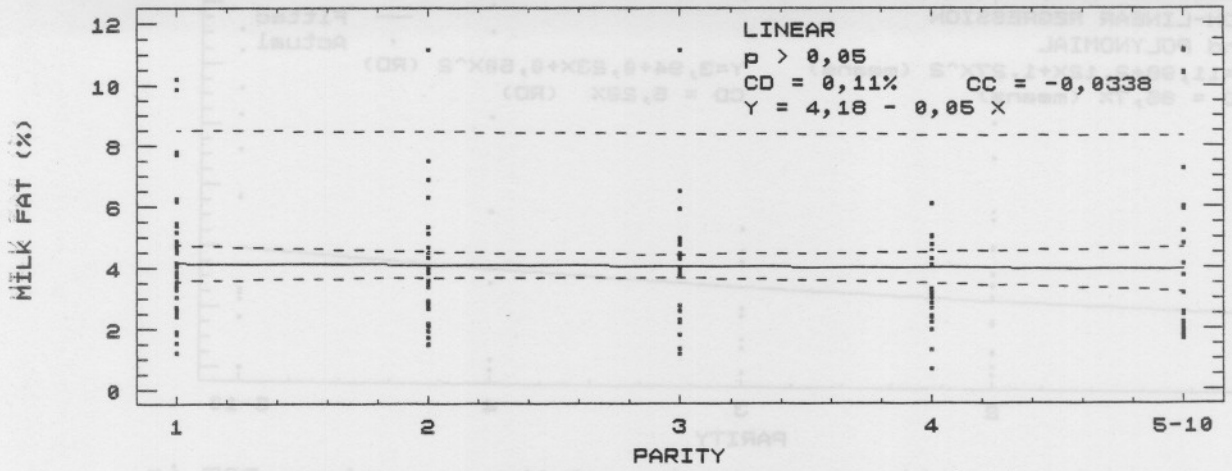

Figure 3.3.21. Linear regression of the percentage milk fat in breast milk of the exposed group on parity. 95% confidence and prediction limits are shown.

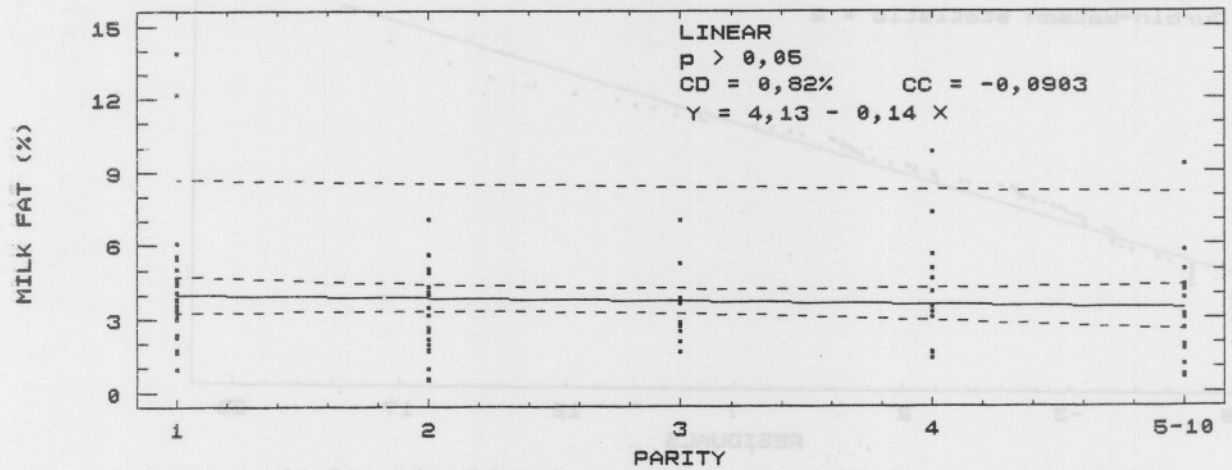

Figure 3.3.22. Linear regression of the percentage DDT in breast milk of the control group on parity. 95% confidence and prediction limits are shown.

The very low coefficient of determination for both the linear regressions (the linear model gave the best coefficients of determination) for the percentage milk fat of the exposed (Fig.3.3.21) and control groups (Fig. 3.3.22), indicated no change in percentage milk fat with an increase in parity.

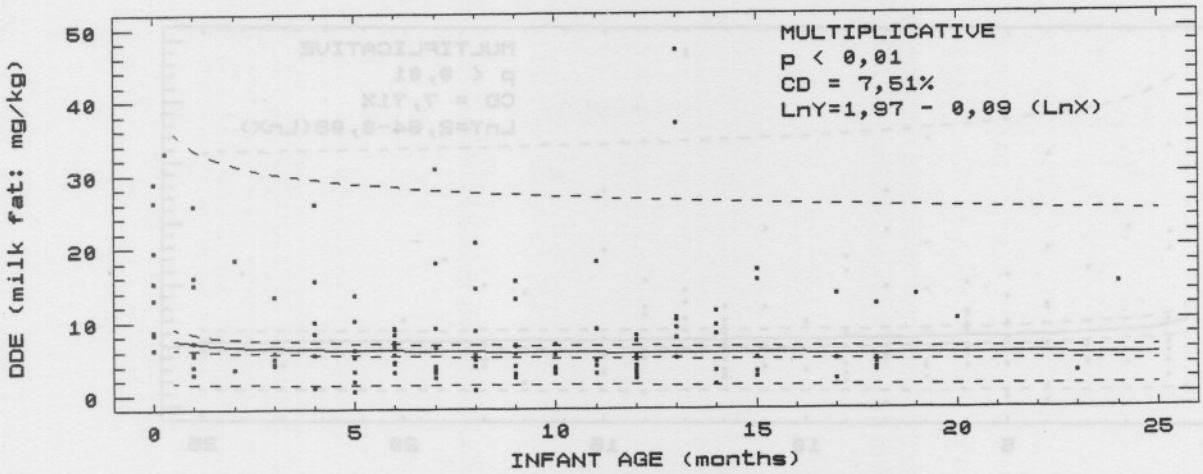

Figure 3.3.23. Multiplicative regression of the DDE in breast milk of the exposed group on infant age. 95% confidence and prediction limits are shown.

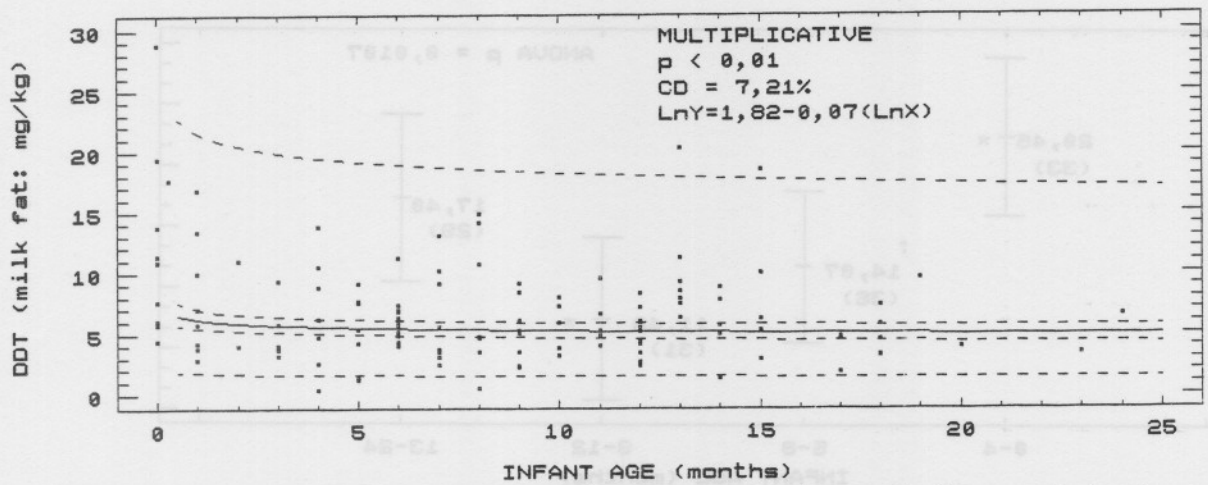

Figure 3.3.24. Multiplicative regression of the DDT in breast milk of the exposed group on infant age. 95% confidence and prediction limits are shown.

As a multiplicative regression model gave the best coefficient of determination, 0,001 months was added to the age variable in order not to discard values for ages less than one month during log transformation. Figs. 3.3.23 and 3.3.24 show almost identical p-values and coefficients of determination for DDT and DDE on infant age. The slope for DDE was somewhat steeper than that for DDT. The p-value for DDD was 0,219 (graph not shown), indicating no change with an increase in the age of the infants.

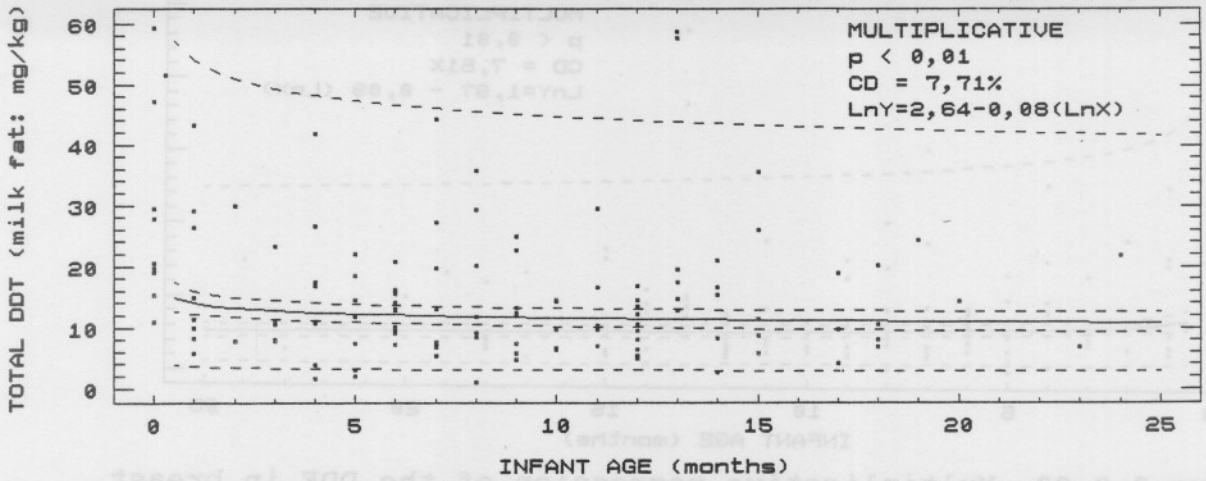

Figure 3.3.25. Multiplicative regression of the ΣDDT in breast milk of the exposed group on infant age. 95% confidence and prediction limits are shown.

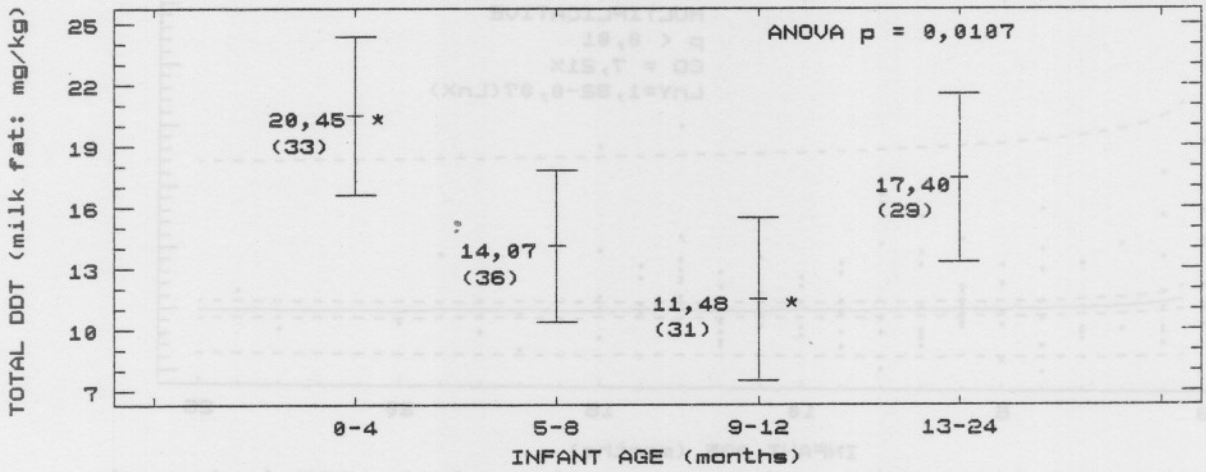

Figure 3.3.26. Means plot for the analysis of variance of the percentage DDT in breast milk of the exposed group by infant age intervals. 95% confidence limits, means and sample size () are shown. Intervals significantly different ($p < 0,05$) from each other are indicated by *.

As ΣDDT is essentially the product of DDE and DDT, the regression analysis of ΣDDT on infant age of the exposed group (Fig. 3.3.25) was not much different from that of DDE (Fig. 3.3.23) and DDT (Fig. 3.3.24). The regression was significant, but the fit of the raw data was not very good, resulting in a poor coefficient of determination. Analysis of variance (Fig. 3.3.26) showed that the ΣDDT exposure for the 9-12 month interval was significantly less than for the 0-4 month interval.

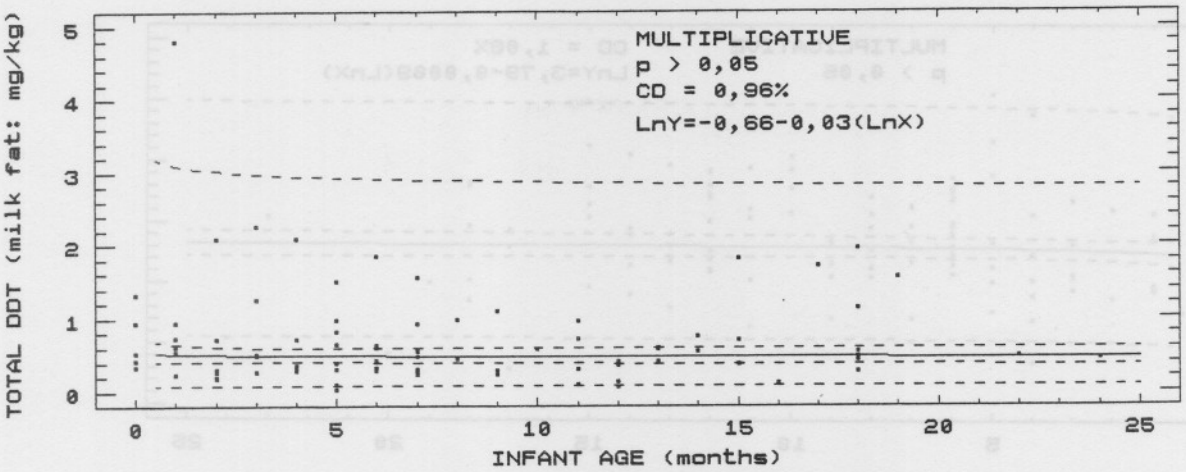

Figure 3.3.27. Multiplicative regression of the ΣDDT in breast milk of the control group on infant age. 95% confidence and prediction limits are shown.

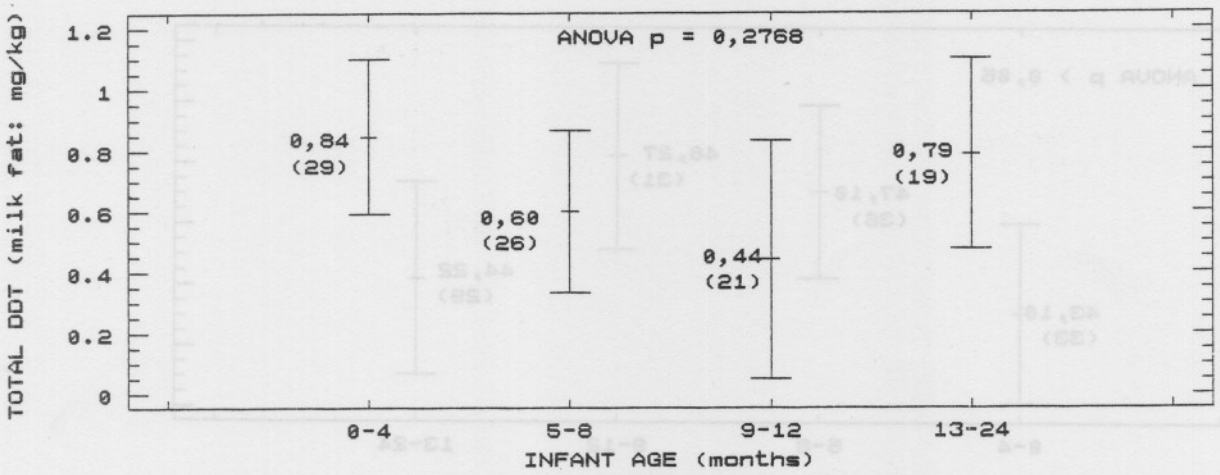

Figure 3.3.28. Means plot for the analysis of variance of the ΣDDT in breast milk of the control group by infant age intervals. 95% confidence limits, means and sample size are shown.

No significant change in the ΣDDT in the breast milk of the control group was indicated by the regression analysis, as the slope was not significantly different from zero (Fig. 3.3.27). The very low coefficient of determination must be noted. The analysis of variance (Fig. 3.3.28) showed a decrease (which was not significant) in the mean exposure during the first twelve months. Mean exposure increased to the initial level during the second year of breast-feeding.

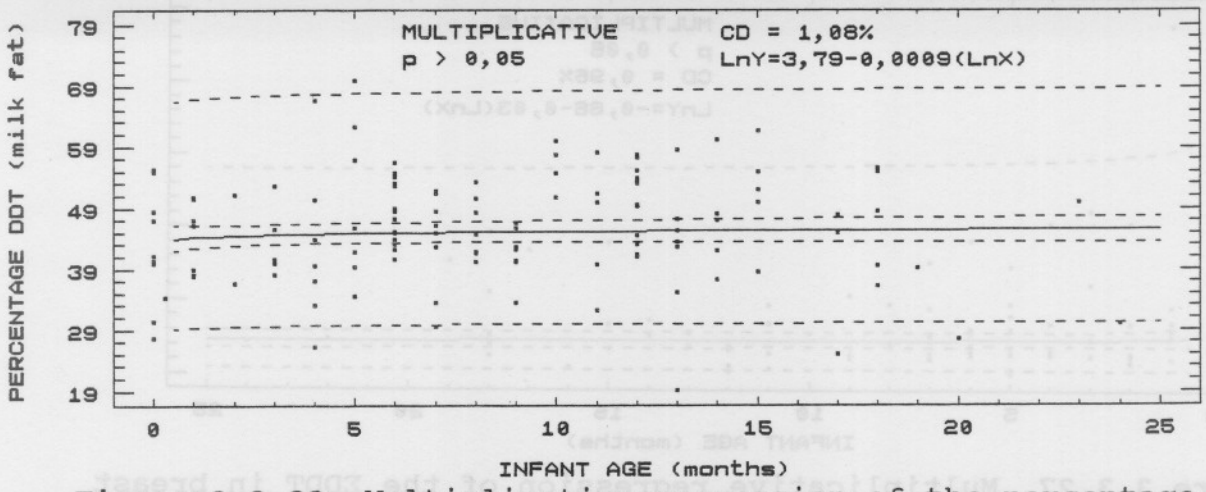

Figure 3.3.29. Multiplicative regression of the percentage DDT in breast milk of the exposed group on infant age. 95% confidence and prediction limits are shown.

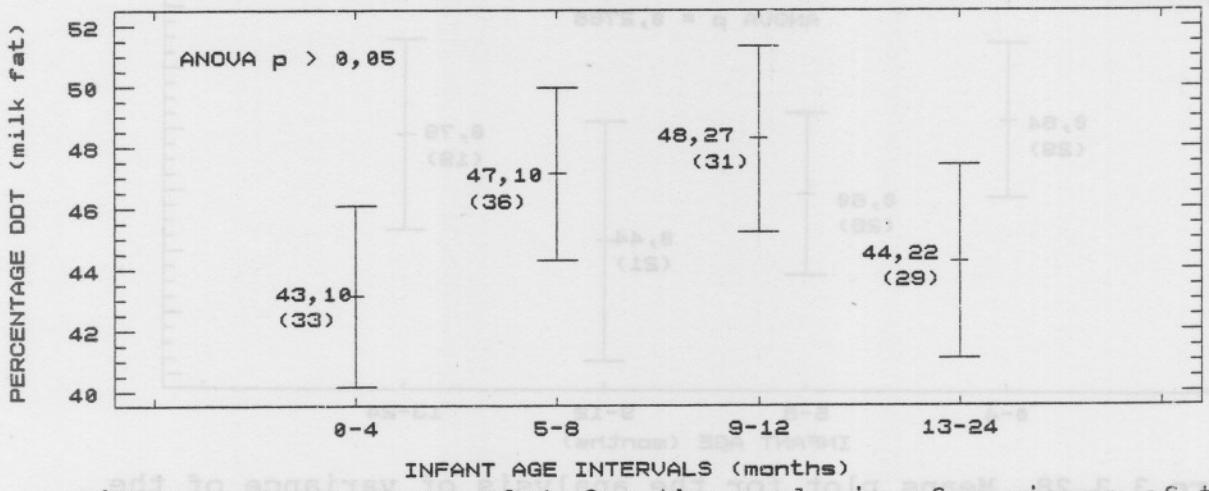

Figure 3.3.30. Means plot for the analysis of variance of the percentage DDT in breast milk of the exposed group by infant age intervals. 95% confidence limits, means and sample size () are shown.

The percentage DDT of the ΣDDT in breast milk of the exposed group remained constant over the breast-feeding period, as indicated by the slope of the multiplicative regression, which was not significantly different from zero (Fig. 3.3.29). A very low coefficient of determination was obtained. The analysis of variance (Fig. 3.3.30) showed a non-significant increase ($p > 0,05$) in the mean percentage DDT during the first twelve months, followed by a drop to almost the initial value. At a confidence level of 10%, the difference between the 0-4 and 9-12 months age interval was significant.

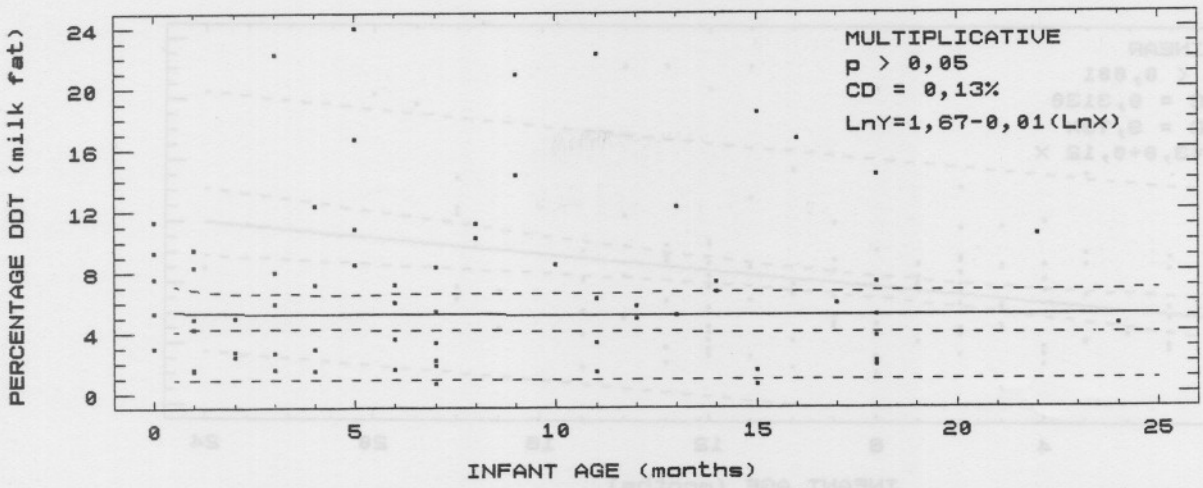

Figure 3.3.31. Multiplicative regression of the percentage DDT in breast milk of the control group on infant age intervals. 95% confidence and prediction limits are shown.

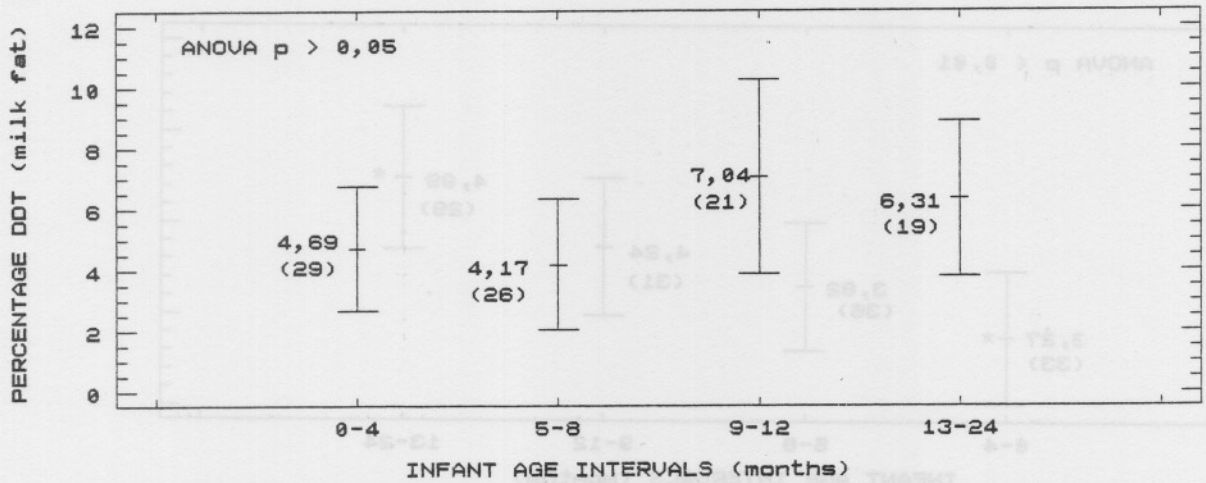

Figure 3.3.32. Means plot for the analysis of variance of the percentage DDT in breast milk of the control group by infant age intervals. 95% confidence limits, means and sample size () are shown.

Significant change in the percentage DDT of the ΣDDT was shown by neither the regression analysis (Fig.3.3.31), nor by the analysis of variation (Fig. 3.3.32) for the percentage DDT of the ΣDDT in breast milk of the control group. The slope of the regression was almost horizontal and a very low coefficient of determination was calculated.

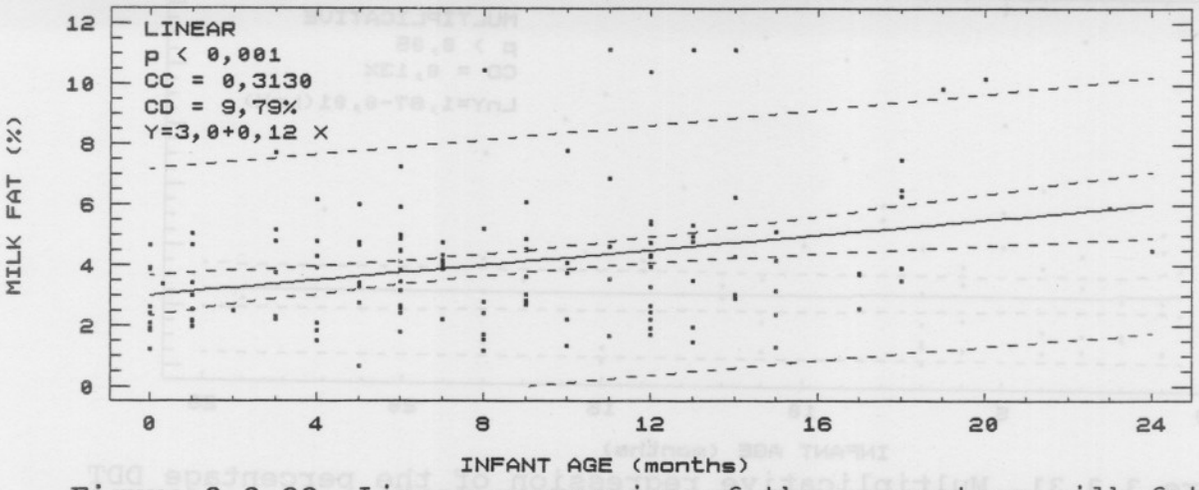

Figure 3.3.33. Linear regression of the percentage milk fat in breast milk of the exposed group on infant age intervals. 95% confidence and prediction limits are shown.

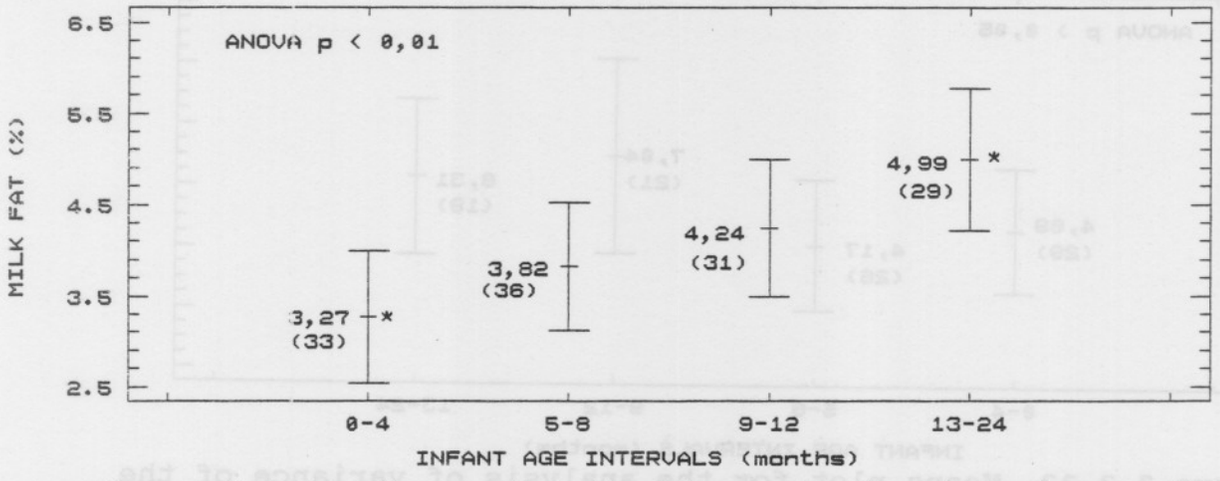

Figure 3.3.34. Means plot for the analysis of variance of the percentage milk fat in breast milk of the exposed group by infant age intervals. 95% confidence limits, means and sample size () are shown. Intervals significantly different ($p < 0,05$) from each other are indicated by *.

Fig. 3.3.33 shows the significant increase in the percentage milk fat during the period of lactation. The coefficient of correlation and coefficient of determination, however, remained low. The analysis of variance (Fig. 3.3.34) showed that the percentage fat during the first four months was significantly lower than for the second year.

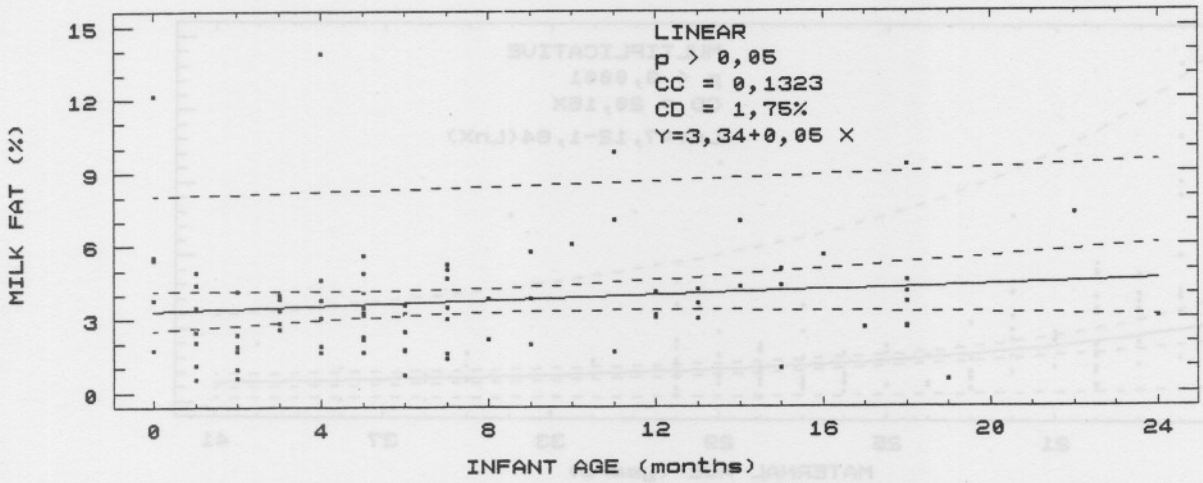

Figure 3.3.35. Linear regression of the percentage milk fat in breast milk of the control group on infant age intervals. 95% confidence and prediction limits are shown.

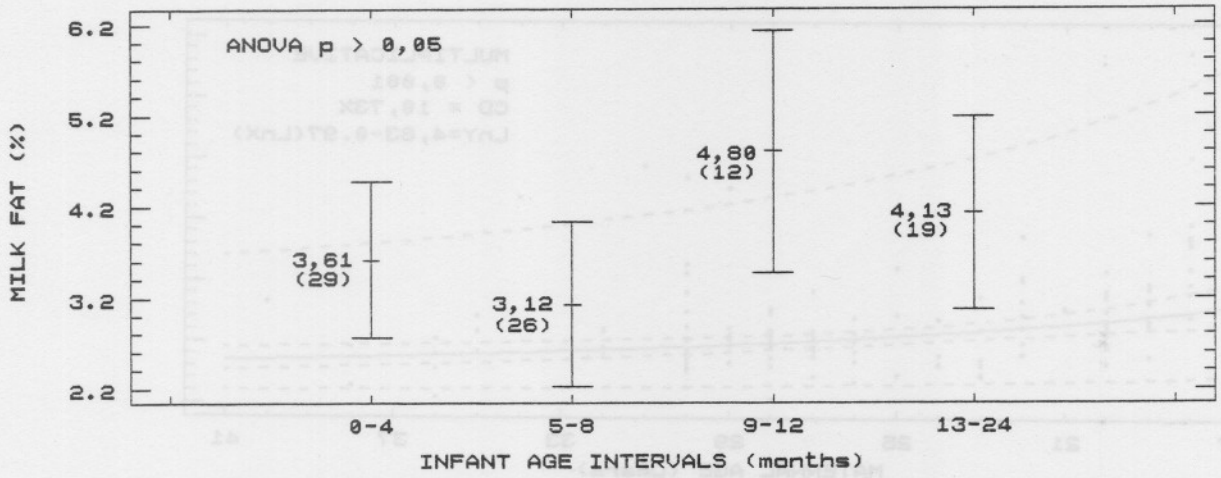

Figure 3.3.36. Means plot for the analysis of variance of the percentage milk fat in breast milk of the control group by infant age intervals. 95% confidence limits, means and sample size () are shown.

No significant increase in the percentage milk fat with infant age was established for the control group (Fig. 3.3.35). The regression also showed a small coefficient of determination. The anova (Fig. 3.3.36) indicated no definite trend, with no significant differences between age intervals.

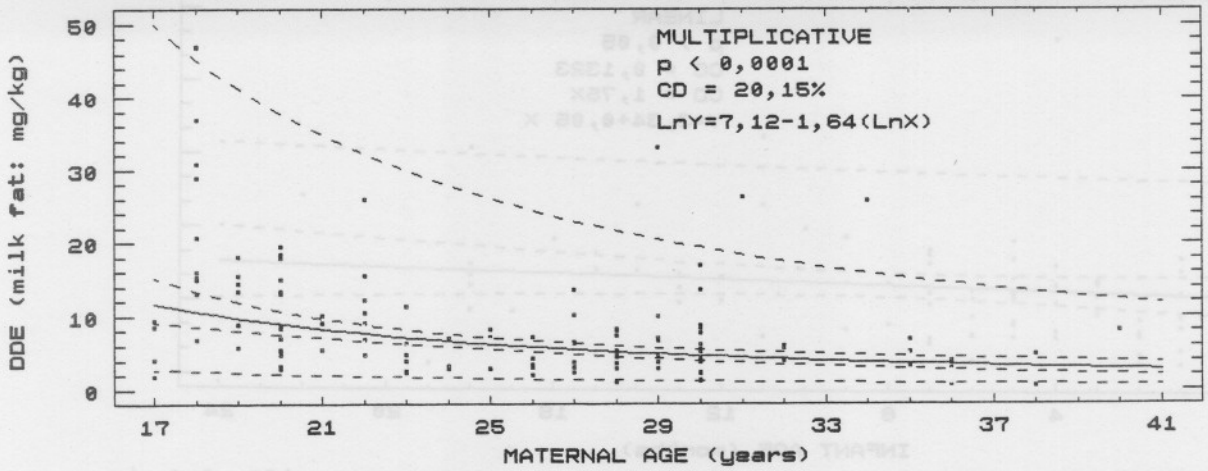

Figure 3.3.37. Multiplicative regression of the DDE in breast milk of the exposed group on maternal age. 95% confidence and prediction limits are shown.

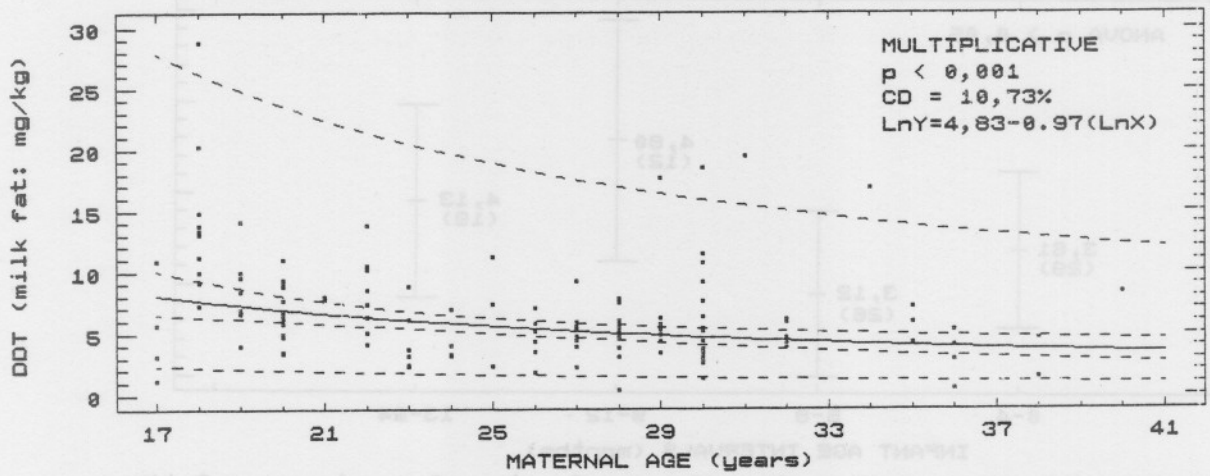

Figure 3.3.38. Multiplicative regression of the DDT in breast milk of the exposed group on maternal age. 95% confidence and prediction limits are shown.

The regression of DDE (Fig. 3.3 37) and DDT (Fig. 3.3.38) in breast milk of the exposed group versus maternal age was comparable, and significant. The slope of the regression for DDT (standard error = 0,28; Fig. 3.3.38) was steeper than for DDE (standard error = 0,25; Fig. 3.3.37). The coefficients of determination were low, with maternal age giving a better explanation for DDE than for DDT. The slope for DDD was -0,774 (standard error = 0,43; graph not shown), with a coefficient of determination of 2,63%. The p-value of 0,0719 indicated no significant change in DDD-levels with maternal age.

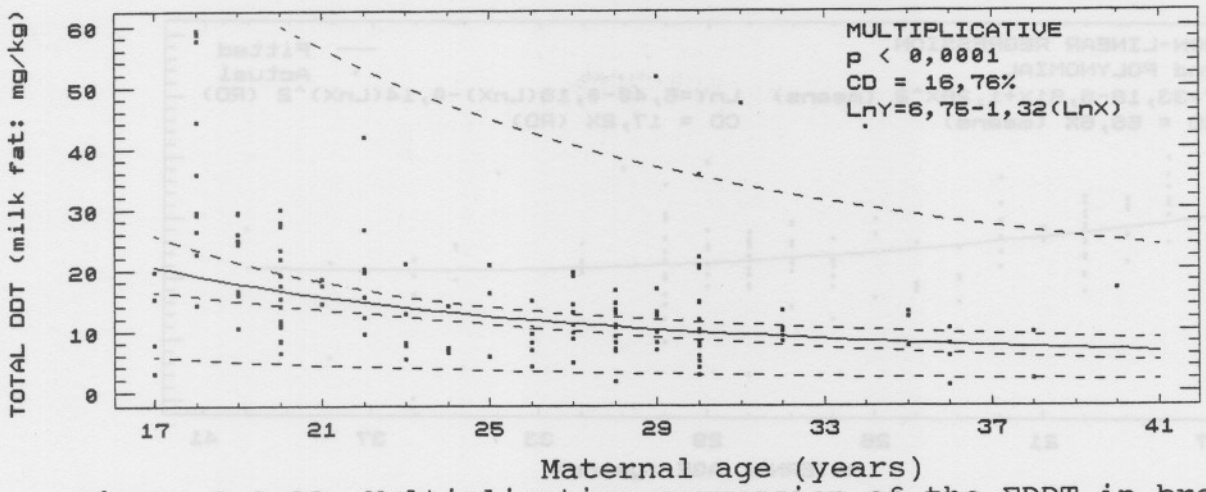

Figure 3.3.39. Multiplicative regression of the ΣDDT in breast milk of the exposed group on maternal age. 95% confidence and prediction limits are shown.

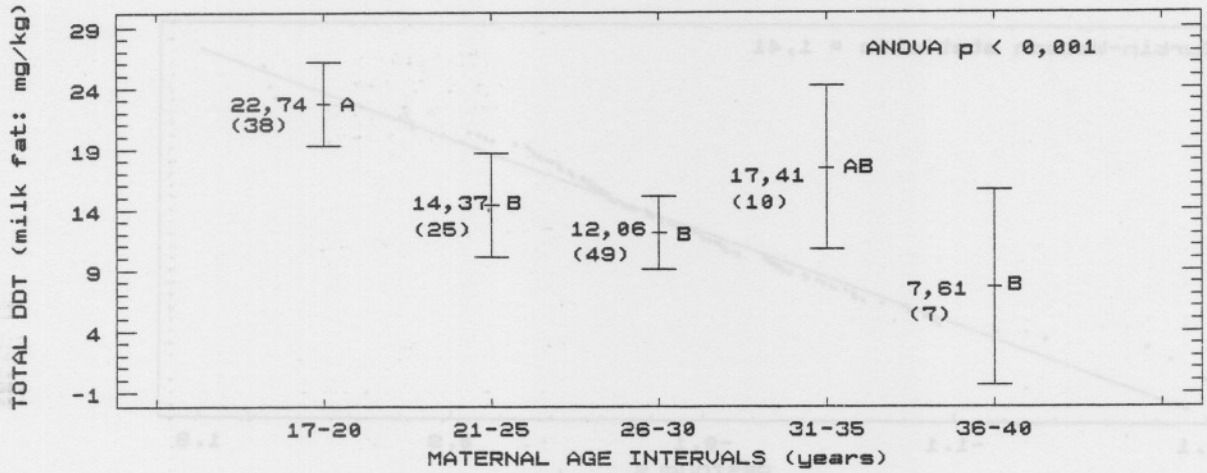

Figure 3.3.40. Means plot for the analysis of variance of the ΣDDT in breast milk of the exposed group by maternal age intervals. 95% confidence limits, means and sample size () are shown. Intervals marked with same symbol did not differ significantly ($p > 0,05$).

The ΣDDT is effectively the sum of DDE and DDT, and the slope of the regression (standard error = 0,26) and coefficients of determination of ΣDDT should therefore lie between the respective statistics of the DDE and DDT regressions. This was shown to be the case by the regression statistics (Fig. 3.3.39). The anova indicated that the ΣDDT-levels for the 17-20 and 31-35 year age intervals were significantly different ($p < 0,05$) from the other intervals (Fig. 3.3.40).

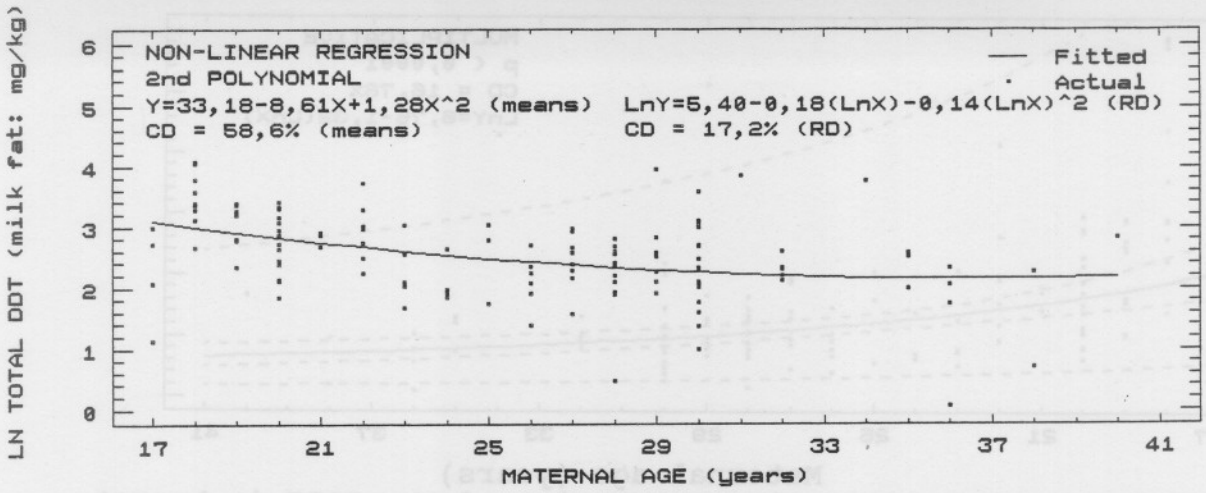

Figure 3.3.41. Non-linear regression of the ΣDDT in the breast milk of the exposed group on maternal age. Information for both the means (calculated separately) and raw data are given.

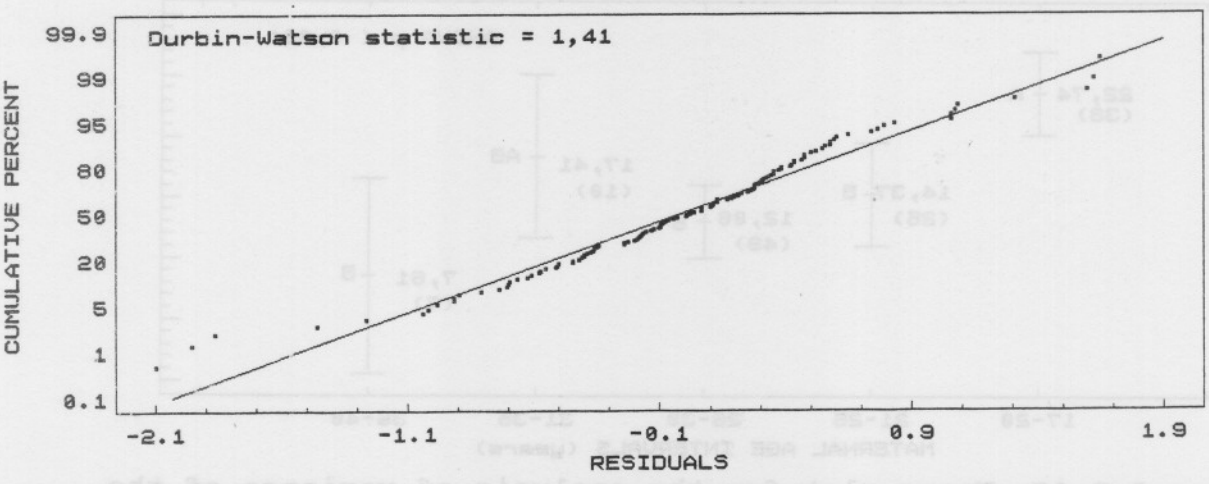

Figure 3.3.42. Probability plot of the residuals of the non-linear regression of ΣDDT in breast milk of the exposed group on maternal age.

The non-linear regression gave a reasonable explanation of the variation of the means of ΣDDT, as indicated by the coefficient of determination (Fig. 3.3.41). The explanation of the log transformed raw data by the regression fell, as a result of the variation of the raw data. The coefficient of determination of this regression was only slightly better than for the multiplicative model (Fig. 3.3.39). The probability plot indicated a systemic influence (Fig. 3.3.42)

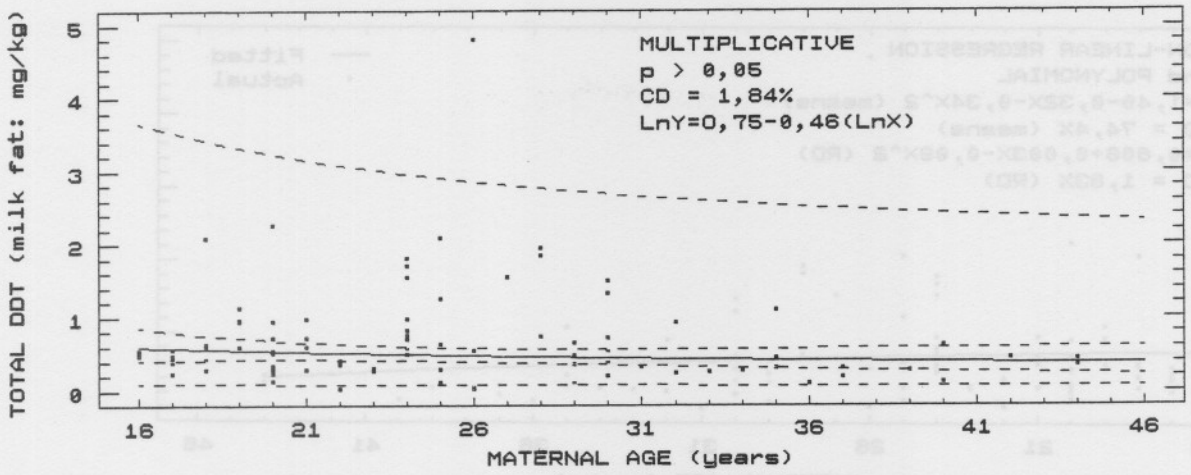

Figure 3.3.43. Multiplicative regression of the ΣDDT in breast milk of the control group on maternal age. 95% confidence and prediction limits are shown.

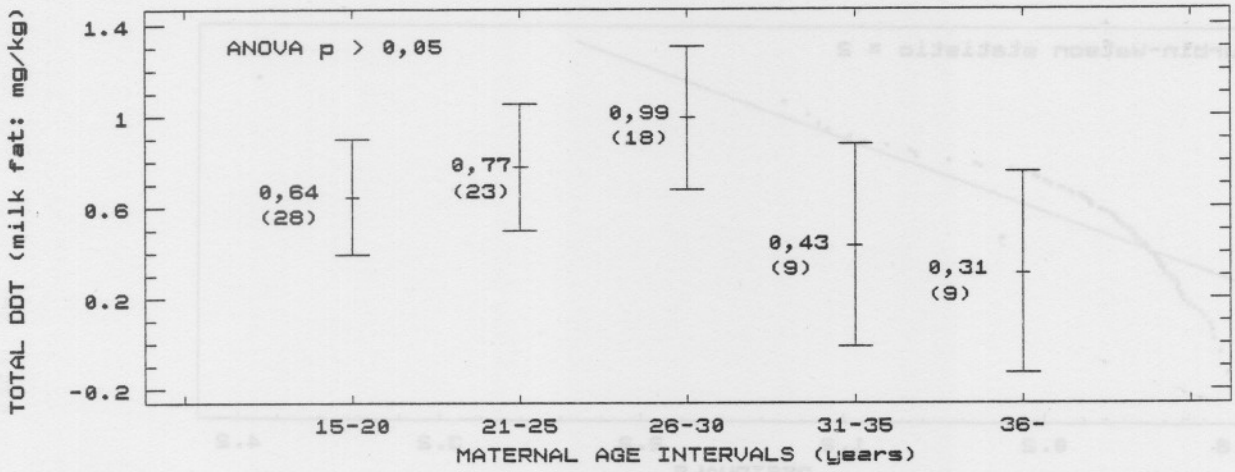

Figure 3.3.44. Means plot for the analysis of variance of the ΣDDT in breast milk of the control group by maternal age intervals. 95% confidence limits, means and sample size () are shown.

A very low coefficient of determination for the regression on maternal age was determined for the ΣDDT in breast milk of the control group (Fig. 3.3.43). The slope (standard error = 0,37) was almost flat, and, together with the insignificant p-value, showed no change in ΣDDT output over the reproductive years. The analysis of variance (Fig. 3.3.44) indicated an increase ($p > 0,05$) in mean values up to 30 years of age, after which it decreased rapidly to below the initial values.

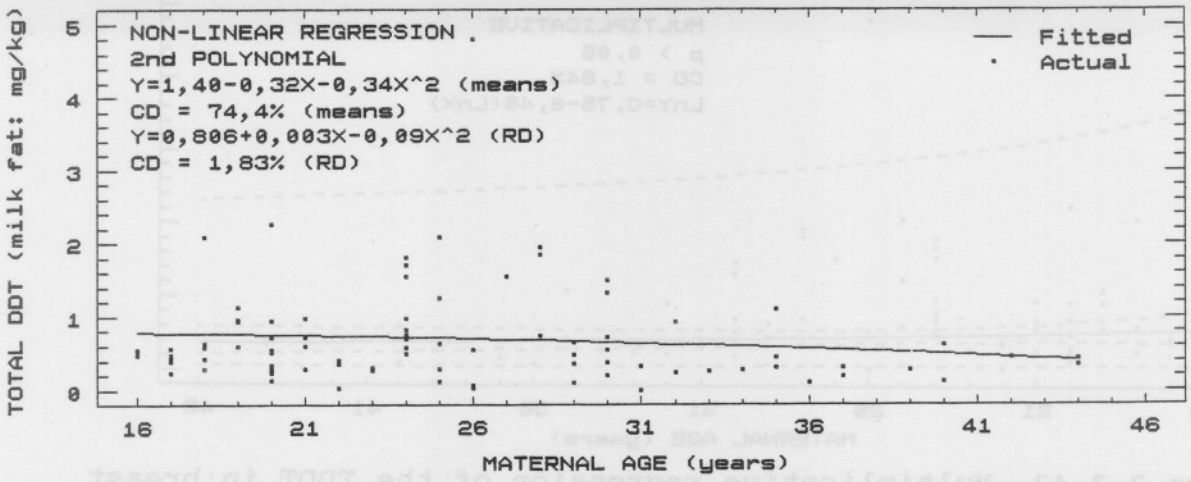

Figure 3.3.45. Non-linear regression of the ΣDDT in breast milk of the control group on maternal age. Information for both the means (calculated separately) and raw data are given.

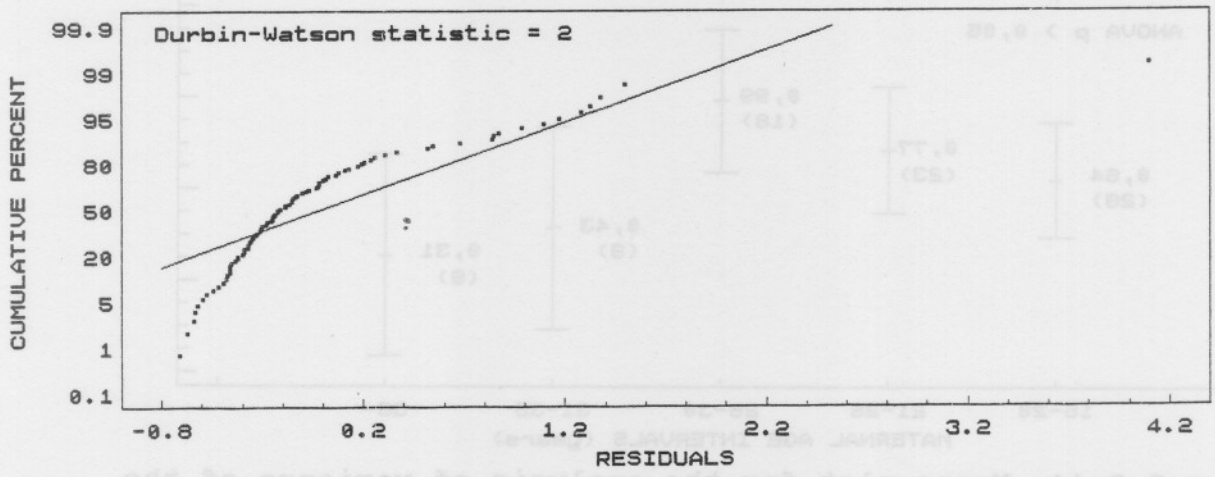

Figure 3.3.46. Probability plot of the residuals of the non-linear regression of ΣDDT in breast milk of the control group on maternal age.

Although the non-linear regression explained the mean ΣDDT of the maternal age intervals satisfactorily (Fig. 3.3.45), the same could not be said of the explanation offered by the non-linear regression of the raw data. This was confirmed by the probability plot (Fig. 3.3.46), which indicated a rather large systemic error.

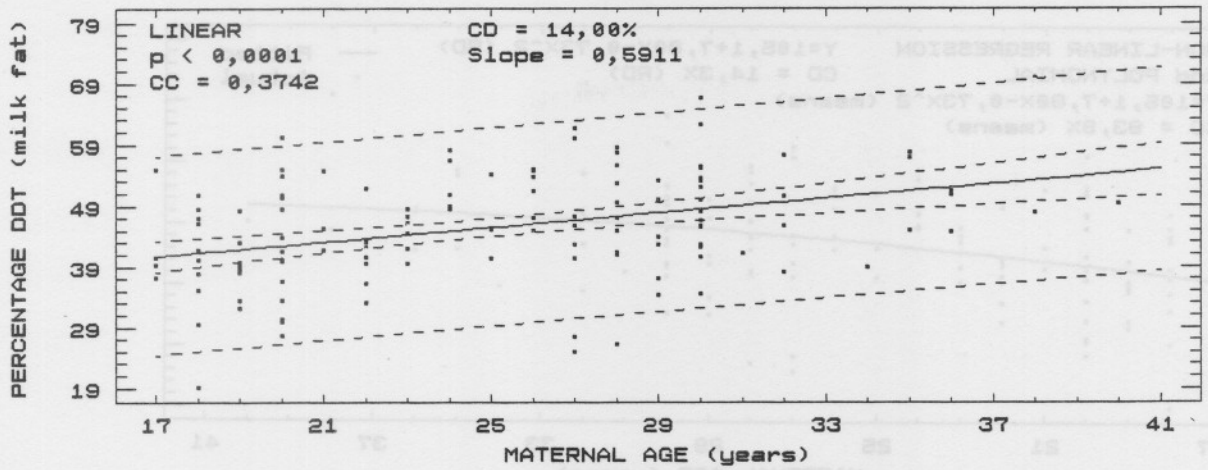

Figure 3.3.47. Linear regression of the percentage DDT in breast milk of the exposed group on maternal age. 95% confidence and prediction limits are shown.

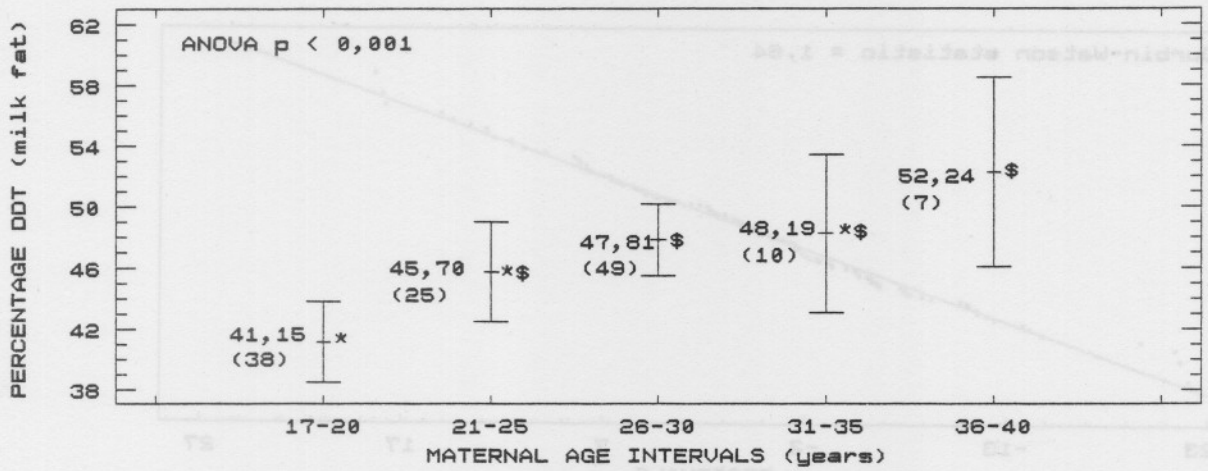

Figure 3.3.48. Means plot for the analysis of variance of the percentage DDT in breast milk of the exposed group by maternal age intervals. 95% confidence limits, means and sample size () are shown. Intervals marked with the same symbols were not significantly different ($p > 0,05$).

The linear regression, which gave the best fit (Fig. 3.3.47), showed a significant increase in the percentage DDT in breast milk from mothers of the exposed group. The correlation coefficient and coefficient of determination was small, probably due to the outliers above and below the 95% prediction limits. The anova (Fig. 3.3.48) showed a significant difference between the 17-20 years interval and the 26-30 and 36+ years age intervals.

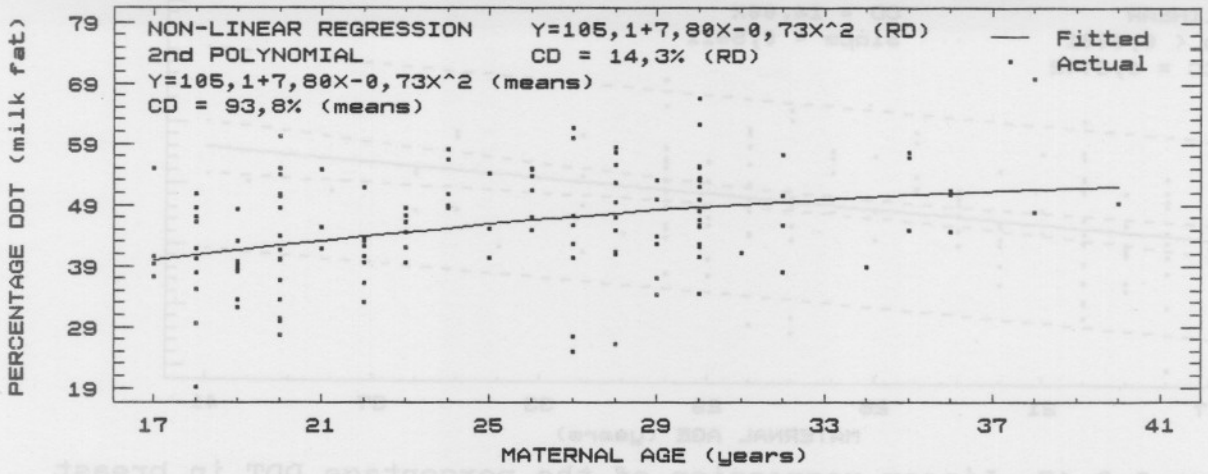

Figure 3.3.49. Non-linear regression of the percentage DDT in breast milk of the exposed group on maternal age. Information for the means (calculated separately) and raw data are given.

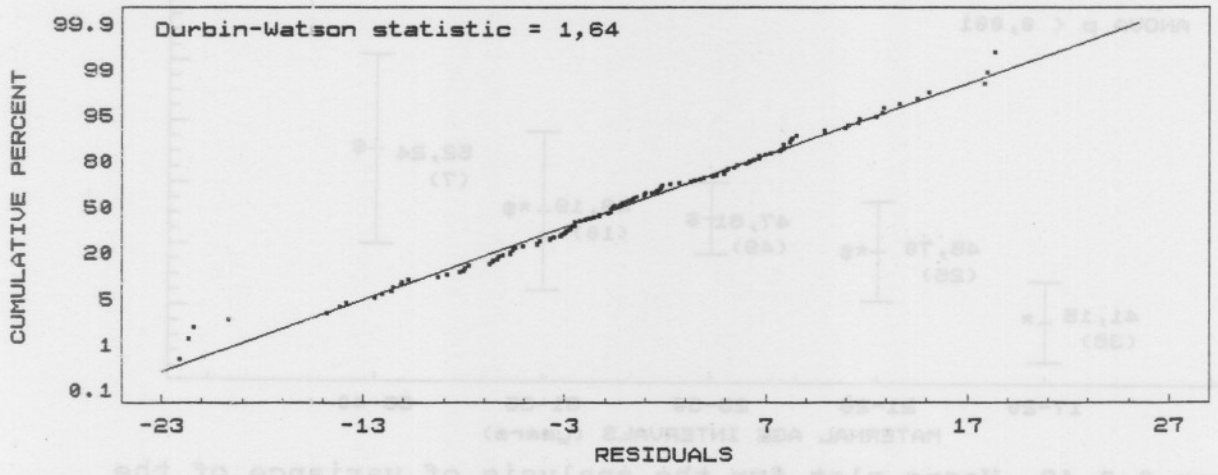

Figure 3.3.50. Probability plot of the residuals of the non-linear regression of the percentage DDT in breast milk of the exposed group on maternal age.

The very good coefficient of determination for the non-linear regression (Fig. 3.3.49) of the means was reduced to almost the same value for the linear regression (Fig. 3.3.48) when fitted to the raw data. The probability plot (Fig. 3.3.50) showed a good fit of the residuals around the mean, with only a slight variation noticeable.

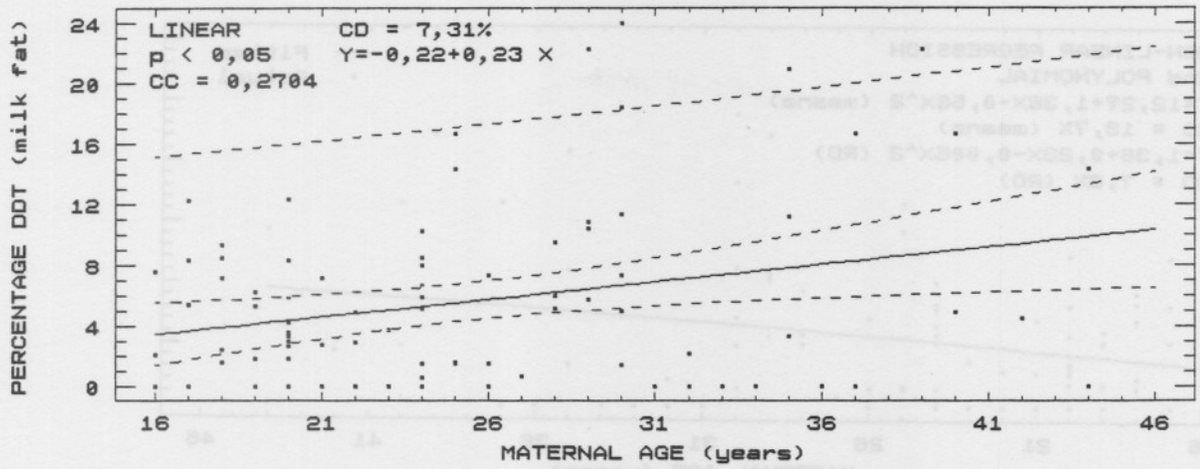

Figure 3.3.51. Linear regression of the percentage DDT in breast milk of the control group on maternal age. 95% confidence and prediction limits are shown.

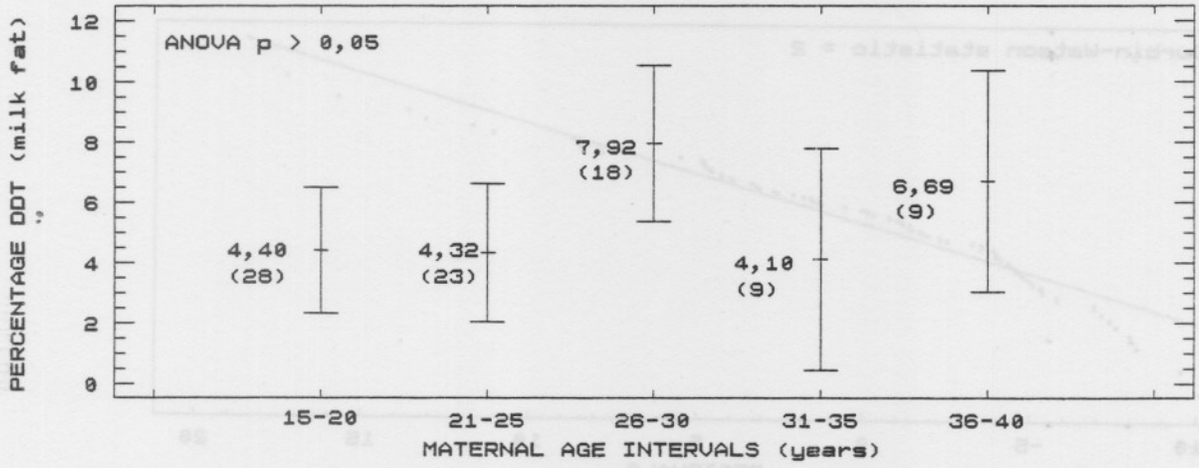

Figure 3.3.52. Means plot for the analysis of variance of the percentage DDT in breast milk of the control group by maternal age intervals. 95% confidence limits, means and sample size () are shown.

A significant p-value (and therefore a significant increase in the percentage DDT), but a low coefficient of determination, characterized the linear regression of the percentage DDT on maternal age for the control group (Fig. 3.3.51). The slope (standard error = 0,088) was less than half the slope of the exposed group (Fig. 3.3.47). The anova (Fig. 3.3.52) showed no significant differences ($p > 0,05$) between the chosen age intervals.

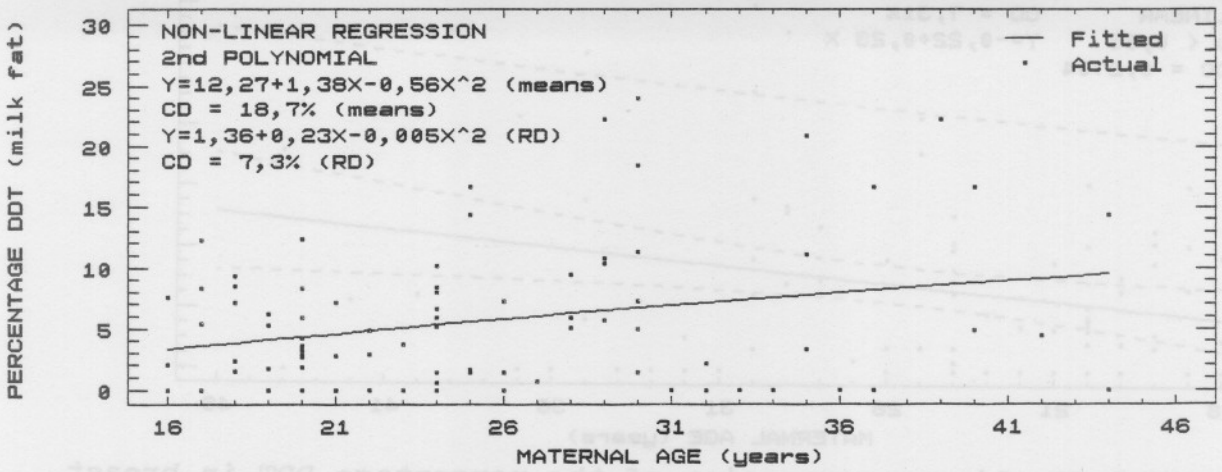

Figure 3.3.53. Non-linear regression of the percentage DDT in breast milk of the control group on maternal age. Information for the means (calculated separately) and raw data are given.

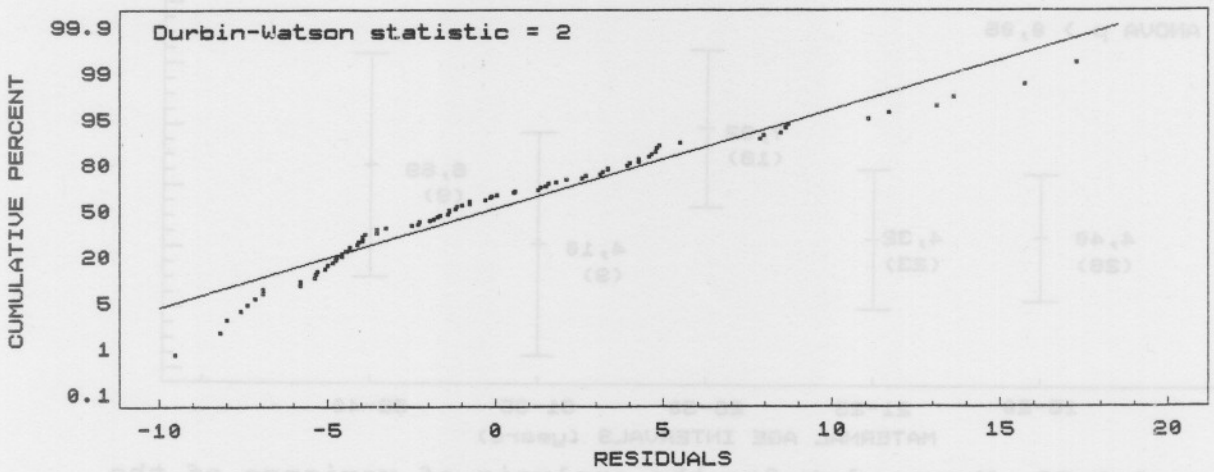

Figure 3.3.54. Probability plot of the residuals of the non-linear regression of the percentage DDT in breast milk of the control group on maternal age.

The non-linear regression model did not explain the relationship between maternal age and the percentage DDT in the breast milk of the control group. This was shown by the low coefficients of determination for both the means and raw data (Fig. 3.3.53). The probability plot (Fig. 3.3.54) also indicated a strong auto-correlation.

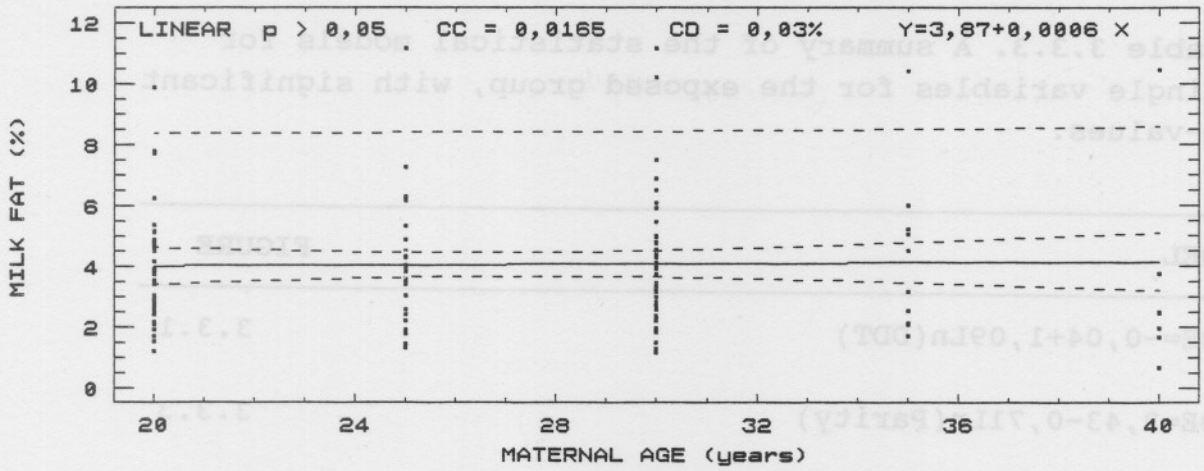

Figure 3.3.55. Linear regression of the percentage milk fat in breast milk of the exposed group on maternal age intervals. 95% confidence and prediction limits are shown.

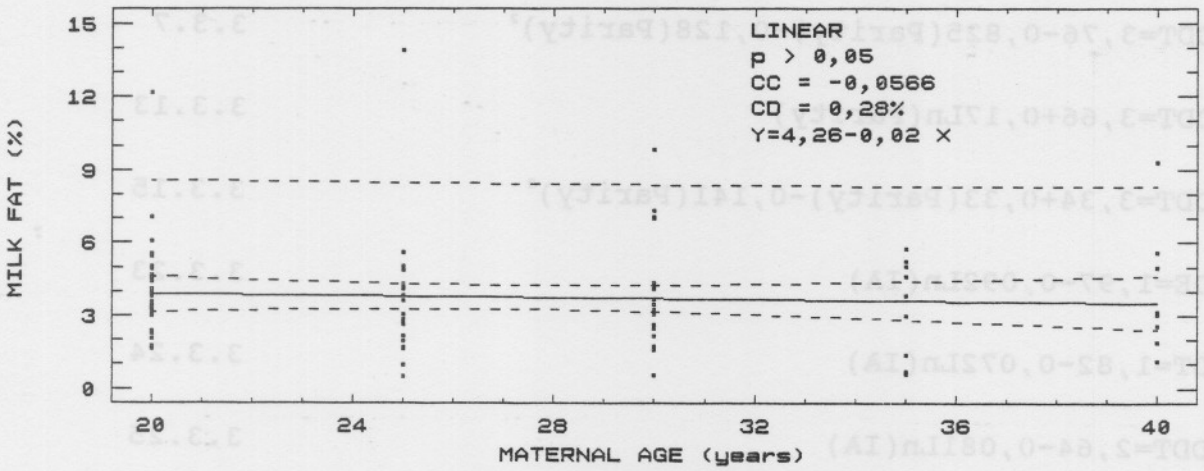

Figure 3.3.56. Linear regression of the percentage milk fat in breast milk of the control group on maternal age intervals. 95% confidence and prediction limits are shown.

No changes were found in the percentage milk fat with an increase in maternal age of the mothers of both the exposed (Fig. 3.3.55) and control groups (Fig. 3.3.56). Very low coefficients of determination were obtained.

Table 3.3.3. A summary of the statistical models for single variables for the exposed group, with significant p-values.

MODEL	FIGURE
LnDDE=-0,04+1,09Ln(DDT)	3.3.1
LnDDE=2,43-0,71Ln(Parity)	3.3.3
LnDDT=2,05-0,39Ln(Parity)	3.3.4
LnΣDDT=2,99-0,56Ln(Parity)	3.3.5
LnΣDDT=3,76-0,825(Parity)-0,128(Parity)^2	3.3.7
Ln%DDT=3,66+0,17Ln(Parity)	3.3.13
Ln%DDT=3,34+0,33(Parity)-0,141(Parity)^2	3.3.15
LnDDE=1,97-0,092Ln(IA)	3.3.23
LnDDT=1,82-0,072Ln(IA)	3.3.24
LnΣDDT=2,64-0,081Ln(IA)	3.3.25
%Fat=2,995+0,125(IA)	3.3.33
LnDDE=7,12-1,64Ln(MA)	3.3.37
LnDDT=4,83-0,968Ln(MA)	3.3.38
LnΣDDT=6,75-1,32Ln(MA)	3.3.39
LnΣDDT=5,40-0,18(MA)-0,14(MA)^2	3.3.41
%DDT=30,70+0,59(MA)	3.3.47
%DDT=105,1+7,80(MA)-0,73(MA)^2	3.3.49

IA = Infant age
MA = Maternal age

Table 3.3.4. A summary of the statistical models for single variables for the control group, with p-values less than 0,022.

MODEL	FIGURE
LnDDT=0,526+0,326Ln(DDT)	3.3.2
LnΣDDT=-0,376-0,427Ln(Parity)	3.3.9
Ln%DDT=1,35+0,43(Parity)	3.3.17
LnΣDDT=0,748-0,458(MA)	3.3.43
%DDT=-0,216-0,229(MA)	3.3.51

MA = Maternal age

The models presented in Tables 3.3.3 and 3.3.4 show that various relationships exist, using either raw or transformed data. A better explanation of the observed relationships was often obtained by using non-linear regression models. For some, the coefficient of determination hardly changed when using linear or non-linear regression. The various relationships will be further discussed in sub-section 4.3.

The foregoing regression analysis for single variables for the exposed and control groups showed that the models, although the fits obtained were reasonable to a certain extent, usually explained only a small portion of the observed data (i.e. small coefficients of determination). This implies that factors such as maternal and infant age, might be nested or confounded with the parity status of the mother in respect of DDT-levels in her milk. This assumption was tested using multiple regression with stepwise variable selection (Statistical Graphics Corporation, 1987). All possible combinations and transformations were tried and the following tables will give only a selection of those models with reasonable adjusted coefficients of determination. Two models will be discussed in detail.

Table 3.3.5. Results of various variable interaction selections as well as single variables for multiple regression models explaining ln ΣDDT-levels in the breast milk of the exposed group. The left part of the table shows the variables and how it is transformed, and the right part shows the results of different combinations of the variables.

VARIABLES	F-ENTER	VARIABLES IN MODEL	CD (adjusted)
1 Ln (PAR)(MA)	37,63	1	22,3%
2 Ln (MA)(IA)	7,82	1 2	23,9%
3 Ln (PAR)(IA)	15,72	1 3	**24,0%[a]**
		1 2 3	23,4%

VARIABLES	F-ENTER	VARIABLES IN MODEL	CD (adjusted)
1 PAR	25,83	1	16,2%
2 MA	23,96	1 2	17,3%
3 IA	2,23	1 3	18,1%
		1 2 3	18,8%

VARIABLES	F-ENTER	VARIABLES IN MODEL	CD (adjusted)
1 Ln PAR	36,92	1	21,9%
2 Ln MA	25,56	1 2	21,7%
3 Ln IA	5,11	1 3	**23,7%[b]**
		1 2 3	23,4%

PAR = parity MA = Maternal age
IA = Infant age CD = Coefficient of determination
a and b = models to be discussed as Model 3.3.1 and Model 3.3.2, respectively.

Models 3.3.1 and 3.3.2 (Table 3.3.5) had the best coefficients of determination (CD). Model 3.3.1 relied on interaction between the variables, and Model 3.3.2 on the log (natural) transformed data. In the following tables and graphs, Model 3.3.1 (with variables 1 and 3) and Model 3.3.2 (also with variables 1 and 3) will be detailed.

Table 3.3.6. Results of the model (Model 3.3.1) for Ln ΣDDT-levels in the breast milk of mothers from the exposed group.

INDEPENDENT VARIABLE	COEFFICIENT	STANDARD ERROR	T-VALUE	P-VALUE
CONSTANT	4,285	0,276	15,50	0,0000
Ln (PAR)(MA)	-0,349	0,071	-4,89	0,0000
Ln (PAR)(IA)	-0,058	0,029	-1,98	0,0495

PAR = Parity MA = Maternal age IA = Infant age

Table 3.3.7. Results of the model (Model 3.3.2) for Ln ΣDDT-levels in the breast milk of mothers from the exposed group.

INDEPENDENT VARIABLE	COEFFICIENT	STANDARD ERROR	T-VALUE	P-VALUE
CONSTANT	3,245	0,166	19,56	0,0000
Ln PAR	-0,501	0,084	-5,93	0,0000
Ln IA	-0,058	0,029	-1,98	0,0501

PAR = Parity IA = Infant age

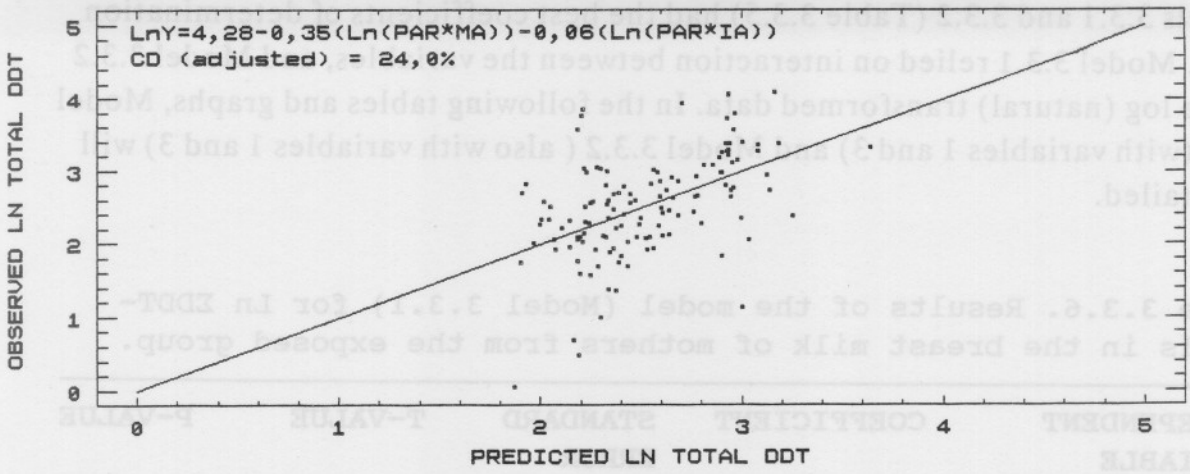

Figure 3.3.57. Plot of the predicted relationship (line) and observed values (squares) for model 3.3.1.

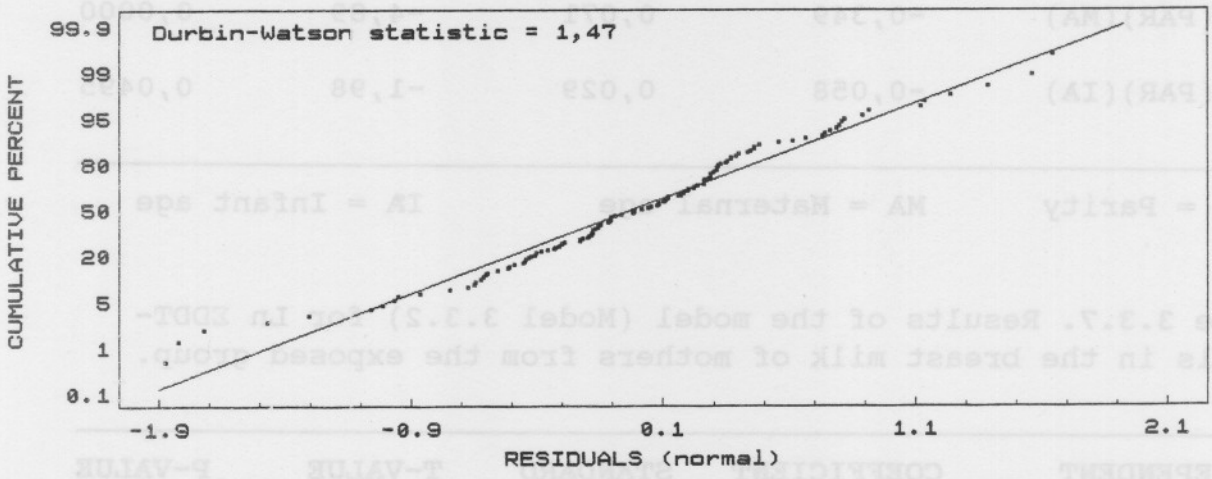

Figure 3.3.58. Probability plot of the residuals of Model 3.3.1.

Fig. 3.3.57 shows the plot of Model 3.3.1 and the observed values. The reason for the low coefficient of determination was the bad fit of the raw data to the predicted model. The probability plot (Fig. 3.3.58) shows strong deviations.

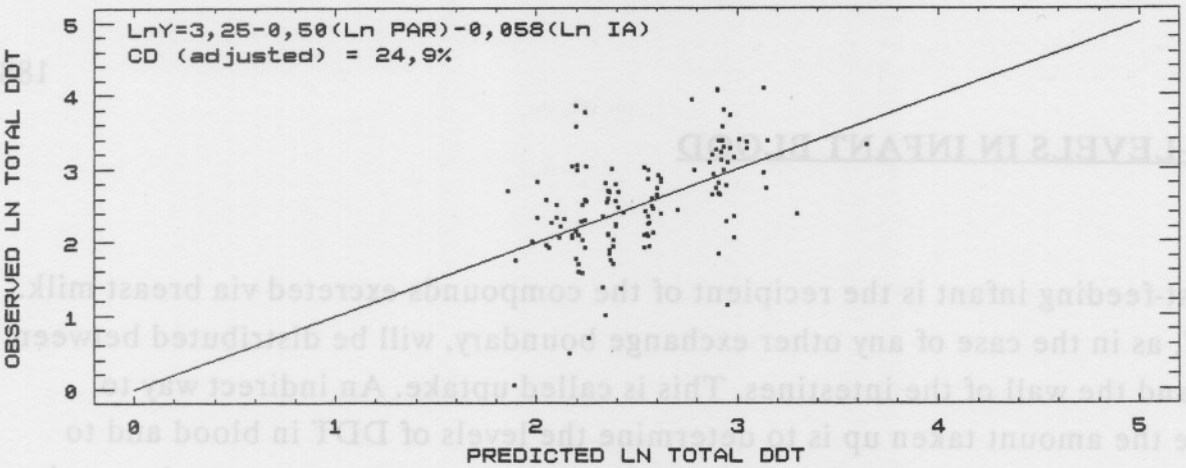

Figure 3.3.59. Plot of the predicted model (line) and observed values (squares) for Model 3.3.2.

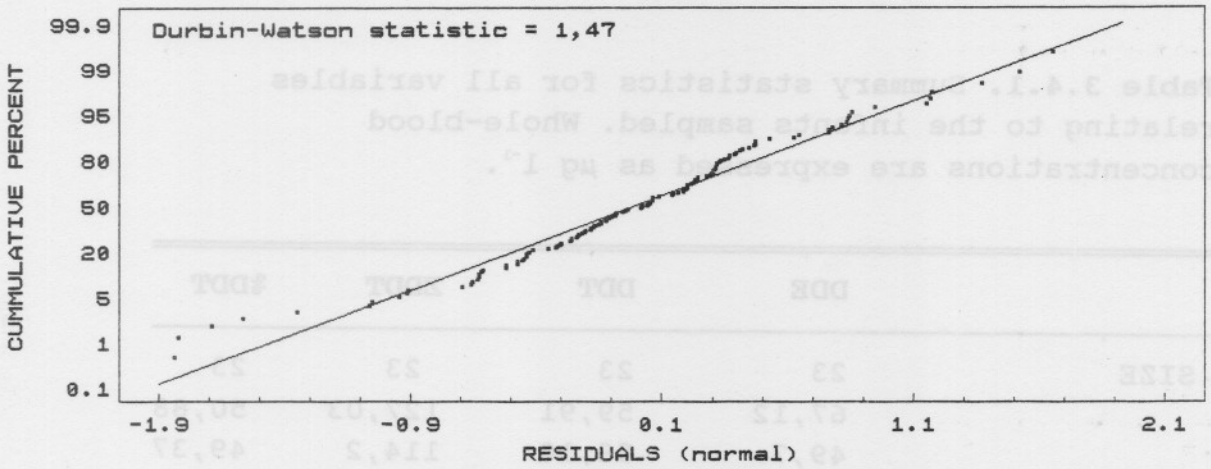

Figure 3.3.60. Probability plot of the residuals of Model 3.3.2.

Fig. 3.3.59 shows very much the same profile as Model 3.3.1 (Fig. 3.3.57). The probability plot (Fig. 3.3.60) also shows the same deviation near the bottom end, when compared with the same plot for Model 3.3.1 (Fig. 3.3.58).

3.4 DDT-LEVELS IN INFANT BLOOD

The breast-feeding infant is the recipient of the compounds excreted via breast milk. The DDT, as in the case of any other exchange boundary, will be distributed between the milk and the wall of the intestines. This is called uptake. An indirect way to determine the amount taken up is to determine the levels of DDT in blood and to extrapolate dosage and body burdens from this. This sub-section presents the results of the measurement of the levels of DDT and its metabolites in whole-blood from 23 infants (exposed group) exposed to DDT through contaminated breast milk.

Table 3.4.1. Summary statistics for all variables relating to the infants sampled. Whole-blood concentrations are expressed as $\mu g\ l^{-1}$.

	DDE	DDT	ΣDDT	%DDT
SAMPLE SIZE	23	23	23	23
MEAN	67,12	59,91	127,03	50,88
MEDIAN	49,7	58,10	114,2	49,37
STANDARD DEVIATION	52,92	30,17	70,84	16,16
STANDARD ERROR	11,04	6,29	14,77	3,37
MINIMUM	5,6	16,9	29,4	22,15
MAXIMUM	218,4	135,3	316,5	80,95
RANGE	212,8	118,4	287,1	58,78

	INFANT AGE (days)	PARITY
SAMPLE SIZE	23	23
MEAN	252,4	3,17
MEDIAN	240	3
STANDARD DEVIATION	144,99	1,85
STANDARD ERROR	30,23	0,39
MINIMUM	5	1
MAXIMUM	540	7
RANGE	535	6

Table 3.4.2. Summary statistics for all variables relating to the mothers of the infants sampled.

WHOLE-MILK ($\mu g\ l^{-1}$)	DDE	DDD	DDT	ΣDDT
SAMPLE SIZE	23	23	23	23
MEAN	297,0	13,60	222,9	535,1
MEDIAN	201,5	9,89	200,9	427,1
STANDARD DEVIATION	345,1	10,34	184,7	519,9
STANDARD ERROR	71,9	2,16	38,5	108,4
MINIMUM	67,1	1,05	35,6	144,1
MAXIMUM	1758,8	43,7	967,9	2743,4
RANGE	1691,7	42,7	932,3	2599,3

MILK FAT ($mg\ kg^{-1}$)	DDE	DDD	DDT	ΣDDT
SAMPLE SIZE	23	23	23	23
MEAN	8,87	0,44	6,74	15,06
MEDIAN	7	0,34	5,9	13,1
STANDARD DEVIATION	7,81	0,36	4,31	11,08
STANDARD ERROR	1,63	0,08	0,90	2,31
MINIMUM	1,9	0,02	1,22	3,1
MAXIMUM	36,9	1,6	20,3	57,6
RANGE	35,0	1,58	19,08	54,5

	%DDT	MATERNAL AGE (years)	MILK FAT %
SAMPLE SIZE	23	23	23
MEAN	45,40	25,83	3,50
MEDIAN	45,06	25	3,15
STANDARD DEVIATION	5,97	5,53	1,58
STANDARD ERROR	1,24	1,15	0,33
MINIMUM	35,24	17	1,43
MAXIMUM	57,28	35	6,29
RANGE	22,04	18	4,86

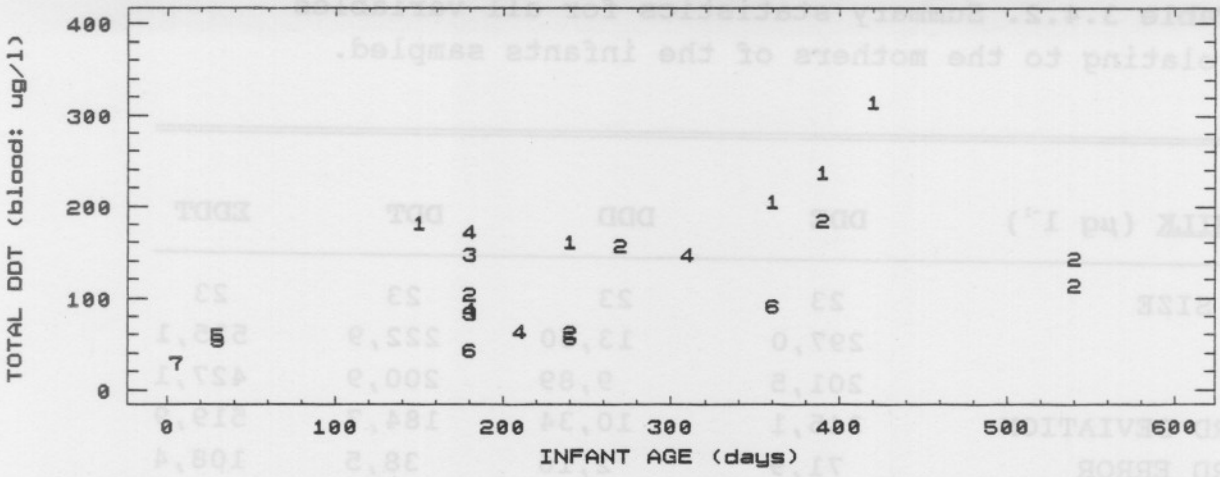

Figure 3.4.1. Scatterplot of levels of the ΣDDT in whole-blood of infants breast-fed by mothers from the exposed group against age. The number indicates the parity of the specific infant.

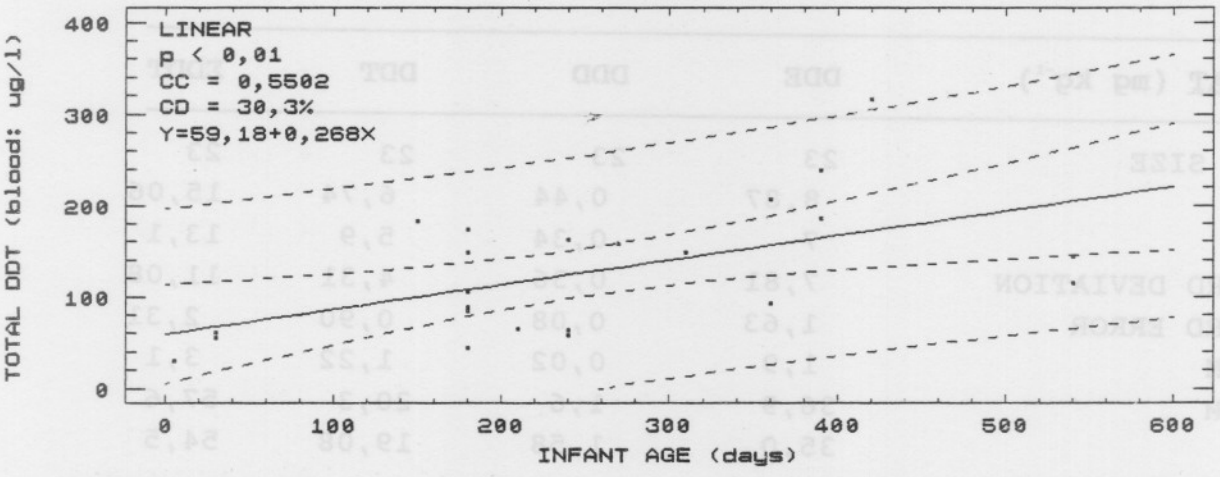

Figure 3.4.2. Linear regression of the ΣDDT in whole-blood of infants breast-fed by mothers from the exposed group. 95% confidence and prediction limits are shown.

A plot of the observed ΣDDT-levels in the infant blood against the age of the infant is given in Fig.3.4.1., and the linear regression in Fig.3.4.2. An increase in levels with age was apparent and significant. The low initial values must be noted. The distribution of the blood concentration of ΣDDT of the firstborn children was limited to the area above the regression line. A firstborn also had the highest value. The others were without any obvious pattern. Also noticeable was the even scattering of the raw data around the regression line.

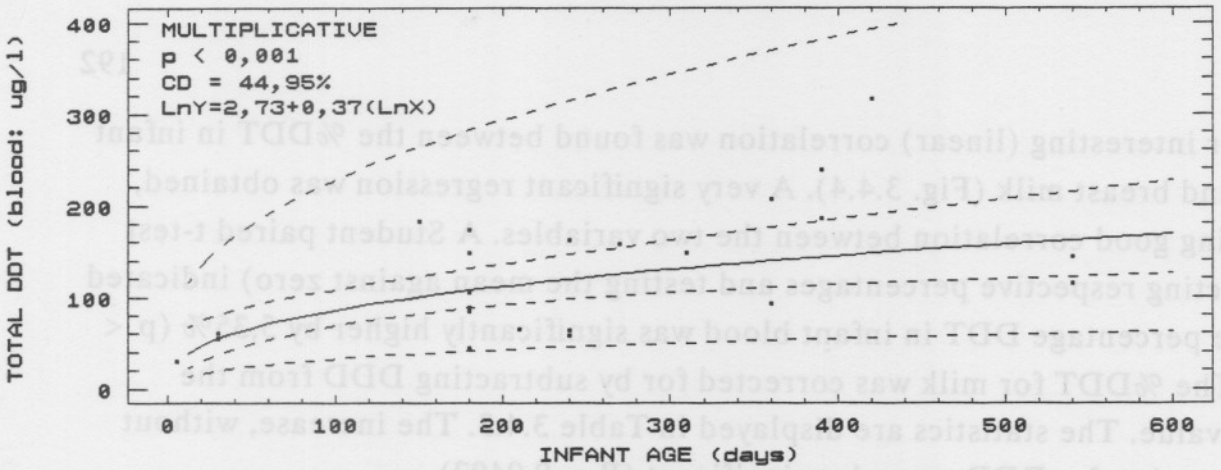

Figure 3.4.3. Multiplicative regression of the ΣDDT in whole-blood from infants breast-fed by mothers of the exposed group . 95% confidence and prediction limits are shown.

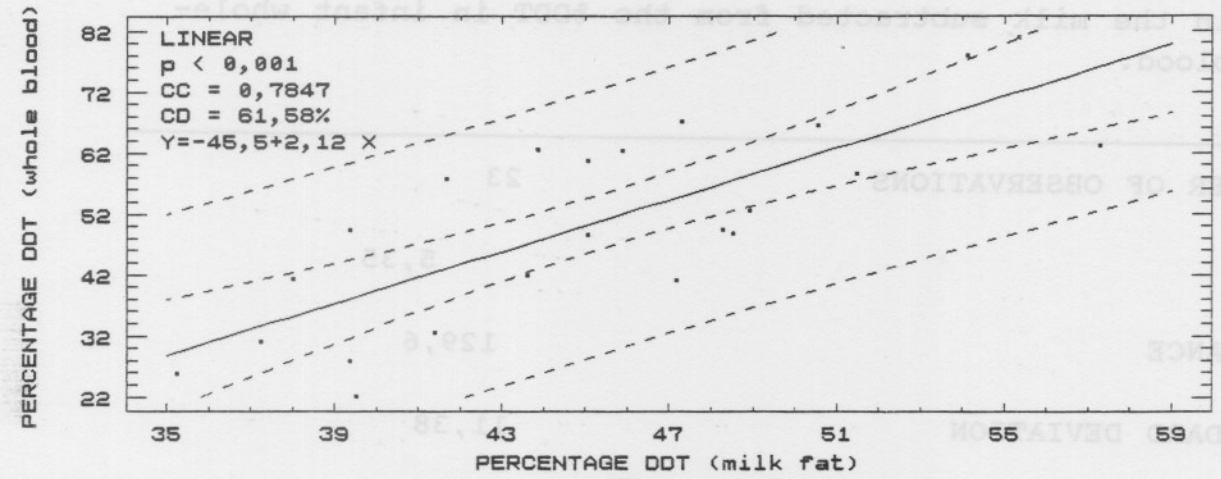

Figure 3.4.4. Linear regression of the percentage DDT in whole-blood from infants breast-fed by mothers of the exposed group. 95% confidence and prediction limits are shown.

The multiplicative regression model (Fig. 3.4.3) increased the significance (p-value), as well as improving the coefficient of determination (CD) over that of the linear regression (Fig. 3.4.2). The prediction lines originated from very close to the Y-intercept. The significance of the multiplicative model also indicated that a log transformation of the dependant values should be used for further modelling.

Another interesting (linear) correlation was found between the %DDT in infant blood and breast milk (Fig. 3.4.4). A very significant regression was obtained, indicating good correlation between the two variables. A Student paired t-test (subtracting respective percentages and testing the mean against zero) indicated that the percentage DDT in infant blood was significantly higher by 5,35% ($p < 0,05$). The %DDT for milk was corrected for by subtracting DDD from the ΣDDT value. The statistics are displayed in Table 3.4.3. The increase, without the correction for DDD, was also significant ($P = 0,0402$).

Table 3.4.3. One sample, two sided t-test for the means and variances of the %DDT (corrected for DDD) in the milk subtracted from the %DDT in infant whole-blood.

NUMBER OF OBSERVATIONS	23
MEAN	5,35
VARIANCE	129,6
STANDARD DEVIATION	11,38
MEDIAN	3,49
95% CONFIDENCE INTERVAL FOR MEAN	0,4279 - 10,28 (22 Degrees of freedom)
NULL HYPOTHESIS:	**MEAN = 0**
T-STATISTIC	2,25
P-VALUE	**0,0344**
AT ALPHA 0,05	**HYPOTHESIS REJECTED**

Modelling, as was the case in the previous section, was done with the different variables that logically could have influenced the eventual DDT-levels in the infant. Multiple regression analysis with stepwise variable selection was done on numerous combinations, interactions and transformations of the variables. Only those models selected on the grounds of good coefficients of determination will be described in more detail. Three models, one each for ΣDDT, DDE and DDT will be discussed.

Table 3.4.4. Results for variables introduced in a multiple model, using stepwise selection, to model Ln ΣDDT in the infant. Two attempts, one based on levels of ΣDDT in whole-milk, and the other on levels of ΣDDT in the milk fat are presented. All variables were log (natural) transformed. The left column shows the variables and how it was transformed, and the right column shows the results of the different variable combinations.

VARIABLES	F-ENTER	VARIABLES IN MODEL	CD (adjusted)
1 Ln PARITY	24,72	1	60,5%
2 Ln INFANT AGE	17,15	1 2	68,5%
3 Ln ΣDDT (milk fat)	2,11	**1 2 3**	**70,0%[a]**
4 Ln MATERNAL AGE	14,18	1 2 3 4	68,3%

VARIABLE	F-ENTER	VARIABLES IN MODEL	CD (adjusted)
1 Ln PARITY	34,72	1	60,5%
2 Ln INFANT AGE	17,15	1 2	68,5%
3 Ln ΣDDT (whole-milk)	3,51	1 2 3	68,9%
4 Ln MATERNAL AGE	14,18	1 2 3 4	67,7%

CD = Coefficient of determination.
a = To be discussed as Model 3.4.1.

Model 3.4.1 had the best coefficient of determination (Table 3.4.4). It relied on three variables, namely parity, infant age and ΣDDT-levels (milk fat) to which the infant was exposed. Introducing the fourth variable reduced the coefficient of determination. Replacing the third with the fourth variable had the same effect. As can be seen, the level of ΣDDT in both cases (based on levels of

ΣDDT in whole-milk or milk fat) contributed less to the final model than the other two variables. A more detailed discussion of Model 3.4.1 will be given in the following tables and figures. Models will also be presented for DDE and DDT levels.

Table 3.4.5. Model fitting results (Model 3.4.1) for Ln ΣDDT-levels in the whole-blood of the infant.

INDEPENDENT VARIABLE	COEFFICIENT	STANDARD ERROR	T-VALUE	P-VALUE
CONSTANT	3,765	0,567	6,645	0,0000
Ln PARITY	-0,528	0,127	-4,161	0,0005
Ln INFANT AGE	0,196	0,077	2,554	0,0194
Ln ΣDDT (milk fat)	0,162	0,115	1,141	0,1747

Table 3.4.6. Analysis of variance for the full regression and the variables for Model 3.4.1.

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	P-VALUE
MODEL	5,855	3	1,952	0,0000
ERROR	2,049	19	0,108	
Ln PARITY	4,925	1	4,925	0,0000
Ln INFANT AGE	0,715	1	0,715	0,0185
Ln ΣDDT (milk fat)	0,214	1	0,214	0,1747

Further statistics of the model were:
Mean Absolute Error = 0,2473
Standard Error of Estimate = 0,3284

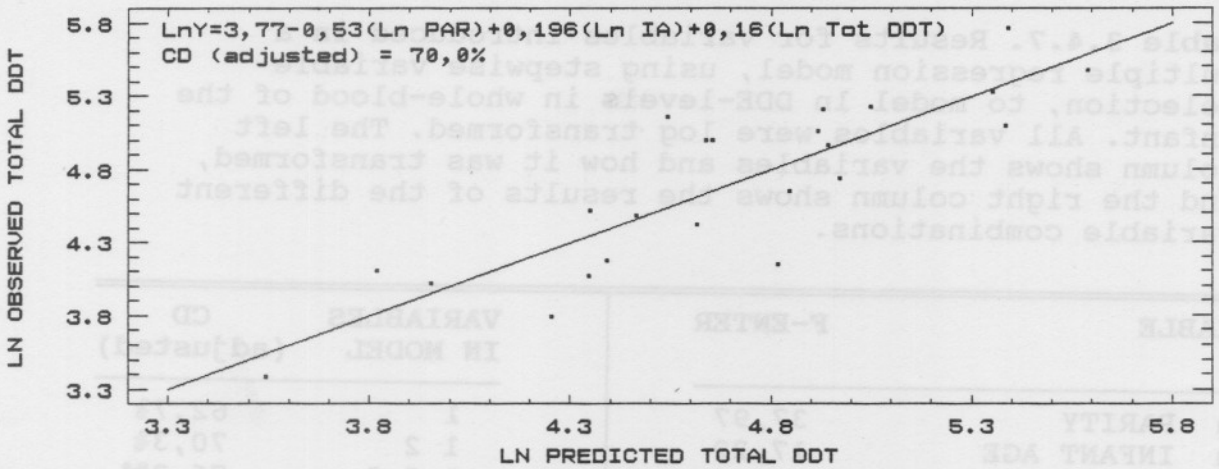

Figure 3.4.5. Plot of model (line) against observed values (squares), as determined by the expression for Model 3.4.1, for the ΣDDT in infant whole-blood.

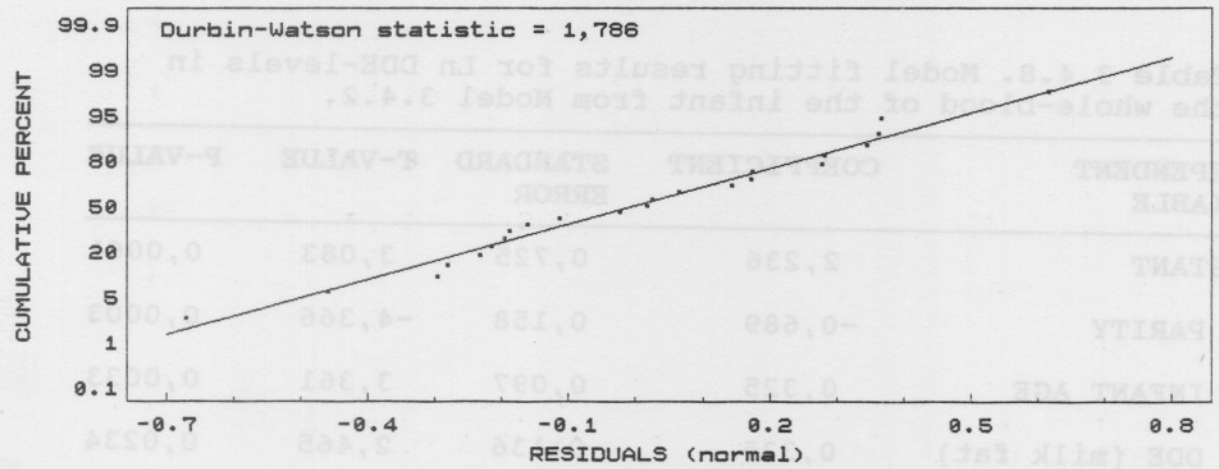

Figure 3.4.6. Probability plot of the residuals of Model 3.4.1 of ΣDDT in whole-blood of the infants sampled.

The plot of the observed values versus the predicted relationship (Fig. 3.4.5) shows a model much improved from that shown in Figure 3.4.2. This was accompanied by a much reduced distribution of the raw data around the predicted relationship (line). The probability plot (Fig. 3.4.6) shows a slight pattern that increases in frequency very tightly around the line. It was unfortunately not possible to identify specific raw data in this plot, and further clarification as to the possible reasons for this was thus not possible. Possible influence measures not included in the model will be discussed later (sub-section 4.5).

Table 3.4.7. Results for variables introduced in a multiple regression model, using stepwise variable selection, to model ln DDE-levels in whole-blood of the infant. All variables were log transformed. The left column shows the variables and how it was transformed, and the right column shows the results of the different variable combinations.

VARIABLE	F-ENTER	VARIABLES IN MODEL	CD (adjusted)
1 Ln PARITY	37,97	1	62,7%
2 Ln INFANT AGE	17,22	1 2	70,3%
3 Ln DDE (milk fat)	1,66	**1 2 3**	**76,3%[a]**
4 Ln MATERNAL AGE	10,91	1 2 3 4	75,4%

CD = Coefficient of determination.
a = To be discussed as Model 3.4.2.

Table 3.4.8. Model fitting results for Ln DDE-levels in the whole-blood of the infant from Model 3.4.2.

INDEPENDENT VARIABLE	COEFFICIENT	STANDARD ERROR	T-VALUE	P-VALUE
CONSTANT	2,236	0,725	3,083	0,0061
Ln PARITY	-0,689	0,158	-4,366	0,0003
Ln INFANT AGE	0,325	0,097	3,361	0,0033
Ln DDE (milk fat)	0,335	0,136	2,465	0,0234

Table 3.4.9. Analysis of variance for the full regression and the variables of Model 3.4.2.

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	P-VALUE
MODEL	11,846	3	3,9486	0,0000
ERROR	3,044	19	0,1602	
Ln PARITY	9,5877	1	9,5877	0,0000
Ln INFANT AGE	1,2849	1	1,2849	0,0107
Ln DDE (milk fat)	0,9732	1	0,9732	0,0234

Further statistics of the model were:
Mean Absolute Error = 0,3024
Standard Error of Estimate = 0,4003

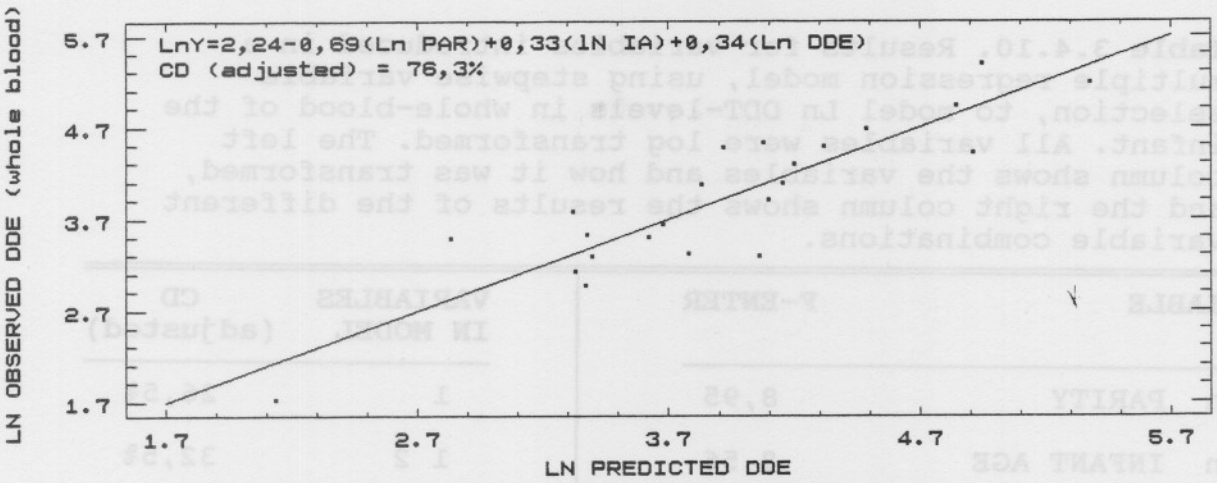

Figure 3.4.7. Plot of model (line) against observed values (squares) as determined by the expression for Model 3.4.2 for the DDE in infant whole-blood.

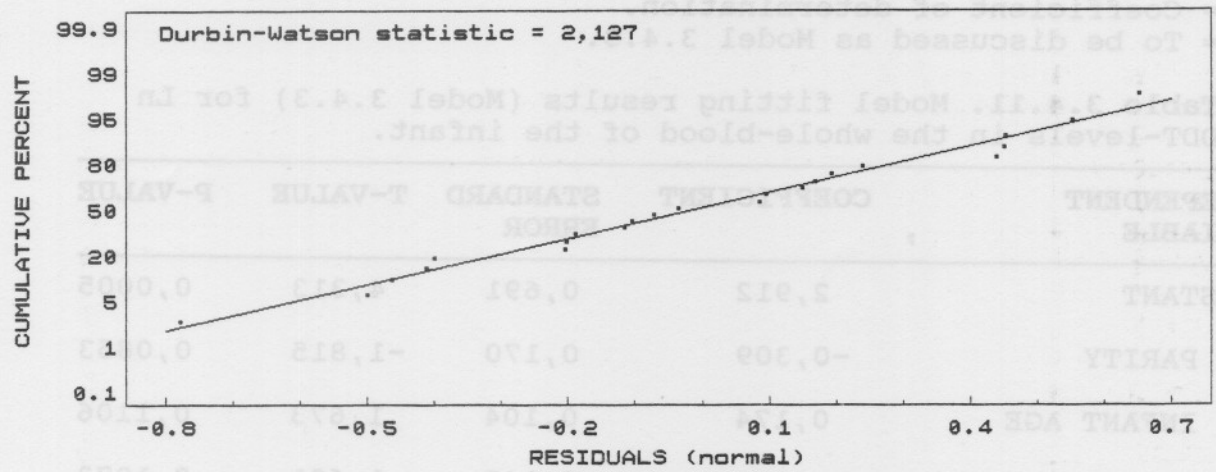

Figure 3.4.8. Probability plot of the residuals of model 3.4.2 of DDE in whole-blood in infants.

The plot of the model for the DDE in infant blood (Fig. 3.4.7) looks similar to the model for the ΣDDT-levels (Fig. 3.4.5). The variation of the data around the model prediction might be slightly less. The probability plot (Fig. 3.4.8) also shows a cyclic pattern similar to the ΣDDT probability plot (Fig. 3.4.6). The Durbin-Watson statistic is larger than 2, indicating auto-correlation between errors.

Table 3.4.10. Results for variables introduced in a multiple regression model, using stepwise variable selection, to model Ln DDT-levels in whole-blood of the infant. All variables were log transformed. The left column shows the variables and how it was transformed, and the right column shows the results of the different variable combinations.

VARIABLE	F-ENTER	VARIABLES IN MODEL	CD (adjusted)
1 Ln PARITY	8,95	1	26,5%
2 Ln INFANT AGE	8,56	1 2	32,5%
3 Ln DDT (milk fat)	2,53	1 2 3	38,2%[a]
4 Ln MATERNAL AGE	7,23	1 2 3 4	36,1%

CD = Coefficient of determination.
a = To be discussed as Model 3.4.3.

Table 3.4.11. Model fitting results (Model 3.4.3) for Ln DDT-levels in the whole-blood of the infant.

INDEPENDENT VARIABLE	COEFFICIENT	STANDARD ERROR	T-VALUE	P-VALUE
CONSTANT	2,912	0,691	4,313	0,0005
Ln PARITY	-0,309	0,170	-1,815	0,0853
Ln INFANT AGE	0,174	0,104	1,673	0,1106
Ln DDT (milk fat)	0,251	0,149	1,691	0,1073

Table 3.4.12. Analysis of variance for the full regression and the variables of Model 3.4.3.

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	P-VALUE
MODEL	3,284	3	1,5318	0,0067
ERROR	3,760	19	0,1979	
Ln PARITY	2,1056	1	2,1056	0,0041
Ln INFANT AGE	0,6128	1	0,6128	0,0945
Ln DDE (milk fat)	0,5655	1	0,5655	0,1073

Further statistics of the model were:
Mean Absolute Error = 0,3112
Standard Error of Estimate = 0,4448

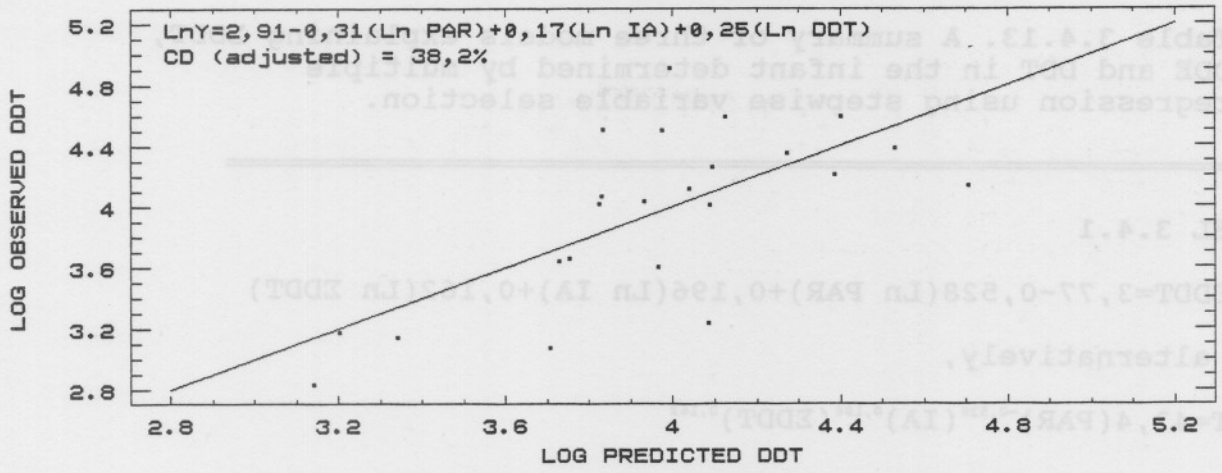

Figure 3.4.9. Plot of the model (line) against observed values (squares) as determined by the expression for Model 3.4.3 for the DDT in infant whole-blood.

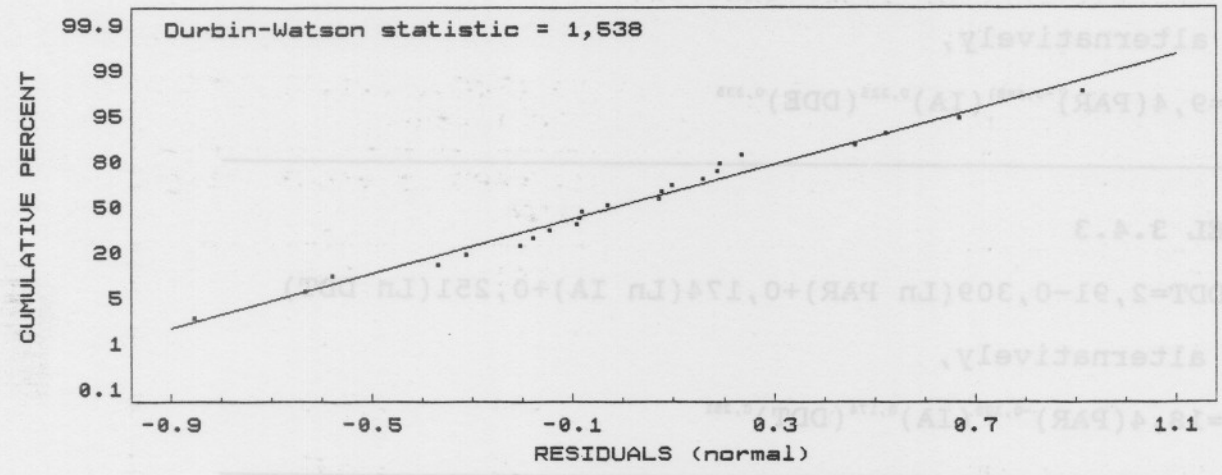

Figure 3.4.10. Probability plot of the residuals of Model 3.4.3 of DDT in whole-blood of the infants sampled.

The plot of the model for DDT in the infant (Fig. 3.4.9) shows a wide distribution of the raw data around the prediction, resulting in a low coefficient of determination. The probability plot (Fig. 3.4.10) shows a tight fit of the residuals around the line, with some slight variations.

Table 3.4.13. A summary of three models explaining ΣDDT, DDE and DDT in the infant determined by multiple regression using stepwise variable selection.

MODEL 3.4.1

Ln ΣDDT=3,77-0,528(Ln PAR)+0,196(Ln IA)+0,162(Ln ΣDDT)

or, alternatively,

$\Sigma DDT = 43,4(PAR)^{-0,528}(IA)^{0,196}(\Sigma DDT)^{0,162}$

MODEL 3.4.2

Ln DDE=2,24-0,688(Ln PAR)+0,325(Ln IA)+0,335(Ln DDE)

or, alternatively,

$DDE = 9,4(PAR)^{-0,688)}(IA)^{0,325}(DDE)^{0,335}$

MODEL 3.4.3

Ln DDT=2,91-0,309(Ln PAR)+0,174(Ln IA)+0,251(Ln DDT)

or, alternatively,

$DDT = 18,4(PAR)^{-0,309}(IA)^{0,174}(DDT)^{0,251}$

PAR = Parity
IA = Infant age (days)

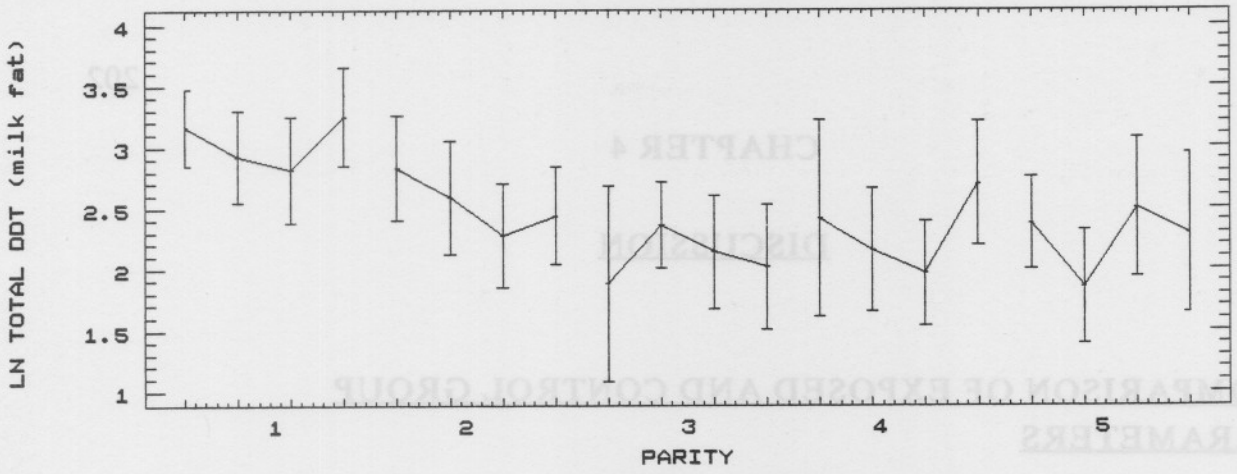

Figure 3.4.11. Means plot of multiple factor analysis of variance, including parity, infant age and levels of ΣDDT in milk (milk fat). Each parity is sub-divided in four age categories, namely, 0-4; 5-8; 9-12 and 13-24 months, and the means for each parity are connected by a solid line.

Fig. 3.4.11 indicates, to a certain extend, the nested nature of the three variables, namely parity, infant age and level of breast milk contamination, in the model. This also shows the difficulty involved in determining the most important predictive factor.

CHAPTER 4

DISCUSSION

4.1 COMPARISON OF EXPOSED AND CONTROL GROUP PARAMETERS

A comparison of two groups can only be done by considering the parameters measured for both groups. This approach might ignore subtle differences, such as possible geographical variation in habits and manners, that could modulate exposure to or uptake of DDT. The history of the Zulu nation tells a story of subjugation of separate tribes, mainly under the renowned king Shaka. The Zulu nation therefore consists of various clans which retained much of their original culture. This could be a source of bias. Furthermore, contact with western civilization did not occur evenly, and some areas saw more development, in a western sense, than others (Torres, 1980) introducing another possible source of bias.

There was a definite difference in level of westernisation between the two populations from which the two groups were drawn (see sub-section 2.1). This was also obvious from the higher percentage of mothers from the control group that practised bottle feeding (Table 3.1.1). In practical terms this means that, given mothers with similar exposure to, and uptake of DDT, the control group could transfer less of the organochlorines to their offspring. On the other hand, it could be argued that the absence of malaria will favour social and economic development more than its endemicity. Consequently, within the boundaries of the Republic, it would have been impossible to find a population comparable to the population living on the Makatini Plain.

To my knowledge there exist few, if any, factors other than bottle feeding that would influence comparisons. The mean maternal age, parity and infant age of the baby clinic populations did not differ significantly (Table 3.1.1). It might be argued that the control group, because of its proximity to Port Shepstone, had

better medical care, thereby decreasing infant mortality. The infant death rate (excluding still births) for Mseleni Hospital was 17,03 per thousand and for Murchison Hospital it was 18,59 per thousand (KwaZulu Department of Health and Welfare, 1985). This indicated comparable medical care to both hospital populations.

Other factors were also comparable between surveys and groups (Tables 3.1.1, 3.1.2 and 3.1.3). The decision to conduct only three surveys of the control group could be seen as a complicating factor for statistical analysis. The rate of sampling between the two groups compared very well (Table 3.1.4), although the lesser numbers for the control group might introduce some form of bias. It is normally expected that a control group should have at least the same, and preferably more subjects than the exposed group. However, the low levels of DDT and its metabolites that were measured for the control group were the only matched criterion (except bottle feeding) that differed in any way from the exposed group. The statistics concerning the DDT parameters (Table 3.2.5) proved that an adequate number of control subjects had been recruited to show significant differences regarding contamination of breast milk.

Age was another factor that could have introduced some bias. The maternal age distribution of the control group was more evenly distributed than that of the exposed group (Figs. 3.1.1 and 3.1.2). Uncertainty was also present with the infant age parameter, although a more careful estimate by the mother, the nurse and myself was possible. This possibly prevented a major distortion of the curve (Figs. 3.1.5 and 3.1.6), as many mothers initially reported the age of their infant as either three, six or nine months.

Perhaps the most important parameter, parity, was the easiest to check. If the mother seemed uncertain, she was asked by the nurse to name the children. The frequency and percentage contribution plots (Figs. 3.1.3 and 3.1.4), indicated two comparable populations, not only in mean parity, but also in distribution.

Frequency distribution of percentage milk fat showed a clustering around the mean, with a long tail to the right (Fig. 3.1.7). A review by Jensen *et al.* (1980)

reported a mean value of 4,2%, for pooled mature milk from five British cities. The highest mean was 4,8% (Bristol) and the lowest 3,7% (Edinburgh). The mean fat content from 33 women from the Ivory coast was 3,07% (standard deviation = 6,5%). Jensen *et al.* (1980) unfortunately did not report maximum values.

Hall (1979) studied the change in fat composition of milk of six mothers during one feed. The mean initial value (fore milk) was 2,42% with a maximum of 5,05%. The mean value for mature milk was 7,48% with a minimum of 3,20% and a maximum of 12,05%. The values reported for the present study compared favourably with these results. It seemed, from the data obtained in the present study, that the assumptions made in sub-section 2.3, concerning sampling time and volume required, were correct and that valid results were obtained.

The maximum fat content determined for the present study was 11,16% for the exposed and 13,87% for the control group. The samples with a high lipid content were yellowish, and were also difficult to extract. The clean-up columns became saturated and a second, and sometimes a third column was needed, to separate the fat from the contaminants. The extraction method used for the present study differed in essence from the other methods in the additional step to disrupt the fat globules and to keep the fat in suspension. Recovery of fat with this method is, however, comparable with others, as the lipid compounds are not chemically altered by this step. Most other methods used combinations of ethyl ether, petroleum ether, ethanol and methanol as solvents to extract milk fat (Jensen *et al.*, 1980), much the same as for the present study.

Distribution of the between-survey parameters were also investigated graphically. As could be expected, the smaller numbers within each survey caused the distribution to be skewed or uneven, in spite of the results of the statistical analysis which analyzed means and variance. Uncertainty about maternal age was much in evidence for the first two surveys of the exposed group (Fig. 3.1.9). The same was true for the infant age profiles (Fig. 3.1.13). The control group profiles (Figs. 3.1.17 and 3.1.21) looked much better, with fewer peaks or valleys. The age factor, especially for the exposed group, was important

in the calculation of exposure, as will be seen later. Not much could be done about this uncertainty, as some births are not recorded, especially in rural areas. (A lack of vital statistics for blacks was noted by Botha and Bradshaw (1985.) Therefore, it could be assumed that, although the age distributions seemed uneven, the actual representation was not. The accuracy of the age variables must therefore be considered as not very good and should be used, where possible, in intervals.

The contribution of the various age intervals of each survey to the total sample also varied. The percentage maternal age interval contribution was relatively constant for the three intervals from 16 to 30 years for both groups (Figs. 3.1.10 and 3.1.18). Large variations were found for the subsequent intervals. The percentage contribution of the older mothers was, however, small and assumed not to have introduced significant bias. The first and second born children were evenly represented between surveys for the exposed group (Fig. 3.1.12). The first three children were equally represented between surveys for the control group (Fig. 3.1.20).

Maternal age, parity and time of survey did not influence the mean fat content of the milk (Tables 3.1.1 and 3.1.2). Some variation in contribution were however present (Figs. 3.1.15 and 3.1.23). A closer look revealed that the majority of the samples fell in the 3-5,9% interval. The only survey not well represented on the percentage contribution charts (Figs. 3.1.16 and 3.1.24), was Mar'87 (exposed group: Fig. 3.1.16). This survey had a higher proportion of samples in the lower milk fat percentage interval. Hall (1979) stated that, except for extreme maternal dietary conditions, the fatty acid pattern remains uniform in general, and is constant for the mother. The minor variation (as the mean for this survey was not statistically significant from the others: $p > 0,05$) that was found, was therefore accepted as a natural variation.

Conclusion: Considering the results of the analysis of the data, and a visual inspection of the graphical representations thereof, it can be concluded that, in terms of the aims of the present study and the geographic actuality, an acceptable and comparable control group was employed for the present study.

4.2 SERIAL CHANGES OF DDT-LEVELS IN BREAST MILK

This sub-section will concentrate primarily on the difference in levels between the exposed and control groups and the serial change in DDT-levels in breast milk of the exposed group. The results will be compared with studies with similar time-based protocols, and with studies that compared different areas or sub-groups in terms of exposure. A more detailed discussion on how the levels determined for this study related to other reports will be given in sub-section 4.4.

4.2.1. DDT-LEVELS IN BREAST MILK OF THE EXPOSED AND CONTROL GROUPS

The respective values for the surveys are given in Tables 3.2.1 to 3.2.4. Apart from the obvious difference in magnitude of contamination with DDT and its metabolites between the two groups, other indicators must also be noted. The fact that all samples of the exposed group had detectable amounts of DDT (as compared to the control group, and the frequencies compared with Fisher's exact test: sub-section 3.3), proved that domiciliary treatment with DDT resulted in exposure, uptake and eventual elimination through milk. The difference between the exposed and control group mean DDT-levels (using a two-sample t-test) is presented in Table 3.2.5. The p-values showed a very significant difference between the exposed and control groups for all the DDT related parameters. Since the only possible source of DDT, other than back-ground levels, was from malaria control operations, it must be concluded that this activity resulted in significantly elevated levels in the exposed group. There are also other indicators, both from the present study and from the literature, that will give more weight to this finding.

Little work has been done on establishing the relationship between the use of DDT for malaria control and the amounts of DDT in breast milk. A number of studies implicated diet as the major route. Some authors have reported on the

DDT-levels in fat of the general population and its relationship with vector control. Reports from Third World countries frequently did not state routes of exposure, quite possibly because it is very difficult to establish. Wherever DDT is available for malaria control, it will usually be available for agricultural purposes, thereby contributing to exposure via the diet. Most of these studies also seem to have been small surveys on poorly defined populations. Some of the studies that investigated vector control and house treatment are discussed below.

Ramachandran *et al.* (1974) attempted to relate malaria control activities (using DDT) with levels of DDT in the fat tissue of the general population in India, but no relationship was detected. House treatment with DDT was also implicated by Wassermann *et al.* (1965) as a source of DDT detected in body fat of people in Israel. Malaria control was mentioned by Hashemy-Tonkabony and Fateminassab (1977), but no correlation between malaria control activities and breast milk levels from Iran was presented (mean ΣDDT-level = 24 $\mu g\ l^{-1}$ for 131 whole-milk samples). Weisenberg *et al.* (1985) mentioned "disease vector control" (Israel), but did not relate their findings to this activity. Stacey and Tatum (1985) established a clear connection between the levels of dieldrin in human milk and house treatment for termite control.

Perhaps the best proof of a positive relationship between malaria control and breast milk levels of DDT came from studies done in New Guinea, Guatamala and Africa. Hornabrook *et al.* (1972) presented evidence that malaria control contributed to elevated levels in whole-milk (246 $\mu g\ l^{-1}$ ΣDDT from a sprayed area, and 3,32 $\mu g\ l^{-1}$ ΣDDT from an unsprayed island). However, the possibility that DDT had been used for crop protection on the control island could not be excluded. Winter *et al.* (1976) sampled 290 milk samples from eight different areas in Guatamala. Livingston (mean of 864 $\mu g\ l^{-1}$ ΣDDT for 30 whole-milk samples), where DDT was applied twice annually intra-domiciliary, was the area with the highest mean level of DDT in milk. DDT might also have been used on a small scale for crop protection around Livingston. Other areas with no malaria control had lower levels of DDT in breast milk, apparently related to its use for cotton crop protection.

The only study from Africa to compare samples from different areas was that of Kanja *et al.* (1986). They only analyzed samples from mothers breast-feeding their first or second child. Nomads from Loitokitok (Ilbarma area: Kenya) had a mean level of 1,96 mg l^{-1} ΣDDT (milk fat[9]). The highest mean value was for mothers from Rusinga Island, with 18,73 mg l^{-1} ΣDDT (milk fat) for 25 milk samples. DDT was apparently used for malaria as well as tsetse fly control (as another source of DDT) on the island. Fish, contaminated by tsetse fly control activities, as a route of exposure, could not be excluded.

The percentage DDT of the exposed group of the present study was much higher than for the control group (Table 3.2.5). This indicated very recent exposure to DDT itself (World Health Organization, 1979). A low percentage DDT means less recent exposure to DDT, or exposure to trace levels in the environment. That the DDT found in the breast milk of the exposed group was derived from malaria control activities, was supported by previous studies. There were no evidence to the contrary. This was perhaps the first study in connection with malaria control to have established DDT-levels in milk, which positively excluded other contributing sources (such as agriculture and tsetse fly control), except for back-ground levels.

All the milk samples of the control group had detectable amounts of DDE (Table 3.2.2). This reflected the ubiquitous nature of this contaminant in the environment. There was only one published study from South Africa on the levels of DDT in cows' milk. Lück and van Dyk (1978) determined DDT in 21 samples of market milk from Natal. The mean level of ΣDDT was 0,07 mg l^{-1} (milk fat), with a maximum of 0,420 mg l^{-1} (milk fat). DDT is certainly still found in measurable quantities in milk in South Africa. Market milk was not available to the exposed population.

The log-normal distributions of the DDT-levels are shown in Figs. 3.2.1 to 3.2.5. Log-normal distributions were also determined for other studies of this nature.

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[9]Some authors express concentration on a mg l^{-1} (milk fat) basis.

Violante *et al.* (1986) used logarithmic transformations of the observed levels in serum of workers exposed to DDT. Woodard *et al.* (1976) showed a distribution that perhaps would have been treated better with a logarithmic transformation. Knoll and Jayaraman (1973) also showed a log-normal distribution plot of DDT in milk. Niessen *et al.* (1984) used both normal and log transformed data in their calculations. Rogan *et al.* (1986a) used logarithmic transformations throughout their analyses. Mussalo-Rauhamaa *et al.* (1988) also showed a skewed distribution, but apparently did not use log transformation.

The distribution of the percentage DDT for the exposed group was normal, but was less obvious for the control group. This was due, not only to the samples with detectable residues, but also to the low levels of DDT, resulting in a skewed distribution. However, no log transformation of percentage DDT values was done, to enable comparison with the percentage DDT parameter of the exposed group.

4.2.2. SERIAL CHANGES OF DDT-LEVELS IN THE BREAST MILK OF THE EXPOSED GROUP

The serial changes in levels of DDT, DDE,DDD (log transformed) and percentage DDT and percentage DDD in breast milk of the exposed group were statistically tested, and the results presented in Tables 3.2.6 to 3.2.15 and Figs. 3.2.7 to 3.2.11 (sub-section 3.2). The most noticeable feature of the series was not the significant differences between surveys, but the lack of it. Excluding DDD from the present discussion, (to be discussed later in this section), only four instances were determined where significant differences between surveys existed.

For DDE and ΣDDT (both for whole-milk and milk fat), no significant changes ($p > 0{,}0083$) were detected. Fig. 3.2.7 shows that there was an increase in DDE-levels (whole-milk) after spraying (Mar'87), and that this increase continued to the next survey, after which it decreased. The increase was not evident for the DDE-levels based on milk fat for Mar'87 (Fig. 3.2.8). It did increase later, as

measured for the Jun'87 survey, after which a slow decrease was noticed. The large variation in values, as a result of various other factors such as maternal and infant age, and particularly parity, resulted in a large variance. The experimental design was either not sensitive enough to detect any significant changes in DDE-levels, or there were none.

Even the increase in ΣDDT did not result in statistically significant differences ($p < 0,0083$) between surveys. The change in ΣDDT-levels was not as would be expected, and it seemed as if the elimination of ΣDDT after application, was much slower. There was, for both whole-milk and milk fat (Figs. 3.2.7 and 3.2.8), a continuous increase in levels up to Jun'87. The Jun'87 levels were 37,2% higher than the Nov'86 levels. This suggests a continues uptake of DDT from the environment of the mother. Results presented later on serum levels of ΣDDT will show that this was not the case for the general population. It is therefore possible that uptake, storage and elimination of ΣDDT in lactating mothers does not follow the pattern associated with a non-lactating population.

There could be several reasons for this. Differential binding of DDT to fat tissue (binding sites with different strengths of binding DDT) and the metabolism of this tissue for breast-feeding might play a rcle. If the proportion of tightly binding sites was much greater than the loosely binding sites, and all the sites were filled, the DDT immediately available would be from the loose sites. With depletion of these sites and metabolism of the body fat reserves, the second store of tightly bound DDT would become available. The hypothesized higher proportion of tightly bound DDT would then result in a continuous increase of serum and milk concentrations. (The possibility of an undetected short-term output just after application will be discussed later.)

As a second possibility, Bakken and Seip (1976) also suggested differential binding. They proposed that loosely bound DDT would explain the initial higher levels at onset of lactation. However, they worked with a mean concentration of 81 $\mu g\ l^{-1}$ ΣDDT (whole-milk), which was much lower than for the present study, and the mothers were not exposed to annual applications of DDT.

A third possibility could be an increased metabolism of the pesticide and its derivatives. Polishuk *et al.* (1970) showed lower levels of DDT and DDD in adipose tissue of pregnant women, but higher levels of DDE when compared to non-pregnant women, indicating enhanced metabolism to DDE. This was, however, not tested for by comparing lactating and non-lactating mothers.

The above speculation is hampered by the fact that there was a large variation in the data. The source of this variation will be discussed in sub-section 4.3. The only possible way of testing the above speculation would be a stringent longitudinal follow up, with frequent surveys of serum, milk and adipose tissue.

A conflicting picture emerged in the case of DDT when comparing the different bases of concentration (Tables 3.2.8 and 3.2.12). The significantly increased whole-milk levels of DDT for Mar'87 over that of Nov'86 ($p < 0,0083$) were not reflected in the analysis of DDT-levels based on milk fat. The only significant change in levels based on milk fat was the increase of the Nov'87 level of ΣDDT over the Nov'86 level ($p < 0,0083$). The ΣDDT-level (milk fat) at the Jun'87 survey was 19,45 mg kg^{-1} ΣDDT, higher, but not significantly ($p > 0,0083$) so, than the Nov'87 level of 18,34 mg kg^{-1} ΣDDT, which was possibly significantly higher ($p < 0,05$) then the Nov'86 level (12,21 mg kg^{-1}). It must also be noted that the p-value for the comparison between Nov'86 and Mar'87 was low (0,0206), but not less than 0,0083, which was required for proof of significance.

No obvious factor was at hand to explain this dichotomy, other than perhaps an over or under representation (bias) of some sub-groups with higher levels of DDT in breast milk (such as primiparous mothers or the younger infants) for the Nov'87 and Nov'86 surveys. The comparison of these variables (Table 3.1.2), and visual inspection of the plots presenting these variables (Figs. 3.1.9 to 3.1.14), did not show any marked contribution of such a group. These changes in DDT-levels, although significant, was considered, when compared to the large actual levels, not to be of clinical significance, since the elevated levels are only in evidence for a part of the year.

DDD has not been discussed up to now. The problem of interpretation of any change of this compound was due to the fact that it was present in very low quantities, and in three samples of the exposed group it was below detection limits. A number of regression analyses and analyses of variance were performed, but no obvious trend or correlation with any parameter was found. The apparent lack of toxicity, and the paucity of any substantial knowledge of this compound, other than as a drug (Hayes, 1982) precludes any further meaningful discussion.

Serial change in levels of DDT, DDE and ΣDDT were not significantly related to surveys for the control group, and no statistics are therefore presented. The small contribution of DDT to the total contamination is evident from Figs. 3.2.10 to 3.2.12.

Comparisons with other reports and maternal health effects of DDT will be discussed in sub-section 4.4. Health implications of the DDT in breast milk to the infant will be discussed in sub-section 4.6.

4.2.3. COMPARISON WITH A LONGITUDINAL SERUM STUDY

As already mentioned, a longitudinal study on serum levels of DDT was carried out concurrently, but separately, with the present study (Bouwman, 1990). The changes in serum levels of all the resident members of twelve families from a sprayed area were determined, using the same sampling schedule as the present study. Breast milk samples from this group (three in total) had levels similar to that of the present study. No factors were apparent that indicated any difference between this group, located about 37 km away from Mseleni, and the Mseleni population to which the mothers of the present study belonged. This assumption, however, was not tested. The major factors of concern were similar living conditions and malaria protection. The change in DDT-levels in serum measured longitudinally is given in Table 4.2.1. The Mar'87 survey for serum was done exactly 10 days after DDT application to the dwellings in which the families resided (Bouwman, 1990).

Table 4.2.1 Mean DDT-levels ($\mu g\ l^{-1}$) in serum of a group of 12 exposed families measured longitudinally over 12 months, using the same survey schedule as for the present study (Bouwman, 1990).

SURVEY	n	DDE	DDD	DDT	ΣDDT
NOV '86	71	103,4	0,21	37,3	140,9
MAR '87	66	127,1	0,45	47,5	174,6
JUN '87	58	109,7	2,57	42,6	155,4
NOV '87	63	107,9	1,60	31,4	142,1

Table 4.2.2. P-values associated with the two sided paired t-test when comparing ΣDDT-levels in serum of an exposed group for the different survey periods. **Bold** indicate significance ($p < 0,0083$ at the 5% level, according to Bonferroni) (Bouwman, 1990).

MONTH	NOV '86	MAR '87	JUN '87	NOV '87
NOV '86	1,0000			
MAR '87	**0,0022**	1,0000		
JUN '87	0,331	0,0197	1,0000	
NOV '87	0,976	**0,00016**	0,6131	1,0000

Table 4.2.1 showed that an increase in serum levels did occur, and that the ΣDDT increased by 23,9% compared to the original value. (The percentage increase for milk levels (milk fat) over the same period was 12,9%.) This increase was followed by a decrease to the original serum levels at the end of the year, and the difference between the Mar'87 and Nov'87 levels was also significant ($p < 0,0083$), as indicated in Table 4.2.2. Half of this group were younger than 21. Further analysis, sub-dividing the group into two age intervals, and correcting for baseline (Mar'87 value - Nov'86 value), showed a difference in kinetics (Table 4.2.3) between the two groups (Bouwman, 1990).

Table 4.2.3. Sequential changes in ΣDDT-levels ($\mu g\ l^{-1}$) in serum of the whole group and sub-divided in two age intervals. Significant changes ($p < 0,05$: paired t-test) are indicated in **bold** (Bouwman, 1990).

PERIOD	WHOLE GROUP	≤20	≥21
NOV'86 - MAR '87	+31,8	+21,5	+46,6
MAR'87 - JUN '87	**-21,6**	-17,9	**-28,8**
JUN'87 - NOV '87	- 5,8	**-29,3**	+36,2
NOV'86 - NOV '87	+ 0,4	-20,7	+28,5

There was a significant difference ($p < 0,05$) in uptake and elimination kinetics between the two groups (Table 4.2.3). For the whole group, the resultant change was a significant uptake after application, followed by a significant decrease ($p < 0,05$: Bouwman, 1990). This change was due to the significant uptake and elimination of the older age interval, not the younger. The younger age interval showed significant elimination ($p < 0,05$) from Jun'87 to Nov'87. Together with other statistical analysis of first order elimination kinetics (not shown here), it means that elimination was the dominant process in the younger age interval, and uptake in the older interval. As the mothers of the present study fell in both categories, uptake and elimination could have been different between the two age intervals. Because of the serial nature of the data of the present study, no such analysis was done.

From the point of view of serum levels of ΣDDT, an increase was detected after application (Bouwman, 1990). Breast-feeding mothers, however, eliminate DDT, DDE and DDD much faster through milk secretion than any other mechanism present in a general population. Therefore it is, with our present knowledge, a matter of conjecture whether or not a short term, elevated output after DDT application did occur. If it did occur, and was present over a longer period, then the results would probably have indicated this. It is, however, a shortcoming of crossectional studies that events with longer duration will have a higher proportion of cases than short term incidents (Kelsey, Thompson and Evans, 1986). The likelihood of detecting such an event would, therefore, be increased by a longitudinal protocol.

4.2.4. COMPARISON OF PERCENTAGE DDT IN BREAST MILK AND SERUM

The percentage contribution of each of the three components of ΣDDT over time presented the clearest picture of the dynamics. Percentage composition is independent of the base of calculation (whole-milk or milk fat). The increase in percentage DDT was significant ($P < 0,0083$) from Nov'86 (42,57%) to Mar'87 (50,87%: Table 3.2.14). A significant decrease ($P < 0,0083$) was determined from Mar'87 to Nov'87 (43,27%). The decrease in percentage DDE (Mar'87: Fig. 3.2.9) was more as a result of the increased DDT than because of a reduction in real DDE-levels.

These changes can be explained in the context of application of DDT, uptake and elimination. Application of DDT resulted in an increase in exposure, that gave rise to an elevated percentage DDT in the milk. DDT was metabolized or excreted, during the following months, so that the percentage composition of DDT and DDE returned to the initial level. The difference in percentage DDT of the samples taken three months after spraying ceased (Jun'87: 45,85%), was not significantly different ($p > 0,05$) from the preceding survey (Mar'87), nor the following survey (Nov'87: Table 3.2.14). A comparison with the serum data, however, showed a slightly different picture (Table 4.2.4: Bouwman, 1990).

Table 4.2.4. Change in percentage DDT of the ΣDDT of an exposed group which was tested longitudinally for serum concentrations (Bouwman, 1990).

MONTH	n	%DDT
NOV '86	71	28,9
MAR '87	66	31,0
JUN '87	58	29,9
NOV '87	63	25,2

The percentage DDT in serum was lower, the change from Nov'86 to Mar'87 was less pronounced than for the respective milk values, and this longitudinal change

was not significant. This again poses the question whether uptake, storage and elimination of DDT and its derivatives in lactating mothers would follow the same pattern compared to non-lactating women of the same population. Differential transfer of DDE and DDT from the blood to the milk was a possible explanation, a discussion of which will follow.

4.2.5. DIFFERENTIAL TRANSFER OF DDE AND DDT TO MILK

None of the studies consulted during this investigation made any reference to differential transport of the various metabolites of DDT. Seven studies in which both blood and milk concentrations of DDT and DDE were determined, are listed in Table 4.2.5. Six of these studies indicated higher percentage DDT in milk than the corresponding blood sample composition. A pair-wise two-sided t-test, using the results from all seven studies (Table 4.2.5), showed that the higher mean percentage DDT in breast milk (4,77%), when compared with serum, was significantly different from zero ($p = 0,0232$).

A good correlation of results was shown by the studies of Eckenhausen *et al.* (1981) and Dyment *et al.* (1971) (Table 4.2.5). The only study reporting a reversed correlation was that of Polishuk *et al.* (1977). None of these studies discussed this aspect. Neither was it reported in the article by Jensen (1983), who reviewed more than 200 articles on this subject. Wolff (1983) gave an extensive summary of the transfer of DDT and PCBs between fat, blood and milk compartments, but did not mention differential transfer of DDT and DDE.

Table 4.2.5. Studies on humans from which percentage DDT of ΣDDT of both blood and corresponding milk samples could be calculated.

	AUTHOR	%DDT BLOOD	%DDT MILK
1	Skaare *et al.* (1988)	16,0	27,1
2	Mes *et al.* (1984)	10,54	15,26
3	Eckenhausen *et al.* (1981)	19,8	21,8
4	Siddiqui *et al.* (1981)	29,2	34,7
5	Polishuk *et al.* (1977)	17,7	16,8
6	Dyment *et al.* (1977)	19,7	21,8
7	Curley and Kimbrough, (1969)	34,23	43,10

The difference in composition of DDT and DDE can be further explored using information concerning other compartments or compounds. Adamovic, Sokic and Smiljanski (1978) mentioned a difference in percentage DDE of the ΣDDT between milk (78% DDE) and body organs (68% DDE) of the general population of Serbia. They called the higher contribution of DDT, a "DDT surplus". The explanation they gave was that DDE was mobilized faster from the fat compartment than was DDT. The logic was unclear, as this would have resulted in lower percentage DDE in body fat of lactating mothers, not the higher values that they found. Quinby *et al.* (1965), using the Schechter-Haller method, analyzed milk and body fat for DDT and DDE content. They found and mentioned a difference in composition between milk and body fat. The percentage DDT in body fat was 32% and in milk it was 40%, but they offered no explanation.

Polishuk *et al.* (1977) found some isomers of PCBs present in plasma, but not in milk. One isomer was present in plasma at a significantly higher ratio than other isomers in milk. The ratios for three other isomers in milk fat were the inverse

of that found in the plasma. Ando, Saito and Wakisaka (1986) analyzed maternal blood, cord blood and milk from 36 mothers for PCBs. They found selective transfer of PCBs from the placenta to cord blood, but no significant correlations of PCB concentrations (per isomer) between milk and maternal blood.

Evidence of differential transfer of chlorobenzenes from fat to milk has also been found. Jan (1983) found differences in the isomer distribution of chlorobenzenes between body fat and milk fat. He explained it on grounds of the water / octanol partition coefficients that differed. He did not elaborate on where the differential transfer was supposed to happen. It could be either from the fat to the blood, or from the blood to the milk compartments. In light of the blood / milk results from other studies, the argument for the latter is better supported.

Further evidence of differential transfer came from an animal study. Woolley and Talens (1971) fed female rats three concentrations of DDT, namely, 25, 100 and 200 $\mu g\ kg^{-1}$ DDT. Milk and blood of lactating rats were analyzed. The percentages DDT in blood was 70,0%, 60,9% and 85,0% at the three concentrations, respectively. The percentages DDT in milk were 91,9%, 94,3% and 98,9%, respectively. The rest of the DDT was made up of DDE and a little DDD.

The fact that serum samples of the mothers participating in the present study were not taken, should be seen against the lack of any mention of such a process in literature. Future sampling protocols have been changed to this effect. More discussion on this aspect will be given in sub-section 4.3.

Conclusions: For a serially sampled baby clinic population of lactating mothers, representing the Mseleni population of lactating mothers that live in DDT-treated dwellings, it was concluded that the recorded change in DDE and ΣDDT-levels in breast milk over a period of a year, was not statistically significant. This was supported by present data.

For a serially sampled baby clinic population of lactating mothers, representing the Mseleni population of lactating mothers that live in DDT-treated dwellings, it was concluded that the recorded change in DDT-levels in breast milk over a period of a year, although statistically significant, was not considered to be clinically significant.

For a serially sampled baby clinic population of lactating mothers, representing the Murchison population of lactating mothers that do not live in DDT-treated dwellings, it was concluded that there was no significant serial change of any measured parameter relating to levels of contamination of DDT and its derivatives. This was supported by the present data.

For a serially sampled baby clinic population of lactating mothers, representing the Mseleni population of lactating mothers that live in DDT-treated dwellings, it was suggested that the percentage DDT in breast milk was higher than the percentage DDT in the blood compartment of the same mother. This was suggested both by the data presented here and by data recalculated from literature.

4.3 FACTORS INFLUENCING LEVELS OF DDT AND ITS METABOLITES IN BREAST MILK

There are several factors modulating the body burden of lactating mothers that could influence eventual exposure to the infant (see sub-sections 1.8.3, 1.8.4 and 1.8.5). Some factors, such as suckling time and volume of breast milk consumed, are difficult to measure, and are inherently variable. These measurements were not undertaken and standard values, derived from literature, will be used where necessary. Maternal age, infant age, parity and percentage milk fat were taken into account for the present study. Parity and maternal age correlated well for the exposed group (linear regression, $p < 0{,}001$: coefficient of determination = 58%). This section will attempt to explain the results presented in sub-section 3.3. The results will also be compared with other studies that report work of a similar nature. One of the main features of this comparison will be the relatively high levels that were found in the breast milk of the Mseleni group.

4.3.1 INFLUENCE OF DDT INPUT INTO THE ENVIRONMENT ON THE RATIO BETWEEN DDT AND DDE IN BREAST MILK

The dependence of DDE on DDT-levels is shown by the good coefficient of determination of the multiplicative model that describes the relationship between these two variables for the exposed group (Fig. 3.3.1). As DDE-levels in any system are a function of the application of DDT (assuming application of pure DDT) and the breakdown processes, this relation must be true. The correlation between the DDE and DDT-levels in a system where it is measured depends on the sources, routes of exposure and metabolism. DDE is more resistant to breakdown, and will persist in the environment longer than DDT. This results in a higher proportion of DDE (lower percentage DDT) with time (World Health Organization, 1979). Malaria control with DDT, because of its magnitude and method of application (intra-domiciliary), was the only source of DDT that was considered for the exposed group. The routes of uptake may be more varied.

The source of ΣDDT for the control group was varied, as there was no deliberate input of (pure) DDT into that area as indicated by the low levels of ΣDDT and the small contribution of DDT to ΣDDT in breast milk. The levels were incurred from background contamination, probably residues of previous agricultural use and drift (conceivably also from malaria control activities). These varied sources of DDT resulted in a lower coefficient of determination of the regression between the two major isomers present in the breast milk of the control group (Fig. 3.2.2). The same arguments also were offered by Knoll and Jayaraman (1973) and Skaare *et al.* (1988).

4.3.2. INFLUENCE OF PARITY ON LEVELS OF DDT AND ITS METABOLITES IN THE BREAST MILK OF THE EXPOSED GROUP

The inverse relationship of ΣDDT-levels in milk with parity was shown by the significantly higher ($p < 0,05$) levels of DDT in milk of primiparous mothers of the exposed group, when compared to milk of mothers with more than one child (Fig. 3.3.6). The mean level of ΣDDT in breast milk to which the first born was exposed (24,82 mg l^{-1} ΣDDT), was almost twice as high as for the following children (mean level of ΣDDT in milk for second child 13,94 mg l^{-1} ΣDDT). Regression analysis also showed significant influence ($p < 0,05$) of parity on DDE, DDT and ΣDDT concentrations (Figs. 3.3.3 to 3.3.5), although the distribution of the raw data resulted in large variation, with poor coefficients of determination. The fit of the second order polynomial regression (Fig. 3.3.7) to the ΣDDT data was only marginally better (coefficient of determination = 25,05%) than for the multiplicative model (22,86%). The increase (although not significant: $p > 0,05$) in levels of ΣDDT from the fourth to the fifth child was, however, indicated by the regression. The probability plot (Fig. 3.3.8) indicated the possibility of a systemic error in the model. The source of this variation will be discussed later. This finding, regarding the association between levels of ΣDDT and parity, confirmed the presence of negative balance of DDT in breast milk of lactating mothers. This process has been described by Bradt and

Herrenkohl (1976), Norén (1983a and 1983b), Stacey *et al.* (1985) and Rogan *et al.* (1986a) (see sub-section 1.8.3).

The slope of the multiplicative regression model for DDE (-0,71) was much steeper than for DDT (-0,39), indicating faster elimination of DDE from the body (Figs. 3.3.3 and 3.3.4). The possibility that DDE was metabolized faster than DDT to water soluble products (as a function of parity) did not necessarily account for this phenomenon, as will be shown later. Coefficients for the rates of elimination (assuming first order kinetics) calculated for the decrease between the first and second, and third and fourth child, showed that the rates decreased in both instances with parity by more than half. The coefficients for DDE were 0,4520 and 0,2023, respectively. They were 0,2877 and 0,1386 for DDT, respectively. The respective rates for ΣDDT were 0,4308 and 0,1832.

That an apparent process of accelerated elimination of DDE was present, was further supported by the results of the second order polynomial regression (which gave a better coefficient of determination than did the multiplicative model) of percentage DDT on parity (Fig. 3.3.15). This selective process might, however, be the manifestation of another underlying aspect which will be addressed a little later in this sub-section. The change in ratios of the two important isomers, DDT and DDE, needs to be considered first.

An increase in percentage DDT was apparent when the milk of primiparous mothers (34,48%) was compared to the milk of mothers with two (46,44%) and three children (50,93%: Fig. 3.3.14). After the third child, a slight decrease, to 50,58% (fourth child), was found. The percentage DDT in milk of primiparous mothers was significantly less ($p < 0,05$) than the percentage DDT in milk from mothers with two or more children. This phenomenon has not been mentioned by any of the articles consulted during this study.

Together with the finding in the previous section, that the percentage DDT in breast milk was likely to be higher than in serum, a confusing picture arose. If DDT was preferentially transferred from the blood compartment to milk, why was the initial percentage DDT lower in milk of primiparous mothers than for

milk from mothers with two or more children, and why did the percentage DDT increase? Preferential transfer of DDT would mean depletion of maternal body burden of DDT, resulting in a decreasing percentage DDT. In the case of the present study, the percentage DDT increased.

One should keep in mind that the actual ΣDDT-levels decreased significantly ($p < 0,05$) with parity (Fig. 3.3.6). DDE decreased by almost half, from 15,06 mg l^{-1} DDE for primiparous milk to 7,36 mg l^{-1} DDE for milk from mothers nursing their second child (Fig. 3.3.3). The respective values for DDT were 9,23 and 6,21 mg l^{-1} DDT; a decrease by a third (Fig. 3.3.4). It was therefore only the ratio of DDT to ΣDDT that increased.

One possible explanation for this finding can be based on the following:

1. The metabolism of DDT to DDE in the human is relatively slow (World Health Organization, 1979).

2. It can also be assumed that DDT is the major component of the spectrum of DDE, DDT and DDD present and bio-available in the immediate environment of the maternal system, especially after application.

The levels of DDE present in the maternal body (ignoring any form of elimination) depends on both external uptake and internal metabolism from DDT. The last process is slow, and the importance of the first process was not established. The levels of DDT in the maternal body, on the other hand, depend entirely on external uptake. If, during lactation, the elimination of DDE was faster than its combined uptake (already proved) and metabolism from DDT, and the relative environmental availability of the two differ (such as regular application of a 75% DDT formulation of which 4% of the active ingredient consists of DDE), then the ratio of DDE to DDT in milk could change in favour of DDT.

Even if DDT was preferentially transferred from the blood compartment to milk, the continued input of DDT to the immediate environment of the mother, concurrent with a depletion of body stores of DDE that are replaced slower than DDT, will result in a change in ratio between DDT and DDE during continuous elimination of both. There are some uncertainties which need to be defined:

1. The assumption relating to both DDT and DDE in the environment, that the combined uptake and formation of DDE (from DDT) by the breast-feeding mother is slower than the uptake of DDT when both are present, needs to be motivated.

No useful information on comparative rates of the human uptake of DDE and DDT through skin, lungs or intestinal tract was available. Two animal studies might, perhaps, throw some more light on this issue. Adams, Coon and Poling (1974) fed rats with fat containing DDT (45%), DDE (29%) and DDD (26%). In the body fat of the rat they found DDT at 50%, DDE made up 37% and about 13% was DDD. The ratios also varied from tissue to tissue. DDT was significantly lower and DDD significantly higher ($p < 0,05$) in weanling carcasses than in adult body fat, but no physiological reason could be suggested by them. However, metabolism of DDT differs between man and rat and the extrapolation would therefore not be valid. Suffice it to say that ratios of the three compounds can vary between food ingested, body fat and transfer media, such as milk and blood.

Sieber (1976) fed rats with the p-p' and o-p isomers of DDT, as well as with p-p'DDD, p-p'DDE and p-p'DDA, and measured lymphatic absorption. Recovery of these compounds from thoracic duct lymph increased with time, but no association was found between the lipid solubilities of the isomers recovered, and the ratios between them. Sieber (1976) ascribed this to variation in rates and routes of excretion between the various isomers, but this was not tested.

These studies only presented indirect evidence supporting the above mentioned assumption. Changes in ratios do occur, but no obvious trend was discernable.

Various factors such as water / octanol partition coefficients, selective uptake and transport, and induced metabolism may play a role.

2. The assumption relating to DDT in the environment only, that DDT is present in relatively large amounts in the immediate environment of the breast-feeding mother, and is taken up and excreted largely unaltered via milk, needs to be motivated.

There are two animal studies which relate to this aspect. Woolley and Talens (1971) fed female rats on three concentrations of pure DDT. DDT in the rat milk made up more than 90% of the ΣDDT, showing that DDT is excreted largely unaltered in rats. Kalra *et al.* (1986) did a similar experiment with eight Indian buffalo, but applied the emulsified DDT topically in a single dose. For the first eight days DDT made up more than half the ΣDDT in milk. The percentage DDT dropped to 36% after 16 days.

These studies present only indirect evidence that DDT is excreted faster via lactation than its biotransformation to DDE, i.e. that it is excreted largely unaltered. This is perhaps the best support for the explanation given on the change of ratios observed with an increase in parity found for the present study. This would also explain the apparent observation that DDE was eliminated faster than DDT, as remarked on earlier.

Proof of these assumptions will be found if the levels of DDT in the different compartments, especially indoor air, can be monitored. Furthermore, the change in ratio of DDE and DDT in serum of the mother over the lactation period should also be established.

4.3.3. INFLUENCE OF PARITY ON ΣDDT-LEVELS IN MILK OF THE CONTROL GROUP

A noticeable feature of the multiplicative model (Fig. 3.3.9.) was that the slope of the ΣDDT on parity (-0,43) was less than the slope of ΣDDT on parity (-0,56)

for the exposed group (Fig.3.3.5). This is perhaps best explained by the fact that the factors governing elimination are present in both groups, but are modulated by concentration. The poor fit of the raw data indicated either a variable exposure to the contaminants, other factors not included in the model, or both. Some of these factors will be addressed during further modelling of the data for the exposed group (sub-section 4.3.9). The multiplicative model gave marginally better results than did the non-linear model for ΣDDT (Figs. 3.3.9 and 3.3.11), and the regression was significant ($p = 0,00587$). The process of negative balance was therefore also present at the control group.

Analysis of variance revealed that the mean ΣDDT-level in milk to which the second child was exposed (0,99 mg l^{-1}) was slightly, but not significantly ($p > 0,05$), higher than for the first born (0,75 mg l^{-1}). There were no other notable variations. This was probably due to the fact that the levels were low and had a large variation.

The multiplicative model (Fig. 3.3.17) was slightly better than the non-linear model (Fig. 3.3.19) in describing the relationship between percentage DDT in the milk of the control group and parity. The p-value (0,01603) indicated a significant increase with parity, but the fit was poor. The results of analysis of variance (Fig. 3.3.18) did not produce any significant increase (or decrease) associated with an increase in parity using 95% confidence intervals. The mean percentage DDT did increase from 4,36 (primiparous mothers) to 7,61 (mothers with five or more children), but this was not significant ($p > 0,05$). This implied that the input of DDT into the environment of the control mothers was in balance with the output of DDT via breast-feeding, with no change in the ratio between the two major isomers. The low levels, together with the large variation observed (a number of samples had no detectable DDT), might conceivably (but unlikely) have concealed any significant change in levels or ratio.

4.3.4. INFLUENCE OF INFANT AGE ON LEVELS OF DDT AND ITS METABOLITES IN MILK OF THE EXPOSED GROUP

The influence of infant age on levels of DDE, DDT and ΣDDT in milk of the exposed group is shown in Figs. 3.3.23 to 3.3.25. The slopes of the multiplicative regressions were negative and significant ($p < 0,05$) for all three parameters, indicating a decrease in levels with an increase in infant age. The slope for DDE (-0,09) was only slightly more than that for DDT (-0,07). The coefficients of determination indicated a very large variation of the raw data, that made interpretation difficult. An obvious source of this variation was parity, but attempts to analyze the effect of infant age for firstborns only did not result in an improved measure of relationship. The p-value for the regression analysis was 0,3756 and the coefficient of determination was 2,25% (analysis not shown).

Analysis of variance (Fig. 3.3.26) indicated that the decrease in levels of ΣDDT started at birth and continued for a year, after which it increased again to slightly less than the original level. The difference between the 0-4 and 9-22 months intervals was significant ($p < 0,05$). Quite a number of the articles that were consulted, referred to this aspect. Only Rogan *et al.* (1986a) and Klein *et al.* (1986) found a reduction in levels associated with an increase in infant age (see sub-section 1.8.4). Rogan *et al.* (1986a) found that the levels were reduced by 24% after six months. The comparable reduction for the present study was about 31%. Curley and Kimbrough (1969), Knoll and Yayaraman (1973), Hagyard *et al.* (1973), Woodard *et al.* (1976), Bakken and Seip (1976) and Eckenhausen *et al.* (1981) reported either no change, or an increase in levels after birth. They, however, reported on relatively low levels of contamination.

Another aspect of Fig. 3.3.26 is the increase in ΣDDT-levels in milk after one year of breast-feeding. This can be ascribed to a reduced intake by the infant, as also suggested by Hofvander *et al.* (1981). They analyzed 18 samples of breast milk three months post partum and 23 samples six months post partum from different mothers. The mean ΣDDT-level for the three month group was 1,388 mg l^{-1} and 1,547 mg l^{-1} (milk fat) for the six month group. The daily intake of milk however, was reduced from 780 ml day^{-1} at three months to 595 ml day^{-1} at six

months, as the infant started eating other food. This probably resulted in a reversal of the negative balance effect that was in force from the onset of lactation. This reversal effect is further supported by the information from Fig. 3.3.30. The percentage DDT increased with infant age for a year, after which it dropped to almost the initial value. Although the changes were not significant ($p > 0,05$), the two trends lend further support to the explanation, given in section 3.3.2, about the reason for the apparent accelerated elimination of DDE.

The consequence of both the significant ($p < 0,05$) regression analysis (Fig. 3.3.25) and analysis of variance (Fig. 3.3.26), together with the findings of Rogan *et al.* (1986a), as well as the general acceptance of the "negative balance" process, seemed sufficient grounds to accept the validity of the reversal of the "negative balance" during the latter part of a lactation period.

This implied that the amount of DDT and metabolites available in the maternal environment was enough to have caused such a reversal, as an increased level of ΣDDT in the milk represents an increased body burden. A debatable indication of this was the decrease in percentage DDT observed in the second year of breast-feeding (Fig. 3.3.30). It could also be argued that the DDT will now remain longer in circulation, with a better chance of being metabolised to DDE, with a return to the normal ratio.

4.3.5. INFLUENCE OF INFANT AGE ON LEVELS OF ΣDDT IN MILK OF THE CONTROL GROUP

There was no significant association ($p > 0,05$) determined for the multiple regression of ΣDDT-levels in breast milk of the control group on infant age (Fig. 3.3.27). The analysis of variance (Fig. 3.3.28), however, showed an almost identical trend compared to the exposed group (Fig. 3.3.26). This finding lends even more support to the findings and suggested explanations in sub-section 4.3.4. The regression analysis (Fig. 3.3.31) and analysis of variance (Fig. 3.3.32) showed some, but not significant ($p > 0,05$), variation in percentage DDT with infant age.

4.3.6. INFLUENCE OF MATERNAL AGE ON LEVELS OF DDT AND ITS METABOLITES IN BREAST MILK OF THE EXPOSED GROUP

The significant reduction ($p < 0,001$) in levels of DDE, DDT and ΣDDT found with an increase in maternal age is shown by the p-values for the multiplicative regression models presented in Figs. 3.3.37 to 3.3.39. The fit of the raw data in the non-linear regression (Fig. 3.3.41: coefficient of determination = 17,2%) was marginally better than for the multiplicative model (Fig. 3.3.39: coefficient of determination = 16,8%), while the probability plot (Fig. 3.3.42) showed the possibility of some other influences. These were, of course, parity and infant age, as already established. A feature of the multiplicative regression was that the slope for DDE (-1,64: Fig. 3.3.37) was more than the slope for DDT (-0,97: Fig. 3.3.38). This can only be seen as verification of previous arguments and suggestions about the apparent accelerated elimination of DDE, as the same data were used, but plotted against a related time factor.

All three possible associations (increase, decrease and no change) with maternal age could be expected (see sub-section 1.8.5). Knoll and Yayaraman (1973), Hashemy-Tonkabony and Fateminassab (1977), Norén (1983b), Wickström *et al.* (1983), Stacey *et al.* (1985), Rogan *et al.* (1986a) and Mussalo-Rauhamaa *et al.* (1988) either found proof or indications of increasing levels of contamination associated with an increased maternal age. Polishuk *et al.* (1977) and Al-Omar *et al.* (1985) found a negative relationship between levels of contamination and maternal age. Woodard *et al.* (1976) did not find any change. Obvious reasons for the variable relationships might be differences in exposure, or a change in exposure and breast-feeding patterns. Another reason could be the relationship between breast and bottle feeding.

The results of this study indicated that the process of "negative balance" continues effectively during the years of lactation. The analysis of variance (Fig. 3.3.40) also supported this. The mean ΣDDT-level for the 17-29 year age interval was significantly higher ($p < 0,05$) than for the 21-25, 26-30 and 36+ years age intervals. The difference between the 17-20 and 31-35 year age intervals was not significant ($p > 0,05$), although the mean was lower. The

maximum reduction of 66,6% of the ΣDDT-levels was between the youngest and oldest maternal age intervals.

If milk constitutes less than a certain percentage of the food intake, or if lactation is only practised for a short period for each child, it is conceivable that the elimination of DDT and its metabolites might exceed the uptake during that period only, and not over the entire reproductive life span, resulting in an increase in overall levels. The effective elimination by the exposed group over the reproductive years by 66,6% is certainly indicative of this assumption. The studies referred to in the review of Jensen (1983), and also those referred to in sub-section 1.8.5, have mostly been carried out in developed countries where lactation is not practised by all mothers, and usually for shorter periods than in the case of the present study.

The finding that the rate of excretion for DDE was twice that of DDT, also translated into a significant positive relationship ($p < 0,001$) of percentage DDT with maternal age (Fig 3.3.47). Non-linear regression did not result in an improved coefficient of determination (Fig. 3.3.49). Analysis of variance (Fig. 3.3.48) indicated that the mean percentage DDT increased by more than 11% from the youngest maternal age interval (17-20) to the oldest age interval. The increase was significant ($p < 0,05$) only for the 26-30 and 36+ years age intervals. The overall trend was also positive, as it was for parity (Fig. 3.3.14). Although effectively plotting the same data against a related variable (maternal age), the effect of an increase in percentage DDT in this instance could be regarded as further proof of the findings made in section 4.3.2 regarding parity.

4.3.7. INFLUENCE OF MATERNAL AGE ON ΣDDT-LEVELS IN MILK OF THE CONTROL GROUP

The multiplicative regression model did not adequately describe the relationship between the ΣDDT-levels in milk and maternal age of the control group (Fig. 3.3.43). Neither did a non-linear regression model show any improvement of the coefficients of determination (Fig. 3.3.45). The probability plot of the non-linear

regression model (Fig. 3.3.46) was very different from that of the exposed group (Fig. 3.3.42), and indicated the presence of a systemic error.

The linear regression of percentage DDT in the breast milk of the control group showed a significant slope ($p < 0,05$) associated with an increase in maternal age (Fig. 3.3.51). The non-linear regression showed no improvement in coefficients of determination (Fig. 3.3.53), and the probability plot (Fig. 3.3.54) indicated the presence of a systemic error. The analysis of variance (Fig. 3.3.52) showed that no single age interval to be significantly different ($p > 0,05$) from any other regarding levels of ΣDDT. No apparent trend was discernable.

4.3.8. INFLUENCE OF PARITY, INFANT AGE AND MATERNAL AGE ON PERCENTAGE MILK FAT

In sub-section 3.3, percentage milk fat was plotted against three other variables, namely parity, infant age and maternal age. Some authors have indicated a relationship between percentage milk fat and DDT-levels (Rappl and Waiblinger, 1975; Wickström *et al.*, 1983). The relationships, using linear regression, will now be more closely examined.

Percentage milk fat did not change significantly ($p > 0,05$) with parity and maternal age of both the exposed and control groups (Figs. 3.3.21, 3.3.22, 3.355, 3.3.56). There was, however, a significant association between infant age and percentage milk fat of the exposed group ($p = 0,0003$: Fig. 3.3.33), but not for the control group ($p = 0,2245$: Fig. 3.3.35). Analysis of variance (Fig. 3.3.34) showed that this was caused by the high percentage fat of the milk ingested by the 13-24 month interval as opposed to the 0-4 month interval. A re-analysis, using analysis of variance, showed that, by excluding milk fat percentages higher than 9,5%, the relationship was not significant ($p > 0,05$) any more. The scatterplot (Fig. 3.3.33) showed that this level excluded all raw data above the 95% prediction limit.

4.3.9. MULTIPLE REGRESSION OF VARIABLES TO EXPLAIN LEVELS OF ΣDDT IN BREAST MILK

Only single variables have been used until now to explain levels of DDT, DDE and ΣDDT in the milk of lactating mothers. It was clear that parity, maternal age and infant age had significant influences on these levels. These relationships have been summarised in Tables 3.3.3 and 3.3.4. Due to the fact that parity and maternal age are related time factors (correlation coefficient = 0,7621), and that infant age could be considered as the time exposed to contaminated milk from a mother with a certain parity status, the effect of all three variables can be inter-related using models. Although a number of models were designed, only two models were presented in full in section 3.3. Quadratic transformations of parity did not improve relationships, neither did the inclusion of percentage milk fat, data on replastering of the hut walls and the use of alcohol.

The logarithmic transformation of the variable data seemed important in improving the models, if judged on coefficients of determination. Models which included variables that were not transformed (Table 3.3.5) had lower coefficients of determination than those for transformed variables. Even after transformation, the results of both models (Models 3.3.1 and 3.3.2) were not encouraging. Model 3.3.1 looked at the interaction between parity and maternal and infant age. The resultant model only explained 24% of the variation. The model is presented in Fig. 3.3.57. This plot showed that the model needed more refining. Some five outliers (near the centre bottom) might be excluded to improve the model, but there still remained a large clustering in the centre.

The graphical representation of Model 3.3.2 (Fig. 3.3.59) looked very much the same as that for model 3.3.1 (Fig. 3.3.57). This model looked at the influence of parity and infant age separately, and gave a coefficient of determination very close to that of model 3.3.1. A wide variety of other transformations was tried, but no improvement using stepwise multiple regression was obtained. The probability plots of both models (Figs. 3.3.58 and 3.3.60) indicated a systemic error or auto-correlation.

Only one article in the literature reported a model (Wickström *et al.*, 1983). The expression for ΣDDT concentrations in breast milk of Finish mothers that they determined is given below:

$$\Sigma DDT = -0,050 + 0,00249(MA) - 0,00971(PAR) + 0,0059(\%\ milk\ fat)$$

They found a significant relationship ($p < 0,05$) between low levels of ΣDDT (31 $\mu g\ kg^{-1}$: whole-milk) and milk fat. It might be possible that, at low levels, the percentage milk fat is a more significant determinant of levels of ΣDDT than other factors. At higher levels, factors such as parity and infant age could have more influence. This would explain the comparable expression of multiple regression (without interaction) determined for the present study (with much higher levels) which, for comparison, is given below.

$$Ln\ \Sigma DDT = 3,25 - 0,050(Ln\ PAR) - 0,058(Ln\ IA)$$

or alternatively

$$\Sigma DDT = 25,79(PAR)^{-0,050}(IA)^{-0,058}$$

The two expressions, because of the transformations, cannot be compared directly. The slope for the expression of Wickström *et al.* (1983) was slightly positive, with a negative Y intercept, whiie the expression derived in the present study had a positive Y intercept and a negative slope.

Uncertainties that must be taken into account were the non-inclusion of other variables that could have influenced results, such as maternal weight or length of each lactation period per previous infant. Improvement of the model, using a wide range of transformations and interactions for the variables for which data was available, was therefore only partially successful.

The results of attempts to do the same kind of modelling for the control group, because of the very low coefficients of determination that were obtained, was not presented.

Conclusions: For a serially sampled baby clinic population of lactating mothers, representing mothers of the Mseleni population of lactating mothers that live in DDT-treated dwellings, it was concluded that:

1 the relationship between concentrations of DDE and DDT in breast milk was best described by a multiplicative regression model. This was supported by analysis of the present data.

2 the process of elimination of ΣDDT via milk significantly exceeded the process of uptake, with increasing parity. This was supported by analysis of present data and by other reports of a similar nature.

3 the firstborn child was exposed to twice the levels of ΣDDT in milk when compared with the following siblings. This was supported by the analysis of present data. Confirmation of this tendency was also found in the literature.

4 the percentage DDT increased significantly ($p < 0,05$) with an increase in parity. This was only supported by present data, not by reports in the literature.

5 the ΣDDT-levels declined significantly ($p < 0,05$) during the first twelve months of breast-feeding. This was supported by the present data and by reports in the literature. A concurrent (but not significant; $p > 0,05$) trend in percentage DDT was also detected.

6 the exposure of infants to ΣDDT through breast milk will be reduced with an increase in maternal age. This was supported by the present data and by some reports in the literature.

7 the rate of elimination of DDT and DDE exceeded the rate of uptake during the years of lactation. This was supported by the present data and by some reports in the literature.

8 the percentage DDT in milk increased significantly ($p < 0,05$) with an increase in maternal age. This was only supported by the present data.

For a serially sampled baby clinic population of lactating mothers, representing mothers of the Mseleni population of lactating mothers that live in DDT-treated dwellings, partially successful models were compiled using parity and infant age as variables.

For a serially sampled baby clinic population of lactating mothers, representing mothers of the Mseleni population of lactating mothers that live in DDT-treated dwellings, it was suggested that:

1 the increase in percentage DDT that occurred with an increase in parity was due to the uptake of DDT and its elimination via milk, and that the recruitment of DDT was faster than the recruitment of DDE. This was supported by analysis of present data and, indirectly, by related animal studies.

2 the increase in levels of ΣDDT observed with an increase in maternal age was caused by a reversal of the "negative balance" effect, due to a reduction of the amount of milk secreted. This was supported by the present data and by reports in the literature.

For a serially sampled baby clinic population of lactating mothers, representing mothers of the Murchison population of lactating mothers that do not live in DDT-treated dwellings, it was concluded that:

1 the process of elimination of ΣDDT via milk exceeded the process of uptake slightly, but significantly ($p < 0,05$), with increasing parity as a time factor. This was supported by present data and reports of a similar nature.

2 increased parity was not significantly ($p > 0,05$) related to a change in exposure of infants to ΣDDT-levels in breast milk. This was supported by analysis of present data only.

3 no significant increase ($p > 0,05$) in percentage DDT was found with an increase in parity. This was supported by present data only.

4 no significant change ($p > 0,05$) in levels of ΣDDT and percentage DDT with an increased infant age was found. This was supported by present data and by reports in the literature reporting low ΣDDT-levels in milk.

5 exposure of the infants to DDT did not change significantly ($P > 0,05$) with maternal age. This was supported by the present data and by some of the findings from the literature reporting on relatively low levels of exposure.

6 percentage DDT changed significantly ($p > 0,05$) with maternal age, but no age interval was significantly different from any other. This was supported by the present data only.

4.4 COMPARISONS OF LEVELS OF DDT IN BREAST MILK AND ASSESSMENT OF ITS RISK TO THE MOTHER

4.4.1 COMPARISONS OF LEVELS WITH THOSE REPORTED FOR OTHER STUDIES

There were four articles listed in Table 1.8.1 with mean ΣDDT-levels in breast milk higher than the mean determined for the exposed group of the present study. Two of them related to Guatamala (Winter *et al.*, 1976; Olszyna-Marzys, 1978), one related to India (Kalra and Chawla, 1981) and one to Africa (Kanja *et al.*, 1986).

Kanja *et al.* (1986) selected only mothers breast-feeding their first or second child (Rusinga Island, mean ΣDDT = 18,73 mg kg^{-1}), but the reason for this was not given. The mean ΣDDT-level for the exposed group of the present study, recalculated for mothers with their first or second child (n=65) was 20,1 mg kg^{-1} (milk fat). This represents the highest value yet determined in Africa. The percentage DDT from Rusinga Island was 51,3% and for the present study it was 41,9%. The source of exposure to DDT of the mothers in the study by Kalra and Chawla (1981) was not stated, making a comparison with the present results difficult. The mean percentage DDT determined by them was also higher than 50%.

The reports from Guatamala, because of some very high values, need to be closely examined. The first report from Guatamala (Olszyna-Marzys *et al.*, 1973) could not be traced in South Africa, but according to Winter *et al.* (1976), the authors reported a maximum ΣDDT-level of 12,2 mg kg^{-1} in whole-milk from Guatamala. This translated to an incredible ΣDDT-level, based on milk fat (divide by 30) of 406,7 mg kg^{-1}. This means that 0,04% of the milk fat consisted of DDT. The mean ΣDDT-level for that report was 2,36 mg kg^{-1} in whole-milk (which represents 78,6 mg kg^{-1}: milk fat) for 46 samples. These high levels were ascribed to extensive use of DDT for cotton protection and semi-annual intra-domiciliary application of DDT for malaria control in the same region. The next

report was from Winter *et al.* (1976). The same communities as for the study mentioned above, as well as others, were included in a survey done during 1973-74. The levels for the three communities (including La Bomba and El Rosario: Table 1.8.1) were significantly less ($p < 0,05$) than for the Olszyna-Marzys *et al.* (1973) study which was done in 1970. These significant decreases in levels were ascribed to the discontinuation of DDT for malaria control (Winter *et al.*, 1976).

The third report from Guatamala, Olszyna-Marzys (1978) contained no reference to the study by Winter *et al.* (1976). The same values as in the first article (Olszyna-Marzys *et al.*, 1973) were repeated, and the possibility mentioned that the levels of DDT, because of legislation introduced as a result of their study, should "*gradually fall*". By that time Olszyna-Marzys must have been aware of the study by Winter *et al.* (1976). His most recent reference was to a published letter from himself, dated 1976. Acta Paediatrica Scandinavica did not list dates of submission or acceptance.

The fourth report (de Campos and Olszyna-Marzys, 1979) also did not include Winter *et al.* (1976) in the list of references. The most recent article in the reference list was dated 1976. The results from 1973 were presented again, but as "*previously published in Spanish*". New results were apparently presented for communities not previously covered, but a comparison with Olszyna-Marzys (1978) showed no new results. The authors should, by this time, have been aware of the similar study in Guatamala, the results of which had been published three years prior to the publication date of this article. The manuscript was received by Archives of Environmental Contamination and Toxicology on January 24, 1977 and accepted on February 28, 1978.

Another unfortunate aspect of all three articles discussed above, was that only ΣDDT was reported. Values for DDT and DDE would have been more informative. No reference as to percentage milk fat could be found. In my opinion, the levels reported by Winter *et al.* (1976) presents a better view of the situation. The failure of de Campos and Olszyna-Marzys (1979) and Olszyna-Marzys (1978) to mention the results of Winter *et al.* (1976), as well as

reporting the same data with different first authors, does not add to the credibility of their data. On the other hand, if the data presented by them could have been mistakenly presented as based on whole-milk instead of milk fat, the range of concentrations reported by them appears more acceptable. However, the possibility exists that their data are valid. Dr C.H. Hansford of the National Institute for Tropical Diseases has been to El Salvador when DDT was still used for both agriculture and malaria control. According to him, the application of DDT, especially in agriculture, was enormous (C.H. Hansford, personal communication, 1989), and could therefore explain the very high levels.

The data from Guatamala and El Salvador have been frequently referred to by other studies, as well as the WHO report on DDT (World Health Organization, 1979). Yet, in the extensive literature list of that publication, only Olszyna-Marzys *et al.* (1973) and Winter *et al.* (1976) were included. On the other hand, Wayland J. Hayes (1982), a very well known figure in the field of toxicology of pesticides, did not include any of the articles on Guatamala in the extensive reference list of his book entitled "*Pesticides studied in man*".

The levels determined for the control group of the present study (Table 3.3.2) fell below most of the values reported from both developing and developed countries included in Table 1.8.1.

4.4.2 ASSESSMENT OF RISK TO MATERNAL HEALTH

The DDT in milk is excreted, and therefore does not contribute to the risk of the mother. It has, however, been shown that the DDT-levels in breast milk compare very well with the levels in maternal body fat (Wolff, 1983). The following discussion will assume that the same relationship is true for the exposed and control groups, and that the maternal body burden is therefore reflected in Tables 3.3.1 and 3.3.2. Since the levels of the control group were significantly less than for the exposed group, the former will be excluded from the present discussion. It would also make it easier to discuss only one concentration with which comparisons can be made. The mean ΣDDT-level in

milk of primiparous mothers of the exposed group in the present study was 24,82 mg kg^{-1} (Fig 3.3.6). The maximum level was 59,3 mg kg^{-1} ΣDDT (Table 3.3.1). A discussion level of 40 mg kg^{-1} will include 93,8% of all (but eight) measurements made. This can be seen as a "*worst case*" evaluation. If this level can be shown to have no observed adverse effect, it can be assumed that lower levels would also be safe.

Perhaps the best way to approach this assessment would be to determine the daily intake of the mothers, and to compare this with other studies. A body fat concentration of 40 mg kg^{-1} ΣDDT translates to a dose of 3,2 mg ΣDDT $woman^{-1}$ day^{-1} (World Health Organization, 1979). Assuming a mean body mass of 60 kg, this represents a dose of 0,053 mg ΣDDT kg^{-1} day^{-1}. This is far below the 285 mg kg^{-1} (single dose), one of the highest confirmed non-lethal dosages (World Health Organization, 1979). The Acceptable Daily Intake (ADI) as set by the WHO is 0,005 mg kg^{-1} day^{-1} (Klaassen *et al.*, 1986). The "worst case" estimated daily intake therefore exceeded the ADI by 10,6 times. The mean dosage for the exposed group was 0,017 mg kg^{-1} day^{-1} This dose exceeds the ADI by 3,4 times. This did not exceed the new ADI of 0,02 mg kg^{-1} day^{-1}, (Coulston, 1985b) but the "*worst case*" daily intake did exceed this ADI 2,7 times.

The daily intake of the "*worst case*" lactating mothers (0,053 mg kg^{-1} day^{-1}), fell within the reported range of no effect levels of the rat and was much less than the NOEL for the monkey and dog (World Health Organization, 1979; Klaassen *et al.*, 1986: see sub-section 1.7.1).

The dosage received by the "*worst case*" mothers was also far below any dosage for which human NOEL could be calculated (1,5 mg kg^{-1} day^{-1}: World Health Organization, 1979). It is generally true, however, that, due to confounding influences such as the use of alcohol, it is difficult to detect adverse effects in general populations that can be ascribed to DDT (Guzelian, 1985).

On the basis of the evidence presented, it can be concluded that the daily dosage of DDT to the mothers of the exposed group, although it exceeded the ADI, did not present a known risk to their health. Heath (1988) listed the limitations of

studies that relate exposure to disease outcomes in human communities exposed to environmental contaminants:

1 There is usually a long and variable latency period between exposure and disease diagnosis.

2 The clinical features of a disease are frequently non-specific.

3 Low frequency of disease, often coupled with a small population size.

These limitations are perhaps offset by the strength of such studies, in that they are conducted on exposed human populations, and therefore do not depend on extrapolation from animal studies. It has the further advantage that action can be taken if a disease is linked to an environmental agent, without knowing its mechanism (Goldsmith, 1988). Assuming that DDT can cause disease, these limitations apply.

Finally, an interesting observation was made by Cameron *et al.* (1979). The long latency period (15-20 years in man) associated with cancer has important survival value in the species, as the peak of the incidence of cancer is prolonged past the reproductive period. The host factors responsible for this are, as yet, not known. Repeated or continuous exposure to known carcinogens, shortens this latency.

CONCLUSIONS: For a baby clinic population of lactating mothers breast-feeding their first or second infant, representing mothers of the Mseleni population that live in DDT-treated dwellings, it was concluded that the results of the present study represents the highest mean ΣDDT-level in breast milk yet determined in Africa. This was concluded after comparison with all known data from Africa, including a Medline literature survey (National Library of Medicine, 1989).

For a baby clinic population of lactating mothers, representing mothers of the Mseleni population of lactating mothers that live in DDT-treated dwellings, it was concluded that the daily intake of a "*worst case*" estimate (0,053 mg kg^{-1} day^{-1}) or body burden of DDT did not pose a health risk to the mother. This was concluded after a risk assessment from the existing literature.

4.5 MODELLING OF DDT IN INFANT BLOOD

The levels of DDE and DDT in whole-blood of infants and the breast milk of their respective mothers were measured, and the results presented in Tables 3.4.1 and 3.4.2. Measurable quantities of DDT and DDE were detected in all samples of whole-blood of infants, as well as in all breast milk samples. The analytical technique was not sensitive enough to determine DDD in infant whole-blood.

Various reports have mentioned DDT in cord blood of neo-nates, confirming the presence of DDT and metabolites in infants before birth. Cord-blood concentration is a measure of trans-placental transfer from the maternal system (Wolff, 1983). Rogan *et al.* (1986a) reported a mean maternal serum level at birth of 12,60 $\mu g\ l^{-1}$ for DDE and a mean cord-blood serum level of 3,95 $\mu g\ l^{-1}$ DDE (both samples taken at birth), a ratio of 0,31. Krauthacker *et al.* (1980) reported a mean maternal serum level of 42,3 $\mu g\ l^{-1}$ ΣDDT at birth and a cord-blood serum level of 10,8 $\mu g\ l^{-1}$ (ΣDDT), a ratio of 0,26. A ratio of 0,46 was calculated from the data of Procianoy and Schvartsman (1981), for a mean ΣDDT level of 30,78 $\mu g\ l^{-1}$ in maternal blood. Kodama and Ota (1980) reported on PCB concentrations in cord-blood and serum during different years. A mean ratio of 0,24 was calculated for a mean concentration of 4,58 $\mu g\ l^{-1}$ PCB in maternal blood. Eckenhausen *et al.* (1981) determined a mean of 9,6 $\mu g\ l^{-1}$ ΣDDT in maternal blood and 3,7 $\mu g\ l^{-1}$ ΣDDT for cord-blood (samples taken 10 days after parturition), a ratio of 0,38.

The placenta therefore, acts as a partially successful barrier to the transfer of organochlorines to the infant's blood supply. Consequently, newborns will have lower levels of organochlorines than their mothers. Fig. 3.4.3 showed, with extrapolation, that infants had about 10 $\mu g\ l^{-1}$ ΣDDT (whole-blood) at birth. This was less than the mean level of 55 $\mu g\ l^{-1}$ ΣDDT calculated for serum from the females in the 20 to 40 year age interval, using the data from the serum study (Bouwman, 1990). This represents a ratio of 0,18. Maternal blood should also have been sampled to allow a proper conclusion to be drawn.

Levels in whole-blood differ from those in plasma, in that plasma contains up to 75% of the ΣDDT in whole blood (Radomski *et al.*, 1971a and 1971b). Morgan, Roan and Paschal (1972) found that less than 18% of DDT and DDE was carried by red blood cells and associated with the low-density and very low-density-lipoproteins. Assuming that the same relationship holds true for infants, as well as a haematocrit of 50%, the levels determined should at least be doubled to obtain a value that can be compared with other serum data. That will bring the mean ΣDDT-level for the infants to 254,06 $\mu g\ l^{-1}$ and the maximum to 633 $\mu g\ l^{-1}$. This may, however, not be true for the infant, as there is a difference in red blood cell / plasma ratio between infants and adults for certain drugs (Morselli, 1976).

The mean values for the levels of DDT and metabolites in serum of the concurrent serum study (Bouwman, 1990) is given in Tables 4.5.1 and 4.5.2. As expected, the levels for serum were significantly lower for the control group compared to the levels of the exposed group. Analysis of variance between age intervals revealed a high level of ΣDDT in the two youngest and three oldest age intervals. The 21-30 and 31-40 years age intervals had the lowest levels. The higher levels found in the children were not expected (Bouwman, 1990).

Kreiss *et al.* (1981) determined a classical relationship of accumulation with age in a black population living down-stream of a defunct DDT factory. Mean ΣDDT-levels (serum) increased from ±30 $\mu g\ l^{-1}$ for the 1-9 month age interval, to ± 350 $\mu g\ l^{-1}$ for the 70+ age interval. The mean ΣDDT-level for the whole community was 76,2 $\mu g\ l^{-1}$. Breast-fed children had higher levels of ΣDDT than did those who were not, but this was determined some years after breast-feeding stopped. When controlled for fish consumption and age, the relation to duration of breast-feeding was no longer significant. This indicated that the infants of the exposed group of the present study were drawn from an infant population with higher than expected uptake or dosage of DDT, when compared to a population with "exceptional" exposure to DDT.

The ΣDDT-levels in infant blood (254,06 $\mu g\ l^{-1}$ after recalculation to serum) exceeded the values determined for the nearby general population used for the

serum study (168,6 $\mu g\ l^{-1}$ ΣDDT for the 3-10 year interval; Table 4.5.2). The elevated levels in serum for the younger children, must therefore be attributed to DDT intake after birth. From the age of three years (lower age limit for the serum study) it seems that a process of negative balance was in force. Further calculations (Bouwman, 1990) indicated that the elimination process (first order kinetics assumed for DDT and DDE from serum) for the younger age groups differed significantly ($p < 0,05$) from that of the older age groups. The coefficient for the rate of elimination of DDT over the period of a year was 0,6371 for people younger than 21, and 0,2639 for people older than 20. The values for DDE were 0,2869 and 0,2434, respectively.

Table. 4.5.1. Summary statistics of levels of DDT and its metabolites in serum taken during November 1986 from families living in DDT-treated dwellings and from the control area (Bouwman, 1990). Levels are expressed as $\mu g\ l^{-1}$ and standard deviations are given in brackets. Percentage samples testing positive for the specific compound are given in sharp brackets.

	EXPOSED GROUP		CONTROL GROUP	
n	71		77	
DDE	103,4	(85,1) <100>	5,95	(7,98) <92,2>
DDD	0,21	(0,7) < 14>	0,015	(0,13) < 1,3>
DDT	37,3	(27,2) <100>	0,077	(0,31) < 6,5>
ΣDDT	140,9	(108,3)	6,04	(8,19)

Table 4.5.2. Serum levels of ΣDDT for age intervals for the exposed and control groups of the concurrent serum study (Bouwman, 1990).

AGE INTERVAL (months)	EXPOSED GROUP n	EXPOSED GROUP MEAN	CONTROL GROUP n	CONTROL GROUP MEAN
3-10	24	168,6	26	4,7
11-20	17	123,1	19	3,0
21-30	5	60,5	8	2,6
31-40	14	84,2	3	1,8
41-50	3	183,1	2	2,5
51-60	4	257,9	5	6,6
61-70	4	200,7	11	13,1
71-80	-	-	2	37,1

There is still no proof that breast milk is the major contributor of DDT in infants, as DDT is also present in air, water, soil and food. Since no attempt was made to analyze levels of DDT in cows' milk or other food consumed by the infants, the contribution attributable to diet could not be quantified. Modelling, using more than one variable, will look more closely at route apportionment.

A major aspect that should be discussed first, is the percentage DDT in peripheral infant blood and milk. The correlation between the ratios of the two compartments was 0,7847, with a significant p-value and a good coefficient of determination (Fig. 3.4.4). By subtracting the percentage DDT in milk from the corresponding percentage DDT in blood, a mean of zero would indicate no difference. However, the two sided t-test showed that the mean obtained was significantly different from the hypothesized zero. The percentage DDT of the infant blood (50,88%), was higher, by a mean of 5,35% (Table 3.4.3), than the percentage DDT in milk (corrected for DDD). A possible explanation might be an argument advanced earlier about the effect of input of DDT into the immediate environment of the mother. (The same, of course, also applies to the infant.) Not only is the percentage DDT in breast milk possibly higher than in the maternal serum; DDT added to the environment of the infant could also have contributed to the elevated percentage DDT in the blood of the infant.

Linear (Fig. 3.4.2) and multiplicative modelling (Fig. 3.4.3) were done. Infant age was the only logical independent variable. The coefficient of determination (multiplicative model) indicated that infant age explained 45% of the variation observed.

Few studies could be found on the measured levels of organochlorines in infants in relation to breast-feeding or, for that matter, any other parameter. Four studies found or indicated increased body burdens of organochlorines with longer lactation (equivalent to infant age for breast-feeding infants). Engst *et al.* (1970) found an increase in levels of ΣDDT in infant body fat from 15 mg kg^{-1} at birth to 16,4 mg kg^{-1} in the following 13 days. After that, the levels dropped to 9,2 mg kg^{-1} ΣDDT, 2-32 months later. The slight increase in levels after birth was attributed to a weight loss, thereby concentrating the organochlorines in the fat.

With the subsequent weight increase of the infant, dilution of the DDT followed. The infant samples were obtained by necropsy, and might thus not be representative of the general infant population from which they were drawn.

Eckenhausen *et al.* (1981) found an increase in DDE-levels in infant blood, from a level of 3,0 $\mu g\ l^{-1}$ at two weeks and 2,9 $\mu g\ l^{-1}$ after two months, to 3,9 $\mu g\ l^{-1}$ after three months. They calculated that this did not constitute a significant increase. Kodama and Ota (1980) determined the change in PCB-levels in 14 breast fed and 6 bottle feeding infants. Levels at birth between the two groups were not significantly different. During the first three months, the blood concentration of the breast-feeding group increased from 1,1 $\mu g\ l^{-1}$ PCB to 3,6 $\mu g\ l^{-1}$ PCB, and increased still more to 4,5 $\mu g\ l^{-1}$ PCB at twelve months. For the bottle feeding group the respective values were 1,1; 1,6 and 0,9 $\mu g\ l^{-1}$ PCB. The values at three and 12 months for the breast-fed group were significantly different. Jacobson *et al.* (1989) found an increase in levels (sera of children) of PCBs (coefficient of determination = 70%), polybrominated biphenyls (PBBs; coefficient of determination = 42%) and ΣDDT (coefficient of determination = 56%) associated with age. Mean levels were 4,36 $\mu g\ l^{-1}$; 2,58 $\mu g\ l^{-1}$ and 4,22 $\mu g\ l^{-1}$, respectively. Percentage DDT was 11%.

The results of Eckenhausen *et al.* (1981), Kodama and Ota (1980) and Jacobson *et al.* (1989) are therefore consistent with the results from the present study. They did, however, apply to relatively low levels (and percentage DDT) as compared to the high levels found in the present study.

Three studies on animals presented additional support for the present findings. The PCB compound, 2,4,5,2',4',5'- hexachloro-biphenyl, was not eliminated from pregnant mice, nor transferred across the placenta to the fetus (Vodicnik and Lech, 1980). After birth, however, almost the entire body burden of the biphenyl was eliminated through lactation within 20 days and accumulated by the offspring. Ando (1978) found that concentrations of DDT in whole suckling rats, exposed to DDT via milk, increased rapidly from birth and followed a sigmoid curve. Tomatis *et al.* (1971) followed a different experimental design and analyzed mouse fetuses from dams exposed to different concentrations of DDT.

The ΣDDT-concentrations in the fetuses were directly related to maternal exposure.

Only one article could be found that predicted age related DDT-levels in infants from the levels in milk. Mes *et al.* (1984) plotted lactation time and a theoretically estimated accumulation of DDT and DDE, derived from milk, in infant body fat. They used sequential analysis of breast milk from 16 women over a three month period. The respective DDE-levels in infant body were estimated at 0,15; 0,40; 1,0 and 1,85 mg kg^{-1} for 1, 2, 4 and 14 weeks post partum, respectively. The respective values for DDT were 0,02; 0,07; 0,12 and 0,25 mg kg^{-1} respectively. They assumed a linear increase of percentage body fat, no provision for adsorption by other organs and no correction for excretion. The levels in milk were not correlated with age or parity. They predicted that the infant body burden would reach adult levels within three months of breast-feeding (Mes *et al.*, 1984). The results of the present study showed that this predicted increase did occur in infants, measured as levels of DDT and DDE in blood, but that the increase continued for more than three months.

Another theoretical approach by Wickizer *et al.* (1981), gave an estimation of infant body burden of PCBs over 12 weeks for infants exposed to 1,5 mg kg^{-1} PCB in milk fat. The infant body burdens at 1, 2, 4, 6, 8, 10 and 12 weeks were 0,25; 0,45; 0,62; 0,75; 0,90; 10,2 and 11,0 mg kg^{-1} PCB body weight, respectively. Mes *et al.* (1984) and Wickizer *et al.* (1981) therefore, presented theoretical confirmation of the positive relationship between levels of ΣDDT and infant age.

The large variation of the raw data (Fig. 3.4.3) might be caused by confounding effects, such as parity and maternal age, which were shown to have an influence on levels of the organochlorines (sub-section 4.3). These effects were modelled for ΣDDT, DDT and DDE. The plots of the models are shown in Figs. 3.4.5, 3.4.7 and 3.4.9. The two major effects that contributed significantly to models relating to ΣDDT and DDE were parity and infant age. Concentration based on milk fat was a slightly better predictor of ΣDDT-levels in whole-blood of the infant compared to concentration based on whole-milk (Table 3.4.4).

The results of the selected models are presented in Tables 3.4.5, 3.4.8 and 3.4.12. The respective coefficients of determination for models 3.4.1 (ΣDDT), 3.4.2 (DDE) and 3.4.3 (DDT) were 70,0%, 76,3% and 38,2%. The regression of all three models was significant (Tables 3.4.6, 3.4.9, 3.4.12).

The respective contribution of each variable in model 3.4.1 was: parity with 60,5%, infant age with 8% and ΣDDT-concentration in milk fat with 1,5%. Parity was therefore the most important determinant of levels of ΣDDT in infant whole-blood. This can also be seen from the high partial correlation values (F-enter) for this variable (Table 3.4.4). The contribution of the other variables was less. Maternal age as a predictor, if included together with the parity and concentration, diminished the accuracy of the model. The reason for this was probably that, as already mentioned, parity and maternal age were related variables. Thus the inclusion of another related variable (even with a high partial correlation; Table 3.4.4) only introduced more variation, without contributing any more descriptive value. The descriptive value of parity in this model corroborates previous findings (sub-section 4.3.2) on the effects of parity on ΣDDT-levels in milk.

The same could be said about the contribution of infant age to this model, and its corroboration of the related findings made in sub-section 4.3.4. The partial correlation was good and the F-enter value was about half that of parity (Table 3.4.5), but still more than maternal age. The contribution made was significant, but small, in a model that already had parity as a variable ($p = 0,0185$).

ΣDDT-levels in milk, contrary to expectations, had a low partial correlation. Its inclusion in the model only contributed 1,5% of the descriptive value. The contribution of this variable to the model, already containing two other variables, was also not significant ($p = 0,1747$). The decision on its inclusion was made because this improved the model (coefficient of determination from 68,5 to 70%) slightly and reduced the mean square error by 30% (not shown). The concentration variable was also significant in the model explaining DDE-levels (Model 3.4.2). This unexpectedly low contribution of concentration to the model could possibly be explained by the following two considerations:

1. Parity was shown to be a major factor in determining ΣDDT-levels in milk.

2. Eventual uptake by the infant depends on time and concentration. The amount present in milk is important, but the amount of milk imbibed by the child depends on the length of the lactation period. This is measured as infant age.

Since the concentration in milk is determined by parity, and the total uptake of the infant depends on its age, the two best predictors would be parity and infant age.

Assuming that maternal exposure to environmental DDT was fairly uniform, the above will explain the small contribution of the concentration of DDT in breast milk. Conversely, if exposure was not uniform, concentration would probably be the main determinant. Partial proof of this is the low levels found in babies exposed to low levels of DDE and PCBs in milk (Kodama and Ota, 1980; Eckenhausen *et al.*, 1981; Niessen *et al.*, 1984).

A very important consequence of the first consideration, and which may be proved by the significance and relative contribution of parity to the model, was that breast milk was the most important source of ΣDDT in the infant. However, the infant was also exposed to other sources of DDT. Possible routes could be inhalation or contact. These sources could also have contributed to the age dependant increase of infant whole-blood levels. Although not presented in the results section, infant age alone explained 42,3% of the variation if used as a single variable in the model. The two major variables together explained 68,5%. It seemed that infant age and parity explained at least part of the same variation. This would imply a relationship between the two variables. Infant age however, reveals nothing about parity and introducing mathematical interaction between these two variables did not improve the model. Although there might have been confounding influences (such as rate of weight gain of the infant, length of lactation period per infant and non-lactating interval between infants),

this indicated breast milk and not the environment, as the major source of ΣDDT present in the infant.

Breast-feeding, as a source of DDT to the infant, was also the subject of a recent report. In a retrospective study by Jacobson *et al.* (1989) it was stated that breast-feeding was "*a significant source of DDT exposure*". For PCBs and PBBs it was found to be the principal source. Infant age, or weeks of breast feeding, was the most important predictor, while parity was not considered by Jacobson *et al.* (1989). Socio-economic status, maternal age and fish consumption were apparently not significant in their model. They did not collect data on breast milk levels of DDT and DDE.

The unknown interval between births, while not lactating, must be seen as an uncertainty. Longer intervals between births would presumably have resulted in more DDT taken up by the mother from the environment, while shorter lactation periods would have resulted in less excretion per period. This could therefore have been partially responsible for variation in levels of contamination in milk. Another factor could be variation in length of lactation period for previous children.

The plot of model 3.4.1 (Fig. 3.4.5) shows more or less an equal distribution of the raw data around the regression line. This plot is much improved on that of the multiplicative model (Fig. 3.4.3). The probability plot (Fig. 3.4.6) showed some auto-correlation, but this was not indicated by the Durbin - Watson statistic.

A better descriptive model (Model 3.4.2: coefficient of determination = 76,3%) was determined for DDE in whole peripheral blood of the infants (Table 3.4.7). Parity explained 62,7% while infant age contributed a further 7,6%. DDE levels in milk added another 6% to the descriptive value of model 6. All three variables were significant in the model ($p < 0,05$; Table 3.4.8) as was the model itself ($p < 0,001$; Table 3.4.9). The plot of the model (Fig. 3.4.8) looks like the plot for ΣDDT (Fig. 3.4.5), with perhaps slightly less variation.

Model 3.4.3, relating to DDT-levels in infant whole blood, on the other hand, had a poor coefficient of determination of 38,2% (Table 3.4.10). Parity only explained 26,5%, infant age 6% and DDT-levels in milk 5,7%. None of the three variables in the model were significant (Table 3.4.11), but the p-value for the model was 0,0067 (Table 3.4.9). This result was probably due to the degrees of freedom (3) by which the sum of squares was divided to obtain mean square from which the p-value was derived. The plot of the model (Fig. 3.4.10) showed that there was some variation of the observed data around the predicted model. The probability plot showed very little variation of the residuals (Fig. 3.4.11).

The reason for the difference between the DDE and DDT models might lie in the arguments made earlier, in sub-section 4.3.2, relating to the respective environmental availability of DDT and DDE. DDT is applied once a year, and this application is independent of parity, infant age and maternal age. There is in other words, a periodic increase in DDT which is independent of other factors, but related to levels in milk. DDE is much more stable and is formed slowly in the body, smoothing out larger variations in both the maternal and infant bodies. The stability of DDE, therefore, lends itself to making better predictions.

Participation in metabolising DDT to DDE by the fetus was mentioned as a possibility by Polishuk *et al.* (1970), who found 22% DDT in cord-blood and 32,7% DDT in maternal blood. This finding was not supported by the present data.

Fig. 3.4.12 shows the difficulties involved in modelling the levels of DDT and its metabolites in infant blood. This graph shows all three variables that have been used in the three models defined above. Two nested trends are apparent. Overall, ΣDDT-levels in breast milk decreased with an increase in parity, while a decrease in levels for the first year was followed by an increase in the second year of breast-feeding. The second trend was apparent for mothers breast-feeding their first, second or fourth infant.

Apart from the variables such as interval between births and length of previous lactation periods, other infant related variables were not included in the model. These were pharmacokinetics (especially organ distribution; Morselli, 1976) and biotransformation of xenobiotics (Klinger, 1982) that differed from the more developed older children and adults. These aspects will be addressed in sub-section 1.9.5. The risk to the infant, associated with these levels, will be discussed in sub-section 4.6.

CONCLUSIONS: For a cross-sectional sampled baby clinic population of breast-feeding infants, representing infants of the Mseleni population of lactating mothers that live in DDT-treated dwellings, it was concluded that:

1 DDT and DDE was present in peripheral whole-blood in measurable quantities. This was supported by present data and inferred from reports in the literature.

2 the mean level of ΣDDT in whole peripheral blood exceeded the mean level of ΣDDT determined in serum of 71 members from 12 families representative of the general population, as well as the 3-10 and 11-20 age intervals of the same group. This was supported by present data only.

3 ΣDDT-levels increased significantly ($p < 0,001$) with infant age in whole peripheral blood. This was indicated by the present data and supported by reports in the literature.

4 70% of the variation of the levels of ΣDDT in whole peripheral blood of the infants was explained by a multiple regression model ($p < 0,001$) that included parity, infant age and ΣDDT-concentration in breast milk as variables. This was supported by present data only.

5 76,3% of the variation of the levels of DDE in whole peripheral blood of the infants, was explained by a multiple regression model ($p < 0,001$) that

included parity, infant age and DDE-concentration in breast milk as variables. This was supported by present data only.

6 38,2% of the variation of the levels of DDT in whole peripheral blood of the infants, was explained by a multiple regression model ($p < 0,01$) that included parity, infant age and DDT-concentration in breast milk as variables. This was supported by present data only.

4.6 THE DDT EXPOSED INFANT: EXPOSURE AND RISK ASSESSMENT

The risk to the infant from the intake of DDT will be discussed by first establishing the daily dosage and then comparing these values with other studies. Since no quantitative risk assessment on any effects in infants has been done, other than a single neurological study, only a tentative attempt will be made to quantify the risk in this thesis. Effects such as immunological and neurological involvement have been discussed under separate headings (sub-sections 1.9.2 to 1.9.10) and their implications will be examined, weighed and compared in sub-section 4.6.2.

4.6.1 DDT EXPOSURE TO THE INFANT

The principal source of DDT in the infant was the breast milk of the mother (sub-section 4.5). The daily dosage (or intake) of DDT and its metabolites by the infant from breast milk can be estimated from its concentration, using certain parameters such as weight of the infant and amount of milk ingested per day. Various authors used different sets of standard values concerning intake and infant weight, but they all correspond fairly closely. Three estimates of intake will be presented.

The following values were used:

The mean weight of the infant was

5 kg (Jensen, 1983)

The mean daily milk intake was

800 ml (Jensen, 1983)

The mean fat content of the milk was

3,95% (the present study)

The mean ΣDDT concentration in breast milk for the exposed group was

15,8 mg kg^{-1} milk fat (the present study)

The mean ΣDDT concentration in breast milk for primiparous mothers of the exposed group was

24,8 mg kg^{-1} milk fat (the present study)

The maximum ΣDDT concentration in breast milk found was

59,3 mg kg^{-1} milk fat (the present study)

Therefore:

The mean daily intake for the infants of the exposed group of the present study was

0,100 mg kg^{-1} day^{-1}

The mean daily intake for infants from primiparous mothers of the present study was

0,157 mg kg^{-1} day^{-1}

The maximum daily intake by an infant calculated for the present study was

0,375 mg kg^{-1} day^{-1}

The mean daily intake for infants of primiparous mothers, assuming constant milk intake by the infant and constant concentration of DDT in the milk, will result in the transfer of 0,559 g of ΣDDT to the gastrointestinal tract (GIT) of the infant over a period of two years. For the maximum value observed, more than one gram will be transferred.

The ADI for ΣDDT, as determined by the WHO (Klaassen *et al.*, 1986), was 0,005 mg kg^{-1} day^{-1}. A new ADI was established at the Joint meeting of the FAO (Food and Agricultural Organization) Panel of Experts on Pesticide Residues in Food and the Environment and the World Health Organization Expert group on Pesticide Residues in Rome in 1984 (Coulston, 1985b). Based partly on two studies of Cabral *et al.* (1982a and b), a new estimate of ADI for ΣDDT of 0,02 mg kg^{-1} day^{-1} was determined.

The exposure to the children in the three categories above was 20; 31,4 and 75 times more than the old ADI. The mean dosage of all the mothers of the exposed group was 0,017 mg kg^{-1} day^{-1}, which exceeded the old ADI by 3,4 times. From the literature (already discussed in sub-section 4.4), these dosages were not expected to result in acute or obvious toxic effects in adults. The above calculated intake by the infant also exceeded the new ADI by factors of 5; 7,9 and 18,75, respectively. This new ADI, however, has not been used in all subsequent papers (Mussalo-Rauhamaa *et al.*, 1988; Jani *et al.*, 1988; Skaare *et al.*, 1988). The new ADI was arrived at by taking more relevant and recent literature into account. Therefore, intake exceeding the new ADI demands even closer attention.

Using the mean ΣDDT-levels determined for infant whole-blood (127,03 μg l^{-1}), and assuming doubling of levels in serum (254,06 μg l^{-1}) according to Radomski *et al.* 1971b; as well as an adipose to serum partition ratio given by Wolff (1983) of 250 (between 200 and 300); the mean body fat concentration in the infant could be calculated as being between 31,8 (derived from serum value) and 63,5 mg kg^{-1} ΣDDT (derived from calculated whole-blood value). This translates further to a mean daily intake by the infant of between 1,3 and 2,1 mg kg^{-1} day^{-1} ΣDDT (World Health Organization, 1979). The maximum intake, based on these

calculations, would be 4,1 mg kg^{-1} day^{-1} ΣDDT. The assumption of these calculations was that the relationships determined for adults hold true for infants as well, and did not take into account the levels in milk. The difference between the values calculated above and those derived from milk intake (sub-section 4.6.1) was considerable. This discrepancy, whether true or not, indicated, however, that there might be differences in the pharmacokinetics of DDT between the infant and adult (sub-section 1.9.2).

4.6.2 RISK ASSESSMENT OF HEALTH EFFECTS OF DDT IN BREAST MILK IN RELATION TO INFANTS

The fact that the ADI (both 0,005 and 0,02 mg kg^{-1} day^{-1} of ΣDDT) was exceeded in the present study, must be seen as significant, since the ADI was derived after considerable discussion of all the known facts. Authors who related the daily dosages they determined to health implications concerning the infant, all stressed the need for continued monitoring and the determination of any possible pathology.

The information on DDT as an abortificant (sub-section 1.9.3) indicated that, at the levels determined, no such effect would be apparent. The data on stillbirths though, warrants some concern. Although not part of the protocol of the study, some information on stillbirth rates at Mseleni Hospital and Murchison Hospital was collected for 1985 (KwaZulu Department of Health and Welfare, 1985). The stillbirth rate at Mseleni was 22,59, slightly higher than the 21,05 rate for Murchison. The still birth rates for the communities (hospital and clinic data combined) were 39,24 and 33,56, respectively. The fact that the levels in cord blood of both stillborn and life born cases were found to be similar (Saxena *et al.*, 1983), indicated maternal factors, rather than feto-toxicity. This aspect needs more attention and can be studied in South Africa.

The studies performed on rat populations (sub-section 1.9.4), on the whole, do not seem to indicate any concern in this respect, as the dosages were in all cases much higher than those determined in the present study. The validity of

extrapolation of these results must include inter-species variation, and can be represented by a safety factor.

The calculated intake of ΣDDT via milk and the intake calculated from the level in infant blood differed, and this should be approached with caution. Uptake via lungs or skin could have contributed to the body burden, but this has not been established. The other possible explanation is the difference in pharmaco-kinetics between adults and infants (sub-section 1.9.5). The smaller percentage of adipose tissue, reduced excretion, reduced bio-transformation, and the possibility of a higher binding of DDT to fatty acids in infant blood, as opposed to adult blood, could contribute towards a significantly higher level in infant blood. Uptake from the GIT also seems to be more efficient in the infant than adult, from where it could be partitioned at a preferential rate higher than that for the adult. This could result in a smaller apparent volume of distribution than would be typical for other, less lipophilic, agents.

Several implications are involved. The high concentration of ΣDDT in infant blood would lead to an increased exposure to organs such as the brain and liver. Because conjugated DDT has been shown to cross the blood-brain barrier and have an effect (and because the presence of such conjugates has not yet been determined in infant blood samples), efforts should be made to determine whether the assumptions made above are correct and to test the implications. In other words, what are the blood-fat partition ratios for the infant, in what form is the DDT present in infant blood, and at what concentration is DDT present in the brain, spinal cord and liver ?

The immune system of the infant, as can be deduced from animal studies, also seem to be a target, possibly reducing the resistance against infections in the early months (sub-section 1.9.6). The studies by Banerjee and his co-workers (1987a and b) were especially relevant, as the dosages they employed were comparable with those determined for the infants of the exposed group of the present study. Unfortunately, the studies were conducted on adult animals. It is, however, paradoxical, that the immune status of the infant in the first few months of life depends on maternal factors derived from milk, which is also

contaminated with DDT. The effect of DDT on both the cellular and humoral immune systems should therefore be determined for infants.

In the field of neurology and behaviour, sufficient evidence from animal studies was presented to indicate that DDT, at the levels and dosages determined for the infants of the exposed group of the present study, may have a detrimental effect on the infant (sub-section 1.9.7). The studies relating to the distribution of DDT in the brain are of special importance. No studies on the distribution of DDT in the brains of humans were found. The fact that conjugated DDT can cross the blood brain barrier must be seen as an aspect that needs further clarification.

It is, however, the studies done on humans, and especially the reports from Misra *et al.* (1984: sub-section 1.9.8) and Rogan *et al.* (1986b: sub-section 1.9.9), that best defined the risk to the DDT exposed infant. That DDE at levels lower than those determined for the present study was associated with hypo-reflexia in infants, must be seen as the most relevant evidence indicating adverse effects (Rogan *et al.*, 1986b). This study was complicated because of the presence of PCBs, and the absence of neuro-physiological measurements, preventing the conclusive establishment of causality. As none of the above mentioned evidence has presented enough relevant data on the health risk involved, a valid and dependable quantitative risk assessment was not possible.

The data of Rogan *et al.* (1986b) can be used to extrapolate to results obtained in the present study. It must, however, be stressed that this is very tentative as the extrapolation goes beyond the levels determined by Rogan *et al.* (1986b). The assumption is therefore made that the relationship between concentration in breast milk and its influence on reflexes remains constant over the region of concentrations to be tested. A multiplicative regression of percentage low reflexes on concentration of DDT (see Table 1.9.1 for values that were used) was significant ($p = 0{,}0200$), with a coefficient of determination of 60%. The percentage low reflexes was estimated for the ΣDDT-levels in breast milk of the control group (0,69 mg kg^{-1}: milk fat), the total exposed group (15,83 mg kg^{-1}: milk fat), the first borns (24,82 mg kg^{-1}: milk fat) and the maximum level (59,3

mg kg^{-1}: milk fat). The calculated percentage infants with low responses were calculated to be 4,28%, 21,60%, 27,25% and 42,74%, respectively. The level where 50% of the infants would show low reflexes was 80 mg kg^{-1} (milk fat). The confidence intervals were very wide, but could not be calculated with any reliability. It can therefore only be concluded that there exists a well founded possibility of risk to the infant. This risk remains to be quantified.

4.6.3 UNCERTAINTIES INVOLVED WITH THE RISK ASSESSMENT

There are quite a number of uncertainties involved with this risk assessment, not least of which is the ever present consideration of inter-species extrapolation. The above assessment relied heavily on data derived from animal models. Recent literature on human studies, that was not available to the commission deciding on the ADI, has been included in the present assessment. It was, however, the lack of well founded data on health effects in infants that prevented the establishment of risk and safe levels. Rogan *et al.* (1986b) found effects, but no information was at hand to determine the significance of the findings in respect of how the infant was affected in relation to, for example, resistance to infection or physiological and psychological development. These questions have generated, and undoubtedly will continue to generate, a lot of speculation.

Another uncertainty was that literature on the effects of DDT might be biased towards adverse effects of this chemical, as authors will have more confidence in submitting positive evidence. Negative evidence could be ascribed to, for example, not enough experimental animals exposed, or too few repetitions, which might influence authors not to submit, or referees not to return an approving judgement.

Evidence of causality was found in numerous laboratory studies listed above. Although conflicting in many respects, most studies, depending on the sensitivity of measurement (compare for example population dynamics with electro-physiological observations), presented well documented evidence of involvement

of the chemical under consideration. The exposure of the subjects of the exposed group to DDT differed drastically from that of the control group in the present study, and this was supported by the recovery of significantly higher levels of DDT in the exposed group (breast milk), which could allow comparative studies.

Dose / response relationships were not determined for the present study. Some of the animal studies consulted did show a marked effect of dosage (and time) on response. This relationship was also indicated to be present in humans by the findings of Rogan *et al.* (1986b) and implicated in the study by Misra *et al.* (1984). That an increase in levels of DDT in the whole-blood of the infant could be modelled, suggests that time / response studies can be done on the infants. The explanation of 70% of the ΣDDT in infant whole-blood should add to the acceptability of such a study, and lend more authority to the finding of any effect, whether negative, positive or none.

CONCLUSIONS:

For a cross-sectional sampled baby clinic population of infants of lactating mothers, representing the Mseleni population of infants of lactating mothers that live in DDT-treated dwellings, it was concluded that:

1. the mean intake of DDT via breast milk exceeded the ADI of 0,005 mg kg^{-1} ΣDDT day^{-1} by a factor of 20 and the ADI of 0,02 mg kg^{-1} ΣDDT day^{-1} by a factor of 5. For first borns this factor was 31,4 and 7,9, respectively and the maximum dosage was 75 and 18,75 times the respective ADIs. These dosages are unlikely to cause acute or obvious toxic responses in adults.

2. there exists a well founded possibility of risk to their health. This was determined from a risk assessment based on relevant literature, specifically integrating knowledge on levels of DDT in infant blood (determined in present study), involvement in cases of stillbirth, increased uptake of DDT via the GIT, the transfer of DDT (and possibly conjugated DDT) through the blood-brain barrier, slower excretion mechanisms,

immature detoxification enzyme systems, possible modulation of a more susceptible immune system and finally the neurological and psychological involvement. Neither the magnitude, nor clinical significance of this risk, could be determined.

4.7 ETHICAL IMPLICATIONS OF THE RESULTS OF THE PRESENT STUDY

The results of the present study must be evaluated, not only from a medical point of view, but also according to the ethical considerations that were mentioned in sub-section 2.2. It was stated that the prime motivator of the present study was the health and welfare of the community. The results must be presented in such a way that the attitudes of the community are not changed in a way that can result in actions that cause stress or adversely affect health. A biased approach, such as that of Ferreira (Sunday Tribune, 27 Jan. 1985) represented one way in which it should not be done. Therefore, a balanced approach, taking into consideration aspects such as malaria control, availability of clean water and the cost of alternative agents, is needed.

4.7.1 MATERNAL HEALTH

The risk to the health of the mother from her body burdens of DDT is, according to present knowledge, less than that from malaria, against which she is protected by DDT. Certainly, the presence of foreign compounds, even when shown not to be harmful at the concentrations determined in the present study, should not be accepted without critical and practical evaluation of each prevailing situation. If at all preventable, steps should be taken to minimize exposure. When not, acceptable risk should be declared only after a careful analysis of available data. This was done in the present study, using the latest information available. The evaluation of risk should be a continuous process. This means that levels or exposure that were considered acceptable in the past may be changed in the light of new discoveries. This is also being done by the joint meetings of the WHO and the FAO (Food and Agricultural Organization) experts on residues.

The risk to the mothers of the exposed group to DDT has been carefully considered in the present study. Therefore,

the mothers, upon request, can be informed that DDT does not pose a risk to their health.

4.7.2 INFANT HEALTH AND BREAST-FEEDING

The possibility that the breast-feeding infant may be harmed by DDT, must be weighed against the known and well documented advantages of breast-feeding when considering acceptable risk. The absence of any overt toxic signs, and the uncertainty about the clinical significance, must also be taken into account. The ethical dilemma revolves around the need to inform the mother about the possible harm that continued feeding might inflict, and the known benefits of malaria control and breast-feeding. Two possible options are open.

The mothers involved could be informed of the possibility of risk to their infants. This might lead to a number of complications. Breast-feeding patterns might change, to the detriment of infant nutrition, as substitute feeds are not always available and can become very costly because of the low monthly income of the family. As infant formula directions cannot be read by illiterate mothers, this might also adversely affect the nutrition of the infants.

A second possibility is the outbreak of diseases such as gastric enteritis in the infants, as clean piped water is not available to the majority of mothers in the northern parts of KwaZulu. Such an event must be prevented. There is no substantiated proof that the risk to the infant attributable to DDT in milk in any way exceeds the risk of losing maternally transferred protection against infections, or exceeds that of malaria itself.

The psychological aspect of breast feeding, in an African context, is unknown. Ignorance of the implications of a risk warning regarding DDT in breast milk could lead either to drastic changes in breast-feeding patterns, or to no change at all. It is, however, an aspect that needs urgent attention.

Any negative information about breast-feeding could pose a problem to the ongoing efforts of the health authorities to promote this form of infant nutrition. The mothers should be advised, upon request, that it is safe to breast-feed their children, and they should be encouraged to do so.

4.7.3 MALARIA CONTROL

The spraying of a bio-active agent in residences poses the question of safety of the inhabitants. The results obtained in the present study show that there may be possible harm to infants. This places the onus on the authorities to determine the efficacy and safety of alternative agents. A major problem with these alternatives is that they were initially developed for agriculture, which required more and more effective and highly toxic compounds, with short half lives. Switching to alternatives will also have medical implications.

After 50 years of research on DDT, new information about sub-clinical effects is still being published. The available knowledge on health effects of alternatives, especially those developed for agriculture, could, conceivably, be less than for DDT. Therefore, the health authorities are under obligation to give attention to identifying safe and effective alternatives, in case the vector develops resistance against DDT, or if future studies indicate that adverse effects caused by DDT can be avoided by using alternatives.

Another aspect of major importance is the cost of alternative agents. DDT is by far the cheapest available, with the closest competitors three to six times more expensive (D.L. Theron, personal communication, 1988). Application might also have to be done more than once a year, which will result in additional pressure on funds and manpower.

Fortunately, research on alternatives is taking place, and much progress has been made by the National Institute for Tropical Diseases in Tzaneen. Aspects that might be considered in a choice of agents can be deduced from the results

of the present study. Attention should be payed to the toxicity of alternative agents to the very young as well as the elderly, as many of the aspects mentioned in the discussion are also relevant to the aged. The need for more information on the effects of DDT on the infant is clear. Therefore, resources should be made available to pursue this matter.

CHAPTER 5

CONCLUSION

DDT, after 50 years of research, can be considered as one of the best known synthetic chemicals. The amount of recent literature on possible sub-clinical effects has shown that researchers and policy makers should never make the mistake of unconditionally declaring any substance as being safe. DDT is used as a pesticide. This means that it interferes with certain *biological* processes to the detriment of the host. Since there are few, if any, species specific agents or strategies that will effectively eradicate or control a single pest or vector, the above consideration, together with the concept of acceptable risk, should always be taken into account. The case of tobacco smoke or ozone depleting chemicals will serve as good reminders that caution, open-mindedness and continued research must be recommended, not only at the introduction of a compound, but also throughout its use.

The need for more information on the sub-lethal effects of DDT on humans has been shown. An approach that takes into account all the factors that are not immediately obvious, is needed. This has recently received attention, as more information on the relative susceptibility of the infant to adverse effects of environmental chemicals has been published. This information has been collated by The International Programme on Chemical Safety (IPCS), a joint venture of the United Nations Environment Programme, WHO and the International Labour Organisation. Its main aim is to evaluate the effects of chemicals on human health and environmental quality. It also develops epidemiological, risk assessment and experimental laboratory methods, and its findings are published by the WHO.

The World Health Organization (1986a) evaluated the health risks from chemicals to infants and young children. Notable persons such as Prof A.A. Jensen, Dr R.D. Kimbrough, Prof W. Klinger and Dr D. Wassermann, all referred to previously in this thesis, were participants at the IPCS meetings that

drew up this document. The summary of this report touched on many of the points mentioned in the discussion. Observations concerning differences between infants on the one hand and young children and adults on the other, included:

- larger body surface / weight ratio
- higher metabolic rate
- larger intake of air relative to body weight
- different body composition
- greater relative energy and fluid requirements
- special dietary needs
- special behaviour characteristics
- organic and inorganic chemicals, in general, are more readily absorbed
- immature detoxification systems
- immature kidney function
- different dose / response relationships

The IPCS (World Health Organization, 1986a) also stressed that developmental toxicology must be promoted, and methodology improved. There is also a need to collect and disseminate epidemiological and clinical data following exposure to chemicals. In the same series, the IPCS (World Health Organization, 1986b) evaluated possible strategies for determining neurotoxicity following exposure to chemicals. It stated that "*..nervous system function should be among the first to be thoroughly assessed in cases of exposure to known or potentially hazardous agents*". Based on this assessment, it was decided that the neurological effects of DDT on six months old infants exposed to DDT via breast milk need to be determined. However, a number of other areas have been identified in the present study that should also receive closer attention. These include immunology, liver function, kidney function and possible conjugation of DDT to fatty acids.

It is clear that an epidemiological approach to effects caused by environmental contaminants should examine groups such as the very young and very old, as they are in many ways probably more susceptible to these agents. In the Republic of South Africa, considerable contributions to the knowledge concerning these aspects can be made, as a wide variety of community types are exposed to widely different environmental conditions. Air pollution from industry or coal and wood fires would expose different populations to different chemicals. In the rural areas, exposure to these agents will be comparatively small, but factors, such as the need to control malaria or bedbugs, will result in exposure to other types of chemicals. Moriarty (1988) said that effects of pollutants on wildlife often pass unnoticed, and that effects that are noticed are often difficult to relate to pollutants. This is, in many cases, also true for human populations.

The amount of work to be done to establish, first of all, the unknown exposure to sometimes unknown agents, is immense. The determination of the risk to health of such chemicals is a task that will require a concentrated effort from highly trained and motivated people.

CHAPTER 6

RECOMMENDATIONS

The following recommendations, based on the results of the present study, are made:

1. Upon request, the exposed mothers should be informed of the lack of any indications of risk attributable to DDT (as used in the present form of malaria control) to their person.

2. Upon request, the exposed mothers should be informed of the lack of any confirmed evidence of risk to their infants, attributable to the presence of DDT in their breast milk. They should be encouraged to continue breast feeding.

3. Note of the results of this study should be taken by the health authorities responsible for malaria control. The need to decide on alternatives to DDT for indoor control of malaria, should be seen, not only from the context of effective control of transmission, but also from the health aspects. These considerations should also include the special situations that characterize the households (specifically breast-feeding of infants) of the protected population.

4. Qualitative risk assessments have shown a distinct lack of information on the health effects of DDT (in breast milk) to the infant. This can be seen as one of the last major questions to be answered about the possible health effects of DDT. Therefore, efforts to establish these effects should be seen as a priority. Many children have been, are, and will be, exposed to this agent.

CHAPTER 7

SUMMARY

The WHO considers DDT as safe to man and environment when applied intra-domiciliary for malaria control. Research into the possible health effects under prevailing conditions, taking social customs into account, have, however, been lacking. This project was undertaken to determine the levels and possible risk of DDT to the health of lactating mothers and their infants.

The aims of the study were:

1. To determine the levels of DDT and its metabolites (DDD and DDE) in the breast milk of mothers from a sprayed and a non-sprayed area, as well as determining changes caused by indoor application of DDT.

2. To determine the uptake of DDT and its metabolites by the infant via breast milk, and to develop a statistical model that describes the dynamics.

3. To determine the risk to the health of the mother and infant that can be ascribed to exposure to DDT and its metabolites.

The experimental design and methods were approved by the Ethics Committee of the Research Institute for Diseases in a Tropical Environment. Lactating mothers visiting the Mseleni (test group) and Murchison Hospitals (control group) were asked to donate milk on several occasions throughout 1986/7. Blood was also taken from 23 infants from the test group. These samples were extracted according to procedures developed for this study and analyzed using gas-chromatography.

DDT was found in all samples from the test group and most of the control group. The mean level of ΣDDT (total DDT) in the breast milk of the test group was 15,83 mg l^{-1} (milk fat), significantly higher ($p < 0,05$) than the 0,69 mg l^{-1} ΣDDT (milk fat) for the control group. This was also the case for DDE, DDD and percentage DDT. Serial changes in levels of DDT were found in the breast milk of the exposed group, but was not considered as clinically significant. No significant changes were observed for the control group.

The levels in breast milk were not considered as posing a risk to the health of the mothers. The ΣDDT level in the milk of the exposed group exceeded the ADI for the infants. The blood levels of the infants was successfully modeled and it was found that parity of the mother and the age of the infant were major factors. Percentage DDT increased significantly ($p < 0,05$) with an increase in parity.

Evidence exists in the literature indicating that immunologic, neurologic and other systems of the infant exposed to high levels via breast milk, may be affected. It was especially a report that correlated an increasing percentage of infants with hypo-reflexia with an increase in levels of DDE in breast milk (Rogan *et al.*, 1986b; lower than the mean ΣDDT determined in the present study), that presented evidence of possible neurological involvement. Determining these sub-lethal effects in infants was identified as a priority for further research.

CHAPTER 8

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TITEL: Neonatal Exposure to DDT Induces increased Susceptibility to Pyrethroid (Bioallethrin) Exposure at Adult Age - Changes in Cholinergic Muscarinic Receptor and Behavioural Variables.

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ABSTRACT: We have recently reported that DDT and the pyrethroid bioallethrin cause similar changes in the brain muscarinic cholinergic receptors (MAChR) and behavioural distrubances in the neonatal and adult mouse when given to neonatal mice during the peak of rapid brain growth. In the present study the interaction between neonatal and adult exposure to DDT and bioallethrin, respectively, is explored. Ten-day-old NMRI mice received a single low oral dose of DDT (0,5 mg/kg body wt). At adult age (5 months) the mice received bioallethrin 0,7 mg/kg body wt/day per os for 7 days. Mice used as controls received 20 % fat emulsion vehicle. The spontanous behavioural tests revealed significant differences, both in mice treated neonatally with DDT and receiving bioallethrin as adults and in mice receiving the vehicle as neonates and bioallethrin as adults, compared with their corresponding controls. However, the behavioural changes developed in mutually opposite directions. Significant changes in MAChR, assayed in the P2 fraction of the cerebral cortex by using the muscarinic antagonist,quanuclidinyl, benzilate ([H-3]QND) and agonist carbochol, was only observed in animals receiving DDT as neonates and bioallethrin as adults. The present study indicates an increased susceptibility in the cholinergic muscarinic receptors and a different behaviour reaction in animals already exposed to DDT (at a physiologically relevant dose), when again exposed to a similar neurotoxic agent as adults.