

The reproductive health effects of long term DDT
exposure on malaria vector control workers in
Northern Province, South Africa.

Mohamed Aqiel Dalvie

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The reproductive health effects of long term DDT exposure on malaria
vector control workers in Northern Province, South Africa.

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Bsc (Physiology, Chemistry); BSc Med Hons (Sportscience),

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DECLARATION

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MA Dalvie

ABSTRACT

Name: Mohamed Aqiel Dalvie

Date: 2 September 2002

Title: The reproductive health effects of long term DDT exposure on malaria vector control workers in Limpopo Province, South Africa.

Summary: Metabolites of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane), used in many developing countries including South Africa for the control of malaria vectors, have been shown to be endocrine disruptors *in vitro* and *in vivo*. The study hypothesis was that male vector control workers highly exposed to DDT in the past should demonstrate clinically significant exposure-related anti-androgenic and/or estrogenic effects reflected in abnormalities in hormone levels, semen, sexual function and fertility.

A cross-sectional study of 60 workers from 3 camps situated near the Malaria Control Center (MCC) in Tzaneen was performed. Tests included a questionnaire, a physical examination, semen analysis (produced via coitus interruptus or masturbation) and blood sampling before and after a gonadotropin releasing hormone (GnRH) challenge (100 µg). Semen count, density and motility using the World Health Organisation's criteria, and morphology using the Tygerberg criteria, were determined. Serum o'p' & p'p' isomers of DDE, DDT and DDD, and basal and post GnRH challenge hormone levels including luteinizing hormone, follicle stimulating hormone, testosterone, sex hormone binding globulin, estradiol (E2) and inhibin were measured.

Forty-eight (81.0%) out of 60 participants produced a semen sample, and 31 (51.8%) completed all tests in full. The mean number of years worked at the MCC was 15.8 ± 7.8 years & mean serum DDT, 94.3 ± 57.1 µg/g of lipid. Mean baseline E2 (mean = 62.4 ± 29.9 pg/ml) levels exceeded the normal reference range.

Associations between DDT exposure measures (years worked at MCC and DDT metabolites) and reproductive outcomes were weak and inconsistent. The most important finding was a positive relationship of baseline E2 and testosterone with DDT metabolites, especially with p'p' DDT and DDD. The strongest association was a linear regression relationship between baseline estradiol and p'p' DDT ($\hat{\beta} = 1.14 \pm 0.33$ pg/ml/µg/g lipid, $p = 0.001$, $R^2 = 0.31$, $n = 46$; adjusted for age and SHBG).

An overall anti-androgenic mechanism best explains the results, but with a number of inconsistencies. Associations might be due to chance as multiple comparisons were made ($n = 175$).

The results therefore suggest that DDT exposure experienced by male malaria vector control workers does not result in clinically or subclinically meaningful adverse reproductive effects.

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GLOSSARY

Acronyms and terms

TERM	DEFINITION
Androgen Index	Index used to indicate unbound testosterone (basal testosterone (nmol/L) X 100/basal SHBG (nmol/L))
Androgen	A hormone involved in developing and maintaining certain male sexual characteristics. It binds to a receptor, termed the androgen receptor (AR)
Anti-androgenic compound	A substance, which prevent binding of true androgens to the androgen receptor (AR).
A priori predictor	A known or obvious predictor
Arylhydrocarbon (Ah) receptor	A common intracellular cytosolic protein involved in gene regulation
Crude /Adjusted association	Bivariate association
DBCP	Dibromochloropropane
Cryptorchidism	Maldescent of the testis
DDD	1,1 dichoro-2,2-bis(p-chlorophenyl)ethane. DDT metabolite consisting of two isomers, p'p'DDD and o'p'DDD
DDE	Dichorodiphenyldichloro dichloroethylene. DDT metabolite consisting of two isomers, p'p'DDE and o'p'DDE.
DDT	1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane. Parent compound. In the text, the term is generally used when referring to one of the DDT metabolites or the sum of all 6 metabolites. Also used to indicate the sum of o'p'DDT and p'p'DDT in which case the term "total DDT " is used for the sum of all six metabolites.
DES	Diethylstilbestrol
DWAF	Department of Water Affairs and Forestry, South Africa
Fecundibility	The monthly probability of conception in the absence

	of contraception outside the gestation period among couples attempting pregnancy
E2	Estradiol
Endocrine system	A major regulatory system in humans and many animals.
Estrogens	Any substance that is involved in producing or maintaining the secondary female sex characteristics in a mammal. These substances binds to the estrogen receptor (ER).
Estrogenic compound	A substance which binds to the ER thereby mimicking natural estrogens.
EPA	Environmental Protection Agency
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
Hydrocele	A testicular tumour with a collection of serous fluid
Hypospadias	Abnormal positioning of the meatus, the opening from which urine passes
LH	Luteinizing hormone
Lipid corrected blood DDT levels	Serum DDT levels corrected for serum lipid
Organochlorine	Chemicals that contain carbon and chlorine, e.g. DDT
SHBG	Sex hormone binding globulin
Stockholm Convention	A treaty to ban 12 persistent organic pollutants signed in 2002 by over 120 countries
Teratogenic	Causes malformed fetuses
Varicocele	Dilatation of the spermatic veins
Vasectomy	Ligation of one or (more commonly) both vasa <i>deferentia</i>
WHO	World Health Organisation
WRC	Water Research Commision, South Africa (part of DWAF)

Statistical symbols and abbreviations

SYMBOL/ABBREVIATION	DEFINITION
$\hat{\beta}$	Linear regression coefficient
95%CI	95 percent confidence interval
n	Sample size, number of observations
r	Correlation coefficient
R ²	Adjusted R ² , variance explained in multivariate model after adjusting for number of variables

Metric unit symbols and abbreviations

SYMBOL/ABBREVIATION	DEFINITION
bpm	Beats per minute
cm	Centimetre
Kg	Kilogram
miu	Micro (10 ⁻⁶) international unit
µg/L	Micro (10 ⁻⁶) gram per litre
µg/g lipid	Micro (10 ⁻⁶) gram per gram of lipid
nmol/ml	Nano (10 ⁻⁹)mole per millilitre
pg	Pico ((10 ⁻¹²)gram
rpm	Revolutions per minute

CHAPTER 1 ENDOCRINE DISRUPTION AND THE MALE REPRODUCTIVE SYSTEM

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Chapter 1: Endocrine disruption and the male reproductive system

1.1 Introduction

There is evidence that the male reproductive system has deteriorated in the last 60-70 years. The most compelling evidence is a systematic meta-analysis of 61 international studies by Carlsen et al., in 1992, that showed a significant decrease in sperm concentration by about 40% and semen volume by 20% over the period 1938-1990 [Carlsen et al., 1992; Keiding et al., 1994; Carlson et al., 1994], although the decline might have stabilised by 1970. Subsequent studies [Auger et al., 1995; Irvine et al., 1996; Van Waeleghem KD et al., 1996; Vierula et al., 1996; WHO Task Force, 1996; Bonde and Storgaard, 2002] have confirmed the decline in semen quality in different regions of the United States, within and among European countries, and developing countries, although it might not be universal. During the same period, there was an increase of 2-4% in the incidence of testicular cancer worldwide as well as substantial increases in the incidences of cryptorchidism (maldevelopment of the testis) and hypospadias in many countries [Toppari, 1996].

The deterioration in male reproductive health has largely been attributed to natural and synthetic chemicals present in the environment, reported to have caused similar effects in wildlife, laboratory animals and humans [Toppari et al., 1996; Crisp et al., 1998]. These chemicals are termed "endocrine disruptors" because of their ability to mimic natural hormones, thereby, disrupting physiological processes. Consequently, a plausible hypothesis that man-made chemicals act as endocrine

disruptors altering development of the male reproductive tract and reducing reproductive health has been made.

1.2 Endocrine/Hormone Disruptors

The concern about endocrine disruption started more than 30 years ago when it was shown in-vitro that certain environmental chemicals such as DDT and polychlorinated biphenyls, can behave like natural estrogens [Bitman et al., 1970; Nelson et al., 1978; McLachlan et al., 1985; Hertz, 1985; Richardson et al., 1985]. They were termed xeno-estrogens. Within the past 10 years this concern has become focused and intensified [Kavlock et al., 1996; Ankley et al., 1997; Colborn et al., 1992; Colborn et al., 1993; Purdom et al., 1994; Rolland et al., 1995]. The potential risks these chemicals may pose for human health and ecological well being (breast and reproductive tract cancers, reduced male fertility, abnormal sexual development, etc.) has been called to attention. [Colborn et al., 1993; Colborn et al., 1996; Birnbaum, 1994; Davis et al., 1993; Kelce et al., 1994; Makela et al., 1994; Sharp et al., 1993; Wolff et al., 1993; Davis et al., 1995].

Originally, these chemicals were known as estrogen mimics or estrogenic chemicals because concern was based almost entirely on perceived effects on the reproductive system which were thought to occur via the estrogen receptor (ER) . Later, it was recognised that these chemicals could effect other elements of the endocrine system, including thyroid, thymic and pituitary hormones and were consequently renamed “endocrine disruptors”. More recently, the term “hormonally active agents” has emerged.

Endocrine disruptors are usually either natural products or synthetic chemicals that mimic, enhance (an agonist), or inhibit (an antagonist) the action of hormones [Crisp et al., 1998], but there are a number of formal definitions in use. The US Environmental Protection Agency (EPA) Risk Assessment Forum's definition which is comprehensive, defines an environmental endocrine or hormone disruptor as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour [Crisp et al., 1998] .

The USEPA further states that impaired hormonal control could occur as a consequence of the following:

- a) *Altered hormone synthesis* which involves the inhibition of steroid biosynthesis for e.g. the inhibition of estrogen biosynthesis through aromatase inhibition by the fungicide fenarimol and the effect on the activity of LH and FSH by environmental estrogens or anti-androgens by disruption of protein hormone synthesis.
- b) *Altered hormone storage and/or release*, for example the inhibition of testosterone release on blockage of the LH receptor or inhibition of the cyclic AMP dependent cascade involved in testosterone synthesis.

- c) *Altered hormone transport and clearance* involving the regulation of blood hormone binding globulin levels which can influence blood hormone levels. An example of this is the estrogen induced increase in testosterone-estrogen binding globulin (TEBG) and androgen induced decrease in TEBG. Certain endocrine disruptors can also alter liver enzymes involved in hormone clearance.
- d) *Altered hormone receptor recognition/binding*, which concerns the alteration of hormone binding to intracellular or membrane-bound receptors by either mimicking the hormone thereby acting as an agonist or by inhibiting binding thereby acting as an antagonist. Examples of these are compounds known to be estrogenic or anti-androgenic, discussed later. Many chemicals classified as environmental estrogens can actually inhibit binding to more than one type of intracellular receptor e.g. DDT and chlordecone can inhibit binding to estrogen and progesterone receptors.
- e) *Altered hormone postreceptor activation* which involves the alteration of cellular responses after activation of hormone receptors e.g. a number of environmental compounds can interfere with a membrane's second messenger systems.

1.3 Endocrine disrupting substances

The compounds which are known as endocrine disruptors in the scientific literature were recognized by means of laboratory tests on animals or cell cultures. They include natural or synthetic hormones found in medicines, phyto-estrogens (plant estrogens), growth stimulants used in livestock farming or synthetic chemicals (other than hormones) such as some pesticides, detergent components and breakdown products, monomers and additives used in the plastics industry, organometals and persistent environmental contaminants from the past [Health Council of the Netherlands, 1997].

The Dutch Health Council Committee [Health Council of the Netherlands, 1997] has compiled a comprehensive list of endocrine disrupting substances and grouped them as follows:

- Pesticides such as p'p'-DDT, p'p'-DDE and o'p'-DDT, chlordecone, beta-hexachlorocyclohexane, dibromochloropropane (DBCP), endosulfan, dieldrin, lindane (gamma-hexachlorocyclohexane), chlorotriazines (atrazine, simazine), vinclozolin, mirex, methoxychlor and toxaphene
- polychlorinated biphenyls (PCBs)
- dioxins including polychlorinated dibenzo-para-dioxins (PCDD) such as TCDD and polychlorinated dibenzofurans (PCDF)
- alkylphenol polyethoxylates (APES) and decomposition products, such as octylphenol and nonylphenol
- bisphenol A

- phthalates, such as diethylhexyl phthalate (DEHP), dibutyl phthalate and butyl benzyl phthalate
- polycyclic aromatic hydrocarbons (PAHs), such as dimethyl benzanthracene and benzo[a]pyrene
- phyto-estrogens, such as coumestrol, zearalenone, isoflavones (including genistein) and lignans
- pharmaceuticals, such as diethylstilbestrol (DES), ethinyl estradiol (in contraceptives) and muscle builders (anabolic steroids).

1.4 The endocrine system

The endocrine system is a major regulatory system in humans and many animals. It is an intricate system of organs (glands) that produce and secrete hormones into the circulation to regulate physiological processes such growth, metabolism and reproduction.

Hormones include glycoproteins, polypeptides, peptides, steroids, modified amino acids, catecholamines, prostaglandins, and retinoic acid. They travel via the bloodstream at very low concentrations (ng or pg/ml), unbound or bound to carrier proteins, exerting effects on tissues or organs. Hormones exert their effects by binding to specific cell surfaces or nuclear receptors. Steroid and thyroid hormones bind to receptors to regulate gene activity (expression) as DNA transcription factors. Protein and peptide hormones function by transmitting an intracellular signal (second messenger) to regulate ion channels or enzymes. Major endocrine organs include the hypothalamus, pituitary, thyroid, parathyroid, pancreas, adrenal,

ovary and testis. Other endocrine tissues include the placenta, liver, kidney, and the gastrointestinal tract. Target organs and tissues include the mammary glands, bone, muscle, the nervous system, and the male and female reproductive organs.

Hormones are also found in invertebrates (e.g. ecdysone) and plants (e.g. auxins). Environmental endocrine disruptors therefore have the potential to influence human health and ecology.

1.5 Male reproductive system

The male reproductive system in mammals requires androgens, which are hormones that bind to androgen receptors (AR), located in the hypothalamus, pituitary, testes and prostate, for normal development. The most important androgen is testosterone, produced by the testis. Other androgens, produced mainly by the adrenal cortex and adrenal glands in small quantities, support testosterone function.

Without injections of testosterone, males that have been castrated prior to adolescence and sexual maturity, do not develop functioning adult reproductive organs [Lavin, 1986]. Androgens given to normal males tend to increase the size of the reproductive organs; castration performed on males that have already reached maturity causes the testis to shrink and to stop functioning [Lavin, 1986].

Androgens are also necessary for the formation of sperm cells and for the maintenance of sexual interest and behaviour.

Other effects of androgens upon the male body are diversified. The growth of pubic hair and of facial and chest hair and the regression of scalp hair, or baldness, are influenced by androgens. During adolescence, androgens lengthen and thicken the male vocal cords, causing voice deepening; they also enhance bone growth and increase the number and thickness of muscle fibres in the male body. Other growth patterns that androgens stimulate are kidney weight and size, the increase of protein in bone tissue, the regeneration of red blood cells, the presence of pigments in the skin, and the increased activity of sweat and sebaceous (oil-producing) glands.

Figure 1.1 shows the organisation and control of the male reproductive system by the hypothalamus-pituitary-gonadal axis. The two main activities of the testes are steroidogenesis and spermatogenesis with androgen biosynthesis occurring in the Leydig cells and spermatogenesis in the seminiferous tubules. These functions are intimately related and are regulated by the pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH).

The decapeptide, gonadotropin releasing hormone (GnRH) is secreted from the hypothalamus in a pulsatile fashion and stimulates the release of both FSH and LH. GnRH and the gonadotropins are in turn affected by feedback of the steroid hormones, testosterone and estradiol at both the anterior pituitary and hypothalamic level. The gonadal peptides inhibin and activin also feed back at the pituitary level to predominantly affect FSH secretion. The Leydig cells are located in the connective tissue stroma of the testes between the seminiferous tubules.

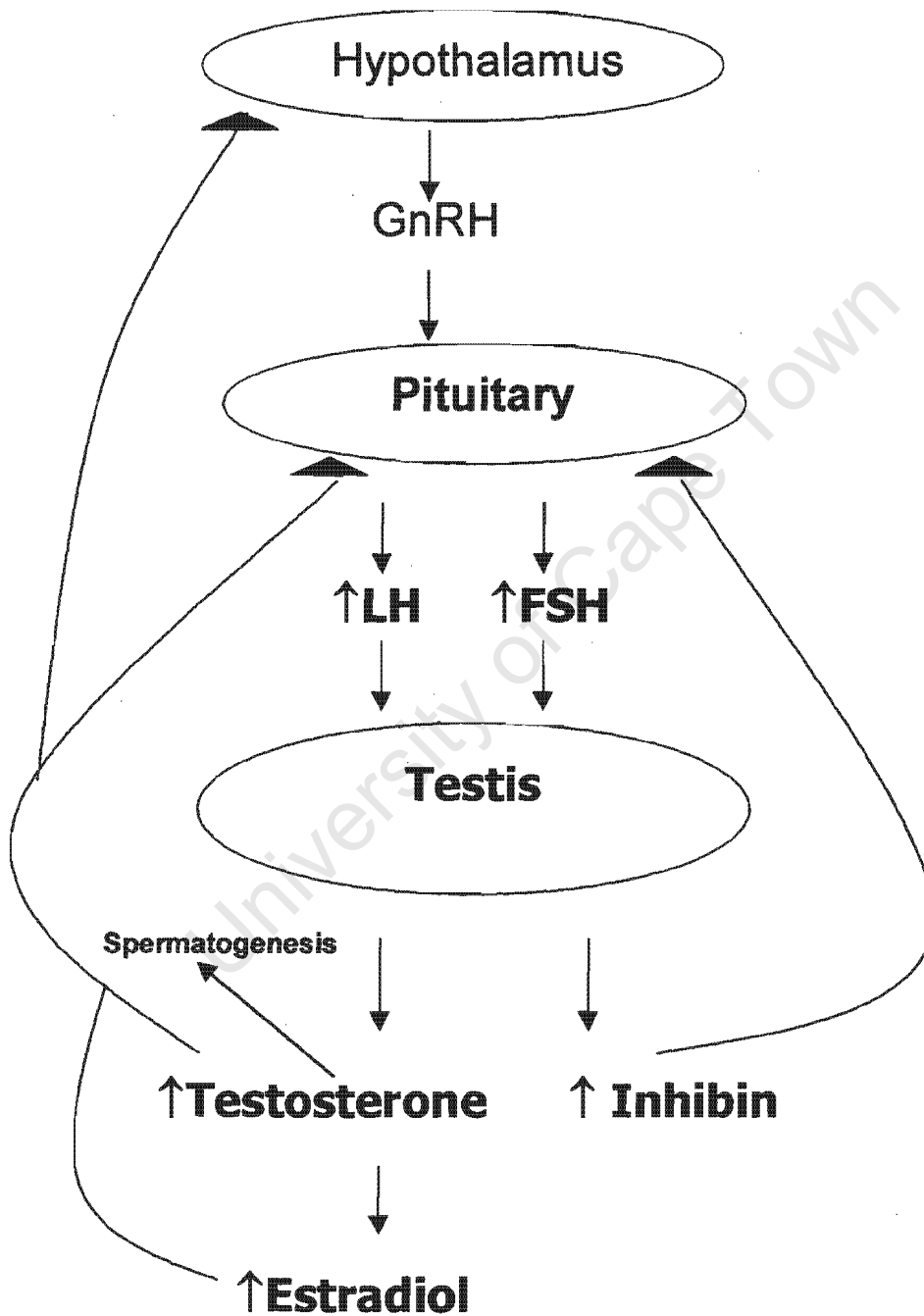
Testosterone biosynthesis takes place in the Leydig cells under LH stimulation. Testosterone in turn has negative feedback action on both the hypothalamus and anterior pituitary. The majority of the circulating testosterone concentration in the serum of normal men originates from the Leydig cells. A percentage of this is converted by 5-alpha-reductase to the potent androgen dihydrotestosterone and some is aromatised peripherally and at target tissues to estrogens.

Spermatogonia (germ cells) in the seminiferous tubules give rise to spermatozoa through a number of stages in the process of spermatogenesis. Approximately 74 days are required for the conversion of the undifferentiated spermatogonia into the spermatozoa and therefore any insult affecting this process may well only become evident some months later [Lavin, 1986]. The Sertoli cells are arranged closely to the spermatogonia and play an important role in their maturation as well as being responsible for the maintenance of the blood-testis barrier and production of estrogens [Lavin, 1986]. Both FSH and testosterone are required for the initiation of spermatogenesis but once normal germinal epithelium is established, testosterone alone can maintain sperm production. Should, however, the epithelium of the seminiferous tubules regress for whatever reason, then reinitiation of spermatogenesis will require both testosterone and FSH stimulation. LH is therefore important for spermatogenesis, mainly through its effect on testosterone production. FSH secretion in turn is inhibited by inhibin secreted from the testes. There is a complex interplay between Leydig cells, Sertoli cells and spermatogonia. Testosterone is therefore a good indicator of Leydig cell function while inhibin,

which is produced by the Sertoli cells, will be decreased if dysfunction of these cells occurs.

Both testosterone and estradiol (E2) are bound to sex hormone binding globulin (SHBG) in the serum and bound steroids are not biologically active. It is therefore important to measure sex hormone binding globulin as well as testosterone and estradiol concentrations when determining hormonal levels as this allows the calculation of the amount of free hormone and therefore the biologically active hormone in serum. Androgens suppress and estrogens increase serum SHBG levels.

Figure 1.1 The hypothalamus-pituitary-gonadal axis in the male reproductive system



1.6 Health effects due endocrine disruptors

Evidence on the health effects of endocrine disrupting chemicals has accumulated in wild and aquatic life, laboratory animals and humans. These include reproductive and developmental effects, cancers (including breast, testicular, prostate, pancreas and liver), CNS effects, thyroid effects, and immunological effects [Crisp et al., 1998].

Most of the health effects due to endocrine disruption which were observed in wildlife and measured in laboratory animals and humans are on the male and female reproductive systems. In wildlife such as fish, birds, gastropods, reptiles and mammals, fertility, demasculinization and feminisation effects have been found [Crisp et al., 1998; Topari et al., 1996].

Effects on the female reproductive system linked to endocrine disruptors, include endometriosis, vaginal, endometrial and breast cancer, structural reproductive tract abnormalities (oviducts and uterus), reproductive dysfunction, and the occurrence of nonneoplastic lesions such as parovarian cysts [Crisp et al., 1998; Health Council of the Netherlands, 1997; Key et al, 1994].

The following section describes the literature on the effect of endocrine disruptors on the male reproductive system.

1.6.1 Endocrine disruptors and the male reproductive system

The male reproductive system can potentially be disrupted at many levels as it involves the hypothalamus and pituitary as well as the testes [Topari et al., 1996]. Chemicals with the ability to affect testosterone production have been identified as particularly important. Although the adult male reproductive system can be affected adversely by disruption of the endocrine balance, the developing male reproductive system pre-and postnatally appears to be particularly susceptible and sensitive.

Endocrine disruption of the male reproductive system in wildlife involves feminisation, demasculinisation, reduced fertility, reduced hatchability, reduced viability of offspring, impaired hormone secretion or activity, and altered sexual behaviour [Topari, et al., 1996].

Three types of male reproductive endocrine disruptors have been identified in the literature [Crisp et al., 1998]. These include estrogens, which interact with estrogen receptors (ER), anti-androgens, which prevent binding of true androgens to the androgen receptor (AR) and those that interact with the aryl hydrocarbon (Ah) receptor, which is a common intracellular cytosolic protein involved in gene regulation. ER's, AR's and Ah receptors are located in the brain, pituitary and periphery. Table 1,1 summarises studies which have investigated male reproductive effects of endocrine disrupting chemicals, based on the reports by the USA's Committee on Hormonally Active Agents in the Environment, [1999], The Health Council of the Netherlands [1997] and other studies in the literature.

Table 1.1 The effects of endocrine disruptors on the male reproductive system

Chemical	Species	Author	Year	Effect
Anti-androgenic effects in humans				
DDE	Man	Bush	1986	No effect on semen quality
Vinclozolin	Men	Zober et al.	1995	No effect on hormonal levels (LH, FSH and testosterone)
p'p' DDE	Man	Cocco	1997	No effect on birth rate, semen count
DDE	Man	Ayotte et al. ¹	2001	Disruption in endocrine function, Reduction in semen quality
DDE	Men	Martin et al.	2002	No effect on androgen levels (testosterone, hydrotestosterone)
Anti-androgenic effects in laboratory animals				
Fenarimol	Rat	Hirsch et al.	1987	Infertility
Vinclozolin (fungicide)	Rat	Gray et al.	1994	Sex differentiation, reproductive tract abnormalities, reduced sperm production
p'p'DDE, DES	Rat	Kelce et al. Kelce et al.	1995 1998	Reproductive tract abnormalities, reduced sperm production
Estrogenic effects in wildlife				
PCBs, alkylphenols, bisphenol-A, TCDD, TCDF, pesticides	Birds, fish, mammals	Crisp et al.	1996	Demasculinisation, reduced fertility
Alkylphenolic chemicals	Rainbow	Jobling et al.	1996	Decrease in testicular growth,

¹ Reported in a letter to a journal lacking detail, but further information was obtained upon enquiry.

	trout			reduced spermatogenesis
DDT, mercury, polychlorinated biphenyls	Panthers	Facemire et al.	1995	Cryptorchidism, reduced spermatogenesis
Estrogenic effects in laboratory animals				
PCB	Rat	Sager	1996	Disruption of fertility hormone levels
PCB (in utero)	Rat	Bouwman et al.	1995	Reduction in sperm concentration, no morphological abnormalities
Octyphenol, octyphenol phenoxyate, butyl benzyl phthalate, DES	Rat	Sharp et al..	1995	Reduction in testicular weight and sperm production
Polyhalogenated aromatic hydrocarbons	Rat/ Hamster	Brouwer et al.	1995	Reduction in sperm production
Dioxins (in utero)	Rat	Mably et al.	1992	Reduction in production of spermatozoa, no effect on sperm morphology or motility
DES	Rat/ mouse	Stillman Linn et al.	1982 1988	Reduction in sperm concentration and motility
DES	Rat	Cooper et al.	1986	Pituitary hormone secretion
Chlordecone and methoxychlor	Rat	Bulger et al.	1985	Reduced fertility
Tamoxifen	Rat	Döhler et al.	1984	Demasculinisation

Estrogenic effects in humans				
DES (in utero)	Man	Palmlund	1996	Reduction of sperm concentration, motility and morphology, increase in testicular cancer
DES (in utero)	Man	Willcox et al.	1995	No evidence of reduced fertility
TCDD	Man	Egeland et al.	1994	Disturbed hormone levels
DDT	Man	Smith	1991	No effect on fertility
DDT	Man	Stachel et al.	1989	No effect on semen density
TCDD	Man	DeStefano et al.	1989	Reduction in sperm concentration and morphology
DES	Man	Bullock et al.	1988	Reproductive tract abnormalities
PCBs	Man	Bush et al.	1986	Reduction in sperm motility and morphology
Chlordecone	Man	Guzelian	1982	Reduction in sperm motility and morphology
DDT	Man	Dougherty et al.	1980	Reduction in semen density
DBCP	Man	Egnatz et al. Olsen et al.	1980 1990	Reduction in sperm concentration and morphology, disturbed hormone levels
β -hexachloro-cyclohexanes	Man	Torniczak et al.	1981	Disturbed hormone levels
DES (in utero)	Man	Gill et al.	1979	Reduction of sperm concentration, motility and morphology, genital malformations and microphallus
Chlordecone	Man	Cannon et al.	1978	Oligospermia

Phthalates	Man	Aldyreva et al.	1975	Disturbed hormone levels
Effects through the Ah-receptor in laboratory animals				
Dioxin (TCDD)	Rat	Gray et al.	1995	Genital malformations impaired spermatogenesis feminisation, decreased fertility
Dioxin	Rat	Whitlock	1994	Decreased fertility
Dioxin (TCDD)	Rodent	Peterson et al.	1993	Genital malformations impaired spermatogenesis feminisation

Initially it was thought that endocrine disruption occurred via estrogenic mechanisms because of the estrogenic properties of suspected compounds including octyphenol, octyphenol phenoxylate, butyl benzyl phthalate methoxychlor, DES, o,p'-DDT, PCB and dioxin (Table 1.1). Effects seen in test-animals include reduction in testicular weight, sperm abnormalities, reproductive tract abnormalities, hypothalamic effects and effects on the gonadotrophins (Table 1.1). In humans, a double-blind clinical trial by Dieckmann and co-workers on 1646 pregnant women in 1953 [Dieckmann et al., 1953] to test the effectiveness of diethylstilbestrol (DES), prescribed as a contraceptive from late 1940 to early 1970, yielded important results in follow-ups of the sons of these women. The follow-up studies found that men exposed to DES in utero, had reproductive defects such as sperm abnormalities, reproductive tract abnormalities including cryptorchidism and hypospadias, genital malformations, microphallus and testicular cancer [Gill et al., 1977; Garci-Rodriguez et al., 1996; Palmlund, 1996], (Table 1.1). Sperm abnormalities were also seen in men exposed to chlordecone, DDT, DBCP, TCDD and PCBs (Table 1.1), with reduction in testis size/weight in men exposed to dioxins

[Roegner,1991]. Men occupationally exposed to chlordecone were found to be oligospermic (Table 1,1). A sex ratio change was noted after a dioxin accident and contamination of food sources in Seveso [Mocerally,1996]. Disturbances in reproductive hormones were also seen in a number of studies where men were occupationally exposed to endocrine disrupting chemicals including phthalates, DBCP, hexachloro-cyclohexanes and TCDD. [Aldyrevva et al., 1975; Olsen et al., 1990; Tomczak et al., 1981; Egeland et al., 1994]

Recently, it has been shown in rodents that exposure to chemicals such as estradiol and DES [Kelce et al., 1995; Kelce et al., 1998], which are highly estrogenic, reduce androgen levels or interfere with androgen action during development causing male reproductive abnormalities such as reduced sperm production capability and reproductive tract defects. These chemicals can bind to the AR without activating it, thereby preventing the binding of true androgens, and are called anti-androgens. Other examples of anti-androgens are the pharmaceutical hydroxyflutamide [Crisp,1998], and the pesticides procymidone [Hosakawa, 1993], vinclozolin [Gray et al.,1994], fenarimol [Hirsch, 1987] and the DDT metabolite p,p'-DDE [Kelce et al., 1995; Kelce et al., 1998]. Effects by anti-androgens on adult and pubertal male rats include infertility and effects on sex differentiation, sexual development and reproductive organ development. Effects observed in male offsprings of pregnant rats exposed to vinclozolin include reduction of the anogenital distance to that characteristic of females, impaired penis development, existence of vaginal pouches, prostate gland agenesis, delayed preputial separation, and reduced or absent sperm production [Gray et al., 1994]. One study (Ayotte et al, 2001) found an effect on the endocrine function and semen

quality of DDE exposed men, but no effects on reproductive hormones, semen quality or birth rate were, observed in men exposed to vinclozolin [Zober,1995] or DDE [Cocco, 1997; Martin et al.,2002]. It would appear that the reproductive effects of anti-androgenic chemicals are similar to those of estrogenic chemicals. Therefore, it is possible that the mechanism by which endocrine disrupting chemicals impair male reproduction, thought to be estrogenic, may be due to their anti-androgenic properties rather than, or in addition to, their property to activate the ER.

Halogenated aromatic hydrocarbons such as dioxin bind to the Ah receptor. Dioxin appears to impair testosterone synthesis and might also impair CNS sexual differentiation thereby causing genital malformations, impaired spermatogenesis, feminisation of male sexual behaviour, and decreased fertility in rodents (Table 1.1).

Table 1.1 also indicates that bio-accumulative organochlorine insecticides, such as DDT, chlordecone and methoxychlor, have been widely implicated as endocrine disruptors [Skakkebaek and Keiding, 1994; Smith, 1991]. Amongst these organochlorine insecticides, DDT metabolites and their isomers are of great interest as possible models for endocrine-disrupting chemicals as will be discussed in the next chapter.

1.7 Concern about endocrine disruption

Concern about the health effects of endocrine disruption have caught the attention of governments, internationally. The U.S. EPA has established a federal advisory working group composed of representatives from environmental groups, industry, academia, and government, called the Endocrine Disruptor Screening and Testing Advisory Committee in 1998 to develop a screening and testing strategy for new and existing chemicals that may act as endocrine disruptors. Also, in 1995, a Committee on Hormonally Active Agents in the Environment was formed to compile a report evaluating the scientific evidence on the effects of endocrine disruption to help policy makers in the USA [Committee on Hormonally Active Agents in the Environment, 1999]. The Health Council of the Netherlands in 1997 also formed a committee that investigated hormone disruptors and their impact on human development. Both reports concluded that although the available evidence does not suggest that these chemicals present acute dangers to human health, there might be subtle chronic effects which need investigation. In South Africa, the Water Research Commission (WRC) has formed an Endocrine Disruptor Group (EDG) comprising of industry, academia and government in 1999, concerned with water research and monitoring of endocrine disrupting chemicals. The group falls under the chemical thrust of health related water research by the WRC. In 2000, the EDC decided on a five-year programme aiming to review international and local literature on endocrine disruptors, research and establish methods of measuring endocrine activities in water, survey health effects and compiling a national database as an inventory of technical and incidence data for researchers. There is, however, no such committee concerned with non-water related issues in South Africa.

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Chapter 2: A review of DDT and a description of the study objectives

2.1 Introduction

DDT, introduced as an insecticide in the 1930's [Tren, 2001; Smith, 1991], was actually first synthesised in 1874. DDT became well known for its dramatic control of malaria and typhus during the Second World War and for its effectiveness in agriculture during 1945-1975. During this period, DDT was used extensively in agriculture, with an estimated worldwide production as high as 60 000 – 70 000 tonnes in 1974 [Smith, 1991; WHO 1979].

During the 1960's it became apparent that DDT persisted in the environment and accumulated in the food chain. [Foran et al., 1989; Atuma, 1985; Evans et al., 1991]. Adverse effects were found especially on wild and aquatic life reproduction including demasculinisation and feminisation of males, and effects on human health were suspected due to toxic effects, including reproductive, cancerous, genotoxic and teratogenic outcomes, shown in the laboratory [Crisp et al., 1998; WHO, 2001]. Many developed countries banned DDT, Sweden being the first in 1969. Global DDT usage dropped. Presently, DDT is banned in most developed countries, but substantial levels of DDT metabolites are still detectable in environmentally exposed citizens [Hunter et al., 1997; Stellman et al., 1998].

DDT is still used in many developing countries for malaria control. There was recently a major public health debate [Roberts, 2000; Smith, 2000; Editorial in Lancet, 2000] amongst more than 120 countries about DDT's inclusion in a treaty (The Stockholm Convention) [United Nations Environment Programme Chemicals (UNEP), 2001] to ban 12 persistent organic pollutants (POPS). The argument was whether DDT should be completely banned or continued as a cheap and effective method for malaria vector control in developing countries. Due to the resurgence of malaria in many developing countries, and the cost and failure of alternative methods, it was decided that DDT use for malaria vector control should be continued.

2.2 Chemical and physical properties

DDT, 1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane is an organochlorine insecticide available in different forms including aerosols, dustable powders, emulsifiable concentrates, granules and wettable powders [WHO, 1979]. Technical DDT normally consists of 77% p'p'-DDT, 15 % o'p'-DDT (15%), 4% p'p' DDE and less than 1 % o'p'-DDD, p'p' DDD and o'p' DDE, all of which are white, crystalline, tasteless, and almost odourless solids [WHO, 1979].

DDT is a chemically stable compound and is highly soluble in lipids (fats and oils) and almost insoluble in water. For this reason it accumulates and persists in the environment (it is resistant to enzymatic breakdown by soil and higher organism)

[WHO,1979]. Some DDT may be degraded in air. The environmental half-life of DDT is approximately 15 years [Exonet, 2001].

2.3 Exposure routes and metabolism

Although DDT is absorbed slowly through the gastro-intestinal tract and poorly via skin and the respiratory system, entry into the body does occur both in occupational and non-occupational settings [WHO, 1979].

Due to its persistence in the environment, DDT residues appear in food and water sources even years after use and this is the most important route of entry into the body in a non-occupational setting. Daily intakes of 0.184 mg/man and DDT levels in the order of 20 mg/kg has previously been recorded in general populations [WHO,1979].

High occupational exposures to DDT occurs amongst formulation, agricultural and malaria vector control workers, and levels in these populations are usually above 100 mg/kg [WHO,1979]. Although DDT is poorly absorbed through the skin, dermal entry usually occurs in an occupational setting from unclothed parts of the skin such as hands, arms, chest, neck and face because workers are frequently in direct contact with DDT for extended periods though handling, mixing and spraying of DDT. Dermal exposure is increased by the fact DDT tends to remain on the hands of workers, days to years after use, particularly in a setting where personal protective

clothing is not used. Small amounts of DDT may also be inhaled and pass through the lungs into the body. Because inhaled DDT particles are generally too large to pass through the lungs into the body, they are more likely coughed-up and ingested.

Once inside the body DDT metabolites, especially p'p' DDE, are stored most readily in fatty tissue due to their lipophilic nature [WHO,1979]. Stored amounts, leave the body very slowly due to slow metabolic degradation. Most p'p' DDT is converted to p'p' DDE [WHO,1979]. DDT metabolites are excreted primarily in the urine.

Levels in fatty tissues may either remain constant over time or can even increase with continued exposure. However, DDT in the body decreases with decreasing exposure [WHO,1979]. They leave the body primarily in urine. Breast milk is another way DDT metabolites may leave the body. The biological half-life of DDT and its dehydrohalogenated form DDE is approximately eight years [Exonet, 2001].

2.4 DDT levels in humans

DDT in humans has been measured in blood, semen, breast milk, urine, bile and adipose tissue [WHO,1979; Smith,1991]. Measurements in adipose tissue where DDT is stored eventually after entering the body can be regarded as the gold standard in representing human exposure, but requires invasive fat biopsy procedures. Blood levels have been shown to correlate well with that in fat ($r = 0.64$, Smith,1991), and

Table 2.1 provides a summary of DDT fat and blood levels measured in previous studies amongst different exposure groups, including control, environmentally exposed and occupationally exposed populations. Studies in which DDT blood levels were corrected for lipids as well as those in which blood DDT levels were not corrected for lipids, are presented in Table 2.1.

DDT levels in the adipose tissue of general populations are usually below 10 µg/g of fat [Smith et al., 1991], while in occupationally exposed workers they may be higher than 60 µg/g of fat [Rivero-Rodriguez et al., 1997; Smith et al, 1991]. Spraying of DDT for malaria vector control (DDT application rate 2 g/active /m²) is less than in agriculture, but it is unclear if the dose delivered to workers is lower. High levels in the order of 100 µg/g of fat have previously been measured in vector control workers [Rivero-Rodriguez et al., 1997]. It is interesting that levels amongst those exposed environmentally are actually similar to those exposed occupationally [Bouwman et al., 1991a; Bouwman et al., 1991b]

Table 2.1 Blood and adipose DDT levels measured in other studies

Author (year)	Study sample	DDT measurement	Average (range)
Uncorrected Bloods ($\mu\text{g/l}$)*			
Nhachi [1989]	40 seasonal sprayers, Zimbabwe	DDE	60
Nhachi [1990]	68 seasonal sprayers, Zimbabwe	DDE	10 (0-150)
Bouwman et al. (1991a)	23 sprayers, Natal, SA	DDT DDE DDD Total DDT	67.7 129.3 11 202
Bouwman et al. [1991b]	Inhabitants of DDT treated dwellings	Total DDT	140.9
	Controls, Natal	Total DDT	6.04
Longnecker [2001]	2380 DDT exposed women in 1959-1966, US	DDE	25 (3-178)
	General US population	DDE	< 15
Hunter et al. [1997]	Women, US	DDE	6.97
Corrected Bloods ($\mu\text{g/g lipid}$)*			
Ayotte et al. [2001]	24 Mexican men	p'p' DDE	77.9 (17-177.2)
Martin et al. [2002]	137 male farm workers	DDE	1.2
DDT in adipose tissue ($\mu\text{g/g}$)			
Rivero-Rodriguez et al. [1997]	40 Mexican sprayers	p'p' DDE	60.98 (9.57-298.42)
		p'p' DDT	31 (0.72-34.5)
		o'p' DDT	2.1 (0.07-29.74)
		p'p'DDD	0.95 (0-3.51)
		Total DDT	104.48 (10.56-665.56)
Smith et al. [1991]	Sprayers	Total DDT	100 -300
	General population	Total DDT	8
* Uncorrected bloods refers to DDT measurements in blood not corrected for lipid content, corrected bloods refers to DDT measurements in blood corrected for lipid content Note: in the table, the terms "DDT", "DDD" and "DDE" is used for the sum of the two respective metabolites and the term "Total DDT" for the sum of all six metabolites.			

2.5 Health effects of DDT

2.5.1 Acute Toxicity

DDT is a WHO class 2 pesticide with an oral LD50 of 500-2500 mg/kg in rats. The acceptable daily intake (ADI) of DDT is 0.02 mg/kg/day and the reference dose in water (RfD) is 0.0005 mg/kg/day [Exonet, 2001].

Acute toxicity occurs mainly orally in laboratory animals at doses of > 50 mg/kg including decreased thyroid function, liver function, central nervous defects and neurological effects [ATSDR, 1994; Van Ert, 1992].

In humans, doses of 280 mg/kg can result in nausea, diarrhoea, increased liver enzyme activity, irritation (of eyes, nose or throat), disturbed gait, malaise and excitability, tremors and convulsions [ATSDR, 1994; Van Ert, 1992].

Chronic toxicity at doses of 4 mg/kg has been shown on the nervous system, liver, kidneys and immune systems in experimental animals [ATSDR, 1994; WHO, 1979], however these effects have not been shown in humans.

2.5.2 The long-term health effects of DDT

Most of the long-term health effects linked to DDT are on the reproductive system, but non-reproductive effects have also been suspected.

NON-REPRODUCTIVE EFFECTS

Teratogenic and genotoxic effects have been shown in test-animals and genotoxic effects in humans [ATSDR, 1994].

DDT has been shown to increase tumour production in the liver, lung and adrenal glands of test animals [ATSDR, 1994]. Garabrant et al. [1992], studied persons involved in the manufacture of DDT and thus exposed to both DDT and chemical intermediaries, and reported significant and substantial associations with pancreatic cancer (risk ratio for ever exposed compared with never exposed = 4.8; 95% confidence interval = 1.3-17.6), rising to a risk ratio of 7.4 for those exposed on average for 4 years.

REPRODUCTIVE HEALTH EFFECTS

DDT metabolites have been shown to produce hormone-like responses in a number of *in vitro* and *in vivo* assay systems [Kelce et al., 1995]. The major stored metabolite of DDT, p'p'-DDE, is a potent inhibitor of androgen-induced AR transcriptional activity and androgen action in developing, pubertal and adult male rats [Kelce et al. 1995]. o'p'-DDT, was also found to be an inhibitor but at much

higher concentrations [Kelce et al., 1995]. o,p'-DDT, p,p'-DDD and to a lesser extent, o,p'-DDD display estrogenic activity in human breast cancer cell line (MCF-7) [Klotz et al. 1996]. o,p'-DDT is the most efficient isomer to bind ER, and p,p'-DDE is the most potent isomer to bind the AR [Kelce et al. 1995]. *In vivo* studies have further confirmed the differential anti-androgenic effects of these isomers. The male pups produced from p,p'-DDE-exposed Long-Evans hooded pregnant rats exhibited reduced anogenital distance at birth and retained thoracic nipples when examined on postnatal day 13, both of which are indicative of prenatal anti-androgenic activity. Moreover, exposure of male rats to p,p'-DDE until after puberty significantly delayed the onset of puberty (the day the prepuce separates from the penis). Finally, treatment of adult male rats with p,p'-DDE significantly reduced androgen-dependent seminal vesicle and ventral prostate weights relative to control rats, despite high serum testosterone levels. [Welch et al. 1969; Duby et al. 1971; Clement and Okey, 1972; Gellert et al. 1972].

Considered in sum, these studies performed using DDT metabolites, provide intriguing evidence that the AR, rather than the ER, is the site of hormonal action by these persistent environmental pollutants.

Reproductive effects have also been shown in wildlife. DDT has been shown to be responsible for developmental effects that indicate demasculinisation of male alligators and "super-feminisation" of females and has been shown to impair reproduction and cause eggshell thinning in birds [Crisp et al., 1998]. DDT has also been linked to cryptorchidism, sperm abnormalities and sterility in Florida panthers [Crisp et al., 1998].

In humans, reproductive health effects by DDT have been shown both in men and women.

Women

A recent study showed that serum levels of the major DDT metabolite DDE were associated with increased risk of premature delivery and small size for gestational age at birth [Longnecker, 2001]. Another recent study shows that maternal consumption of fish contaminated with DDT was associated with reduced fecundability (the monthly probability of conception in the absence of contraception outside the gestation period) among couples attempting pregnancy [Buck et al. 2000]. Maternal consumption of fish for 3-6 years was associated with a fecundability ratio (a ratio >1 indicates improved fecundability, whereas a ratio <1 indicates sub-fecundability when comparing 2 groups) of 0.75 (95% CI = 0.59-0.91). Another study, showed a significant association between maternal DDT blood levels and miscarriage, although the presence of other organochlorines in maternal blood were not controlled for [Wasserman et al., 1982]. Two other studies however, found no association between maternal DDT blood and miscarriage, nor with premature rupture of foetal membranes [Leoni et al., 1989; Ron et al., 1988]. Problems with breast-feeding were seen in women exposed to DDE [Rogan et al., 1987].

DDT has been suspected of causing breast cancer based on vitro studies [Payne et al., 2001], but so far only one epidemiological study on women eating DDT contaminated food, has shown an effect [Key et al., 1994]. No effect on breast cancer was found amongst North Vietnamese women who had raised serum DDT concentrations after exposure to anti-malarial sprays [Schechter, 1997], and no raised levels of DDE were found in breast cancer patients when compared to controls in another study [Hunter et al., 1997].

Recently, a possible relationship between exposure to p'p' DDE and sexual precocity has been suggested in a study of Belgium immigrants and children, but the study had a poor design, and the results are likely to be incorrect. [M. Krstevska-Konstantinova et al., 2001].

Men

Few epidemiological studies investigating health effects of DDT on the male reproductive system have been identified in the literature (Table 2.2) and these are limited and inconclusive [Dougherty et al., 1980; Bush et al., 1986; Stachel et al., 1989]. Most have been small in size, conducted among relatively low exposed populations in the U.S. and Europe, and, in part owing to these low exposures, likely to be confounded by other exposures or risk factors.

Table 2.2 Summary of epidemiological studies which have investigated the health effects of DDT on the male reproductive system

Author (year)	Study sample	Exposure measure	Outcome Measure	Relationship (statistical significance)
Dougherty et al. (1980)	US university students (n = 132)	Semen DDT	Sperm density	Negative (not reported)
Bush et al. [1986]	US men (n = 170)	Blood DDE	Sperm motility and count	None
Stachel et al. [1989]	German men (n = 89)	Semen DDT & DDE	Sperm density	None
Smith [1991]	U.S. DDT factory workers		Number of pregnancies fathered	None
Cocco [1997]	Ecological study of 27 Countries and 17 U.S. states	Tree bark p,p'DDT	Birth rate, Semen count	Negative (p > 0.05) Negative (p > 0.05)
Ayotte [2001]	Mexican men (n = 24)	Blood p,p'DDE	Free testosterone Serum SHBG Semen Volume Semen count	Negative (p < 0.05) Positive (p < 0.05) Negative (p < 0.05) Negative (p < 0.05)
Martin [2002]	North Carolina farmers (n=137)	Blood DDE	Free testosterone Total testosterone Hydrotestosterone	None (p > 0.05) Negative (p > 0.05) None (p > 0.05)

The only study that reported significant reproductive effects by DDT was that by Ayotte [2001] who measured endocrine levels as well as semen parameters in a group of non-occupationally exposed Mexican men with high DDT levels (although the letter published did not state that associations were statistically significant, this was confirmed upon enquiry). The other epidemiological studies investigating the relationship between DDT exposure and the male reproductive system had limitations such as low exposures, insensitive exposure measurements, limited outcome measurements and insensitive study design. These studies did not show any DDT effects on reproductive outcomes including hormone levels, semen parameters and fertility measures. Stachel et al. [1989] did not find an association between semen DDE or DDT and semen density. Dougherty et al. [Dougherty et al., 1980], found a – 0.52 correlation between semen organochlorines including DDT and density but did not report on the statistical significance of the relationship. Bush et al. [1986] who found that 47% of US men had detectable blood levels of p'p'DDE, did not find a negative relationship between the blood DDT and semen density or motility. In an ecological study of several countries and US states by Cocco [1997], a non-significant negative relationship was found between fertility outcomes measured as birth or pregnancy rate and 1987-1990 sperm counts and DDT exposure as represented by 1992-1995 tree bark DDE. In one study of DDT exposed factory workers, there was no effect found on their ability to father children [Smith, 1991], but the limitation in this study was that the reproductive end-point was crude (number of pregnancies reported) and that there was no investigation of the relationship with different exposure levels. Recently, Martin et al. [2002], found no effect of DDE on androgen

levels, including total testosterone, bioavailable testosterone and hydrotestosterone, of North Carolina farmers, however, blood DDE levels (median = 1.2 µg/g lipid), were substantially lower than in those measured in other exposed populations (Table 2.1).

In order to fully investigate the reproductive health effects of DDT on men, large studies of highly exposed workers using sensitive DDT exposure measures such as adipose DDT or blood DDT and sensitive reproductive outcome measures such as endocrine levels and sperm quality are needed.

2.6 The use of DDT in South Africa

As is true in many countries having regions with tropical or sub-tropical climates, in South Africa malaria vector control programs still routinely use DDT. Rural dwellings are sprayed internally to reduce mosquito populations. This results in high exposures for spray applicators who in many cases have been doing this type of work for several decades.

DDT has been used for malaria vector control since 1946 in three provinces, KwaZulu-Natal, Mpumalanga and the Limpopo. It was banned for agricultural use in South Africa in 1974. There was an attempt by the South African Department of Health to substitute pyrethroids for DDT in KwaZulu-Natal and Mpumalanga in 1996 and in Limpopo in 2000. Shortly thereafter malaria infection rates increased substantially. While some of this increase may be attributable to increased cross-border movement from Mozambique and unusually heavy flooding, a substantial fraction appears likely to have resulted from the change in pesticides, especially in view of the fact that the critical transmission species of mosquito (*Anopheles funestus*) developed widespread resistance to pyrethroids [Hargreaves et al., 2000]. South Africa, therefore, reverted to the use of DDT for malaria vector control in 2001.

The amount of DDT used in South Africa for malaria control is considerable, 57.2 tons on average was used per annum between 1990-1999, with a total of 400.5 tons during

that period. In the 1997/98 season, 82 791 kg was used in the Limpopo to spray 900 024 structures.

Table 2.1 shows that DDT blood levels in the order of 100 µg/l (not corrected for lipids) have previously been measured amongst South African malaria control applicators (DDT application rate: 2 g/active ingredient /m²) and amongst those environmentally exposed. Unfortunately, they cannot be compared to levels in applicators from non-African countries where measurements were made either in adipose tissue or blood corrected for lipids. Nevertheless, these levels are substantially higher than those measured in control populations in South Africa and the US (Table 2.1) and in epidemiological studies that have investigated health effects of DDT (Bush et al., 1986; Longnecker, 2001]. They are also higher than levels measured in Zimbabwean sprayers. The DDT levels measured in the breast milk of women in Kwazulu-Natal have been shown to be 31 times the acceptable daily intake in primiparous mothers [Bouwman et al., 1990].

An important factor contributing to high occupational exposures in South African workers could be the non- use of respirators. DDT is applied during the hottest months of the year and workers therefore tend not to use respirators because of discomfort and exertion due to manual operation of spraying pumps [Bouwman, 1991a].

2.7 The purpose, aims, hypothesis and objectives of this study

2.7.1 Study purpose and aim

In view of the continued use of DDT for malaria vector control, the documented associated high body burdens of metabolites (Table 2.1) and the fact that DDT metabolites have been shown to have endocrine disrupting effects in wildlife and laboratory animals, it is therefore important to know whether there are health consequences for humans. Overall, there is a lack of sufficient data from epidemiological studies investigating a number of plausible adverse human health outcomes to draw any conclusions.

There are, however studies such as the carefully conducted one by Garabrant et al. [1992] (Section 2.5) showing a significant increased rate of pancreatic cancer amongst DDT manufacturer workers, Longnecker et al.'s study (Section 2.5) showing an association of maternal serum DDT to premature pregnancy and small size at birth and age 7, and Buck et al.'s study (Section 2.5) showing an association between maternal consumption of DDT contaminated fish and fecundability (Section 2.5), that raise serious concerns about adverse health outcomes in exposed humans.

The few epidemiological studies investigating health effects of DDT on the male reproductive system (Table 2.2), are limited and inconclusive.

The continued study of the chronic health effects of DDT in humans is essential at this time as it will provide important missing information pertinent to the weighing of risks and benefits of different possible approaches to malaria control.

DDT is also important as a possible model for endocrine disruption. A number of agricultural and industrial chemicals have characteristics similar to DDT metabolites, including bioaccumulation in human tissues and evidence of estrogenic or anti-androgenic activity. Some of these chemicals, such as PCBs and dioxins, are similar to DDT in that, despite substantial reductions in current exposures, they remain in measurable quantities in tissue specimens from general population. Pesticides structurally related to DDT still in current use include dicofol, methoxychlor, and vinclozolin. The latter two, along with other industrial chemicals such as ocyphenol and nonylphenol, have been shown to have anti-androgenic or estrogenic properties in animal models. [Gray et al., 1989; Gray, 1992; Gray et al., 1994; Sharpe et al. 1995]. Some other chemicals in current use which have been found to have endocrine activity include alkylphenols, used in the manufacture of detergents; the antioxidants t-butylhydroxyanisole (BHA) and t-butylated hydroxytoluene (BHT), commonly used as food preservatives; and phthalate esters, widely used as plasticizers [Soto et al., 1995].

Many of these endocrine disrupting chemicals have reproductive effects particularly in males and DDT metabolites has been shown to have estrogenic and anti-androgenic effects in animal models (Chapter one).

The ideal study design to address the relevance of reproductive effects observed in animal models for exposed human populations, would focus on a sizable male population with a wide range of exposures to DDT, including high level exposures, and would incorporate measures of semen quality, sexual function and fertility together with measures of baseline and dynamic hormonal function. A unique opportunity exists in South Africa to investigate malaria control workers who have substantial current and historical occupational exposure to DDT. No previous epidemiological study investigating reproductive health effects due to DDT has previously been performed in South Africa.

This study intended to measure reproductive health endpoints consequent on long-term occupational exposure to DDT amongst malaria vector control workers in South Africa. It was expected to provide comprehensive and reliable data to determine whether DDT exposure among male South African malaria vector control workers has resulted in measurable adverse reproductive health effects. This information was intended to be useful in making decisions about whether and how DDT and similar chemicals should be used in the future in South Africa and other parts of the world. Moreover, the results of this study could be important for the design of studies intended to look for more subtle effects among currently lower exposed populations in developed countries.

This was the first study to apply sensitive measures of endocrine function in a field study of human reproductive health effects of potentially endocrine-disrupting

chemical exposures. As such it holds out the possibility of contributing to the construction of a human model of reproductive endocrine disruption for a class of chemicals (persistent organochlorines). Organochlorines continue to be of interest both in developed countries because of residual environmental burdens, as well as in other parts of the world where they continue to be freely used, contributing to an increased global environmental burden. Such a model is also useful for studying other environmental chemical exposures and their impact upon the reproductive endocrine system. A second expected result involves demonstration of the validity and utility of the GnRH stimulation test as a dynamic test for human reproductive endocrine system disruption.

2.7.2 Study hypothesis

The hypothesis of this study is therefore that past DDT exposure causes anti-androgenic and estrogenic effects which are reflected by abnormal semen quality and reduced fertility (figure 2.1).

Figure 2.1 Hypothesis

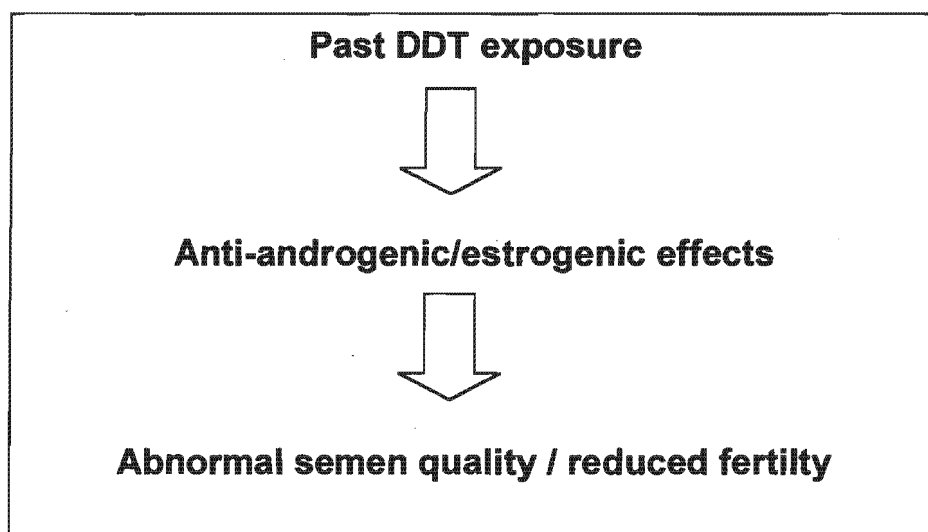
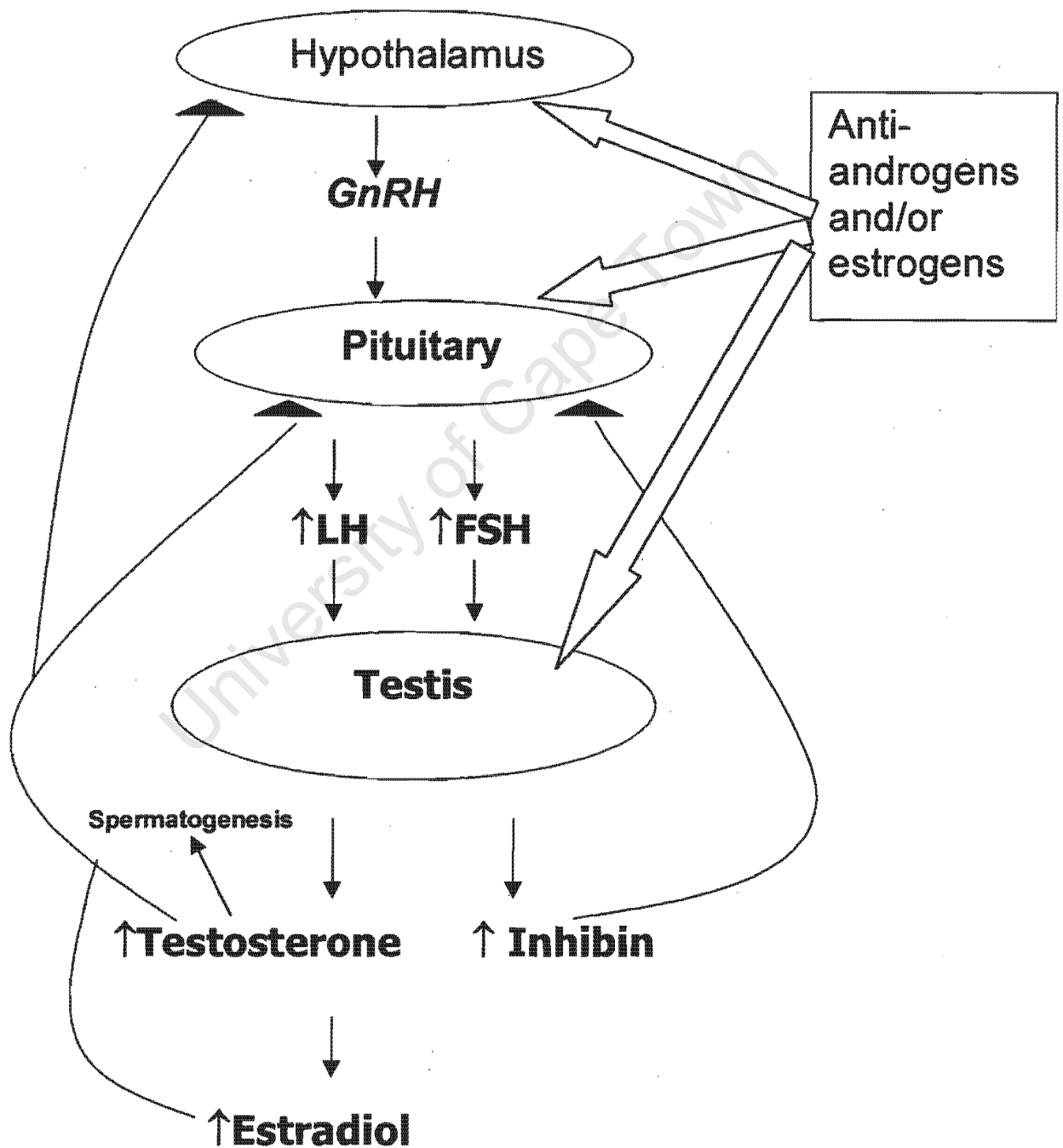


Figure 2.2 outlines where on the hypothalamus-pituitary-gonadal axis, estrogen agonists and anti-androgens such as DDT metabolites might act to cause endocrine disruption in the male reproductive system. A detailed description of the male reproductive system has been given earlier in Chapter 1 (Section 1.5). It can be summarized as follows:

Normally the hypothalamus secretes GnRH which stimulates pituitary release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH stimulates release of testosterone which leads to sperm production. Testosterone can also be aromatised to estradiol (E2). Both testosterone and estradiol have negative feedbacks on the pituitary and hypothalamus. FSH, also concerned with the regulation of spermatogenesis, stimulate release of inhibin which have a negative feedback on pituitary FSH release.

Endocrine disrupters can act at hypothalamic, pituitary and testicular levels.

Figure 2.2 Hypothesised effects of anti-androgens and /or estrogens on the hypothalamus-pituitary-gonadal axis



2.7.3 Study objectives

- 1) To characterise demographic and general health information of subjects.
- 2) To characterize historical DDT exposure and current levels of DDT metabolites in an occupational group with a broad range of exposures and substantial representation of high and low exposure levels.
- 3) To perform a brief reproductive system examination.
- 4) To characterize baseline reproductive pituitary and gonadal endocrine levels.
- 5) To determine the physiological integrity of the hypothalamic-pituitary-gonadal axis by measuring dynamic changes in endocrine levels after a gonadotropin releasing hormone (GnRH) challenge.
- 6) To characterize semen quality parameters in the participating men.
- 7) To assess fertility and sexual function by the number of pregnancies fathered and symptoms of sexual dysfunction.

- 8) To measure potential confounders and effect modifiers such as alcohol consumption and smoking for the relationship between DDT exposure and diverse reproductive outcomes.
- 9) To investigate relationships between specific measures of DDT exposure and specific measures of hypothalamic-pituitary-gonadal function, semen quality and sexual function and fertility.
- 10) To evaluate the sensitivity and utility of the GnRH challenge test in field conditions, as a measure of reproductive effects resulting from endocrine disruption.

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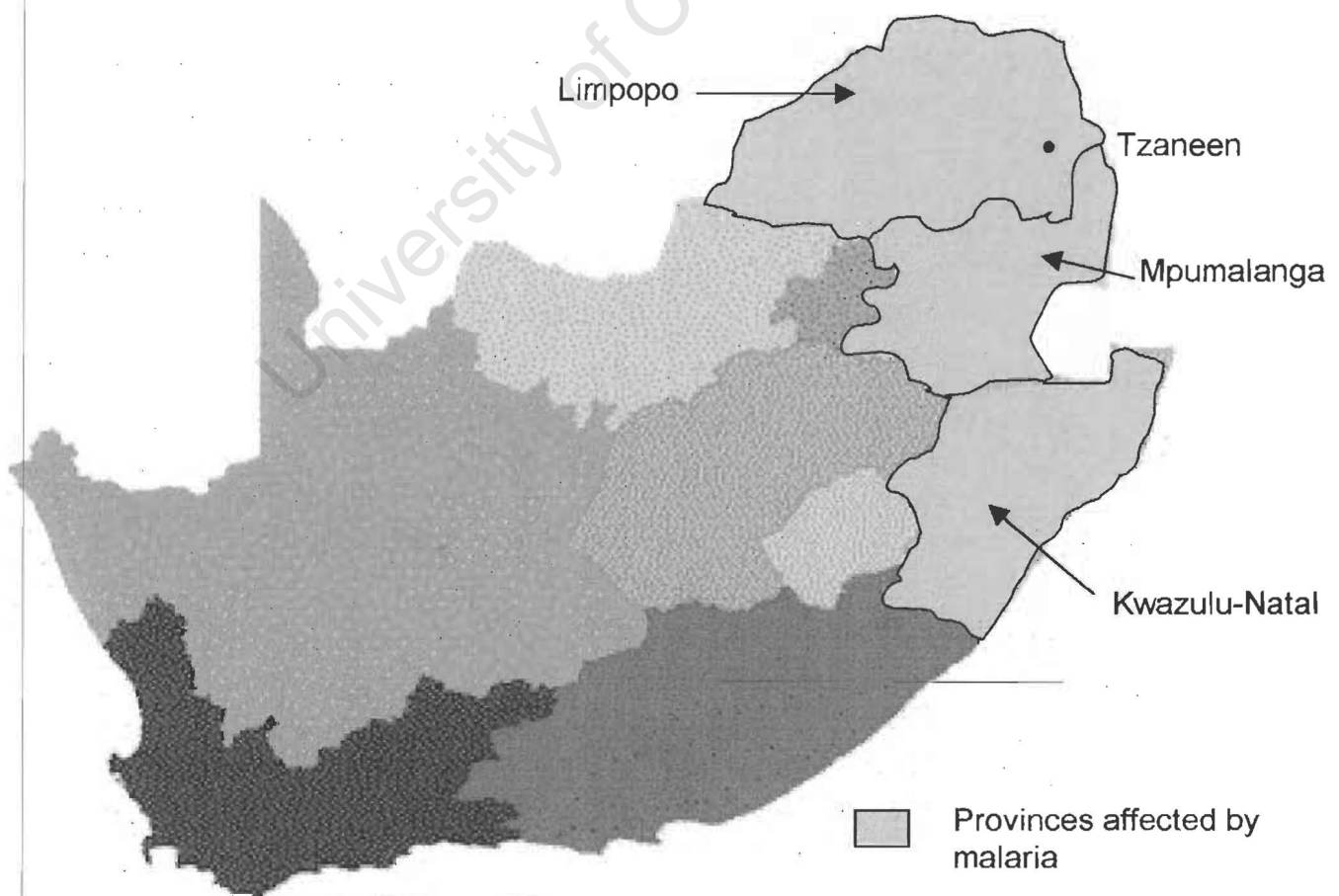
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Chapter 3: Methods used in the study

3.1 Study area and population

The population of interest was DDT-exposed South African malaria vector control workers. As mentioned in chapter one, DDT has been used for malaria vector control in South Africa since 1946 in three provinces including Kwazulu-Natal, Mpumalanga and the Limpopo (Figure 3.1).

Figure 3.1 Malaria provinces in South Africa



The study investigated malaria vector control workers employed by the Limpopo Department of Health (DOH), South Africa. There are currently about 900 of these workers in approximately 42 malaria vector control camps spread throughout the province with approximately 15-30 workers per camp. Most of the camps are concentrated in the highly populated rural areas. There are three language groups; Venda, Shangaan and Pedi, represented among the workers.

3.2 Study design and sampling

The study was conducted in June, 1999 and focused on camps in the vicinity of the Provincial Department of Health Malaria Control Centre (MCC) in Tzaneen (Figure 3.1), located in the south of the province, where the language spoken is Pedi. Three camps, Mamitwa, Bellevue and Riverside, located close to the control center, were selected. A total of sixty-eight workers are located at these three camps. Potential participants were identified through records maintained by the MCC.

The study was cross-sectional in design. Internal controls were used because participants were expected to have widely varying levels of serum DDT metabolites owing to marked differences in both the length of employment as a malaria vector control worker and the likelihood of exposure in varying job categories that range from highly exposed spraymen to less exposed case investigators, active surveillance teams, drivers and team leaders yielding internal exposure contrast. There were no external controls.

Owing to the long half-life of several DDT metabolites, lipid-corrected serum levels of these represent a time- integrated measure of biological dose [Smith, 1991, WHO, 1979). The major thrust of the study was to relate these measures of exposure/dose to fertility and reproductive function outcomes, measures of semen quality, and baseline and dynamic measures of endocrine function.

3.3 Pilot studies

Prior to the main study, two pilot studies were conducted at the Malaria Control Center in Tzaneen on workers from three other nearby camps, Sunnyside, Thabina and Metz.

During the first pilot in May 1998, three investigators informed participants about the project and field-tested the logistics of questionnaire administration and collection of biological specimens. Parameters assessed included acceptability, understandability and cultural sensitivity of questionnaire items for participating men and their partners; likely participation rates of pilot subjects solicited; and accessibility of subjects and internal and external controls.

During the second pilot in August 1998, revised questionnaires were administered and biological samples collected (semen and serum pre- and post-GnRH challenge) among 43 registered workers from Thabina and Sunnyside as well as 10 administrative workers at the Malaria Control Centre.

Participation rates were modest (34%), especially with collection of semen samples (11%). It was clear that the motivational and educational aspects of the study, which involved a one-hour group briefing session to all potential participants performed by a sociologist previously employed at the MCC, would require modification and improvement.

3.4 Motivation of workers

Due to difficulties encountered in recruiting workers to participate in the pilots, a programme to motivate workers to participate in the study was implemented. Pedi speaking male and female psychology honours students also proficient in English, from the University of the North, located outside Tzaneen were chosen to motivate workers and their partners to participate in the study. Both participated in the second pilot as interviewers/translators and had student-counseling experience. Their training involved attendance at the fertility clinic of a local hospital and familiarisation with the study protocol and material relating to health effects of pesticides and DDT.

The motivators had an understanding of local cultures, belief systems and languages, and a familiarity with study aims and methodology in addition to counseling skills. Due to the nature of local culture and religion, the workers, who were all male, were expected to respond best to a male motivator not previously known to them. Similarly, a female motivated the partners of the workers to participate in assisting their partner to produce semen samples.

The two motivators developed a motivation programme under the supervision of the study co-ordinator. The motivation programme lasted one week, with each camp visited 3 times and sessions held in the form of group meetings conducted in Pedi.

During the first visit, the possible health effects of DDT, the study aims, requirements for tests to be performed, risks and benefits and the voluntary and confidential nature of participation were explained in detail with ample time for any questions to be answered. The latter included explaining to workers that semen production would be via masturbation or coitus interruptus and emphasising the importance of abstinence. This was followed by a group discussion aimed to explore fears regarding participation.

During the second visit, a group discussion was held to emphasize the benefits of participation which included, a reproductive health examination for males and the potential importance of study results for DDT use without providing much detail about the reproductive health effects of DDT. They were also informed about a small monetary compensation (R 100) for participation costs and a free meal during the survey.

The final visit involved introductions to the field team and the signing of informed consent forms (Appendix A).

3.5 Main study measurements

3.5.1 Questionnaire

The final study questionnaire (Appendix B), which was back-translated into English, resulted from versions used in the two pilots. Many of the questions were thus pretested in the pilots. Questions were generally kept short, closed and clear to enhance validity and reliability.

Two trained male interviewers, who were both sociology students in their early twenty's from a neighboring town, administered questionnaires to workers in face-to-face, 45-60 minute interviews. The interviews were conducted in Pedi, but reported on the questionnaire in English.

The questionnaire was structured to first cover information on demographics and general medical and health history, followed by the more sensitive sections on sexual functioning history (including assessment of erectile function and libido) and reproductive history including an estimate of time to pregnancy. The pilot studies had indicated that questions in the latter sections would not pose a problem in the study. Questions in these sections were constructed with the assistance of two experienced reproductive health epidemiologists and were based on those used in previous major local studies in similar populations [Cooper et al, 1991 (a) and (b) Cooper et al, 1996, Cooper et al, 1999, Hoffman et al 1997]. Questions in the final two sections, history of alcohol, tobacco and cannabis use and work history, were

based on previous local pesticide studies [Dalvie et al, 1999, London, 1995]. The work history questions were last to blind interviewers as to the exposure status of the subject.

History of tobacco use included smoking of cigarettes and pipe, as well as chewing tobacco and snuff. Smoking of dagga (cannabis) was also included.

The work history questions included: years of employment at the malaria control centre; job titles, duration and activities including exposure to DDT; and activities during the non-spray season in current and previous MCC jobs, pesticide exposure before working at the MCC; and exposure to other relevant industrial agents suspected of being endocrine disruptors.

Spraying years were calculated as the total number of years worked in jobs in which spraying or mixing duties were reported.

Although the questionnaire included questions on time to pregnancy, these responses could not be analysed because insufficient information was provided by respondents.

3.5.2 Physical examination

An abbreviated physical examination related to the reproductive system (Appendix C) was conducted by a doctor, who was also on standby for any possible adverse reaction by workers to any of the tests. The purpose of the physical examination was to identify obvious confounders like infection, previous injury, hernias or tumors.

Height, weight and secondary sexual characteristics (Tanner stage) and any genital anatomical abnormalities were assessed.

3.5.3 Semen analysis

The andrology laboratory of the Department of Obstetrics and Gynaecology at UCT conducted the semen analyses. In the pilot study, masturbation participation rates were low with only 6 (11%) out of a total 50 workers producing a semen sample. Upon further discussion of cultural beliefs and practices with participants, a decision was made to allow coitus interruptus as an alternative method for sample production in the main study. Subjects were instructed to abstain for at least 2 days prior to semen sample collection by masturbation or coitus interruptus. They were asked to produce the sample into a clean plastic container previously given to them, and to keep this at body temperature. Ejaculation was to have been less than an hour before 6 a.m. when the male motivator came to collect the samples. The containers were labeled and placed in a bag held at room temperature with the

aid of hot water bottles and transported immediately to the laboratory at the MCC for analysis. The method, dates and times of current and previous ejaculations, and were recorded.

An experienced reproductive biologist performed analyses which included, sperm count (millions), sperm density (millions/ml), quantitative sperm motility (% motile), as well as liquefaction, consistency, pH, and agglutination, following established World Health Organisation protocols [WHO, 1992]. Period of abstinence (hours) and time lag from sample production to analysis were also recorded. Slides were prepared and later air shipped back to the University of Cape Town for morphology determination (% normal) using the strict Tygerberg criteria [Kruger, 1988]. Qualitative motility was also assessed at the MCC using the Tygerberg method.

3.5.4 Collection of blood samples

Six 10 ml blood samples were collected from participants by two nurses and a medical technologist for estimation of serum DDT and hormones. Whole bloods were collected in heparinised 10 ml test tubes using butterfly needles. The sample for DDT analyses was taken first, followed by those intended for hormone analyses at -15, 0, 30, 90 and 120 minutes after the administration of 100 µg of GnRH.

Samples were allowed to clot for 20 minutes, then centrifuged at 3000 rpm after which the supernatant (serum) was split into two 5 ml Greiner tubes and stored

frozen (-20° centigrade). Blood samples were transported on dry ice to the respective laboratories.

3.5.5 Biological exposure measurement

Potential for inhalation and skin exposure to DDT among the malaria vector control workers occurs during hand-held spraying inside of domiciles. Potential exposures also occur through the handling and mixing of pesticides and the cleaning and maintenance of spray equipment. During the pilot studies, it was established that respiratory protection or special clothing or gloves during spray operations is not used (The workers said that personal protective clothing or equipment is not supplied, while management said that they would not be worn even if supplied).

Spraying of structures occurs during August to December, while during the rest of the year the workers are engaged in active surveillance and various other duties at the MCC.

The malaria control workers are organized into local work units. Overall work activities of these work units are very similar; however, there is substantial variation in job duties and potential for differential exposure among individual members of a work unit. The job categories include sprayers, foremen, malaria case investigators (who collect blood samples), field workers (who collect mosquitoes),

field leaders (who supervise fieldworkers) and health and safety officers (who inspect houses).

Whole blood samples from participants were allowed to clot and the serum stored frozen (-20° centigrade) in solvent precleaned containers (blood sample collection and preparation were described earlier in section 3.5). One of two split samples was shipped to a laboratory highly experienced in the measurement of organohalogenes in the serum maintained by the State of Michigan, USA. The DDT isomers, o'p'-DDT, p'p'-DDT, o'p'-DDE, p'p'-DDE, o'p'-DDD, and p'p'-DDD were determined with a single step extraction and clean-up procedure and analysed by gas chromatography with electron capture detection [Bouwman et al 1989].

Lipid analyses were performed at the South Africa Institute for Medical Research (SAIMR) using the other split samples. Postprandial increases in serum lipids have been demonstrated to increase the serum levels of DDT metabolites [Phillips et al 1989] by increasing the amount of lipid in the serum. However, when serum levels are expressed per unit of lipid weight (e.g. µg/g lipid) fasting and eating do not affect the metabolite concentration. The equation: $TL = 1.677 (TC-FC) + FC + TG + PL$, where TL = total lipids, TC = total cholesterol, FC = free cholesterol, TG = triglycerides, PL = phospholipids, has been shown to provide the most reliable measure of total lipids and the best method of correction [Phillips et. Al., 1989]. Therefore, serum concentrations of isomers of DDT and their metabolites were calculated as µg/g total lipid. The Liebermann-Burchard method for cholesterol determination, Fluorometric-Hantzsch reaction method for triglycerides and a

colorimetric method for phospholipids were utilized for the measurements used in the calculation of total serum lipid [Cooper et al 1982, Takayama et al 1977]. Lipid analyses were performed (under the direction of Dr. S. Omar) at the South African Institute for Medical Research (SAIMR).

It should be noted that in most part of this document, the term "DDT" refers to the sum of all six metabolites, but in certain tables it is used for the sum of o'p'DDT and p'p'DDT and the term "total DDT " is then used for the sum of all six metabolites. The terms "DDD" and "DDE" is used for the sum of the two respective isomers for each of these metabolites.

3.5.6 Endocrine measures including the gonadotropin releasing hormone (GnRH) challenge test

Section 1.5 in chapter one, which outlines the male reproductive system, provides a background to this section detailing the endocrine measurements made in this study.

Consenting subjects also underwent a GnRH stimulation test. This is a dynamic test for human reproductive endocrine function which has previously been used to detect abnormalities in men with significant gonadal dysfunction due to testicular disorders such as cryptorchidism, varicocele, testicular torsion and vasectomy [Fisch et al 1990, Fisch et al 1989, Fisch et al 1988, Lipshultz et al 1976, Hudson et al 1980]. The GnRH test has been used to indicate disruption in the normal

hypothalamic-pituitary-testicular axis (HPT) [Besser, 1972; Fisch, 1990; Lavin, 1986]. Increased serum endocrine hormone responses, especially of LH and FSH, to GnRH stimulation reflects decreased negative feedback at the level of the hypothalamus-pituitary as a result of diminished testicular function. An altered response might indicate H-P-T dysfunction secondary to exposure to hormonally active pesticides.

The test could therefore provide additional insight into the functioning of the male reproductive system of the malaria vector control workers studied, over and above that given by measuring basal endocrine levels. The study offered the opportunity to test the validity and utility of this test as a dynamic test for reproductive dysfunction.

Baseline and post-GnRH (100 µg) challenge test levels of pituitary and gonadal hormonal levels FSH, LH, testosterone, estradiol (E2), SHBG and Inhibin-B were measured.

A butterfly catheter was used to collect blood for measuring baseline and post-GnRH challenge (100 µg) [Lavin, 1986] levels of FSH, LH, testosterone, E2 and β -inhibin. With respect to the timing of the challenge, samples were drawn at -15, 0, 30, 60, 90 and 120 minutes after the GnRH challenge.

The Department of Chemical Pathology at UCT was responsible for the analysis of all the hormonal measures except inhibin B. One of two serum aliquots (blood collection and preparation described earlier in section 3.5) was transported to the Medical Research Council's Reproductive Health Unit in Edinburgh, United Kingdom for measurement of inhibin B. The other was utilised for the measurement of FSH, LH, testosterone, E2 and sex hormone binding globulin in Cape Town by means of commercial assay kits routinely used in the laboratory. These assays are standardised and tested by the manufacturer for high specificity (low cross-reactivity), consistency and precision.

LH was measured with the MAIAclone IRMA kit [Biochem Immuno Systems, 1985 a; Biochem Immuno Systems, 1985 b], which is a highly specific immunoradiometric assay with a high degree of sensitivity using I_{125} labelled monoclonal antisera directed against the ligand. (Laboratory intra- and inter-assay variation of 3.3% and 5.8%, respectively)

Serum FSH and total testosterone was measured using the ACS-180 competitive chemiluminescent automated systems [Bayer Corporation, 2000] which are also both highly specific and sensitive (Laboratory intra- and inter-assay variation of 1.9% and 5.6%, respectively for FSH and 6.8% and 8.7% for testosterone).

Serum E2 was measured utilising an in-house radio immunoassay [Biosource-Europe SA, 1994]. Plasma serum samples were extracted with diethyl ether. A highly specific anti-serum raised in house and tritiated E2 label (from Amersham)

was used as the radioactive tracer (Laboratory intra- and inter-assay variation of 6.6% and 13% respectively).

SHBG was measured with the IRMA kit from Orion Diagnostica [Orion Diagnostica, 1990] which is a non-competitive “liquid-phase” immunoradiometric assay, using a labelled monoclonal antibody. (Laboratory intra- and inter-assay variation of 5.8% and 7.9% respectively.)

Inhibin B was measured in the Centre for Reproductive Biology in Edinburgh, using a two site enzyme-linked immunoassay which utilises a capture antibody directed against the C-terminal portion of the human B-subunit and the F(ab) fraction of a mouse monoclonal antibody (R1) to the N-terminal portion of the inhibin-A subunit conjugated to alkaline phosphatase [Anawalt et al, 1996; Illingworth et al, 1996; Crofton et al 1997; Plant et al, 1997]. The assay has been previously validated for measurement of inhibin-B in the human in studies conducted within the MRC Reproductive Biology Unit [Illingworth et al, 1996; Anawalt et al. 1996]. The assay is available in kit form from Serotec and is in routine use within the MRC Reproductive Biology Unit.

3.6 Logistics

Most of the motivation programme was carried out a week before the study, with only the final visit occurring after arrival of the field team. During the week when

motivation was implemented, a working programme was constructed for the next week.

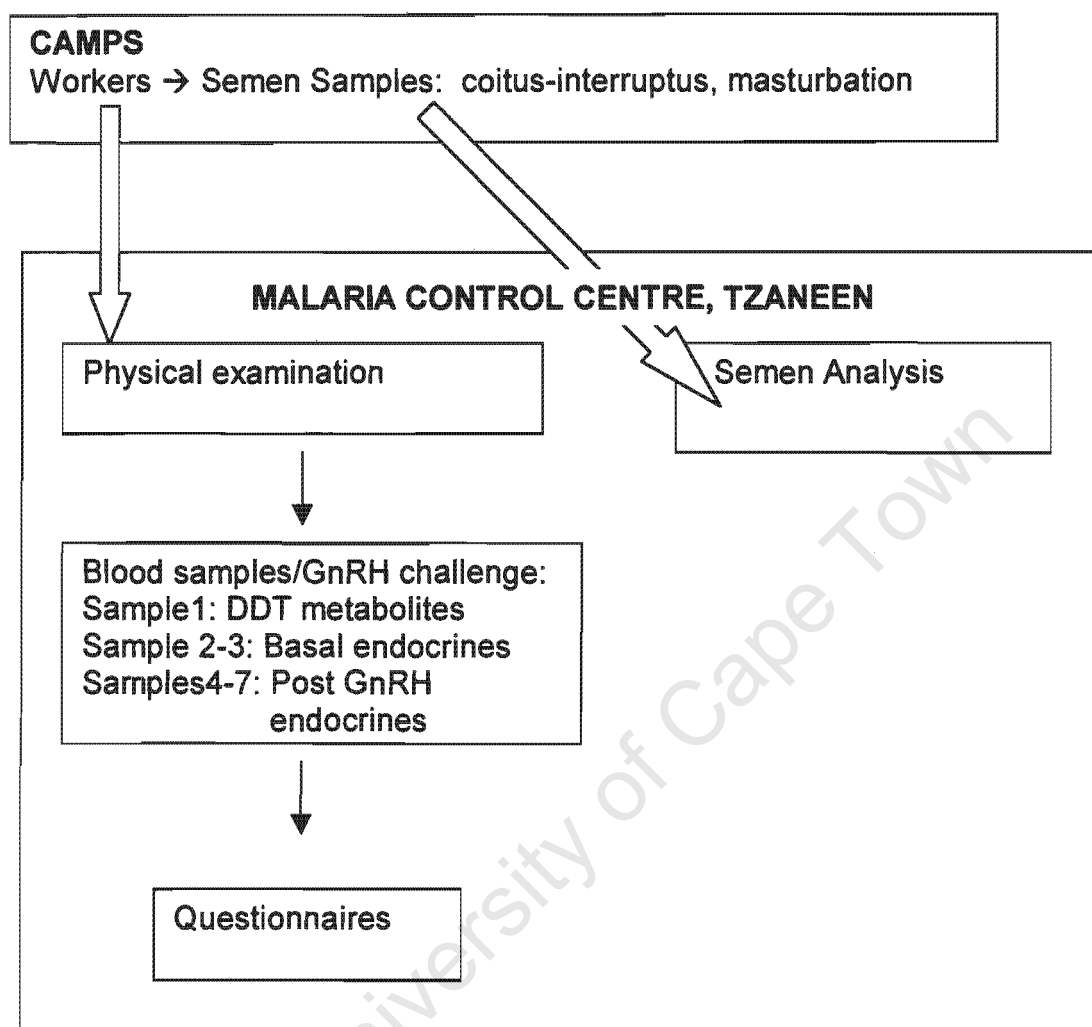
The rest of the research team including the project co-ordinator, the reproductive biologist, the medical doctor, the medical technologist, two nurses, the three interviewers from the University of the North and a second driver arrived in Tzaneen on the 21 June 1999.

The research team visited the three camps to introduce themselves to the workers on Tuesday, 22 June. The tests were performed on the three days that followed, one camp per day, at the MCC in Tzaneen. Two technologists from the MCC assisted with blood centrifugation and splitting of sera.

Workers were transported to the MCC offices after the male motivator had collected the semen samples. Workers who did not produce a semen sample were also included.

The physical examination was done first, followed by blood taking and GnRH challenge test and then the questionnaire interview (Figure 3.2).

Figure 3.2 Outline of the order of tests performed in the study



3.7 Data management

At first contact during motivation, an identification number was assigned to each subject. All data collection instruments, as well as all biological specimen containers, were labeled with their identification number.

These included consent forms, questionnaires, physical examination report sheets, semen analysis report sheets, holding envelope, semen collection containers and test tubes used during blood collection.

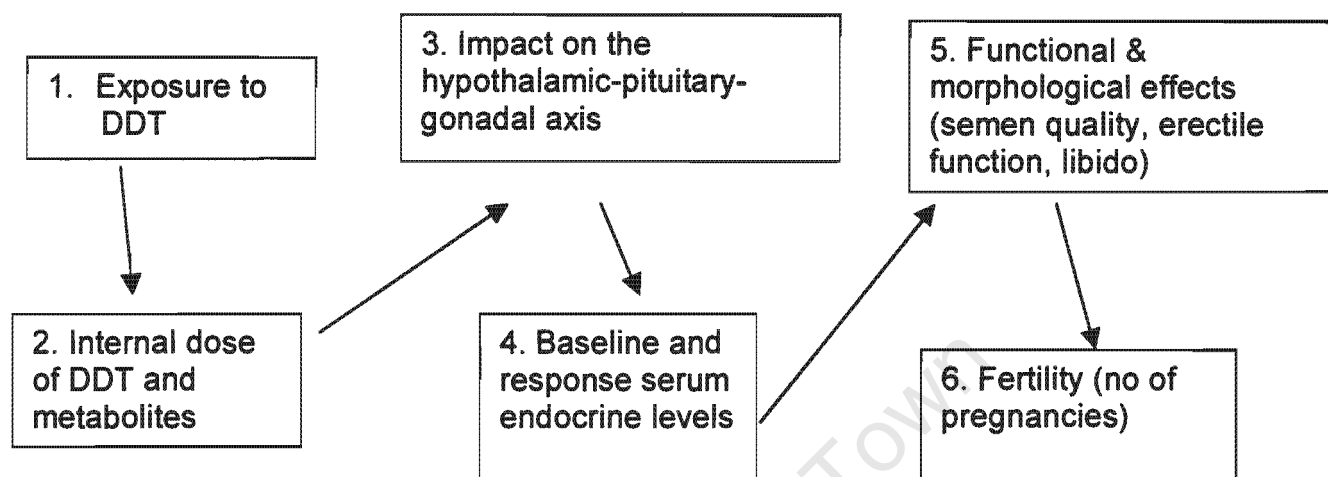
Data was double punched by the data capturing service at UCT. Laboratory results were included afterwards. (The number of samples analyzed were compared to the number of samples recorded during collection of samples).

3.8 Data analysis

3.8.1 The causal chain.

The analysis was guided by a conceptual model of the potential causal chain leading from exposure to DDT to adverse fertility outcomes, outlined in Figure 3.3.

Figure 3.3 Causal chain



Key associations of interest involved the internal dose of DDT and its direct effects on endocrine function (stages 3-4 in the above model) and/or their effect on measures of function, morphology and fertility (stages 5 and 6).

3.8.2 Outcome measures

The primary outcomes of interest included measures of semen quality (sperm count, density, motility, and morphology), function (lack of interest in sex, lack of ejaculation, early ejaculation) and fertility (total pregnancies fathered). Semen quality variables were analysed both as continuous and dichotomous variables. Dichotomous cut-offs, which were based on WHO [WHO, 1992] and/or Tygerberg

criteria [Kruger, 1988], were 20 million/ml for sperm density, 40 million for sperm count, 50 % for motility and 2% (Tygerberg infertility cut-off) and 6% (Tygerberg fertility cut-off) normal for morphology.

Baseline and post-GnRH challenge test serum hormonal levels were also primary outcome measures.

While total serum testosterone was measured in the laboratory, exposure effects on free plasma testosterone, normally 1-4 % of total testosterone [Södergard, 1982], were estimated by controlling for the effect of SHBG on total testosterone during multivariate analysis using linear regression analysis (described in section 3.9.5). The free androgen index: basal testosterone (nmol/L) X 100/basal SHBG (nmol/L), which indicates free plasma testosterone [Nanjee and Wheeler, 1985] was not used as an outcome, because ratios are problematic when using linear regression analysis [Kronmal, 1993]. However, further sub-analyses were done to investigate if there was a discrepancy between the exposure effect on the two indices of free testosterone. This can be summarised as follows:

Model 1: $btst = b_0 + b_1*ddt + b_2*age + b_3*bshbg + error$

Model 2: $(btst/bshbg) = c_0 + c_1*ddt + c_2*age + error (2)$

or, equivalently $btst = bshbg*(c_0 + c_1*ddt + c_2*age + error)$

Model 3: $btst = b_0 + b_1*ddt + b_2*age + b_3*bshbg + b_4*ddt*bshbg + b_5*age*bshbg + error$

Where $btst$ = total serum testosterone, b and c = regression coefficients, $bshbg$ = basal SHBG, ddt = DDT metabolite

In model 1, total testosterone is adjusted for basal SHBG to account for free testosterone. Coefficient b_1 estimates the effect of DDT on basal testosterone among people of the same age and basal SHBG levels, i.e. basal SHBG is held fixed.

In model 2 the testosterone/SHBG ratio is used as an outcome variable. However, the problem with model two is that one is essentially looking at an INTERACTION, i.e. the coefficient c_1 for DDT measures the extent to which DDT and basal SHBG have an interactive effect on basal testosterone, but it's a strange model that has no main effect for DDT.

Model 3 is a full version of model 1 and 2 including interaction terms and was used to test if there were significant interaction between exposure and basal SHBG which would mean that model 1 is not valid. Models 1 and 2 are both special cases of model 3: model 1 is a special case with $b_4 = b_5 = 0$ and model 2 is a special case with $b_0 = b_1 = b_2 = 0$.

Basal and post-GnRH total testosterone, LH, FSH, inhibin-B and total E2, were mostly analysed as continuous variables. GnRH challenge test effects on hormones were calculated as the absolute change at the different time points from the mean of the two pre-challenge baseline measures. The peak change from all time points and the summed change at each time point was used. Various

dichotomous variables were also generated from basal levels by using the upper and lower limits of normal ranges as well as upper and lower quartiles as cut-offs. The GnRH responses were dichotomised using upper and lower quartiles as well as zero, as cut-offs. These same variables were also treated as independent (exposure) variables of interest in the analyses of semen quality and fertility variables as outcomes.

3.8.3 Exposure measures

The primary measures of exposure were serum levels of DDT metabolites. Although lipid corrected levels were used, analysis using uncorrected levels controlled for lipids were also conducted. Those of greatest interest were p,p'-DDE because of its demonstrated potent anti-androgen effects. o,p'-DDT was of interest because of its estrogenicity. Years working at the MCC was used as secondary measures of exposure. Spraying years, which was calculated as the total number of years in jobs in which the respondents reported performing spraying tasks, could not be used because 95% of workers reported spraying history. Binary exposure variables were generated from malaria years (number of years worked at the MCC) and DDT metabolites levels by using the median as cut-off.

3.8.4 Variables included in multivariate models

Covariates for multivariate modelling were selected a priori (variables which were obvious or shown in the literature to be confounders) or were based on bivariate analysis where associations had p-values < 0.1. Table 3.1 lists these covariate variables. Body mass index was not included in multivariate models, because it was not found to be significantly associated ($p < 0.1$) to any reproductive outcomes or DDT.

Table 3.1 Covariates included in multivariate modelling of the effect of exposure on reproductive outcomes

Outcome group	Covariates included in multivariate models	Criterion for inclusion		
		Apriori	Bivariate p value < 0.1	
		Yes, No	Yes, No	Outcomes associated to
Semen parameters	Age	Yes	Yes	Density
	Abstinence from sex	Yes	Yes	Motility
	Structural or pathological abnormalities	Yes	Yes	Motility
	Fever in the last 2 months	Yes	No	
Hormones	Age	Yes	No	
	Baseline SHBG	Yes	Yes	Basal testosterone &E2 Post GnRH LH & FSH
Number of pregnancies	Age	Yes	Yes	Number of pregnancies
Sexual dysfunction	Age	Yes	No	
	Schooling	Yes	No	

3.8.5 Statistical analysis

Statistical analyses was conducted using Stata [Stata Corporation,1997].

Univariate, bivariate and multivariate analyses were performed for relevant variables.

Univariate analyses summarised the distribution of each measured variable. Exploratory bivariate analyses were used to assess the nature of the associations between outcomes, exposure and other covariates. Relationships between outcomes and exposure, as well as between different outcome indices and different exposure indices, controlling for other covariates were investigated during multivariate analysis using multiple linear regression. Models were assessed for normality of residuals, uniformity of variances, and co linearity of variables. There was evidence of skewness in the distribution of the residuals which was alleviated by logarithmic transformations, but because the transformed models did not change the nature of associations, the untransformed models are presented. Co linearity was assumed for variables with a correlation of > 0.9 or a variance inflation factor > 10 . The effect of outliers/influential points, identified by DFBETA's > 1 , Cook's $D > 0.5$ or student residuals > 2.5 , on relationships was also assessed.

Multiple logistic regression analysis was also used to confirm and further explore relationships between exposure and dichotomous outcomes.

The adjusted R^2 is presented in the results to indicate the total variance explained by a multivariate model after adjusting for the number of variables in the model. The term " R^2 " is, however, used to avoid confusion with term "adjusted effect" and "adjusted $\hat{\beta}$ ".

3.8.6 Statistical power

Power calculations are presented in Table 3.2 below, for a total sample size of 54. The table shows the power associated with detecting a 60% change in mean levels of the baseline unexposed group relative to the high exposure group, which are equal in size. The power calculations were done using a two sample-test of equality of means [Stata Corporation, 1997], with $\alpha = 0.05$. A 60 % difference in mean levels between the groups was used because this is the approximate mean difference in semen parameters between WHO fertile and infertile categories [WHO, 1992]. The semen parameters represent a critical outcome for which good data is available in the literature to estimate means and standard deviations in this study population. Means and standard deviations used in these calculations are derived from a recent study [Robins et al, 1997] of a group of lead exposed South African workers of similar cultural and economic background to the current study population.

These figures indicate good power ($> 75\%$) for testing hypotheses of dose-response effects on sperm density and motility, but less than optimal power for sperm count (53%) and morphology (60%).

**Table 3.2 Power to detect > 60 % change* in semen parameters
of sample size = 54**

<u>Semen Parameter</u>	<u>Power</u>
Sperm density	77%
Sperm count	53%
% Motile	100%
% Abnormal morphology	60%

* $100 \times [\text{unexposed group mean} - \text{high exposed group mean}] / \text{unexposed group mean}]$

3.9 Ethics

Workers were informed in detail in their native language about the study purposes, study protocol and of risks and benefits of the study. A consent form (Appendix A) detailing these in Pedi had to be signed before participation.

Potential participants who wished to decline certain aspects of the study such as semen analysis or GnRH challenge testing were encouraged to participate in other aspects (questionnaire, baseline hormonal levels) so that the maximum possible information was available to investigate any potential for selection bias.

Data were treated confidentially, with personal identification codes assigned to each participant.

A doctor was on standby for any adverse effects resulting during testing such as during the GnRH challenge and backup at the nearby hospital was available.

The study protocol was approved by the University of Cape Town's Ethics Committee and by the University of Michigan Internal Review Board.

Individual reports were made available to workers. The workers were informed if reproductive abnormalities were found or not. Where an abnormality was found ($n = 3$), a detailed account was given of their condition and they were advised to go to the nearest clinic for further examination.

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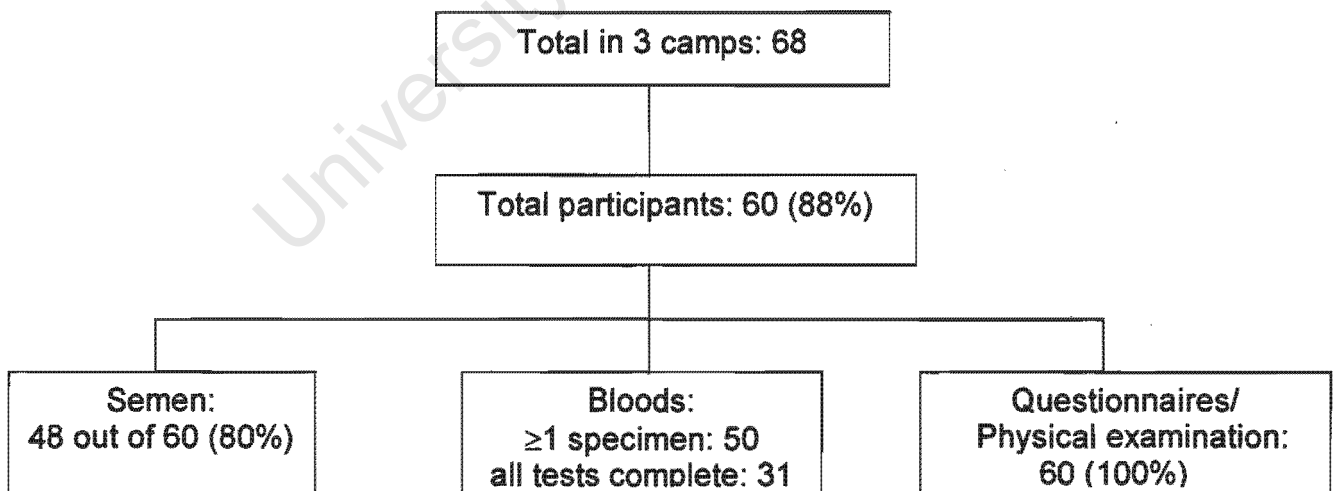
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Chapter 4: Results

4.1 Study participation

Figure 4.1 summarises participation in the study and in the individual tests. Sixty workers (88%), including 18 each from Mamitwa and Bellview camps, 23 from Riverside camp and the manager of the three camps, participated. Forty-eight (80%) produced semen samples, four through masturbation at the MCC. Fifty subjects (83%), including 47 who donated semen, donated at least one sample of blood. All the subjects completed the physical examination and the questionnaire (one partially completed the questionnaire). Thirty-one (52%) completed all the tests in full and an additional 12 partially completed the GnRH challenge test.

Figure 4.1 Summary of participation in the study



4.2 Univariate results

4.2.1 Descriptive characteristics of workers

Table 4.1 shows that the participants had a high mean age of 45 ± 9 years and a low level of education (mean number of schooling years = 6.9 ± 2.7 years).

The mean number of pregnancies fathered (from all partners) including stillbirths, miscarriages, abortions and tubal pregnancies was 5.6, with no one reporting zero pregnancies. All the participants ($n = 60$) were currently either married or had a partner.

Table 4.1 Descriptive characteristics of study participants

Variable (unit)	n	Mean (SD)	Median, (25 th –75 th Percentile)
Age (years)	60	45.3 (9.1)	47 (39.5-52)
Schooling (years)	60	6.9 (2.7)	7 (5-8)
Height (cm)	60	172.8 (8.0)	172 (167-178.5)
Weight (kg)	60	71.7 (11.7)	70.5 (64-81.5)
Pulse (bpm)	50	68.7 (11.3)	68 (60-76)
Total number of partner pregnancies *	53	5.6 (3.4)	5 (3-7)
* Includes stillbirths, miscarriages, abortions and tubal pregnancies; includes all partners			

4.2.2 Medical histories, sexual problems and lifestyle factors

Table 4.2 summarises self - reported medical histories, sexual problems and lifestyle factors of the participants. The most common medical problems reported were headaches and back problems. Of the chronic conditions many reported high blood pressure, while prevalences of diabetes, asthma and tuberculosis were low.

Self-percieved current problems with sexual function ranged from 10 % reporting inability to ejaculate to 20% reporting difficulty obtaining full erection. Nine subjects (15%) reported one problem, 3 (5%) reported 2 problems, 6 (10%) reported 3 problems and 1 reported all four problems. No one reported sexual problems other than the ones listed in the questionnaire and shown in Table 4.2.

All subjects reported having consumed alcohol and 58% have smoked during their lifetime.

Table 4.2 Medical history, sexual and lifestyle factors

Variable	n	(%)
Self-reported history of:		
Diabetes	59	1.7
TB –past/current	59	3.4
Fits	59	0
High blood pressure	59	23.7
Asthma	59	1.7
Headaches	59	37.3
Heart problems	59	8.5
Back problems	59	23.7
Reported current sexual problems (always, usually or often)		
Lack of interest in sex	59	18.6
Difficulties in full erection	59	20.3
Early ejaculation	59	13.6
Inability to ejaculate	59	10.2
Lifestyle factors		
Ever smoked cigarettes	59	58.3
Ever drank alcohol	59	100
Fever in last 2 months	52	42.3

4.2.3 Physical sexual characteristics

Table 4.3 outlines the abnormalities detected on examination of the genital region of the participants. Abnormal testis disposition was by far the most prevalent (71%) abnormality and might have been due the cold winter weather during which the study was performed. There were two subjects who showed evidence of testicular tumors and they were referred to have a biopsy. Forty-four subjects (73%) had at least one abnormality when including testis disposition, and 12 (20%) excluding testis disposition. The majority (97%) of subjects had a Tanner age score of 5 out of 6 indicating normal pubic hair growth.

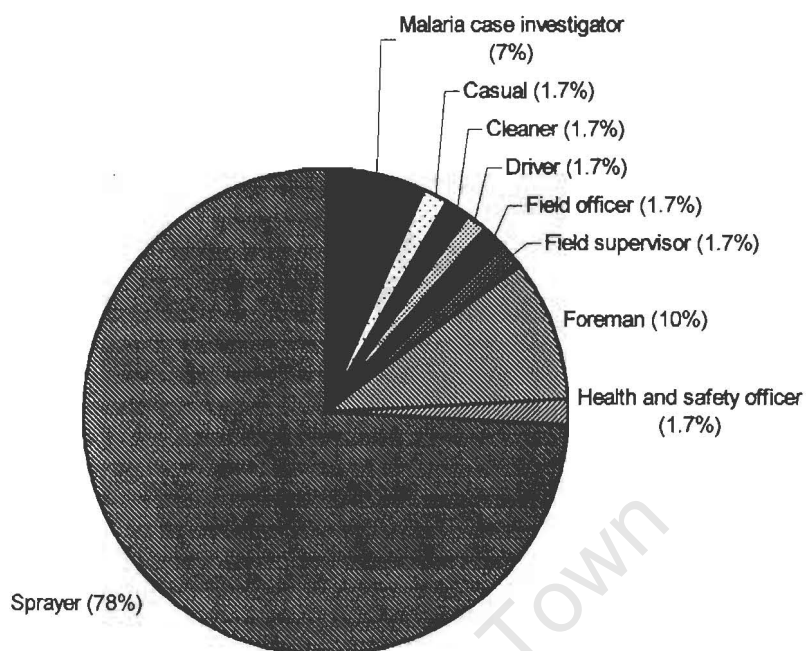
Table 4.3 Physical examination results

Abnormality (either left, right or both)	Number (%)*
Abnormal penis (penile ulcers)	5 (8.3)
Abnormal testis disposition (not low in scrotum)	43 (71.1)
Clinical evidence suggestive of testicular tumors	2 (3.3)
Abnormal epididymis (tender or enlarged)	5 (8.3)
Abnormal vas deferens (palpable)	1 (1.7)
Hydrocele	4 (6.7)
* n = 60	

4.2.4 Exposure

Most of the study participants had a long work history at the MCC (Table 4.4). Virtually all (n=57) reported that they had held only one job at the MCC, while three reported 2 jobs, thus showing little job change within the MCC. Although 8 different MCC job titles were reported (Figure 4.2 shows jobs ever held at the MCC) with 78% current or past sprayers, only three (5%) had no spraying or mixing tasks in any job at the MCC showing that most job categories involve tasks associated with direct exposure. Mean p'p'DDE in non-sprayers ($85.0 \pm 89.9 \mu\text{g/g lipid}$, n= 13) was higher than in sprayers ($64.1 \pm 39.8\mu\text{g/g lipid}$, n= 41), but not significantly. Years working at the MCC (malaria years) correlated with p'p' DDE ($p < 0.05$), and this was therefore used as a secondary measure of exposure rather than job category.

Figure 4.2: Different job titles held by participants at the MCC



DDE levels were the highest of the three metabolites, and the p'p' isomer was more abundant than the o'p' isomer for all three metabolites. p'p' DDE levels were the highest of all metabolites.

Correlations between individual metabolites were low to modest (< 0.6), except for that between o'p' DDT and o'p'DDD (correlation coefficient = 0.9) and that between p'p' DDT and p'p'DDD (correlation coefficient = 0.7).

Table 4.4 Work history and serum DDT levels in workers

Variable (unit)	n	Mean (SD)	Median, (25 th –75 th Percentile)
Malaria years (years)	59	15.8 (7.8)	17 (8-21)
Serum DDT metabolites (µg/g lipid)			
o'p' DDT	47	2.21(1.98)	1.57 (1.09 –2.44)
o'p' DDD	47	0.062(0.092)	0.033(0-0.08)
o'p' DDE	47	0.053(0.064)	0.051(0-0.089)
p'p' DDT	47	26.1(13.7)	26.9 (15.7-34.0)
p'p' DDD	47	0.91 (0.68)	0.70(0.44-1.15)
p'p' DDE	47	65.0 (48.8)	52.3 (36.0 – 83.1)
DDT	47	28.1(15.1)	28.2 (17.1-36.7)
DDD	47	0.98 (0.74)	0.74 (0.45-1.25)
DDE	47	65.0 (48.8)	52.4 (36.0-83.1)
Total	47	94.3 (57.1)	83.3 (61.8-113.0)

4.2.5 Semen parameters

The participants had good compliance in producing semen samples, with a median abstinence period of about 4 days (Table 4.5) and 80% higher than the recommended 2 days.

Upon semen collection, donors were asked about the frequency of their sexual activity in the week before. Twenty-seven donors responded to the question, of

which a very high proportion (77.8%) reported a frequency of $\geq 1/\text{day}$. The reason why other donors did not report on their sexual activity was because they were shy or felt awkward to discuss the topic in the presence of the partner. All samples reached the field laboratory within the recommended one-hour of production, with those not analysed immediately kept incubated. The interval between ejaculation and start of analysis ranged between 90 to 370 minutes, with an average of 231.9 ± 60.6 minutes.

Table 4.5 summarises semen parameters in the study and shows a high prevalence of samples with values below WHO and Tygerberg criteria [Kruger, 1988; WHO, 1992]. Morphology scores were especially low with the highest score of 6%, in the Tygerberg subfertile range [Kruger, 1988], and only 7 above the infertile point of 4%. The strict Tygerberg criteria probably contributed to the high abnormal morphology proportions. Eighty-four percent ($n = 45$) of semen donors had a poor prognosis according to the Tygerberg classification.

One subject, who reported having fathered 4 pregnancies in the past, had a zero sperm count.

Table 4.5 Semen parameters in the study

Variable (unit)	n	Mean (SD)	Range	Median, (25 th – 75 th Percentile)	% Subjects < criterion
Semen volume (ml)	48	1.6 (1.2)	0.05–4.5	1.5 (0.5–2.5)	60 < 2 ml ^a
Sperm Density (million/ml)	48	74.6(85.1)	0–386	36 (19–119.5)	29 < 20 million/ml ^a
Sperm Count (million)	46	93.8(130.3)	0–552	58.8 (17.2–96.8)	41 < 40 million ^a
Motility (% motile)	46	54.3(18.7)	0–70	60 (45–70)	26 < 50% ^a
Morphology (% normal)	44	2.5(1.8)	0–6	3 (1–4)	84 < 5% ^b
Abstinence (days)	41	3.8 (2.9)	0.2–14.3	3.0 (2.3–5.1)	22 < 2 days
^a WHO criteria [WHO, 1992]					
^b Tygerberg infertile range [Kruger, 1988]					

Table 4.6 compares semen parameters in the study produced via masturbation with those produced via coitus interruptus (CI). With only 4 masturbation samples, no firm conclusions can be drawn.

Although the semen volumes produced via CI were higher than those produced via masturbation, densities were significantly ($p > 0.05$) lower, resulting in lower counts. The mean age and proportion of physical abnormalities of the masturbation group was higher than the CI group, and they had a longer abstinence period.

Table 4.6 Comparison of semen parameters and other characteristics with respect to method of sperm production in the study and pilot

Variable(unit)	Mean $\pm$ SD (range)/proportion, by method	
	Masturbation (n=4)	CI (n=44)
Sperm volume (ml)	0.9 $\pm$ 0.9	1,7 $\pm$ 1.2
Sperm density (million/ml)*	153.5 $\pm$ 77.9 (10-246)	67.4 $\pm$ 82.9 (0-386)
Sperm count (million)	159.3 $\pm$ 173.6 (7.1-409)	87.5 $\pm$ 126.3 (0-552)
Age (years)	52.8 $\pm$ 4.7 (48-59)	44.1 $\pm$ 8.8 (26-60)
Abstinence (days)	137.7 $\pm$ 76.2 (55-205)	89.5 $\pm$ 68.6 (5-342)
Presence of physical abnormalities, including testis disposition (%)	100%	73%
Presence of physical abnormalities, excluding testis disposition (%)	50%	18%
* P value for difference between 2 groups < 0.05		

4.2.6 Blood hormones

Table 4.7 summarises baseline serum hormone levels, as well as levels after the GnRH test. All baseline values, except for SHBG, are compared to normal values published by the respective manufacturers (these reference ranges are derived from healthy adult males and were in use for reference purposes in the respective laboratories). For testosterone the mean age of the reference population was 34

years. Although these reference values might not be directly applicable to the study population, they do offer a useful standard and are used routinely in the respective laboratories.

For SHBG, the laboratory reference range was different from the manufacturers' range. (11-71 nmol/L).

Median baseline LH, FSH, SHBG and testosterone of workers were within normal ranges, while baseline E2 was above the upper limit (50 pg/ml) of normal.

Sixty five percent of participants' baseline E2s, 2% of LHs and FSHs, 14% of testosterones, 18% of SHBGs and 0% inhibin were above the upper limit of normal (Table 4.7), while 23% of baseline LHs and FSHs, 2% of E2s and SHBGs and 4% of testosterones and 45% inhibin were below the lower limit of normal (Table 4.7).

The median free androgen index of the participants was 55(n=49) ranging from 10 to 130 (25th-75th range: 38-69).

Hormone levels in response to the GnRH challenge are indicated by the baseline to peak and the sum of all differences at each time point in Table 4.7. GnRH stimulation of pituitary LH and FSH secretion was clearly shown by the increased levels of these hormones, but stimulation of E2, testosterone and inhibin in response to increased gonadotrophin release was not clear, with many subjects not responding positively.

Table 4.7 Blood hormone levels

Variable (unit)	n	Mean $\pm$ SD	Median, (minimum, 10, 25 th , 75 th , & 90 th Percentile, maximum)	Normal values
Baseline serum:				
LH (miu/ml)	49	2.59 $\pm$ 1.8	2.3 (0.65, 1, 1.55, 3.25, 4.5, 11.7)	1.5-9.2
FSH (miu/ml)	49	3.57 $\pm$ 3.15	3.05 (0.1, 0.7, 1.65, 4.9, 7.2, 19.9)	1.4-18.1
E2 (pg/ml)	49	62.4 $\pm$ 29.87	56 (22.5, 45.5, 75.5, 115.5, 208.5)	10-50
SHBG (nmol/L)	49	40.8 $\pm$ 23.4	34 (12, 17, 25, 50, 66, 136.5)	12.7-55
Testosterone (nmol/L)	49	19.8 $\pm$ 9.0	18 (4.7, 9.7, 13, 26, 32.2, 45.1)	8.4-28.8
Inhibin (pg/ml)	49	115.5 (53.4)	115.5 (25.1, 49.1, 67.1, 151.9, 202.8, 244.7)	100-400
GnRH Peak:				
LH (miu/ml)	42	21.8 $\pm$ 15.7	17.8 (4.8, 7.2, 12.9, 28.8, 35, 83.3)	
FSH (miu/ml)	42	4.9 $\pm$ 4.6	3.55 (0.6, 1, 2.3, 6.9, 8.4, 27.6)	
E2 (pg/ml)	42	9.5 $\pm$ 27.7	8 (-70.5, -16, -2.5, 22, 37.5, 95)	
Testosterone (nmol/L)	42	3.2 $\pm$ 6.6	2.5 (-11.8, -5.3, -0.2, 6.5, 10.2, 21.6)	
Inhibin (pg/ml)	42	18.8 $\pm$ 50.0	8.6 (-61.4, -33.2, -11.8, 43.6, 54.6, 188.7)	
GnRH Sum:				
LH (miu/ml)	30	63.5 $\pm$ 43.2	52.7 (12.6, 19.1, 28.3, 85.2, 118, 210.3)	
FSH (miu/ml)	30	17.8 $\pm$ 16.6	13.9 (1.7, 2.4, 6.6, 24.6, 35.7, 86.7)	
E2 (pg/ml)	26	-14.0 $\pm$ 97.8	10 (-318, -143, -36, 47, 88, 113)	
Testosterone (nmol/L)	30	3.3 $\pm$ 23.2	6.3 (-51, -27.1, -12.5, 17.9, 32.8, 54.8)	
Inhibin (pg/ml)	28	-3.5 $\pm$ 152.2	11.4 (-331, -215, -94.4, 89.5, 189, 380)	

4.3. Bivariate and multivariate associations

Details of statistically significant associations ($p < 0.05$) are mostly presented because details of non-significant associations were largely non-contributory. Multivariate analysis using serum DDT uncorrected for serum lipid but controlled for lipids as exposure measures in the model did not yield results that were different to that found when using DDT levels corrected for lipids.

4.3.1 Symptoms of sexual dysfunction and exposure

There were no statistically significant associations ($p < 0.05$) found between symptoms of sexual dysfunction (lack of interest in sex, difficulties in full erection, early ejaculation and inability to ejaculate) and malaria years both in bivariate analysis and multiple logistic regression analysis, adjusting for age and school years.

Of the DDT metabolites, only p,p'DDE was positively associated with reported problems obtaining a full erection in bivariate analysis ($p = 0.0486$), but not when adjusted for age and schooling ($p = 0.219$).

4.3.2 Pregnancies

The number of pregnancies fathered was not significantly associated with malaria years or any of the DDT metabolites in bivariate and multivariate analyses (with age as the only covariate).

Pregnancies increased significantly with age in bivariate analysis ($p < 0.05$)

(Unadjusted $\hat{\beta} = 0.18 \pm 0.05$ pregnancies per year, $R^2 = 0.22$, $n = 53$).

4.3.3 Semen parameters

There were few statistically significant associations between semen parameters and measures of exposure. In bivariate analysis, only sperm density was significantly associated with p'p' DDE (Table 4.8), but in the opposite direction to what was expected. Nor were other covariates such as age, abstinence period, the presence of one physical abnormality and fever in the last 2 months significantly associated with any of the semen parameters at bivariate level.

Nor were semen parameters strongly associated with exposure in any of the multivariate analyses, with only sperm count negatively associated with p'p' DDT ($p = 0.044$, Table 4.8). None of the covariates were significant predictors of semen parameters in multivariate models that excluded exposure.

Table 4.8 Significant associations of semen parameters with DDT metabolites

Semen Parameter (Unit)	Exposure	Unadjusted Associations			Adjusted Associations *		
		$\beta \pm SE$	P	R^2 (n)	$\beta \pm SE$	P	R^2 (n)
Count (million)	p'p'DDT	-2.16 $\pm$ 1.5	0.146	0.03 (42)	-3.65 $\pm$ 1.7	0.044	0.047 (38)
Density (million/ml)	p'p'DDE (μ g/g of lipid)	0.47 $\pm$ 0.22	0.035	0.08 (43)	0.39 $\pm$ 0.32	0.233	0.04 (38)
* Adjusted for age, abstinence period, the presence of one or more physical abnormalities and fever in the last 2 months							

4.3.4 Endocrine function (basal hormones, SHBG and GnRH responses)

4.3.4.1 Relationships between endocrine function with semen parameters and fertility outcomes

There were very few statistically significant relationships between basal and post GnRH hormone levels and semen parameters. Semen density was negatively associated with basal testosterone ($\hat{\beta} = -3.8 \pm 1.83$, $p = 0.046$, $R^2 = 0.04$, $n = 45$) when adjusted for age, abstinence period, the presence of one or more physical abnormalities and fever in the last 2 months, and positively with basal inhibin, both

when adjusted ($\hat{\beta} = 0.76 \pm 0.276$, $p = 0.010$, $R^2 = 0.12$, $n = 39$) and not adjusted ($\hat{\beta} = 0.63 \pm 0.22$, $p = 0.006$, $R^2 = 0.14$, $n = 48$) for the same covariates.

The number of pregnancies fathered was also positively associated with the sum inhibin response after the GnRH challenge both unadjusted ($\hat{\beta} = 0.012 \pm 0.01$, $p = 0.035$, $R^2 = 0.17$) and when adjusted for age ($\hat{\beta} = 0.13 \pm 0.004$, $p = 0.004$, $R^2 = 0.47$, $n = 26$).

4.3.4.2 SHBG

Baseline SHBG was a significant positive predictor of baseline testosterone ($\hat{\beta} = 0.26 \pm 0.46$, $p = 0.000$, $R^2 = 0.39$, $n = 26$), but a negative predictor of post GnRH challenge peaks of testosterone ($\hat{\beta} = -0.12 \pm 0.42$, $p = 0.016$, $R^2 = 0.16$, $n = 26$) and E2 ($\hat{\beta} = -0.51 \pm 0.18$, $p = 0.006$, $R^2 = 0.14$, $n = 26$) when adjusting for age.

Age was not a significant covariate of endocrine hormones, but was positively associated with baseline SHBG ($\hat{\beta} = 0.86 \pm 0.36$, $p = 0.021$, $R^2 = 0.09$, $n = 49$).

There was no significant association of baseline SHBG with malaria years or any of the DDT metabolites during bivariate and multivariate analysis (adjusted for age).

4.3.4.3 Relationships between endocrine hormones

The only significant relationships among basal and post GnRH challenge hormone levels of the same hormone when adjusted for age and baseline SHBG, were between basal FSH and both the peak FSH ($\hat{\beta} = 0.269 \pm 0.107$, $p = 0.017$, $R^2 = 0.11$, $n = 42$) and the sum FSH ($\hat{\beta} = 0.086 \pm 0.038$, $p = 0.029$, $R^2 = 0.27$, $n = 26$) GnRH response, and also between basal E2 and the sum E2 ($\hat{\beta} = -0.167 \pm 0.077$, $p = 0.042$, $R^2 = 0.11$, $n = 26$) GnRH response.

Relationships among basal endocrine hormones were adjusted for age and basal SHBG. Basal E2 and testosterone were not significantly related to basal LH. Basal LH and basal FSH were positively associated. (Adjusted $\hat{\beta} = 0.42 \pm 0.53$, $p = 0.000$, $R^2 = 0.62$, $n = 49$). Basal testosterone had a strong positive association (Adjusted $\hat{\beta} = 2.58 \pm 0.65$, $p = 0.000$, $R^2 = 0.31$, $n = 49$) with basal E2. Basal FSH had a significant negative relationship to basal inhibin (Adjusted $\hat{\beta} = 0.03 \pm 0.007$, $p = 0.000$, $R^2 = 0.27$, $n = 49$)

4.3.4.4 Basal and post GnRH hormone levels and exposure

Relationships between hormones and exposure were adjusted for age and baseline SHBG¹.

¹ Including body mass index as a covariate in multivariate models, did not change associations.

Malaria years were not strongly associated with hormone levels during both bivariate and multivariate analysis, and the only significant relationship was with peak testosterone level after the challenge (Adjusted $\hat{\beta} = 0.38 \pm 0.173$, $p = 0.035$, $R^2 = 0.24$, $n = 42$).

There were no significant associations between DDT metabolites and basal or post GnRH LH or FSH both unadjusted or when adjusted for age and basal SHBG.

There were also few relationships between DDT metabolites and inhibin with only p'p'DDE significantly associated with peak levels after the challenge (Adjusted $\hat{\beta} = 0.39 \pm 0.17$, $p = 0.031$, $R^2 = 0.07$, $n = 41$) and o'p'DDD to basal levels (Adjusted $\hat{\beta} = -175.8 \pm 82.9$, $p = 0.04$, $R^2 = 0.09$, $n = 46$).

There were positive relationships between most DDT metabolites and basal E2 and testosterone (Table 4.9). Table 4.9 summarises the relationships between all the DDT metabolites and basal levels of the two hormones after adjusting for age and baseline SHBG, and shows the influence of outliers and influential points on these relationships.

Table 4.9 shows that p'p' DDT and p'p'DDD were positively associated ($p < 0.08$) with basal E2 and testosterone both when including and excluding outliers or influential points. Basal E2 was significantly ($p < 0.05$) related to p'p' DDE when including outliers and influential points, and o'p' DDT, o'p' DDE and o'p' DDD when excluding the points.

The details of the outliers and influential points, determined by student residuals > 2.5 , Cook's $d > 0.5$ and Dfbeta's > 1 are summarised in Table 4.10. Two points (numbered 1 and 2 in Table 4.10) influenced all relationships between basal E2 and DDT metabolites and had substantially raised basal E2 levels, with most DDT metabolite levels also being high. One of these two points (numbered 2 in Table 4.10), which also had a high testosterone level, also influenced the relationship between basal testosterone and o'p' DDE. Another point (numbered 3 in Table 4.10) which influenced the relationships between basal E2 and o'p' DDT and o'p'DDD had very high levels of these two metabolites. The fourth point (numbered 4 in Table 4.10) which influenced all relationships between basal testosterone and DDT metabolites had a high level of basal testosterone. As an example, Figure 4.3 demonstrates that excluding outliers slightly weaken the bivariate linear relationship between basal E2 and p'p' DDT.

Although exclusion of outliers and influential points was considered important in determining the consistency of relationships, this does reduce power and therefore relationships including all data points are discussed in detail in the thesis.

Table 4.9 DDT metabolite linear regression predictors of baseline E2 and testosterone including and excluding outliers and influential points

DDT metabolite ($\mu\text{g/g}$ lipid)	Including outliers & influential points				Excluding outliers & influential points			
	n	$\beta \pm \text{SE}$	P	R^2	n	$\beta \pm \text{SE}$	P	R^2
Baseline E2 (pg/ml)								
p'p'DDT	46	1.14 ± 0.33	0.001	0.31	44	0.49 ± 0.27	0.079	0.25
p'p'DDE	46	0.24 ± 0.11	0.038	0.19	44	-0.16 ± 0.11	0.167	0.17
p'p'DDD	46	22.0 ± 6.9	0.003	0.32	44	10.5 ± 5.5	0.061	0.21
o'p'DDT	46	2.03 ± 2.55	0.431	0.11	43	4.76 ± 2.15	0.033	0.22
o'p'DDE	46	110.3 ± 81.2	0.182	0.14	44	148.6 ± 53.1	0.008	0.27
o'p'DDD	46	50.1 ± 54.8	0.358	0.12	43	101.6 ± 46.7	0.036	0.22
Baseline Testosterone (nmol/L)								
p'p'DDT	46	0.17 ± 0.67	0.014	0.51	45	0.17 ± 0.062	0.011	0.53
p'p'DDE	46	0.26 ± 0.023	0.269	0.45	45	0.29 ± 0.021	0.186	0.47
p'p'DDD	46	2.88 ± 1.43	0.050	0.48	45	3.05 ± 1.33	0.027	0.51
o'p'DDT	46	-0.117 ± 0.5	0.816	0.43	45	-0.026 ± 0.47	0.957	0.45
o'p'DDE	46	13.68 ± 15.95	0.396	0.44	44	19.47 ± 14.08	0.174	0.48
o'p'DDD	46	-0.64 ± 10.74	0.958	0.43	45	1.26 ± 10.15	0.902	0.45
Note: Relationships adjusted for age and basal SHBG								

Table 4.10 Details of outliers and influential points (O/I) for the linear regression relationships between DDT metabolites and basal E2 and testosterone

O/I point	Age (yrs)	Serum DDT(μ g/g lipid)						Basal endocrines		
		p'p'DDT	p'p'DDE	p'p'DDD	o'p'DDT	o'p'DDE	o'p'DDD	E2 (pg/ml)	TST* (nmol/L)	SHBG nmol/L
1	54	38.98	273.5	1.08	1.26	0.095	0.036	159.5	23.1	34
2	51	63.3	135.9	2.43	3.83	0	0.144	208.5	39.5	64.5
3	41	38.3	171.75	1.76	10.3	0	0.443	22.5	12.6	30.5
4	36	29.7	25.5	0.90	1.31	0.051	0.027	55.5	39.4	50

* TST : Testosterone

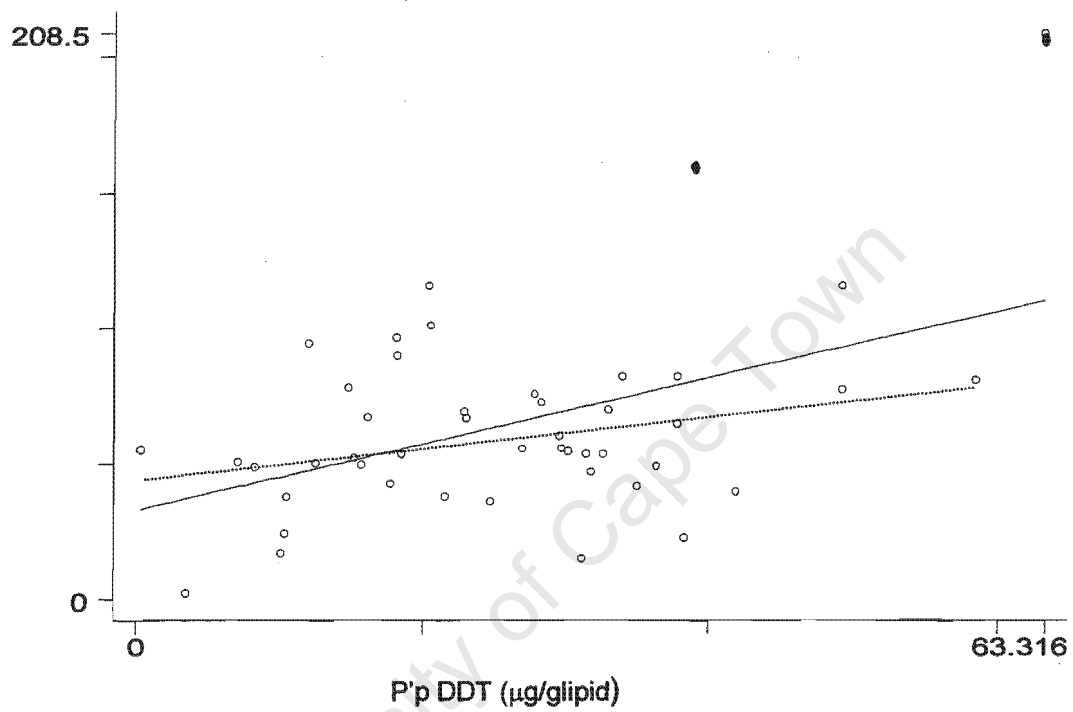
Points 1 and 2 were outliers for all relationships between basal estradiol and DDT metabolites adjusted for age and basal SHBG

Point 2 was also an outlier for the relationship between basal testosterone and o'p'DDE

Point 3 was an outlier for the relationship between E2 o'p' DDT and o'p'DDD

Point 4 was also an outlier for all the relationships between basal testosterone and DDT metabolites

Figure 4.3 Simple linear regression relationship between basal E2 and p'p'DDT including and excluding outliers (n = 46)



● Outliers. There were two outliers for this relationship (Points labelled 1 and 2 in Table 11)

— Regression line for relationship including outliers

..... Regression line for relationship excluding outliers

Table 4.11 shows both the crude associations and the full linear regression models for relationships of basal E2 and testosterone with p'p' DDT, which had a strong association with both hormones.

Table 4.11 Linear regression models showing the crude and adjusted associations of p'p' DDT with basal E2 and testosterone

Variable (unit)	$\hat{\beta} \pm (SE)$	P
Basal E2 (pg/ml)		
p'p' DDT ($\mu\text{g}/\text{glipid}$)		
Crude ($R^2 = 0.22$, $n = 46$)	1.22 ± 0.35	0.001
Adjusted	1.14 ± 0.33	0.001
Age (years)	0.74 ± 0.54	0.175
Basal SHBG (nmol/L)	0.38 ± 0.20	0.064
Constant	-13.6 ± 23.8	0.571
$R^2 = 0.31$ (Mutivariate model), $n = 46$		
Basal testosterone (nmol/l)		
p',p' DDT ($\mu\text{g}/\text{glipid}$)		
Crude ($R^2 = 0.09$, $n = 46$)	0.20 ± 0.09	0.023
Adjusted	0.17 ± 0.07	0.014
Age (years)	-0.19 ± 0.11	0.093
Basal SHBG (nmol/L)	0.26 ± 0.04	0.000
Constant	-13.6 ± 4.9	0.009
$R^2 = 0.51$ (Mutivariate model), $n = 46$		

FREE AND BOUND TESTOSTERONE

With regard to testosterone, the linear regression models used in Table 4.9 correspond to “model 1” mentioned earlier (Data analysis, section 3.9.2 in Chapter 3) whereby the effect of DDT metabolites on free testosterone is measured by adjusting total serum testosterone for SHBG. The results therefore indicate an effect of p'p'DDT and p'p'DDD on free testosterone. When “model 2” was used, no significant relationship was found between the testosterone/SHBG ratio and DDT metabolites. “Model 2” suggests an interaction between DDT metabolites and SHBG. When using “model 3”, basal SHBG was not found to interact significantly with DDT metabolites and therefore demonstrating that “model 2” is not the appropriate model to use. This is also supported by the fact that that none of the DDT metabolites were found to be significantly associated with SHBG. Although basal testosterone was not found to be significantly related to any of the DDT metabolites, when using “model 3”, this could be due to a lack of power when including too many terms in the regression model.

The individual effects of the various DDT metabolites on baseline E2 and testosterone, were explored in a multivariate model by firstly including all the metabolites except o'p' DDT and o'p'DDD which had a correlation of > 0.9 ($VIF > 10$) simultaneously into the same model (including outliers and influential points) and then by additionally not including p'p' DDT and p'p'DDD, which had a correlation of 0.7 into the same model. In models that included either p'p' DDT or p'p' DDD, but not both, these were the only significant DDT metabolites ($p < 0.05$), with small changes in

the correlation coefficients and model variance explained. When both p'p' DDT and p'p' DDD were included in the same model, the association between p'p' DDD and the two hormones disappeared, while that between p'p' DDT and the two hormones remained when controlling for o'p' DDT, with minimal effects on the regression coefficient and variance explained (R^2). In the latter case o'p' DDT became a significant negative predictor of the two hormones. The highest model R^2 when including DDT metabolites simultaneously into the same model, was 0.35 for basal E2 and 0.56 for basal testosterone showing that the other metabolites added little to the variance explained by p'p' DDT alone for these hormones (0.31 for basal E2 and 0.51 for basal testosterone, Table 4.11).

Multiple logistic regression analysis confirmed that abnormally high basal E2 and testosterone levels were related to high p'p' DDT and p'p' DDD levels (Table 4.12).

With regard to relationships between DDT metabolites and the GnRH response of testosterone and E2, multiple logistic regression analysis showed that poor testosterone responders were strongly related to p'p' DDT (Table 4.13). The poor testosterone responders had significantly higher ($p = 0.05$) basal testosterone even when controlling for basal SHBG and age, but not significantly abnormal basal or post GnRH E2, LH, FSH, inhibin or SHBG or abnormal semen, fertility or sexual function.

Those with abnormally high basal E2 had significantly lower semen morphology ($p < 0.05$).

Table 4.12 Relationships between dichotomous basal E2 and testosterone with dichotomous p'p'DDT and p'p'DDD using multiple logistic regression analysis

DDT metabolite*	Odds Ratio	95 % CI	P-value
Outcome = E2 [#] , n = 46			
p'p' DDT	2.47	0.66-9.2	0.178
p'p' DDD	3.40	0.93-12.3	0.0650
Outcome = Testosterone [#] , n = 46			
p'p' DDT	74.1	0.75-7332.1	0.066
p'p' DDD	27.5	0.75-1001.3	0.071
Note: Model includes age and basal SHBG as continuous variables			
* DDT metabolite variable dichotomized at the median			
[#] Outcomes dichotomized at the upper limit of the normal range (refer to Table 4.7)			

Table 4.13 Relationships between poor testosterone GnRH responders with dichotomous p'p' DDT using multiple logistic regression analysis

DDT metabolite *	Odds Ratio	95 % CI	P-value	^s Model R ²
	Outcome = E2 [#] , n = 41			
p'p' DDT	6.9	1.03-46.7	0.046	0.25
^s Model includes age and basal SHBG as continuous variables * P'p' DDT dichotomized at the median [#] Maximum testosterone GnRH response dichotomized at 0				

4.3.4.5 Relationship between different exposure indices

The relationship between DDT metabolites and malaria years was assessed using the KSM procedure in Stata [Stata Corporation,1997]. This procedure uses weighted and locally unweighted smoothing of any two variables being analysed, and thereby produces an exploratory relationship by joining the points in a scatter plot. The KSM procedure showed the above relationship to be linear, as best illustrated by the plot between p'p' DDE and malaria years shown in Figure 4.4. A multiple linear regression model was therefore used to determine the association between DDT metabolites and malaria years, with age as a covariate (Table 4.14). Although the residuals were not normally distributed and this was alleviated by square-root transformation, the

untransformed results are presented because transformation did not change the nature of associations substantially.

Malaria years was significantly, strongly and positively associated (Table 4.14) with p,p' DDE, but not significantly and mostly negatively associated with the other DDT metabolites. Outliers did not have an effect on these relationships. Table 4.14 summarises the multiple linear regression relationship between p,p' DDE and malaria years.

Figure 4.4 KSM plot between p,p'DDE and malaria years

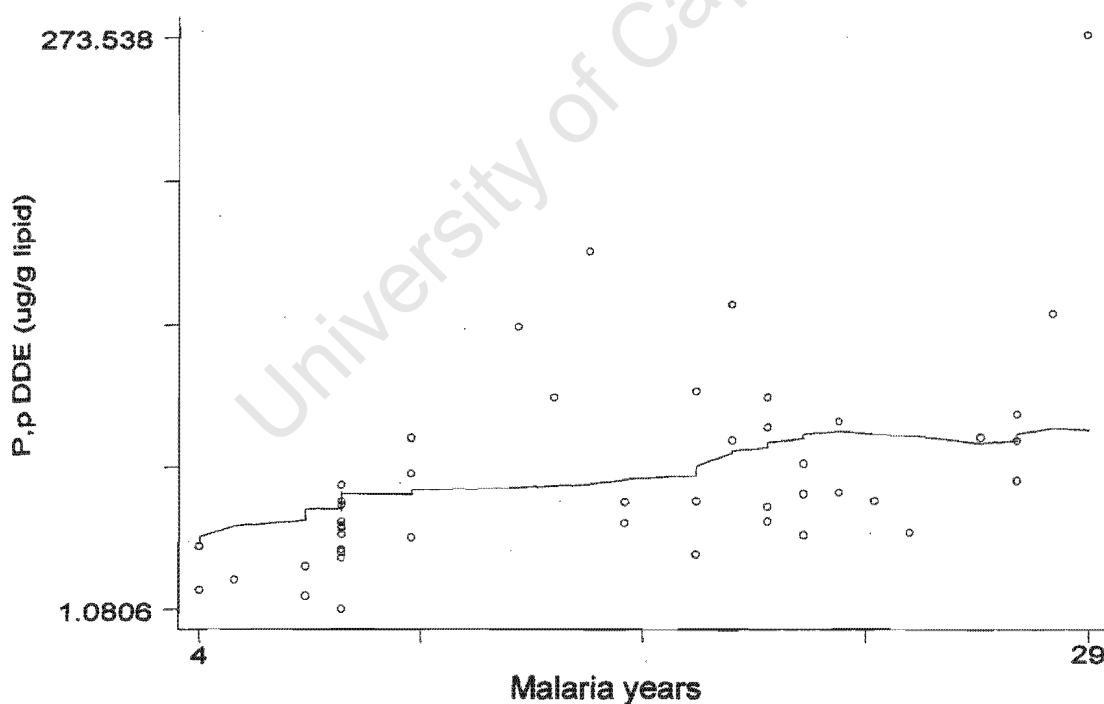


Table 4.14 Multiple linear regression relationship between p'p' DDE and malaria years

Variable (unit)	$\beta \pm (SE)$	P
p'p' DDE ($\mu\text{g/g}$ lipid)		
Malaria years (years)	3.0 ± 1.19	0.016
Age (years)	0.65 ± 1.0	0.522
Constant	-9.2 ± 33.9	0.013
$R^2 = 0.27, n = 47$		

4.3.4.6 Workers with infection

Three workers were found to have infections as indicated by their increased white cell counts in semen during semen analysis, but the exact cause was not known. The physical examination indicated two of them to have signs of epididymoorchitis and they were given referral letters by the doctor to the nearest clinic. Excluding these participants from the dataset, had a minimal effect on exposure outcome relationships.

4.4 References

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Chapter 5: Discussion

5.1 Important findings in the study

In this study, associations of any of the selected hormonal and reproductive health endpoints with measures of exposure, either based on years working at the MCC or levels of DDT metabolites, were weak and inconsistent. For malaria years the only statistically significant association was with the peak testosterone level after the challenge ($\hat{\beta} = 0.38 \pm 0.173$, $p = 0.035$, $R^2 = 0.24$).

For DDT metabolites, the only reasonably strong and consistent associations were with the hormones, E2 and testosterone, especially baseline measures, but also some dynamic measures in response to GnRH challenge. The most important finding in the study was that basal E2 and testosterone levels were positively associated with p'p' DDT and p'p'DDD levels (Table 4.9). For testosterone, there appears to be an effect on the unbound portion, because there was no evidence that the association was modified by SHBG. The effect of p'p' DDD might have been due to a high correlation with p'p' DDT ($r = 0.7$), because when both were included in the same multivariate model, the effect of p'p'DDD disappeared and there was little added to the variance explained of the two hormones when including other metabolites simultaneously into the same model than when including p'p'DDT alone. Both p'p'DDT and p'p'DDD are short to medium measures of exposure, suggesting that the effects found were due to exposure experienced recently by workers. p'p' DDE, the long term DDT exposure

measure, was found to be neither consistently nor significantly related to these hormone levels or to any reproductive endpoint measured.

An interesting finding was also that poor testosterone GnRH responders had significantly higher p'p'DDT (Table 4.13) and significantly higher basal testosterone.

The effect of DDT metabolites on semen, sexual function and fertility outcomes was not consistent.

Another interesting finding was that those with abnormally high basal E2 had significantly poorer semen morphology ($p < 0.05$).

It is perhaps also worth noting that mean baseline E2 levels (Table 4.7) were substantially above the upper limit of the normal range reported for the assay (50 pg/ml) for adult males and that most participants (65%) had levels higher than this value. Baseline levels of testosterone, however, which would also be expected to be high since E2 is produced from testosterone at the periphery (Figure 4.5), tended towards the high end of the normal range. The normal reference range was derived from a different population to that of the study population, and is used routinely in clinical laboratories in South Africa. It is uncertain whether the reference values are applicable to this particular study population. Testosterone has been shown to decrease with age [Bremner et al., 1985, Vermeulen et al., 1995] and the high mean age of the participants when compared to the manufacturer's reference population

(mean = 34 years), would indicate the high median testosterone levels in the study population. One would expect an age effect on E2 levels as well, but this has not been reported previously. Age was not found to have an effect on the basal levels of the two hormones in this study, but this might also be due to the relatively narrow age range of the participants. The free androgen index ratio's (median = 55, interquartile range¹: 38-69) of workers (mean age = 44.3 ± 9.0 years, n = 49) in this study was, however, similar to that reported for North Carolina farmers [Martin et al., 2002] in the age groups < 45, 45-54 and 55-64 years (for e.g. median androgen index for the 45-54 years age group was 57.4 and interquartile¹ range: 49.0-71.5) with substantially lower blood DDE levels.

Inhibin has previously been shown to be a marker of male reproductive ability and it is interesting that a high proportion (45%) of inhibin levels amongst participants were found to fall below the normal range. However, mean levels were not outside the normal range and inhibin levels were not found to be related to DDT exposure. Again the issue of appropriate normal reference values is raised.

The bivariate linear regression model for basal E2 predicts that an increase in serum p,p'-DDT equivalent to the mean (26.9 $\mu\text{g/g}$ lipid) level found in the workers in this study, will produce an increase of 32 pg/ml ($\hat{\beta} = 1.22 \pm 0.35$ pg/ml/ $\mu\text{g/g}$ lipid) in basal E2 and 5.4 nmol/L in basal testosterone ($\hat{\beta} = 0.2 \pm 0.9$ nmol/L/ $\mu\text{g/g}$ lipid). This could explain why basal E2 levels in the study were above the reference range and basal

¹ 25th – 75th percentile

testosterone levels were not. The R^2 (proportion of variance explained, 0.22 for basal E2 and 0.09 for basal testosterone) in these models is, however, indicative of substantial (unexplained) variability about the mean levels.

5.2 Possible biological mechanisms explaining results

The biological mechanisms that can be used to explain the results based on known *in vitro* and *in vivo* effects of DDT are: 1) anti-androgenic effects on the hypothalamus and pituitary 2) estrogenic effects on the hypothalamus and pituitary 3) Alteration in SHBG biosynthesis and 4) Alteration of testosterone and E2 biosynthesis [Crisp et al., 1998; Topari et al., 1996; Health Council of the Netherlands, 1997]. Another possibility 5) is that there is a mixture of estrogenic and anti-androgenic effects. There is also a possibility that the results may be due to unknown non-hormonal mechanisms.

5.2.1 Evaluation of known biological mechanisms

- 1) The most likely explanation for the positive association of basal E2 and testosterone with DDT metabolites is an overall anti-androgenic effect whereby DDT metabolites block androgen receptors in the pituitary and hypothalamus, resulting in increased pituitary release of LH stimulating increased testosterone secretion. Peripheral aromatisation of testosterone results in raised E2. Figure 5.1 outlines this hypothesis and also shows that there are inconsistencies.

2) INCONSISTENCIES IN ANTI-ANDROGENIC HYPOTHESIS:

- Firstly, DDT metabolites were not positively associated with FSH and LH. An explanation for this could be that the blood levels of these hormones are very variable because they are secreted in pulsatile fashion. There was also no association between basal LH with testosterone or E2 as one would expect in the male gonadal axis, indicating that relationships with basal LH are difficult to measure perhaps for the same reason i.e.pulsatile secretion. LH and FSH levels are time dependent, although the coefficients of variance (Table 4.7) of LH and FSH were not much higher than that of testosterone and E2.
- The second inconsistency is that, although the aromatisation of testosterone to E2 is suggested by the strong positive association of basal levels of these two hormones, associations between DDT metabolites and E2 were stronger than those between testosterone and DDT metabolites (Table 4.9).
- Thirdly, although p'p'DDT was negatively associated with sperm count, the effect of DDT metabolites on semen quality were not consistent. The expectation was that peripheral blockage of androgen receptors will inhibit the effect of testosterone, and will result in semen abnormalities.

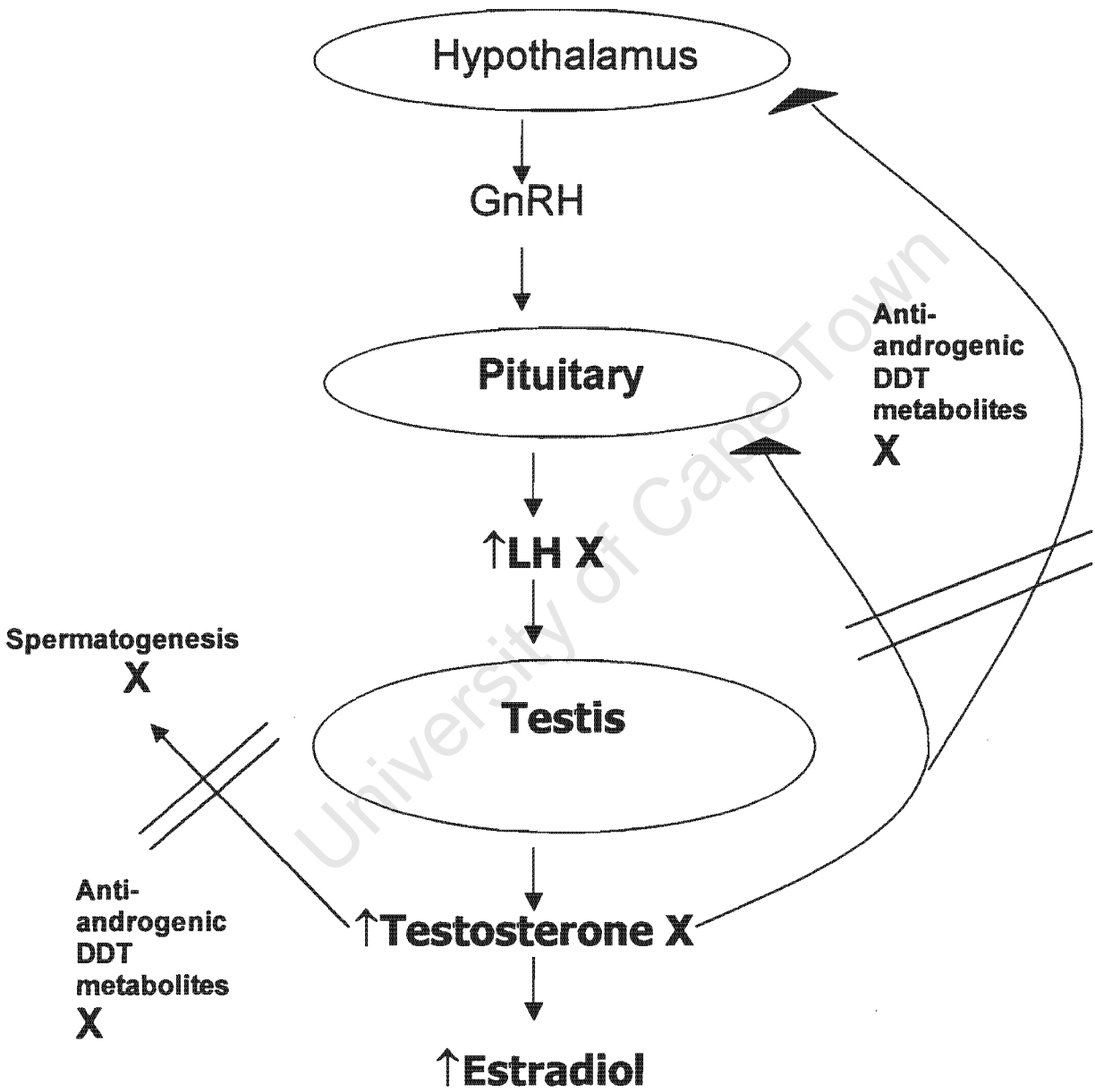
- *In vitro* and laboratory studies with rats have shown anti-androgenic effects [Kelce, 1995; Welch et al., 1969; Duby et al., 1971; Clement and Okey, 1972; Gellert et al., 1972]. but *in vitro* studies cannot be easily compared to studies on humans where many physiological processes are occurring simultaneously and laboratory rats are also not easily comparable because of differences between species. No previous epidemiological study have found that DDT has an effect on semen [Dougherty et al., 1980; Bush et al., 1986; Stachel et al., 1989; Smith, 1991; Cocco 1997], while other endocrine disrupting chemicals have been shown to have negative effects [Crisp et al., 1998; Topari et al., 1996; Health Council of the Netherlands, 1997].
- Fourthly, the strongest associations of basal E2 and testosterone were with p,p'DDT, which is not known to be strongly anti-androgenic or estrogenic, and p,p' DDD which has been shown to be estrogenic [Kelce, 1995]. In the literature, p,p' DDE, has been shown to be the most potent anti-androgen of the DDT metabolites [Kelce, 1995] and o,p' DDT, was shown to be estrogenic and anti-androgenic, but neither finding was reproduced in this study. This could again be due a difference between *in vitro* and *in vivo* systems. There were no previous studies found in the literature that investigated the reproductive health effects of all the DDT metabolites. p,p' DDE has been found to be associated with adverse male reproductive effects in one small study [discussed later, Ayotte, 2001].

- An increase in post-GnRH hormonal levels due to DDT were not found.

Blockage of androgen receptors in the pituitary would result in the inhibition of negative feedback from androgens and thereby in an increase in hormonal secretions. However, as discussed later in the chapter (Section 5.8), this might have been due to the fact that the GnRH test is not suitable for measuring the reproductive effects of DDT.

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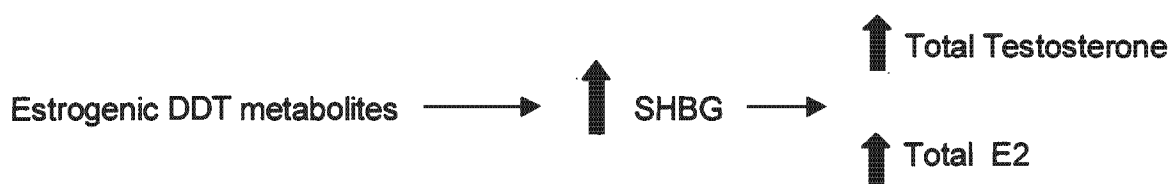
Figure 5.1 Possible effects of anti-androgens on hypothalamus-pituitary-gonadal axis



X : Inconsistencies in study results (see text)

- 2) Estrogenic effects (on the hypothalamus and pituitary) would have resulted in a decrease in basal testosterone and E2 and our results do not support this mechanism.
- 3) DDT metabolites have previously been shown to be stimulators and inhibitors of SHBG synthesis [Edmunds et al., 1990]. The positive association between DDT metabolites and basal E2 and testosterone could also have resulted from estrogenic effects of DDT metabolites which increases basal SHBG leading to increased total testosterone and E2 (Figure 5.2). There was, however no association found between DDT metabolites and basal SHBG. It might be that a small effect on SHBG could result in large effects on testosterone and E2.
- 4) Raised testosterone and E2 could be due to increased biosynthesis, or decreased metabolism and/or binding to SHBG (Figure 5.2). o'p'DDT is known to inhibit glucocorticoid biosynthesis [Crisp, 1998], it might be that p'p'DDT stimulates the synthesis of testosterone. There is, however, no literature that supports this proposed mechanism.
- 5) There is also a possibility that there was a mixture of effects between estrogenic and anti-androgenic DDT metabolites. When all DDT metabolites were included in the same multivariate model, p'p'DDT had a positive effect on the basal levels of testosterone and E2, while o'p'DDT had a negative effect.

Figure 5.2 Possible estrogenic effects of DDT metabolites



5.3 Comparison of DDT reproductive effects found in this study with those found in previous studies

The few previous epidemiological studies investigating the health effects of DDT on the male reproductive system have been discussed in chapter 2 and summarised in Table 2.2. As mentioned in chapter 2, the only previous study that reported reproductive effects of DDT (partially reported in a letter)², was that by Ayotte et al. [2001] who measured endocrine levels as well as semen parameters in a group of non-occupationally exposed Mexican men with high DDT levels. In that study, p'p'DDE seems to raise SHBG thereby decreasing unbound testosterone which in turn had a negative effect on semen quality. This is not consistent with the results of this study which found neither DDT effects on SHBG levels nor on semen quality parameters. Also in this study, significant associations was found with a different metabolite, i.e. p'p'DDT and testosterone. Recently, Martin et al. [2002], also found no effect of DDE on the testosterone levels of North Carolina farmers, but exposure

² Although the statistical significance of relationships were not reported upon in the letter, further enquiry confirmed this.

levels in that study were substantially (about 30 times) lower than in this study. The other epidemiological studies investigating the relationship between DDT exposure and the male reproductive system had limitations such as low exposures, insensitive exposure measurements, limited outcome measurements and insensitive study design, as discussed in chapter 2, and were not able to show that DDT had any effects on reproductive outcomes including semen parameters, hormone levels, sexual function and fertility measures. On the other hand, endocrine disrupting chemicals such as DES in utero, chlordecone, DBCP, vinclozolin, phthalates and TCDD have been shown to cause abnormalities in semen and hormonal balance (chapter one).

In summary, our findings on the effects of DDT on the male reproductive system, are consistent with the literature which does not provide evidence for a clear effect of long-term DDT exposure on male reproductive health in human subjects.

5.4 Multiple comparisons

The lack of consistency between a priori predicted patterns and observed patterns of associations of specific DDT metabolites with hormonal endpoints raises some question about the likelihood of associations being due to chance in view of the multiple comparisons carried out in the data analysis. A total of 175 comparisons (not all of which were independent e.g. some DDT metabolites correlated with others) were made between exposure ($n = 7$) and outcome indices ($n = 25$) of which 14 (8%) were

significant at the 5% level using multiple linear regression or multiple logistic regression or bivariate analysis. Of these, only the positive bivariate association between sperm density and p'p' DDE was clearly in the unexpected direction, whereas the other 13 (7 % of total) did not contradict the study hypothesis. Eleven (6%) associations were significant at the 5% level when using only multiple linear regression analysis (excluding bivariate and multiple logistic regression analysis). The percentage of significant associations found in the study was therefore not substantially more than that expected by chance (5%). On the other hand, one should bear in mind that the total number of participants was modest so that some associations may not have been identified owing to suboptimal study power especially for endocrine, sexual function and fertility endpoints. Also, although calculations made before the study indicated that a sample of 54 participants would have adequate power to detect changes greater than 60% in certain semen parameters (100 % for motility and 77% for density), potential effects on semen and other reproductive endpoints from this study appear to be more subtle. The number of sperm donors was also less than 54.

5.5 Bradford Hill criteria

Most of the Bradford Hill [Bradford Hill, 1965] criteria, used as a guideline for assessing causality in epidemiological studies, are not fulfilled with regard to reproductive health effects of DDT on the male reproductive system:

As discussed before, significant associations found between outcomes and exposure in the study results, (which included dose dependent analysis in the form of linear and logistic regression analysis), could have been due to chance and were not consistent despite the fact that the study sample had good exposure contrast, including very highly exposed workers. **Strength** (1) and **biological gradient** (2) were not demonstrated because of weak DDT effects translating to uncertain exposure response relationships.

Experimental evidence (3) is not applicable to this study. The study hypothesis is, however, **biologically plausible** (4) because of the anti-androgenic and/or estrogenic effects by DDT shown in the laboratory.

Consistency (5) is not applicable due to the few comprehensive DDT studies done so far. Reproductive effects by **analogous** (chemically similar) (6) endocrine disrupting chemicals for e.g. chlordecone have been found.

Despite the cross-sectional design of the study, **temporality** (7) would not have been a problem for any DDT effects found because DDT exposure could be safely assumed to precede the reproductive outcome in question.

There is a problem with **specificity** (8), because many other risk factors can cause reproductive health problems.

The evidence that DDT causes male reproductive health effects via endocrine disruption does therefore not have coherence (9).

5.6 Exposure levels measured in the study

In view of the animal and cellular models which demonstrate that metabolites of DDT have potent anti-androgenic or estrogenic effects, it was plausible that malaria control workers with years of occupational exposure to DDT would demonstrate measurable effects on baseline reproductive hormone levels, dynamic hormone responses to GnRH, semen quality, sexual functioning, and fertility. The selected study population had a further advantage of substantial variability in years of DDT spraying, so that a substantial contrast in exposure could be expected within the population. This expectation was borne out in the range of measured levels of serum DDT metabolites (Table 4.4). The relative proportions of the six measured metabolites were in keeping with a priori expectation, i.e. that p'p' DDE would be highest owing to it being the main metabolite of p'p' DDT and the most highly bioconserved, followed by concentrations of p'p' DDT and then o'p' DDT as these are the two main components present in commercial grade DDT with the former at a substantially higher concentration than the latter. The concentrations of the other three metabolites, o'p' DDE, p'p' DDD and o'p' DDD, which are minor components of commercial grade DDT and are not highly bioconserved, were as expected, substantially lower than the other three metabolites. Moreover, absolute levels of DDT metabolites were high as found previously in other

studies on malaria control applicators both within South Africa and elsewhere (Table 5.1), suggesting that exposure measures in this study were valid, and that this was a suitable group for investigation of the study hypothesis.

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Table 5.1 Comparison between DDT levels measured in this study with that measured in other studies

a) DDT levels measured in the study

	Uncorrected DDT ($\mu\text{g/L}$)	DDT corrected for total lipids ($\mu\text{g/g lipid}$)
DDT metabolites	Median, (range)	Median, (range)
o'p' DDT	13.1 (0-72.9)	1.57 (0-10.3)
o'p' DDD	0.32 (0-3.07)	0.033 (0-0.43)
o'p' DDE	0.46 (0-2.47)	0.051(0-0.260)
p'p' DDT	195.2(2.5-828.3)	26.9 (0.32-63.33)
p'p' DDD	6.1(0-31.86)	0.70 (0-3.03)
p'p' DDE	378.8(8.46-4134.8)	52.3 (1.08-273.5)
DDT	206.5 (2.47-878.4)	28.2 (0.3-67.1)
DDD	6.3 (0-33.75)	0.740 (0-3.1)
DDE	379.7(8.46-4136.24)	52.4 (1.1-273.6)
Total DDT	642.79 (10.9-4761.4)	83.3 (1.40-315.00)
Note: the terms "DDT", "DDD" and "DDE" is used for the sum of the two respective metabolites and the term "Total DDT" for the sum of all six metabolites		

b) Blood and adipose DDT levels measured in other studies

Author (year)	Study sample	DDT measurement	Average (range)
Uncorrected Bloods ($\mu\text{g/L}$)*			
Nhachi [1989]	40 seasonal sprayers, Zimbabwe	DDE	60
Nhachi [1990]	68 seasonal sprayers, Zimbabwe	DDE	10 (0-150)
Bouwman et al. (1991a)	23 sprayers, Natal, SA	DDT DDE DDD Total DDT	67.7 129.3 11 202
Bouwman et al. [1991b]	Inhabitants of DDT treated dwellings	Total DDT	140.9
	Controls, Natal	Total DDT	6.04
Longnecker [2001]	2380 DDT exposed women in 1959-1966, US	DDE	25 (3-178)
	General US population	DDE	< 15
Hunter et al. [1997]	Women, US	DDE	6.97
Corrected Bloods ($\mu\text{g/g lipid}$)*			
Ayotte et al. [2001]	24 Mexican men	p'p' DDE	77.9 (17-177.2)
Martin et al. [2002]	137 male farm workers	DDE	1.2
DDT in adipose tissue ($\mu\text{g/g}$)			
Rivero-Rodriguez et al. [1997]	40 Mexican sprayers	p'p' DDE	60.98 (9.57-298.42)
		p'p' DDT	31 (0.72-34.5)
		o'p' DDT	2.1(0.07-29.74)
		p'p' DDD	0.95(0-3.51)
		Total DDT	104.48(10.56-665.56)
Smith et al. [1991]	Sprayers	Total DDT	100 -300
	General population	Total DDT	8
* Uncorrected bloods refers to DDT measurements in blood not corrected for lipid content, corrected bloods refers to DDT measurements in blood corrected for lipid content Note: in the table, the terms "DDT", "DDD" and "DDE" is used for the sum of the two respective metabolites and the term "Total DDT" for the sum of all six metabolites.			

used as an estimate for recent exposure, was highly correlated to p'p'DDE in that study, although this was not reported. In this study p'p'DDE and p'p'DDT were not highly correlated ($r = 0.41$). The use of personal protective equipment amongst South African workers is generally low, and would probably not have influenced exposure calculations in the study, while recent weight loss, reflecting changes in fat metabolism and found to have a small effect in the Mexican study, might be another limitation in this study, but not a major one.

Other factors that could have influenced correlations between DDT levels and exposure history in this and the Mexican study [Rivero-Rodriguez et al., 1997] are non-occupational exposures including inhabiting houses and huts sprayed with DDT and contamination of the environment including food and water sources. Incorrect recall by workers of spraying activities and substantial inter-worker variability in accumulating DDT metabolites could also have influenced correlations (misclassification).

In summary, the job history in this study was not a particularly useful measure of exposure when compared to biological exposure measures. However, the significant statistical relationship between malaria years and p'p'DDE levels as well the 27% variance explained, shows that more detailed job history data and weighting thereof using accurate occupational hygiene data, could be a valid long-term exposure measure. Detailed job recent history could similarly be a more valid measure of recent exposure.

5.8 Endocrine measures in the study

5.8.1 The utility of the GnRH test

The GnRH test was not found to add much information to that provided by basal endocrine hormone levels with respect to the reproductive endocrine effects of DDT on the malaria vector control workers.

The test has been used previously to investigate men with clinically significant gonadal dysfunction (cryptorchidism, varicocele, testicular torsion and vasectomy) who show an exaggerated LH and FSH response after GnRH stimulation due to reduced negative feedback on the hypothalamus and pituitary [Fisch et al., 1989; Fisch et al., 1988; Lipshultz et al., 1976; Hudson et al., 1980; Rosette et al., 1994]. It has also been used to investigate patients with gonadotrophin deficiency who show an increased FSH and LH response [Moore and Eastman, 1996]. For patients with gonadal dysfunction, the GnRH test is a sensitive test which can detect LH and FSH abnormalities even in patients who have normal basal levels, and was therefore included in this study to detect the possible disruption of the hypothalamus-pituitary-gonadal axis due to anti-androgenic and estrogenic effects of DDT.

However, it would seem from the results found in this study, that possible anti-androgenic and estrogenic effects due to DDT are not detected more effectively by GnRH testing than associations between DDT metabolites and basal endocrine levels.

The reason for the difference could be due to the fact that in patients with gonadal dysfunction or hypogonadism, pituitary release of LH and FSH is affected and that GnRH testing is effective in detecting changes in these two hormones which typically respond within 30 min of stimulation. When it comes to gonadal effects, the relevant hormones, do not respond as quickly to GnRH stimulation, and are not shown as effectively by GnRH testing. The GnRH test used in this study was based on the 2-hour test used in clinical testing. Fisch et al. [1990] has previously shown that the test could even be simplified to two points over 30 minutes, to test for changes in LH and FSH.

5.8.2 The validity of endocrine measurements in the study

Although most baseline and post GnRH hormone values were not strongly related to semen parameters or measures of fertility, both semen density and the number of pregnancies fathered increased significantly with increasing basal inhibin levels, supporting the view that inhibin, which is a direct product of the Sertoli cells in the testis, may be a marker of male fertility [Vernaeve, 2002].

The decrease in semen density found with increased basal testosterone levels could be due to suppression of gonadotropin levels and sperm counts by testosterone [McLachlan, 2002].

Overall, however, the study did not find a consistent relationship between endocrine status as measured by basal and post GnRH endocrine hormones, and semen parameters of the workers, but a lack of a relationship has also been reported in many other studies [Crisp, 1998 ;Toppari, 1996].

Baseline hormone levels in most cases were not associated with responses to GnRH stimulation with only basal FSH significantly related to peak and sum responses and E2 to the sum response. This is consistent with a previous study that did not find significant relationships between basal LH and FSH and peak post GnRH values in a study of English men [Besser, 1972]. Peak LH and FSH levels after GnRH stimulation in workers which were respectively 10 and 2 times more than basal levels, and consistent with that found in normal US and English males [Fisch, 1990; Besser, 1972]. In the study of English men, testosterone and E2 were found to have a slow response to GnRH stimulation and did not change significantly over a 2 hour period [Besser, 1972]. This is consistent with the indistinct response of testosterone, E2 and inhibin to GnRh stimulation found in the study.

Most of the expected relationships between individual hormones in the male hypothalamus-pituitary-gonadal axis, as well as relationships between endocrine hormones and SHBG were found in this study. Significant relationships between LH and testosterone, and between LH and E2 were not found, but this may be due to the variability of LH.

An expected positive relationship between basal SHBG and age [Vermeulen et al., 1995] was found. As mentioned earlier, age was not found to a significant negative predictor of basal testosterone as expected [Bremner et al., 1985, Vermeulen et al., 1995], but this might have been due to the relatively narrow age range of the participants.

Although this study did not directly measure the free testosterone levels of participants, effects on free testosterone were estimated by considering the outcome from two valid methods. The mean free androgen index of the participants (54.8 ± 23.8) corresponds with that measured amongst North Carolina farmers exposed to DDT.

5.8.3 Summary

In summary, baseline hormone and post GnRH test measurements in the study were valid, but the latter was not found to be more sensitive for measuring the reproductive health effects of DDT, as initially thought. Baseline hormone levels do not correlate with GnRH hormone responses, and neither was associated with semen parameters.

5.9 Validity of semen measurements

5.9.1 Semen analysis

Semen quality in the participating men was assessed through routine semen analysis done in the field. Comprehensive studies have confirmed that these traditional semen parameters are predictive of pregnancy outcome [Bostofte et al., 1982; Bostofte et al., 1990; Barrat et al., 1998]. There is limited, if any, evidence that computer-assisted semen analysis of motility parameters improves correlation with fertility over traditional motility measures [Krause, 1995].

The methods of semen analysis followed WHO recommendations [WHO, 1992] with the exception of sperm morphology for which the Tygerberg criteria [Kruger, 1988], which are used routinely in our laboratory, were followed as there is evidence that this is a reproducible measure which better correlates with a well-defined biological endpoint, *in vitro* fertilization success rates, than the WHO recommended approach [Menkveld and Kruger, 1995; Kruger et al., 1996]. However, with 84% semen samples in this study and 71% of those in a study of lead exposed workers [Table 5.2; Robins, 1997], falling in the narrow interval of 0-6% normal morphology using Tygerberg criteria, there could be an argument that these criteria are inappropriately strict for an epidemiological setting where the aim is to measure more subtle changes. The other set of criteria used, those by the WHO [WHO, 1992], are less stringent, but are also characterized on the other hand by normal morphology scores, which are

perhaps too high. An example of a study in which WHO criteria were used, is one investigating pesticide exposures amongst greenhouse workers [Abell, 2000], where more than two thirds of the semen samples had normal morphology scores above 70%.

Semen volume, motility and morphology (but not density) have previously been found to decrease with age [Kidd et al., 2000], but this has not been found in this study which had a relatively restricted age range. Other a priori covariates (abstinence, physical abnormalities and fever in the past 2 months) were also not found to be significantly associated with any of the semen parameters.

5.9.2 Methods of semen production

Masturbation is the standard method used to collect semen in studies investigating semen quality and at fertility clinics, although other methods including coitus interruptus and a number of clinical methods, are also used [Gerris, 1999; Sofikitis, 1993; Carlsen, 1992]. Most of the sperm samples in this study were produced using coitus interruptus, because it was culturally more acceptable in this particular rural population. The use of a specialised condom has been shown to produce excellent results, but has not been found to be generally acceptable [Gerris, 1999], and would also not have been appropriate in this setting.

With coitus interruptus there might be problems with semen production such as loss of ejaculate and mixing of partner fluids. Also, it is usually the first part of the ejaculate which has the highest sperm densities which is lost, resulting in lower sperm densities. Table 4.6 indicates that the semen quality of samples in this study obtained from masturbation, compares well with that obtained from coitus interruptus, although with only 4 masturbation samples, one would be cautious in drawing any conclusions. The higher semen volume of coitus interruptus samples could be due to increased fluid secretion from greater sexual stimulation, and/or fluid from the partner, and/or a greater loss of ejaculate in masturbation samples, while the lower semen densities could be due to the loss of some of the early part of the ejaculate, which usually have the highest densities.

Table 5.2 compares semen parameters in this study with that of urban South African lead workers [Robins, 1997] in which samples were obtained using masturbation (this study, which reported only very modest effects of lead on sperm quality, was chosen for comparison because it is the group which appears most similar ethnically and culturally for which data is available in the literature). Overall the semen quality in the two studies are comparable. As can be expected, the mean semen volume ($n = 48$) in this study, was lower than that found in study of lead workers [Robins, 1997], but sperm densities were similar. As a result, sperm counts were also lower ($p < 0.05$). Sperm motility was also significantly lower ($p < 0.05$). Prevalence of abstinence less than 1 day was lower than in the lead workers [Robins, 1997]. The prevalence of low (close to WHO subfertility levels) sperm counts (< 40 million), was much higher than

that in the study of lead workers [Robins, 1997], but low sperm densities (< 20 million) and motilities (< 30%) in this study were not much higher. The mean sperm morphology score in this study was much lower ($p < 0.05$) than in the study of lead workers [Robins, 1997] and the prevalence of very low morphology scores (Tygerberg infertile level = 5%) were also slightly higher. Differences in morphology scores, might be accounted for by variability in measurements amongst the two laboratories in the two studies.

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Table 5.2 Comparison of semen parameters in study and a study of South African lead workers [Robins, 1997]

Variable (unit)	N	Mean (SD)	Range	Percent of subjects
Semen volume (ml)				<2 ml ^a
Robins Study	97 48	2.3 1.6 (1.2)	Not reported 0.05-4.5	Not reported 60
Sperm Density (million/ml)				<20 million/ml ^a
Robins Study	97 48	72.7(59.9) 74.6(85.1)	0-300 0-386	20.6 29.0
Sperm Count (million)				< 40 million ^a
Robins Study	97 46	178.6(193.8) 93.8(130.3)	0-957 0-552	22.7 41.0
Motility (% motile)				< 50% ^a
Robins Study	97 46	60.8(17.3) 54.3(18.7)	33-100 0-70	22.7 26.0
Morphology (% normal)				< 5% ^b
Robins Study	97 44	4.3(4.3) 2.5(1.8)	0-17 0-6	71.1 84.1
Abstinence (days)				≤ 1 day
Robins Study	97 41	7.2(14.1) 3.8(2.9)	0.5-90 0.2-68	4.3 2.0
^a WHO criteria, [WHO, 1992]				
^b Tygerberg infertile range, [Kruger, 1988]				

5.9.3 Summary

The semen outcome measures in this study were therefore considered to be reasonably valid.

5.10 Other limitations in the study

The association between reported erectile dysfunction and o'p' DDE could be due reporting bias, because the subjects knew that the study was on the health effects of DDT and, there is a high level of concern about sexual performance in the local male population for cultural reasons.

Selection bias due to the small (< 10% of study population) study sample is not thought to be a major factor in the study, as the subjects from the three selected camps were typical of the target population. DDT levels were high as found previously in male vector control workers in South Africa [Bouwman et al., 1991a] with similar characteristics.

Generalisability of the study results is limited by the exclusion of women and children from the study. Although women and children are not known to be employed in work involving DDT spraying at the MCC, it has been shown previously in South Africa that DDT levels amongst hut dwellers [Bouwman, 1991b], are substantial. Women and children may also be more susceptible to the effects of DDT. The study population is

unlikely to differ substantially from malaria vector control sprayers in other less developed countries.

During data collection, there is no way that responders could have influenced blood results, therefore excluding bias. The possibility that a semen donor might intentionally have given someone else's sample is not strong in this community where these matters are very personal. There is also a lack of opportunity and incentive as everyone in the camp had an opportunity to participate. People who did not produce a semen sample were also studied. Technicians and other members of the research team were blinded as to the exposure status of participants as exposure questions were administered last.

The healthy worker effect is not thought to be important due to high unemployment and people being unlikely to give up a job and the fact that reproductive or any other health effects due to DDT are not likely to have an effect on employment status.

Table 4.4 shows that there was exposure contrast in the study with regard to both DDT metabolites and spraying years. The absence of an external control group in the study, was therefore not thought to be important with regard to health effects at low exposure levels, particularly as it was difficult to find a similar unexposed group of people due to DDT spraying of dwellings in the local population from which sprayers are drawn.

5.11 Applicability of study results to policy decisions and future studies

In considering the results of this study and their applicability to policy decisions, the small sample size may have led to missed effects. The population under study may also be inappropriate. We chose to study male workers conducting spraying because this was a well-defined, easily locatable group of individuals to whom we had access through their employer, the provincial government. The literature shows that measured levels of serum DDT metabolites in women living in domiciles sprayed for malaria vector control of South Africa are in a range somewhat lower than, but still comparable to, that of applicators. Women and children may be a more sensitive population for DDT effects.

As the normal development of the fetus is known to be highly dependent on hormonal influences and these lipid soluble DDT metabolites would be expected to readily cross the placenta, the offspring of women living in domiciles sprayed during pregnancy may be an especially sensitive target group. In particular, there might be increased risk for congenital anomalies of the reproductive tract associated with low androgen/high estrogen environments such as hypospadias and cryptorchidism.

Longnecker et al. [2001] have recently found a relationship between DDT and the risk of preterm delivery and intrauterine growth retardation in women. The researchers compared DDE levels in blood samples, taken from 2380 pregnant US women between 1959-1966, to recorded information about their infants, including date of

birth, gestational age and weight. They found that the adjusted odds ratios (OR) of preterm birth (ORs = 1, 1.5, 1.6, 2.5, 3.1; trend $p < 0.0001$) and small-for-gestational-age (Ors = 1, 1.9, 1.7, 1.6, 2.6; trend $p = 0.04$) increased steadily with increasing concentrations of serum DDE. South Africans exposed to DDT, sprayed for malaria vector control since 1946, can be expected to have substantially higher exposure levels (total DDE levels of 141 $\mu\text{g/L}$ previously measured in inhabitants of DDT treated dwellings, Table 5.1) than those reported by Longnecker (25 $\mu\text{g/L}$). A comprehensive study along similar lines to Longnecker would be quite important in developing a fuller understanding of adverse health effects associated with the use of DDT for malaria vector control in the entire exposed population.

5.12 References

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Chapter 6: Conclusions and Recommendations

6.1 Conclusions

6.1.1 DDT effects on male reproductive health

- a) No DDT-related overt clinical reproductive abnormalities were found amongst a group of South African malaria vector control workers.
- b) Subclinically, there were few and inconsistent etiological associations with respect to DDT exposure, and these could be due to chance as multiple comparisons were made. Additionally, the strongest associations were with metabolites (p,p'-DDT and p,p'-DDD) which have not been shown *in vitro* to be strong endocrine disruptors (whether anti-androgenic or estrogenic in action), while associations with metabolites (p,p'-DDE and o,p'-DDT) which have been shown to be stronger endocrine disruptors *in vitro*, were few and weak.

The most notable finding in the study was the abnormally high levels of basal E2 prevalent amongst the workers studied, and the consistent positive association of these levels, as well as basal testosterone levels, with DDT metabolites. These associations were especially evident for metabolites (p,p'-DDT and p,p'-DDD) known to reflect short-to-medium-term exposures. There is no clear biological mechanism that explains these associations. An overall

anti-androgenic mechanism (Figure 5.1) best explains the results, but with a number of residual inconsistencies.

These results suggest that long-term exposures to DDT experienced by currently employed male malaria vector control workers do not result in meaningful clinical or subclinical adverse reproductive health effects, despite the high exposure levels measured (orders of magnitude higher than in developed countries).

6.1.2 Measurements of male reproductive health

The study has also provided some insight into research methods for measuring male reproductive function.

- a) The GnRH challenge test, which was anticipated to be more sensitive to detect exposure effects, appeared to add little value to information provided by baseline levels of reproductive hormones alone, as far as potential endocrine disrupting effects are concerned.
- b) Production of semen via coitus interruptus seemed to be a valid alternative in communities where masturbation is not culturally practiced, because the quality of semen produced via coitus interruptus in this study was acceptable when compared to a previous local study.

- c) The critical importance of a good motivation programme to enhance participation of workers in a study of reproductive health in non-urban communities is highlighted.

6.1.3 Measurement of DDT exposure

Job-history variables were not strongly correlated with DDT metabolite levels due to misclassification resulting from an insufficiently detailed job-history. Measurement of DDT exposure should not exclude analyses of DDT metabolites in blood when job-history is insufficiently detailed.

6.2 Recommendations

6.2.1 Further study of DDT and other endocrine disrupting chemicals' effects on human health

There are still insufficient scientific studies in human populations that have investigated the health effects of DDT. There are also insufficient epidemiological data on endocrine disruption, in general. Thus further epidemiological study investigating the health effects of DDT and other endocrine disrupting chemicals is needed.

6.2.2 DDT use for malaria vector control

The Stockholm Convention [United Nations Environment Programme Chemicals (UNEP), 2001] banning 12 persistent organic pollutants, including DDT, was signed in 2001 by more than 120 countries. A limited exception was made for DDT use for malaria control. Countries, will be allowed to use DDT for malaria vector control while being urged to pursue alternatives.

The environmental persistence and harm were weighed up against DDT's cost-effective insecticidal properties and the lack of evidence of adverse human health effects.

The findings of this study are consistent with the limited number of previously conducted studies that found little evidence of adverse DDT-related effects on semen, hormones or fertility. This study similarly does not provide evidence against the use of DDT for malaria vector control.

Given the evidence for malaria rebound in many developing countries following cessation of DDT spraying of domiciles, and the current economic realities faced by such countries in using alternatives which have been shown to be less efficient, more costly and more prone to insecticide resistance, the right of developing countries to use DDT specifically for purposes of malaria control must be protected. The absence of DDT-related human health effects must be considered in the context of its considerable effectiveness in controlling morbidity and mortality from malaria, an extremely serious public health problem and contributor to the burden of disease in tropical countries.

However, given the high biological exposure levels found in this and other studies, and the environmentally persistent nature of DDT, research into alternative methods for malaria vector control should be actively pursued as a precautionary measure.

6.2.3 Highly exposed populations need to be studied and monitored.

Non-agricultural use of DDT is not a low exposure situation as shown in this and previous studies. The high DDT levels amongst malaria workers and high levels

previously measured amongst environmentally exposed persons despite exclusively non-agricultural use of DDT, suggest that there is widespread and substantial environmental contamination exclusively from malaria control programmes, with considerable human absorption.

With likely continued use of DDT in less developed countries for malaria vector control for some time yet, the small number of studies that have so far investigated human health effects, and the high biological exposures resulting from anti-malarial spraying especially in women and children, there is considerable need for further study to ensure that long-term reproductive and other effects in men, women and children are not missed.

This study, using sensitive exposure and reproductive end-points, did not find convincing adverse effects amongst malaria vector control workers. However, the findings are based on small subject numbers, and further exploration in a larger group of exposed workers and community residents would be needed to provide more definitive conclusions.

In addition to adverse effects on reproduction, other health effects such as those on cancer should also be investigated.

6.2.4 Occupational health and safety issues

The potential effects of spraying DDT for malaria control should be closely monitored. The Department of Health should monitor DDT levels amongst malaria vector control workers and compile an accurate register for each worker with regard to DDT exposure and metabolite levels including detailed job history information. This will generate better data for future investigations into health effects of DDT.

The Department of Water Affairs and Forestry (DWAF), should actively monitor water quality and wild and aquatic life in areas where DDT is sprayed. Currently, there is no monitoring of water for pesticides in South Africa, but the DWAF through the Water Research Commission (WRC), has recently funded projects investigating pesticide water pollution. One study found consistent low-level pollution of surface and ground water including drinking water in Western Cape rural waters (London et al, 2000), and this has prompted further investigation by the WRC into the development of screening methods for water pollution by pesticides. Given, the persistence of DDT in the environment and its past history of adverse environmental effects, monitoring of water and wild and aquatic life for DDT in sprayed and neighbouring areas is important.

At the moment, the use of personal protective equipment (PPE) for vector control workers may not be practical due to extremely hot and humid weather conditions in malaria-infested areas. This area needs further attention at various levels including the development of more practical PPE, investigation into behavioural and social

barriers to the use of PPE, and the training and education of workers on the importance of using PPE. Low education levels make the workers more vulnerable to exposure as a consequence of their limited ability to read and understand health and safety information.

6.2.5 More attention from stakeholders

In line with international developments, there should be greater focus by government, industry, academia and other stakeholders in South Africa on the topic of endocrine disruption. After a strategic meeting held in 2000 by the Endocrine Disruptor Group formed by the Water Research Commission (part of the Department of Water Affairs in South Africa), a five-year working programme was adopted. However no further developments took place. The involvement of the Department of Health, is especially important.

6.2.6 Exposure measurements

A better understanding of the relationships between environmental exposure, work history, body stores and blood levels of DDT and its different metabolites is needed.

Investigations into the health effects of DDT in less developed countries where laboratory measurements are costly require the use of a validated job exposure history. Detailed work history information is required to fully disentangle short and

long term DDT exposures. Biological monitoring is, however, the recommended exposure measure as it reflects more accurately the internal dose likely to result in human health effects, and integrates the different routes of exposure.

Correcting blood DDT levels for lipid content compares well with adipose tissue levels (Table 5.1).

6.2.7 Further elucidation of biological mechanisms

With regard to possible biological mechanisms explaining the results found in this study, it would seem that much still needs to be done both *in vitro* and *in vivo* to clarify the basic mechanisms involved in potential and actual endocrine disruption of the male reproductive system by DDT metabolites.

6.3 References

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APPENDICES

Appendix A: Consent Form

University of Cape Town

Consent to participate in a study of reproductive effects of exposure to DDT

1. Title of research project

Reproductive health effects of long-term DDT exposure on male malaria control
Control workers in South Africa

2. Purpose of research

The University of Cape Town is conducting this important study which will investigate the effects of DDT on the reproductive system. This is the system which is responsible for a man's ability to father normal, healthy children. By the study of men who have had work exposure to DDT, as well as a comparison group of unexposed men, we hope that we can determine whether DDT exposure has any effects on reproductive health. The investigation will include the wives and partners of the men in the study. However, the women are only asked to fill out a questionnaire.

3. Description of the research project

If you are a man and agree to participate you will be asked to complete the following during a single day:

A: Have a blood test. The end of a small plastic tube (called a butterfly) will be inserted into a blood vein in your arm and left in for a period of two and a half hours so that a medication (GnRH) may be given once and blood may be removed several times. A total of six samples of blood will be drawn (less than one half cup of blood in total). We will measure in your blood the levels of DDT and DDT metabolites and several reproductive hormones (special substances produced by your body to regulate reproduction) including LH, FSH, estradiol, testosterone, and inhibin. These are the same types of tests as are routinely done for men with a fertility problem. You will be given a medication (GnRh) so that we can see whether it changes the levels of hormones in your blood. This is a very sensitive way to test if the reproductive system is behaving in the right way.

B: complete a questionnaire. A male member of our study team will interview you in privacy to complete the questionnaire. You will be asked questions about your education, general health, reproductive health, work history, sexual history, success in fathering children, and use of cigarettes and alcohol.

C: produce a semen sample by masturbation. You will be given instructions and a collection cup by a male member of our study team and asked to produce and collect semen by masturbation in a private room. This semen sample will be examined under a microscope to see whether there are normal numbers of healthy sperm.

4. Expected benefits to you and others

You will be given a written copy of all your test results along with an interpretation of their meaning. You may wish to show these to a doctor if you are having any problems. This information could be useful in making decisions about whether and how DDT and similar chemicals should be used in the future in South Africa as well as many other parts of the world and help to protect those working with such substances at home or at work.

5. Costs to you resulting from participation in the study

The study is offered at no cost to you. In the event a problem is discovered and you wish to be seen by a doctor for it, we can recommend to you who to see. However, any additional costs of such medical visits or treatment will not be the responsibility of the study team.

6. Confidentiality of information collected

You will not be personally identified in any reports on this study. Your name will not appear on any of the records or forms. Instead each person will be given a survey number which will be written on the forms. Only the chief researcher will have the list which will link the numbers to your name.

7. Documentation of the consent

One copy of this document will be kept together with our research records on this study. A second copy will be given to you to keep.

8. Contact person.

You may contact the following person for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

Name of person _____ Telephone _____

9. Consent of the participant

I have read the information given above. I understand the meaning of this information. Dr./mr./ms _____ has offered to answer any questions concerning the study. I hereby consent to participate in the study.

Printed name of participant

signature

Interviewers (print)

signature

IS IT YOUR INTENTION THAT THE INTERVIEWERS BE THE WITNESS?

Date: _____

At: _____

MALE QUESTIONNIARE

Subject Number.....4

Interviewer Number.....6

Camp Name_____

Camp Code.....8

Date of interview (DD/MM/YY) 14

Thank you in advance for agreeing to complete this questionnaire. Your answers to these questions will be coded with your ID number and not your name. All of the answers you give to me will be kept confidential and used for research only. If any of the questions I ask are not clear, please stop me, and I will try to make them clear.

A. Background information

A1. What is your date of birth?.....20

A2. What is the highest standard that you passed at school?.....22

A3. Did you have any additional training after you left school (for example, vocational, institutional or apprenticeships)? Yes ___ No ___ 23

If "yes", how many months/years at (interviewer to convert years to months by multiplying by 12)

1. Vocational Institution. _____
2. Apprenticeship. _____
3. Other, Specify. _____

☐☐ 25
☐☐ 27
☐☐☐ 29

A4.a) Are you either married now or do you have a steady girlfriend or partner? ☐☐ 30

- Married/living with someone as married ☐ 1
Widowed ☐ 2
Divorced/separated ☐ 3
Single ☐ 4

A4b) If married, how long are you married? _____ (years)

☐☐

B. GENERAL MEDICAL HISTORY

B1. How do you judge your own health in general?

- Excellent ☐
Very good: ☐
Good: ☐
Bad: ☐

☐

B2. Have you had/do you have:

	No	Yes	DK	Year diagnosed
a). Diabetes	☐	☐	☐	19____ ☐ ☐ ☐ ☐
b). TB	☐	☐	☐	19____ ☐ ☐ ☐ ☐
c). Fits	☐	☐	☐	19____ ☐ ☐ ☐ ☐
d). High blood pressure	☐	☐	☐	19____ ☐ ☐ ☐ ☐
e). Asthma	☐	☐	☐	19____ ☐ ☐ ☐ ☐
f). Headaches	☐	☐	☐	19____ ☐ ☐ ☐ ☐
g). Heart problems	☐	☐	☐	19____ ☐ ☐ ☐ ☐
h). Back problems	☐	☐	☐	☐ 19____ ☐
☐ ☐ ☐				
i). Others _____	☐	☐	☐	19____ ☐ ☐ ☐ ☐

B3.a) Have you had/do you have any other chronic illnesses (longer than three months)?

- Yes ☐
No ☐

☐

b) If yes, describe _____

☐☐

B4. Have taken any daily medication during the last 3 months?

Yes ☐

No ☐

☐

If "Yes", fill in the schedule below:

For conventional medication

Name of medicine

Against disease

How long did
you take it for
4 weeks

E.g., Ibuprofen

Muscle pain

☐	☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐	☐

For traditional medication

Name of medicine

Against disease

How long did
you take it for

Example: Ibuprofen

Muscle pain

☐	☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐	☐

(Yes = 1, No = 2, Don't know = 3)

B5. In the last 2 months, have you had
infections or flu that caused a fever

Yes

No ☐

☐

B6. If "Yes", how long ago was this? ☐ (days)
Describe the infection. _____

☐

_____ ☐

B7. Do you usually or frequently
suffer from colds and fever?

Yes

No ☐

☐

- B8. Do you usually or frequently suffer from coughing? Yes ☐
No ☐
- B9. Do you usually or frequently suffer from Ringing in your ears? Yes ☐
No ☐

C. GENITAL HEALTH HISTORY

- C1. Have you ever had mumps?
Yes ☐
No ☐
D/K ☐
- C2. If yes, how old were you when you had mumps? _____ years old ☐☐
- C3. Have you received treatment because one or both testicles were not in the scrotum?
No ☐
Yes, operation: _____ (Year of operation: 19____)
Yes, hormonal treatment: _____ (Year the treatment started: 19____)
D/K ☐☐☐☐☐
- C4. Were you born with one or both of your testicles outside the scrotum, but they went down by themselves?
Yes, one testicle: _____
Yes, both testicles: _____
No: ☐
D/K ☐
- C5. Have you ever had an injury, resulting in swelling/dicolouring of the scrotum?
Yes: ☐
No: ☐
- C6. Have you ever had an operation for one of the following? ☐
- | | Yes/No/DN | Year | |
|---------------------------|-----------|--------|--|
| Inguinal hernia: | _____ | 19____ | ☐ ☐ ☐ |
| Testes swelling: | _____ | 19____ | ☐ ☐ ☐ |
| Torsion of the testis: | _____ | 19____ | ☐ ☐ ☐ |
| Testicular cancer: | _____ | 19____ | ☐ ☐ ☐ |
| Other deseases of the | | | |
| Penis, urethra or scrotum | _____ | 19____ | ☐ ☐ ☐ |

If Yes, specify: _____

☐☐☐

C7. Have you been sterilized?

Yes ☐

No ☐

☐

C8. Have you ever suffered from the following:

	<u>Yes, No, D/K</u>	<u>Last time Diagnosed</u>	<u>If more than once how many times</u>
a. Gonorrhoea & Chlamydia - Drop/burning discharge from penis	☐	19 _____	☐ ☐ ☐ ☐
b. Epididymitis - Painful & Swollen inflamed testicles	☐	19 _____	☐ ☐ ☐ ☐
c. Cystitis - burning and frequent urination	☐	19 _____	☐ ☐ ☐ ☐
d. Prostatitis - difficulty & discomfort passing water when the bladder is full	☐	19 _____	☐ ☐ ☐ ☐
e. Varicocele - painless swelling of the testicles (balls).	☐	19 _____	☐ ☐ ☐ ☐
f. Hernia - swelling that is sometimes painful & gets bigger when coughing or straining in the groin.	☐	19 _____	☐ ☐ ☐ ☐
g. Diabetes - sugar sickness	☐	19 _____	☐ ☐ ☐ ☐
h. Penile ulcers (sores on or near penis).	☐	19 _____	☐ ☐ ☐ ☐

☐

C9a) Have you ever had any other diseases of the penis, urethra or scrotum?

Yes:

Year:

No:

Don't know

☐☐☐

C9b) If "Yes", specify

☐

D. SEXUAL HISTORY

The following questions are particularly sensitive, and for this we apologise, we however will appreciate your forthright and helpful response to the questions. If you rather not answer these questions then we will understand.

D1. With how many different women have you had sexual intercourse in the past 3 months? 0 __, 1 __, 2 __, 3-5 __, 6 or more __ ☐

D2. If not 0, is this sex partner, or one of these sex partners, your usual or main, or steady sex partner? (this can include your wife) Yes __ No __ ☐

D3. If "Yes", for how long has she been your main partner (years) __ ☐☐

D5. How often do you have any of the following problems?

Always Usually Often Rarely Never

a) Lack of interest in sex	☐	☐	☐	☐	☐	☐
b) Difficulties in obtaining a full erection	☐	☐	☐	☐	☐	☐
c) Too early ejaculation	☐	☐	☐	☐	☐	☐
d) Lack of ejaculation	☐	☐	☐	☐	☐	☐
e) Other problems	☐	☐	☐	☐	☐	☐
Describe _____						☐ ☐

D6. How satisfied are you with your sex life? Very satisfied __, Somewhat satisfied __, Somewhat dissatisfied __, Dissatisfied __ ☐

D7. How often do you usually have sexual intercourse?

Every day __, Several times per week __, Once every second week __, About once a month __, Less than once a month __ ☐

D8. How frequently do you feel a sexual drive? This feeling may include wanting to have sexual experience (intercourse), planning to have sex, feeling frustrated due to lack of sex, etc

Every day __, Several times per week __, Once every second week __, About once a month __, Less than once a month __ ☐

D9. During the past month, have you awakened from sleep with a full erection? Yes __, No __ ☐

E. REPRODUCTIVE HISTORY

E0. How many years in total have you had a sexual relationship with your current main partner __ (years) ☐☐

E1. Have you ever made your current main partner pregnant? (This includes current pregnancy, livebirths, any miscarriages, induced abortion, ectopic pregnancy and stillbirth) Yes __ No __ ☐

E2. How many times have you made your current main partner pregnant? __ ☐☐

E3. Do you have a partner who is pregnant now? Yes __ No __ D/K __ ☐

E4. Have you ever made any previous or other partner pregnant? Yes __ No __ D/K __ ☐

E5. How many partners in total have you ever made pregnant? ____ ☐☐

E6. How many liveborn children have you fathered? (from all your partners) ____ ☐☐

E7. How many other pregnancies have you fathered that resulted in, stillbirth, miscarriage, induced or elective abortion and tubal (ectopic) pregnancy? ____ ☐☐

- | Child
Number | Partner
Number | Main
Partner

(yes, no) | Sex, M=male
F=female | Birth
Year/ |
|-----------------|-------------------|----------------------------------|-------------------------|----------------|
|-----------------|-------------------|----------------------------------|-------------------------|----------------|

I would like to ask you questions concerning the last liveborn child you fathered with your main current partner (who gets the woman's questionnaire).

- 193

- F4. Were you and your partner using a contraceptive method before you began to try for that lastborn child
- | | | |
|-----|---|--------------------------|
| Yes | 1 | |
| No | 2 | |
| D/K | 3 | ☐ |

[IF NO GO TO G8]

- F5. What kind of contraceptive did you/or your partner use? (specify) ☐

(Prompt for condom, vaginal foam, oral contraceptives, injectable contraceptives such as depot provera or nuristerate, rhythm, IUD)

- F6. Did you and/or your partner decide to stop using this method in order for her to become pregnant?
- | | | |
|-----|-----|--------------------------|
| Yes | ___ | |
| No | ___ | |
| D/K | ___ | ☐ |

- F7. When did you and/or your partner stop using this method? _____
(MM/YY) ☐☐☐☐

SOME COUPLES MAY HAVE TIME INTERVALS IN THEIR LIFE WHEN THEY DON'T DO ANYTHING TO AVOID PREGNANCY, BUT THEY DO NOT GET PREGNANT ANYWAY

- F8. Have you ever had any time-intervals of at least one year, (including this past year) when you had intercourse without doing anything to avoid pregnancy, but your partner did not become pregnant?

Yes ___ ☐
No ___ If No, GO TO QUESTION G13

THE FOLLOWING QUESTIONS CONCERN THE TIME-PERIOD, WHEN YOU TRIED TO ACHIEVE A PREGNANCY FOR MORE THAN 1 YEAR.

- F9. For how long did you and your partner attempt to achieve a pregnancy all together?
- a) _____ years and/or _____ month before we succeeding in a pregnancy that led to a live birth ☐☐☐
- b) Gave up after _____ years and/or _____ months. ☐☐☐
- F10. Have you or your former/present partner ever sought examination to find the reason for your partner not becoming pregnant? ☐

Yes ___

No ☐ GO TO QUESTION G13

If "Yes"; then what was the reason found for the problem?

_____ ☐

- F11. Did you or your partner receive any treatment because of problems with achieving pregnancy?

Yes ☐

No ☐ GO TO QUESTION 13

If "Yes", which kind of treatment did you or your partner receive, and did it succeed?

<u>You/Partner</u>	<u>Treatment</u>	<u>Succeed (Yes/No)</u>
_____	_____	_____ ☐ ☐ ☐ ☐ ☐
_____	_____	_____ ☐ ☐ ☐ ☐ ☐
_____	_____	_____ ☐ ☐ ☐ ☐ ☐
_____	_____	_____ ☐ ☐ ☐ ☐ ☐
_____	_____	_____ ☐ ☐ ☐ ☐ ☐
_____	_____	_____ ☐ ☐ ☐ ☐ ☐

- F12. For how long had you and your partner attempted to achieve a pregnancy before you received the treatment?

_____ years and/or _____ months

☐☐☐

(If had more than one livebirth child)

I am going to ask you the same questions as above, but now concerning the 2nd last liveborne child pregnancy you fathered with your main current partner (who gets female questionnaire.

- F13. What was the date of birth of your 2nd last liveborn child? _____ (birthdate).

☐☐☐☐☐

☐

- F14. Were you and your partner actively trying to have a child before the birth of your 2nd to last liveborn child?

Yes ☐

No ☐

D/K ☐

☐

- F15. For how long did you and your partner attempt to get pregnant with that 2nd to last liveborn child before you succeeded?

_____ months and or _____ years.

☐☐☐

- F16. Were you and your partner using a contraceptive method before you began to try for that 2nd to last liveborn child?

Yes ____
No ____
D/K ____

☐

If "Yes"; go to question 17

- F17. What did you/or your partner use? (specify)

☐

(Prompt for condom, vaginal foam, oral contraceptives, injectable contraceptives such as depot provera or nuristerate, rhythm, IUD)

- F18. Did you and/or your partner decide to stop using this method in order for her to fall pregnant?

Yes ____
No ____
D/K ____

☐

If "Yes"; go to question 19.

If "No", go to Section H, question 1

- F19. When did you and/or your partner stop using this method? _____

(MM/YY)

☐☐☐☐

G CIGARETTE AND ALCOHOL HISTORY

The next are a few questions about cigarette smoking:

- G1. Have you ever smoked cigarettes? Yes __, No __

☐

If you had ever smoked, answer the following other wise skip box

- G2a) Do you smoke cigarettes (as of one month ago) Yes __, No __

☐

- b) How old were you when you first started regular cigarette smoking? Age in years _____

☐☐

- c) Over the years, what is the average number of cigarettes you smoked per day?

_____ cigarettes per day?

☐☐☐

- d) If you have stopped smoking cigarettes completely, how old were you when you stopped? _____ age in years

☐☐

- e) How many cigarettes do you smoke per day now? _____ cigarettes per day

☐☐☐

The next are a few questions about smoking a pipe:

G3. Have you ever smoked a pipe? Yes __, No __

☐

If you had ever smoked , answer G4

G4a) Do you smoke pipe (as of one month ago) Yes __, No __

☐

b)How old were you when you first started smoking pipe regularly?

Age in years ____

c) Over the years, what is the average number of bowls you smoked per day?

_____ bowls per day?

d) If you have stopped smoking pipe completely, how old were you when you stopped? ____ age in years

e) How many bowls do you smoke per day now? ____ bowls per day

The next are a few questions about chewing tobacco:

G5. Have you ever chewed tobacco? Yes __, No __

☐

If you had ever chewed tobacco, answer G6

G6a) Do you chew tobacco (as of one month ago) Yes __, No __

☐

b)How old were you when you first started chewing tobacco regularly?

Age in years ____

c) Over the years, what is the average number of rolls you chewed per day?

_____ rolls per day?

d) If you have stopped chewing tobacco completely, how old were you when you stopped? ____ age in years

e) How many rolls do you chew per day now? ____ rolls per day.

The next are a few questions about snuffing:

G7. Have you ever snuffed tobacco? Yes __, No __

☐

If you had ever snuffed tobacco , G8

G8a) Do you snuff tobacco (as of one month ago) Yes __, No __

☐

b) How old were you when you first started snuffing tobacco on a regular basis? Age in years _____

c) Over the years, what is the average number of snuffs you took per day?
_____ snuffs per day?

d) If you have stopped taking snuffs completely, how old were you when you stopped? _____ age in years

e) How many times do you snuff per day now? _____ snuffs per day

G9. Have you drunk any alcoholic beverages (liquor, wine, beer, traditional beer) in your life? Yes ___ No ___

If yes, have you drunk in the last year

The table below concern the type and quantity of alcoholic beverage you consume/consumed regularly.

If past drinker (stopped for more than one year previously)

G10. How many years did you drink?
Interviewer to circle time period

- a. <1 year
- b. 1 - 4 years
- c. 5 - 9 years
- d. 10 - 19 years
- e. 20 + years

G11. How many years has it been since your last drink?

G12. How much did you drink?

22

Mondays to Thursday

- a. did not drink the week
- b. 1 - 2 drinks
- c. 3 - 4 drinks
- d. 5 + drinks

G13. **Fridays to Sunday evenings (weekends)**

23

- a. do not drink on weekends
- b. 1 - 2 drinks on weekends
- c. 3 - 4 drinks on weekends
- d. 5 + drinks on weekends

If Present (has drunk sometime during the past year

G14. How many years have you been drinking?

☐24

Interviewer to circle time period

- a. <1 year
- b. 1 - 4 years
- c. 5 - 9 years
- d. 10 - 19 years
- e. 20 + years

G15. How much did you drink?

☐22

Mondays to Thursdays

- a. do not drink the week
- b. 1 - 2 drinks
- c. 3 - 4 drinks
- d. 5 + drinks

G16. Fridays to Sunday evenings (weekends)

☐23

- a. did not drink on weekends
- b. 1 - 2 drinks on weekends
- c. 3 - 4 drinks on weekends
- d. 5 + drinks on weekends

KEY

1 DRINK =

1 GLASS OF WINE

1 TOT OF SPIRITS

1 GLASS OF BEER

1 GLASS OF TRADITIONAL BEER

G15. Do you have any comments about this survey?

☐☐☐

H. WORK HISTORY

First I will ask you questions about jobs you have held as part of the Malaria Control Programme

H0. When did you begin working in the malaria control programme? _____ month _____ year

H1. Are you currently working in the malaria control programme?

☐

Yes __, No __, Don't know __

H2. If yes, what is your current job title?

☐

H3 a). What do you do in this job?
(Describe your usual duties in a sentence).

H3 b) When did you start in this particular job _____ month _____ year

H4. Have you had any exposure to pesticides in this job?

Yes _____

No _____

☐

If yes, describe these duties _____

☐

If "No", go to question H11

H5. On this job, do you apply or mix pesticides?

Yes _____

No _____

☐

If "No", go to question H11

H6.

a) What are the names of the pesticide of the pesticides that you usually apply or work with?

☐

H7.a) Do you spray houses? Yes __, No __

☐

b) What type of houses do you work with when spraying?

Traditional (mud/daub surface) _____

Westernised _____

Both..... ☐

H8. What are your job duties during the months when there is no spraying? (usually between January and July)?

_____ ☐ ☐

H9. Do your job duties between January to July expose you to pesticides? Yes _____ No _____ ☐

H10. If "Yes" describe the duties which expose you to pesticides?

_____ ☐ ☐

H11. What was your job title just before your current job? _____ ☐

H12. What did you do in that job?
(Describe your usual duties in a sentence). _____ ☐ ☐

H13. When did you begin in that job? Month _____ Year _____ ☐ ☐ ☐ ☐

H14. Have you had any exposure to pesticides in that job? Yes _____ No _____ ☐

If yes, describe these duties _____ ☐

If "No", go to question 21

H15. On that job, did you apply or mix pesticides? Yes _____ No _____ ☐

If "No", go to question 21

H16.

b) What are the names of the pesticide of the pesticides that you usually applied or worked with?

_____ ☐

H17.a) Did you spray houses? Yes __, No __ ☐

b) What type of houses did you work with when spraying? Traditional (mud/daub surface) __
Westernised __
Both..... ☐ ☐

H18. What were your job duties during the months when there was no spraying? (usually between January and July)?

_____ ☐ ☐

H19. Did your job duties between January to July expose you to pesticides? Yes __ No __ ☐

H20. If "Yes" describe the duties which exposed you to pesticides?

_____ ☐ ☐

H21. What was your job title just before your that job?

_____ ☐

H22. What did you do in that job?
(Describe your usual duties in a sentence).

_____ ☐ ☐

H23. When did you begin in that job? Month ____ Year ____

☐ ☐ ☐ ☐

H24. Have you had any exposure to pesticides in that job? Yes __ No __

☐

If yes, describe these duties _____

☐

If "No", go to question 31

H25. On that job, did you apply or mix pesticides? Yes __ No __ ☐

If "No", go to question 31

H26.

- c) What are the names of the pesticide of the pesticides that you usually applied or worked with?

_____ ☐

H27.a) Did you spray houses? Yes __, No __

☐

- b) What type of houses did you work with when spraying?

Traditional (mud/daub surface)___

Westernised

Both..... ☐ ☐

H28. What were your job duties during the months when there was no spraying? (usually between January and July)?

_____ ☐ ☐

H29. Did your job duties between January to July expose you to pesticides?

Yes

No

☐

H30. If "Yes" describe the duties which exposed you to pesticides?

_____ ☐ ☐

H31. What was your job title just before that job?

_____ ☐

H32. What did you do in that job?

(Describe your usual duties in a sentence).

_____ ☐ ☐

H33. When did you begin in that job? Month ____ Year ____

☐ ☐ ☐ ☐

H34. Have you had any exposure to pesticides in that job?

Yes

No

☐

If yes, describe these duties

☐

If "No", go to question 41

H35. On that job, did you apply or mix pesticides?

No ☐ Yes ☐

If "No", go to question 41

H36.

d) What are the names of the pesticide of the pesticides that you usually applied or worked with?

_____ ☐

H37.a) Did you spray houses? Yes ☐, No ☐

b) What type of houses did you work with when spraying?

Traditional (mud/daub surface) ☐

Westernised ☐

Both..... ☐

H38. What were your job duties during the months when there was no spraying? (usually between January and July)?

_____ ☐ ☐

H39. Did your job duties between January to July expose you to pesticides?

Yes ☐
No ☐

☐

H40. If "Yes" describe the duties which exposed you to pesticides?

_____ ☐ ☐

H41. What was your job title just before your current job?

_____ ☐

H42. What did you do in that job?

(Describe your usual duties in a sentence).

_____ ☐ ☐

H43. When did you begin in that job? Month _____ Year _____

☐ ☐ ☐ ☐

H44. Have you had any exposure to pesticides in that job? Yes ☐ No ☐

If yes, describe these duties ☐

If "No", go to question H51

H45. On that job, did you apply or mix pesticides? Yes ☐ No ☐

If "No", go to question 51

H46.

e) What are the names of the pesticide of the pesticides that you usually applied or worked with? ☐

H47.a) Did you spray houses? Yes ☐ No ☐

b) What type of houses did you work with when spraying? Traditional (mud/daub surface) ☐ Westernised ☐ Both..... ☐

H48. What were your job duties during the months when there was no spraying? (usually between January and July)? ☐

H49. Did your job duties between January to July expose you to pesticides? Yes ☐ No ☐

H50. If "Yes" describe the duties which exposed you to pesticides? ☐

H51. Have you ever applied pesticides or worked with pesticides in any other job on a farm or at your home or farming at home before? Yes 1 ☐ No 2 ☐

H52. If "Yes", please give dates when you did such work, where you did this work? If possible list the pesticides you used then.

Began		Ended	Where	Pesticide used							
year	year										
a)	_____	_____	_____	_____	☐	☐	☐	☐	☐	☐	
b)	_____	_____	_____	_____	☐	☐	☐	☐	☐	☐	
c)	_____	_____	_____	_____	☐	☐	☐	☐	☐	☐	
d)	_____	_____	_____	_____	☐	☐	☐	☐	☐	☐	
e)	_____	_____	_____	_____	☐	☐	☐	☐	☐	☐	

H53. Have you ever worked with the substances listed below/

	Several times per week	Once a week to once a mnth	Less than once a mnth	Not at all
Industrial lacquer	☐	☐	☐	☐
House painting	☐	☐	☐	☐
Welding of metal	☐	☐	☐	☐
Metal turning or similar	☐	☐	☐	☐
Degreasing of metal	☐	☐	☐	☐
Cleaning with organic solvents	☐	☐	☐	☐
Welding of plastics	☐	☐	☐	☐
Spraying/laying out chemicals	☐	☐	☐	☐
Photo development	☐	☐	☐	☐
Working with anaesthetics	☐	☐	☐	☐
Working in laboratories	☐	☐	☐	☐
Working at temperatures > 50 C	☐	☐	☐	☐
Radiation exposure	☐	☐	☐	☐
Working with pesticides	☐	☐	☐	☐
Work with pesticides on farms	☐	☐	☐	☐
Work with pesticides at home	☐	☐	☐	☐
Work with pesticides in a factory	☐	☐	☐	☐
Work with lead or car batteries	☐	☐	☐	☐

H79. Do you think exposures in your work place affect a person's sex drive or fertility?

Yes ☐

No ☐

DK ☐

H80. Is there any difference in your sexual drive on holidays or weekends or both you have been away from work one or more days? Yes ☐

No ☐

Appendix C: Physical Examination Report Sheet

University of Cape Town

PHYSICAL EXAMINATION

ID-CODE: _____

DATE: _____

PHYSICIAN: _____

Measures/evaluations of height, weight, testes disposition, varicocele and hydrocele have been performed with the man in standing position.

Evaluation of pubic hair should be according to the stages of Tanner, for which illustrations have been provided.

For evaluation of testes size, the orchidometer provided has to be used.

HEIGHT: _____ cm

WEIGHT: _____ kg

GENITAL REGION:

Scars due to surgery: No: _____ Yes: _____
(describe as "other remarks")

Pubic Hair: Tanner age: _____
(1-6)

Penis: Normal: _____ Abnormal: _____
(describe as "other remarks")

Testes disposition: Left: _____ Right: _____
(HS = high in scrotum, LS = low in scrotum, I = inguinal canal, N = non palpable)

Testes size: Left: _____ ml Right: _____ ml

Testes consistency: Left: _____ Right: _____
(N = normal, S = soft, H = hard)

Testes tumour: Left: _____ Right: _____
(N = no, Y = yes)

Epididyrstes: Left: _____ Right: _____
(N = normal, A = abnormal. And describe as "other remarks")

Vas deferens: Left: _____ Right: _____
(P = palpable, N = non-palpable)

Varicocele: Left: _____ Right: _____
(0 = no varicocele, 1 = only surely detected during Valsalvas procedure, 2 = palpable without Valsalvas procedure, 3 = visible)

Hydrocele: Left: _____ Right: _____
(Y = yes, N = no)

OTHER REMARKS