A COHORT STUDY OF THE EFFECTS OF PENTACHLOROPHENOL ON MALE REPRODUCTIVE FUNCTION

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This study evaluated the reproductive function of men who manufactured the wood preservative pentachlorophenol (PCP).

The fertility of the exposed men was compared to that of unexposed male workers at the plant. The fertility of both groups of workers was assessed using the Standardized Fertility Ratio (SFR) analysis program developed by Dr. Richard Levine and co-workers at the Chemical Industry Institute of Toxicology. Six study groups were evaluated, differentiated on the basis of the type of exposure (only exposed to PCP, or ever-exposed to PCP), source of data on children's' date of birth (birth certificate or worker recall), and whether all exposed workers or only those with chloracne were included in the analyses.

At all parities, the fertility of workers exposed to PCP was reduced by 14 to 18 percent relative to the workers' pre-employment fertility. For PCP-exposed workers with chloracne, fertility was reduced by 30 percent relative to the workers' pre-employment fertility. These findings were all statistically significant (α=0.10). When the analysis was limited to the parity experience greater than or equal to one, the fertility of the workers exposed to PCP was slightly greater than that of the workers' pre-employment fertility. For PCP-exposed workers with chloracne, fertility was reduced by 16 percent relative to the workers' pre-employment fertility. None of these findings were statistically significant (α=0.10).

The live-birth sex ratio analysis of the offspring of all exposed workers indicated no statistically significant reduction in the proportion of boys conceived pre- or post-employment, compared to the

expected proportion based on national vital statistics data. However, the proportion of sons conceived by exposed workers with chloracne after employment was reduced markedly, although not significantly. Among the unexposed workers, there was no reduction in the proportion of boys conceived pre-employment, but there was a statistically significant reduction in the proportion of boys conceived post-employment. This reduction appears to be due to older paternal age and higher maternal parity. There was no statistically significant difference in the proportion of boys conceived pre-employment between the exposed and unexposed workers.

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CERTIFICATE OF APPROVAL

I hereby recommend that the thesis prepared under my supervision by

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I concur with this recommendation

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Recommendation concurred in

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THESIS

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This thesis is dedicated to my wife, Lidia, and my two sons, David and Martin. It is also dedicated to the memory of my parents, James and Audrey, and to my maternal great-aunt, Daisy Warren, who taught me to believe that there was no such word as "can't" in the English language.

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LIST OF ABBREVIATIONS

EPA	U.S. Environmental Protection Agency
HpCDD	Heptachlorodibenzo-p-dioxin
HpCDF	Heptachlorodibenzofuran
HxCDD	Hexachlorodibenzo-p-dioxin
HxCDF	Hexachlorodibenzofuran
HCB	Hexachlorobenzene
I-TEF	International Toxicity Equivalency Factor
LD_{50}	Lethal Dose to 50% of the population
OCDD	Octachlorodibenzo-p-dioxin
OCDF	Octachlorodibenzofuran
PCP	Pentachlorophenol
NaPCP	Sodium Pentachlorophenate
NTP	National Toxicology Program
PCDD	Polychlorinated Dibenzo-p-dioxin
PCDF	Polychlorinated Dibenzofuran
PeCDD	Pentachlorodibenzo-p-dioxin
PeCDF	Pentachlorodibenzofuran
TCDD	Tetrachlorodibenzo-p-dioxin
TCDF	Tetrachlorodibenzofuran
2,3,7,8-TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TEQ	Toxicity Equivalents (to 2,3,7,8-TCDD)

SUMMARY

A cohort study was conducted of the reproductive experience of male pentachlorophenol (PCP) manufacturing workers at a large chemical plant. Data from personal interviews with workers and their spouses, medical examinations, and employment history were collected and reviewed.

From the unexposed and pentachlorophenol-exposed workers in the cohort, six study groups of exposed and unexposed workers were evaluated. These groups were differentiated on the basis of the type of exposure (only exposed to PCP, or ever-exposed to PCP), source of data on children's' date of birth (birth certificate or worker recall), and whether all exposed workers or only those exposed workers with chloracne were included in the study groups with unexposed workers.

The first dichotomy was made to avoid confounding from the significantly different pattern of polychlorodibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) contamination in penta *versus* the phenoxyherbicide esters and lower chlorinated phenols to which ever-exposed penta workers were also exposed. The second dichotomy was introduced to evaluate the accuracy and effect of worker recall of reproductive events on the fertility evaluations. The third dichotomy was introduced to evaluate whether workers with the highest level of PCDD/PCDF exposure or susceptibility (*i.e.*, those with chloracne) incurred a greater degree of impairment of reproductive function than exposed workers overall.

The six study groups included either 175 or 176 unexposed men, depending on the data sources used. The two study groups that included men ever-exposed to pentachlorophenol contained 225 and 226 workers, depending on the data sources, while the two study groups that included men only-exposed to PCP had 162 and 163 workers. The two remaining study

groups including ever-exposed men with chloracne both had only 30 workers.

The fertility of the exposed men was compared to that of unexposed male workers at the plant. The fertility of both groups of workers was assessed using the Standardized Fertility Ratio (SFR) analysis program developed by the Dr. Richard Levine and co-workers at the Chemical Industry Institute of Toxicology. The SFR program calculates observed to expected numbers of live births pre-employment and post-employment. The expected number of live births is calculated from national vital statistics birth probabilities for women, adjusted for maternal age, year of birth, parity, and race.

At all parities, the fertility of workers exposed to PCP was reduced by 14 to 18 percent relative to the pre-employment fertility of all workers. For two PCP-exposed subgroups with chloracne, fertility was reduced by 30 percent relative to the workers' pre-employment fertility. These findings were all statistically significant at a 10 percent level of significance. When the analysis was limited to the parity experience greater than or equal to one to compensate for the marital status artifact, the fertility of the workers exposed to PCP was slightly greater than that of the workers' pre-employment fertility. For the two PCP-exposed subgroups with chloracne, fertility was reduced by 16 percent relative to the workers' pre-employment fertility. None of these findings were statistically significant at a 10 percent level of significance.

The live-birth sex ratio analysis of the offspring of all exposed workers indicated no statistically significant reduction in the proportion of boys conceived pre- or post-employment, compared to the expected proportion based on national vital statistics data. However, the proportion of sons conceived by exposed workers with chloracne after employment was reduced markedly, although not significantly. Among the

unexposed workers, there was no reduction in the proportion of boys conceived pre-employment, but there was a statistically significant reduction in the proportion of boys conceived post-employment. This reduction appears to be due to older paternal age and higher maternal parity. There was no statistically significant difference in the proportion of boys conceived pre-employment between the exposed and unexposed workers.

I. INTRODUCTION

A. Purpose

The primary purpose of this study was to assess whether a cohort of males occupationally-exposed to pentachlorophenol experienced a significant reduction in fertility. The secondary purpose of this study was to evaluate whether the sex ratio of the children conceived after exposure was altered from expected values. The study workers had engaged in the manufacture of pentachlorophenol and its sodium salt, sodium pentachlorophenate (hereafter abbreviated as PCP and NaPCP, respectively; or generically referred to as penta) at a chemical plant in the midwestern United States between 1938 and 1978.

This study is derived from data collected as part of an occupational morbidity study of a cohort of workers potentially exposed to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in the production of chlorophenols and chlorophenoxy herbicides at the chemical plant. The main study collected data from worker and spouse questionnaires, clinical tests, and physical examinations of plant workers. The main study constitutes the largest morbidity study of penta production workers ever conducted.

B. Literature Review of Pentachlorophenol

1. Pentachlorophenol Background Information

a. Synthesis, Properties and Contaminants

Pentachlorophenol was first synthesized in 1841 by Erdmann, and in 1843 by Laurent, but commercial use did not begin until the 1930s. (Grimm, Schaller & Valentin, 1985) Pentachlorophenol and its sodium salt are the most important commercial forms of pentachlorophenol. The potassium salt

and the lauric acid ester are pentachlorophenol derivatives of minor commercial importance, and were not synthesized at the study chemical plant. Consequently, they will not be discussed further. Tetrachlorophenol (TCP) and its sodium salt sodium tetrachlorophenate (NaTCP) also figure in as TCP is the major chlorophenolic contaminant of PCP, as PCP is for TCP.

Pentachlorophenol is produced by the direct catalytic chlorination of phenols or by the hydrolysis of hexachlorobenzene. Only the former synthetic route has been used commercially in the United States. (Crosby *et al.*, 1981) Commercial pentachlorophenol is sold as a technical-grade material of roughly 85 percent purity. (IARC, 1986) Pure pentachlorophenol has a white to light tan needlelike crystalline appearance at room temperature, while the technical-grade material appears as tan to dark brown, or even gray flakes. (Crosby *et al.*, 1981; National Toxicology Program, 1989) Technical-grade NaPCP appears as cream-colored beads. (National Toxicology Program, 1989)

PCP has a pungent odor when heated. It has a vapor pressure of 0.00017 mm of Hg at 20°C. (Crosby *et al.*, 1981; National Toxicology Program, 1989) For instance, 30-80 percent of PCP may evaporate within a year following dip- or brush-treatment of coniferous wood. (WHO, 1987) NaPCP is nonvolatile, but has a sharp odor at room temperature due to slight hydrolysis. (Crosby *et al.*, 1981) PCP is soluble in many organic solvents, but is poorly soluble in water. At low pH (<1 percent ionized at pH=2.7), but readily soluble at a pH 6.7 (the pH of many freshwater surface water bodies) it is 99 percent ionized. (Crosby *et al.*, 1981) The nominal and numerical descriptors, and chemical and physical properties of PCP and NaPCP are summarized in Tables I through IV.

Technical-grade penta is a complex chemical mixture, with its specific composition dependent upon a number of variables related to the

batch synthesis process. The impurities that arise during the synthesis of penta vary significantly with the method and conditions of synthesis, date of synthesis, degree of post-production "clean-up", and analytical methodology. (Crosby *et al.*, 1981; National Toxicology Program, 1989)

While improperly controlled batch synthesis has been a prime factor responsible for the level and types of penta impurities in any particular batch, secular changes in synthesis have occurred. Thus, the date of synthesis is an important factor in penta composition because of the changes in the penta manufacturing and purification processes over time that have likely resulted in significant changes in the phenolic and nonphenolic composition of penta.

For instance, a 1938 patent was apparently the basis of penta manufacturing processes up to the mid-1950s. Manufacturing processes of today are based upon patents issued in the late 1950s and early 1969s, and purification processes patented in the late 1970s. (U.S. Environmental Protection Agency, 1987c)

In addition, post-manufacturing processes may be significant. For example, in 1970 the penta finishing process at the chemical plant was changed and block production began. (Carpenter, O'Malley, Haring Sweeney, Fingerhut & Marlow, 1991) Large (2,000 pound) blocks of penta were removed from molds after loosening with a hammer and chisel, and occasionally a blow torch. (Carpenter *et al.*, 1991) Both methods may have resulted in potentially significant worker exposures, the former from the release of penta-contaminated particulate matter, the latter from the generation of penta fumes and *de novo* generation of PCDDs/PCDFs. This supposition is supported by a sharp increase in chloracne incidence after 1970. (O'Malley *et al.*, 1990)

TABLE I

CHEMICAL IDENTITY OF PENTACHLOROPHENOL

CHEMICAL IDENTITY	PENTACHLOROPHENOL INFORMATION
Synonyms:	chlorophen; PCP; penchlorol; penta; pentachlorofenol (Dutch); pentachlorofenolo (Italian); pentachlorophenol; 2,3,4,5,6-pentachlorphenol
Trade Names:	Acutox; Chem-Penta; Chem-Tol; Chlorpher; Cryptogil oil; Dowicide 7; Dowicide EC-7; Dow Pentachlorophenol DP-2 Antimicrobial; Durotox; Duvotoec; EP-30; Fungifen; Fungol; Glazd Penta; Grundier Arbezol; Lauxtol; Lauxtol A; Liroprem; Moosuran; NCI-C 54933; NCI-C 55378; NCI-C 56655; Pentacon; Penta-Kil; Pentasol; Penwar; Peratox; Permacide; Permagard; Permasan; Permatox; Priltox; Permite; Santophen; Santophen 20; Sinituho; Term-i-Trol; Thompson's Wood Fix; Weedone; Witophen P
Chemical Formula:	C_6HCl_5O
CAS Registry Number:	87-86-5
Reference	WHO, 1987; Clement, 1989

TABLE II

PHYSICAL AND CHEMICAL PROPERTIES OF PENTACHLOROPHENOL

PROPERTY	PENTACHLOROPHENOL VALUES		REFERENCES
Molecular Weight	266.35		Clement, 1989
Melting Point (C)	190-191	(anhydrous)	Clement, 1989
	174	(monohydrous)	
Boiling Point (C)	310	(decomposes)	Clement, 1989
Density (g/cm^3)	1.978		Clement, 1989
Solubility			WHO, 1987;
Water (mg/L)	5	(0C, pH 5)	Clement, 1989
	14	(20C, pH 5)	
	2,000	(20C, pH 7)	
	8,000	(20C, pH 8)	
	15,000	(20C, pH 10)	
Organic Solvents (mg/L)			
Acetone	500,000	(25C)	
Benzene	150,000	(25C)	
Ethanol (95%)	1,200,000	(25C)	
Ethylene Glycol	110,000	(25C)	
Isopropanol	850,000	(25C)	
Methanol	1,800,000	(25C)	
pK_a	4.7	(25C)	Crosby, 1981
Log K_{ow} (mL/g)	5.01		Clement, 1989
Vapor Pressure (mm Hg)	0.000017	(0C)	Crosby, 1981
	0.00017	(20C)	
	0.0031	(50C)	
	0.14	(100C)	
	25.6	(200C)	
	758.4	(300C)	
Henry's Law Constant	0.0000034		Clement, 1989
Flash Point	Not Flammable		Clement, 1989
Flammability Limits	Not Flammable		Clement, 1989

ns = Not Specified

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TABLE III

CHEMICAL IDENTITY OF SODIUM PENTACHLOROPHENATE

CHEMICAL IDENTITY	SODIUM PENTACHLOROPHENATE INFORMATION
Synonyms:	penta-ate; pentachlorophenate sodium; pentaclorophenol,sodium salt; pentachlorophenoxy sodium; pentaphenate; phenol, pentachloro-, sodium derivative monohydrate; sodium PCP; sodium pentachlorophenate; sodium pentachlorophenolate; sodium pentachlorophenoxide
Trade Names:	Albapin; Cryptogil Na; Dow Dormant Fungicide; Dowicide G; Dowicide G-St; Napclor-G; Santobrite; Weed-beads; Xylophene Na; Witophen N
Chemical Formula:	C_6Cl_5ONa $C_6Cl_5ONa \cdot H_2O$ (as monohydrate)
CAS Registry Number:	131-52-2 27735-64-4 (as monohydrate)
Reference	WHO, 1987

TABLE IV

PHYSICAL AND CHEMICAL PROPERTIES OF SODIUM PENTACHLOROPHENATE

PROPERTY	SODIUM PCP VALUES	REFERENCES
Molecular Weight	288.3 306.3 (monohydrate)	WHO, 1987
Melting Point (C)	Decomposes	Crosby, 1981
Boiling Point (C)	Decomposes	Crosby, 1981
Density (g/cm^3)	2	WHO, 1987
Solubility		
Water (mg/L)	22,400 (20C, pH ns)	Crosby, 1981
	>200,000 (20C, pH 10)	WHO, 1987
	330,000 (25C, pH ns)	WHO, 1987
	33,000 (30C, pH ns)	Crosby, 1981
Organic Solvent (mg/L)		WHO, 1987
Acetone	350,000 (25C)	
Benzene	Insoluble (25C)	
Ethanol (95%)	650,000 (25C)	
Ethylene Glycol	400,000 (25C)	
Isopropanol	250,000 (25C)	
Methanol	250,000 (25C)	
pK_a	N/A	
Log K_{ow} (mL/g)	N/A	
Vapor Pressure (mm Hg)	N/A	
Henry's Law Constant	N/A	

ns = Not Specified
N/A = Not Available
Log K_{ow} = Log Octanol/Water Partition Coefficient

Analytical methodology is another important factor in estimating the concentration and composition of penta and its related impurities in biological and environmental samples. For instance, a hydrolysis treatment step is necessary for biological fluid specimens to cleave the glucuronide acid conjugated with pentachlorophenol. (Edgerton & Moseman, 1979) Most early studies failed to include this step. Consequently, the results of these early studies are suspect as they would have reported only the concentration of primarily "free" (unconjugated) pentachlorophenol.

In addition, some of the major impurities in PCP, such as the polychlorodiphenyl ethers, polychlorophenoxyphenols, and cyclohexadienones may undergo thermal or photochemical ring closure and be converted to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) during analysis by gas chromatography. (Crosby *et al.*, 1981) The ability of some polychlorodiphenyl ethers to undergo ring closure has been known since a study in 1972. (Jensen & Renberg, 1972)

Those polychlorodiphenyl ethers that can undergo ring closure to form PCDDs are generically referred to as "predioxins", the isomers that cannot are termed "isopredioxins". (Jensen & Renberg, 1972) The early (pre-1975) analytical work with penta tended to overestimate the values for these nonphenolic impurities, especially for OCDD (e.g. (Johnson, Gehring, Kociba & Schwetz, 1973)), because of its spontaneous generation in the gas chromatograph from other impurities. (Crosby *et al.*, 1981) This analytical artifact is avoided today by removing the predioxin phenoxyphenols from the sample by one of several clean-up techniques prior to analysis (e.g., column chromatography on an alumina oxide column) or by treating the extract with concentrated sulfuric acid. (Jensen & Renberg, 1972; Nilsson, Norström, Andersson & Rappe, 1978)

The phenolic impurities found in technical penta are the lower chlorinated phenolic components, tri- and tetrachlorophenols, and the higher chlorinated phenoxyphenols formed by condensation of two PCP

molecules. The neutral nonphenolic contaminants consist of: (1) polychlorinated dibenzo-p-dioxins (PCDDs) formed by ring-closure of predioxins; (2) polychlorinated dibenzofurans (PCDFs) formed by reaction of PCP with hexachlorobenzene; (3) polychlorodiphenyl ethers; (4) hexachlorobenzene; and (5) chlorinated cyclohexenones and cyclohexadienones. (Crosby *et al.*, 1981; WHO, 1987).

The most important of the nonphenolic contaminants, from a toxicological standpoint, are the PCDDs and the PCDFs. However, until recently the most potent of these congeners, 2,3,7,8-TCDD, had never been detected in pentachlorophenol at detection levels as low as 1 ppb. (Brantner, 1989; Crosby *et al.*, 1981; National Toxicology Program, 1989; WHO, 1987) In 1987, Hagenmaier and Brunner published the results of analyses of two PCPs and two NaPCPs for 2,3,7,8-substituted PCDD and PCDF congeners. (Hagenmaier & Brunner, 1987) Neither of the two European PCPs contained 2,3,7,8-TCDD at a detection limit of <0.05 ppb, but both of the NaPCP samples did. One of the NaPCP samples, Dowicide G, contained 0.23 ppb, and the other NaPCP, Preventol PN manufactured by Bayer AG, contained 0.51 ppb. In addition, the 1,2,3,7,8-PeCDD congener (which also has never been measured in penta before) was measured in all four samples at concentrations ranging from 1 ppb in PCP to 18 ppb in Dowicide G.

Hexachlorobenzene (HCB) is another important nonphenolic impurity whose presence cannot be overlooked. HCB may be present due to unreacted starting material (for the HCB hydrolysis synthetic route), or it may arise as the principal product resulting from heating PCP above 300°C. (Crosby *et al.*, 1981) At slightly lower temperatures, two PCP molecules will spontaneously condense to form octachlorodibenzo-p-dioxin (OCDD) on prolonged heating, or at temperatures above 200°C. (WHO, 1987)

Table V presents a summary of the phenolic and nonphenolic composition of PCP from various manufacturers. Table VI presents a summary of the PCDD and PCDF congener-specific analyses, and 2,3,7,8-TCDD toxicity

TABLE V

IMPURITIES IN TECHNICAL GRADE PENTACHLOROPHENOL PRODUCTS PREVIOUSLY MANUFACTURED IN THE UNITED STATES

	PRODUCT GRADE SPECIFICATION, MANUFACTURER, PCP CONTENT (%)								
COMPONENT	Technical Monsanto 84.6%	Technical Dow 88.4%	Purified Dow 98.0%	Technical Dow EC-7 90.4%	Technical Dow ns	Pure Aldrich 98.6%	Technical Dow EC-7 91.0%	Technical Dow DP-2 91.6%	Technical AWPI Composite 90.4%
Phenols:									
Tetrachlorophenols	30,000	44,000	2,700	104,000	ns	14,000	94,000	70,000	38,000
Trichlorophenols	ns	<1,000	500	<1,000	ns	<100	70	440	100
Higher Chlorinated Phenoxyphenols	ns	62,000	5,000	ns	ns	6,400	ns	40,500	62,100
Dibenzo-p-dioxins:									
Tetrachloro-	<0.1	<0.05	<0.05	<0.05	<0.2	<0.08	<0.04	ns	ns
Pentachloro-	<0.1	ns	ns	ns	<0.2	ns	ns	ns	ns
Hexachloro-	8	4	<0.5	1	9	<1	0.19	0.59	10.1
Heptachloro-	520	125	<0.5	6.5	235	ns	0.53	28	296
Octachloro-	1,380	2,500	<1.0	15	250	<1	0.69	173	1,386
Dibenzofurans:									
Tetrachloro-	<4	ns	ns	ns	<0.2	ns	ns	ns	ns
Pentachloro-	40	ns	ns	ns	<0.2	ns	ns	ns	1.4
Hexachloro-	90	30	<0.5	3.4	39	ns	0.13	12.95	9.9
Heptachloro-	400	80	<0.5	1.8	280	ns	0.15	172	88
Octachloro-	260	80	<0.5	<1.0	230	ns	ns	320	43
Hexachlorobenzene:	ns	ns	ns	400	ns	10	65	15	50
Reference	1	2	2	2	3	4	4	4	4

ns = Not Specified

AWPI Composite = American Wood Preservers Institute industry composite prepared from PCP from Monsanto, Reichhold Chemicals, Inc., and Vulcan Materials Co.

1 = Goldstein, 1977

2 = Schwetz, 1974

3 = Buser, 1975

4 = NTP, 1989

TABLE VI

CONCENTRATION OF 2,3,7,8-TCDD TOXICITY EQUIVALENTS IN TECHNICAL AND EC-7 GRADE PENTACHLOROPHENOL USED IN THE NTP CANCER BIOASSAY STUDIES

CONTAMINANT	I-TEF/89	2,3,7,8-Substituted PCDD/PCDF Congener Concentrations in NTP PCP (mg/kg)	
		TECHNICAL GRADE PCP	EC-7 GRADE PCP
PCDDs			
2,3,7,8-TCDD	1	ND	ND
X,2,3,7,8-PeCDD	0.5	ND	ND
X,2,3,7,8-HxCDD	0.1	10.9	ND
X,2,3,7,8-HpCDD	0.01	182.0	1.9
OCDD	0.001	970.0	ND
PCDFs			
2,3,7,8-TCDF	0.1	0.063	ND
X,2,3,7,8-PeCDF	0.05	0.14	ND
2,3,4,7,8-PeCDF	0.5	0.31	ND
X,2,3,7,8-HxCDF	0.1	1.59	ND
X,2,3,7,8-HpCDF	0.01	22.4	0.07
OCDF	0.001	154.0	ND
I-TEQ/89		4.6	0.02
References	EPA, 1989	Brantner, 1989	Brantner, 1989

CDD = chlorodibenzo-*p*-dioxin; CDF = chlorodibenzofuran

T = tetra; Pe = penta; Hx = hexa; Hp = hepta; O = octa

X = chlorine atom(s) at any other position on the rings

I-TEF/89 = International Toxicity Equivalency Factors for PCDDs/PCDFs

I-TEQ/89 = International Toxicity Equivalents to 2,3,7,8-TCDD

equivalents (TEQs) contained in the purified technical-grade PCP (EC-7) and composite technical-grade PCP used in the recent NTP carcinogen bioassays of pentachlorophenol. (National Toxicology Program, 1989) As one can see, the composition of commercial PCP varies significantly. The 2,3,7,8-TCDD TEQs calculations were based on the International Toxicity Equivalency Factors (I-TEF) for PCDDs and PCDFs developed and adopted by the North Atlantic Treaty Organization member nations. (U.S. Environmental Protection Agency, 1989b) It is important to note that even absent 2,3,7,8-TCDD, a purified technical PCP like EC-7 is estimated to contain the equivalent of 20 ppb 2,3,7,8-TCDD (*i.e.*, 20 ppb TEQ), and a less purified PCP is calculated to contain 4.6 ppm TEQ -- 230-fold more.

b. Pentachlorophenol Usage and Exposure

Pentachlorophenol and its sodium salt were among the most effective and widely used biocides in the world because of their toxicological potency, persistence in the environment, ready availability, and relatively low cost. (Crosby *et al.*, 1981; National Toxicology Program, 1989) Wood treatment has always been the predominant use for penta. This term can be further divided into two categories: wood protection and wood preservation. "Wood protection is short term control for sapstain produced by molds and fungi which discolor the wood and decrease its value," and "wood preservation is used for the long term prevention of rot and may involve much higher concentrations of PCP or TCP." (Horstman, Rossner, Kalman & Morgan, 1989)

With its versatility, penta has found other applications in industry, agriculture, and in the home. In the United States, penta's broad-spectrum biocidal properties have led to diverse non-wood uses. The major domestic applications for which penta has been used are summarized in Table VII. Overseas, penta has been used as an herbicide in rice paddies (Japan), and as a molluscicide for control of the intermediate (snail) host of

TABLE VII

INDUSTRIAL, AGRICULTURAL, AND DOMESTIC USES OF PENTACHLOROPHENOL

CATEGORY	APPLICATION
Wood Uses:	Wood preservation, sapstain control
Non-Wood Uses:	
Herbicidal Uses:	greenhouses; ornamental lawns and edging; rights of way; commercial and industrial non-crop areas; domestic dwellings; public facilities; wasteland and aquatic areas; golf courses
Antimicrobial Uses:	evaporative condensers, air washers; adhesives, sealants, and canning cements; gaskets; photographic solutions; other uses including latex paints, rubber defoaming agents, paper coatings, polyvinyl chloride emulsions, zinc-silicone dioxide coatings and feathers; textiles and cordage; leather tannery; marine caulking/paints; slime control in the pulp and paper industry; petroleum drilling muds
Disinfectant Uses:	mushroom houses; construction materials
Mossicide Uses:	lawns, roofs
Defoliation:	rights of way
Reference	U.S. EPA, 1987a; WHO, 1987

schistosomiasis and human bilharziasis and of liver fluke in cattle. (Blair, 1961; WHO, 1987)

Widespread human exposure to penta has occurred, and continues to occur. Evidence of penta residues may occur following exposure to the parent molecule because of the chemical's ubiquitous use, its vapor pressure of 0.00017 mm of Hg at 20°C (PCP), its high water solubility (NaPCP), or it may arise from metabolism of other commonly occurring chlorinated xenobiotics such as hexachlorobenzene, pentachlorobenzene, and beta-hexachlorocyclohexane (BHC, lindane). (Engst, Macholz, Kujawa, Lewerenz & Plass, 1976; Crosby *et al.*, 1981; National Toxicology Program, 1989; WHO, 1987) Preliminary data from the National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Study conducted from 1980-1983 revealed that 26,463 workers (85 percent of them males) in 1,490 plants were potentially exposed to penta in United States workplaces. (Clement, 1989, p. 93)

Among approximately 6,000 urine specimens collected as part of the NHANES II national probability survey (1976-1980), 79 percent had detectable concentrations of pentachlorophenol. (Murphy, Kutz & Strassman, 1983) PCP was by far the most frequently detected pesticide or pesticide residue in urine. The estimated geometric mean concentration of PCP in the urine was 6.25 µg/L. (Kutz, Carra, Cook, Stroup & al., 1983) A more recent survey of chlorophenolic and phenoxy herbicide residues in the urine of 197 Arkansas children reported that PCP was the most prevalent (at 100 percent detection frequency) and had the highest median concentration of any of the chemicals analyzed. (Hill *et al.*, 1989) All of these urine samples had measured PCP concentrations greater than 2 µg/L.

Recent estimates of the long-term daily intake of PCP in nonoccupationally exposed members of the general public range from 16 to 19

µg/d, with the food chain accounting for nearly all of the exposure. (Geyer, Scheunert & Korte, 1987; Hattemer-Frey & Travis, 1989)

Up until about 1980, penta usage in the United States was such that approximately 80 percent of pentachlorophenol was used for commercial wood treatment (primarily utility poles), 6 percent was used as a slime control agent in the pulp and paper industry, and 3 percent was used in various non-industrial applications, as an herbicide and defoliant agents, fence post treatment, and as a paint preservative. (Crosby *et al.*, 1981) The remaining 11 percent of PCP was converted to the NaPCP form, with this balance used primarily in wood protection, primarily as a sapstain control agent. (WHO, 1987) Thus, roughly 95-98 percent of U.S. production was used directly or indirectly in wood treatment. (WHO, 1987)

c. Pentachlorophenol Production and Regulation

Following nearly 14 years of regulatory review and rulemaking pursuant to its statutory authorities under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Resource Conservation and Recovery Act (RCRA), the United States Environmental Protection Agency (EPA) actions ultimately led to the cessation of PCP and NaPCP manufacturing in the United States in January 1992, when the last manufacturer voluntarily halted production and later applied for voluntary cancellation of its product registrations under (FIFRA). (U.S. Environmental Protection Agency, 1992b)

The regulatory scrutiny of PCP began in 1978, when the EPA exercised its authority under FIFRA and initiated a Rebuttable Presumption Against Registration (RPAR -- now termed a Special Review) of penta. (U.S. Environmental Protection Agency, 1978) The EPA initially justified this action against pentachlorophenol on the basis of evidence of its

fetotoxicity and teratogenicity in experimental animals. (U.S. Environmental Protection Agency, 1978)

In the following decade, the EPA subsequently expanded the scope of its health concerns to penta workers and members of the general public handling penta, and end-uses of treated materials due to the presence of the penta impurities hexachlorodibenzo-p-dioxin (HxCDD) and hexacnlorobenzene (HCB). These chemicals have been classified by EPA as Group B2 (probable) human carcinogens on the basis of animal laboratory experiments, and both chemicals are fetotoxic and teratogenic. (U.S. Environmental Protection Agency, 1981; U.S. Environmental Protection Agency, 1984a; U.S. Environmental Protection Agency, 1984b; U.S. Environmental Protection Agency, 1986; U.S. Environmental Protection Agency, 1987a; U.S. Environmental Protection Agency, 1987b; U.S. Environmental Protection Agency, 1988b)

EPA's final actions under FIFRA for penta used on wood proscribed: penta contaminant concentrations in excess of specific values (in accordance with a defined schedule); penta work practices; analytical and record keeping requirements; and end-uses for both wood preservative and non-wood uses of PCP and its salts. (U.S. Environmental Protection Agency, 1987b; U.S. Environmental Protection Agency, 1988b) For instance, indoor treated wood and virtually all non-wood uses were prohibited, and sale and application of penta was restricted to only certified pest control applicators, meaning that sales and use of penta by members of the general public were banned. Certified compliance limits for PCP impurities have been promulgated. Since February 2, 1989 HxCDD impurities had to have been reduced to the point that monthly batch averages could not exceed 2 ppm, with no batch exceeding 4 ppm. Also, the reduction in HxCDD levels could not result in an increase in HCB concentrations above 75 ppm, with no 2,3,7,8-TCDD at a detection limit of 1 ppb.

In 1988, Congress amended FIFRA to require pesticide registrants pay an annual fee to the EPA to continue pesticide production. Based on these cumulative actions three of the last four penta products were voluntarily canceled in early 1992 (U.S. Environmental Protection Agency, 1992b), and the sole remaining penta product had its registration canceled for nonpayment of the annual registration fee. (U.S. Environmental Protection Agency, 1992a)

In 1980, pursuant to its authority under the Resource Conservation and Recovery Act (RCRA), EPA promulgated regulations that among other things listed as "hazardous wastes" numerous wastes from specific and nonspecific industrial sources, and mandated strict "cradle-to-grave" requirements for the treatment, storage, and disposal of the identified hazardous wastes. (U.S. Environmental Protection Agency, 1980) Included in this regulation was a hazardous waste listing (K001) for two types of waste streams from the wood preserving industry. This listing applied to "bottom sediment sludge from the treatment of wastewaters from wood preserving processes that use creosote and/or pentachlorophenol." (U.S. Environmental Protection Agency, 1980)

In 1985 EPA expanded its regulatory control over the disposal of penta-containing wastes under the RCRA program by creating three new hazardous waste listings covering: "wastes...from the production or manufacturing use...of pentachlorophenol, or of intermediates used to produce its derivatives," as F021; "discarded unused formulations containing tri-, tetra-, or pentachlorophenol or discarded unused formulations containing compounds derived from these compounds...," as F027; and "residues resulting from the incineration or thermal treatment of soil contaminated with EPA Hazardous Wastes Nos. F020, F021, F022, F023, F026, and F027," as F028. (U.S. Environmental Protection Agency, 1985)

In 1988, EPA proposed to classify and regulate under RCRA two additional chlorophenolic waste streams (F032 and F033) from wood preserving processes. (U.S. Environmental Protection Agency, 1988a) Final action on this proposal was recently taken by EPA. (U.S. Environmental Protection Agency, 1990b) Finally, EPA recently promulgated a new regulation that characterizes as RCRA hazardous waste D037 any waste whose leachate contains more than 100 mg/L of pentachlorophenol, according to the specific laboratory "toxicity characteristic leaching procedure" (TCLP). (U.S. Environmental Protection Agency, 1990a)

Throughout the 1980s, the PCDD and PCDF impurities in penta were the focus of the most controversial aspects of PCP use, and the rationale behind EPA's stringent regulatory control strategy over its manufacture, use, and disposal. (Crosby *et al.*, 1981; National Toxicology Program, 1989; U.S. Environmental Protection Agency, 1984b; U.S. Environmental Protection Agency, 1985; U.S. Environmental Protection Agency, 1987a; U.S. Environmental Protection Agency, 1987b; U.S. Environmental Protection Agency, 1988a; U.S. Environmental Protection Agency, 1988b)

In 1989, pursuant to its authority under the Safe Drinking Water Act (SDWA), EPA proposed a drinking water maximum contaminant level goal (MCLG) and a maximum contaminant level (MCL) for penta in public water supplies of 200 mg/L based on the 1978 study of Schwetz (U.S. Environmental Protection Agency, 1989) EPA analysis of the NTP carcinogenic bioassay of penta led the Agency to classify penta as a Group B2 (probable) human carcinogen, independent of its PCDD and PCDF contamination, and to repropose an MCLG and MCL for penta at 0 and 0.001 mg/L (*i.e.*, 1 µg/L), respectively. (U.S. Environmental Protection Agency, 1991b) EPA recently promulgated the final drinking water MCLG and MCL for penta at 0 and 1 µg/L, respectively. (U.S. Environmental Protection Agency, 1991a)

No completely reliable estimates of pentachlorophenol production in the United States are available prior to the cessation of production in 1992. Disparate production values have been reported over the years. One secondary reference source summarized the following worldwide production estimates in metric tons (million pounds): 50,000-60,000 (110-132) in 1981, 90,000 metric tons (198 million pounds) in 1983, and 30,000 tonnes (66 million pounds) in 1987. (WHO, 1987) Another secondary reference source cites the following production values (presumably for the United States) from a single document: 45 million pounds in 1983, 42 million pounds in 1984, 38 million pounds in 1985, and 32 million pounds in 1986. A third reference cites current annual world production of pentachlorophenol as an estimated 17,000 metric tons (37.4 million pounds), with the United States accounting for almost 75 percent, or 12,700 metric tons (28 million pounds) of the world total supply. (National Toxicology Program, 1989)

One thing is clear about these estimates, they are internally consistent in demonstrating a trend toward decreased production of penta in the 1980s. Vulcan Chemical, Wichita, Kansas was believed to be the only remaining penta producer in the United States (National Toxicology Program, 1989), however Chapman Chemical Company enjoys that distinction. (The Bureau of National Affairs, 1994) Other large domestic producers of penta ceased manufacturing the chemical years ago, including such companies as Monsanto Chemical Company, Sauget, Illinois (1978), Dow Chemical Company, Midland, Michigan (1980), and Reichhold Chemicals, Inc., Tacoma, Washington (1985). (WHO, 1987)

2. Toxicokinetics of Pentachlorophenol

a. Absorption

i. Animal Studies

Animal studies have demonstrated that pentachlorophenol is well-absorbed following oral (Ahlborg, Lindgren & Mercier, 1974; Braun & Sauerhoff, 1976; Braun, Young, Blau & Gehring, 1977; Meerman, Sterenborg & Mulder, 1983), inhalation (Hoben, Ching, & Casarett, 1976b), or dermal exposure (Wester *et al.*, 1993). In general, gastrointestinal absorption was rapid and nearly complete as indicated by recovery of greater than 90 percent of administered radioactivity in urine, feces, expired air, plasma, and tissues. Females absorbed the compound faster than males. Approximately 70-75 percent of a single 20 minute inhalation exposure was absorbed by rats. Table VIII summarizes the results of several studies of the absorption and elimination kinetics of PCP in several species, including man, after short-term exposure.

In the first well-conducted absorption study, female NMRI mice were administered a single intraperitoneal or subcutaneous injection of a 97 percent radiochemically pure ^{14}C-labeled PCP in olive oil. (Jakobson & Yllner, 1971) Rapid absorption of the PCP was determined by the high specific activity in a whole-body autoradiogram four hours after subcutaneous injection of the ^{14}C-labeled PCP. At that time, the highest specific activities was found in the "fundus wall of the stomach and in the stomach and intestinal contents." (Jakobson & Yllner, 1971) Indeed, the radioactivity was distributed throughout the gastrointestinal tract including the liver.

Braun and Sauerhoff investigated the pharmacokinetics of PCP in monkeys. (Braun & Sauerhoff, 1976) Three male and three female rhesus monkeys (*Macaca mulatta*) were administered a single oral dose of 10 mg/kg

TABLE VIII

TOXICOKINETICS OF PENTACHLOROPHENOL AFTER A SINGLE EXPOSURE

SPECIES	SEX	N	ROUTE (vehicle)	DOSE (mg/kg)	PEAK BLOOD CONC. (ppm)	TIME TO PEAK CONC. (hrs)	PLASMA HALF-LIFE (hrs) ABSORPTION	PLASMA HALF-LIFE (hrs) ELIMINATION	URINARY EXCRETION RATE (hrs)	REFERENCE
Mouse	F	11	i.p. (c.o.)	15-37	nd	nd	nd	nd	alpha=24* 45-60% of dose after 24 hours	Jakobson and Yllner, 1971
	ns	ns	i.p. (o.o./p.g.)	25	nd	nd	nd	nd	66% of dose after 24 h	Ahlborg et al., 1974
	ns	ns	p.o. (o.o./p.g.)	25	nd	nd	nd	nd	17% of dose after 24 h	Ahlborg et al., 1974
Rat	F	?	oral (?)	31-40	nd	nd	nd	nd	alpha=10; beta=2448# $t_{1/2}$ 10 h	Larsen et al., 1972
	ns	ns	i.p. (o.o./p.g.)	25	nd	nd	nd	nd	70% of dose after 24 h	Ahlborg et al., 1974
	ns	ns	p.o. (o.o./p.g.)	25	nd	nd	nd	nd	20% of dose after 24 h	Ahlborg et al., 1974
	M	?	inhal. (w)	5.7	nd	nd	nd	nd	alpha=24* $t_{1/2}$ 24 h	Hoben et al., 1976
	M	3	oral (c.o.)	10	50	4-6	0.36	nd	alpha=17; beta=40# 80% of dose after 9 days	Braun et al., 1977
	F	3	oral (c.o.)	10	50	4-6	0.46	nd	alpha=13; beta=33# 78% of dose after 9 days	Braun et al., 1977
	M	3	oral (c.o.)	100	nd	nd	nd	nd	alpha=13; beta=121# 72% of dose after 8 days	Braun et al., 1977
	F	3	oral (c.o.)	100	nd	nd	nd	nd	54% of dose after 8 days $t_{1/2}$ 27 h*	Braun et al., 1977
Monkey	M	3	oral (c.o.)	10	10 - 30	12-24	3.64	72	25.6% of dose after 24 h $t_{1/2}$ 40.8 h*	Braun and Sauerhoff, 1976
	F	3	oral (c.o.)	10	10 - 30	12-24	1.81	83.5	8.3% of dose after 24 h $t_{1/2}$ 92.4 h*	Braun and Sauerhoff, 1976
Man	M	4	oral (w)	0.1	0.245	4	1.3	30	$t_{1/2}$ 33 h*	Braun et al., 1979
	M	1	oral (eth)	0.016	0.185	nd	nd	16 days	$t_{1/2}$ 18 days*	Uhl et al., 1986
	M	1	oral (eth)	0.308	nd	nd	nd	nd	$t_{1/2}$ 20 days*	Uhl et al., 1986

ns = not specified; nd = not determined

c.o. = corn oil; o.o. = olive oil; eth = ethanol; p.g. = propylene glycol; w = water

* = monophasic excretion

= biphasic excretion

of an at least 99.9 percent radiochemically pure ^{14}C-labeled PCP in corn oil.

Concentrations of PCP in plasma and urine were determined at periodic intervals for up to 360 hours after administration of the PCP. Peak ^{14}C-labeled PCP plasma concentrations of 10-30 µg/g (mg/L) were reached 12-24 hours after administration of the dose. Although the distribution patterns were similar in both sexes, females had uniformly higher plasma and tissue PCP concentrations than males. Absorption kinetics appeared to be first order, with calculated plasma absorption rate constants for male and female monkeys of 0.215 and 0.383 hr^{-1}, respectively, corresponding to plasma absorption half-lives of 3.64 and 1.81 hours.

Braun and his coworkers subsequently evaluated the pharmacokinetics of PCP in Sprague-Dawley rats. (Braun *et al.*, 1977) A single oral dose of either 10 mg/kg or 100 mg/kg of 99.9 percent radiochemically pure ^{14}C-labeled PCP in corn oil was administered to male and female Sprague- Dawley rats. The PCP administered at both dose levels was readily absorbed. The peak ^{14}C-labeled PCP plasma concentration (C_{max}) of approximately 50 µg/g (mg/L) in both sexes was reached between 4 and 6 hours after administration of the 10 mg/kg dose. In contrast to monkeys, rats achieved higher peak ^{14}C-labeled PCP plasma concentrations, and attained the peak concentration more quickly. Again, assuming first order absorption kinetics, the plasma absorption rate constants were 1.95 and 1.52 hr^{-1}, corresponding to absorption half-lives of 0.36 and 0.46 hours, for male and female rats, respectively.

Hoben *et al.* conducted inhalation exposure experiments with male Sprague-Dawley rats to determine the distribution and excretion of sodium pentachlorophenate, and to investigate whether this substance might accumulate in tissues upon multiple exposures. (Hoben, Ching, & Casarett, 1976a; Hoben, Ching, & Casarett, 1976b; Hoben, Ching, & Casarett, 1976c;

Hoben, Ching, Casarett, & Young, 1976; Hoben, Ching, Young, & Casarett, 1976) The experiment to evaluate the distribution and excretion of sodium pentachlorophenate involved exposing four groups of eleven rats each to sodium pentachlorophenate aerosol in a specially designed exposure chamber. The rats received a single 20 minute exposure which resulted in a calculated dose of 5.7 mg/kg. At prescribed intervals after the exposure, animals were sacrificed and lung and liver tissues, and urine and plasma were analyzed for PCP residues.

The authors plot of the data show rapid pulmonary uptake of PCP. Immediately after exposure ceased approximately 35 percent of the dose was found in the plasma, about 25 percent was found in the liver, and slightly less than 2 percent was found in the lung. At the sacrifice 24-hours after the brief exposure, approximately 55 percent of the dose was found in the urine collected from the animals, and about 9 and 7 percent of the dose was found in the liver and plasma, respectively. Less than about 0.80 percent of the dose remained in the lung. After 24-hours, these four sites indicate that about 70-75 percent of the original dose could still be accounted for as PCP.

Meerman *et al.* studied the gastrointestinal absorption of PCP and NaPCP from drinking water or food in male Wistar rats. (Meerman *et al.*, 1983) Drinking water containing 1.4 mM (≈320 mg/L) NaPCP, or food containing 350 ppm (17.5 mg/kg bw) PCP or NaPCP were provided *ad libitum* for 1 week. Based on an analysis of plasma levels and toxicokinetic parameters, the authors concluded that "...NaPCP is almost completely absorbed from the intestinal lumen...". Plasma concentrations of PCP were slightly lower among the rats receiving PCP and NaPCP in food. Bioavailability was estimated to be 0.90.

Reigner *et al.* conducted an investigation of NaPCP toxicokinetics in male Sprague-Dawley rats after low-dose (2.5 mg/kg) intravenous and oral

administration. (Reigner, Gungon, Hoag & Tozer, 1991) They reported that NaPCP intravenous and oral absorption were similar, yielding estimates of bioavailability of 0.91 and 0.97 via two different estimation methods.

ii. Human Studies

Like the animal studies, the human studies have demonstrated either quantitatively or qualitatively that pentachlorophenol is well-absorbed following oral (Braun, Blau & Chenoweth, 1979; Uhl, Schmid & Schlatter, 1986), inhalation (Casarett, Bevenue, Yauger & Whalen, 1969), or dermal exposure (Enarson, Chan-Yeung, Embree, Wang & Schulzer, 1986; Horstman *et al.*, 1989; Jones, Winter & Cooper, 1986; Wester *et al.*, 1993). The first quantitative human absorption study was a multifaceted occupational study conducted by Casarett *et al.* (Casarett *et al.*, 1969) It assessed the importance of inhalation exposure to penta absorption in the workplace.

Casarett *et al.* monitored the ambient air and respiration rate of two subjects with no previous occupational exposure to PCP who spent 45 minutes in an enclosed area while applying PCP to lumber with a brush. No description of any personal protective equipment worn by the two men was provided. The concentration of PCP in urine was measured for the two days prior to exposure, and the 7 days (Subject A) and 5 days (Subject B) immediately following exposure.

Based on these measurements and tidal volumes from handbook data, the authors estimated that 88 and 76 percent of the inhalation exposure dose was recovered from the urine of the two subjects. While a contribution from dermal absorption cannot be excluded, it appears that inhalation exposure predominated in this exposure scenario. The authors noted a one-day lag period between the time of exposure and an increase in urine PCP concentrations.

Braun *et al.* later conducted an experiment on the pharmacokinetics of orally administered PCP (>99 percent purity) in humans. (Braun *et al.*, 1979) Four healthy male volunteers of normal weight, ranging in age from 21 to 55 years of age, ingested 0.1 mg/kg of NaPCP (>99 percent purity) dissolved in 25 mL of water. Food was withheld for a period of 8 hours before and 1 hour after PCP administration. The plasma PCP concentration increased quickly, with an average C_{max} of 0.2 µg/mL (mg/L) occurring 4 hours after administration of the NaPCP. The absorption rate constant was 1.16 hr^{-1}, and the corresponding absorption half-life was 1.3 hours.

Uhl *et al.* orally administered PCP (>99 percent purity) dissolved in 40 percent ethanol to three volunteers at doses of 3.9, 4.5, 9, and 18.8 mg. No dietary restrictions were placed on the volunteers. (Uhl *et al.*, 1986) One of the volunteers received a second single dose of PCP. The second dose was 0.016 mg of ^{13}C-labeled PCP/kg of body weight (^{13}C-PCP >99 percent isotopic purity) dissolved in an ethanol solution. That individual had a PCP plasma concentration of 0.185 mg/L two days after ingesting the PCP. This study indicates that the oral absorption of PCP is enhanced when it is administered in an organic solvent, like ethanol, instead of water.

While the food chain appears to be the primary exposure route for the nonoccupationally exposed general population, dermal and inhalation exposure are the predominant exposure pathways for pentachlorophenol workers. However, only a single well-documented case report exists that quantitatively demonstrates the significance of dermal exposure to pentachlorophenol.

Bevenue *et al.* relate the sequence of events that followed after a worker immersed his hands in a 0.4 percent solution of pentachlorophenol while cleaning a paint brush. (Bevenue, Haley, & Klemmer, 1967) Pain and reddening of his hands forced the man to remove his hands from the solution within ten minutes. Two days after the incident, a 24-hour urine specimen

was submitted for analysis for PCP. The sample was reported to contain 236 ppb PCP. First morning voids periodically submitted for penta analysis over the next several weeks revealed a slow (one month) return to urinary PCP levels normal for the Hawaiian male population (23 ppb).

Four other studies have examined percutaneous absorption of chlorophenate mixtures. The first study examined chlorophenol exposure among Finnish sawmill workers who used the sodium salt of 2,3,4,6-tetrachlorophenol (containing approximately 5 percent penta) (Kauppinen & Lindroos, 1985) The authors found low levels of chlorophenols in the air, but high concentrations of chlorophenols in urine. This finding led the authors to conclude that "...the skin is the prominent route of exposure."

Another study assessed dermal exposure to chlorophenates of timber mill workers in Washington State using Permatox 100™ (Reichhold Chemical Company) as a sapstain control agent. (Fenske, Horstman & Bentley, 1987) The sapstain formulation contained 20 percent 2,3,4,6-tetrachlorophenol (TCP), 3 percent PCP, and less than 0.4 percent other chlorophenol isomers, with all chlorophenols present as their water soluble sodium salts. (Fenske *et al.*, 1987) Based upon the use of a fluorescent dye tracer, wipe samples, and air sampling that confirmed the presence of low chlorophenol concentrations, dermal exposure was estimated to account for 95 percent of total exposure. This finding was concordant with the earlier Finnish study. In a follow-up study at this same Washington State mill, Bentley *et al.* reported that air sampling and surface sampling of wood "...confirmed skin as the major route of exposure." and found a significant reduction in sawmill workers' urine chlorophenol levels after counseling on personal protective equipment and personal hygiene. (Bentley, Horstman & Morgan, 1989)

Enarson *et al.* evaluated health effects among sawmill workers exposed to chlorophenates in Canada. (Enarson *et al.*, 1986) Results from personal

airborne samplers, urine and serum indicated the dermal exposure group had the highest levels of chlorophenates in serum and urine when compared to either the control group or the airborne exposure group.

Among nonoccupationally exposed populations, concentrations of PCP in urine and plasma have been correlated with ambient air concentrations in three environmental studies of residents of U.S. homes built of PCP-treated wood. (Cline, Hill, Phillips & Needham, 1989; Hosenfeld *et al.*, 1986; Sangster, Wegman & Hofstee, 1982) While this indicates a possible role for inhalation absorption, a contribution from dermal contact cannot be excluded since urinary PCP concentrations (presumably a surrogate measure of skin contact) were significantly greater among children under 12 years of age than among older children and adults.

Gerhard *et al.* examined 90 women with histories of either habitual abortion, idiopathic infertility, menstrual disorders, or climacteric symptoms and evaluated the correlation of these various symptoms with serum concentrations of PCP and lindane. (Gerhard, Derner & Runnebaum, 1991) Among the 22 women with the highest serum PCP and lindane concentrations, PCP and lindane concentrations were highest in those with infertility. Endocrine (adrenocortical, dehydroepiandrosterone, or thyroid) or immunologic function depression was more than twice as prevalent as among a control group of women with normal serum PCP or lindane concentrations. The authors noted that the symptoms disappeared in 12 of the women who ceased exposure to the PCP and lindane.

Jones *et al.* evaluated the absorption of penta in people working with treated wood in the United Kingdom. Their analysis of urine penta levels and job categories led them to conclude that "...measurable PCP absorption occurs as the result of the handling of wood treated with PCP-containing preservatives." (Jones *et al.*, 1986)

Finally, the potential importance of dermal absorption is evidenced by two recent *in vitro* dermal absorption studies. (Horstman *et al.*, 1989; Wester *et al.*, 1993) In the first study, human cadaver abdominal skin (dermis and epidermis) was obtained at autopsy from a single individual. The dermal penetration of PCP, NaPCP, and sodium tetrachlorophenate (NaTCP) were determined for pieces of sectioned skin placed into 1.0 cm^2 Franz diffusion chambers. Two test solutions were used. The first consisted of Permatox 100™, an aqueous-based commercial wood protection product for sapstain control, containing NaPCP and NaTCP at concentrations very close to those commonly used in the wood treatment industry (0.44 percent NaPCP and 1.54 percent NaTCP). The second test solution consisted of PCP in diesel oil. Again, the concentrations used in the experiment were very close to those commonly used in the wood treatment industry (approximately 15.4 percent PCP and 0.94 percent TCP).

The skin samples were each exposed for 24 hours. Plasma receptor fluid accumulated only 0.14 to 3.0 percent of the dose. Recovery in the epidermis was higher, 0.88 to 5.3 percent, and 2.5 to 5.8 percent in the dermis. Dermal absorption of PCP and TCP was approximately two- to three-fold higher from the aqueous solution as from the diesel oil solution.

Wester *et al.* evaluated the dermal absorption of PCP in soil and acetone *in vivo* in the Rhesus monkey, and *in vitro* in human cadaver skin. (Wester *et al.*, 1993) In the Rhesus monkey percutaneous absorption of PCP was 24.4±6.4 percent from the applied dose in soil and 29.2±5.8 percent from acetone vehicle over a 24-hour period with unoccluded skin. These differences were not statistically significant. *In vitro* absorption was poor. Plasma receptor fluid accumulated 0.11 to 1.5 percent of the NaPCP dose, while skin concentrations were between 0.11 and 3.7 percent depending on the vehicle (higher absorption with the acetone vehicle) and skin

source. Wester *et al.* conclude that *in vitro* data greatly underestimates *in vivo* dermal absorption.

In contrast, dermal absorption values for PCP and NaPCP of 50 percent and 10 percent, respectively, were assumed by U.S. EPA in its regulatory actions against penta under FIFRA. (U.S. Environmental Protection Agency, 1984c) These default dermal absorption values were based upon an analysis of rat oral LD_{50} data.

b. Distribution

i. Animal Studies

Laboratory studies have relied almost exclusively upon single exposures to PCP to characterize tissue distribution. Those experimental studies indicated that most pentachlorophenol is rapidly excreted in the urine with only a small percentage of the dose found in the tissues. This conclusion is supported by studies which have treated animals with radiolabelled PCP, and then followed the time course of urinary and fecal PCP excretion and organ residues at sacrifice.

The previously mentioned study of Jakobson and Yllner was the first animal study to administer radiolabelled PCP to monitor the excretion, tissue distribution, and metabolism of pentachlorophenol. (Jakobson & Yllner, 1971) In this study, female mice (NMRI) were administered a single intraperitoneal or subcutaneous injection of 15-37 mg/kg ^{14}C-labeled PCP in olive oil. Regardless of the method of PCP administration, most of the activity (72-83 percent) was found in the urine. Among the organs, whole-body autoradiography and analysis of individual body organs revealed that "the highest specific activity was found in the gall bladder and its contents, the wall of the stomach fundus, the contents of the gastro-intestinal tract and the liver." (Jakobson & Yllner, 1971) Only a nominal amount of specific activity was found in lung, heart, and brain tissue. A

negligible fraction of the PCP dose, less than 0.05 percent, was found in expired air as $^{14}CO_2$. Based on the cumulative evidence the authors concluded that there was both gastric and biliary secretion of PCP or its metabolites, as well as fecal excretion.

Shortly after publication of the Jakobson and Yllner paper, Larsen *et al.* published a study on pentachlorophenol excretion and tissue distribution in rats. (Larsen, Kirsch, Shaw, Christian & Born, 1972) Male and female rats of an unidentified strain were administered a single oral dose of 99.5 percent radiochemically pure ^{14}C-labeled PCP dissolved in olive oil equivalent to 31-40 mg/kg of body weight.

The study reported the average total percentages of PCP excreted into urine 24, 48, and 72 hours after administration of the penta were 50.2, 63.4, and 65.2 percent, respectively, and the greatest amount of activity was measured in the urine sample collected 16 hours after dosing. Ten days following the dosing an average of 68.3 percent of the administered activity had been recovered in the urine Again, this study demonstrates that urinary excretion is the predominant route of excretion for pentachlorophenol. Benzene extraction of fecal material for PCP resulted in recovery of between 6.5 and 8.2 percent of the administered dose. Ignoring the fecal elimination pathway, the authors computed estimates of the tissue dose distribution. The greatest activity was found in the liver, followed by the kidney and blood. Low levels were estimated for the brain, fat, heart, lung, muscle, and gonads.

The previously described NaPCP inhalation exposure experiments conducted by Hoben *et al.* provide a similar pattern of distribution and excretion of pentachlorophenol compared to oral exposure. (Hoben, Ching, & Casarett, 1976b) After receiving a single 20 minute exposure, animals were sacrificed and lung and liver tissues, and accumulated urine and plasma were analyzed for PCP residues. The authors plot of the data show that

immediately after exposure ceased approximately 35 percent of the dose was found in the plasma, about 25 percent was found in the liver, and slightly less than 2 percent was found in the lung. At the sacrifice 24-hours after the brief exposure, approximately 55 percent of the dose was found in the urine collected from the animals, and about 9 and 7 percent of the dose was found in the liver and plasma, respectively. Less than about 0.80 percent of the dose remained in the lung. After 24-hours, data for these four sites indicated that about 70-75 percent of the original dose could still be accounted for as PCP.

In one of the two multiple dosing experiments conducted by Hoben *et al.*, two groups of 10 rats were exposed in the inhalation chambers for 20 minute periods daily for 5 days. (Hoben, Ching, & Casarett, 1976b) The estimated dose was 1.04 mg/kg. As in the earlier single dose experiment, animals were serially sacrificed and lung and liver tissues, and urine and plasma analyzed for PCP residues.

The plot of the data show that immediately after the fifth exposure session was concluded, approximately 32 percent of the PCP dose was found in the plasma, about 22 percent was found in the liver, and less than 2 percent was found in the lung. At the sacrifice 24-hours after the exposure, approximately 75 percent of the dose was found in the urine collected from the animals, and about 5 percent of the dose was found in each of the liver and plasma. Less than about 0.30 percent of the dose remained in the lung. These results are remarkably similar to those obtained from the single PCP inhalation exposure, showing little indication for bioaccumulation of PCP in rats following several repeated exposures.

Larsen *et al.* evaluated the transfer of pentachlorophenol through the rat placenta. (Larsen, Born, Kessler, Shaw & van Sickle, 1975) Charles River CD strain pregnant rats were orally administered a dose of 60 mg/kg of a 99.54 percent radiochemically pure ^{14}C-labeled PCP dissolved in olive oil on day 15 of gestation. The animals were serially sacrificed after

dosing, and the amount of PCP in maternal blood, placental tissue, and the fetuses determined. The amount of PCP in maternal blood peaked 8 hours after dosing at approximately 1.1 percent of the dose. The amount in the placenta and the fetuses peaked at 12 hours, with approximately 0.3 and 0.1 percent of the dose, respectively. This experiment indicates that very little PCP passes through the placenta to reach the fetus.

ii. Human Studies

Sparse human data is available regarding the tissue distribution of PCP in the general population. Most of the data that human data that exists is based on autopsy data of victims of fatal PCP intoxication due to either accidental overexposure or suicide. (Armstrong, et al., 1969; Blair, 1961; Gordan, 1956; Menon, 1958; Robson, Kissane, Elvick, & Pundavela, 1969; Wood, Rom, White, & Logan, 1983) Consequently, lacking accurate quantitative information on dosage, the inferences one may make regarding these data are quite limited. Nonetheless, consistent with the animal data, urine, blood and tissue PCP concentrations are elevated in cases of fatal PCP poisoning. In particular, PCP concentrations in the liver and kidneys, and occasionally the lungs (from inhalation exposure) are greatly increased. When compared to tissue and fluid PCP concentrations from the medical and toxicological literature, including data based on general population autopsy data from Germany (WHO, 1987), PCP concentrations in cases of fatal poisoning are two to five orders of magnitude higher than the general nonoccupationally-exposed public. Aside from several surveys of urinary PCP concentrations, scant other data exist to characterize the distribution of PCP in other body fluids and tissues.

c. Metabolic Transformation

i. Animal Studies

The biotransformation of PCP in warm-blooded animals, including humans, occurs via the following metabolic pathways: conjugation (PCP to PCP-glucuronide); reductive dechlorination, hydrolytic dechlorination, and oxidation. (Renner & Mucke, 1986; Renner & Hopfer, 1990) Other metabolic reactions for PCP and PCP-metabolites exist which are species-dependent, such as methylation (PCP to pentachloroanisole). (Renner & Mucke, 1986) PCP has been discovered to be a metabolite of pentachlorobenzene, hexachlorobenzene, and pentachloronitrobenzene (Renner & Hopfer, 1990) and lindane (Engst *et al.*, 1976). Tetrachlorophenols, a major contaminant of penta, are also metabolites of PCP, pentachlorobenzene, hexachlorobenzene, and pentachloronitrobenzene. (Renner & Hopfer, 1990) In general, there is a tendency towards metabolism of these compounds to lower chlorinated phenols.

Up to the 1970s, it was widely accepted that most PCP was excreted unchanged in the urine. More recent studies have demonstrated that the sample preparation methodology commonly employed in PCP analyses of biological fluids destroyed conjugated PCP and its metabolites in urine and serum prior to analysis. For instance, prior to analysis of urinary PCP samples are typically acidified to low pH before extraction with an organic solvent, derivatized to improve instrument response, and analyzed by gas chromatography. "This procedure, performed to get PeCP (PCP) in its unionized form and therefore increase the extraction recovery, leads to hydrolysis of PeCPG (PCP-glucuronide), as shown with rat urine in a previous study." (Reigner, Bois & Tozer, 1992, p. 21) In fact, Lilienblum reported that PCP-glucuronide was unstable at 37 C (roughly the normal human body temperature) at pH less than 7.4. (Lilienblum, 1985) Given that urine has an average pH of 6.3, significant amounts of conjugated PCP may

be hydrolyzed *in vivo* in the bladder prior to voiding. (Reigner *et al.*, 1991)

Nonetheless, hydrolysis is an important step in preparing a biological fluid sample prior to analysis for PCP. Hydrolysis of PCP-glucuronide in the samples is an important step because the small and highly variable interindividual proportion of free PCP is not a good indicator of absorbed dose. Edgerton and Moseman analyzed urine from the general population and exposed workers with and without an acid hydrolysis step. The hydrolyzed urine samples contained about 4 to 6 times as much PCP as the unhydrolyzed samples, and the difference could be as much as 17-fold higher. (Edgerton & Moseman, 1979) Thus, hydrolyzing the samples yields a repeatable measure of total PCP concentration that removes a major contributor to sample variability. Unfortunately, it also destroys information about the presence and concentrations of the various PCP conjugates and metabolites.

In general, only a relatively low percentage of PCP is metabolized, as opposed to conjugated, which is manifested by the large percentage excreted in the urine as PCP in all species tested. This low rate of metabolism may be due, in part, to its inaccessibility given the high percentage of PCP bound by plasma proteins.

Deichmann *et al.* were the first researchers to posit that pentachlorophenol was metabolized, based upon their experimental oral administration of NaPCP to rabbits, and intraperitoneal administration to rats. (Deichmann, Machle, Kitzmiller & Thomas, 1942) However, they did not identify any metabolites or conjugates in rat or rabbit urine. It was assumed that PCP was metabolically inert until decades later when evolving analytical chemistry techniques permitted the identification of pentachlorophenol residues in human food in the late sixties. (Renner & Mucke, 1986) These findings prompted the research initiative of the 1970s and 1980s to more critically define the metabolism of pentachlorophenol.

Jakobson and Yllner were the first researchers to show that PCP was metabolized to tetrachlorohydroquinone (TCH) in animals. (Jakobson & Yllner, 1971) After administering ^{14}C-labeled PCP to mice they collected and analyzed urine during the next 24 hours. About 21 percent of the administered activity was recovered as TCH, about one-third of the dose was excreted in the form of unchanged PCP. Some PCP conjugation occurred. However, the nature of the PCP and TCH conjugation could not determined because of the absence of a urine PCP hydrolysis pretreatment step from their experimental design.

Ahlborg *et al.* investigated the metabolism of PCP rats and mice by analyzing urine for metabolites following oral or intraperitoneal administration of a 100 percent isotopically pure ^{14}C-labeled PCP. (Ahlborg *et al.*, 1974) They confirmed the results of the Jakobson and Yllner study that PCP is metabolized by dechlorination to TCH in mice, and extended that finding to rats.

Unconjugated PCP comprised approximately 40 percent of the activity excreted in the urine of both rats and mice. Twenty-four hours after dosing unconjugated TCH was the only metabolite identified, representing 24 percent of the activity in the mouse, but only 5 percent in the rat. Attempts to hydrolyze the conjugates of PCP and TCH were unsuccessful due to inhibition of the hydrolytic activity of two different enzymes. Two minor components were also identified, tetrachlorophenol, a known impurity of pentachlorophenol, and trichlorohydroquinone which they speculated was a metabolite of tetrachlorophenol. Curiously, when PCP was administered orally the radioactivity recovered in urine of mice was only one-half of that recovered in rats.

Braun and Sauerhoff failed to demonstrate any urinary metabolites of PCP in rhesus monkeys. (Braun & Sauerhoff, 1976) One might conclude that perhaps PCP metabolism in monkeys is unique, not only is pentachlorophenol excreted essentially unmetabolized, it is also excreted unconjugated.

However, a more likely explanation is the fact that PCP glucuronide and TCH are unstable in urine or plasma, especially at low pH (Reigner *et al.*, 1991; Renner & Mucke, 1986), and Braun and Sauerhoff extracted urine after acidification thus destroying the penta conjugates and TCH. PCP glucuronide was reported to be unstable at a pH less than 7.4 (Lilienblum, 1985) and PCP sulfate (another PCP conjugate) is easily hydrolyzed under acidic conditions. (Reigner *et al.*, 1991) More recent metabolic studies add ascorbic acid to prevent TCH degradation. (Reigner, Rigod & Tozer, 1990) Also, research has demonstrated that conjugated PCP is released during storage at room temperature, or upon repeated thawing of frozen samples. (Norén & Sjövall, 1987, p. 61)

A year later, Braun *et al.* published the results of experiments designed to evaluate the metabolism of PCP in Sprague-Dawley rats. (Braun *et al.*, 1977) They reported that unchanged PCP in the urine accounted for 48 percent of the administered dose and TCH for 10 percent. The remainder of the radioactivity in the urine accounted for 6 percent of the administered dose, which the authors ascribe to PCP-glucuronide. No TCH-glucuronide was reported to be present. The absence of TCH and the low percentage of conjugated PCP is likely again an artifact of the sample preparation methodology and the instability of these substances at low pH. In this study, Braun *et al.* extracted PCP and TCH after acidification to a pH of 2. (Reigner *et al.*, 1991, p. 1556)

In a subsequent study, Ahlborg *et al.* were able to provide indirect evidence (using two different hydrolysis techniques) that PCP and TCH conjugate with glucuronic acid in the urine of rats administered 99.9 percent pure PCP intraperitoneally. (Ahlborg, Larsson & Thunberg, 1978) The authors presented the following breakdown on the composition of compounds excreted in the urine of PCP-treated rats: PCP, 60 percent; PCP-glucuronide, 9-16 percent; TCH, 7 percent; TCH-glucuronide, 16-22 percent.

In addition, based on the work of another part of the overall study, the authors conclude that dechlorination of PCP to TCH is mediated by microsomal enzymes that can be induced by phenobarbital. In this study, Ahlborg *et al.* extracted PCP and TCH after acidification to a pH of 2 (Reigner *et al.*, 1991, p. 1556), thus reducing the percentage of TCH and conjugated PCP recovered in urine.

In a related study published the same year, Ahlborg and Thunberg reported on the effects on PCP (99.9 percent purity) metabolism in rats of pretreatment with phenobarbital (PB), 3-methyl-cholanthrene (3-MC), or 2,3,7,8-tetrachlorodibenzo-p-dioxon (2,3,7,8-TCDD). (Ahlborg & Thunberg, 1978) They reported that pretreatment by either 3-MC or 2,3,7,8-TCDD led to (approximately two-fold) greater tetrachlorohydroquinone formation than did pretreatment with PB. In addition, pretreatment with 3-MC and 2,3,7,8-TCDD led to (about four-fold) greater trichlorohydroquinone formation than did PB pretreatment.

A more recent study by Lilienblum contradicted this work of Ahlborg *et al.* (Lilienblum, 1985) Lilienblum reported that while investigating PCP glucuronides synthesis in rat and human liver microsomes and their stability at urinary pH, they found that glucuronidation of PCP was not inducible by either phenobarbital or 3-methylcholanthrene. This conclusion directly conflicts with the reports of Ahlborg *et al.* (Ahlborg *et al.*, 1978; Ahlborg & Thunberg, 1978) Lilienblum further states that based upon the instability of PCP conjugates at urinary pH, determinations of PCP-glucuronide in urine underestimate the amount excreted by the kidneys. Thus, the estimates presented above regarding the proportion of penta excreted as PCP-glucuronide may be biased downward by an indeterminate amount that vary by sample preparation method, animal species, and animal treatment.

It is important to note that all of the controlled studies on the metabolism of PCP in mammals have been conducted with high purity PCP, not commercial grade PCP. An experiment in fish revealed that compared to commercial PCP, fish exposed to purified PCP conjugated a greater proportion of the PCP before eliminating it as the glucuronide. (Huckins & Petty, 1983) To the extent that conjugation is a rate-limiting step in detoxification, impurities in commercial PCP may alter both the metabolism and toxicity of PCP. However, metabolism studies in humans, described below, where exposure was to commercial PCP containing impurities lends confidence that the better animal studies accurately model human metabolism.

In a recent and perhaps most definitive study of PCP metabolism in (female Sprague-Dawley) rats, Renner and Hopfer identified tetrachlorohydroquinone as the major PCP metabolite. (Renner & Hopfer, 1990) This metabolite was excreted principally in conjugated form. The following are the other confirmed PCP metabolites identified by Renner and Hopfer: tetrachlorocatechol, trichlorohydroquinone, 2,3,4,5-tetrachlorophenol, 2,3,4,6-tetrachlorophenol, 2,3,5,6-tetrachlorophenol, tetrachloro-1,4-benzoquinone, tetrachlororesorcinol, and traces of trichloro-1,4-benzoquinone.

In another recent well-conducted PCP metabolism study in male Sprague-Dawley rats, Reigner *et al.* refuted a number of findings of Braun *et al.* (Braun *et al.*, 1977) and (Ahlborg *et al.*, 1974) both of whom also used Sprague-Dawley rats. Braun *et al.* had reported unconjugated PCP accounted for 48 percent of the dose and unconjugated TCH accounted for 10 percent of the dose. The corresponding values were 41 to 43 percent for PCP, and 5 and 24 percent for TCH in rats and mice, respectively. In contrast, the values for the current study are about 5 percent and 1 percent, respectively. These discrepancies are believed due to now recognized errors in sample preparation methodology discussed above that

Reigner *et al.* were able to avoid. Therefore, quantitatively it appears that metabolites (such as the most abundant TCH) are not important. Their toxicological importance, however, is a separate issue that is discussed below.

ii. Human Studies

The Ahlborg *et al.* study discussed above presented the earliest work on the metabolic transformation of PCP in humans. (Ahlborg *et al.*, 1974) Twenty-four urine specimens were collected from two spraymen who had occupational exposure to pentachlorophenol as well as to other chlorophenolic compounds.(WHO, 1987) Tetrachlorohydroquinone was detected in the urine of both workers. This is the only human *in vivo* study that has detected TCH as a metabolite of PCP.

As previously mentioned, Braun *et al.* administered a single 0.1 mg/kg of body weight (purity >99 percent) dose of NaPCP to each of four healthy male volunteers. (Braun *et al.*, 1979) Within 7 days (168 hours) of administration approximately 74 percent and 12 percent of the dose were excreted as PCP and PCP-glucuronide, respectively, and 4 percent was eliminated as PCP and PCP-glucuronide in the feces. Of this four percent, half (2 percent) was eliminated as PCP and half as PCP-glucuronide. The fate of the remaining 10 percent of the administered dose was unexplained. The results of their analysis led them to conclude that the pharmacokinetic profile of orally ingested PCP in humans was more similar to that of rats than of monkeys. This is not surprising since the same analytical protocol was followed, including the addition of HCl to acidify urine and plasma. Consequently, the low estimates of PCP-glucuronide and the absence of tetrachlorohydroquinone are unremarkable given what is now known as the inappropriate sample preparation of these materials for penta and PCP metabolite analyses by this study's authors.

Janssens and Schepens supplied one of the first hints of significant PCP conjugation. They reported that long-term penta exposure from treated homes results in a large percentage of it being excreted as the glucuronide. (Janssens & Schepens, 1984) In particular, they reported that in over 50 percent of the urine samples the fraction of free (unconjugated) PCP was "...very low (below 30%)." (Janssens & Schepens, 1984) These PCP-glucuronide values are far higher than the 12 percent figure reported in the Braun *et al.* (1979) study, although they are likely underestimates of conjugated PCP given the acid extraction step performed on every sample.

Juhl *et al.* confirmed the metabolism of PCP to tetrachlorohydroquinone in rat and human liver homogenates *in vitro*. (Juhl, Witte & Butte, 1985) The rate of transformation was similar in both species' homogenates. However, the rate of PCP metabolism was an inverse function of the concentration and was 1,000 times lower at 1.0 mmol/L (266 mg/L) than at 0.01 mmol/L (2.66 mg/L). More recently, however, the results of the human *in vivo* pharmacokinetics experiments by Uhl *et al.* cast doubt regarding the applicability of the Juhl *et al. in vitro*, and the Braun *et al.* (1979) *in vivo* study results. (Uhl *et al.*, 1986)

Within the detection limits of their instrumentation, Uhl *et al.* observed no tetrachlorohydroquinone or 2,3,4,5- and 2,3,4,6-tetrachlorophenol were found in urine or blood plasma. In contrast to the results of the Braun *et al.* (1979) study, Uhl *et al.* reported that the percentage of PCP excreted as the glucuronide increased daily after the administration. By the end of the second week post-administration, the percentage of PCP-glucuronide excreted in the urine attained the "normal" range for nonoccupationally exposed populations of approximately 61-70 percent (median 65 percent). However, Uhl *et al.* made the same sample preparation errors as many others who have researched PCP metabolism in animals and human fluids and who have acidified these fluids prior to

analysis. To 5 mL of urine Uhl *et al.* added 2 mL of concentrated HCl, and to each 1 mL of plasma they added the same volume of concentrated HCl. Thus, Uhl *et al.* effectively guaranteed that no TCH would be found.

Gómez-Catalán *et al.* analyzed 50 urine specimens from individuals with no known PCP or HCB exposure in Barcelona, Spain. (Gómez-Catalán, To-Figueras, Planas, Rodamilans & Corbella, 1987) Samples were analyzed with and without acid hydrolysis. Free PCP in the urine amounted to only about 13 percent of the total urinary PCP; conjugated PCP amounted to about 87 percent of the total PCP.

Finally, Norén and Sjövall measured the concentrations of free and conjugated PCP in urine from 12 individuals without occupational exposure to the chemical. They reported that "…PCP was present almost exclusively as conjugates in our samples…" (Norén & Sjövall, 1987, p. 61) Reigner *et al.* calculate that about 98.5 percent of the PCP was present as PCP-glucuronide. (Reigner *et al.*, 1992, p. 21)

d. Elimination and Excretion

i. Animal Studies

The studies discussed above demonstrate that PCP and its metabolites, when present, are relatively rapidly cleared from the body of most animals, including man, after short-term exposure. Clearance occurs via two major routes: urine and feces. Urinary elimination dominates over fecal excretion. Only small amounts of PCP or its metabolites are found in tissues, indicating that most of these substances leave the body soon after short-term exposure. However, repeated exposure may lead to accumulation in tissues that are slow to reach equilibrium.

Three studies in rats reported similar amounts of PCP excreted in urine after administration. Braun *et al.* recovered 64 percent of the high

(100 mg/kg) oral dose and 80 percent of the low oral (10 mg/kg) dose (Braun *et al.*, 1977) within 8 and 9 days of administration, respectively; and Reigner *et al.* recovered an average of about 58 percent of the intravenous dose (2.5 mg/kg) and 52 percent of the oral dose (2.5 mg/kg) in the urine as PCP and metabolites within 3 days of administration (Reigner *et al.*, 1991). Ahlborg *et al.* reported 70 percent of an intraperitoneal dose (10-25 mg/kg) was recovered in urine after 24 hours, but about 50 percent of the oral dose (10-25 mg/kg) was recovered after 4 days. (Ahlborg *et al.*, 1974)

Three studies in rats reported similar amounts of PCP excreted in feces. Larsen recovered between 9.2-13.2 percent of the oral dose after 10 days (Larsen *et al.*, 1972) and Braun *et al.* recovered 18.6 ± 3.7 percent of the low oral dose (Braun *et al.*, 1977) within about 9 days of administration, and Reigner *et al.* recovered an average of 10.1 percent of the intravenous dose and 9.3 percent of the oral dose in the feces as PCP and metabolites within 3 days of administration (Reigner *et al.*, 1991). Thus, biliary excretion of PCP contributes to the elimination of PCP from the body. *E. coli* in the rat gastrointestinal tract secrete ß-glucuonidase and sulphatase, which would be expected to hydrolyze much of the conjugated PCP and TCH that enter the gastrointestinal tract of the animals, except that TCH inhibits ß-glucuonidase activity (Reigner *et al.*, 1991) thus confounding the data interpretation of PCP and its metabolites in feces.

ii. Human Studies

The data indicate that the renal excretion of unconjugated PCP "...is a minor pathway of elimination." (Reigner *et al.*, 1992, p. 21) Two lines of evidence support this belief. First, if the kidneys were principally responsible for PCP clearance from the body one would expect individuals

with impaired renal function to higher concentrations of PCP in their plasma. Pearson *et al.* investigated this possibility. (Pearson, Schultz, Rivers & Gonzalez, 1976) They evaluated 14 normal individuals and 23 patients undergoing chronic hemodialysis. The plasma concentrations of PCP, DDT, and DDE in the normal, predialysis, and post-dialysis groups were virtually identical and were not statistically different. The authors concluded that the fate of pesticides that are both lipid soluble and protein-bound is not governed by renal function.

Second, Cline *et al.* analyzed serum and urine samples from 34 controls and 123 residents of PCP-treated log homes. The measured values varied widely. However, when urine concentrations were corrected for creatinine levels the normalized urine concentrations correlated well with the serum concentrations (r=0.92). (Cline *et al.*, 1989) This finding further supports the evidence discussed above that it is PCP-glucuronide, not PCP, that is excreted in urine. Reigner *et al.* argue that PCP-glucuronide like creatinine (which are both polar compounds) would be expected to be poorly absorbed by the renal tubule, whereas a nonpolar compound like PCP would be expected to be absorbed. (Reigner *et al.*, 1992)

Thus, the kidneys and to a lesser extent the liver (through enterohepatic circulation) play the greatest roles in the clearance of PCP from the body. Table IX summarizes the key results of animal studies of PCP elimination and excretion. Reigner *et al.* reported that about 10 percent of the PCP dose was excreted in the feces. (Reigner *et al.*, 1991) The amount excreted in the feces was about the same for both intravenous and oral administration. The authors felt that this low amount may have been due to either "...low capacity of for biliary transport of the conjugates or enterohepatic cycling via hydrolysis of the conjugates within the alimentary canal." (Reigner *et al.*, 1991, p. 1556)

TABLE IX

RECOVERY OF PENTACHLOROPHENOL FOLLOWING A SINGLE EXPOSURE

SPECIES	N	DOSE (mg/kg)	TIME (hrs)	% RECOVERY FROM URINE	% RECOVERY FROM FECES	% RECOVERY FROM OTHER TISSUES	% TOTAL DOSE RECOVERED	REFERENCE
Mouse								
Female	1	14.8	96	83.0	7.8	2.3	98.1	Jakobson & Yllner, 1971
	1	18.2	96	72.0	5.0	10.0	87.6	
	1	37.2	96	73.1	3.8	5.9	84.8	
	1	35.2	168	81.5	9.7	1.3	93.9	
	1	36.8	168	80.4	11.5	0.4	92.4	
Rat								
Male	3	10	216	80.1	18.8	0.8	99.8	Braun et al., 1977
Female	3	10	216	78.1	19.3	0.8	99.8	
Male	3	100	192	72.2	24.5	nd	97.6	Braun et al., 1977
Female	3	100	192	54.4	42.8	nd	97.6	
Monkey								
Male	2	10	168	75.2	12.3	nd	nd	Braun & Sauerhoff, 1976
Female	2	10	360	70.3	18.3	11.4	100	
Monkey								
Male	1	30	144	2.0	24.0	nd	nd	Ballhorn et al., 1981
	1	50	144	3.1	12.3	nd	nd	
Human								
Male	4	0.1	168	86	4	nd	nd	Braun et al., 1979

The experimental human exposure studies discussed above involved administration of a single oral dose of NaPCP or PCP to male volunteers while the occupational studies involved individuals with repeated exposures. By comparing these human studies, it appears that the excretion kinetics of penta is significantly altered by repeated exposure *versus* a single exposure. Thus, the validity of the pharmacokinetic models based on acute exposure studies is questionable. Three papers have evaluated the literature to develop pharmacokinetics models for long-term penta exposure. These studies will be discussed in the next subsection: retention and turnover.

Braun *et al.* reported that within 168 hours after administration, the men eliminated 74 percent of the total dose as the parent compound and 12 percent as the conjugated PCP-glucuronide. (Braun *et al.*, 1979) Approximately 4 percent of the total dose was excreted in the feces, half as free PCP and the other half as PCP-glucuronide. In the other human experimental PCP exposure study, Uhl *et al.* reported that up to 96 hours after administration of a single 0.31 mg PCP/kg body weight approximately 30 percent of the PCP was eliminated as the glucuronide. (Uhl *et al.*, 1986)

e. Retention and Turnnover

i. Animal Studies

Several of the experimental studies discussed earlier have investigated the retention or biological half-life of PCP and its metabolites in the body. As previously discussed, the pharmacokinetics of PCP and its metabolites varies by sex and species.

Larsen *et al.* analyzed the data from their excretion and distribution study of PCP in rats and concluded that the urinary excretion appeared to follow a two-component biphasic model. (Larsen *et al.*, 1972) The first

component had a half-life of 10 hours (during which 50 to 60 percent of the dose was eliminated), the second component had a half-life of 102 days.

The accuracy of the sigma-minus plot used by Larsen *et al.* to generate these estimates has been criticized by Braun *et al.* because it depends upon subtracting the cumulative amount of PCP excreted in urine from the dose, and thus is highly sensitive to the total recovery of PCP in the study. (Braun *et al.*, 1977) The total dose recovery value was not determined in the study. Failure to obtain total dose recovery approaching 100 percent can result in gross errors in elimination rate estimates, particularly for the longer duration second-component. (Braun *et al.*, 1977)

In their 1976 study of the pharmacokinetics of PCP in rhesus monkeys Braun and Sauerhoff posited that the long half-life of PCP was attributable to biliary excretion and enterohepatic circulation. (Braun & Sauerhoff, 1976) The authors reached this conclusion from the observation that 11.5 percent of the dose still remained in monkey tissues 15 days after the PCP was administered, and 80 percent of that amount was located in the intestines and liver. (Braun & Sauerhoff, 1976) Braun and Sauerhoff calculated that steady-state concentrations of PCP would be reached in monkey plasma after the ten consecutive daily doses. Their model assumed linear kinetics, no saturation of excretion or metabolism mechanisms, or induction or inhibition of metabolic pathways. (Braun & Sauerhoff, 1976)

Braun and Sauerhoff computed that the clearance of PCP from plasma was monophasic in rhesus monkeys, and from the observed first order kinetics, calculated a half-life 80 hours. There were sex-related differences in the rates of clearance of PCP from urine and plasma, with shorter PCP clearance half-lives found in males than in females for both plasma and urinary removal. For instance, for male and female monkeys the average plasma elimination rate was 0.0103 and 0.0083 hr^{-1}, respectively, corresponding to elimination half-lives of 72.0 and 83.5 hours. For male

and female monkeys the average urine excretion rate was 0.017 and 0.0075 hr^{-1}, respectively, with corresponding half-lives of 40.8 and 92.4 hours.

Subsequent studies with monkeys have used therapeutic cholestyramine treatment to confirm that hypothesis that enterohepatic circulation of PCP plays a critical role in the long observed half-life in nonhuman primates. (Ballhorn, Rozman, Rozman, Korte & Greim, 1981; Rozman, Ballhorn, Rozman, Klaassen & Greim, 1982) Among control monkeys receiving 30 mg PCP/kg/d, 93.2 percent of the penta was excreted in urine and 7.7 percent in feces. (Ballhorn *et al.*, 1981) The monkeys receiving the cholestyramine following the penta excreted 12.1 percent in urine and 86.9 percent in feces. The control monkeys receiving 50 mg PCP/kg/d excreted 79.9 percent of the dose in urine and 20.1 percent in feces. In comparison, the cholestyramine-treated monkeys excreted 15.4 and 84.6 percent in urine and feces, respectively. (Ballhorn *et al.*, 1981)

Apparently cholestyramine binds PCP in bile, resulting in decreased reabsorption and increased fecal elimination. (Rozman *et al.*, 1982) In addition, the study by Rozman *et al.* of the therapeutic effects of cholestyramine on PCP excretion included a separate evaluation of the half-life of PCP prior to remedial treatment of three male rhesus monkeys. The PCP was administered as a single dose of 50 mg ^{14}C-labeled PCP/kg body weight in 50 percent ethanol administered to the animals by stomach tube. Compared with PCP-treated controls, cholestyramine treatment reduced urinary excretion from 35 to 5 percent of the administered dose, and increased fecal excretion from 3 to 54 percent of the administered dose.

The half-life of PCP in blood and urine was calculated to be 32 and 30 hours, respectively. The shorter half-life values from this study compared with the Braun and Sauerhoff (1976) study may be due to the different carrier solvents used (ethanol *versus* corn oil). Enhanced PCP absorption also was observed in humans administered PCP in ethanol compared

with those administered NaPCP in water. (Braun *et al.*, 1979; Uhl *et al.*, 1986) This difference might also be due to the five-fold higher dose Rozman *et al.* administered to the monkeys *versus* the dose administered by Braun *et al.*

In a subsequent study, Braun *et al.* showed dramatic interspecies differences between the pharmacokinetics of PCP in rats and monkeys. For instance, the clearance of PCP from plasma was biphasic in the rat, not monophasic like the monkey. The alpha-phase elimination half-life was only 13 (in males) to 17 hours (in females), much faster than in the monkey, and a much slower but variable beta-phase elimination (half-life of 40 hours in male rats dosed at 10 mg/kg and 121 hours at 100 mg/kg, but 32 hours in female rats at the higher dose). They concluded that rats, like mice (and unlike monkeys), do metabolize PCP. (Braun *et al.*, 1977)

Thus, in addition to these interspecies differences, there are pronounced sex-related intraspecies differences. As in monkeys, the clearance rates of PCP from urine and plasma were faster in males than for females of these species. The pharmacokinetic estimates of Braun *et al.* have been criticized on the grounds that the periodic sampling methodology was flawed. Specifically, Braun *et al.* "…did not take multiple blood samples from a same animal, but killed two animals at different times to get the kinetic profile. Analysis of such data, called by others 'naive pooled data', has been criticized because it can lead to the use of a wrong model and/or to incorrect parameter estimates." (Reigner *et al.*, 1991, p. 1555)

It is important to note the observation by Braun *et al.* that "retention of PCP in the plasma in preference to uptake by tissues other than the liver and kidneys appears to be associated with the tenacious binding of PCP to plasma protein." (Braun *et al.*, 1977) In fact, approximately 99 percent of the PCP in plasma was bound to protein. The

authors suggest that this factor, and not extensive renal reabsorption, is responsible for the low tissue-to-plasma ratios and low renal clearance rate. (Braun *et al.*, 1977)

Four days after the PCP was administered, over 90 percent of the radioactively-labelled PCP was eliminated during the rapid phase, that is, within 3 days, and only 0.5% of the administered dose remained in the rats. This pharmacokinetic profile led the authors to conclude that "the toxicity of PCP was not cumulative upon repeated ingestion." (Braun *et al.*, 1977) The variable nature of the beta-phase elimination is puzzling. At the 100 mg/kg dose female rats exhibited a monophasic elimination pattern with a half-life of 27 hours, still much faster than in rhesus monkeys. However, the strength of this conclusion is somewhat tenuous. If the last sample data point (at 192 hours) were eliminated, a biphasic elimination profile could easily be fit. (WHO, 1987) In a study of the distribution of PCP following inhalation exposure, Hoben *et al.* likewise reported a monophasic (first-order) elimination pattern for PCP in male rats, and estimated a comparable half-life in rats of 24 hours. (Hoben, Ching, & Casarett, 1976b)

Meerman *et al.* evaluated the pharmacokinetics of PCP or NaPCP administered in food, and NaPCP administered in drinking water for a period of one week. (Meerman *et al.*, 1983) They reported that the concentration of penta in the plasma of their Wistar rats was much lower than the level expected based on the earlier pharmacokinetic study in Sprague-Dawley rats conducted by Braun *et al.* Their analysis of the data indicated that "the distribution and elimination of PCP was best described by a two-compartment, open system model with half-live values of 2.17 and 7.24 hr for the two phases of the plasma disappearance curve of PCP." The elimination rate for the male Wistar rats (receiving approximately 17.5 mg/kg) was 0.192 hr^{-1} *versus* 0.0343 hr^{-1} for the male Sprague-Dawley rats (receiving 10 mg/kg). In addition, Meerman *et al.* point out that the

volume of distribution for Wistar rats is 58 percent greater than that for Sprague-Dawley rats. They conclude that a much faster elimination rate in Wistar strain rats explains their unexpectedly low plasma penta levels.

Leighty *et al.* demonstrated that PCP underwent esterification with palmitic acid *in vitro* in a rat liver enzyme system. (Leighty & Fentiman, 1982) They speculated that the toxic effects of penta exposure may stem from its *in vivo* retention in the body after esterification with fatty acids. According to this hypothesis, penta would enter lipid membranes of mitochondria and microsomes and alter their function.

Reigner *et al.* evaluated the pharmacokinetics of PCP in rats, using the same strain as Braun *et al.* Reigner *et al.* fit an open two-compartment model to data from a rats administered 2.5 mg/kg of PCP via i.v. Their half-life estimate for the first phase, 0.7 hours, is dwarfed by the 17.4 hours of Braun *et al.*, and about one-third of the estimate of 2.2 hours of Meerman *et al.* Their half-life of the second phase, 7.1 hours, is still a fraction of the 40.2 hours of Braun *et al.*, but nearly identical to the estimate of 7.2 hours of Meerman *et al.* Given the greater care and sophistication of the most recent experiment, and their consistency with the work of Meerman *et al.* these findings should carry greater weight.

ii. Human Studies

As discussed earlier, Bevenue *et al.* reported on the case of an individual who was exposed to PCP from cleaning a paint brush with a solution that contained pentachlorophenol. (Bevenue, Haley, & Klemmer, 1967) The man had immersed his hands in the solution for ten minutes. From sequential measurements of urinary PCP concentrations an elimination half-life of approximately 15 days can be derived. (WHO, 1987)

In the same year Bevenue *et al.* reported the results of a survey of urinary PCP concentrations among 541 individuals, most of whom were males, residing in Honolulu or adjacent areas. (Bevenue, Wilson, Casarett, & Klemmer, 1967) Of these people, 130 (24 percent) were pest control operators (Group A), presumably with a greater opportunity for exposure. The remainder of the sample consisted of individuals either randomly selected from households or industry (Group B), or from a large cohort involved in a health study (Group C).

Between one and five urine samples were collected from each individual, at periods ranging from 1 to 115 days apart. Not surprisingly, those individuals with potential occupational exposure to PCP exhibited far greater mean urinary PCP concentrations than those nonoccupationally exposed. The mean urinary PCP concentration was 1,802 ppb in Group A, and 465 ppb among occupationally exposed members of Group C. The mean value was 40 ppb in Group B, and 44 ppb among nonoccupationally exposed members of Group C. (Bevenue, Wilson, Casarett, & Klemmer, 1967) The mean urinary PCP value for the nonoccupationally exposed members of Group C is nearly 7 times higher than mean concentration measured in the NHANES II study of the general U.S. population.

From the sequential urinary PCP measurements Bevenue *et al.* recorded and plotted what appears to be biphasic elimination pattern, with a rapid initial elimination phase followed by a slower rate of excretion. However, the authors were acknowledged the possibility that intermittent occupational exposures were responsible for the observed excretion patterns, or that high body PCP loadings in excess of some threshold might trigger the higher elimination rate. Following occupational penta exposure, they reported mean decrements in urinary PCP concentrations of 34.7 percent per day 1-2 days following exposure, 3.3 percent per day 10-23 days following exposure, and 0.85 percent per day 64-115 days following exposure.

Casarett *et al.* measured blood and urine PCP concentrations in wood treatment workers from two plants that used PCP. (Casarett *et al.*, 1969) In one instance, the urinary elimination rates of PCP for two workers who spent 45 minutes in a confined space painting wood were presented. The authors noted a time lag of approximately one day before urine PCP concentrations significantly increased, with peak concentrations attained between one and two days after the exposure. The authors report an average urinary excretion half-life of 10 hours for the two subjects.

In other observations from among employees of these same two wood treatment plants, Casarett *et al.* reported upon the mean decrease in urine PCP after cessation of exposure. (Casarett *et al.*, 1969) Again, considerable variation in the urine PCP values was evident among individuals absent PCP exposure for the same number of days. For instance, after a single day's absence an average decrease of 39 percent in urine PCP was found. After 2 days, the average decrease was 71 percent, it was also 71 percent after 5 days, 82 percent after 7 days, 78 percent after 14 days, and 60 percent after 18 days.

These data led the authors to conclude that "the failure of the PCP concentration to decrease more than about 60 to 80% even after a long absence from exposure suggests yet another constant in the compartmental equilibrium controlling excretion rate." (Casarett *et al.*, 1969) Casarett *et al.* proposed that PCP bound to plasma proteins was the possible identity of this missing compartment. They also inferred that there are differences in kinetics between single and repeated exposures.

Begley *et al.* made periodic measurements of blood and urine PCP, renal clearance values for phosphorus and creatinine, and calculated a phosphate reabsorption index, for workers of a company that treated lumber, furniture and other wood products with a 5 percent solution of PCP. (Begley, Reichert, Rashad & Klemmer, 1977) Five blood and urine samples

were collected from each of 18 male employees of the firm who had volunteered for the study on the last day of work before a 20-day vacation, and on the morning of the third, sixth, thirteenth, and twentieth day of vacation.

Although there was considerable interindividual variation in the data, by the twentieth day of vacation, average PCP concentrations in blood and urine declined to less than half of their pre-vacation values. PCP concentrations increased on the sixth day of vacation for 11 of the 18 participating workers, indicating to the study authors that PCP was being released from some secondary corporeal storage compartment. The estimated half-life in blood and urine was approximately 9 days when the entire data set was used (WHO, 1987, p. 111), about 12 days for blood and urine when the data from the sixth to twentieth days post-vacation were used (Uhl *et al.*, 1986), and 15.6 days when it was estimated from five mean plasma concentration measurement times. (Reigner *et al.*, 1992, p. 20)

In their experimental treatment of four healthy male volunteers, Braun *et al.* noted a significant time lag between plasma T_{max} (4 hours) and urinary T_{max} (42 hours) which they ascribe to the "strong enterohepatic recirculation" of PCP, similar to that observed in reports from laboratory animal experiments. (Braun *et al.*, 1979) Their analysis of the data led them to judge that "absorption and elimination in humans could be described by a one-compartment open system model with first-order absorption, enterohepatic recirculation and first-order elimination." (Braun *et al.*, 1979) Braun *et al.* computed elimination half-lives of 30.2 ± 4.0 hours for PCP from plasma, and 33.1 ± 5.4 and 12.7 ± 5.4 for excretion of PCP and PCP glucuronide from urine, respectively. These values for plasma (32 hours) and urinary (30 hours) elimination are nearly identical with those from a subsequent experiment in monkeys by Rozman *et al.*

Braun *et al.* concluded that the rat appeared to be a better model for PCP pharmacokinetics in man than the monkey due to its closer similarity for a number of parameters. Based upon a model simulation of the pharmacokinetics of PCP in man, Braun *et al.* concluded that near steady-state concentrations of PCP would be reached in 8.4 days, with a maximum plasma concentration of 0.0491 mg/L. Braun *et al.* concluded that from this analysis that the current occupational health air guideline value of 0.5 mg/m^3 was acceptable even if exposure were to occur on a repeated basis.

The results of the experimental treatment of three healthy human male volunteers by Uhl *et al.* differs in many respects from those obtained from the human study of Braun *et al.* For instance, Uhl *et al.* failed to observe the time lag between the maximal blood and urine concentrations. This may be the result of the more rapid absorption of PCP in the ethanol carrier solvent. In addition, Uhl *et al.* estimated an elimination half-life of 20±3.4 days in urine for one of the men administered 18.8 mg of unlabeled PCP/kg body weight, and an elimination half-life of 18±2.4 days in urine, and 16±2.5 days in blood for the same individual administered 0.98 mg ^{13}C-PCP/kg body weight. (Uhl *et al.*, 1986) Uhl *et al.* discuss several possible explanations for their extremely long elimination half-life estimates.

First, Uhl *et al.* considered and discarded the possibility that enterohepatic recirculation is responsible for this phenomenon because of the results of a side investigation they conducted of PCP concentration in bile of cholelithiasis patients with postoperative T-drains *versus* plasma and urine levels. PCP concentrations in bile were found to be comparable to those in urine and plasma. In addition, no significant changes in PCP concentrations in the three body fluids were observed during the drainage of bile via a catheter, leading them to conclude that enterohepatic circulation is not a PCP reservoir in humans. No further details about this experiment were reported. Clearly, the conclusions Uhl *et al.* reach

about enterohepatic circulation directly conflict with data and interpretations of data from several of the animal studies discussed above.

Second, Uhl *et al.* considered the possibility that blood could serve as a reservoir for PCP due to its strong binding affinity for plasma protein. They judge this reservoir a likely candidate based upon measurements they performed as part of this study wherein greater than 96 percent of the PCP in plasma was bound to protein. Citing the similar measurements of PCP binding in rat plasma (99 percent) by Braun *et al.* (1977), they conclude that protein-bound PCP in plasma is partially responsible for this phenomenon.

Finally, Uhl *et al.* considered the protein binding effect of PCP on renal clearance. Based upon renal clearance measurements of their volunteers they calculated that PCP clearance was "extremely low (0.07 mL/min)," far below normal clearance rates of 90-160 mL/min. (Uhl *et al.*, 1986) Uhl *et al.* conclude that "more than 99% of the filtered PCP is reabsorbed in the renal tubules. With a pK_a of 5.26 PCP is present almost totally in the phenolic form at normal urinary pH (5-6). Under these conditions, reabsorption of PCP occurs readily." (Uhl *et al.*, 1986) As a test of this hypothesis, *in vivo* alkalinization of urine (with sodium bicarbonate) was performed to observe the effect on PCP excretion. As pH increased from 5.4 to 7.8, urinary excretion of PCP increased more than eight-fold. No further details about this experiment were provided.

Another possible explanation for the disparate half-life estimates for humans from the Braun *et al.* and Uhl *et al.* studies is the volunteer dosing methodology. Braun *et al.* withheld food from their volunteers for a period beginning 8 hours prior to dosing and ending 1 hour after ingestion of the penta. On the other hand, the volunteers in the Uhl *et al.* study were not restricted in their dietary practices prior to, or after, dosing. Food in the intestinal tract may have slowed penta absorption in the Uhl *et*

al. study, and possibly accounting for the observed differences in half-lives.

Uhl *et al.* calculated that repeated exposure to PCP for 3 months would be necessary in order to attain steady-state conditions in humans. They concluded that the existing laboratory animal pharmacokinetic data are a poor match for humans and that the human body burden of PCP is some 10-20 times higher than what would be expected from extrapolation from the animal data. (Uhl *et al.*, 1986) The occupational exposure studies conducted by Bevenue *et al.*, Casarett *et al.*, and Begley *et al.* supply supporting evidence for the slower PCP elimination rate estimates in humans developed by Uhl *et al.* and thus repudiate the rapid PCP elimination estimates developed by Braun *et al.*

A study of workers exposed to chlorophenol mixtures shows similar long elimination half-lives. For instance, Kalman and Horstman observed urinary elimination rates among 40 woodworkers chronically exposed to a Permatox 100™ sapstain formulation (3 percent PCP, 21 percent tetrachlorophenol) and a control group of 40 unexposed workers from the same plant during a 16-day vacation and plant shutdown. (Kalman & Horstman, 1983) No clear elimination pattern of PCP was observed during the 16-day period when the authors assumed a one-compartment first-order decay and that initial urine PCP levels were at steady-state in computing the estimates of the urinary excretion half-life of PCP.

Their estimates of half-lives for individual workers ranged from 4 days to 72 days, with an average half-life of 28 days and a standard error of 25 days. It is important to note that half-life estimates could not be computed for 11 other exposed participants because either urine PCP concentrations actually increased over the 16 day vacation (n=9), or they barely diminished at all (n=2). These inconsistent results led the authors to conclude that PCP exposure outside the workplace was probably occurring.

It should be noted, however, that single grab urine samples were collected and that no density correction was applied to the sample results.

Reigner *et al.* evaluated 15 human studies of individuals with occupational or nonoccupational exposure to PCP that also evaluated PCP clearance in urine and blood. (Reigner *et al.*, 1992) Eleven of the studies were judged to have appropriate sample preparation methodology and to have provided data on individuals. These 11 studies provided sufficient data to calculate 20 plasma clearance values on a total of 604 individuals. An overall weighted average PCP plasma clearance value of 0.0177 Lh^{-1} (0.425 Ld^{-1} was calculated. The clearance values calculated from these studies were independent of average plasma concentration, the slope of the clearance value *versus* average plasma concentration was not significantly different from zero.

Reigner *et al.* compared their overall clearance value from the only two human pharmacokinetic papers. While they calculated an overall clearance value of about 0.018 Lh^{-1}, Braun *et al.* (Braun *et al.*, 1979) give a value of 0.51 Lh^{-1} (over 28-fold higher), and Uhl *et al.* (Uhl *et al.*, 1986) give a value of 0.0042 Lh^{-1} (over 4-fold lower). While they find a computational error in the work of Uhl *et al.* that brings the adjusted clearance estimate much closer to their overall clearance value, no completely satisfactory reason was offered why the Braun *et al.* study should be so markedly at odds with 11 other studies.

Using the clearance concept methodology, Reigner *et al.* proceeded to calculate average daily PCP intake for the general nonoccupationally exposed U.S. population from the various nonoccupationally exposed study groups they evaluated for PCP clearance. Their estimates of 12 to 22 µg/day are consistent with the estimated values of 16 µg/day for Americans (Hattemer-Frey & Travis, 1989) and 19 µg/day for Germans (Geyer *et al.*, 1987). The similar values give confidence in the accuracy of the

methodology used by Reigner *et al.*, and support for the belief that PCP has a longer (~16-20 day) rather than shorter (~7 day) biological half-life. Reigner *et al.* estimated PCP intake for PCP plant workers from three occupational studies. Their estimates were 1,788, 2,449, 3,791 µg/day, or 2.5 to 5.4 x 10^{-2} mg/kg/day for a 70 kg man.

Related to the pharmacokinetics of PCP are those of its PCDD/PCDF contaminants. Pirkle *et al.* estimated the median half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) from 36 Air Force veterans of Operation Ranch Hand at 7.1 years (95 percent confidence interval 5.8-9.6). (Pirkle *et al.*, 1989) Gorski *et al.* estimated the biological half-life of the higher chlorinated 2,3,7,8-substituted PCDD/PCDF congeners present as contaminants in penta at 2-6 years, based on a case of penta poisoning in a 7 year old girl, and two adipose tissue biopsies collected 31 months apart. (Gorski, Konopka & Brodzki, 1984) However, this value may underestimate the half-life of these congeners in adults, as a study of Yusho oil poisoned children has shown that the half-life of a similar class of lipophilic compounds, PCBs, may be two- to three-fold less than the value in adults simply due to dilution of body burden from growth. (Yakushiji *et al.*, 1984)

The range of estimated half-lives for the higher chlorinated PCDDs/PCDFs is shorter than the half-life of 5 to 11 years inferred for the 2,3,7,8-TCDD congener. (Kang *et al.*, 1991) Moreover, the half-life estimates for 2,3,7,8-TCDD in humans are significantly larger than the estimate of 2-years obtained from a rhesus monkey given a single oral dose of the compound (McNulty *et al.*, 1982), and far greater than the range reported for rodents (rats, mice, guinea pigs, and hamsters) of 10.8-31 days. (U.S. Environmental Protection Agency, 1985b) More importantly, the biological half-life of the PCDD/PCDF contaminants of PCP far exceed the half-life of PCP in the human body.

Several factors confound these half-life estimates. First, the determination of a half-life from only two sampling events cannot discriminate between a one- or two-compartment elimination model. Second, the exposure pattern (*i.e.*, acute or chronic), and the continued presence of a background exposure source may significantly alter half-life estimates. In particular, the latter condition can bias half-life estimates upwards for ubiquitous xenobiotics like PCBs and PCDDs/PCDFs. (Phillips, 1989) Third, the lability to degradation of members of a class of compounds may bias the results. For instance, persons with recent exposure to xenobiotics like PCBs tend to have a larger proportion of the more readily biodegradable congeners. (Phillips, 1989)

f. Binding with Body Components

i. Animal Studies

As described above, a large number of *in vivo* and *in vitro* experiments have been performed in an attempt to determine the pharmacokinetics of penta in the body, and its interaction with body components. For instance, as previously mentioned, the study of Braun *et al.* reported that about 99 percent of the PCP in rat plasma was bound to protein. (Braun *et al.*, 1977) The binding of PCP to plasma proteins was greater at very low plasma concentrations, presumably because of the preferential binding to a smaller number of higher affinity sites. (WHO, 1987) Also, Hoben *et al.* demonstrated the importance of protein binding in a series of *in vitro* experiments. (Hoben, Ching, Young, & Casarett, 1976) Those authors reported that human plasma had a greater binding capacity for PCP than rat plasma. They postulated that this difference may be responsible for the longer retention and higher blood PCP concentrations observed in humans.

In vitro work by Leighty and Fentiman showed that PCP is esterified with palmitic acid in a rat liver microsomal system. (Leighty & Fentiman, 1982) The authors speculated that the toxic effects of PCP may result from its conjugation with fatty acids and thus to intercalate into lipid membranes and disrupt the function of an enzyme system.

Several studies have consistently documented the presence of PCP, or an esterified form, palmitoylpentachlorophenol (PPCP) in human adipose tissue at concentrations ranging from 4 to 250 ppb. [Shafik, 1973 #1042; Ohe, 1979 #386; Morgade, 1980 #658; Ansari, 1985 #294; From adipose tissue surveys of the general population, the average concentration of PCP typically ranges from 26 ppb [Shafik, 1973 #1042] to 35 ppb (Williams, LeBel & Junkins, 1984); Ohe, 1979 #386] Ansari et al demonstrated the presence of PPCP in a human fat randomly obtained from a cadaver at a concentration of 240 ppb. (Ansari, Britt & Reynolds, 1985) The storage of a more lipophilic form of PCP by humans is significant in that these reservoirs are not static, and during lipid mobilization potentially toxic levels of these materials may be released into the circulation.

Weinbach and Garbus earlier had reported that from their work in an *in vitro* rat liver test system, mitochondrial protein is the site of action of uncoupling phenols. (Weinbach & Garbus, 1965) PCP has a great affinity for mitochondrial protein.

Arrhenius *et al.* examined the subcellular distribution and effects of PCP. (Arrhenius, Renberg & Johansson, 1977; Arrhenius, Renberg, Johansson & Zetterqvist, 1977) They showed that PCP accumulated in the microsomes and cytosol at concentrations three to six times higher than those in mitochondria. While mitochondria were about four times more sensitive to PCP than the microsomes, the differential accumulation indicated to the authors that both sites may be injured from PCP exposure -- the uncoupling of mitochondrial oxidative phosphorylation and the detoxification ability

of the endoplasmic reticulum. (Arrhenius *et al.*, 1977) The authors conclude that the selective blocking of P450 metabolism of electrophilic compounds may lead to reactive electrophilic intermediates. Thus, it is possible that "PCP can synergistically increase the toxic and carcinogenic action of other chemicals to which man and animals are exposed…". (Arrhenius *et al.*, 1977)

ii. Human Studies

As mentioned above, Uhl *et al.* concluded that plasma-protein binding of PCP was the likely cause of the long urinary excretion pattern observed in humans. (Uhl *et al.*, 1986)

Dougherty *et al.* were first to report the presence of pentachlorophenol in human semen. (Dougherty, 1978) Later, Dougherty collaborated on a study that showed there was a nine-fold enrichment of pentachlorophenol concentration in human sperm *versus* semen levels -- after centrifuging semen samples and separately analyzing the cells and supernatant for PCP, 40 percent of the PCP was bound to the cells that contained only 4 percent of the sample mass. (Kuehl & Dougherty, 1980) The concentrations reported by Dougherty *et al.* are in the range that Weinbach found were effective for inhibiting oxidative phosphorylation. (Weinbach, 1957; Weinbach & Garbus, 1965) However, no studies have been conducted that assess the motility of sperm of PCP-exposed workers.

3. Health Effects of Pentachlorophenol Exposure

Just as there have been a remarkably large number of toxicokinetic studies of pentachlorophenol, an even larger number of studies have investigated the health effects of penta exposure. However, a large proportion of these studies have focused on lethality as the endpoint of

interest, and only two have evaluated reproduction the topic of interest to this thesis. A survey of the penta health effects literature is described below.

Because of research published in the last 23 years, the toxicological importance of the nonphenolic impurities in penta described earlier is more clearly understood. It appears that the inherent acute toxicity of the chlorophenols (especially pentachlorophenol) is greater than that associated with the trace levels of the impurities. However, with one apparent exception, this situation is reversed for longer-term exposures. Under such conditions, these impurities are far more toxic than the chlorophenols, and they are responsible for most of the toxic effects manifested in subchronic and chronic experiments. The sole exception appears to be the fact that the recent NTP carcinogenicity bioassays of two commercial penta mixtures resulted in greater carcinogenic responses in a wider variety of organ systems than would be expected on the basis of HxCDD contamination levels alone.

a. Acute Toxicity

i. Animal Studies

A number of animal studies have investigated the acute toxicity of pentachlorophenol and sodium pentachlorophenate, many in the 1930s and 40s. (Deichmann, 1943; Deichmann, Machle, Kitzmiller, & Thomas, 1942; Kehoe, Deichmann-Gruebler, & Kitzmiller, 1939; Keplinger, Lanier, & Deichmann, 1959; McGavack, Boyd, Piccione, & Terranova, 1941; Noakes & Sanderson, 1969; Schwetz, Keeler, & Gehring, 1974a; Schwetz, Keeler, & Gehring, 1974b) From the studies discussed in Section 2, above, it follows that pentachlorophenol is readily absorbed by all routes of exposure, and is distributed throughout the body. Likewise, pentachlorophenol is toxic by all routes of exposure (oral, dermal, inhalation, subcutaneous, and

intravenous). However, a greater exposure is required by the dermal route to achieve a toxic effect equivalent to that of exposure by any of the other routes, probably due to the relatively poorer absorption from this route of exposure than the others. In addition, in rats the respiratory route of exposure has been reported to be at least 10 times more toxic than an equivalent oral exposure. (Hoben, Ching, & Casarett, 1976a)

Symptoms of acute penta poisoning vary somewhat between species, however, hyperthermia is universal finding among mammals. Other typical mammalian responses include vomiting, elevated blood pressure, increased respiration rate and amplitude, tachycardia, and hyperglycemia. (Knudsen, Verschuuren, Den Tonkelaar, Kroes & Hellman, 1974) In rodents, hyperthermia, tremors, muscle spasms, and loss of righting reflex occur. (National Toxicology Program, 1989) Respiratory paralysis is the usual proximate cause of death. (National Toxicology Program, 1989) A rapid rigor mortis occurs within 3 to 5 minutes of death, about 45 minutes earlier than rats sacrificed by a lethal concentration of ether. (WHO, 1987) The liver is often enlarged in exposed mammals, although overt toxicity may be absent. (Greichus, Libal & Johnson, 1979) Common symptoms among humans include a loss of appetite and body weight, hyperpyrexia (temperatures up to 109°C), headache, respiratory irritation, nasal and ocular mucosa congestion, and weakness. (Knudsen *et al.*, 1974)

Although not presented here, pentachlorophenol is the most acutely toxic of the chlorinated phenols, regardless of the route of exposure or species. (Ahlborg & Larsson, 1978; Borzelleca, Hayes, Condie & Egle, 1985) Rabbits appear more sensitive than rats to the effects of acute dermal *versus* acute oral exposure to pentachlorophenol. Yet the acute toxicity from sodium pentachlorophenate appears comparable for the two species.

The acute oral toxicity of penta to rats and mice appears roughly comparable. The oral LD_{50} values for rats range from 27-330 mg PCP/kg body

weight while the corresponding LD_{50} values range from 36-177 mg/kg for mice. It is interesting to note the effect of ambient temperature on the LD_{50} of intraperitoneally administered pentachlorophenol. The LD_{50} of PCP in adult rats is 620 mg/kg of body weight at 8°C (46°F); 420 mg/kg at 26°C (79°F); and 120 mg/kg at 36°C (97°F). (Keplinger, Lanier, & Deichmann, 1959)

Pentachlorophenol appears to be more acutely toxic than NaPCP when administered to rats and rabbits orally or dermally, but appears almost equitoxic when administered intraperitoneally or subcutaneously (*i.e.*, when then is no physical barrier to absorption). Neither sex is consistently more susceptible than the other, although technical PCP appears to be more toxic for females than males (Schwetz, Keeler & Gehring, 1974), and young animals appear more susceptible to the effects of penta than do adult animals. (Schwetz, Quast, Keeler, Humiston & Kociba, 1978)

At least some of the differences in the acute toxicity values may be ascribed to the different or unknown carrier solvents used to dose the animals, the frequently unknown or unreported purity of the pentachlorophenol and sodium pentachlorophenate administered to the animals, the duration of dermal contact with the material (for dermal toxicity testing), and the number, strain, and age of the animals used in the toxicological evaluations (which are frequently unreported).

The acute toxicity associated with exposure to pentachlorophenol is believed to be due to its uncoupling of oxidative phosphorylation in mitochondria (the subcellular organelles responsible for energy conversion in cells). (Corbett, Wright & Baillie, 1984; Weinbach, 1957; Weinbach & Garbus, 1965) These effects occur at exquisitely low concentrations, 10^{-6} to 10^{-4} M. (Weinbach, 1957) At these low concentrations, cells may survive for a limited time through use of glycolytic phosphorylation energy yielding reactions (which are unaffected).

At still higher concentrations, 10^{-3} M or higher, "glycolytic as well as oxidative enzymes are completely suppressed, with rapidly fatal results." (Weinbach, 1957) Some research suggests that pentachlorophenol uncouples oxidative phosphorylation at low concentrations and inhibits it at high concentrations, and that Na^+, K^+-ATPase is the site of action of the toxin. (Corbett *et al.*, 1984)

ii. Human Studies

There have been a large number of case reports of acute PCP intoxication due to accidental poisoning or suicide attempts. At present, at least 56 cases have been reported in the world literature with at least 32 fatalities (57 percent fatality rate). "In these cases, absorption of several hundred mg of pentachlorophenol has usually occurred in severe or fatal poisoning and pentachlorophenol blood levels as high as 10-20 ppm (mg/kg) have been measured soon after exposure. On the other hand, workers in wood-treating plants may reach pentachlorophenol blood and urine concentrations as high as 10-20 ppm without symptoms of toxicity." (Kimbrough, 1980, p. 392)

Blair was the first to note that a disproportionate number of the accidentally fatal penta poisonings occur in the summer months, or during other warm periods.(Blair, 1961) This observation is concordant with the results of animal experiments by Keplinger *et al.* reported above wherein raising the ambient temperature increased the acute toxicity of the PCP. (Keplinger, Lanier & Deichmann, 1959) Confirmatory results were found in an *in vitro* experiment by Buffa *et al.* (Buffa, Carafoli & Muscatello, 1963)

The health of effects from acute PCP exposure in humans are similar to those manifested in animals, including the almost immediate onset of rigor mortis. It is important to note that many of the penta-related fatalities have been from case reports of individuals exposed principally

or exclusively through dermal exposure. Infants and children in particular, appear to be very sensitive to dermal exposure of pentachlorophenol. Twenty newborn infants became ill, 9 seriously and two died from the improper use of penta as a germicidal agent in diaper laundering for the nursery of a hospital. (Armstrong *et al.*, 1969; Robson, Kissane, Elvick & Pundavela, 1969) A case report from England tells of a preschool girl who suffered from nonfatal penta poisoning after bathing on 13 days in water contaminated with PCP from a tainted cold water storage tank. (Chapman & Robson, 1965)

b. Subchronic Toxicity

i. Animal Studies

Aside from the developmental and reproductive toxicology studies, which will be discussed separately, less than a handful of investigations have been conducted of the health effects of short-term or subchronic pentachlorophenol exposures to animals that have also accounted for the PCP impurities. This is a sharp contrast with the plethora of acute toxicity studies.

Ten animal studies have been conducted which have evaluated the subchronic toxicity of either technical grade or purified penta. (Debets, Strik, & Olie, 1980; Fleischer, Meib, Robenek, Themann, & Eckard, 1980; Forsell, Shull, & Kately, 1981; Greichus, Libal, & Johnson, 1979; Hillam & Greichus, 1983; Kerkvliet, Baecher-Steppan, Claycomb, Craig, & Sheggeby, 1982; Kerkvliet, Baecher-Steppan, & Schmitz, 1982; Kinzell, et al., 1981; Knudsen, Verschuuren, Den Tonkelaar, Kroes, & Hellman, 1974; Savolainen & Pekari, 1979) Several noteworthy conclusions can be drawn from these studies. First, technical grade PCP, and not purified PCP is responsible for the wide variety of toxic endpoints identified. This implies that contaminants present in technical grade PCP and not the PCP itself are

responsible for many of the toxic effects observed. Second, subclinical effects of toxicological significance may occur at technical grade PCP doses at which there are no outward manifestations of illness. Finally, immunological disruption appears to be the most sensitive toxicological endpoint altered by technical grade PCP exposure.

ii. Human Studies

No health effects studies of humans subchronically exposed to PCP or NaPCP were found.

c. Chronic Toxicity

i. Animal Studies

Aside from the two subcutaneous and four oral administration carcinogenicity studies, only five high quality studies involving prolonged exposure to PCP have been conducted that have also accounted for the level of impurities in the PCP. (Goldstein, Friesen, Linder, Hickman, & al., 1977; Kimbrough & Linder, 1978; McConnell, et al., 1980; Parker, Jones, Matthews, McConnell, & Hass, 1980; Schwetz, Quast, Keeler, Humiston, & Kociba, 1978) Like the subchronic animal studies, these chronic studies observed that it is the impurities present in technical grade PCP, and absent from purified PCP, that principally account for the systemic toxicity. The target organs of chronic pentachlorophenol exposure are primarily the liver, kidney, and bone marrow. (National Toxicology Program, 1989)

ii. Human Studies

Numerous case reports have been published and several epidemiological investigations conducted that have assessed various health endpoints in

PCP-exposed workers. It appears clear that penta workers do suffer from a higher incidence of conjunctivitis, chronic sinusitis, and low-grade chronic upper respiratory infection (Embree *et al.*, 1984; Gilbert *et al.*, 1983; Klemmer *et al.*, 1980), chloracne (Bond, McLaren, Brenner & Cook, 1989; Carpenter *et al.*, 1991; Cole, Stone, Gates & Culver, 1986; Leet & Collins, 1991; O'Malley *et al.*, 1990; Sehgal & Ghorpade, 1983), and aplastic anemia (Roberts, 1963; Roberts, 1981; Roberts, 1983; Rugman & Cosstick, 1990) In addition, a substantial number of case reports of nonoccupational PCP exposures and illness have been published. The nonoccupational and occupational symptomatology of these studies (generalized itching or burning of the skin, nausea, vomiting, loss of appetite, headache, dizziness, and fatigue (Sangster *et al.*, 1982)) are consonant for some effects, but differ on others.

Unfortunately, relatively few controlled epidemiological investigations of workers chronically exposed to penta have been conducted. The best of these studies and case reports evaluated a persistent and disfiguring skin condition known as chloracne, which is also an endpoint relevant to this thesis investigation. The epidemiological literature concerning PCP exposure and chloracne is discussed below.

Epidemiological studies have shown that a number of halogenated aromatic hydrocarbons (e.g., chlorinated naphthalene, hexachlorobenzene, PCBs, and PCDDs/PCDFs - especially 2,3,7,8-TCDD) may cause chloracne. This disfiguring condition appears to be a very sensitive biological indicator of exposure to these types of chemicals. Chloracne has been found in some individuals without other evidence of toxicity. (Suskind, 1985)

Numerous case reports and two recent epidemiological studies provide strong support for a causal association between PCP exposure and adverse dermal effects, including chloracne. Baader and Bauer were the first to suggest an association between penta exposure and dermatological effects.

(Baader & Bauer, 1951) The authors reported on a review of compensation claims of 17 penta manufacturing workers in West Germany. All of the workers supplied had manifested severe skin eruptions and furunculosis, and all but one man still had extensive acne despite the termination of exposure more than a year earlier. The first cases of chloracne occurred approximately 5 months after penta production began. Additional details from this study were later published in German. (Bauer, Schulz & Spiegelberg, 1961)

Seghal and Ghorpade published a case report of a 20-year old male NaPCP manufacturing worker who developed chloracne after 10 months of employment. (Sehgal & Ghorpade, 1983) The patient was described as being exposed to NaPCP fumes (and presumably dusts) while packing the material.

Cole *et al.* reported on a single case of chloracne diagnosed in a 32-year old white male. (Cole *et al.*, 1986) The patient was co-owner of a pier construction company. His work often involved lying on top of penta treated wood to make refined measurements while wearing only shorts and shoes (*i.e.*, without protective clothing). This case report stresses the potential importance of dermal exposure to penta. The symptoms of chloracne appeared within 9 months of beginning the work, and resolved after several months of medical treatment and adopting the use of protective clothing to prevent dermal absorption.

Bond *et al.* assessed the incidence of chloracne among chemical workers who had potential exposure to PCDDs at Dow Chemical Company facilities. (Bond *et al.*, 1989) The authors reported that of eight production areas evaluated "the largest share of cases occurred among employees who were assigned to Chlorophenol Production and Finishing Areas, where pentachlorophenol was the principal product and H/OCDDs (hexa- to octachlorinated dioxins) would have been the major chloracnegens encountered." (Bond *et al.*, 1989, p. 773) In fact, the incidence rate was

marginally higher among these chlorophenol production and finishing workers than among 2,4,5-trichlorophenol production workers. Chloracne incidence rates were substantially lower among other areas involved in the production of chlorophenoxy herbicides.

O'Malley *et al.* conducted a investigation of the incidence of chloracne among penta manufacturing workers at the W.G. Krummrich plant of the Monsanto Company. (O'Malley *et al.*, 1990) Workers ever having been listed as an hourly worker in a penta department between 1938 and 1978 were considered exposed. Company medical records, medical surveillance reports, and workmen's compensation claim reports were used to identify individuals who had developed chloracne. Approximately one-third of the workers had no medical records and thus could not be identified as cases. Consequently, these workers were excluded from the analysis.

Other exclusionary criteria included: individuals who were employed in 2,4,5-T ester or PCB manufacturing departments at the time of a chloracne diagnosis; individuals employed in these departments within 2 years of a chloracne diagnosis, even if they were employed in the PCP department at that time; or if the individual were diagnosed with chloracne more than 2 years after leaving the PCP department. After these exclusionary criteria 648 workers remained with medical records employed on or after 1953 who satisfied all of the study criteria.

A total of 47 cases had a diagnosis of chloracne associated with exposure to PCP. "The overall cumulative incidence of chloracne was 7.2% (47/648), and the 2.1% (47/2,252 PYAR)." (O'Malley, et al., 1990) The annual incidence rate varied from 0 to 1.46. Duration of exposure did not appear to be associated with an increased risk of chloracne. However, workers with reported to have had direct skin contact with penta had a cumulative incidence ratio of 4.6 [95% confidence interval 2.1-8.1] for developing chloracne compared with workers who had no record of direct dermal contact with PCP.

Several other references indicating a causal relationship between chloracne and PCP exposure exist. For instance, Fielder *et al.* conducted a literature review of penta toxicology, and listed a number of overseas cases of chloracne in penta-manufacturing workers. (Fielder, Sorrie, Bishop, Jones & Van Den Heuvel, 1982) Baxter reported 25 cases of chloracne among 40 regularly exposed workers who manufactured PCP and Santobrite™ in the United Kingdom, and 2 cases among 25 intermittently exposed workers. (Baxter, 1984) Sterling *et al.* reported a high frequency of self-reported skin disorders on questionnaires supplied as part of a survey of sawmill workers. (Sterling, Stoffman, Sterling & Maté, 1982)

Lambert *et al.* published three case reports describing the presence of severe skin lesions among individuals exposed to pentachlorophenol. The first two cases involved diagnoses of pemphigus vulgaris among a man and woman with chronic nonoccupational exposure to PCP. The third case involved a diagnosis of chronic urticaria in a man who had treated a lot of wooden framework with PCP. In each case the symptoms worsened or ebbed in step with serum PCP concentrations.

In their attempt to find biological markers of exposure to 2,3,7,8-TCDD among residents of Seveso, Del Corno *et al.* evaluated chloracne. (Del Corno, Montesarchio & Fara, 1985) After discussing the ideal qualities of a marker of exposure, Del Corno *et al.* discuss the utility of chloracne as such a marker among children of the Seveso area. They reported that:

a) "the conditions of exposure reported in the questionnaires on subjects with skin lesions were not substantially different from those reported on subjects without skin lesions but living in the same contaminated area.
b) nevertheless, the exposure of subjects with chloracne is more frequently described by all risk indicators." (Del Corno *et al.*, 1985, p. 143)

This suggests that there may be some intrinsic and variable degree of susceptibility among individuals responsible for the development of chloracne aside from merely being exposed to a chloracnegenic agent.

Neuberger *et al.* measured blood levels of 2,3,7,8-TCDD in nine chemical production workers and in 21 other individuals without chloracne, four from the same company and the remainder from other companies. (Neuberger, Landvoigt & Derntl, 1991) Current and historically extrapolated levels of 2,3,7,8-TCDD in blood lipid from these nine chemical workers with exposure to dioxin-contaminated products dwarfed those from the 21 referents. Moreover, the current and historically extrapolated (assuming a 7-year biological half-life) levels of 2,3,7,8-TCDD in blood lipid from the three chemical workers with "medium" chloracne (current median 498 pg/g, extrapolated serum concentration 2,682 pg/g) greatly surpassed the levels of the six men with "light" chloracne (current median 305 pg/g, extrapolated serum concentration 1,640 pg/g). The mean concentration of 2,3,7,8-TCDD in blood lipid from this German plant and those from U.S. chlorophenol and chlorophenoxyherbicide manufacturing plant workers, when adjusted for biological half-life, were judged to be comparable. However, the levels in Seveso residents exceeded those of even the chloracne-afflicted workers.

Neuberger *et al.* report a possible threshold for chloracne from a worker with an extrapolated 2,3,7,8-TCDD blood lipid concentration of 526 pg/g (*i.e.*, 526 ppt), which they note was less than levels estimated for three other workers without chloracne. Neuberger *et al.* suggest that direct skin contact with 2,3,7,8-TCDD-contaminated materials may be a more important predictor of an individual's likelihood of developing chloracne than the blood lipid level.

d. Genetic Toxicology

i. Animal and *in vitro* Studies

Five bacterial assays for induction of gene mutation and one for growth inhibition due to DNA damage by pentachlorophenol have been negative. (National Toxicology Program, 1989) One Salmonella-microsome test, however, did yield positive results when the chemical was present along with phenobarbital or 5,6-benzoflavone-induced rat liver S9. (National Toxicology Program, 1989) In addition, tests of major impurities in PCP: OCDD, hexachlorobenzene, and three tetrachlorophenols - in Salmonella tests conducted by NTP were negative. (National Toxicology Program, 1989) However, 2,3,5,6-tetrachlorophenol was positive for both increased SCEs and chromosomal aberrations.

Exposure of Adult male Drosophila to sodium pentachlorophenate did not induce sex-linked recessive lethal mutations, nor did hexachlorobenzene (a major contaminant of PCP) induce dominant lethal mutations in rodents. (National Toxicology Program, 1989) PCP has been tested for genetic toxicity *in vivo* in two mammalian bioassays. PCP was positive in a mammalian spot test in mice that were administered 0-100 mg/kg of 99 percent pure PCP. (Fahrig, Nilsson & Rappe, 1978) PCP tested negative in the mouse sperm morphology test in which mice were exposed to pentachlorophenol (purity unstated) at doses up to 400 mg/kg for 5 days. (National Toxicology Program, 1989) The utility of the mouse sperm morphology test is questionable, and a five day exposure period is far too short to ensure that all phases of sperm production had been exposed.

ii. Human Studies

Several studies have been performed that have examined the effects of pentachlorophenol exposure on human chromosomes, but the results are far

from clear. (Bauchinger, Dresp, Schmid & Hauf, 1982; Schmid, Bauchinger & Dresp, 1983; Wyllie, Gabica, Benson & Yoder, 1975; Ziemsen, Angerer & Lehnert, 1987) In general, they tend to support the negative findings from the *in vitro* analyses reviewed above, but there remains the possibility of chromosomal damage from PCP exposure.

Sister-chromatid exchange (SCE) is a popularly evaluated marker of genetic damage in humans since SCEs are quicker and easier to score than chromosome aberrations, and will often reveal positive indications of damage when other tests are negative. (National Research Council, 1989) "SCE is the reciprocal exchange of DNA between chromatids at one locus and does not result in alteration of chromosomal structure. SCEs reflect repair of several types of lesions; they are more efficiently induced by compounds that form DNA adducts or otherwise intercalate into DNA (e.g., alkylating agents) than by agents that break the DNA backbone (e.g., radiation)." (National Research Council, 1989)

The interpretation of elevated SCEs is difficult. It is important to bear in mind that SCEs are not indications of mutational events, and there are no known adverse health consequences of having SCEs. (National Research Council, 1989) However, patients with Bloom syndrome (BS) have a "dramatically increased rate of SCE and a high frequency of quadriradial formation, indicative of homologous chromosome exchange." (Schottenfeld & J.F. Fraumeni, 1982) BS victims have a very high cancer rate - all individuals surviving to age 40 have had at least one malignant tumor. (Schottenfeld & J.F. Fraumeni, 1982)

Chromosomal aberrations are measured in somatic cells. Alterations in the chromosomal structure are unambiguous evidence of genetic damage. The fundamental lesion believed to cause chromosomal aberrations is a break in the chromatid fiber. (National Research Council, 1989) Chromosomal aberrations are believed to be less sensitive markers of chemical exposure, as most chemicals induce aberrations "only when the cell is in the S-phase

(*i.e.*, chromatid aberration detectable as SCEs)." (National Research Council, 1989)

The study by Wyllie *et al.* reported no chromosome damaging effect of PCP in the blood of six workers, as measured by analysis for chromosome aberrations and sister chromatid exchange (SCE). (Wyllie *et al.*, 1975)

In a cytogenetic study of 20 healthy PCP exposed workers, Bauchinger *et al.* found no significant effect of PCP exposure on the frequency of SCEs in smoking PCP workers as compared to smoking controls. (Bauchinger *et al.*, 1982) However, there was a small but significant increase in the frequency of dicentrics and acentrics.

In a brief abstract of another cytogenetic study, this one of 22 PCP manufacturing workers, Schmid *et al.* reported significant increased yields of dicentrics and acentrics, but no significant difference in the SCE frequency between PCP-exposed workers who smoked and smoking controls. The results led the authors to conclude that PCP induced a "weak clastogenic effect which is S-independent and similar to that of ionizing radiation." (Schmid *et al.*, 1983)

Finally, Ziemsen *et al.* performed an *in vivo* and *in vitro* cytogenetic study. (Ziemsen *et al.*, 1987) They did not use a comparison group for the results of the blood samples from 20 healthy PCP workers. They reported "...no correlation between SCE frequency and serum concentration of PCP or time of employment." (Ziemsen *et al.*, 1987) Also, they found no effect of PCP exposure on the frequency of chromosomal aberrations. Importantly, they failed to observe the well-known effect of smoking on SCE frequencies. They ascribe the failure to observe this well-known effect to the small number of nonsmokers. The *in vitro* work substantiated the findings of the *in vivo* analyses.

e. Reproductive Effects Studies

i. Animal Studies

Only one animal study evaluated the possibility of adverse effects of pentachlorophenol exposure on the fertility of the male. Following standard Food and Drug Administration Segment I testing protocol, Schwetz *et al.* fed groups of 10 male and 20 female Sprague-Dawley rats Dowicide EC-7. (Schwetz *et al.*, 1978) EC-7 was a purified pentachlorophenol, once manufactured by Dow Chemical Company, characterized by its low nonphenolic contaminant content. The rat groups were fed 0, 3, or 30 mg of PCP/kg of body weight/day for 62 days prior to mating, during 15 days of mating, and throughout gestation and lactation until they were sacrificed and necropsied.

This study design evaluated, at least in part, the effects of penta on male reproduction, as well as developmental toxicity. (Schwetz *et al.*, 1978) No significant effects on several reproduction indices was noted among animals in the low dose group. However, statistically significant deficits were noted in four of the six reproduction indices among the high dose group animals, including liveborn pups; 7-day, 14-day, and 21-day survival. The percentage of pregnant rats and the 24-hour survival among rat pups was apparently unaffected by the PCP treatment. The study concluded that pentachlorophenol exposure had no apparent effect on the fertility of the exposed mating pairs. (Schwetz *et al.*, 1978)

Limitations in the study design (e.g., small numbers of animals tested, failure to use continuous breeding protocol to increase sensitivity of the test for male reproductive toxicity, and poor representativeness of rodent models of human reproductive toxicity) prohibit one from formulating definitive conclusions whether PCP is a male animal reproductive toxin. For instance, it should be borne in mind that the sensitivity of animal experimental models of human reproduction are quite poor. Many animal

species, including rodents and rabbits, are superfertile when compared with the human male. For instance, in mice almost a 90% reduction in sperm count is necessary before fertility is affected. (Amann, 1982) In rats, fertility is present at 1 percent of normal sperm counts. (Meistrich, 1989) New Zealand white rabbits would require a reduction of >99.6 percent in the number of motile sperm before any decrease in fertility could be detected. (Williams *et al.*, 1990) Thus, the absence of an effect on the fertility of treated male laboratory rats, mice, and rabbits is not sufficient evidence to conclude there is no reproductive risk to exposed men.

This discussion would not be complete without a review of the literature of the animal male reproductive toxicity studies with PCDDs/PCDFs. For the most part, that means a review of those experiments with 2,3,7,8-TCDD since the higher chlorinated PCDDs/PCDFs present in PCP have not been well-characterized toxicologically.

Khera and Ruddick evaluated the developmental effects of maternal 2,3,7,8-TCDD exposure, the extent of placental transfer of 2,3,7,8-TCDD between dam and fetuses, and dominant lethal effects in Wistar rats. (Khera & Ruddick, 1973) Only the last test, which assessed the effect of TCDD exposure on pregnancies, viable embryos, and percentage of pregnant females are relevant to this study.

Groups of 20 male Wistar rats were administered 0, 4, 8, or 12 µg/kg/day of 2,3,7,8-TCDD for seven consecutive days after which the surviving males under went seven consecutive 5-day mating trials. For each mating trial, a male cohabited a cage with two untreated virgin females. The females were sacrificed 9 days after the trial ended, and viable embryos, resorption sites, and corpora lutea were counted. At the end of the experiment the surviving males were sacrificed, and their testes and epididymis examined for histological alterations.

All of the high dose (20/20), 55 percent of the intermediate dose (11/20), and 10 percent of the low dose males died from the treatment. No control animals died. The authors concluded from the absence of effects in two of their three reproductive indices (viable embryos and resorption sites) that no dominant lethal mutations occurred. However, the incidence of pregnancies was much lower in the low and intermediate dose groups than in the control group (usually one-half to one-third). No formal statistical comparison was made by the authors. The study was limited by the limited treatment period, which corresponded to the postmeitotic stages of spermatogenesis, the small number of animals treated, and the high mortality rate in several treatment groups.

Murray *et al.* evaluated the reproductive effects of low dose 2,3,7,8-TCDD treatment in a 3-generation study using Sprague-Dawley rats. (Murray *et al.*, 1979) Male and female rats in the study were continuously maintained on diets corresponding to 0, 0.001, 0.01, or 0.1 µg/kg/day. The parental group (the F_0 generation) were dosed for 90 days prior to mating. Each treated male cohabited a cage with two females from the same dose group for 15 days (three rat estrous cycles).

The F_0 rats were bred approximately one month after the F_{1a} generation was born because of the unusually low pregnancy rates in all groups, including the controls. For the F_{1b} and subsequent generations, the mating protocol was modified to improve the pregnancy rate. In the modified protocol, each female cohabited a cage with a male of the same dose group for 6 days, and then after a 6 day wait, the procedure was repeated with a different male. Selected members of the F_{1b} and F_2 generations were mated at approximately 130 days of age to produce the F_2 and F_3 generations, respectively.

In addition, the authors included a crossover design in the experiment to assess, if possible, whether there were differential

reproductive effects because of greater sensitivity of one sex. As one part of this study, after 12 months on the test diet, 20 treated male or female F_0 rats receiving 0.1 µg/kg/day of 2,3,7,8-TCDD were each mated with an untreated rat of the opposite sex for 10 days. Subsequently, the females were sacrificed and examined solely to determine the number of pregnancies, number of implants per dam, and the percentage of implants resorbed.

No significant signs of toxicity were observed in the F_0 generation's 90 day exposure period prior to mating. A significant reduction in fertility and neonatal survival in the F_{1a} generation led to the discontinuation of this dose. F_{1b} and F_2 rats dosed at 0.01 µg/kg/day also experienced a significant decrease in fertility ($p < 0.05$). Furthermore, litter size, gestational survival, neonatal survival, and growth were all significantly reduced at this dose. No effect on any reproductive parameter was observed at the 0.001 µg/kg/day dose level.

The incidence of pregnancies among untreated females mated with treated males was not significantly different from the untreated males bred with the untreated females group or the control group (12/20, 18/20, 3/10, respectively). In contrast, the fertility index for the two F_0 generation matings were 10 and 3 percent. Only 3 of 20 treated F_0 females mated with untreated males became pregnant, which the authors ascribe to the dams advanced age. Neither the number of implantations per dam, or the percentage of resorbed implantations was significantly different from the control group values for cross-mated F_0 males. While the former also was true for the for cross-mated F_0 females, the percentage of resorptions was significantly elevated compared to the control group values. From the crossover study results, the authors concluded that ingestion of 2,3,7,8-TCDD did not impair the fertility of males, but it did interfere with the

fertility of females, perhaps by increasing the incidence of late resorptions and decreasing copulatory activity.

Lamb *et al.* divided 200 5-week old male C57BL/6N mice into eight groups of 25 mice. (Lamb, Marks, Gladen, Allen & Moore, 1981) Half of the mice (four groups of 25) were used in toxicity experiments), the other half were used in studies of fertility and reproductive performance. Dosing began when the mice were 8 weeks old. The mice were assigned to control or experimental dosing regimens in such a way as to reduce weight differences between groups. The mice were then placed on one of three chemical treatment or control diets for 8 weeks, that is, for the entire period of spermatogenesis.

The control group mice (Group I) were administered a diet containing 2 percent corn oil. The experimental treatment groups administered diets containing 2,4-D, 2,4,5-T, and 2,3,7,8-TCDD (equal doses in mg/kg/d for 2,4-D and 2,4,5-T; dosage in µg/kg/d for 2,3,7,8-TCDD) as follows: Group II (40; 2.4); Group III (40; 0.16); and Group IV (20; 1.2), respectively.

After 8 weeks of dosing, the mice were restored to the control diet. The following day, each treated male was caged with three virgin female mice for up to 5 days per week for 8 weeks. Mated females were segregated together in another cage. The authors defined as fertile matings those "…resulting in implantation sites, fetuses, or live offspring." (Lamb *et al.*, 1981) The endpoints evaluated by the authors included: mating frequency, percent of fertile matings, sperm number, sperm motility, and percent of abnormal sperm. The pregnant females were subsequently used in teratology experiments. (Lamb, Moore, Marks & Haseman, 1981)

There was a statistically significant decrease in mating frequency (defined as the number of vaginal plugs divided by the total number of females cohabiting with males) in Group III, but not in Group IV, or in Group II whose phenoxyherbicide dosage was equivalent and its TCDD dosage

15-fold higher than in Group III. Neither the fertility index (defined as the number of fertile matings divided by the number of females with plugs), nor total fertility (defined as the number of fertile matings divided by the total number of females cohabiting with males) were significantly reduced in any of the experimental groups. When weekly or individual male fertility was assessed, again no treatment-related effects were seen. No treatment-related changes were observed in sperm concentration, sperm motility, or percent abnormal sperm. In addition, the development and viability of the offspring of rat pups sired by treated males was not significantly different from those of control males. (Lamb *et al.*, 1981)

In contrast, research in West Germany suggests that high dose exposure to male rats may impair fertility. (Chahoud, Krowke, Schimmel, Merker & Neubert, 1989; Neubert, Krowke, Chahoud & Franz, 1987) In this study, mature male Wistar rats were given one of two 2,3,7,8-TCDD dosing regimens by subcutaneous injection. Groups of 30 male rats were administered either an initial (loading) TCDD dose of 25 µg/kg, followed by weekly injections of 5 µg/kg (TCDD-25), or an initial TCDD dose of 75 µg/kg, followed by weekly injections of 15 µg/kg (TCDD-75). Since the TCDD-25 and TCDD-75 weekly dosages were administered in a single injection, they correspond to doses of approximately 0.71 and 2.1 µg/kg/day, respectively. The control group consisted of 40 male Wistar rats. The rats were maintained on this regimen for 10 weeks before mating, and throughout the mating period.

Each male was caged with three virgin females for two hours each weekday. This practice was repeated until one of the females demonstrated the presence of sperm in a vaginal smear. At this point, the sperm-positive testing female was replaced with another virgin. This protocol was repeated until each male had the opportunity to mate with six females.

Sperm-negative females were mated with untreated males of known fertility to verify the females fecundity.

As with the Lamb *et al.* study above, male fertility was indirectly assessed by evaluation of female fertility. On day 21 of the pregnancy, the females were sacrificed and the number of viable fetuses, dead fetuses, and resorptions were determined for each litter. At the end of the study all of the males were sacrificed and their organs visually inspected and weighed. Light microscopic examination was performed on testes for all males, and electron microscopic evaluations were conducted on testes from six males from each group. The selection process was not explained.

The high dose group suffered 93 percent mortality within 16 weeks, the first high dose mouse died after 4 weeks, and the LD_{50} was attained after 8 weeks of treatment. Only 30 percent of the high dose group mice survived up to the beginning of the mating period. The low dose group did not suffer any morality within the first 12 weeks, that is, all of the mice survived up to the beginning of the mating period. In the 13-20 week period, 10 percent of the mice succumbed to the effects of the TCDD. The body weight was significantly depressed in both TCDD treatment groups. However, in the low dose group, body weight stabilized 4 weeks after TCDD treatment was initiated at about 90 percent of the initial body weight. Thus, while control mice gained on average 83 grams, low dose TCDD-treated mice lost 26 grams on average.

The impregnation distribution of the 26 males surviving the TCDD-25 treatment regimen shows some interesting differences with the control group. For instance, there was no significant difference between the proportion of control and TCDD-25 males able to impregnate all six females 55 percent *versus* 62 percent, respectively. However, a marked difference is apparent when lower impregnation rates are examined. A much higher impregnation rate was seen for males in the control group able to

impregnate four or five females than in the TCDD-25 group, 45 percent (*i.e.*, all of the remaining males) *versus* 23 percent.

Finally, 15 percent of the surviving TCDD-25 males were unable to impregnate a single female *versus* none in the control group. This failure was not attributable to the females as subsequent matings with untreated males were successful two-thirds of the time. The overall mating index (defined as the number of "positive" females divided by the number of mated females) for the control and TCDD-25 groups was 98 percent and 84 percent, respectively. This difference was statistically significant ($p < 0.01$).

The pregnancy index (defined as the number of pregnant females divided by the number of positive females) did not differ between the control and TCDD-25 groups. Interestingly, the fertility index (defined as the mean and standard deviation of time in which one male fertilizes three females) was nearly twice as long in TCDD-25 mice as in control mice (14 *versus* 8 days). This has some relevance to the time-to-pregnancy endpoint discussed in Chapter 2.

Paradoxically, the relative weight of the testes in the TCDD-25 group exceeded those in the control group, and there was no decrease in absolute weight. Light microscopic examination of the testes revealed pathological changes in the testes of all TCDD-treated males in the tubules and Sertoli cells.

Moore *et al.* studied the effects of 2,3,7,8-TCDD administration on endocrine function. (Moore, Potter, Theobald, Robinson & Peterson, 1985) In the time-course experiment, single oral doses of either 4.5 or 15 µg 2,3,7,8-TCDD/kg of body weight in a corn oil/acetone vehicle were administered by gavage to sexually mature Sprague-Dawley rats. *Ad libitum*-fed and pair-fed controls received the dosing vehicle. In order to discriminate between the hypophagia of wasting syndrome effects of 2,3,7,8-TCDD and any endocrine effects, food intake by pair-fed controls was

limited to the daily consumption measured in the corresponding TCDD treatment group.

Plasma testosterone and dihydrotestosterone (DHT, the more potent metabolite of testosterone) concentrations, and testicle, seminal vesicle, ventral prostate, and epididymis weights were measured in the control and treatment groups. The dose-response experiment was similar, except that groups of 12 rats were given single doses of either 0 (*ad libitum*-fed or pair-fed controls), 6.25, 12.5, 25, 50, or 100 µg TCDD/kg of body weight.

The time-course experiment revealed that 2,3,7,8-TCDD exposure resulted in an androgenic deficiency in male rats early in the light cycle, and a more modest reduction in androgen concentrations in the pair-fed controls. No clear pattern was seen in androgen concentrations late in the light cycle. Seminal vesicle weights in the TCDD-treated rats and their pair-fed controls were significantly reduced compared with the *ad libitum*-fed rats. However, the effect in pair-fed controls was only about one-half of that experienced by their TCDD-treated counterparts. Ventral prostate weights were only affected in rats receiving the high dose TCDD. There was no effect on testis weight.

TCDD treatment caused a dose-related increase in hypophagia, and decreases in body weight, plasma testosterone concentrations and DHT, and androgen-dependent male sex organ weights (*i.e.*, seminal vesicle, ventral prostate, testis, and caput epididymis). Pair-fed controls experienced these same changes, but the degree of reduction was typically only one-half of that observed in their TCDD-treated counterparts. The authors estimated that on day 7 following treatment with TCDD, the effective dose for 50 percent of the population (ED_{50}) for the reductions in plasma testosterone and DHT was approximately 15 µg TCDD/kg-bw, and the no observed effect level was 25 µg/kg.

The depression of plasma testosterone concentration was noted one day after initiation of TCDD treatment at sublethal doses (15 µg/kg), but maximal depression was attained 7 days after dosing. The maximum effective dose, leading to plasma androgen concentrations one-tenth to one-third of those in control animals, was in the range of the LD_{50} for TCDD (50-100 µg/kg).

Since evidence indicates that TCDD exposure impairs testicular heme synthesis and microsomal enzymes it has been postulated that it may also reduce testosterone synthesis. (Tofilon & Piper, 1982) However, experimental data have shown that TCDD exposure does not effect steroid metabolism or luteinizing hormone concentrations. (Moore *et al.*, 1985) The authors posited that the TCDD-induced reduction in plasma testosterone levels may account for the reproductive pathology observed, although the mechanism of this process was unknown. (Moore *et al.*, 1985)

Two recent and elegant studies into the mechanism of 2,3,7,8-TCDD interference in hormonal function have led to findings directly related to this investigation. Bookstaff *et al.* probed the mechanism whereby TCDD treatment lowers testosterone concentrations, but somehow interferes with the compensatory rise in luteinizing hormone (LH). (Bookstaff, Moore & Peterson, 1990) "LH is the primary hormone which regulates testosterone synthesis and secretion in male rats." (Bookstaff *et al.*, 1990) LH is secreted by the pituitary gland in response to gonadotropin-releasing hormone (GnRH). A feedback mechanism based on plasma androgen and estrogen concentrations maintains androgen levels in the normal physiological range.

Sexually mature male Sprague-Dawley rats were used in all experiments. The rats were administered single oral doses of 2,3,7,8-TCDD while pair-fed controls received the vehicle (corn oil/acetone). *Ad libitum* controls were also used to establish baseline values for different parameters. The authors investigated several hypothesized mechanisms by

which TCDD exposure might block plasma LH concentrations from rising to expected levels in order to compensate for the subnormal androgen concentrations.

The authors investigated the possibility that TCDD treatment affects the rate at which LH is removed from plasma by injecting rats with TCDD (100 µg/kg) or vehicle, and 7 days later administering exogenous LH to rats under barbiturate anesthesia by i.v. Since barbiturates suppresses endogenous LH secretion, the comparison of the exogenous LH disappearance rate in TCDD-treated and control rats tested the validity of the hypothesis. No difference was noted in the time-course of disappearance of plasma LH in the two groups of rats. This suggests that the TCDD does not affect the clearance rate of LH from plasma.

Next, the authors probed the possibility that the presence of gonadal steroids was necessary for TCDD to exert its effect on plasma LH concentrations by castrating rats from the treatment and control groups, and then administering either TCDD (50 µg/kg) or the vehicle to them. After the TCDD dosing, there was no significant difference between the postcastration rise in plasma LH concentrations, except pair-fed controls on day 5. This suggests that the absence of a compensatory increase in LH concentrations in TCDD-treated rats, despite declining androgen concentrations, is mediated through the presence of gonadal steroids, and not due to a decrease in the rate of LH synthesis. Moreover, there was no affect on plasma DHT and estradiol concentrations which suggests there is no enhanced metabolism (clearance) of testosterone to DHT or estradiol that might explain the absence of the compensatory plasma LH response (since both DHT and estradiol are more potent inhibitors of LH secretion than testosterone).

The authors then evaluated the possibility that TCDD treatment increases the potency of testosterone, dihydrotestosterone (which cannot be

metabolized to an estrogen), and estradiol (another metabolite of testosterone) as feedback inhibitors of LH secretion. Rats were dosed with either TCDD (100 μg/kg) or the corn oil/acetone vehicle, castrated, and implanted with capsules containing varying amounts of the three hormones. Since implanted steroid hormone sources are unaffected by TCDD treatment, the response of LH secretion with this experimental design provides information on whether the potency of these hormones is accentuated by TCDD. No increase in metabolism of testosterone to estradiol was detected.

The results of the testosterone, DHT, and estradiol implant experiments demonstrated that TCDD treatment raised the potency of all three hormones as feedback inhibitors of LH secretion. As testosterone, DHT, and estradiol concentrations rose plasma LH concentrations fell. This indicates that metabolism of androgens to estrogens is not essential for the heightened potency of androgens to inhibit the compensatory LH response. At physiological testosterone concentrations, plasma LH concentrations in the TCDD-treated rats were no different from those of the control rats. At subphysiological testosterone concentrations, plasma LH concentrations in the TCDD-treated rats were significantly lower than those of control rats. Still unknown is the relative contribution of DHT and estradiol to the enhanced potency of testosterone.

In addition, TCDD treatment had only a minor effect on the weights of the ventral prostate and seminal vesicles (two organs which are androgen-dependent) of rats who received the testosterone implants. This indicates that the enhanced potency of testosterone to suppress the compensatory increase in LH is not a general phenomenon that affects all androgen-sensitive organs.

Finally, the authors determined the ED_{50} for TCDD treatment that increased testosterone effectiveness to block the compensatory increase in plasma LH concentrations by administering TCDD or vehicle to castrated rats which then received implants that released subphysiological amounts of

testosterone. Seven days later (when the TCDD response is fully developed), the ED_{50} was found to be 10 μg TCDD/kg. Interestingly, the high doses of TCDD dramatically reduced the plasma concentration of LH but not the pituitary LH content.

Since reduced feed consumption is known to decrease LH concentrations, the use of pair-fed controls is noteworthy. In some cases significantly reduced plasma LH concentrations were noted, however, the effects of TCDD treatment far exceeded those from dietary deprivation alone. Thus, while food reduction may appear to enhance the potency of androgens and estrogens, it is not sufficient to explain the marked responses observed in these experiments.

The authors speculated on the mechanism for the increased potency of gonadal steroids in inhibiting the compensatory increase in plasma LH concentrations to subphysiological testosterone levels. Since gonadotropin-releasing hormone (GnRH) is the hypothalamic hormone responsible for stimulating LH secretion, low plasma androgen levels in control rats leads to a compensatory increase in pituitary GnRH receptor cell numbers and increased sensitivity of the pituitary to GnRH secretion. However, these changes are absent in TCDD-treated rats.

In a second investigation published in the same year, Bookstaff *et al.* explored whether the inhibition of the compensatory LH response to subphysiological androgen concentration was mediated by the pituitary gland, the hypothalamus, or both. (Bookstaff, Kamel, Moore, Bjerke & Peterson, 1990) The methods were the same as above (*i.e.*, intact, castrated, and castrated rats with testosterone implants with *ad libitum* and pair-fed controls) with the exception that a third (positive) control group was included, consisting of *ad libitum* rats which were castrated and implanted with testosterone-containing capsules. These animals are referred to as hypoandrogenic ALC rats.

The authors assessed the effect of TCDD treatment on the number of pituitary GnRH receptors. TCDD treatment prevented the compensatory increase in the number of GnRH receptors, confirming an earlier study. Pituitary responsiveness to GnRH stimulation was assessed in both groups of ALC control, pair-fed control, and TCDD-treated (100 µg/kg) rats. One week after dosing with TCDD or vehicle, the rats were anesthetized with a barbiturate, which suppresses hypothalamic GnRH secretion while not affecting pituitary responsiveness to exogenous GnRH, and infused with GnRH.

In response to the GnRH infusion, the hypoandrogenic ALC rats experienced the normal increase in plasma LH concentrations. In contrast, TCDD-treated rats had a plasma LH response identical to intact ALC rats with normal androgen concentrations that was far lower than that experienced by the hypoandrogenic ALC rats. This suggests that TCDD treatment inhibited the normal compensatory rise in pituitary responsiveness to GnRH that accompanies subphysiological testosterone concentrations. Pair-fed controls had nearly an identical number of GnRH receptors as ALC control rats, a number that was marginally larger than that for the TCDD-treated rats. This suggests that feed restriction had a small effect on the number of pituitary GnRH receptors.

In another experiment in which castrated rats received either the vehicle or the same dose of TCDD (100 µg/kg) as before, TCDD "…did not affect plasma LH concentrations, hypothalamic GnRH content, or pituitary GnRH receptor number of binding affinity." (Bookstaff *et al.*, 1990) Thus, gonadal hormone mediation is essential for TCDD to affect the compensatory responses of the hypothalamic/pituitary axis.

In a related experiment, castrated rats were infused with GnRH under barbiturate anesthesia (to suppress endogenous GnRH response). No difference in the time-response of plasma LH concentrations was observed

between ALC and TCDD-treated rats. This suggests that the presence of testicular hormones is necessary for TCDD to block the compensatory increase in responsiveness to GnRH release.

In order to determine whether the presence of androgens is required to inhibit pituitary feedback responses, castrated rats received testosterone implants that imbued them with subphysiological plasma testosterone concentrations (30 percent of normal) prior to castration and TCDD treatment. While ALC and PFC rats manifested the normal increase in plasma LH concentration and an increase in pituitary GnRH receptor number, TCDD-treated rats showed neither response (the deficits were statistically significant from the values for both control groups), although testosterone concentrations were nearly identical in the three groups.

To determine the ED_{50} for TCDD treatment that would block the feedback increase in plasma LH concentration and GnRH receptor number, rats were treated with the vehicle or graded doses of TCDD. The ED_{50} was estimated to be 20 µg/kg for both responses. These values compare favorably with earlier estimate by Bookstaff *et al.* of 10 µg/kg as the ED_{50} for inhibition of plasma LH response by testosterone.

This study reaffirmed that TCDD treatment increases the potency of testosterone as a regulator (inhibitor) of plasma LH concentrations. More importantly, it deduced the mechanism of this response. That is, TCDD exerts an effect on the hypothalamus (by regulating pituitary GnRH receptor number) and a pituitary effect (by altering GnRH responsiveness). Still, the ultimate biochemical mechanism by which TCDD increases the potency of androgens remains unknown.

Kleeman *et al.* investigated the mechanism by which 2,3,7,8-TCDD decreases testosterone secretion by the testes. (Kleeman, Moore & Peterson, 1990) Their experiments followed the same general design as the Bookstaff *et al.* (Bookstaff *et al.*, 1990; Bookstaff *et al.*, 1990) The authors also

discovered that TCDD treatment diminished intratesticular testosterone content. This led them to conclude that inhibition of testosterone synthesis, rather than failure of testosterone secretion, is the basis of the reduced testosterone secretion. Moreover, these effects were not secondary to undernutrition, the anorexia "wasting syndrome" characteristic of high-dose TCDD exposure.

From further experiments the authors concluded that TCDD inhibited pregnenolone synthesis. Pregnenolone is a metabolic product of cholesterol and the initial steroidogenic intermediate of testosterone synthesis. Since the conversion of cholesterol to pregnenolone is dependent upon activity of cytochrome $P450_{scc}$ in mitochondria, then perhaps impairment of this enzymatic activity, or of the process for mobilizing cholesterol to this enzyme is responsible for the reduction in testosterone secretion following TCDD treatment.

Umbreit *et al.* administered soil contaminated by PCDDs/PCDFs from a former 2,4,5-T manufacturing plant to male C57B/6 mice by gavage. (Umbreit, Hesse & Gallo, 1988) Comparison groups of mice received decontaminated soil, recontaminated soil, and one of two 2,3,7,8-TCDD-contaminated industrial soils. Ten male mice were used in each treatment group, except the decontaminated soil group which consisted of 50 males and 50 females. The females served as the mating partners of the treated mice. Males were dosed beginning at six weeks prior to mating, and continuing for 30 weeks. This protocol should have ensured that all stages of spermatogenesis were exposed to the presence of TCDD.

The low doses of 1 to 10 µg/kg/week administered for 30 weeks resulted in no significant toxicity, and as important, no effect on reproductive function. However, only a limited number of reproductive parameters were measured including: number of pregnancies; number of litters born dead; births; pups born; live pups born; pups/litter; live

pups/litter; and number of pups surviving until weaning. Unfortunately, the number of animals treated (10 per group) was very small, statistical power was further reduced due to the death of several mice in the course of the experiment from causes judged by the authors to be unrelated to the treatment. The study design failed to assess early fetal deaths by sacrificing pregnant dams midway through pregnancy to determine the number of resorptions. Also, since rodents are superfertile compared to humans, the absence of data on sperm counts is noteworthy. As a result of these deficiencies the significance of this study's findings are quite limited.

No studies were found which evaluated the male reproductive consequences of PCDF exposure, or exposure to the other trace contaminants of PCP (*e.g.*, polychlorodiphenyl ethers, chlorinated cyclohexenones and cyclohexadienones), with one exception. Oishi *et al.* conducted experiments to compare the toxicity of PCB and PCDF mixtures. (Oishi, Morita & Fukuda, 1978) The congeneric identity of the PCB and PCDF mixtures was not determined.

Male Sprague-Dawley rats were administered very high doses of these compounds in their feed for four weeks. Rats in the high dose PCDF (10 ppm) group experienced a statistically significant reduction in food consumption, and dramatic reductions in body weight gain compared to the control group. In addition, absolute and relative seminal vesicle and ventral prostate weights were depressed in comparison with the control group values. Finally, serum testosterone concentrations were significantly depressed in rats in the high dose PCDF group. One precautionary note is essential in interpreting this study. That is, that the obvious acute toxicity of the high dose PCDF mixture could easily have been responsible, through secondary mechanisms, for the effects on the accessory sex glands and the serum testosterone levels.

ii. Human Studies

Four studies from the epidemiological literature, two case reports and two analytical studies, have evaluated male workers exposed to pentachlorophenol for potential adverse reproductive health effects. All four report possibly suggestive adverse reproductive effects on male fertility, but neither individually nor collectively are they entirely persuasive.

One case study from Germany of pentachlorophenol manufacturing workers reported "disturbances of libido" among four of 10 (40 percent) PCP manufacturing workers stricken ill within months of the beginning of penta production. (Baader & Bauer, 1951, p. 287) The other case report from Germany is a follow-up investigation to the 1951 penta manufacturing plant investigation of Baader and Bauer. (Bauer *et al.*, 1961) Bauer *et al.* note a "decrease in libido or potency" among seven of 17 (41 percent) pentachlorophenol-exposed workers. (Bauer *et al.*, 1961, p. 538) These case reports, although provocative, suffer from a lack of standardized comparison of rates of decreased libido or potency among other plant workers.

Gilbert *et al.* conducted two studies of wood treatment workers. (Gilbert *et al.*, 1983; Gilbert, Minn, Duncan & Wilkinson, 1990) They conducted a cohort study, which they termed a "case control study" (actually somewhat of a cohort study) in their published paper, that included an external group of workers to use for comparison purposes in an evaluation of the health status of wood treatment workers. They also conducted a "historical prospective study" (actually a cross-sectional study) of all former or current wood treatment workers with more than 3 months experience.

For the "case-control study", 182 wood treatment workers were identified who had had long-term exposure to wood treatment chemicals since

1960. Records of only 125 of the 182 men (69 percent) were available for follow-up investigation. Of the 125 eligible workers, only 88 were alive and willing to participate (70 percent of the eligible, only 48 percent of the original cohort). Thirty-one former or current workers were known to be alive and declined to participate. Sixty-six of the wood treatment workers enrolled into the study were still employed in the industry, the remaining 22 workers were either retired or in a different line of work. These 88 workers were compared to 58 age-, sex-, race-, physical activity-, and weight-matched "controls" (some of whom had an opportunity for PCP exposure)

Gilbert *et al.* reported that there were no significant differences between the wood treaters and the "control" group with respect to "educational level, smoking history, alcohol consumption, frequency of flu-like symptoms, number of symptoms, number of children fathered, or marital status. The pentachlorophenol-exposed wood treatment workers had slightly fewer children than male "controls", but the difference was not statistically significant. (Gilbert *et al.*, 1983) However, this assessment of male reproductive effects was based on a single and very simplistic endpoint, namely the number of children born to married men during the exposure period. The lead author could not remember if the results were race- or age-adjusted. (Personal Communication with Fred Gilbert, December 6, 1990)

The Gilbert *et al.* study suffers from several deficiencies that make it inadequate for assessing either the presence or absence of a risk for a variety of endpoints, especially impaired male fertility. First, a cross-sectional design is an inappropriate study design for most investigations. In addition, the small sample size problem was accentuated by the poor follow-up and low recruitment rates. The statistical analysis was naively simplistic and could not have adjusted for the expected number of births

during the exposure period. Finally, there was no clear definition of exposure.

For her Master of Science thesis, Corddry, conducted an investigation of pregnancy outcomes among wives of male employees of a sawmill, where the workers were exposed to aqueous solutions of sodium tetrachlorophenate and sodium pentachlorophenate. Her study population consisted of a sample of 42 women who had experienced 60 pregnancies, and 40 control pregnancies. The study found no statistically significant differences in demographic characteristics or pregnancy outcomes between the two groups. Corddry concluded that there was a "…greater rate of spontaneous abortions among the exposed pregnancy group than among the control group, but this difference was not statistically significant, and the results appeared to influenced by confounding from maternal alcohol consumption." (Corddry, 1981).

Collectively, the results of these four studies is at best suggestive of the potential for adverse male reproductive effects to follow occupational exposure to pentachlorophenol. Consequently, the purpose of this thesis is to specifically test the hypothesis that occupational exposure to PCP may adversely affect quantitative and objective measures of male fertility.

This review would be incomplete without some discussion of the epidemiological studies that have evaluated the reproductive function or fertility of men who may have been exposed to PCDDs/PCDFs.

The evidence of a human hazard to male reproduction from PCDDs/PCDFs is far less clear than the animal data. A number of occupational and environmental studies of men exposed or potentially exposed to PCDDs/PCDFs have been conducted with conflicting results.

One study reported on the condition of 3 young scientists accidentally exposed to 2,3,7,8-TCDD in the course of laboratory

experiments. Although two of the three cases reported chloracne, none had porphyrinuria or a loss of libido. (Oliver, 1975)

Suskind and Hertzberg conducted a follow-up investigation to a Nitro, West Virginia chemical plant that had an explosion in its 2,4,5-T manufacturing area in the late 1940s. (Suskind & Hertzberg, 1984) The explosion resulted in the release of PCDDs/PCDFs in the operations area, and many workers developed chloracne. The differences in miscarriage rates between the exposed and unexposed plant workers were reported to be not statistically significant. The stillbirth and neonatal mortality rates in the exposed group exceeded those of the unexposed group, but again these rate differences were not statistically significant.

The Vietnam War and the aerial spraying of phenoxyherbicide defoliants has led to numerous health studies of both the Vietnamese population and U. S. soldiers potentially exposed to the spraying. Vietnam veterans have been vocal in expressing a myriad number of health complaints and concerns which many attribute to possible exposure to PCDD/PCDF impurities contained in defoliants (principally Agent Orange) used in the Vietnam War. Some studies have reported that Vietnam War Veterans have slightly altered rates of reproductive outcomes (Stellman, Stellman, & Sommer, 1988), while others do not (Centers for Disease Control, 1988b; Wolfe, et al., 1995). Two studies have measured altered semen characteristics among Vietnam veterans (Centers for Disease Control, 1988a; DeStafano, Annest, Kresnow, Schrader, & Katz, 1989), but found no reported difference in the number of offspring of Vietnam and non-Vietnam veterans.

The Centers for Disease Control conducted a multidimensional health assessment study of Vietnam veterans called the Vietnam Experience Study. As part of that study, a subsample of 571 participants had semen samples evaluated. "Vietnam veterans had lower sperm concentrations and lower mean proportions of morphologically 'normal' sperm cells. Despite differences

in sperm characteristics, Vietnam and non-Vietnam veterans have fathered similar numbers of children." (Centers for Disease Control, 1988a, p. 2708)

Subsequently, DeStafano *et al.* reported that Vietnam veterans had significantly lowered mean sperm counts (64.8 x 10^6 sperm/mL for Vietnam veterans *versus* 79.8 x 10^6 non-Vietnam veterans), were twice as likely to be oligospermic (*i.e.*, sperm counts <20 x 10^6 sperm/mL), and have a significantly lowered mean proportion of morphologically normal sperm heads. (DeStafano *et al.*, 1989) However, despite these differences, both groups of veterans reported fathering similar numbers of children.

After dismissing confounders as a possible explanation for their findings, DeStafano *et al.* concluded that dioxin exposure was unlikely to be the explanation for the findings since no studies have demonstrated reduced human male fertility following exposure to dioxins, and serum dioxin levels of Army ground troops was low. They do, however, cite a secondary reference to an 11-year old unpublished study which reported a 40 percent decrease in average sperm counts and decreased plasma testosterone levels in railroad workers involved in cleaning up a dioxin-contaminated material spill was mentioned. This study, apparently a reference to a pentachlorophenol spill which occurred in southern Illinois some years ago, has never appeared in the peer-reviewed literature.

The U. S. Air Force Health Study (formerly termed the "Ranch Hand Study") found no difference in the reproductive function among Air Force personnel potentially exposed to Agent Orange during military service in Vietnam. (Lathrop, Wolfe, Albanese & Moynahan, 1984) The distributions of semen characteristics (mean sperm concentrations and of the percentages of abnormally-shaped sperm) between Ranch Hand and a comparison group of military service veterans were similar. The number of children was similar as well. The only significant difference was the small excess number of reported spontaneous abortions for post-Vietnam service conceptions among

Air Force personnel potentially exposed to Agent Orange, but the inconsistent results of reports from husbands and spouses suggests some sort of systematic recall bias among these servicemen. Further study has revealed no increased risk for spontaneous abortions, stillbirth, or biologically meaningful birth defects due to paternal dioxin exposure. (Wolfe, et al., 1995)

f. Proposed Mechanisms of Male Reproductive Toxicity

The pentachlorophenol pharmacokinetic and toxicity studies reviewed above suggest mechanisms by which penta might reduce male fertility. The literature on its PCDD/PCDF impurities suggest mechanisms by which penta exposure might cause either reduce male fertility or induce an alteration in the sex ratio of the children born to workers. The *in vivo* and *in vitro* studies cited below under each heading reference the literature findings supporting each of the hypothesized mechanisms of male reproductive toxicity. It should be noted that the hypothesized mechanisms enumerated below are not necessarily mutually exclusive.

i. Pentachlorophenol-induced Loss of Libido

Two case reports in the medical literature describe a high incidence of loss of libido among male pentachlorophenol production workers in Germany. (Baader & Bauer, 1951; Bauer *et al.*, 1961) It is possible that an observed reduction in PCP worker fertility is due to loss of libido, and not to an adverse effect on spermatogenesis. Recently, vanhoorne reported on the results of investigations into the endocrine and reproductive effects of carbon disulfide exposure on men. (Vanhoorne, Comhaire & de Bacquer, 1994; Vanhoorne, Vermeulen & de Bacquer, 1993) They reported "...a

significant effect of carbon disulfide on libido and potency, but no effects were noted on fertility nor on semen quality."

ii. Pentachlorophenol-induced Hyperthermia

It is well-known that acute pentachlorophenol poisoning induces hyperthermia, which may or may not be fatal. Numerous case reports and experimental studies in the past century have explored the adverse effects of hyperthermia and hyperpyrexia on spermatogenesis. Mammalian testicular temperature is maintained at several degrees below normal body temperature for optimal sperm production. Elevated boby temperature, for whatever reason, results in degeneration of the seminiferous epithelium that nourishes and maintains sperm production. (Lähdetie, 1995) The key findings from this research is that the adverse effects of hyperthermia or hyperpyrexia on semen characteristics and fertility are transient and reversible. (Mieusset, Bujan, Mansat, Grandjean & Pontonnier, 1991)

Some researchers have examined the adverse effects on spermatogenesis resulting from experimental heat exposures to humans or animals. (Griffiths, 1893; MacLeod & Hotchkiss, 1941; Procopé, 1965; Reid, Mason, Withers, & West, 1981; Robinson & Rock, 1967; Robinson, Rock, & Menkin, 1968; Rock & Robinson, 1965; Venkatachalam & Ramanathan, 1962; Watanabe, 1959) Other researchers have assessed the effect disease-induced hyperpyrexia on spermatogenesis. (Koentjoro-Soehadi, 1982; MacLeod, 1951; Marmor, Elefant, Dauchez & Roux, 1986; Mills, 1919) Still others have evaluated the effects of occupational (Dukes-Dobos, 1981; Mortensen, 1988) or environmental heat stress (Levine, 1988b; Levine *et al.*, 1988; Saint Pol, Hermand, Beuscart, Jablonski & Leroy-Martin, 1989) on semen quality. In addition, there is anecdotal evidence of the use of hot baths as a contraceptive measure in some cultures. Several studies have correlated low sperm counts with elevated scrotal temperature due to anatomical

anomalies such as varicoceles or cryptorchidism. (Zorgniotti & MacLeod, 1973; Zorgniotti & Sealfon, 1988; Zorgniotti, Sealfon & Toth, 1980)

The medical and scientific evidence is convincing that elevated intrascrotal temperatures in a mammalian male, for whatever reason (whether internally- or externally-mediated), can impair spermatogenesis, and can under appropriate circumstances reduce or eliminate fertility. Furthermore, this action does significantly affect libido. A review of this literature follows.

Studies have documented the temperature differential that exists between the scrotum and the core body temperature in the male of mammalian species Several mechanisms are known to be involved in maintaining testicular temperatures below the core body temperature. This temperature gradient is highest in the rat and mouse (8.3 and 8.5°C), intermediate in the rabbit and ram (6.2 and 7.1°C), and lowest in human and nonhuman (rhesus monkey) primates (1.4-2.38°C and 2°C, respectively). (Kandeel & Swerdloff, 1988; Robinson, Rock & Menkin, 1968) Moreover, as studies have consistently demonstrated, the maintenance of this temperature gradient is essential for normal fertility.

The testis rely upon thermoregulation for proper spermatogenesis, being so readily accessible they have proven to be very vulnerable to the damaging effects of heat from experimental or environmental sources. A number of experimental techniques have been used to study the effect of heat on spermatogenesis, these include: artificial cryptorchidism, scrotal insulation, acute febrile illness, elevated ambient temperature, experimental varicocele, dry heat, wet heat, infrared or microwave radiation, and ultrasound waves. (Kandeel & Swerdloff, 1988) All of these techniques have been successful, under appropriate conditions, in inhibiting spermatogenesis. (Kandeel & Swerdloff, 1988)

In humans, most of the data on the effect of heat on spermatogenesis stems from studies of patients with two anatomical anomalies, varicoceles (varicose veins in the scrotum) and cryptorchidism. Varicoceles cause "...retrograde flow in the spermatic vein which may be due to insufficiency of a valve at the confluence of the spermatic with the renal vein." (Clarke, 1966) Presumably poorer blood flow in the scrotum impairs the body's thermoregulatory control over testicular temperature. Varicoceles are also quite common, occurring in approximately 8 percent of a sample of otherwise normal Navy and Marine Corps reservists. (Clarke, 1966) Their causative role in infertility is now considered dubious since infertile men both with and without varicoceles have higher intrascrotal temperatures.

Nonetheless, the relationship between varicoceles and infertility remains controversial. For instance, Mieusset *et al.* found that in comparisons of 150 infertile, nonazoospermic men and 37 euspermic men, the mean scrotal temperatures of the infertile men were significantly higher than those of the fertile men. (Mieusset, Mansat, Bujan & Pontonnier, 1987) In the former, the higher the scrotal temperature the more altered the seminal characteristics. Yet, while the scrotal temperatures of infertile men with varicoceles were greater than those of fertile men, they did not differ from other infertile men without varicoceles. A similar set of conclusions was reached by Zorgniotti and MacLeod in a study of 35 euspermic men, 50 men with varicoceles seeking treatment for infertile marriages, and 44 men without varicoceles who also sought treatment for infertile marriages. (Zorgniotti & MacLeod, 1973)

Cryptorchidism results from the failure of the testicles to descend into the scrotum early in life. Men with cryptorchidism tend to have poorer fertility. Higher intrascrotal temperature alone may not be the causative factor because of two lines of evidence. (Snyder, 1990) First, boys with unilateral cryptorchidism have abnormalities of the seminiferous tubules in the descended testicle. Second, men who have had surgery as

children to correct unilateral cryptorchidism have lower sperm counts and higher FSH concentrations as adults than do normal men. Thus, even unilateral cryptorchidism may conceal bilateral abnormalities. Whether these abnormalities are congenital, or acquired due to damage from higher abdominal temperatures is unknown.

The results of several medical case reports are presented next, in chronological order. Although not completely definitive, nonetheless, these natural experiments are informative. Moreover, their results are completely consistent with the results of animal experiments and observations that demonstrate adverse effects on spermatogenesis from elevated body temperatures. Like the animal studies, plasma FSH, LH, and testosterone levels are not significantly affected unless there is prolonged exposure to very extreme ambient temperature conditions (>45°C, 113°F). (Kandeel & Swerdloff, 1988)

Mills performed autopsies on 60 United States army soldiers who died of epidemic pneumonia. (Mills, 1919) The men ranged in age from 18 to 40 years old. Mills described the pathological changes in the testicles which accompanied the disease. The degree of damage correlated with the duration of the illness prior to death. Among the pathological changes observed by Mills were: "(1) cessation of spermatogenesis; (2) degeneration of preformed spermatocytes, spermatids, and spermatozoa; (3) desquamation of altered cells and fragments of the same; (4) formation of giant cells in the tubule walls with subsequent liberation into the lumen; (5) disappearance of all desquamated cells and all those derived from the spermatogonia by mitosis; (6) in some instances thickening of the hyaline layer of the basement membrane." (Mills, 1919) For instance, in eleven cases of streptococcus haemolyticus, spermatogonia were often damaged and reduced in number. In addition, spermatozoa were absent from nine of the

cases, and "…mere traces…" were represented in the other two cases. (Mills, 1919)

MacLeod published case reports of three male medical students who had routinely submitted semen samples to MacLeod's research laboratory prior to hospitalization for moderately high to high fevers, and who experienced startling decreases in sperm count. (MacLeod, 1951) The first case involved a 27 year old man with chickenpox who developed "…a spiking fever for five days, ranging from 101° F on third and fourth days, falling to 99.5° F on the fifth and sixth days."

On the third day of hospitalization the patient began receiving the antibiotic aureomycin. He was discharged on the ninth day with no sign of secondary infection. A semen sample submitted approximately one month after his discharge from the hospital had a sperm count of only 9 million/cm^3 (5.6 percent of his average prehospitalization value). In addition, the motility of the sperm were greatly depressed and the proportion of abnormal sperm increased. These findings were in stark contrast to the results from the 35 ejaculate samples provided by the student in the five months prior to his illness, at which time his average sperm count was 160 million/cm^3.

In this case, recovery to normal sperm counts was rapid. Twenty-two days after the initial discovery of the low sperm count (approximately 7 weeks after being discharged from the hospital), his sperm counts exceeded average control levels and motility and morphology of the sperm had returned to normal.

The second case of chickenpox struck a 22 year old male who was admitted to the hospital with a fever of 103.5° F. During the subsequent three days his body temperature steadily returned to normal, and he was afebrile on the fourth day. He was discharged four days later with no complications.

Nineteen days after his admission (ten days after his discharge), a semen sample was submitted for analysis and the sperm count found to be 73 million/cm^3, but 70 percent were motile and morphology was little affected. Subsequently, a progressive decline in his semen quality took place with the nadir occurring on the thirty-fourth day following his admission to the hospital. At that time, the sperm count had fallen to 3 million/cm^3 (2 percent of his baseline sperm count), the sperm were virtually immotile, and there was an extremely high proportion of morphologically abnormal cells.

Again, recovery was fairly rapid. Three days after the nadir, the sperm count was unchanged but they had regained normal motility and the morphologically abnormal cells were beginning to disappear. After the passage of another six days (43 days after admission to the hospital) the sperm count had risen to 20 million/cm^3. Sperm count recuperation was fairly rapid, though not as rapid as with the first case. Twenty days later (sixty-three days after hospitalization) his sperm count had risen to only 39 million/cm^3. Fifteen days later (day 78 after hospitalization) his sperm count was 211 million/cm^3, exceeding even his baseline value.

The third case was a 23 year old man hospitalized with pneumonia who had a temperature of 104°C the previous day. Despite massive antibiotic treatment, the patient's body temperature fluctuated between 99.5°F and 104°F. He became afebrile during his third week in the hospital, and was discharged on his thirtieth day. Thirty-eight days after his admission to the hospital (eight days after his discharge), his sperm count was only 2 million/cm^3, and they were completely immotile with a large proportion (58 percent) of abnormal cells. Five days later only a few immotile sperm cells were found. After eight additional days the man was azoospermic. The passage of another week (fifty-eight days after admission and nearly one month after discharge from the hospital) saw the return of small

numbers of "feebly motile cells...all of which showed very poor morphology…". (MacLeod, 1951)

Sperm counts remained low, although there was a slow progressive improvement in sperm motility and morphology until a dramatic increase occurred 90 days after discharge. The sperm count had suddenly risen to 152 million/cm^3 with normal cell motility and morphology. This value exceeded even the baseline sperm count of 130 million/cm^3.

The kinetics of effect of fever on sperm count, motility and morphology are important determinants of its affect on fertility. Reduction in sperm motility seem to parallel reductions in sperm count, with motility recovering more rapidly than sperm counts.

The time from the onset of the fever to the lowest level of semen quality can only be guessed at in case one due to the sparse data. However, in the second case this time period appears to be about 40 days, and in the third case it appears to be 51 days. The duration of the depressed sperm count and recovery period from the nadir of semen quality were, respectively: approximately 20 days depression and 21 days recovery for case one; 30 days depression and another 30 days recovery period for case two; and at least 34 days depression and approximately 18 days recovery period for case three. Thus, a single episode of hyperpyrexia may directly impair spermatogenesis, and hence adversely affect male fertility for periods of three to four months, with the duration and degree of sperm count depression dependent upon the duration and magnitude of the fever.

Koentjoro-Soehadi reports on a case of azoospermia caused by typhoid fever in Indonesia. (Koentjoro-Soehadi, 1982) A 23-year old male who had been married for two years sought medical attention for "involuntary childness." The physical findings were essentially normal, but his sperm count was only 1.8 million/cm^3. After one month of treatment(steroid hormone and multivitamin), this value rose to 6.4 million/cm^3.

Three months later, the patient was hospitalized for 10 days for Typhoid fever. During his illness, his temperature ranged from 39-41.2°C (*i.e.*, 102-106°F). About one month after his release from the hospital the patient was tested and found to be azoospermic, despite continued fertility treatment. This condition was reaffirmed in a specimen obtained two weeks later. Three weeks later (approximately three months after the onset of the fever) sperm reappeared and a count of 1.6 million/cm^3 was recorded. Motility was reduced. In addition, after the hospitalization, the patient's coital frequency reportedly fell to once per week even though coital activity was unaffected. Thereafter, the patient was lost to further follow-up.

The author cites andrological research indicating the sensitivity of the testicles' production of sperm to elevated temperature due to damage to the tubules. Further, he incorrectly states that the Leydig cells outside the tubules are unaffected by temperature changes, hormone production continues and libido is normal. In fact, high temperatures can alter Leydig cell function and result in diminished testosterone production. (Levier & Spaziani, 1968) The author notes that the observed recovery time of 3 months closely matches the approximate period necessary for the process of spermatogonia maturing to spermatids (70 days) and appearing in an ejaculate (an additional 20 days).

Marmor *et al.* discuss the influence of hyperpyrexia on semen quality in Hodgkin's disease patients prior to treatment. (Marmor *et al.*, 1986) Semen samples were collected from 57 men. Although mean results were normal for a fertile population, 19 had abnormal results, 12 of whom had been feverish for at least two weeks, or had recently recovered from fevers.

Of these 12 men, four had slight fevers with body temperatures less than 38.5°C (101°F). The only semen abnormality in these men was a marked

reduction in sperm motility (asthenospermia). However, in all cases it exceeded 25 percent. The other eight men had higher fevers. Their sperm counts were more seriously affected Three were azoospermic, the others were asthenospermic or oligoasthenospermic. Only five febrile men were noted to have normal semen quality, and all had suffered from mild fevers of about 38°C (100°F). The rapidity (approximately two weeks) with which sperm counts decrease after a high fever confirms earlier experimental studies. (MacLeod & Hotchkiss, 1941; Procopé, 1965)

Eight studies in which human subjects were experimentally subjected to hyperthermic conditions, in order to measure the effect on spermatogenesis, were located in the literature. They are reviewed next, in chronological order of their publication. It should be noted that two basic approaches to artificially inducing the effects of fever on spermatogenesis have been taken. The first, rather crude, approach was to elevate whole body temperature through exposure to diathermy machine radiation, steam (sauna) baths, or immersion in hot water. The second approach was to use localized heating of the scrotum with either wet or dry heat sources, or scrotal insulation. All approaches have successfully resulted in significant reductions of sperm counts.

MacLeod and Hotchkiss subjected 6 healthy young unmarried male volunteers to controlled hyperpyrexia for the purpose of monitoring the effect of high temperature on spermatogenesis. (MacLeod & Hotchkiss, 1941) After establishing baseline semen quality (sperm counts, motility, and morphology) for these individuals, values that were within reported norms, the volunteers were administered one or two heat treatments.

The men were placed within a "fever cabinet," actually a diathermy machine which heats tissue by short-wave electromagnetic induction. The machine contained a humidifier and air conditioner which were used to maintain relative humidity within the cabinet at between 95 and 100 percent. Thus, the exposure was to dry heat since it was the

electromagnetic radiation that caused the heated the volunteers tissues. The cabinet was designed so that the volunteer's head protruded through an opening during the course of the heat treatment.

The cabinet was preheated to 43°C (109°F), and maintained at this temperature until the naked volunteer's oral body temperature reached within approximately 2°C of the maximum targeted body temperature of 40°C (104°F), although in most cases the maximum temperature attained was 40.5-41°C (105-106°F). On average, it took 45 minutes for the volunteers body temperature to reach the targeted temperature. The authors state that the mean chamber temperature during a experimental "fever" treatment was 43°C, so that "…for at 32 minutes the surface of the scrotum was exposed to this high temperature, and that, for a considerably longer period (up to 3 hours), the blood temperature was elevated to points 0.5° to 4°C above normal." (MacLeod & Hotchkiss, 1941)

The volunteers provided semen samples every 3 to 6 days. In every case, the sperm count of the subject fell immediately after the heat treatment, and reached their lowest levels 25 to 55 days after the treatment. The sperm count depression persisted for between 15 and 50 days, after which there was a relatively swift return to pretreatment values in approximately 20 to 30 days. For volunteers first and second heat exposure, the mean time for total sperm counts to decrease to the middle of the lowest value was 42 days and 50 days, respectively. The mean time for return to normal sperm count values was 25 days for both the first and second heat treatments. The average duration of the sperm count depression following the first and second heat treatment was 32 days and 37 days, respectively.

Watanabe conducted experiments with 18 healthy, unmarried male medical student volunteers to ascertain the effects of external heat treatment on spermatogenesis. (Watanabe, 1959) The students ranged in age

from 21 to 26 years of age. Three to five semen samples were provided by each volunteer to provide baseline values. The average sperm count was normal for Japanese males, being somewhat lower than that reported for Americans. After a recovery period, some students were used repeatedly for different experiments.

Unlike the MacLeod and Hotchkiss experiment in which the entire body except the head was exposed to elevated ambient temperatures, in this study, only the scrotum of the volunteers was heated. This was accomplished with a specially-shaped, thermostatically-controlled electrically heated warm water bath. The acceptable treatment temperature range of the water bath used in the experiments was 43-47°C (109.4-116.6°F), although usually it was between 44 and 46°C (111.2-114.8°F). The scrotal heat treatment was conducted for periods of 30 continuous minutes duration.

Nine different heat treatment regimens were developed, six involved only a single course of heat application (Group A) although all but one resulted in serial heat exposures, the remainder involved multiple heat applications at various time intervals (Group B). Two to six individuals were assigned to each heat treatment group. Group A-1 received a single heat application. Group A-2, A-3, A-4, and A-5 volunteers received heat applications on 2, 3, 6, and 12 consecutive days. Group A-6 received heat applications on 6 alternated days. Group B-1 individuals began by receiving scrotal heat application for 4 consecutive days, followed by 3 consecutive days at 4 week intervals. Group B-2 individuals received an identical treatment regimen, followed by 3 consecutive days of heat application at 3 week intervals. Group B-3 individuals received the same treatment regimen as Group B-1 individuals, followed by 4 consecutive days of heat application at 3 week intervals.

Among volunteers receiving Group A heat applications certain clear trends in the data can be discerned. First, among individuals receiving "mild" (single) heat application treatment the results were somewhat equivocal. No consistent effect on sperm count was observed.

Second, among individuals who received "moderate" heat application (2 or 3 days of consecutive treatment) a decrease in sperm counts was apparent approximately 2 to 6 weeks. Sperm counts remained depression for 2-7 weeks. Recovery of sperm counts to pretreatment levels was completed by 8-13 weeks after the treatment stopped.

Interestingly, among volunteers who received longer-term treatments (Groups A-4, -5, and -6), there was an initial steep rise in sperm concentrations 2 to 3 weeks after the treatment to sperm counts as much as 150-310 percent of baseline values. This preceded a sharp decline in the sperm counts in the following 1 to 5 weeks. Depression of sperm counts below 20 million/cm^3 was seen in all but one man, and this depression persisted for 1 to 9 weeks. This was followed by a "rebound" effect in which sperm counts exceeded pretreatment values by 170-560 percent for 1 to 5 weeks. It should be noted that sperm motility decreased as the severity of the treatment (number of consecutive days of treatment) increased. Also, the proportion of males affected and the consistency of those findings rose rapidly as the severity of the treatment regime increased.

Among Group B volunteers, the effect of repeated periodic heat applications on spermatogenesis were dramatic. The heat applications inured by Group B-1 volunteers resulted in a sustained depression of sperm counts below 20 million/cm^3 for at least 9 weeks. Recovery to approximate pretreatment values was confirmed in three of the four men 3, 7, and 7.5 months after the third and final series of heat applications. However, in the two longer follow-up cases, the motility and sperm counts were still "somewhat lower" than the pretreatment values.

The two volunteers in Group B-2 failed to demonstrate a reduction in sperm counts with the Group B-1 regimen. When the intervals between heat applications was shortened, however, a progressive and long lasting depression of sperm counts below 20 million/cm^3 occurred. Since the volunteers left the area after the study, no data on the recovery of their sperm counts was collected, but the depression persisted throughout the 12 week treatment period.

The two volunteers in Group B-3 also failed to respond to the heat application regimen of Group B-1 as expected. They also did not respond to the shorter interval heat application regimen of Group B-2. The sperm counts of these individuals did decrease when the duration of the repeated heat applications was increased to four consecutive days. For these two men, recovery to pretreatment values took 4 and 6 months.

These experiments demonstrate that there is individual variability in susceptibility to impairment of spermatogenesis by heat. Nonetheless, repeated heat treatments at suitable intervals effectively suppressed sperm counts to very low levels, for long periods of time, in all men tested. In all cases with follow-up, the effect of repeated heat applications for several months on sperm counts was reversible.

Rock and Robinson investigated the effects of intrascrotal hyperthermia primarily from its potential role as a cheap, harmless, and reversible means of birth control. (Rock & Robinson, 1965) It appeared to show promise in this respect, and paradoxically, as a therapeutic technique for oligospermia.

The study was divided into two distinct research components. The objective of the first component was to determine the differences in intrascrotal and rectal temperatures among individuals (oligospermic, euspermic, and those with varicoceles) in different postures, and in different environments (room temperature and immersed in hot water). The

results of this first study component are not relevant to this project and will not be discussed.

The objective of the second component was to describe the effects of intrascrotal hyperthermia on euspermic and oligospermic men. For the first experiment, seven euspermic men were fitted with various types of underwear with insulation in the scrotal area. Six of the men wore this insulated underwear constantly for about six weeks, the seventh wore it constantly for about 14 weeks.

The authors noted a decline in sperm concentration approximately 3 weeks after the men began wearing the insulated underwear. The semen volume, like the earlier studies, remained virtually unchanged. The authors interpreted the absence of an effect on semen volume as confirmation of experimental data that showed no deleterious effect of testicular heating on the Leydig cells.

The lowest concentration of sperm was measured between the fifth and ninth weeks after the men began wearing the insulated underwear. The sperm counts at this time ranged from 5 to 25 million/cm^3. The authors note that the minimum concentration was a function of the individual's normal pretreatment sperm count. Interestingly, the authors noted no adverse effect on semen quality except for the individual who wore the insulated underwear for 14 weeks. This man showed a "high degree of dyspermia." (Rock & Robinson, 1965)

All of the men were oligospermic for periods ranging from 3 to 8 weeks after they ceased wearing the insulated underwear. In all but one case the men's sperm counts returned to their pretreatment values. The one exceptional individual his recovery level sperm count exceeded his pretreatment value. Also, two other men demonstrated the "rebound" effect after they stopped wearing the insulated underwear. No adverse effects on libido or function were reported in interviews of three men.

In a preliminary report, the authors detail that the effects of intrascrotal hyperthermia on 20 oligospermic men. The treatment was accomplished by immersion of the scrotum in hot water (43-45°C, 109-113°F) for 30 minutes on 6 alternate days. Like the euspermic men, hyperthermic treatment of the scrotum resulted in a decrease in sperm concentrations, but over a much longer period of time 11 to 112 days. However, nearly one-half of the oligospermic men exhibited a rebound effect after the cessation of treatment to higher sperm counts than they possessed initially. The peak of the higher sperm counts occurred, on average, about 8 weeks after the discontinuation of treatment. In addition, wives of six of these nine men became pregnant and had normal pregnancies.

Procopé investigated the effects of repeated hyperthermia treatments on spermatogenesis. (Procopé, 1965) The 12 volunteers in his study were all married medical students who had normal sperm and genitalia. The men ranged in age from 23 to 30 years of age. At least two semen samples were collected from each man before the experiment, as well as during the experimental treatment, and for a period of two months afterward. Compliance by the men in providing semen specimens was reported as good.

The hyperthermic treatment was carried out in a Finnish sauna bath, where each man bathed 6 to 8 times over a period of two weeks. The temperature range in the bath was measured at between 77 and 90°C (171-194°F). The men were instructed to remain in the sauna as long as they could endure it. The average duration in the sauna was 15.3 minutes, and the average amount of time spent in the sauna in a two week period was 2 hours and 24 minutes. The average increase in rectal temperature from the sauna treatment was 0.93°C (1.6°F).

For some unexplained reason, the observational periods were demarcated in 10 day increments. The lowest total sperm counts were observed during the period 30-39 days after the initiation of the sauna

hyperthermia treatment. The median count during this period was 68 million. Unfortunately, no semen volume data was recorded so sperm counts in millions/cm^3 cannot be compared with data generated by other studies. Assuming the average semen volume were 3-4 mL, then these men would have been rendered either oligospermic or borderline oligospermic. At the end of the observation period the sperm counts had returned to essentially pretreatment values.

A slight increase in the proportion of morphologically abnormal sperm was observed during the course of the study. At its peak, during the period 40-49 days into the experiment, the median proportion of abnormal sperm was 59.5 percent. Three months later, 11 of the men had a lower proportion of abnormal sperm, one man had a higher proportion. No motility data were obtained because of their sample collection technique (*i.e.*, condoms).

In a follow-up study to their 1965 study, Robinson and Rock again evaluated the effect of intrascrotal hyperthermia induced by scrotal insulation on spermatogenesis. (Robinson & Rock, 1967) Ten healthy euspermic males between the ages of 19 and 43 volunteered to wear an insulated athletic supporter during waking hours for six to 11 weeks.

Eight of the ten men agreed to a pretreatment experiment to determine the effect of scrotal insulation and posture on the scrotal-rectal temperature differential. There was a mean decrease of 0.8°C in scrotal-rectal temperature between nude men in supine and standing positions and men wearing only an insulated athletic supporter for 30 minutes while supine and in standing position.

Four of the ten men wore an insulated athletic supporter for 8 weeks or less, the remaining six men wore them for nine weeks or more. Among the men wearing the supporters for 8 weeks or less, the average minimal sperm ratio (actually a percentage, is the lowest total sperm count in any post-

treatment specimen divided by the baseline total sperm count) was 13.9. Among the six men who wore the insulated supporters for longer duration, the average minimal sperm ratio was 8.2.

When the results from the two groups were pooled a clear dip in total sperm counts was apparent in the third week after initiation of the treatment. The nadir in sperm counts was reached in the seventh week. A pronounced recovery in sperm counts appeared in the fourth week after the experimental treatment was discontinued. No significant variation in semen volume with treatment was discerned. Subjective reactions were mild. Five men reported no reduction in libido, the other half reported minor changes in libido, either increased or decreased.

In yet another study published the following year, Robinson and Rock further explored the effects of intrascrotal hyperthermia on spermatogenesis in euspermic and oligospermic men. (Robinson *et al.*, 1968) Unlike the earlier experiments, the authors replaced the insulated athletic supporter with a 150-watt light bulb as the means to raise the intrascrotal temperature. In addition, the authors evaluated the effect of intrascrotal hypothermia on spermatogenesis in euspermic and oligospermic men. The results of these tests follows.

In the first experiment, 14 euspermic men (most were medical students) volunteered to participate in the experiment. Their euspermic status was confirmed by analysis of two or three semen samples provided prior to the study. Euspermia was defined as sperm concentrations greater than 40 million/cm^3. Specific continence intervals were stipulated for the semen specimens provided before, during, and after the treatment.

According to the study protocol, the men had their scrota heated by the 150-watt light bulb for 30 minutes on 14 consecutive days. Preliminary studies indicated that the light bulb had to be positioned rather closely to the scrotum (8 cm, 3.1 inches) in order to attain temperatures of a

heating bath found to be effective in reducing spermatogenesis, 40-43°C. Ten of these men were also evaluated to determine the effect of a single such heat treatment on the scrotal-rectal temperature differential. Four additional euspermic men were tested to determine the effect on spermatogenesis from 30 minutes treatment sessions conducted over 28 consecutive days.

The authors discussed at some length the literature on what constitutes the normal intrascrotal-rectal temperature differential. This range appears to be from 1.4-4.8°C (1.4°C in their study), with the intrascrotal temperature always being lower than the rectal temperature. The scrotal light bulb heat treatment, like others before it, effectively reversed this temperature differential. After treatment, the intrascrotal temperature was, on average, 1.5°C higher than the rectal temperature. This change represented a relative increase of 2.9°C in the scrotal temperature *versus* the core body temperature. It is important to note that the rectal temperature was stable during these treatments. Thus, this increase truly represents the elevation in intrascrotal temperature.

The nadir of the group mean minimum sperm ratio 43.3, occurred during the fifth week. No significant "advantage" was found in reducing sperm counts by extending the duration of treatment to 28 consecutive days. Recovery of sperm counts was evident 8 weeks after the treatment was concluded. No conclusive effect on sperm morphology was possible.

The complimentary part of this study assessed the effect of this 14-day intrascrotal hyperthermia treatment on five oligospermic men. Oligospermia was defined as sperm concentrations less than or equal to 20 million/cm^3. All five men were married and possessed no physical defects or had previous illnesses that were thought could account for their condition. The oligospermic exhibited a rapid and remarkably large rebound effect in which post-treatment mean sperm ratios rose to as much as 500,

more than double the recovery period mean sperm ratio of euspermic men. However, there was wide variability in the response of the few men in this experiment, and the authors considered the response less predictable than that of euspermic men.

Brown *et al.* five men a single exposure to hot (85°C, 185°F) dry (<10 percent humidity) air in a sauna for 20 minutes, and then assessed the effect spermatozoa. (Brown-Woodman, Post, Gass & White, 1984) No information on the ages or health status of the men was provided. Their comprehensive assessment included sperm count, morphology, and motility as well as sperm metabolism, the percentage of viable sperm, an ultrastructural examination of sperm. This study was the only one of the seven thus far reviewed that included statistical comparisons of the treatment effects. The Student's t-test designed for repeated measures was used.

The brief sauna exposure increased rectal temperature by 0.4-0.7°C, and resulted in a statistically significant reduction in mean sperm counts within one week in all of the men, from a pretreatment average of 250 million to 160 million ($p < 0.05$). The lowest count was observed one week after the treatment. The depression in sperm counts persisted for five weeks in four of the men, the youngest man was less affected by the sauna treatment. Sperm counts returned to normal by the fifth week after the treatment, and fluctuated until they exceeded the baseline values at 10 weeks.

The sauna treatment resulted in a small, but not statistically significant, reduction in the proportion of morphologically normal sperm that continued through the end of the observation period. It had no effect on the percentage of viable sperm or the volume of the semen. However, glucose metabolism and motility increased one week after the treatment only to return to values slightly higher pretreatment values for the remainder

of the observation period. There were some ultrastructural changes at the end of the first week that persisted until the sixth week. Among these changes were swollen plasma membrane (2-5 weeks), an increase in the number of immature sperm present in ejaculates (3-5 weeks), and some disorganization of the mitochondria in the neck region of some sperm. These changes disappeared six weeks after the treatment.

The onset of the reduction in sperm counts (one week) occurred more rapidly than in other studies that induced greater and more prolonged heat stress. For instance, the single hyperthermia treatment MacLeod and Hotchkiss subjected their volunteers to resulted in an increase in oral temperature by 0.5-4°C for 3 hours, yet sperm counts did not decrease until the 18th day after treatment, although they remained depressed for 50 days. Likewise, the repeated sauna treatment used by Procopé each resulted in an average increase in rectal temperature of 0.93°C, yet the nadir of sperm counts was observed 30-39 days after the termination of treatment.

The authors offered no explanation for the differences in the timing of the effects in their study *versus* earlier studies. They did speculate that the early depression in sperm counts may have been due to damage to epididymal sperm, and that the continued depression of sperm counts was possibly due to "...damage to spermatozoa in the lumen of the seminiferous tubules and to spermatozoa during their later stages of development in the testis." (Brown-Woodman *et al.*, 1984)

Finally, Sanger and Friman evaluated the effect of underwear type on spermatogenesis in a self-described pilot study. (Sanger & Friman, 1990) The two study participants were healthy euspermic adult males in their early thirties who were participants in a sperm bank program. Their participation in the program required regular production of semen specimens. Commercially available tight-fitting bikini style, or loose-fitting boxer short style underwear was alternately worn by the men. Sperm

density, total number of sperm, total number of motile sperm and the total number of sperm per hour of abstinence from ejaculation were analyzed in the study.

The experimental design was to use "...a single blind, randomized ABAB withdrawal design," in order to control for the cyclical factors controlling spermatogenesis. (Sanger & Friman, 1990) The authors describe the ABAB design as the most conservative design for this sample size. The first man began by wearing tight underwear in Nebraska in July, the other man began wearing the tight underwear three months earlier in April. Every three calendar months for one year, the men alternated the wearing of tight and loose-fitting underwear.

The authors reported that sperm counts gradually declined for both men during the periods when they wore the tight-fitting underwear and gradually increased when they wore the loose-fitting boxer shorts. In fact, changes in all of the measured semen parameters mirrored those of the sperm counts, namely gradually improving after the men switched from tight-fitting to loose-fitting underwear and gradually declining after they switched from loose-fitting to tight-fitting underwear. An index developed by the authors, the total number of motile sperm per hour of abstinence (TM/h), showed the greatest response to of any parameter measured. TM/h increased by 21 percent and 23 percent in the first and second man, respectively, as they proceeded from wearing tight-fitting to loose-fitting underwear. The authors observed changes in the semen parameters within two weeks following changes in the style of underwear. The authors considered but dismissed other potential explanatory factors (season and stress) as being responsible for the observed effects.

Finally, the authors discussed the conceptive and contraceptive qualities of underwear style. The evidence they present indicates that the effect, though real, was relatively small for the euspermic men. Thus, while changing to loose-fitting underwear may have some clinical

significance for oligospermic men, given the significant variation within each "treatment," tight-fitting underwear is unlikely to be a reliable contraceptive method for euspermic men, or to significantly impair their fertility.

Rachootin and Olsen conducted a case-control study in Denmark to evaluate male and female occupational factors that are associated with infertility or delayed conception. (Rachootin & Olsen, 1983) A total of 1,069 infertile couples who were examined or treated for infertility at their hospital during 1977-1980 constituted the eligible cases for the study. The control group for the study was obtained from 4,305 couples who had a healthy child born at the hospital during the period 1977-1979. Socioeconomic, demographic, medical, and reproductive history data were obtained by a self-administered, mail-in, questionnaire. Of the eligible couples, a total of 927 cases and 3,728 control couples responded to the questionnaire.

The authors reported that heat exposure to the male partner was one of the few occupational factors consistently identified as resulting in a statistically significant increase in infertility or delayed conception. Odds ratios and 95 percent confidence intervals for these two endpoints were 1.9 [1.2-2.8] and 1.9 [1.5-2.6], respectively. (Rachootin & Olsen, 1983)

Finally, Figa-Talamanca *et al.* tested the hypothesis that high heat can effect male fertility. (Figà-Talamanca *et al.*, 1992) Their study consisted of two parts. The first part involved interviews with 92 healthy male ceramics oven operators with long exposure to high temperatures and 87 controls from the shipping department of the same industry. The second part involved collecting and analyzing semen samples from 46 ceramics oven operators and 14 comparison group members. Sperm concentration, morphology, and motility were evaluated.

The questionnaire responses indicated a higher frequency of self-reported childlessness and difficulty in conceiving among the high temperature-exposed oven operators than among the shipping department males. Although and unexposed men had the same average number of children, a larger proportion of the wives of high temperature-exposed men were childless (7.6 percent) compared to 1.1 percent among the wives of the unexposed group. Moreover, 4.3 percent of the high temperature-exposed men desired more children compared to 2.3 percent in the unexposed group. Based on questionnaire data, the time to pregnancy for the wives of high temperature-exposed men (3.3 months) was two and one-half times longer (1.3 months) than that for the unexposed men.

Sperm velocity was the only semen parameter that was significantly altered between the two groups. The authors concluded that overall, sperm profiles from the high temperature-exposed oven operators had a higher prevalence of "pathologic" characteristics than those from the unexposed comparison group based on a review by an andrologist blinded to the exposure status of the men. The "pathologic" classification was assigned to the sperm samples of high temperature-exposed men 4.6 times more frequently than that of the unexposed men.

The small sample size of this study is unfortunate, as was the poor participation in the semen sampling protocol. Nonetheless, the nonparticipants did not differ significantly from participants in social or demographic characteristics, work history, or smoking and drinking habits. This study reinforces the findings of the literature above, namely that high temperatures can effect male fertility.

iii. Uncoupling of Oxidative Phosphorylation

Animal cells are able to survive by making adenosine triphosphate (ATP), the chemical energy supply for each cell, by oxidative

phosphorylation. Oxidative phosphorylation is a process that involves the consumption of fats, carbohydrates, and oxygen, "…the process takes place at specialized membranes such as the inner membrane of the mitochondrion, the cellular organelle where energy production and storage takes place." (O'Brien, 1994) It is hypothesized that sufficiently high levels of exposure may have occurred as to trigger the biochemical mechanism of acute toxicity from pentachlorophenol (metabolic uncoupling or inhibition of oxidative phosphorylation) that have been discussed earlier. Specifically, this poisoning of cellular mitochondria may have affected fertility directly as the motility of sperm is dependent upon the energy-intensive efforts of the flagellum (tail).

Evidence supporting this mechanism is found in the research of Dougherty *et al.* who first reported the presence of pentachlorophenol in human semen. (Dougherty, 1978) Later, Dougherty collaborated on a study that showed there was a nine-fold enrichment of pentachlorophenol concentration in human sperm *versus* semen levels -- 40 percent of the PCP was bound to the cells that contained only 4 percent of the sample mass. (Kuehl & Dougherty, 1980) The concentrations reported by Dougherty *et al.* are in the range that Weinbach found were effective for inhibiting oxidative phosphorylation. (Weinbach, 1957; Weinbach & Garbus, 1965) More recently, Wagner *et al.* conducted analyses of PCP and nonachloro-2-phenoxyphenol (NCPP) in human tissues (testes, kidneys, prostate gland, liver, and fat) obtained from autopsies of eight men. (Wagner, Durand, Inman, Kiigemagi & Deinzer, 1991) Their analyses showed that "…on the average the highest residues of both PCP and NCPP were found in the testes and other non-fatty tissues." (Wagner *et al.*, 1991, p. 600) Of the tissues tested, the authors calculated that the testis had the highest bioconcentration factor (BCF=6.2) for PCP, with significant enrichment of prostate PCP concentrations (BCF=3.5). (Wagner *et al.*, 1991)

In the early 1970s, the G.D. Searle Company of Skokie, Illinois, was researching new spermicidal agents. One particular compound was noted to be a potent spermicide. Upon further investigation, it became clear that like pentachlorophenol, the new compound was a potent oxidative phosphorylation uncoupler. (Personal Communication with Karl Mackerer, July 1989) Further research revealed that this compound had unacceptable systemic toxicity side-effects, and thus further research was abandoned.

iv. Disturbance of Microsomal Detoxification

Like many other halogenated aromatic hydrocarbons (e.g., PCDDs, PCDFs, and PCBs), pentachlorophenol induces microsomal enzymes. (Vizethum & Goerz, 1979) However, an *in vitro* study of rat liver microsomes has shown that penta inhibits microsomal detoxification enzymes by disturbing electron transport from flavins to cytochromes. (Arrhenius *et al.*, 1977) Thus, it is hypothesized that workers' exposure to pentachlorophenol during manufacture may have resulted in a synergistic increase in the toxicity of other toxicants to which they were exposed, either those PCDD/PCDF impurities present in the penta, or those they may have been exposed to in other occupations or the general environment.

v. Perturbation with Lipid Membranes

A number of studies have evaluated different aspects of penta toxicity to cell membranes. (Duxbury & Thompson, 1987; Packham, Thompson, Mayfield, Inniss & Kruuv, 1981; Smejtek, 1987) Cells exposed to sublethal doses of PCP resulted in membrane damage to the viable cells. (Duxbury & Thompson, 1987) It is hypothesized that workers' exposure to pentachlorophenol during manufacture may have resulted in the attainment of cumulative damage to the integrity of germinal cell membranes, resulting in subfertility.

vi. PCDD/PCDF-Induced Toxicity

It is possible that the PCDD/PCDF impurities present in the pentachlorophenol may possibly have effected the fertility of male employees. Several of the early studies on this subject noted alteration in testicular morphology, or sterility in animals fed 2,3,7,8-TCDD. However, in each case these changes were observed in dead or moribund animals. (Lamb *et al.*, 1981) The implication has been that effects on male fertility are only observed if the animals are gravely or fatally ill from the TCDD exposure. However, more recent animal research on the adverse effects of 2,3,7,8-TCDD on male endocrine function and fertility lends one to pause to consider the possibility the effects of low level exposure on humans. Despite the provocative findings of male reproductive impairment in pre- or perinatally exposed rats, these test systems are not believed relevant and are not discussed since the cohort members in this study are adults. Also, the reproductive system of adult rats appears to be about 100-fold less sensitive to 2,3,7,8-TCDD toxicity (ED_{50}=15 µg/kg-bw) (Moore *et al.*, 1985) *versus* prenatal or perinatal exposures (ED_{50}=0.16 µg/kg-bw) (Mably, Moore, Bjerke & Peterson, 1991). Although there are extremely low concentrations of 2,3,7,8-TCDD in penta, assuming that the I-TEF approach for PCDDs/PCDFs adopted by EPA is valid, then dosing adult rats at approximately 3.3 mg PCP/kg-bw should give a TEQ dose of dioxins and furans equivalent to the ED_{50} identified by Moore *et al.* However, Schwetz *et al.* dosed male rats at 3 and 30 mg PCP/kg-bw for 62 days and observed no effect on male fertility. This raises questions concerning the legitimacy of the TEQ approach as currently formulated.

Following a paper by researchers at the National Institute for Occupational Safety and Health (NIOSH) regarding levels of serum testosterone and gonadotropins in dioxin-exposed workers, James suggested dioxin exposure may alter the ratio of the number of baby boys to girls,

with a shift in the ratio toward a greater number of girls. (James, 1995) James bases his hypothesis on the literature findings of occupational and environmental influences on the sex ratio, and his own work which hypothesizes there is extragametal parental hormonal control over the sex ratio of offspring. (James, 1986) He conjectures that men with lower serum testosterone concentrations are more prone to father girls than boys, and that since dioxin-exposed men have reduced serum testosterone concentrations they should also have a lower than expected sex ratio of boys to girls.

C. Review of Reproductive Toxicology

"Reproduction is a complex process that begins with gametogenesis: continues through gamete interaction, implantation, and embryonic development, growth, and parturition; and is completed with sexual maturation." (Mattison & Thomford, 1989) Reproductive processes occur in environments where xenobiotics are ubiquitous, further complicating the process of identifying and characterizing agents that may be potential hazards to human reproduction.

Interference with normal reproductive processes may stem from disruption of the hypothalamic-pituitary-gonadal interaction. Reproductive toxins may exert an adverse effect by either direct or indirect means. (Mattison & Thomford, 1989) In fact, a xenobiotic may induce reproductive toxicity by one or more of several distinct mechanisms. Direct-acting reproductive toxins, may cause their effects by either their chemical reactivity (e.g., destruction of gametes by alkylating agents), or by their structural similarity to endogenous substances (e.g., ersatz hormones or inhibitors). Indirect-acting reproductive toxins may produce effects by way of their metabolism to a direct-acting toxin (ultimately acting by virtue of chemical reactivity or structural similarity), or by altering

physiological control mechanisms (e.g., enzymatic induction and inhibition, or steroidal pharmacokinetics). (Mattison & Thomford, 1989)

Animal reproductive toxicology studies have tended to focus on a handful of general readily measurable endpoints: fertility (e.g., mating, female fecundity, male fertility, and gestation indices), epididymal sperm characteristics, tissue (testicle, seminiferous vesicle, and epididymis) weights and histology, and serum hormone profiles. (Hurtt, 1989; Lamb, 1989; Meistrich, 1989; Williams *et al.*, 1990) For obvious ethical reasons, only a few of these endpoints can be assessed in humans (e.g., testis size in situ, serum hormone profiles). (Williams *et al.*, 1990) Other endpoints that have been used to assess damage to the integrity of the human male reproductive system include measurements of semen quality (e.g., sperm count, percentage of motile sperm, and percentage of morphologically normal sperm), sperm viability (stain exclusion and hypoosmotic swelling); and more recently developed techniques like computer-linked video microphotography to determine sperm velocity. (Schrader, Turner & Ratcliffe, 1988) However, recruitment of men into such studies is problematic and variation in and enforcement of collection protocols difficult. The poor predictability of sperm counts has led some to question the utility of the measurement. (Badenoch, Evans & McCloskey, 1989) A point of semantics is that the commonly used term sperm "count" is in fact not a count of sperm per se, but a measure of sperm concentration or density in a given volume of seminal fluid.

Animal toxicology experiments have yielded important information regarding hazard identification and mechanisms of toxicity of a number of reproductive toxins. Classical rodent breeding experiments (single and multigeneration) have, on occasion, provided useful information on the effects of various substances on male libido, ejaculation, fertilization, and embryonic development. However, the results of fertility measurements in animals are too often unsuitable for extrapolation to humans because of

their insensitivity to even dramatic reductions in sperm production and function. (Blazak, 1989)

There is poor concordance between fertility measurements in animals and humans because most laboratory and domesticated animal species have been highly selected for reproductive performance and males of these animal species produce far more spermatozoa than are needed for fertilization. (Meistrich, 1989) Several studies have been performed that illustrate the relative superfertility of male animals compared with the men.

In surgically altered male rats a 90 percent reduction in sperm count was necessary before an affect on breeding performance was detected, and fertility was even reported at 1 percent of normal sperm counts. (Aafjes, Vels & Schenck, 1980) Likewise, Blazak *et al.* noticed only a slight impairment on fertility in male rats having an 80 percent reduction in the number of morphologically normal, motile sperm produced after treatment with nitrobenzene. (Blazak *et al.*, 1985) Almost a 10-fold reduction in sperm count is necessary in mice before fertility is affected. (Meistrich, 1982) Williams *et al.* report their experiments with the New Zealand white (NZW) rabbit "…suggest that a reduction of >99.6% in the number of motile sperm would have to occur before any decrease in fertility could be detected in the NZW rabbit." (Williams *et al.*, 1990)

In contrast to many mammalian species, many men produce only about twice the number of sperm necessary before fertility begins to decline. (Amann, 1982) Moreover, human semen has relatively high proportions of morphologically abnormal and nonmotile sperm which further reduces potential human male fertility. (Blazak, 1989) If the subject were not already complicated enough, rodents and humans have different dose-response curves to known testicular toxins. (Meistrich, 1989) Altogether, interspecies differences in a number of reproductive parameters and the impracticality of obtaining some types of data in humans are significant

obstacles that make animal models of reproductive risk to human males unreliable. (Working, 1988) Thus, the fact that the animals most commonly used for reproductive toxicity testing produce 10- to 100-fold more sperm than is required for normal fertility, the smaller proportion of normal sperm produced by humans, and the different dose-response curves following toxicant exposure, leads one to conclude that although animal fertility studies are an important research and screening tool, they remain relatively insensitive for making interspecies comparisons and risk assessment predictions. Or as Hurtt put it, "Reproductive toxicological assessment has obviously not developed to the point where a single animal end point is indicative of reproductive risk in the human. The need to assess multiple end points of reproductive function in experimental animals is clearly evident. Development of end points that are less subjective and more sensitive and that are attentive to the physiologic differences between the species is critical for eventual risk assessment." (Hurtt, 1989)

It is important to recognize that variation in species' response to potential reproductive toxins may arise from: (1) differing pharmacokinetics; (2) differential dose-response patterns of similar target cells among species; (3) the toxicant affecting different target cells in different species; and (4) the species possessing different testicular responses to exposure. (Meistrich, 1989) Three principal elements that may affect the interspecies extrapolation of reproductive toxins include: (1) the choice for expressing administered dose (milligram of chemical per kilogram of body weight per day (mg/kg/d), or milligram of chemical per unit body surface area per day ($mg/m^2/d$); (2) the end point selected for evaluating response; and (3) the time delay after exposure at which responses are evaluated. (Meistrich, 1989)

The selection of the appropriate units for expressing administered dose is quite controversial. In the past, humans were considered to be

more vulnerable to the effects of a variety of chemicals because of the manner in which the dose was expressed - mg/kg/d. This approach fails to account for interspecies differences in pharmacokinetics and pharmacodynamics which tend to reduce the interspecies differences. Interspecies extrapolation of the systemic toxicity of cancer chemotherapeutic agent dosage was discovered to be better estimated by expressing dosage in terms of body surface area (*i.e.*, mg/m^2). Thus, while the ratio of the administered dose to the target tissue dose is likely to vary significantly between species it may not be as large as what one might estimate on the basis of expressing dosage in terms of mg/kg/d. (Meistrich, 1989)

The time delay following exposure is important because of the differences in spermatogenesis between rodents and humans. There exist two spermatogonial cell populations in the testes: stem cells and differentiating cells. (Meistrich, 1989) The relatively small number of stem cells periodically replicate to maintain their numbers, with a fraction differentiating to continue along the process of spermatogenesis. (Meistrich, 1989) In rodents the stem cells are referred to as type A spermatogonia, whereas in primates two stem cell populations have been identified: inactive type A_{dark} and the proliferating type A_{pale} spermatogonia. (Meistrich, 1989)

Once spermatogonia have begun to differentiate, the diploid primary spermatocytes proceed through four to 10 more mitotic divisions. After the last mitotic division they undergo meiosis to become haploid secondary spermatocytes with half of the complement of chromosomes as a normal somatic cell (*i.e.*, 23 chromosomes in humans). Secondary spermatocytes develop into spermatids. The spermatids undergo an amazing transformation to become spermatozoa following reorganization of the nucleus and cytoplasm. (Dixon, 1986) It is only after the spermatozoa leave the testes

and enter the epididymis that they complete maturation and acquire the ability to fertilize. (Meistrich, 1989)

The seminiferous tubules contain germ cells at different stages of development and Sertoli cells. (Dixon, 1986) Periodically, spermatogonia of certain areas of the tubules become committed to differentiation, and cohorts of same stage germ cells develop synchronously. (Dixon, 1986) If one were to view this process from any particular point in the tubules, the length of time it takes for one complete series of germ cell associations to pass by is referred to as the cycle of the seminiferous epithelium. The duration of this cycle is a function of, and is equivalent to, the stem cell replication rate. (Dixon, 1986) This cycle is approximately 8.6 days in the mouse, 12.9 days in the rat, and 16.0 days in man. (Dixon, 1986) However, it takes approximately 4.5 cycles from the beginning of stem cell differentiation to completion of sperm maturation. This maturation period is about 40 days in the mouse, 60 days in the rat, and 80 days in man. (Meistrich, 1989) Thus, if a reproductive toxin were to directly affect libido (e.g., (Vanhoorne *et al.*, 1994)), the effect may be seen immediately, but if it were to affect sperm, an effect may not be observable until roughly a three month latency period has passed, and depending upon the type and rate of damage repair may still be observable 3 or more months after exposure ceased. (Levine, 1988a, p. 225)

From a male reproductive toxicity testing standpoint, one would want to ensure that duration of dosing of male animals was for this period of time and preferably at least six cycles of the seminiferous epithelium (53 days in mice and 77 days in rats) to ensure that damage to different stages of spermatogenesis could be observed. (Amann & Berndtson, 1986) From this perspective, it can be seen that human males may require up to twice as long as male mice to recover from damage to spermatogonium. With regard to the present study, the typically brief intermittent exposures the exposed workers received poses some difficulty for interpretation.

D. Review of Reproductive Epidemiology

Unlike cancer or heart disease, reproduction is not a disease entity. Rather, fertility, as measured by the birth of a liveborn child, is a function of the social processes influencing conception as well as the biological processes affecting the reproductive competence of a couple. Most of the reproductive epidemiology studies that have been conducted have focused upon fertility and adverse pregnancy outcomes in females, and birth defects in offspring, rather than decrements in sexual function. Relatively few male reproductive epidemiological studies have been conducted in the workplace. On the toxicological side, this historical neglect regarding the susceptibility of the male reproductive system resulted in part from ignorance, with attention focused instead on the female system and her progeny (teratology). (Zenick, 1984)

This oversight was perhaps due to concern over avoiding birth defects tragedies like the thalidomide-induced phycomelia ("flipper arms") disaster in Western Europe resulting from use of a prescription morning sickness medication in 1961. On the epidemiological side, the reason lies in large part with the small number of tools available for studying adverse effects on fertility and the absence of any such major male reproductive incidents to raise awareness and to compel support for more male reproductive epidemiology research.

In 1977, Donald Whorton *et al.* published an occupational epidemiology study that ignited this moribund field of inquiry. (Whorton, Krauss, Marshall & Milby, 1977) California workers at a pesticide plant involved with manufacturing the nematocide 1,2-dibromo-3-chloropropane (DBCP) requested a fertility evaluation through their union. The request was allegedly prompted after wives of DBCP-exposed workers at a company-sponsored softball game discussed the near absence of children despite active efforts to have children. Whorton *et al.* determined that most of

the men were either azoospermic or oligospermic as a consequence of their exposure to DBCP. Whorton *et al.* and others published a series of follow-up studies of these and DBCP workers, rendering DBCP the distinction of being the archetypal male reproductive toxin.

Prior to the Whorton study, clinical studies were the sole male reproductive epidemiology information source. Such research demonstrated the adverse reproductive effects of certain pharmaceutical agents on males, namely a positive association between impotence and consumption of such medicines as: antihypertensive, antihypertensive with diuretic combination, antianxiety, antidepressant, antipsychotic, and anorexic drugs. (Quinn *et al.*, 1990) In fact, the principle source of information in the literature on the reproductive hazards of substances to humans is derived from chemical and physical agents administered for medicinal purposes. Other agents which have been identified as male reproductive agents in this manner include ionizing radiation, and antineoplastic agents such as chlorambucil, cyclophosphamide, procarbazine, and doxorubicin. (Meistrich, 1989)

"The clinical assessment of reproductive toxicity can be an extremely difficult task." (Mattison & Thomford, 1989) Reproductive impairment may be manifested in many ways, from loss of libido to sterility. For one endpoint, infertility, difficulty is encountered in the research field because of the absence of a uniform definition, the variety of scientific disciplines involved (because "infertility encompasses a heterogeneous group of problems," (Marchbanks, Peterson, Rubin, Wingo & Group, 1989)), the sociological and biological complexity of the processes involved in the successful birth of a live child, and the different etiologies that may interfere with these processes. The major problem in human fertility research is that there is no "gold standard" for evaluating outcomes, and as seen below the apparently simple choice of an infertility definition may dramatically affect research findings.

Marchbanks *et al.* contrasted the effect of five different commonly used infertility definitions on the demographic characteristics, prevalence of history of infertility, age at classification as infertile, and cumulative incidence of conception after diagnosis of infertility. (Marchbanks, Peterson, Rubin, Wingo, & Group, 1989) They concluded that women classified as infertile according to the definitions of "unprotected intercourse for 12 months," or "unprotected intercourse for 24 months" were "...more likely to be black, less educated, and classified as infertile at younger or older ages than women classified by alternative definitions. The prevalence of a history of infertility ranged from 6.1% (*physician diagnosis*) to 32.6% (*unprotected intercourse for 12 months*)." (Marchbanks, Peterson, Rubin, Wingo, & Group, 1989) Cumulative incidence of conception 120 months after diagnosis of infertility also differed among the various definitions. The authors concluded that the choice of infertility definition may dramatically affect research findings.

"Fecundity is the physical ability of a woman or couple to presently have children and refers to women or couples with any number of children (unless classified by parity)." (Mosher & Pratt, 1990, p. 8) However, "fecundity is difficult to measure since it refers to the theoretical ability of a woman to conceive and carry a fetus to term." (Last, 1988, p. 48)

The term "fertility" relates to "the actual production of live offspring. Stillbirths, fetal deaths, and abortions are not included in the measurement of fertility in a population." (Last, 1988, p. 48) Thus, fertility is a subset of fecundity. That is, not all woman or couples who are fecund have actually demonstrated this through the birth of a liveborn child. "Infertility is a medical concept; it is used by physicians to identify couples who may need to be evaluated to see whether they need medical services to help them have a baby." (Mosher & Pratt, 1990, p. 8) Traditionally, it is defined by an arbitrary dichotomy, namely, those

continuously married couples who have not used any contraception (and have not had a sterilizing operation), and have not become pregnant in the 12 months immediately preceding the interview. (Mosher & Pratt, 1982, p. 3)

The distinction between infertility and fecundity is useful because the factors responsible for conception may be unrelated to those responsible for carrying a pregnancy to term. (Belsey, 1984, p. 256) Furthermore, they may require different types of treatment or prevention methods. (Belsey, 1984, p. 256) Unfortunately, a crude classification scheme like infertility can cause confusion between clinicians and epidemiologists, and miss subtle effects such as subfecundity. For instance, women who halted the use or oral contraceptives have been shown to have short-term increases in conception waits (expressed by the decreased probability of pregnancy in the first few cycles). This effect would have been missed had the authors used only the clinical definition of infertility in their analysis since the overall proportion failing to achieve pregnancy at one year was no different from women using not using oral contraceptive. (Harlap & Baras, 1984)

Working definitions of infertility that are both related to couples and relevant to use in epidemiologic investigations were developed by the World Health Organization's Scientific Group on the Epidemiology of Infertility in 1975 follow. (Belsey, 1984, p. 256)

- "*Primary infertility*: The woman has never conceived despite cohabitation and exposure to pregnancy (this term was never defined) for a period of two years.
- *Secondary infertility*: The woman has previously conceived, but is subsequently unable to conceive, despite cohabitation and exposure to pregnancy for a period of two years; if the woman has breast-fed a previous infant then exposure to pregnancy should be calculated from the end of the period of lacatational amenorrhoea.
- *Pregnancy wastage*: The woman is able to conceive, but unable to produce a live birth. Loss of pregnancy during the first 28 weeks is referred to as early and intermediate fetal death, or abortion, and may be spontaneous or induced. Beyond 28 weeks of gestation and up to term, such losses are referred to as late fetal deaths, or stillbirths.
- "*Unproven infertility*" or "*unproven fertility*" refers to problems sometimes perceived by individuals or couples as

> infertility or included as infertility in demographic surveys, whereas in fact, the woman is virtually not at risk of conception. The problem may be biological, such as among lactating women who are anovulatory, or couples practising contraception; or circumstantial, when there is the absence of cohabitation or coitus (*e.g.*, women whose consort is temporarily away)."

Fecundity appears closely related to the term "fecundability" coined by the demographer Corrado Gini in 1924. (Leridon, 1977, p. 22) The Frenchman Leridon refined the term and related concepts as:

- "total (or physiological) fecundability, when including *all* conceptions;
- recognizable fecundability, when excluding pregnancies ending within 2 weeks after conception (first missed menses)…;
- apparent fecundability, when including all pregnancies recognized and declared (on the occasion of an interview) by the woman;
- effective fecundability, when including only pregnancies ending in a live birth…." (Leridon, 1977, pps. 22-23)

A central concept in reproductive epidemiology is the recognition of the couple as the unit of analysis. The rationale for studying the fertility of couples is that it "…may be the simplest way to detect adverse effects of exposures on the biologic processes that affect conception and early human development," and [b]ecause couple fertility integrates the effects of several biologic processes in the male and female partners as well as the developing conceptus…that cannot be monitored independently in normal human reproduction." (Baird & Wilcox, 1986a, p. 362) Integration of measurement means that it may be easier to detect an adverse effect on reproduction when the exposure interferes with more than one biological process. In addition, there may be no practical means of monitoring individual processes in humans, even if it were technically feasible, given the barriers to researching human intimacy. Thus, by monitoring one outcome, one is indirectly monitoring many biological processes involved with successful fertility. (Baird & Wilcox, 1986a)

The major limitation of monitoring couple fertility is its potential insensitivity to detecting adverse effects on individual processes. (Baird

& Wilcox, 1986a) For instance, if an agent were to affect only a single process in the chain of events that leads to successful pregnancy and delivery, and it were not a particularly potent agent, then there may be no measurable effect on the couples fertility. (Baird & Wilcox, 1986a) A good example of this phenomenon can be seen with oral contraceptives. Women who halted the use of oral contraceptives have been shown to have short-term increases in conception waits (expressed by the decreased probability of pregnancy in the first few cycles). This subtle effect would have been missed had the authors used only the clinical definition of infertility in their analysis since the overall proportion failing to achieve pregnancy at one year was no different from women using not using oral contraceptive. (Harlap & Baras, 1984)

As discussed above, animal reproductive toxicologists have developed a number of methods to measure male fertility, and most of these methods are invasive such as the posthumous weighing of male gonadal and accessory sex gland tissue, or upon the direct assessment of semen quality parameters (e.g., sperm count (concentration), morphology, motility, serum hormone levels, and sperm viability. Relatively, noninvasive techniques are used by animal reproductive toxicologists, however, such as counting the number and weights of live born and stillborn pups, and measuring serum hormone levels. Of these noninvasive endpoints, only pregnancy outcome, children's birthdates, serum hormone and testicular size measurements were made of the workers examined as part of this study.

In view of the logistical and compliance problems associated with semen collection, and ethical constraints for the collection of data on the other parameters, several indirect, unintrusive and noninvasive assessment methods to evaluate couples' fertility were developed in the late 1970s and early 1980s. The literature review in the next section will focus exclusively on noninvasive male reproductive epidemiological methods.

1. Noninvasive Measures of Adverse Effects

a. Live Birth Rate

Polednak investigated the potential oocyte toxicity of ionizing radiation by using the number of liveborn children as a measure of fertility of 199 women employed as radium (luminous) watch-dial painters. (Polednak, 1980) These women received significant doses of both external radiation (gamma rays) and internal radiation (alpha particles). This group consisted of women employed in the trade between 1916 and 1929, principally at the Luminous Processes Inc. factory in Ottawa, Illinois, whose radium body burden had been measured at least once between 1951 and 1975. (Polednak, 1980) Exclusion criteria included: women whose year of first marriage preceded their year of first employment in the industry (30); women whose year of marriage was unknown (26); women who were never married (13); and women who were married for less than three years (6).

Data on marriages, number of live births (except the few reported out-of-wedlock live births), and number of "fetal deaths" (which included the total number of "miscarriages" and "stillbirths") were obtained from questionnaires on medical and family history. A live birth rate index was defined as "the number of reported live births divided by the number of years of marriage (until surgical menopause or age 45) times 100." (Polednak, 1980) Data on the fertility of cohorts of women in the United States were not used for the analysis because the author felt that these working women were likely dissimilar from other women of the same birth cohort.

As there was no internal or external comparison groups, prior to analysis the group was stratified into four groups by estimated ovarian-dose (<5 , 5-19 , ≥20, and ≥100 rem). Multiple linear regression was used to analyze the live birth rate data alone, or as it varied with other variables.

The analysis revealed there were no significant differences in the percentages of childless women (*i.e.*, live birth rates of zero) when the women were categorized by ovarian dose group. However, among women with more than one live birth, the mean log live birth rate decreased with increasing ovarian-dose level, attaining statistical significance in the two highest dose groups. Using the lowest dose group as the baseline, the pairwise comparison of the mean log live birth rates achieved statistical comparison with the ≥20, and ≥100 rem dose groups. However, a separate analysis for the 32 women with 20-99 rem (a subset of the ≥20 rem group) was not significant. This implies that the results for the 25 women with the highest ovarian doses (≥100 rem) may have been responsible for the statistically significant findings of the ≥20 rem group.

The author used multiple linear regression to evaluate the contribution of radium intake dose and duration of employment to predictions of live-birth rates. Only the log of the dose, and not the log of employment of duration was a significant predictor of the live-birth rate. In addition, an assessment of the live-birth rates stratified by duration of employment (three levels) and radium intake (two levels) failed to manifest an effect of duration of employment.

The author postulated that the reduction in live-birth rate observed among women in the higher ovarian-dose groups (≥20 rem) may have been due to: (1) oocyte destruction, or fetal loss due to dominant lethal mutations; (2) differences in contraceptive practices among the dose groups; or (3) differences in other confounding variables. (Polednak, 1980).

b. Standardized Birth Ratio and Standardized Fertility Ratio

The SBR was originally proposed by Otto Wong *et al.* as a technique for retroactively investigating the reproductive performance of workers from four plants who were exposed to ethylene dibromide (EDB) during the manufacturing process. (Wong, Utidjian & Karten, 1979) In many respects it is analogous to the Standardized Mortality Ratio (SMR) methodology for investigating causes of death, as they are both variations on the calculation of an indirectly adjusted standardized rate ratio.

Wong *et al.* recognized that unlike females, data on fertility rates and reproductive performance of males does not exist in the United States. Consequently, the authors saw that the national data pertaining to female fertility presented an opportunity to perform an indirect assessment of adverse effects of EDB on the males. The authors made the explicit assumption that the male workers were the exclusive sexual partners of their designated spouses. Single men were excluded from the study because the authors felt that unreliable data would be obtained from them or their sexual partners.

The calculation of adverse reproductive impacts was relatively straightforward. The authors computed a standardized birth ratio (SBR) for the exposed men in each plant. The SBR was equal to the observed number of live births for each couple after exposure (actually 9 months after first exposure to accommodate the normal human gestation period) divided by the expected number of births. The expected number of live births to each spouse was derived from national fertility rates for successive birth cohorts of U.S. women. These rates have been published since 1917, and are currently published by the National Center for Health Statistics (NCHS). The pre-exposure reproductive history is ignored by the SBR method.

The women-years at risk of pregnancy of the spouses nine months after initial exposure to EDB, or marriage (whichever was later), was summed through September 1977. Periods between divorce and remarriage were ignored from computations of periods at risk of conception. The confounding effects of maternal age, birth cohort, parity, and race, were controlled for by using the age-, parity-, and race-specific birth probabilities of successive birth cohorts from the NCHS, and applied to the women-year reproductive experience of each spouse.

The results from the computation of this ratio was used as an index of fertility. An SBR greater than one was considered evidence of greater than normal fertility; an SBR less than one was considered indicative of subnormal fertility. Statistical significance was evaluated assuming the SBR followed a Poisson distribution. An alpha value of 0.05 was used as the level of significance.

A total of 297 couples from the four plants were included in the analysis, with the spouses contributing 1,092 person-years of post-exposure observation time. Analysis of the SBR for each of the four plants revealed a significant effect on fertility in only one plant. White male workers exposed to EDB in Plant D experienced a 50 percent reduction in the number of births. However, when the reproductive experience of all four cohorts was combined, no significant changes in the SBR were observed despite pooled SBRs of 0.82 and 0.56 for whites and nonwhites, respectively. No dose-response trend in fertility was observed. The authors computed that with an alpha of 0.05, and a 20 percent reduction in fertility, the power of their study was 0.90. Consequently, they dismiss the idea that the lack of statistical significance in their results was due to small numbers.

Wong *et al.* discussed several potential biases and limitations in the data and their methodology. First, they used births among only married women while the NCHS data are based on births among both married and unmarried women. The authors acknowledge that their exclusionary protocol

may have slightly underestimated the expected number of births. Thus, the SBRs may be slightly overestimated. Adopted children and stepchildren were included in establishing parity, but not in births. The operating assumption was that family size is controlled principally by economic rather that biological influences.

Various ethnic groups have fertility ratios that are significantly different from the national average, but the NCHS data includes only the dichotomous variable "white/nonwhite". Wives of workers were assumed to be of the same ethnic group/race as their exposed worker husband. The absence of data that may also influence fertility, such as religious orientation, socioeconomic status and other cultural values. For instance, contraceptive practices, employment status of wives, educational attainment by the couple, income level of the family, family size, and regional differences in fertility were ignored because of the absence of corresponding data from the national rates. Again, the authors felt that the inability to address these factors in their analysis resulted in a slight underestimation of the expected number of births, and a slight overestimation of the SBR. The authors recommend the use of an in-plant control group to address these influential variables that cannot otherwise be directly assessed.

Levine *et al.* modified and extended the protocol described by Wong *et al.*, renaming the calculated ratio the Standardized Fertility Ratio (SFR) (Levine *et al.*, 1980) In addition, they described in far greater detail the mechanics of computing the SFR. (Levine *et al.*, 1980) The most important contributions Levine *et al.* made towards advancing the SBR/SFR methodology was the discrimination between exposed and nonexposed periods for the workers, and the use of each individual as essentially his own control by separately evaluating each individual's pre- and post-exposure fertility. It is important to note that Levine *et al.* characterized their

methodology as a "…screening device to select situations which warrant further in-depth study." (Levine *et al.*, 1980)

While Levine *et al.* assumed also that the observed number of births follows a Poisson distribution they included a useful twist. For births occurring prior to exposure the number of such births observed, O_1, has a mean value of ϕE_1, where E_1 is the expected number of births based on U.S. national fertility rates. The constant phi (ϕ) is included to account for differences in fertility between the plant population and the standard rates derived from the U.S. general population. The value of phi may take into account factors such as socioeconomic and marital status, religious preference, or ethnicity that may differ between the two populations. Thus, in theory the SFR is able to adjust for more potentially confounding factors than does the SBR. (Levine, 1981)

The observed number of births occurring after exposure to an agent, O_2, is considered to be independent of O_1 and to also follow a Poisson distribution with a mean value of $\theta\phi E_2$. The constant theta (θ) is now the parameter of interest as it represents the change in fertility related to the exposure. Originally, phi was to be estimated by O_1/E_1, and theta (if there were two groups, one exposed and another unexposed), by the ratio the SFRs for exposed and unexposed workers', that is by $[(O_{2e}/E_{2e}) \div (O_{1e}/E_{1e})]/[(O_{2u}/E_{2u}) \div (O_{1u}/E_{1u})]$. (Levine *et al.*, 1980, p. 784)

Subsequently, Levine *et al.* refined the definitions of ϕ and θ. (Levine, Blunden, DalCorso, Starr, & Ross, 1983) For the situation described above with two groups, one exposed and another unexposed, phi was defined as O_u/E_u, where the subscript *u* refers to the pooled pre-employment fertility experience of the exposed and unexposed workers. Theta was defined as the ratio of the post-employment fertility (SFR) of the exposed group to the collective pre-employment fertility (SFR) of the exposed and unexposed groups, that is, $[(O_e/E_e) \div (O_u+1/E_u)]$. One was added to O_u in

the denominator to give it finite variance. Levine *et al.* state that the bias this introduces is small for even small samples sizes.

In a pilot investigation, Levine *et al.* implemented this methodology with data collected from workers at two small chemical plants. (Levine *et al.*, 1980) Twenty-seven ever been married men qualified for participation at the first plant, however, only 14 men qualified from the second plant. The workers in Plant A manifested significantly greater fertility than the workers in Plant B, or the general U.S. population. In addition, post-employment SFRs exceeded pre-employment SFRs, although the difference was not statistically significant. The power to detect a 50 percent reduction in fertility (θ=0.5) with alpha equal to 0.10 was a very low, 0.15 and 0.22 for the two plants, respectively. As assumed, θ did not vary significantly by age, parity, birth cohort, or race.

One peculiar aspect of this methodology should be noted, that is, the increasing proportion of women-years contributed by ever-married women from the pre- to post-employment periods. Like Wong *et al.* only ever-married men (or women) are included in the study, thus as the birthing experience is traced backward in time, "…the distribution of never-married and ever-married person-years will approach more closely that of the general population." (Levine *et al.*, 1980) Since ever-married women have greater fertility than never-married women and the birth probabilities are based on the experience of women regardless of their marital status, "SFRs for intervals closer to the date of interview will be exaggerated somewhat as the result of including a larger proportion of ever-married person-years." (Levine *et al.*, 1980) The authors ascribed the nonsignificant increases in fertility from pre- to post-employment to this phenomenon.

Levine *et al.* identified what they believed were two flaws in the SBR methodology proposed by Wong *et al.* First, Levine *et al.* noted that the method of Wong *et al.* sums birth probabilities for individual years to

yield the expected number of births. This method assumes that "consecutive birth probabilities within parity levels are unconditional probabilities, a supposition which can lead incorrectly to an expected number of births exceeding one for a given parity level." (Levine *et al.*, 1980) Second, they thought the method of Wong *et al.* magnified the differences between the cohort and the general population by excluding unmarried person-years from the analysis.

Levine *et al.* noted that if cohorts under investigation had fewer than 300 births, and only 30 percent of those births occurred during the father's exposure period at risk, then it is unlikely that reductions in fertility smaller than 20 percent (theta) would have sufficiently high power to be detected. (Levine *et al.*, 1980) However, "more important to power than the total number of births is p, the proportion of births expected to occur during the period at risk from exposure. The larger the p, the greater the power that can be achieved from a given number of births." (Levine, 1981)

Levine *et al.* evaluated their methodology in an investigation of workers exposed to the known (and potent) male reproductive toxin DBCP. (Levine, Symons, Balogh, Milby & Whorton, 1981) Using a cross-sectional study design, all 39 members (36 male and 3 female) of a group of workers who were currently employed in an agricultural chemical division where the nematocide DBCP was made were initially included the study. The reproductive performance of 33 males remained for study after excluding three males who were never married, and the three females who constituted a group too small to be able to detect a reduction in fertility.

The results of their analysis indicated that the post-exposure fertility of the 33 men ranged from 0.2-0.40 of their baseline fertility. (Levine *et al.*, 1981) This means that they suffered a 60 to 80 percent reduction in fertility during their period of exposure. The authors

calculated that the power to detect this range of reduction in fertility varied from 0.63 to 0.94. (Levine *et al.*, 1981) No reduction in fertility was seen among the quality control laboratory workers with the lowest levels of DBCP exposure. In fact, their SFRs were highest during their supposed period at risk of exposure. In contrast, the other agricultural chemical workers experienced significant reductions in fertility during their period at risk. Finally, their assumption that phi is a constant, independent of female age, birth cohort, parity or race did not appear to be violated.

As mentioned above, the original methodology described by Levine *et al.* in 1981 was later modified in 1983. This was done to eliminate positive bias (*i.e.*, an overestimation bias) in the calculation of the relative fertility parameters phi and theta that results from a negative bias introduced in the calculation of expected births. (Starr & Levine, 1983) The authors, Starr and Levine, reported that the method developed by Wong *et al.* for calculating the expected number of births eliminates this bias, but its use with the pre- and post-exposure groups introduces two penalties. First, it leads to an estimate of theta that does not have a finite variance. Secondly, the approach of Wong *et al.* discards from the analysis the reproductive experience of the study cohort prior to marriage. (Levine *et al.*, 1980)

The solution to the first problem was to derive a modified estimate of θ by arbitrarily adding one birth to O_u. (Starr & Levine, 1983) This eliminated the problem while resulting in "...minimal negative bias in the estimate of θ for moderate ϕE_u." (Starr & Levine, 1983) The solution to the second problem was to agree with the position of Wong *et al.* and to restrict the analysis to the reproductive experience of only married men. However, they decided to revise their methodology for doing so to remove negatively biased estimates of phi.

The approach was "…to disaggregate married experience by parity and restrict attention to the experience at parity one or greater (married/P1+). An ancillary advantage is that spouses are then all of proven fertility. This approach is successful because the bias introduced into theta by the dependence of marital status on age in the reference population is confined almost exclusively to parity zero experience…Thus, restricting the analysis to married experience at parity one or greater avoids the bias inherent in parity zero birth rates that are not specific to marital status." (Starr & Levine, 1983) The authors proceeded to illustrate the effects of parity and proportion of surgically sterilized couples on the differential estimates in birth rates. The authors concluded that controlling for sterilization in the analysis confounds the results and should not be performed.

In another fertility investigation of men involved in the production of DBCP, Levine *et al.* included 182 men who had ever been exposed to DBCP at the plant. (Levine, Blunden, DalCorso, Starr & Ross, 1983) As part of the study, 87 of the men (47 percent) completed a self-administered medical and marital questionnaire. Of the 87 men, 20 were excluded from the analysis because of missing data regarding their marital or reproductive histories, and seven were eliminated from the analysis because they never married. The medical, personal, and work history data for the remaining 60 men were analyzed according to the modified method of Starr and Levine. (Starr & Levine, 1983)

The results of this study indicated a significant reduction ($p < 0.05$) in fertility among these men following exposure to DBCP at all parities combined, and at parity 1+, $\theta=0.51$. In addition, the post-exposure fertility experience was divided into two periods so that there was an equal number of expected births in each group. The analysis showed large differences between the short-term (≤1.8 years since the cessation of

exposure) and long-term (≥1.8 years) post-exposure SFRs. The SFR values for parity 1+ were 0.86 and 1.47 for these two time periods, respectively, indicating some degree of recovery in male fertility with the increasing passage of time.

An analysis of the worker reproductive questionnaire data indicated that after DBCP production began in 1955, marked deficits in fertility would have been apparent in 1959 or 1960 had this methodology existed and been applied. This is as much as 18 years before the first preliminary human report of an adverse effect on male fertility by DBCP. (Whorton *et al.*, 1977)

In addition, the authors evaluated exposure-related fertility during three intervals that yielded at least 5 births at parity 1+. They discovered that the earliest period (1956-1962) had the lowest, albeit nonsignificant, reduction in fertility. When the exposure-duration relationship to fertility was assessed, statistically significant dose-response trends were manifest for all parities and parity 1+, but not for parity zero.

Finally, the mean ages of the comparison groups were adjusted to determine if fertility was reduced regardless of the length of exposure, and whether the reduction in fertility with longer duration exposure was due to increasing age or the effect of the chemical. Increasing age, with or without adjustment, did not effect fertility in the unexposed years. In contrast, with or without adjustment, duration of exposure in excess of 3.5 years resulted in a significant reduction in fertility ($p < 0.05$ and $p < 0.01$, respectively). Fertility did decrease with lengthening duration of no exposure, but the magnitude of the change is dwarfed by that induced by the chemical exposure. Fertility decreased with increasing duration of exposure, with or without mean-age adjusted fertility.

In 1985, Wong *et al.* published another methodological paper which built upon their previous work, as well as the papers of Levine *et al.* (Wong, Morgan & Whorton, 1985) Perhaps the most important contribution of this work, which is directly relevant to this dissertation project, is its articulation of analytical issues for which there are no scientifically ideal solutions, and its discussion of modifications of the methodology in instances where an internal comparison group is available.

The Wong *et al.* paper began with a discussion of nomenclature, specifically why the term they adopted, the standardized birth ratio (SBR), was more appropriate than the term standardized fertility ratio (SFR) coined by Levine *et al.* Basically, the authors argued that the definition of fertility is more encompassing than what is actually being measured by either methodology. Fertility is related to a variety of successful reproductive events and outcomes only one of which, live births, actually is being evaluated.

Wong *et al.* recommended avoiding comparisons of the fertility of exposed workers and an internal comparison group with the indirect standardization approach engendered in the SBR approach. That is, they felt it inappropriate to compute an SBR for the exposed group *versus* the U.S., and an SBR for the unexposed group *versus* the U.S., and then compare the ratio of the two SBRs. This was a direct attack on the foundation of the SFR methodology, and its calculation of theta. Wong *et al.* cited several literature references (Kilpatrick, 1962; Kilpatrick, 1963; Kitagawa, 1955; Kitagawa, 1964; Liddell, 1960; Silcock, 1979; Wong, 1977; Wong & Decoufle, 1982) that detail the problems engendered with the use of an internal comparison group (as called for in the SFR methodology), arguing that problems may arise if the age-, race-, parity-, year-specific distributions are very different between the exposed and internal comparison groups. (Wong *et al.*, 1985, p. 303)

As an alternative to indirect standardization to national rates, Wong *et al.* advocated the use of an internal comparison group as providing a group more comparable to the exposed group. In cases where a large internal comparison group exists (and presumably the birth rates are stable), the authors suggested using the comparison group as the standard in the indirect standardization process. They referred to this statistically as a "one-sample problem." (Wong *et al.*, 1985)

On the other hand, if the internal comparison group were small, their birth rates can no longer be assumed stable. Instead, the expected number of births would be considered a random variable rather than a constant, a condition the authors referred to as a "two-sample problem." (Wong *et al.*, 1985) In this case, the authors suggested a direct comparison between the two groups using a traditional statistical technique such as the Mantel-Haenszel chi-square. Nonetheless, one would need to ensure the comparability of the two groups to avoid introducing bias (e.g., age bias from evaluating older less fertile populations against younger more fertile populations). One could evaluate the corresponding fertility experience of both the exposed and internal comparison groups prior to, during, and after exposure. Assuming the populations were comparable, Wong *et al.* suggested using a test based on the Poisson distribution which is designed to detect deviations in the SBR ratio from unity at different alpha values. (Bailar & Ederer, 1964)

Wong *et al.* also pointed out that the presence of an internal comparison group allows for greater control in the analysis. For instance, a variety of potential confounders (such as volitional or disease-induced sterility, contraceptive practices, religion, educational level, socioeconomic status, number of adopted children and stepchildren) could be controlled for with this technique which could not with ordinary SBR because no corresponding national data are available. If this information

were collected from members of both the exposed and internal comparison cohort, one could adjust for each confounder in the analysis. Otherwise, one is forced to include the entire period of married exposure time in the analysis even though it may be known that the husband had a vasectomy or mumps orchitis, and thus his spouse was not at risk of pregnancy for some period.

Finally, Wong *et al.* recommended relaxing the alpha value from the traditional 0.05 to 0.15 or 0.20, in order to improve the sensitivity of the technique. When the alternative alpha values, but not the traditional alpha value, were used deficits in male fertility among EDB and DBCP worker cohorts were statistically significant.

Starr *et al.* commented upon the methodological paper by Wong *et al.* discussed above. (Starr, Levine & Boyle, 1986) Starr *et al.* recounted six weaknesses of the SBR methodology. A point-counterpoint summary follows. First, Starr *et al.* argued the accumulation of person-year experience of only the spouses of married men results in an upward bias in the SFR because the national birth rates do not adjust for marital status. The national birth rates represent a weighted average of the births of married and unmarried women. Starr *et al.* argued that since married women have higher birth rates than unmarried women, the use of the national rates for indirect standardization will tend to underestimate their fertility, especially for age and parity classes at which relatively smaller proportions of women are married. Consequently, use of the SFR would inflate the worker population's SFR relative to the national rates, and make the worker population appear more fertile than the norm.

In rebuttal to this commentary, Wong argued that marital status is irrelevant since the SBR is both age- and parity-adjusted. (Wong, 1986) What he found important was the extent to which the proportion of married women significantly differs between the study group and national rates.

Wong countered that while Starr *et al.* identify a problem they fail to offer any solutions to it, and have made the same assumption in at least one of their own fertility studies.

The second criticism of the SBR methodology by Starr *et al.* was the fact that it ignored information on surgical sterilization of workers and their spouses that may be collected in the course of the study. Starr *et al.* argued that ignoring such data would lead to underestimates of the birth rates for couples in which neither mate has been sterilized as sterilization rates rise with increasing age and parity. Wong responded that since national rates do not incorporate this information and adjust for sterilization, there is no basis for doing so for the study population. Again, he noted that the issue is irrelevant because the SBRs are age- and parity-adjusted.

The third criticism of the SBR by Starr *et al.* was of the argument by Wong *et al.* that socioeconomic, rather than biological, factors are the prime determinants of parity. They proceeded to question the method developed by Wong *et al.* of adding the number of adopted or stepchildren to the parity of a male workers wife. They argued that this practice, instead of merely introducing a positive bias in the SFR, as alleged by Wong *et al.*, can actually result in a positive or negative bias. Wong responded that no ideal solution exists, and reiterated an earlier suggestion to analyze the data both ways. That is analyze the data with and without adopted and stepchildren being counted toward determining parity of the wife.

In their fourth criticism of the SBR, Starr *et al.* pointed out that the assumption that fertility is restored to a women immediately after delivering a child is clearly false. Wong simply responded that little is known about the duration and degree of infertility related to childbirth. Furthermore, he adds that the national statistics make the same assumption.

In their fifth criticism of the SBR, Starr *et al.* pointed out that some biases introduced into the SBR estimates are eliminated by calculating the ratio (theta) of the pre-exposure to the post-exposure reproductive experience (SFRs) of individual workers, an idea they developed. Wong retorted that comparing the exposed and unexposed reproductive experiences of the same workers "…is flawed because of built-in bias: the pre-employment experience is heavily characterized by younger maternal age, lower parity, and earlier calendar time…and no statistical adjustment procedures will be able to remove the bias completely." (Wong, 1986) He concluded that the post-exposure fertility will tend to appear lower because of the age bias in the procedure.

Finally, Starr *et al.* argued that the concern raised by Wong *et al.* regarding errors introduced by direct comparisons of groups after indirect standardized is overblown. They cited a paper by Breslow *et al.* which they interpreted to indicate that rarely does the ratio of two indirectly standardized rates behave aberrantly. (Breslow, Lubin, Marek & Langholz, 1983) Starr *et al.* claimed that the direct standardization method preferred by Wong *et al.* is "…subject to much greater sampling variability and hence is a much less sensitive method for detecting exposure effects in most applications." (Starr *et al.*, 1986)

Wong responded to this position by pointing out that theta, as defined by Levine *et al.*, had infinite variance - an undesirable statistical property. Wong viewed the solution proposed by Levine *et al.*, to add one extra birth to the actual number of births, dimly since it biased theta by inflating the number of births in the unexposed period. As a result, one would be more likely to conclude that there was lower reproductive performance following exposure. Furthermore, Wong criticized Starr *et al.* for seriously misinterpreting the conclusions of an example provided in a paper by Silcock.

Recently, two additional studies have been published that have applied the SFR methodology to occupational cohorts of male employees. The first study investigated the possible effects of sewer treatment plant chemical exposures on male fertility. (Lemasters, Zenick, Hertzberg, Hansen & Clark, 1991) There were 317 married male sewage treatment plant employees eligible for participation, 231 of whom (72.9 percent) agreed to participate. After exclusions and dropouts, the final groups consisted of 219 workers, 133 exposed and 86 unexposed.

The authors manually applied the SFR methodology to determine whether there was a deficit in the number of live births. In addition, they prepared an analysis of reproductive history data collected from a questionnaire by telephone interview with the workers' spouses. This information was used to evaluate whether there were periods in which there was difficulty in becoming pregnant (*i.e.*, increased time-to-pregnancy). A separate study, to be reported later, involved the analysis of semen samples. No adverse effects of employment on fertility were revealed by either fertility monitoring approach.

Welch *et al.* prepared the most recent paper to apply the SFR approach to investigate the effects of chemical agents on male fertility. (Welch, Plotkin & Schrader, 1991) This paper is the fourth in a series of articles assessing different health endpoints in painters at a shipbuilding facility exposed to glycol ethers. In this study, two techniques for assessing male reproductive function (analysis of workers' semen samples and SFR analysis using the CIIT fertility analysis package) were implemented and the results compared.

A total of 900 painters, 600 of them men, were employed at the shipyard where the study was conducted. Of these men, 94 lived within a 1-hour commute (20 mile radius) of the clinical site used to perform the semen analyses. Semen samples were provided by 73 of the 94 exposed workers, and 40 of 55 unexposed control workers at the shipyard. The semen

analysis was quite extensive, and included "…sperm viability, density, motility, and morphology and pH and volume…". (Welch *et al.*, 1991) In a somewhat unusual character protocol, medical and reproductive history data were collected from questionnaires administered to the male workers. After excluding unmarried men, there were 74 exposed men and 51 unexposed men who completed reproductive questionnaires for the SFR analysis. However, the SFR analysis included only the 50 exposed and 50 unexposed workers with complete questionnaire data.

Most of the semen sample comparisons achieved marginally statistical significance. The mean sperm count and count per ejaculate were lower in the exposed than the unexposed workers (p=0.10 and 0.11, respectively). In addition, the proportion of men classified as oligospermic (*i.e.*, sperm count <20 million/cm^3) was higher in the exposed than in the unexposed workers (p=0.12). Finally, four of the exposed workers, but none of the unexposed workers was azoospermic (or essentially azoospermic).

The SFR analysis indicated that the comparison of exposed workers pre- and postemployment SFRs was not statistically different (1.66 and 1.58, respectively). In contrast, the postemployment SFR for the unexposed workers was smaller than their preemployment SFR (1.36 and 1.70, respectively). Based on some power calculations, the authors estimated that the SFR component of the study had approximately 60 percent power to detect a 20 percent reduction in fertility, and nearly 100 percent power to detect a 40 percent reduction. The authors reviewed the only two previous studies to have performed both semen and SFR analyses. (Hamill *et al.*, 1982; Levine *et al.*, 1980) In both cases, the results of both the semen and SFR analyses were concordant. One found no effect of DNT or TDA on male fertility (Hamill *et al.*, 1982), the other found an effect of DBCP exposure on male fertility. (Levine *et al.*, 1980) The results of their study led Welch *et al.* to conclude that the SFR questionnaire approach to

fertility assessment "…is less sensitive than semen analysis as a screening tool for male reproductive function." (Welch *et al.*, 1991)

c. Survival and Linear Regression Models

In a 1983 paper, Starr *et al.* evaluated the use and performance of logistic regression *versus* indirect standardization in the SFR computations of workers at three chemical plants. (Starr, Dalcorso & Levine, 1986) One reason for the authors' efforts to explore alternative analytical methods was their recognition that "…indirect standardization using the national birth rates might yield erroneous results because that standard does not account completely for the influence of a woman's past reproductive history on subsequent events. Specifically, each person-year contributes the same expected number of births whether or not a woman gave birth to a child in the previous year, two years prior, or last gave birth ten years prior." (Starr *et al.*, 1986)

The indirect standardization analysis was performed as described earlier. (Starr & Levine, 1983) The logistic regression model was constructed by assuming that a person-year of exposure occurred if a worker was employed in an area of the plant where there was potential exposure to the agent of interest for at least six months. The dependent variable in the model was a birth or the failure of a birth to occur during each person-year. Since it is known that maternal age, birth cohort, and parity affect fertility, these variables were included in each model. In addition, in each model, exposure status was included as a dichotomous variable, and three dichotomous lag variables were included. The logistic regression parameter estimates were obtained by running the BMDP software PLR in backward elimination.

The SFRs obtained by indirect standardization and the odds ratios obtained by logistic regression compared very well. The two methods were

concordant in accepting or rejecting the null hypothesis of no effect on fertility of workers at two of the three chemical plants. However, at the third plant a significant interaction term between exposure and one of the lag variables was found. Notwithstanding this result, the authors concluded this was a spurious finding.

Starr *et al.* expressed some concern at the disagreement of the two approaches at the third chemical plant, and at the sensitivity of the logistic regression approaches to the inclusion of extraneous variables. They reported that inclusion of some additional variables not originally included in the logistic regression model for the chemical plant workers potentially exposed to DBCP resulted in a reduction in the main effects of exposure below a level of statistical significance ($p=0.05$).

In 1985, Boyle and Starr presented two survival model approaches to fertility evaluation. (Boyle & Starr, 1985) "In the first model, U.S. birth rates specific to maternal age, race, parity, and birth cohort are used as underlying hazard rates. Covariate effects are estimated by maximizing the full likelihood. In the second model, covariate effects are estimated via Cox regression with stratified underlying hazard rates regarded as unknown nuisance parameters." (Boyle & Starr, 1985) Thus, the first model can be used to derive the SFR described by Starr and Levine (Starr & Levine, 1983) or the SBR of Wong *et al.* (Wong *et al.*, 1979) assuming the random time variable u is set equal to unity. (Boyle & Starr, 1985)

One important distinction between this proportional hazards modeling approach and the SFR or SBR is that only the proportional hazards approach terminates a woman's risk of childbirth in the year in which she delivers. The other two models assume a women is immediately fertile after delivery.

In the first survival model, each women's fertility experience is accumulated on an annual basis with the assumption that the birth outcome in any given year is independent of prior fertility experience. In

addition, each women's birth experience is considered independent of the birth experience of every other woman in the cohort. Therefore, the likelihood of a birth is simply the continuous product of the individual birthing probabilities. The second model uses a partial likelihood approach provided by Cox regression to obtain alternative estimates of covariate effects while stratified underlying hazard rates are regarded "...as unknown nuisance parameters." (Boyle & Starr, 1985) In recognition that parity and maternal age are major factors affecting fertility, Boyle and Starr recommend stratifying the fertility hazard rate on these two variables. Women of the same age and parity would be compared to each other, while birth cohort and race are treated as covariates.

Fertility data from a reproductive health study at a chemical plant were used to demonstrate the application of the two models. A total of 226 male employees were in the occupational cohort. The authors followed the same protocol used by the National Center for Health Statistics in devising the U.S. fertility tables, and assumed that women are only fertile during a 35-year period from age 15 to age 49. (Boyle & Starr, 1985) "Women were withdrawn from the study at the earliest occurrence of one of the following three events: the fiftieth birthday, the interview day, or divorce/separation from the male worker." (Boyle & Starr, 1985) Approximately 24 percent of the total person-year experience of the workers was accumulated while "not married," and four percent of the births occurred during these person-years.

In the test of the data from this cohort with the first model of Boyle and Starr, worker exposure, marital status, parity zero, and birth spacing were considered as dichotomous covariates. Linear covariates were maternal age, birth cohort (relative to 1900), and parity greater than zero. Birth spacing variables were also included. The model predicted a 39 percent reduction in the fertility hazard rate, but this reduction was not statistically significant (p=0.131).

In the test of the data from this cohort with the second model of Boyle and Starr, the hazard rate was "…stratified by 35 single years of maternal age and 9 parity groups." (Boyle & Starr, 1985) The same covariates were used as described for the first model above. Again, a reduction in fertility was computed (42 percent) that was not statistically significant (p=0.094).

The authors urged caution in formulating the underlying hazard rate for the second model so as to minimize information loss. This is essential since in this model, a stratum without a birth does not enter the partial likelihood function. Therefore, a compromise was judged necessary between the need for specificity and the loss of information. Finally, the authors reconfirmed the findings of Starr and Levine (Starr & Levine, 1983) that the national fertility rates overestimate the fertility of unmarried women regardless of parity, and underestimate it for married women of demonstrated parity (*i.e.*, parity greater than one). (Boyle & Starr, 1985)

d. Time-to-Pregnancy

The time-to-pregnancy measure, developed by workers from the National Institute for Environmental Health Sciences (NIEHS), has been advocated as a sensitive gauge of adverse reproductive impairment causing subfecundity. (Baird, 1988; Baird & Wilcox, 1985; Baird & Wilcox, 1986a; Baird, Wilcox & Weinberg, 1986) The time-to-pregnancy measure was originally devised for use in prospective studies, although most of the studies published to date are retrospective studies of currently pregnant women identified at obstetrical clinics. (Baird & Wilcox, 1985; Baird & Wilcox, 1986b; Joesoef, Beral, Rolfs, Aral & Cramer, 1990; Olsen, 1991; Weinberg, Wilcox & Baird, 1989; Wilcox, Weinberg & Baird, 1988) However, the method has been applied to retrospectively collected data from previous pregnancies (Joffe, 1989;

Schaumburg & Olsen, 1989), even though retrospective studies select against low fertility and sterile couples. (Baird, 1988)

The time-to-pregnancy method is based on analysis of the number of menstrual cycles or months required before a couple achieves a pregnancy, and is seen as a way to increase the sensitivity for the timely detection of adverse effects of agents on fertility. (Baird *et al.*, 1986) The major drawback to the method appears to be the collection of accurate data on the time-to-pregnancy, and other confounding variables that affect fertility. (Baird & Wilcox, 1986a)

Only three studies have attempted to assess the validity of the data collected with a time-to-pregnancy questionnaire (Baird, Weinberg & Rowland, 1991; Baird *et al.*, 1986; Joffe, 1989), and only one has discussed the effect on power from use of self-administered questionnaires. (Baird *et al.*, 1991) Since time-to-pregnancy data cannot be objectively confirmed by other data sources (e.g., medical records), Baird *et al.* used two indirect methods to assess the validity of the self-reported data. (Baird *et al.*, 1986) First, the distribution of time-to-pregnancy of pregnant women from a then ongoing retrospective study were compared to time-to-pregnancy values reported by these women for previous planned pregnancies, and to those of women followed prospectively after removal of intrauterine contraceptive devices. Second, the authors evaluated the observed and expected digit preference of responses of 6-cycle, 12-cycle, and 18-cycle delays before becoming pregnant.

The distribution of time-to-pregnancy from currently pregnant women participating in the then ongoing study prospective fertility study closely matched those of women in the prospective study. However, the distribution of time-to-pregnancy recalled from earlier pregnancies was different from that of the prospective study, a larger proportion of the pregnant women reported becoming pregnant in the first month and the digit preference was

obvious. The number of women reporting 6-cycle, 12-cycle, and 18-cycle delays before becoming pregnant, after correcting for a variety of confounders, was not statistically greater than expected.

Joffe has documented the increasing unreliability of questionnaire responses to reproductive outcome data with the passage of time since the event. (Joffe, 1989) As one might expect, there was a strong inverse relationship between the time elapsed and the concordance with records. Perfect concordance varied on a set of questions designed to glean information about past episodes of infertility or subfertility, from 60.5 percent for reproductive events occurring within 10 years to 14.8 percent for events occurring more than 30 years earlier, the values for minor discrepancies for the same two reporting periods were 13.2 and 48.1 percent, and 2.6 and 11.1 percent for serious discrepancies, respectively. (Joffe, 1989, pps. 270-271) Plots of male and female workers' recalled time-to-pregnancy for planned conceptions agreed very well with literature values.

Finally, Baird *et al.* recently reported on the effects of errors in self-administered questionnaire responses in time-to-pregnancy data on power. (Baird *et al.*, 1991) This was assessed by comparing responses to a short reproductive questionnaire and to a subsequent extensive telephone interview conducted by trained staff. After assuming the responses to the lengthier telephone interview were correct, a correlation of 0.82 was found between the two types of questionnaires. Errors in most important variables were nondifferentially distributed, biasing the results towards the null hypothesis. Power was significantly lost for weak effects. However, the authors calculated that exposure to a moderately potent agent, one that reduced fecundity by 50 percent ("…equivalent to adding about three cycles to the median time to pregnancy…") would be detected with 80 percent power. Baird *et al.* concluded that the short self-administered reproductive questionnaire was still a useful surveillance tool.

5. Testicular Volume

The testicular volume is an important measure of gonadal health. (Takihara, Sakatoku, Fujii & Nasu, 1983) It seems reasonable to measure testicular size since the seminiferous tubules account for over 90 percent of testicular volume. (Handelsman & Staraj, 1985) However, the two most common measurement techniques -- measurement of the length and diameter with calipers, or by comparative palpation using testicular models -- are either difficult or fraught with potential errors. An orchiometer has been shown to give better results. (Takihara *et al.*, 1983) Surprisingly, a review of the reproductive epidemiology literature revealed that testicular measurement techniques have been used in less than a handful of occupational epidemiology studies, and all for DBCP-exposed workers.

In the first study , Lipshultz *et al.* investigated the reproductive status of DBCP workers and controls at two Shell Oil Company plants. (Lipshultz *et al.*, 1980) The authors stated that a physical examination of the participating workers was conducted, an examination that "...focused on the genitalia and included precise testicular measurements." (Lipshultz *et al.*, 1980, p. 465) However, the method used to measure testicular size was not specified. While not displaying the data nor providing details on the statistical analysis, Lipshultz *et al.* report finding no significant difference in testicular size between the exposed and control workers, but some decrease in testicular size among the azoospermic men compared to the "expected norms" of testicular diameter.

Egnatz *et al.* evaluated the reproductive status of DBCP-exposed and unexposed workers at the Dow Chemical Company. A physical examination of the participating workers was conducted that included an estimation of testicular volume by "...visual comparison with four models of a testicle graduated in size and using a 13-interval recording scale." (Egnatz, Ott, Townsend, Olson & Johns, 1980, p. 728) Egnatz *et al.* performed regression

analysis for testicular volume on the total duration of exposure. Only workers with direct contact with DBCP had a statistically significant reduction in testicular volume ($p<0.005$).

More recently, Olsen *et al.* evaluated the reproductive recovery of former DBCP-exposed workers at a Dow Chemical Company. (Olsen, Lanham, Bodner, Hylton & Bond, 1990) Testicular size of the workers was measured by a single physician with machinist's calipers. There was a statistically significant trend toward reduced testicular size with lower sperm counts.

f. Sex Ratio

The sex ratio describes the relative proportions of males and females in a population. Computationally, it is either defined as the number of males in the entire population, or as the number of males divided by the number of females, times 100. (Teitelbaum, 1972) Using the latter definition, the normal sex ratio is between 104 and 107. A higher than normal sex ratio implies a greater number of males relative to the number of females in the population, and a lower sex ratio means the converse.

Several different measures of the sex ratio are use in demography, genetics, and epidemiology. According to convention, as described by Teitelbaum (Teitelbaum, 1972, p. 90):

- "The *primary sex ratio* is defined as the sex ratio at either 'conception' or 'fertilization', although these two terms are not completely synonymous.
- The *secondary sex ratio* normally refers to the sex ratio of live births, but stillbirths are sometimes included.
- The *tertiary sex ratio* is even more indeterminate; it refers to the sex ratio of a cohort at some age after live birth, possibly at 'marriage age' or at age of 'independence'."

A number of demographic factors are known to influence the sex ratio in man. For instance, there is a significant linear inverse relationship between parity level and paternal age and the sex ratio (Chahnazarian, 1988; James & Rostron, 1985), and a positive relationship with

socioeconomic status (Chahnazarian, 1988; James & Rostron, 1985; Teitelbaum & Mantel, 1971). The sex ratio appears to follow a curvilinear relationship with respect to maternal age. (James & Rostron, 1985) Among the myriad number of other factors that have been investigated include family size, sex of the first born child, sex of the last prior pregnancy, time of conception in the menstrual cycle, frequency of intercourse, and artificial insemination.

A number of articles in the medical and epidemiological literature have noted aberrations in the secondary sex ratio in humans associated with certain medical conditions or occupational exposures. For instance, Alperovitch and Feingold first noted a deficit of sons among male multiple sclerosis (MS) patients (n=109) and a statistically significant excess of daughters among female MS patients (n=272). (Alperovitch & Feingold, 1981) James reviewed the literature (five papers) concerning the biased sex ratio of MS patients and provided additional analyses from the pooled data (n=4,429 children of male MS patients; n=9,570 children of female MS patients). (James, 1994) He reported that the sex ratio of male and female MS patients before disease onset closely approximates the ratio for Caucasian live births. The sex ratio after disease onset is a different story. Male MS patients have a statistically significant reduction in the proportion of sons after disease onset, while the sex ratio of female MS patients after disease onset is not significantly altered. James concludes that the altered sex ratio is a consequence of the disease and not a cause.

Olsson and Brandt reported on biased sex ratios among male and female non-Hodgkin's lymphoma (NHL) patients. (Olsson & Brandt, 1982) Men and women diagnosed with NHL in their thirties of forties had a decreased sex ratio (33 percent sons *versus* expected value about 51 percent, sex ratio 0.49 *versus* expected value about 1.05, n=109 children). The lowest sex ratio was observed among young men with NHL (30 percent sons, sex ratio 0.43, n=70 children). These differences were statistically significant as

compared to the sex ratio of NHL patients diagnosed in their fifties and to a set of control patients (n=266 children among NHL patients in their fifties).

Chahnazarian *et al.* evaluated the sex ratio at birth of offspring of hepatitis B parents among four populations scattered around the world where the virus is endemic. (Chahnazarian, Blumberg & London, 1988) Their data analysis "...suggest that parents who carry the hepatitis B virus tend to have an unusually high probability of bearing sons...but there is no evidence that immunity in parents has any effect." (Chahnazarian *et al.*, 1988, p. 368)

Lyster noted that abalone divers on the southeast coast of Australia had a dramatically lower sex ratio (35 percent sons, sex ratio of 0.53, n=130 children). (Lyster, 1982) He provides a brief recap of other literature of occupations associated with low sex ratios, and hypothesized about the role gonadotrophic hormones may play citing a Romanian infertility clinic's experience in raising the sex ratio in men treated with testosterone or gonadotrophin.

Potashnik *et al.* discussed the testicular function and reproductive experience of 15 DBCP-exposed workers in Israel. (Potashnik, Goldsmith & Insler, 1984; Potashnik & Yanai-Inbar, 1987) Among all (including normospermic) men, the proportion of male births during exposure dropped to 35.2 percent, and dropped even further in the recovery period to 21 percent. Among the azoospermic and oligospermic men, there were only two sons born out of 12 children born during exposure ($p<0.025$). Testosterone levels were noted to have been normal at all times. The authors hypothesize that the reduced sex ratio "...might be a reflection of the early effect of DBCP on male reproductive performance before a state of severe testicular dysfunction and infertility is reached." (Potashnik *et al.*, 1984, p. 213)

James has published a number of papers exploring this and related literature, and attempted to develop and refine a coherent biological explanation for this phenomenon. (James, 1986; James, 1987a; James, 1987b; James, 1989; James, 1990a; James, 1990b; James, 1992) James hypothesizes that the sex of human offspring is controlled, in part, by the hormone levels of the parents at the time of conception. According to his hypothesis in humans "...high levels of testosterone and oestrogen favouring the production of males, and high levels of gonadotrophin and progesterone favouring females. It is suggested that these hormones may also bias sex ratios (proportion of males at birth) in other mammalian species, but not necessarily in the same direction." (James, 1992, p. 121) On the basis of the Egeland *et al.* article, which reported an inverse relationship between measured serum dioxin and hormone levels in workers from two plants in the National Institute for Safety and Health (NIOSH) dioxin registry (Egeland *et al.*, 1994), James recently hypothesized (James, 1995) that since men exposed to dioxins have lower testosterone concentrations, they should also have a lower sex ratio (*i.e.*, a lower than expected proportion of sons).

E. Potential Confounders in Reproductive Epidemiology

It is important to remember the precise definition of a confounder. According to Schlesselman, "a confounder (confounding variable) is an extraneous variable that satisfies both of two conditions: (1) it is a risk factor for the study disease; and (2) it is associated with the study exposure but is not a consequence of exposure." (Schlesselman, 1982, p. 58) Schlesselman added that in practice any extraneous risk factor which is not a consequence of exposure may be regarded as a confounder if controlling for it in the analysis appreciably alters the risk estimates of an exposure.

1. Male Reproductive Confounders

A number of factors have been reported that affect male gonadal and gonadotropin function, and thus may serve as potential confounders in male reproductive epidemiology studies. These include: occupational and nonoccupational exposures to reproductive toxins, age, health status, alcohol consumption, cigarette smoking, medications, and licit and illicit drug use. With the exception of the occupational and nonoccupational literature, a brief review of this literature follows.

a. Age

Dai *et al.* evaluated factors affecting plasma testosterone levels in 243 middle-aged male participants in the Multiple Risk Factor Intervention Trial (MRFIT) at the University of Pittsburgh. (Dai *et al.*, 1981) In their study population, total and free testosterone levels decreased significantly with increasing age in these men selected from the general population because of high risk factors for coronary disease (high blood pressure, high cholesterol, and cigarette smoking). This finding is consistent with other studies that have found that mean plasma testosterone concentrations are relatively stable from age 20-50, but are significantly lower from age 70 onward. (Dai *et al.*, 1981)

Tsitouras *et al.* examined the relationship between serum testosterone and alcohol, cigarette smoking, percentage body fat, and coronary heart disease in a group of 183 healthy married men 60-79 years of age. (Tsitouras, Martin & Harman, 1982) Serum testosterone did not decrease significantly with age, nor was it significantly correlated with age. However, sexual activity declined rapidly and nonlinearly after age 60. When testosterone levels were compared after adjustment for age group and sexual activity level, older men with higher testosterone tended to be more sexually active, and men with lower testosterone to be less active.

Rudman *et al.* measured plasma testosterone levels in 44 long-term male nursing home residents. (Rudman *et al.*, 1988) The average age of the study participants was 76.4. Forty-six percent of the men had plasma testosterone levels below 300 ng/dL (*i.e.*, hypogonadism). Men possessing low serum testosterone levels also had low free testosterone concentrations. Thirteen of the men had elevated LH levels which the study authors' interpret as evidence of "peripheral hypogonadism." Hemoglobin, cholesterol, and seizures were significantly and directly correlated with serum testosterone level.

Recently, Gray *et al.* conducted a meta-analysis of the effects of aging on testosterone (T) levels. (Gray, Berlin, McKinlay & Longcope, 1991) The authors reviewed 88 published studies and abstracts on the subject, but only 44 of the studies were included in the meta-analysis because of methodological flaws. They concluded that "…overall, there is a moderate relation between T (testosterone) and aging, but this relationship is considerably weaker for studies reporting correlations," and "general health status predicts both the level of T and the slope of the relation between T and aging.". (Gray *et al.*, 1991, p. 681) They found general health status to be a good predictor of testosterone concentration and for the slope of the relation between testosterone and aging.

Finally, as mentioned above, paternal age appears to be inversely related to the secondary sex ratio. (Chahnazarian, 1988; James & Rostron, 1985) That is, with advancing age, a man is more likely to father a daughter than a son.

b. Cigarette Smoking

A number of researchers have investigated the potential adverse effects of tobacco smoking on male fertility parameters or endocrine function. Several of the studies published to date have demonstrated that

cigarette smoking in males adversely effects sperm or endocrine function. Some studies have indicated that smoking causes a dose-response reduction in sperm density, others have found a reduction in the proportion of motile sperm and an increased proportion of abnormal sperm in male cigarette smokers as compared to nonsmokers. As always, conflicting studies exist in the literature. Some have reported no adverse effects, others a beneficial effect of cigarette smoking on semen quality, and still others have reported an increase in testosterone concentration. A brief review of the literature follows.

Evans *et al.* evaluated sperm density ("counts") and sperm morphology among smokers and matched nonsmokers attending a subfertility clinic in Edinburgh. (Evans, Fletcher, Torrance & Hargreave, 1981) Patients with medical or occupational risk factors for sperm abnormalities were excluded from the analysis. A total of 43 smokers and 43 nonsmokers participated in the study.

The authors found no significant association between smoking and sperm count or sperm morphology, so each variable was assessed separately. Overall, the average proportion of morphologically normal sperm in nonsmokers (57.7±1.18 percent) was significantly higher than in smokers (52.9±1.47 percent, p=0.01). This relationship was preserved when only males with sperm counts over 60 million/mL were considered. However, no dose-response relationship was found between the proportion of morphologically abnormal sperm and the number of cigarettes smoked per day.

In contrast to the results of the study of Evans *et al.*, Godfrey found no significant differences between smokers and nonsmokers in sperm morphology, sperm motility, or sperm count among male patients at an infertility clinic in Australia. (Godfrey, 1981) The significance of Godfrey's study is questionable given its brief description in the letter to the editor, and inconsistency with prior or subsequent literature, albeit its sample size was nearly twice as large as the Evans *et al.* study.

In order to address the question raised by Evans *et al.* of whether cigarette smoking adversely effects semen quality, Rodriguez-Rigau *et al.* assessed semen quality among 437 consecutive male partners of infertile couples. (Rodriguez-Rigau, Smith & Steinberger, 1982) A total of 159 men remained in the "normal" group after excluding 181 men who were predisposed to infertility due to specific illnesses or exposures, a separate study considered the 97 men with varicoceles. Of these 159 men, 101 were nonsmokers. There were no significant differences in the average ages of the men in all the groups.

Among the 159 "normal" men, there were no significant differences between cigarette smokers and nonsmokers in the proportion of normal sperm, average sperm motility, mean sperm count, or the frequency distribution of sperm counts. These results differed little when the smoking group was subdivided into those men smoking less than 20 cigarettes per day, and those smoking more than 20 cigarettes per day. Among the 97 men with varicocele, again no significant differences were found between cigarette smokers (n=59) and nonsmokers (n=38) in sperm motility, proportion of normal sperm, sperm count, or frequency distribution of sperm. However, when the men with varicocele were compared to the "normal" men, it was apparent that the former group had a significantly higher proportion of men with sperm counts less than 20 million/cm^3 than the "normal" group (32.3-35.4 percent *versus* 23.5-25.8 percent, respectively). The authors call for further study to clarify the role, if any, of cigarette smoking on male fertility.

Handelsman *et al.* examined testicular and endocrine function in 119 consecutive healthy males visiting a sperm donor bank in Australia for a screening examination. (Handelsman, Conway, Boylan & Turtle, 1984) Current and former smokers were classified as "smokers" for the purposes of their analysis. Smokers were comparable to nonsmokers in physical parameters and

prevalence of varicoceles. Semen samples were collected in the laboratory after 2 days abstinence.

Handelsman *et al.* found no statistically significant effects on endocrine function by smoking. Sperm density was much higher in nonsmokers (95.5±8.0 million) than in smokers (64.1±9.2 million), but not significantly so after cube root transformation to normalize the distribution. However, total sperm output, sperm motility, motile sperm density, total motile sperm, oval sperm density, and total number of oval sperm all were significantly higher in nonsmokers than smokers ($p=0.01-0.001$). No significant differences between smokers and nonsmokers were found for plasma FSH, LH, or testosterone concentrations.

Hoidas *et al.* used a scanning electron microscope (SEM) to evaluate sperm from fertile and infertile smokers and nonsmokers in Edinburgh. (Hoidas, Williams, Tocher & Hargreave, 1985) The authors found significant differences in sperm abnormalities between fertile and infertile men, but not between smokers and nonsmokers. The authors speculate that the SEM measures features not seen by the light microscope used in standard semen quality assessments.

Kulikauskas *et al.* evaluated the effects of cigarette smoking on semen. quality of 238 male patients visiting a infertility clinic. (Kulikauskas, Blaustein & Ablin, 1985) The authors excluded from their study those men with medical (e.g., chronic alcoholism) or physical conditions (e.g., orchitis, prostatitis) that could influence semen quality, but no actual physical examinations were conducted that may have identified men with other conditions (e.g. varicoceles). Men were classified as smokers if they smoked at least 4 cigarettes per day for the last 5 years. The semen samples were collected in the laboratory after 3 to 7 days of abstinence from alcohol, barbituates, and sexual activity.

The results of their study showed that sperm density and motility were significantly lower in smokers than nonsmokers ($p<0.001$). Average

sperm density in smokers (n=103) was less than one-half of that in nonsmokers (n=135). However, no significant differences in sperm morphology were found.

Sueldo *et al.* study investigated the relationship between seminal prolactin (PRL) concentration and semen quality in 63 men from infertile marriages. (Sueldo, Berger, Kletzky & Marrs, 1985) Two of the men were oligospermic (sperm density <20 x 10^6/mL), 38 were asthenospermic (<60 percent motility), 6 were oligoasthenospermic (sperm density <20 x 10^6/mL and <60 percent motility), and 17 had a normal semen profile. The fertilizing capacity of semen from 49 men was assessed with the zona-free hamster ovum penetration test, and in 14 men it was assessed by *in vitro* fertilization of human oocytes.

Mean seminal and serum PRL concentrations were not statistically different, nor were they correlated (r=0.11). The eight oligospermic individuals had a mean seminal PRL concentration significantly higher than that of the 55 normospermic men ($p<0.05$). The 44 men with asthenospermia also had a significantly higher mean seminal PRL concentration than the 19 men with normal sperm motility ($p<0.01$. A comparison of the results from the hamster ovum penetration test revealed that the nine men with abnormal test results (*i.e.*, <15 percent penetration) had significantly higher seminal PRL levels than the 40 men with normal test results. The comparison of *in vitro* human oocyte fertilization test results revealed no significant differences in seminal PRL concentrations between the three men who failed the test and the 11 men who passed the test.

The authors cited several studies that report smaller testes and prostate glands in hyperprolactinemic men, perhaps, they postulate, due to "…decreased Leydig-cell steroidogenesis and/or down-regulation of PRL receptors in the secondary sex organs." However, the authors admitted that the absence of a correlation between seminal and serum PRL concentrations

in their study would eliminate the proposed mechanisms as explanations for the observed effect of PRL on sperm density and motility. The authors concluded that the higher levels of PRL in seminal plasma of men with low sperm density, low sperm motility, or poor penetration in the hamster ovum test suggests that PRL "...may have a negative effect on the functional capacity of spermatozoa."

Referring to the Kulikaukas *et al.* study, Klevene and Balossi suggested in a letter to the editor that prolactin may be responsible for the reduced fertility among smoking males. (Klevene & Balossi, 1986) The authors cited an unexpected result of a recent cardiovascular risk factor study they had conducted in Argentina in a group of 73 normotensive men. The authors found that PRL concentrations were significantly increased in smokers *versus* nonsmokers ($p<0.001$), and a dose-dependent increase in serum PRL concentrations was observed ($p<0.001$). In his response to the comments by Klevene and Balossi, Ablin identifies the fact that "PRL acts on the male accessory sexual glands of reproduction independently and synergistically with luteinizing hormone (LH) and testosterone (T). While LH is the principal pituitary gonadotropin the Leydig cells require for androgenic activity, PRL (and follicle-stimulating hormone) also possesses the ability to directly activate Leydig cells, and under physiologic conditions, it potentiates the stimulatory effect of LH on testicular steroidogenesis." (Ablin, 1986)

Vogt *et al.* evaluated the effects of cigarette smoking on semen quality of 333 healthy male volunteers 19-40 years of age in Germany. (Vogt, Heller & Borelli, 1986) After an evaluation process consisting of a clinical examination which focused on the genitalia, and completion of a questionnaire on medical history, sexual behavior, radiation exposure, and tobacco, alcohol and drug use, 239 men were evaluated further and requested to deliver a semen sample. Men were classified as "smokers" if they reported smoking at least one cigarette per day for at least 1 year prior

to the examination, as "ex-smokers" if they had smoked regularly for a year but had discontinued smoking at least 1 year prior to the examination, and if they had never smoked. The men were requested to refrain from sexual activity for 5 days prior to providing the semen sample.

Vogt *et al.* found no significant differences between smokers, ex-smokers, and never smokers in sperm motility, sperm morphology, or serum FSH and LH levels. Sperm density displayed a marginally significant trend of decreasing concentration from never smokers to ex-smokers to smokers ($p=0.07$). In addition, among smokers there was a significant linear trend toward decreased sperm density with increasing smoking ($p=0.05$), and a significantly higher concentration of serum testosterone in smokers than in nonsmokers. The authors ascribe the latter difference to personality differences between smokers and nonsmokers

In a literature review of smoking and reproduction published in the same year, Stillman *et al.* enumerate 11 of 12 studies (several from foreign researchers) that found smokers had reduced sperm density compared to nonsmokers. (Stillman, Rosenberg & Sachs, 1986) Two studies attempted to assess whether cigarette smoking induced a dose-dependent effect on sperm density. One found a strong relationship, the other found an equivocal response. The authors tally 12 studies that have evaluated the effects of cigarette smoking on sperm motility. Eight of these studies found a lower proportion of motile sperm among smokers *versus* nonsmokers, two an opposite effect, and the remaining two essentially no difference between the two groups. Finally, Stillman *et al.* cite 11 studies that have evaluated sperm morphology among smokers and nonsmokers. Five of the studies found an decreased proportion of morphologically normal sperm among smokers compared to nonsmokers, one found the opposite effect, and the remaining four reported approximately equal proportions of normal sperm amongst smokers and nonsmokers.

Stillman *et al.* discuss one important potential source of bias, the health status of volunteers for the semen quality studies. They hypothesize that if nonsmokers volunteering for these studies are healthier than smokers, their semen quality may be better for health status reasons unrelated to smoking. The found only the Kulikauskas *et al.* study included both fertile and infertile men, and it demonstrated reduced sperm density, motility, and impaired morphology among smokers of both groups. (Kulikauskas *et al.*, 1985)

Effendy and Krause evaluated the association between six environmental risk factors (trauma, heat, noise, smoking, pesticides, and plastics) and history of infertility in 93 consecutive male patients visiting their infertility clinic in Germany. (Effendy & Krause, 1987) The authors reported that exposure to none of these six environmental factors was significantly associated with differences in semen quality parameters when compared to those patients without the exposure. However, male smokers with exposure to heat had the lowest sperm count and sperm motility proportion of any environmental risk factor/smoking status group.

Dikshit *et al.* evaluated the effect of tobacco consumption (chewing and smoking) on the semen quality of consecutively seen men, 20 to 35 years of age, from idiopathic infertile marriages who were undergoing initial medical screening in India. (Dikshit, Buch & Mansuri, 1987) A total of 929 men were originally investigated, but 303 men were eliminated from the study due to a variety of apparently valid exclusionary criteria. The 626 patients selected for further study constitute the largest blinded and matched study population investigated on the topic of tobacco effects on semen quality. Of these men, 288 did not consume tobacco products, 119 were tobacco chewers, and 219 were cigarette smokers (>10 cigarettes per day).

Semen samples were obtained from each of the men in the laboratory after 3 to 5 days of abstinence. The samples were analyzed for semen quality parameters (volume, viscosity, sperm density, total count, motility, and morphology) by standard techniques by a clinician blinded to the purpose of the study. Tobacco users had a smaller volume of ejaculate, and lower sperm density and total sperm count. However, none of these differences were statistically significant. There were no appreciable differences in the sperm motility and percent morphologically normal sperm among the three groups. The authors concluded that chewing or smoking tobacco is unrelated to impaired semen quality in men who had idiopathic hypofertility.

Klaiber *et al.* evaluated the interrelationships between cigarette smoking, testicular varicoceles, and semen quality. (Klaiber *et al.*, 1987) The authors paid 160 healthy young volunteers (Sample 1) to complete a physical examination and provide a semen sample. Another group (Sample 2), consisting of 94 husbands of infertile marriages, underwent the same screening process.

Their analysis showed that cigarette smoking in the presence of varicoceles was a highly statistically significant factor associated with the incidence of oligospermia in both the Sample 1 and Sample 2 populations. The percentage of oligospermic smokers with varicoceles was 17 times greater than in the remaining subgroups in Sample 1, and 3 times greater than in the remaining subgroups in Sample 2. When both populations were combined, 93 percent of the men with varicoceles and oligospermia were smokers.

In addition, a comparison of smokers with nonsmokers found that overall smokers had lower sperm density, percent motility, and motility grade than nonsmokers. Limiting the comparison to only smokers and nonsmokers without varicoceles established the independent role of smoking as a risk factor for impaired semen quality. Smokers in Sample 2 had

significantly lower sperm density, percent motility, and grade of motility. No significant differences were found between these two subgroups in Sample 1. However, when the semen quality parameters of the Sample 1 subgroups of smokers and nonsmokers without varicoceles who did not smoke marijuana were compared, smokers had a lower average sperm density and grade of motility. No firm conclusions could be drawn on the effect of alcohol on semen quality because of the insufficient number of abstainers.

The authors hypothesized that the combined effects of smoking and varicoceles on the testes may stem from increased levels of catecholamines documented to occur from smoking. Adrenal catecholamines may enter the testes from the spermatic vein via retrograde flow, and thus damage the seminiferous vesicles epithelium. Elevated concentrations of catecholamines have been found in the spermatic vein of men with varicoceles, and animal experiments have shown that elevated concentrations of these substances may damage the seminiferous epithelium.

Barrett-Connor and Khaw conducted a population-based study in Southern California to evaluate the effect of cigarette smoking on plasma estrogen, androgen, and sex hormone-binding levels in men with and without heart disease. (Barrett-Connor & Khaw, 1987) The study participants were all original members of the early 1970s Lipid Research Clinic Prevalence study. A total of 590 men 30-79 years of age participated in Visit I of that study, and 198 participated in Visit II. Data from both groups were used in their study.

The authors reported that current male smokers without cardiovascular disease (CVD) had higher estradiol, estrone, and androstenedione levels than former or never smokers, but that there was no difference between these groups in testosterone or sex hormone binding levels. Adjusting the analyses for age, body mass, alcohol consumption, and regular exercise did not alter these conclusions. The increase in these hormones was dose-dependent with the hormone levels increasing with reported smoking rate.

Dai *et al.* assessed the relationship between cigarette smoking and serum sex hormones in men by reanalyzing data from two groups of male participants in the Multirisk Factor Intervention Trial (MRFIT) cohort. (Dai, Gutal, Kuller & Cauley, 1988) The one group consisted of 121 male MRFIT participants from the Pittsburgh area, the other group was comprised of 163 men from the entire MRFIT cohort who ultimately developed CVD and 163 matched controls. The authors reported a positive correlation between cigarette smoking and serum total androstenedione level in both studies. Likewise, this association was independent of a number of potential confounding factors. The total serum and free testosterone concentrations were positively correlated with cigarette smoking among the first group and controls, but not among the second group of men who subsequently developed CVD. Unlike the Barrett-Connor and Khaw study, Dai *et al.* found no association between cigarette smoking and either serum estradiol or estrone.

Klaiber and Broverman assessed the pharmacodynamics of estradiol (E_2) and testosterone in 43 paid male college students (22 smokers and 21 nonsmokers). (Klaiber & Broverman, 1988) For each man, a medical history was obtained, a complete physical examination was performed, and blood, urine, and semen samples were obtained for analysis. Serum levels of estradiol and testosterone, and the production and metabolic clearance rates for both steroid hormones were determined.

The authors found no statistically significant differences between smokers and nonsmokers for mean testosterone concentration, the production rate of testosterone, or the metabolic clearance rate of E_2. However, E_2 concentrations were significantly higher in smokers than in nonsmokers. The higher E_2 concentrations was determined to result from higher E_2 production rates rather than decreased clearance. The elevated mean levels of E_2 and E_2 production rate were inversely related to decreased sperm

density in smokers. The authors postulate that the decreased sperm density observed in smokers may be due to E_2 inhibition of testicular testosterone synthesis, a key factor in spematogenesis.

Marshburn *et al.* assessed the effects of coffee drinking, cigarette smoking, and alcohol consumption on semen quality of 546 consecutive men who sought treatment at a North Carolina fertility clinic between 1978 and 1982. (Marshburn, Sloan & Hammond, 1989) The medical history and consumption level of each of these three products was collected. The data from 445 men remained after screening out those men with urologic abnormalities and other reasons. Semen samples were collected after 3 days abstinence from ejaculation.

The authors reported the following results: "…smoking was associated with diminished semen volume, coffee drinking was correlated with increases in sperm density and percentage of abnormal forms, while alcohol consumption appeared to have no effect." The effects attributed to smoking and coffee consumption did not appear dose-dependent. Men who drank coffee had a borderline statistically significant increase in the percentage of motile sperm. Men who drank more than four cups of coffee per day and who smoked more than one pack of cigarettes per day had a lower proportion of motile sperm, and a higher percentage of dead sperm compared to men who did not consume either product. "Alcohol consumption was not correlated with any alteration in semen quality in either univariate or multifactorial analysis." (Marshburn *et al.*, 1989, p. 163)

Attia *et al.* investigated serum hormone levels in male cigarette smokers and nonsmokers in Egypt. (Attia, El-Dakhly, Halawa, Ragab & Mossa, 1989) There were 50 heavy smokers and 35 never smokers in the two study groups. Study group members completed a questionnaire, clinical examination, and laboratory measurement of serum estradiol (E_2), prolactin, and testosterone levels. The authors, found that smokers had significantly elevated E_2 and prolactin levels, but that testosterone concentrations were

unaffected. This latter finding is consistent with the findings of Handelsman *et al.* (Handelsman *et al.*, 1984) However, the authors caution, this finding does not mean that the biologically active (free) testosterone fraction is unaffected by smoking. This matter was recommended for further research.

c. Drugs and Medications

Numerous studies have identified the fact that certain licit and illicit drugs and medications can induce sexual dysfunction in males. The best studied and most widely abused drug, ethyl alcohol (ethanol), has clearly demonstrated adverse effects on the male reproductive system. It appears that with acute ethanol intake "…the primary effect of alcohol is on the testicular synthesis and secretion of testosterone. Both alcohol and its major metabolite, acetaldehyde, inhibit the testicular enzymes involved in testosterone synthesis. This testicular effect of alcohol is worsened by the increased metabolic clearance rate of testosterone by the liver…". (Smith & Gilbeau, 1985, p. 262)

Chronic alcohol consumption has a more complicated pattern. Multiple organ and endocrine abnormalities may be involved. Hypogonadism and gynecomastia are commonly associated with alcoholic cirrhosis, testimony to the disruption of normal reproductive hormone function and to the importance of the liver in maintaining sexual function. (Smith & Gilbeau, 1985, p. 262) Estrogenic hormone concentrations are increased in alcoholic men. This results in suppression of gonadotropin and testosterone production, and inhibition of accessory gland function. (Smith & Gilbeau, 1985)

Turner *et al.* reviewed the literature regarding the health effects of chronic alcohol ingestion. (Turner, Mezey & Kimball, 1977) They cite the folklore that sexual potency is reduced in males consuming excessive

amounts of alcohol. The authors note several limitations in studies of this topic. For instance, the timing of blood sampling for testosterone is important. More recent studies, however, suggest the timing of blood sampling is not so important to obtaining valid results. (Bain, Langevin, D'Costa, Sanders & Hucker, 1988; Schrader, Turner, Breitenstein & Simon, 1993) Testosterone is secreted in pulses throughout the day, plasma testosterone levels vary several-fold depending upon circadian and seasonal fluctuations. In addition, some studies measured total plasma testosterone, while only the free, nonprotein-bound testosterone (about 3 percent of the total amount) is biologically active in that it can enter cells. Despite these shortcomings, Turner *et al.* summarize the pertinent literature. In addition, since alcoholics are a rather heterogeneous group, the duration of high alcohol intake (*i.e.*, acute or chronic), the age, and nutritional and health status of the affected individuals are important covariates.

Turner *et al.* related one study of 30 males with a history of heavy drinking, half of the males reported no sexual dysfunction, but the other 15 reported impotence which disappeared in 12 during periods of sobriety. (Turner *et al.*, 1977) The authors noted that serum testosterone levels have been reported to be depressed in males with liver cirrhosis, regardless of the cause.

Turner *et al.* describe two separate studies, one of male alcoholics, the other of male nonalcoholics, both indicated that heavy alcohol intake leads to reductions in plasma testosterone levels. In the nonalcoholic males, a 29-55 percent decline in plasma testosterone was observed if daily alcohol intakes was 220 grams, but not at 80 grams daily. These studies suggest there may be a threshold toxic effect on the testes, and that testicular toxicity may be apparent without cirrhosis or nutritional deficiencies. Also, one study of two alcoholic and two nonalcoholic

volunteers who consumed 220 g of alcohol daily for 4 weeks reported an average increase of 222 percent in testosterone reductase compared with levels prior to the experiment. Thus, accelerated metabolism of testosterone removal may also play an important role in explaining the effect of alcohol on male reproductive function.

Dai *et al.* found no significant association between a patient's history of alcohol intake and plasma testosterone level. (Dai *et al.*, 1981) In fact, the authors' reported an increase in both free and total testosterone levels with mild to moderate alcohol consumption. These findings are somewhat at odds with other studies that have shown that "cirrhosis of the liver is associated with hypogonadism and gynecomastia. Plasma testosterone levels have been found to be lower in these patients especially with alcoholic cirrhosis." (Dai *et al.*, 1981) In addition, elderly men consuming more than 4 ounces of alcohol daily had diminished sexual activity, but not lower serum testosterone. (Tsitouras *et al.*, 1982)

A review article by Van Thiel neatly summarizes the *in vitro* and *in vivo* studies of the effects of alcohol on the male reproductive tract. (van Thiel, 1983) Van Thiel interprets the literature to suggest that chronic alcohol abuse itself, and not the accompanying liver disease, is responsible for inducing impotence, loss of libido, and testicular atrophy. He concludes the following (van Thiel, 1983, p. 31):

- "Ethanol is a primary Leydig cell toxin both *in vivo* and *in vitro*.
- Such toxicity is manifested by reduced testosterone biosynthesis and secretion, resulting in endocrine failure of the testes.
- Such testicular endocrine failure is initially compensated for *in vivo*, at least in part, by enhanced gonadotropin secretion.
- With prolonged ethanol exposure, such central compensation ultimately fails and hypothalamic-pituitary (central) failure also occurs.
- *In vitro*, acetaldehyde, the first metabolic product of ethanol metabolism, is also a Leydig cell toxin.
- Recovery from alcohol-induced injury is possible early, but with repeated or chronic injury, a state of irreversible injury develops and is manifested by overt testicular atrophy."

In a study of semen and endocrine function in male patients at an infertility clinic, Handelsman *et al.* failed to find any adverse effects of moderate alcohol consumption on testicular function. (Handelsman *et al.*, 1984)

Iranmanesh *et al.* reported abnormally high concentrations of the gonadotropins LH and FSH in the plasma of chronic alcoholics during a brief abstinence from alcohol. (Iranmanesh *et al.*, 1988) Also, free testosterone and estradiol levels were elevated, while total testosterone levels were normal. The authors interpreted these findings as evidence that alcohol impairs the normal functioning of the hypothalamic-pituitary-gonadal axis.

Alvarez *et al.* performed *in vitro* fertilization experiments with media containing different concentrations of ethanol. (Alvarez *et al.*, 1988) They reported that the addition of ethanol to the culture medium induced a dose-dependent increase in the percentage and rate of acrosomal loss in sperm cells. While the loss of acrosomes and other structures would preclude gamete fusion, apparently the presence of ethanol in the culture medium had no effect on viability or motility. (Alvarez *et al.*, 1988) The loss of acrosomes observed in the cultures was induced at concentrations comparable to blood alcohol levels constituting the legally drunk limit for driving a motor vehicle (e.g., 22 mM is equivalent to 100 mg percent).

Laboratory experiments have confirmed that marijuana and its principal psychoactive component, Δ^9-tetrahydrocannabinol (THC) "…inhibit the secretion of the pituitary hormones, LH and FSH as well as prolactin. These changes in pituitary hormone levels produce decreases in sex steroid hormones. In male laboratory animals, acute or chronic administration of THC results in lower serum concentrations of testosterone." (Smith & Gilbeau, 1985, p. 250) The results of marijuana use on human males is far less clear. One study of chronic heavy users found a reduction of plasma

testosterone levels compared to age-matched controls who had never used the drug, while another study found no such effect. Both animal and human studies have shown that chronic marijuana use leads to decreased sperm production. (Smith & Asch, 1987) There is some tolerance development after habitual use of this drug. It is unknown what role this phenomenon plays in ameliorating the effects, if any, of marijuana on male fertility since no epidemiological studies have been conducted. In addition to marijuana, opiates have been found to adversely effect male fertility. "Chronic narcotic users have decreased fertility and atrophy of male accessory sex organs. Similar findings were observed in laboratory animals…". (Smith & Gilbeau, 1985, p. 254)

In a review article, Story cited 118 brand name prescription medications that induced adverse effects on male sexuality. (Story, 1974) The author divided the medications into six major groupings by their intended pharmaceutical use. These were: antihypertensive, antihypertensive with a diuretic combination, antianxiety, antidepressant, antipsychotic, and anorexics. The side effects included impotence, decreased libido, aspermia, and premature or delayed ejaculation.

Levi *et al.* describe four cases of young male patients who had been treated for ulcerative colitis with sulphasalazine and had developed oligospermia and infertility. (Levi, Fisher, Hughes & Hendry, 1979) No other causes for their condition were found. When the men were withdrawn from the drug their sperm counts rapidly improved, and spouses of three of the four men became pregnant. Semen quality rapidly deteriorated after resuming sulphasalazine therapy in two men with follow-up records.

As previously discussed, clinical studies have demonstrated adverse reproductive effects of certain pharmaceutical agents on males, namely a positive association between impotence and consumption of such medicinals as: antihypertensive, antihypertensive with diuretic combination,

antianxiety, antidepressant, antipsychotic, and anorexic drugs. (Quinn *et al.*, 1990)

From the toxicological and medical literature, Schlegel *et al.* identified adverse effects on spermatogenesis or spermatozoa associated with 23 commonly used antibiotics from seven major antibiotic classes, including: nitrofurans, macrolides, aminoglycosides, tetracyclines, sulfa drugs, penicillins, and miscellaneous. (Schlegel, Chang & Marshall, 1991) "For humans, infertility or significant alterations in semen parameters have been well documented for the nitrofurans and for patients on sulfasalazine. Other commonly used antibiotics, such as minocycline, have been shown to be toxic to sperm at any concentration." In general, the mechanism of action of antibiotics on spermatogenesis is unknown, but appears related to the ability of the compound to cause spermatogenic arrest. The authors advised physicians to consider the likelihood of antibiotic treatment causing a detrimental effect on male fertility, at least during the duration of treatment. They admonished physicians to remember that "…sulfasalazine was in widespread use for over 40 years in many patients before an association with infertility was recognized."

In a review article on impotence, Turnbull and Weinberg remind physicians of iatrogenically induced cases of erectile dysfunction. (Turnball & Weinberg, 1983) They cite 10 medications known to induce impotence in males.

Slag *et al.* screened 1,180 men for impotence at a Veteran's Administration outpatient clinic. (Slag *et al.*, 1983) Of these men, 401 (34 percent) were impotent, and 188 (47 percent) of the impotent men volunteered to be referred for further evaluation. The cause of the impotence in 25 percent of the volunteers was medication, and psychogenic disturbances in 14 percent. Significantly, 38 percent was ascribed to endocrine dysfunction (10 percent primary hypogonadism, 9 percent secondary

hypogonadism, 9 percent diabetes, 5 percent hypothyroidism, 4 percent hyperprolactinemia, and 1 percent hyperthyroidism).

In their meta-analysis of the relationship between testosterone and aging, Gray *et al.* concluded that "...medication is a significant predictor of T level in the univariate setting but it does not affect the age-associated change in T." (Gray *et al.*, 1991)

d. Body Fat

Several studies have evaluated the role of body fat on male reproduction. Consistent with previous studies, Dai *et al.* reported that obesity was strongly correlated with lower free and total testosterone levels. (Dai *et al.*, 1981) However, no reduction in sexual activity with increasing percentage of body fat was found in one study of 183 healthy married men 60-79 years of age. (Tsitouras *et al.*, 1982)

e. Varicoceles

As discussed at length earlier in this Chapter, varicoceles generally are no longer considered to be causally associated with male infertility since infertile men both with and without varicoceles have higher intrascrotal temperatures. However, provocative findings like those of Klaiber *et al.* indicate that there may be an interactive effect of smoking and varicoceles that impairs male infertility. (Klaiber *et al.*, 1987)

f. Psychosocial Factors

Several articles have examined the role of psychological factors affecting sexual functioning. For instance, Turnbull and Weinberg describe loss of libido as "...one of the cardinal signs of a major depression." (Turnball & Weinberg, 1983)

Bents conducted a literature survey of 53 articles published between 1948 and 1985 on the psychology of male infertility. (Bents, 1985) Contrary to the Turnbull and Weinberg quotation regarding the adverse effect of depression on fertility, Bents cited two well-controlled studies that found better fertility characteristics among depressed patients than men with higher sociability and extroversion scores. Bents found the literature contained 14 references on the adverse effects chronic stress or traumatic psychosocial stress on male fertility. Bents finds the consistent findings of impaired endocrine and testicular function from this literature to be the most convincing.

Giblin *et al.* conducted a longitudinal study of the interrelationships among semen quality, stress, and personal adaptability. (Giblin, Poland, Moghissi, Ager & Olson, 1988) Twenty-eight healthy male volunteers provided semen samples every 2 weeks for 6 months. The volunteers were not asked to abstain from ejaculation prior to providing a sample. They were, however, asked to complete a questionnaire with each sample denoting: the time since the last ejaculation, number of ejaculations in the prior 2 weeks, alcohol, coffee, and cigarette consumption in the 2 days prior to sample collection, and any illnesses or drug use. A self-appraisal of stress social support, and life events was collected monthly. Semen samples were split and analyzed independently by two technicians for sperm density, volume, motility, and morphology.

Two novel findings from this study were that "…semen measures were unrelated to both changes in health status and health behaviors, as well as unrelated to averaged life event and social support scores." However, there was a significant positive correlation between abnormal sperm morphology (tapered heads) and all measures of self-reported stress, and a negative association with "ego resiliency" as measured by a subscale of the MMPI.

2. Female Reproductive Confounders

A large variety of factors affect a woman's reproductive experience. Factors such as her age, parity, socioeconomic status, the frequency of sexual intercourse, a woman's knowledge of her fertile period, use of birth control, previous reproductive outcomes, prior reproductive disease or surgery, lactational experience, and chemical exposures during pregnancy, may affect the probability of successful conception, development, and delivery of a normal child. (Howe, Westhoff, Vessey, & Yeates, 1985; James, 1979; Källén, 1988; Kline & Stein, 1984; Potter & Millman, 1985; Selvin & Garfinkel, 1976) Because of the intense focus on maternal factors affecting normal development and delivery, a far greater number of factors have been identified that may confound epidemiology studies of female reproduction and adverse pregnancy outcomes than have been identified for males. Since many of the factors that may impair male fertility can be measured only indirectly it is especially important to identify the maternal factors (exposures and behavior patterns) that may reduce a woman's fertility, and control for them, if possible, during the data analysis.

a. Age

Unlike men who produce sperm continually after puberty, women have a more limited reproductive period. Women are born with their full complement of eggs, although only a small fraction mature and are released during their reproductive years. Fertility for women begins at menarche, which normally starts by the early teens, and ends with menopause, typically between the ages of 45 and 55.

In perhaps the largest women's fertility study conducted, Howe *et al.* reported on the results of the Oxford Family Planning Association contraceptive study. (Howe, Westhoff, Vessey & Yeates, 1985) This

prospective study recruited 17,032 white, married women, between the ages of 25 and 39 from family planning clinics in England and Scotland during the period 1968-1974. The women were observed for an average of 11.5 years. Each woman was interviewed at enrollment about her reproductive, medical and social history, and at follow-up clinic visits about changing contraceptive practices.

Of the enrolled women, 4,104 stopped using birth control methods to plan a pregnancy on 6,199 occasions. All the periods of risk of pregnancy were included in the analysis, and each occasion of birth control discontinuation was treated as an independent observation during the analysis (although the authors admit it is not strictly correct).

Howe *et al.* reported that the increasing age of both nulliparous and parous women was inversely related to decreasing fertility (p trend= 0.0001). (Howe *et al.*, 1985) Compared to 25-27 year old women, 36-37 year old nulliparous women had a relative fertility rate of 0.48 (95 percent confidence limits: 0.31-0.73). The corresponding relative fertility rates for 36-37, 38-39, and ≥40 year old parous women *versus* 25-27 year old women were: 0.97 (95 percent confidence limits: 0.80-1.19), 0.64 (0.48-0.86), and 0.49 (0.30-0.81), respectively. Of great interest to this study was the fact that towards the end of the reproductive period the probability of conception diminishes, and the time to pregnancy rapidly increases for women as they age. (Howe *et al.*, 1985).

The authors were reluctant to ascribe all these observed changes to declining ovarian function. They relate that 75 percent of the women had indicated that they had completed their families at the time of enrollment, and note the general decline in frequency of intercourse with age. Howe *et al.* accepted the precipitous decline in relative fertility for parous women 38 years of age or older as more likely due to declining ovarian function. The decline in relative fertility for nulliparous women, they note, begins

from age 28 onward, a phenomenon likely due to "...more powerful selective influences operating on nulliparous women than on parous women."

With increasing maternal age, "...the risk increases for miscarriage, for having an infant with a low birth weight, or an infant who dies perinatally." (Källén, 1988) Furthermore, the incidence of pregnancy complications like hypertension, low birth weight, diabetes mellitus, abruptio placentae, and placenta previa have been reported to increase with increasing maternal age, (Cunningham & Leveno, 1990), although the effects of some of these complications have been mitigated in recent years by medical advances. While the risk of trismomy 21 (Down's syndrome) increases exponentially with advancing maternal age, a recent population-based study of 26,859 children with birth defects of unknown etiology (*i.e.*, those not known to be of chromosomal origin or due to maternal factors) in British Columbia reported no positive association between the incidence of such birth defects and increasing maternal age. (Baird, Sadovnick & Yee, 1991)

b. Cigarette Smoking

Cigarette smoking by women has been associated with spontaneous abortion, congenital malformations, preterm birth, and intrauterine growth retardation. (De Mouzon, Spira & Schwatz, 1988; Himmelberger, Brown & Cohen, 1978; Stillman *et al.*, 1986) In addition, women cigarette smokers have been shown to have premature menopause. (Jick, Porter & Morrison, 1977) For every two-year age group between 44 and 53, never smokers were less likely to be post-menopausal compared with current smokers of one-half or ≥1 pack per day. Of course the proportion of post-menopausal women increased with age, but it increased more rapidly for women smokers. Too, there was a dose-response relationship wherein never smoking women in any age group had a lower proportion of post-menopausal women than one-half

pack per day women smokers, who in turn had a lower proportion of post-menopausal women than ≥1 pack per day women smokers. In the older age groups, up to 22 percent more women smokers were post-menopausal than the corresponding never smoking women.

While the mechanism of this action was not understood at the time, subsequent animal experiments demonstrated that polyaromatic hydrocarbons (PAHs) present in tobacco smoke are metabolically activated in the ovaries, where they destroy oocytes, and perhaps induce ovarian cancers. (Mattison, Shiromizu & Nightingale, 1983; Mattison & Thorgeirsson, 1978) Premature ovarian senescence has appeared in every species or strain studied.

A recent review of the epidemiological literature on this topic found 14 published papers that met their entry criteria. (Midgette & Baron, 1990) Their review confirmed the relationship between smoking and premature menopause. The authors found that the difference in median or mean ages between smoking and nonsmoking women ranged from 0.3-1.7 years, and that cigarette smoking raises the odds of menopause for women between the ages of 44-55 by approximately two-fold.

Olsen *et al.* conducted a case-control study in Denmark to investigate the relationship between cigarette smoking, alcohol consumption, and infertility. (Olsen, Rachootin, Schiodt & Damabo, 1983) A total of 1,069 infertile couples (cases) and 4,305 fertile couples (controls) were selected for the study. Approximately 87 percent of both groups responded to the self-administered mailed questionnaire for a total of 927 cases and 3,728 controls.

Cigarette smoking (classified by a simple dichotomy "yes" or "no") and alcohol consumption were significantly greater in cases than controls. There was a potential selection bias in that only couples who sought infertility treatment were selected as controls. These couples may not be representative of all infertile couples. A second potential bias stems

from the fact that the control group couples were queried about their smoking habits in the year a child was delivered. Since these couples may have ceased smoking.

A separate analysis of the control group revealed a statistically significant association between smoking and delayed conception (>1 year). The odds ratios and 95 percent confidence limits were 1.8 (1.3, 2.5) and 1.3 (1.0, 1.8) for primary and secondary subfecundity, respectively. No association was found for alcohol consumption and delayed conception.

Baird and Wilcox from the National Institute for Environmental Health Sciences conducted an epidemiological study that reported an association between cigarette smoking and delayed conception in women. (Baird & Wilcox, 1985) In that study, conducted in the Minneapolis/St. Paul area, a total of 678 pregnant women who were noncontracepting at the time they were attempting to become pregnant were recruited from area obstetric clinics and offices. After adjusting for potential confounding variables by using Cox (proportional hazards) regression, smokers fertility was approximately 72 percent of nonsmokers. Moreover, a dose-response relationship was found with light and heavy smokers having fertility estimated at 75 and 57 percent of the pregnancy rate of nonsmokers, respectively. "Smokers were 3.4 times more likely to have taken greater than a year to conceive compared with nonsmokers." (Baird & Wilcox, 1985)

In the Howe *et al.* Oxford Family Planning Association contraceptive study described earlier, fertility was also inversely related to maternal social class (p trend=0.0005), parity (p trend=0.0001), and cigarette smoking (p trend=0.0001). (Howe *et al.*, 1985) The authors stated that "…the newest and most important finding…" of this study was the confirmation that cigarette smoking adversely effects fertility. Not only did maternal cigarette smoking reduce fertility, it also increased the time to pregnancy.

Of relevance to the present study is the fact that neither height, weight, or Quetlet's index "...showed significant or consistent associations with fertility." (Howe *et al.*, 1985) Only women who reported "gynaecological problems" experienced a significant reduction in relative fertility rates. Aside from the six variables already described which had a significant effect on fertility (*i.e.*, social class of husband, age at marriage, parity, age of nulliparous women, age of parous women, and cigarette smoking), "no evidence of other important confounding was found...". (Howe *et al.*, 1985)

Two studies of tubal infertility and IUDs by Daling *et al.* (Daling *et al.*, 1985) and Cramer *et al.* (Cramer *et al.*, 1985) have demonstrated that cigarette smoking is an independent and significant risk factor for tubal infertility. In the Daling *et al.* case-control study, 159 nulligravid women with primary tubal infertility were compared to 159 matched controls who had delivered a child. Cases were far more likely to be a current cigarette smoker than controls, 38 percent *versus* 15.7 percent. Stillman *et al.* calculate the odds ratio for smoking induced infertility from this study as 3.3 (95 percent confidence limit 1.9-5.6). (Stillman *et al.*, 1986) No other confounders were examined.

Cramer *et al.* conducted a case-control study that included 283 nulligravid white women diagnosed with primary tubal infertility, and 3,833 white women controls who were admitted for delivering a baby. Two controls were selected for each case. Three variables exerted a significant effect on the risk of tubal infertility independent of the use of an IUD, these were: level of education, number of sexual partners, and smoking. After adjusting for a variety of potential confounders (e.g., education, number of sexual partners, use of oral contraceptives, IUD, or barrier methods of contraception), the authors reported that the relative risk for primary infertility according to smoking status (ever, former, or current). A

statistically significant risk of cervical factor and tubal disease infertility was seen only for current smokers. The relative risk for these two disease entities were 1.5 (p-value 0.04, 95 percent confidence limit 1.0, 2.1) and 1.6 (p-value 0.006, 95 percent confidence limit 1.1, 2.1), respectively. No significant effects were seen for ovulatory factor or endometriosis. A significant trend of increasing risk of tubal disease infertility with increasing number of pack-years smoked was observed. The authors concluded that only certain types of primary female infertility were significantly associated with cigarette smoking.

De Mouzon *et al.* conducted a prospective study of the relation between cigarette smoking and fertility. (De Mouzon *et al.*, 1988) Of 2,022 questionnaires returned by couples along with at least one month's temperature curve, 1,887 (93 percent) satisfied entry criteria, and 1,164 (62 percent) achieved a pregnancy during the study period. Women were classified as smokers if they smoked more than one cigarette per day. However, "the vast majority of smoking women consumed fewer than 10 cigarettes daily." (De Mouzon *et al.*, 1988, p. 379) Only 20 women smoked more than one pack per day. Also, women smokers tended to be in a lower social class than nonsmoking women.

Using Cox proportional hazards regression, the researchers found that nonsmokers had a significantly larger number of pregnancies than the nonsmoking group (65 percent *versus* 52 percent, $p<0.001$). Moreover, the pregnancy rate among smoking women was significantly lower than among nonsmokers for the first cycle as well as the for the entire curve ($p<0.001$) At the end of one year of trying, 85 percent of the nonsmoking women had conceived *versus* 70 percent of the smoking women. Smoking women were twice as likely to take more than one year to conceive as nonsmoking women.

There were some confounding factors which complicate the interpretation of these results. For example, 70 percent of the couples tried to conceive prior to entering the study, and these couples had a significantly poorer pregnancy rate than those couples that deferred attempting a pregnancy until after they entered the study. Also, women whose husbands smoked had a significantly lower pregnancy rate.

Additional analyses were run on the 30 percent of the couples who deferred attempting a pregnancy until after they entered the study. Again, smoking women had a significantly lower pregnancy rate during the first cycle of trying ($p<0.05$), but this relationship was only marginally significant for the cumulative pregnancy curve ($p<0.08$). The authors argue against the role of smoking as having an appreciable effect on women's fertility, and instead ascribe at least part of the observed effects to "…behavioral factors…".

The population-based Danish study by Olsen described above also examined the effect of cigarette smoking on subfecundity. (Olsen, 1991) Consistent with most earlier studies on the topic, maternal smoking resulted in statistically significant increases in time to pregnancy as defined by delays in achieving pregnancy exceeding either 6 months or 12 months. No dose-response effect was observed. It should be noted that only a small percentage (5 percent) of the women smoked one or more packs of cigarettes per day, although this group included 556 women.

Interestingly, a paternal smoking effect was reported. This effect was seen for subfecundity defined as time to pregnancy exceeding either 6 months or 12 months. Olsen reported that paternal smoking behavior affected subfecundity in a dose-dependent fashion with nearly the same degree of increase in the odds ratios for increased times to pregnancy as seen with maternal smoking. He concluded that this study, despite its selection bias against sterile couples, confirmed the results of his

earlier study. Olsen acknowledged his identification of a paternal effect was inconsistent with the results of the Baird and Wilcox paper.

c. Caffeinated Beverages

Several groups of researchers have examined the relationship between a woman's consumption of caffeinated beverages and her fertility or pregnancy outcome experience. The first paper was designed as a prospective investigation of the frequency of very early pregnancy loss among healthy women who were planning to become pregnant. (Wilcox *et al.*, 1988) The women who volunteered for the study were predominantly white, college graduates, in their late twenties and early thirties. A total of 221 women were recruited into that study. These women were interviewed regarding their consumption of cigarettes, and caffeinated or alcoholic beverages, and personal behavior (*i.e.*, dates of menstrual periods and sexual intercourse). The study described in this paper investigated the fecundability of the 104 women who had failed to conceive within three months after recruitment, and who were reinterviewed regarding exposures after enrollment while they were trying to conceive.

The authors assessed the fertility of each woman by calculating the fecundability (*i.e.*, the probability of conceiving in each menstrual period) of each woman, exposed and unexposed. Cox's proportional hazards model was used to estimate the fecundability ratios and control for confounding factors. Over a span of 13 menstrual cycles, the women consuming higher amounts of caffeinated beverages were consistently less likely to conceive than women consuming lower amounts of caffeine. The weighted mean fecundability ratio over the 13 cycles was 0.59 (95 percent confidence limits 0.40-0.87). When reinterview information on caffeine consumption from a survey conducted 6 months into the study was included, this association became even more pronounced. The fecundability ratio

using the updated information was 0.53 (95 percent confidence limits 0.35-0.79).

Multivariate analysis was used to examine the effects of potential confounders on the caffeine:fecundability relationship. Neither the woman's age, frequency of intercourse, age at menarche, or in utero exposure to her mother's cigarette smoking behavior affected the association. In addition, no significant effects from vitamin, analgesic, alcohol, or marijuana use on fecundability were seen, either in the crude analysis, or after controlling for caffeine intake. Women's weight, with or without height, was unrelated to fecundability.

When the fecundability ratio was analyzed by daily caffeine consumption rate divided into quintiles, a clear dose-response relationship was observed. Women in the highest quintile were only 26 percent as fecundable as women in the lowest quintile. Using the 13 cycle (one year) period as the clinical definition of infertility, women in the highest caffeine intake quintile faced a relative risk of infertility 4.7 times greater ($p<0.005$) than that of women in the low caffeine quintile. When the authors examined the caffeine-impaired fertility relationship by using data on the caffeine consumption from enrollment and the three month reinterview, only the data from the latter source were significantly associated with fecundability. This led the authors to speculate that the effects of caffeine on fertility are short-lived.

In a letter to the editor, Christianson *et al.* describe a reanalysis of interview data from pregnant women they collected between 1959 to 1967. (Christianson, Oechsli & van den Berg, 1989) Their questionnaire included questions on prepregnancy and current caffeine, alcoholic beverage, and tobacco consumption, and whether the women had difficulty becoming pregnant. While admitting their data were "...less refined..." than that of Wilcox *et al.*, their reanalysis indicated that heavy coffee drinkers (>7

cups per day) reported nearly twice the chance of having had difficulty in becoming pregnant as compared to women drinking <1 cup of coffee per day.

Christianson *et al.* suggest that undetected pregnancies at the time of entry to the study may have resulted in systematic bias in the results of the Wilcox *et al.* study due to the fact that one of the earliest effects of pregnancy is aversion to coffee. (Christianson *et al.*, 1989) Wilcox responded by arguing that if the bias were present, then women who were unknowingly pregnant at the time of interview should have reported less caffeine consumption, and that this effect should be most apparent in the first cycle attempt at pregnancy. (Wilcox, 1989) However, previously unreported data from the Wilcox *et al.* study showed that an unusually high proportion of women conceived in the first cycle of trying (41 percent). Wilcox concludes that there is no evidence of a greater reduction in fecundability during this first cycle, and thus no basis for concluding that the suggested bias is operating to any significant degree.

Approximately one year after the Wilcox *et al.* paper, Joesof *et al.* published another study that examined the relationship between caffeine consumption and increased time to pregnancy. (Joesoef *et al.*, 1990) The Joesof *et al.* study was a case-control study performed with data collected several years earlier by Cramer *et al.* for a study designed to investigate the association of IUD use and tubal infertility. (Cramer *et al.*, 1985) A total of 2,817 women without a history of infertility who delivered a child between April 1981 and September 1983 at one of seven institutions as a result of a planned pregnancy served as the controls. Cases consisted of women diagnosed with primary infertility at these same institutions in the same time period.

Interviews with the same questionnaire were conducted with both cases and controls. The questionnaire probed topics on personal behavior, socioeconomic factors, medical history, and consumption of legal or illicit

drugs. Unfortunately, the questionnaire asked about caffeine intake at times when the women were not pregnant. (Weinberg & Wilcox, 1990) This may be significant given the efforts many women take to reduce caffeine consumption and generally live healthier while attempting to become pregnant. (Wilcox *et al.*, 1988)

Fertility effects were evaluated four ways. The first three measures were based on the interview reported time to pregnancy of the 2,817 women controls for the last birth. First, the mean time to pregnancy was computed for women consuming different amounts of caffeinated beverages. Second, a monthly fecundability ratio was computed for conception rates. Third, a fecundability ratio (risk ratio) based on a proportional hazards model was computed to examine the independent effects of several factors. Finally, case-control comparisons of caffeine consumption between women with primary infertility and primiparous controls were performed.

The authors reported that caffeine intake had no effect on mean times to conceive. Time to conceive was significantly related to a number of other factors, such as age, weight, years of education, number of previous pregnancies and miscarriages, and cigarette smoking. Many of these factors correlated with caffeinated beverage consumption. Total caffeine intake increased with increasing age, weight, and cigarette and alcoholic beverage consumption. Finally, caffeine consumption was not associated with infertility in infertile women and their primiparous controls.

More recently, Olsen evaluated the effects of cigarette smoking and caffeinated beverage consumption on subfecundity in a population-based survey in Denmark. (Olsen, 1991) During the period April 1984 to April 1987, a questionnaire was provided to all pregnant women in two Danish cities during their 36th week of pregnancy. (Olsen, 1991) The goal of the survey was to identify the effects of social condition and life-style factors on pregnancy outcomes. The survey included questions on maternal

and paternal smoking and drinking habits (alcohol, coffee, and tea), and time to pregnancy.

The questionnaire reached almost all of the targeted women, and 86 percent of them (n=11,888) responded. After removing questionnaires from women who had been treated for infertility or who had not responded to that portion of the survey, 10,886 women's responses remained for analysis.

Only after adjusting for a large number of covariates did maternal consumption of coffee or tea result in a statistically significant (α=0.05) increase in the time to pregnancy, and then only among woman smokers who consumed 8 or more cups of these beverages daily, and where subfecundity was defined as a delay in becoming pregnant exceeding 12 months. The odds ratio for this group of women was 1.35 (95 percent confidence limits: 1.02-1.48)

Although some studies have reported associations between coffee consumption and birth defects, spontaneous abortion, stillbirth, and prematurity, no definitive conclusions can be drawn due to methodological problems with the studies (Hogue, 1981) In a large study of late birth outcomes in Boston, Linn *et al.* found no association between coffee consumption and low birth weight or prematurity. (Linn *et al.*, 1982)

d. Drugs and Medications

A number of licit and illicit drugs and medications contain powerful pharmacological agents whose use or abuse may affect a woman's reproductive function. Smith has performed two comprehensive reviews of this topic. (Smith & Asch, 1987; Smith & Gilbeau, 1985)

Perhaps the most widely abused licit agent that affects the reproductive system is ethanol. Unfortunately, nearly all of the studies of the reproductive effects of ethanol have been conducted in males. However, a few studies have been conducted with females. In female rodents

alcohol appears to inhibit gonadal activity. Primates appear to be less sensitive to such effects from acute exposure. In human clinical studies of the acute effects of ethanol ingestion no significant effect on sex steroids or gonadotropins were observed. (Smith & Gilbeau, 1985, p. 262) Likewise, no effect was seen in rhesus monkeys when alcohol was administered during different phases of the menstrual cycle. (Smith & Gilbeau, 1985, p. 262) In contrast to the situation with acute alcohol intake, chronic alcohol abuse by human females has been associated with infertility and menstrual abnormalities, and by disruption of reproductive function in female monkeys. (Smith & Gilbeau, 1985, p. 262)

The consumption of alcohol by pregnant women results in a constellation of adverse effects known as fetal alcohol syndrome (FAS). (Smith & Asch, 1987) This syndrome was first recognized in 1973. Children with this syndrome suffer from: "(1) prenatal growth deficiency in length and weight; (2) microcephaly; and (3) short palpebral fissures. Infants with FAS are small for their gestational age and, unlike other small infants, they remain small for their age through childhood." (Smith & Asch, 1987, p. 365) FAS is the leading cause of mental retardation in the United States today. (Streissguth *et al.*, 1991)

Several studies have investigated the effects of marijuana or its active ingredient THC on the menstrual cycle. Most of these experiments have been performed on female rhesus monkeys. Short-term dosing at levels that lead to serum THC concentrations corresponding to those seen in habitual marijuana smokers, THC disrupted the monkeys' menstrual cycle. (Smith & Gilbeau, 1985, p. 262) These changes persisted for several months after treatment ceased. Long-term treatment with THC rendered all of the female monkeys anovulatory and disrupted their hormone levels. (Smith & Gilbeau, 1985, p. 262) Tolerance eventually developed over a period of 3 to 4 months and the monkeys reestablished normal ovulatory menstrual cycles.

he monkey data is consist[illegible] with human data. In a study of the
of marijuana use on the [illegible] reproductive system, 26 young women
orted using marijuana at [illegible] four times per week were compared to
matched controls who repo[illegible] never using the drug. (Smith & Asch,
The women who used marij[illegible] had shorter menstrual cycles and
luteal phases. Another [illegible] of women who habitually used
na showed that, like the m[illegible]ys, tolerance developed and hormonal
rations returned to norma[illegible] Smith & Asch, 1987)
recent matched case-cont[illegible] study in Washington State demonstrated
men who smoked marijuana [illegible] elevated relative risk (RR) of
infertility due to ovula[illegible] abnormality (RR=1.7, 95 percent
nce limits: 1.0-3.0). (Mue[illegible] Daling, Weiss & Moore, 1990) This
s much greater if marijua[illegible] occurred within 1 year of attempting
me pregnant (RR=2.1, 95 pe[illegible] confidence limits: 1.1-4.0).
r *et al.*, 1990) The dura[illegible] or frequency of marijuana use did not
in consistently detected e[illegible]s, and these reported effects were
to women who smoked mari[illegible] infrequently.
he use of marijuana by pre[illegible] women has been shown to increase the
adverse effects on the fe[illegible] (Smith & Asch, 1987) For instance,
l use of marijuana was ass[illegible]ed with infants showing more symptoms
ral nervous system immatu[illegible] significantly shorter gestational
significantly lower birt[illegible]ght, length, and head circumference,
reased incidence of mecon[illegible] in the amniotic fluid of newborns.
& Asch, 1987, p. 364)
ther illicit drugs like th[illegible] narcotics cocaine and heroin have been
o significantly complicate[illegible]nancies or deliveries, but clinical
emiological data is sparse[illegible] Narcotics do disrupt female
ctive hormones in both ani[illegible]s and humans, resulting in significant
ons in serum LH and FSH co[illegible]rations. (Smith & Asch, 1987, p. 359)
ent case-control study rep[illegible] that women who used cocaine had an

infertility, and couples who used a combination of mechanical and chemical barrier contraceptives had the greatest protection from tubal infertility. (Cramer *et al.*, 1987)

"Pelvic inflammatory disease (PID) is a serious complication of sexually transmitted disease (STD). It is a major cause of infertility, ectopic pregnancy, and chronic pelvic pain in women." (Aral, Mosher & Cates, 1991) In fact, "tubal factors are responsible for approximately 15% of infertility…". (Sellors, Mahony, Chernesky & Rath, 1988) The STDs Chlamydia trachomatis and Neisseria gonorrhoeae are responsible for the majority of proven cases of PID. (Rice & Schachter, 1991) However, the contribution of other bacterial species (e.g., Streptococcus, Escherichia coli, and Haemophilus influenzae), which may account for 25 to 50 percent of some case series, cannot be ignored. (Kessel, 1989; Rice & Schachter, 1991)

Beginning with three studies in the early 1970s, a large body of epidemiological literature has accumulated, building toward a consensus, that implicates intrauterine devices (IUDs) as a leading cause of PID, hence tubal infertility. (Cramer *et al.*, 1985; Daling *et al.*, 1985) In particular, the Dalkon Shield has been identified as the IUD whose use leads to the highest risk of PID. (Cramer *et al.*, 1985; Daling *et al.*, 1985)

More recently, however, a reanalysis of the evidence by Kessel calls into question the studies which have found an association between IUD use and PID. (Kessel, 1989) Based upon his review of the differences between prospective and case-control studies, limitations in the diagnosis of PID, bias in case-control studies, and other factors, Kessel concludes that the association between IUDs and PID is likely an artifact of clinician diagnostic bias. (Kessel, 1989) Others assert that insertion of an IUD places a women at higher risk of PID for the first 4 months, probably due

to the introduction of vaginal or cervical organisms into the uterus. (Washington, Cates & Wasserheit, 1991)

f. Breastfeeding

It has been known since antiquity that breastfeeding impairs a woman's fertility, even as she provides nutrition for her child. (Thapa, Short & Potts, 1988) Aristotle noted this fact, which has since been confirmed by modern science. (Habicht, Davanzo, Butz & Meyers, 1985; Thapa *et al.*, 1988) "Lactational infertility takes two forms. There is a period of complete infertility during lactational amenorrhoea when ovulation is suppressed, and this is followed by a variable period of lowered fecundity after the resumption of ovulatory menstrual cycles." (Thapa *et al.*, 1988)

The length of this anovulatory period is dependent upon the frequency and duration of breastfeeding, the degree of supplementation of breast milk, and the mother's age and nutritional status. (Gray *et al.*, 1990; Habicht *et al.*, 1985; Thapa *et al.*, 1988) For example, a 20-year old woman who partially breastfeeds her child will ovulate, on average, 12.7 months after delivery. (Habicht *et al.*, 1985) For a 34-year old women, this anovulatory period would extend so long as she breastfeeds.

It appears that the lactational infertility induced by breastfeeding is an important natural regulator of birth spacing in both Third World countries (Habicht *et al.*, 1985), and in developed countries. (Lewis, Brown, Renfree & Short, 1991) From a study of women in Baltimore and Manila, researchers concluded that women who breastfed exclusively during the first 6 months postpartum reduced their risk of pregnancy to levels corresponding with failure rates of IUDs or oral contraceptives (≈2 percent). (Gray *et al.*, 1990) Partial breastfeeding yielded protection from pregnancy better than those achieved by barrier or rhythm methods (≈6

percent). (Gray *et al.*, 1990) Two recent Australian studies have supported the conclusion that lactational amenorrhea can provide excellent protection from pregnancy during the first 6 months after delivery. (Lewis *et al.*, 1991; Short, Lewis, Renfree & Shaw, 1991)

g. Other Factors

Bullen *et al.* a small prospective cohort study to examine the effects of strenuous exercise on the induction of menstrual disorders in untrained women. (Bullen *et al.*, 1985) Their sample size consisted of 28 women of "...documented ovulation and luteal adequacy...". The women were randomly assigned to weight-loss and weight-maintenance treatment groups so as to be able to discern the possible independent effects of exercise from weight loss. Only one of the 16 women (6.25 percent) in the weight-loss treatment group had a normal menstrual period, as compared to three of the 12 women (25 percent) in the weight-maintenance group. In addition, the incidence of luteal surge abnormalities increased over time in the weight-loss treatment group ($p<0.01$). The authors concluded that vigorous exercise can alter reproductive function in women.

Green *et al.* conducted a case-control study of infertility related to ovulatory dysfunction. (Green, Weiss & Daling, 1988) A total of 376 nulligravid women with evidence of this disorder were compared to fertile controls. The authors reported that nulligravid women who were at either extreme from the ideal height:weight ratio were at greater risk for ovulatory infertility. For nulligravid women whose body weight to height ratio was 85 percent or less of the ideal value, the odds ratio was 4.7 (95 percent confidence ratio 1.5, 14.7) for increased risk of ovulatory dysfunction. For nulligravid women whose body weight to height ratio was 120 percent or greater than the ideal value, the odds ratio was 2.1 (95 percent confidence ratio 1.0, 4.3) for increased risk of this type of

infertility. These associations were not observed among women who had been pregnant.

The authors argue that the infertility observed among underweight women supports Frisch's critical weight theory that states that a women must attain a critical percentage of body fat in order to reach menarche, and one might conclude, to continue ovulating. Obese women are more likely to have amenorrhea and be anovulatory, perhaps due to "...decreased serum levels of sex steroid-binding globulin (SSBG) and increased adrenal androgen secretion." (Green *et al.*, 1988)

As mentioned earlier, maternal parity level is inversely related to the secondary sex ratio, and maternal age displays a curvilinear relationship. (Chahnazarian, 1988; James & Rostron, 1985)

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II. METHODS AND MATERIALS

The overall main study design was that of a retrospective cohort study. Additional details on the original cohort study are described below.

A. Study Hypotheses

The primary study hypothesis was that male workers exposed to pentachlorophenol (and its PCDD/PCDF contaminants) during its chemical synthesis incurred reduced fertility as manifested by a value of theta (θ, the ratio of the Standardized Fertility Ratio (SFR) from the exposed workers to the SFR of all workers prior to employment at the plant) less than one. The secondary study hypothesis was that the sex ratio among the live born children of exposed workers would be altered from the expected proportion of boys to girls. Specifically, the *a priori* hypothesis was that the proportion of boys born to the exposed workers after employment at the plant would be smaller than the expected value based on national vital statistics data.

B. Definition of the Study Cohort

1. Selection of Exposed Workers

To qualify as a candidate for the original main study, an individual was defined as having worked in a department, either exposure or exclusionary, if they had worked in that department for a (cumulative) total of at least three days. This period was selected because three days was the minimum length of employment consistently required by the company's employee health information (EHI) database for classification into any specific department.

2. Definition of the Exposed Cohort

The exposed cohort was defined as all past and present plant employees employed after 1931 who were alive as of November 7, 1985 who met one or more of the following criteria:

1. Hourly production workers who had worked in Departments 236, B-821, 237, or 268 between January 1, 1931 and December 31, 1983 as these departments produced chlorophenols or chlorophenoxy herbicides, the substances of interest known also to contain PCDD/PCDF contaminants;
2. Maintenance workers who appeared on the plant Chloracne Registry, because chloracne is strongly associated with exposures to various halogenated chemicals, including chlorophenols, chlorophenoxy herbicides, and PCDDs/PCDFs;
3. Workers who were noted to have chloracne on the plant medical records with the exception of workers who were exclusively engaged in the production of PCBs or chlorinated benzenes because these substances, too, can cause chloracne and other specific health outcomes under investigation.

Only hourly production workers in exposed departments were included in the main study. Salaried production workers were not included because of their small numbers. They were, however, recruited for a small pilot study. Hourly production workers who became salaried employees after leaving an exposed department and salaried unexposed workers were included in the main study. Production workers who met the selection criteria were included in the main study. The definition of the exposed cohort was presented to company epidemiologists who then identified exposed employees through their EHI database. The exposed cohort was identified in the Fall of 1985. On November 7, 1985, 785

workers had been identified who met the entry criteria for the exposed cohort.

3. Definition of the Unexposed Workers

The unexposed cohort was defined as all past and present plant employees who were alive as of November 7, 1985 who met all of the following criteria:

1. Never worked in Departments 236 or B-821 (the penta production departments, henceforth referred to as Dept. 236), 237, or 268;
2. Did not appear on the Chloracne Registry;
3. Were not listed on company documents as having chloracne;
4. Never worked in Maintenance;
5. Never worked in Department 224, 233, or 273 (Chlorinated Benzenes);
6. Never worked in Department 218 (Muriatic Acid/Chlorinated Benzene);
7. Never worked in Department 262 (2,4-D);
8. Never worked in Department 246 (PCBs);
9. Never worked in Department 239 (Santophen);
10. Never worked in Department 313, 508, or 861 (Laboratory); and
11. Did not work in Department 226 (Salt) after November 27, 1932

Workers who had worked in any of these exclusionary departments were omitted from the unexposed worker group due to: 1) potential exposure to the study compounds (maintenance; 2,4-D; Santophen; or laboratory); 2) work in an area adjacent to an exposed department; or 3)

exposure to chemicals possibly affecting specific health outcomes under investigation (PCBs or chlorinated benzenes). The definition of the unexposed cohort was presented to company epidemiologists who then identified unexposed employees through the EHI database. The unexposed cohort was identified in the Fall of 1985. On November 7, 1985, 1,704 employees had been identified who met the entry criteria for the unexposed worker cohort.

4. Selection of the Unexposed Cohort

The goal in the selection of the unexposed cohort was to select a group of such workers as similar to the exposed group as possible with the exception of exposure.

The comparison of the characteristics of the exposed and unexposed cohorts revealed major imbalances on employment status, length of employment, and percent of vital status unknown. A cross-tabulation of employment status by age revealed that terminated workers comprised a majority of available unexposed workers in the age strata 55 years and older. Furthermore, a cross-tabulation of the percent vital status unknown by age among the terminated unexposed workers revealed that the vital status of a majority of these workers age 60 years and older was unknown. Some of these workers were undoubtedly deceased and thus ineligible for study.

Time constraints did not permit the ascertainment of vital status among the 757 unexposed whose vital status was unknown even though this would have simplified the selection of the unexposed workers. Unfortunately, such an effort would have delayed the start date of the medical examinations until the summer of 1986, and the company indicated the strong possibility of layoffs at the plant during early 1986. Layoffs would have seriously hindered recruitment of the study

populations, and thus jeopardized the entire study. As a result, the decision was made to concurrently track and recruit a stratified random sample of the unexposed cohort.

The stratified random sampling strategy was designed to minimize the imbalance on these potential confounders while providing a sufficient oversample of (older age) strata where deaths were more likely to have occurred. The complete final sample of unexposed workers selected on November 7, 1985 consisted of 153 active, 32 transferred, 52 retired, and 528 terminated workers for a total of 765 unexposed workers for a potential comparison group.

5. Comparison of Main Study Exposed and Unexposed Workers

The mean age of the main study exposed group (58.3 years) is slightly older than the mean age of the main study unexposed group (56.5 years). The age, sex, race, length of employment, employment status, pay type, and unknown vital status distributions of the final main study sample of unexposed workers are far more similar to the exposed than were these characteristics in the population of unexposed workers from which the sample was drawn. Nevertheless, serious imbalances remain with respect to employment status and unknown vital status. In addition to having a higher percentage of terminated workers with unknown vital status in the unexposed group, 58% of terminations among the unexposed workers were involuntary compared to 39% among the exposed.

C. Measurement of Exposure

Data on the exposure of individuals in the study were available from three sources: 1) the EHI database; 2) an individual's self-reporting of exposure on the administered Occupational and Environmental

Questionnaire; and 3) chloracne, which has historically served as a marker of exposure, as diagnosed by dermatologic exam or plant medical records.

1. The Employee Health Information Database (EHI)

The Occupational Exposure Module of the EHI contains the detailed work histories of each worker ever employed at the plant. These data were abstracted by company coders from the plant's personnel files. The data include the start date, end date, Department number, job title, and percent time for each job that an employee held at the plant. Workers were identified as having worked in a specific department if they had spent at least 3 days in that department. More recently, EHI recorded departmental assignments as short as several hours; three days, however, appears to have been a more consistent minimum threshold over the first six decades of plant operation. The Occupational Exposure Module of the EHI covered the time period from January 1, 1918 to December 31, 1986.

A company audit of a sample of 143 plant employees indicated an overall error rate of 2.3% for the EHI Occupational Exposure variables when compared to the original personnel records. The error rate for Department (used to classify exposure) was 2.5%. An independent audit of the EHI work history database, based on a sample of 193 employee records, concluded that the error rates estimated in the internal audit were accurate, but that approximately 9% of the exposed employees may not be identified through EHI.

While EHI provided a reasonably accurate record of the departmental work history of hourly production workers, it was much less precise for maintenance and salaried workers. Personnel records identified maintenance workers through their assignment to a general maintenance department. While these records contained maintenance job titles, they did not begin recording the specific production departments

in which an individual maintenance worker had actually worked until January 1, 1982. Plant workers often moved back and forth from production to maintenance jobs. Measures of cumulative lengths of exposure cannot accurately measure the contribution of time spent in an exposed department as a maintenance worker prior to 1982.

The Personnel Department also did not consistently record the departmental assignments of salaried workers. The main study investigators attempted to identify all salaried exposed (i.e. workers who were salaried at the time of their exposure), through interviews with active and retired management personnel. Salaried workers who had worked in exclusionary departments may have been missed by EHI and inadvertently included in the unexposed group. The extent of misclassification is believed to be small, as only 4.1% of unexposed workers achieved salaried status throughout their tenure of employment, and of these only a fraction might have rotated through exclusionary departments while salaried. As a precaution, unexposed salaried workers who self-reported having worked in exclusionary departments were excluded from the analysis.

Another limitation of the EHI Occupational Exposure Module is that between 1969 and 1978 it did not clearly or consistently separate Departments 236 (pentachlorophenol) and 237 (chlorophenols). This confusion is due to the fact that after 1969 these adjacent departments were, for personnel purposes, considered to function as a single unit. Between 1969 and 1978 EHI misclassified Department 237 operators to Department 236. These workers could fortunately be identified by their "237-operator" job title. After the shutdown of Department 236 in 1978, many Department 237 workers were still coded as working in Department 236. These misclassifications were corrected prior to analysis.

2. Occupational and Environmental History Questionnaire

Each participant was asked to respond to an Occupational and Environmental History Questionnaire prepared and administered by the University of Illinois Survey Research Laboratory. The purpose of this questionnaire was to obtain information on work history independent of the EHI Occupational Exposure Module. This questionnaire was composed of a: 1) Plant-specific section; 2) Non-plant section; 3) Contacts section; and 4) Last Job Held section. The Plant-specific section asked questions about dates of hire and termination for each term of employment at the study plant; date of employment, length of employment, job title, and personal protective equipment worn in each exposed department; and employment in exclusionary departments. The Non-plant specific section asked questions about hire date, termination date, place of employment, product, and department for each job held before and after employment at the plant. The Contacts section asked questions about occupational exposures to chlorophenols, chlorophenoxy acids, and PCBs outside the study plant and nonoccupational exposures to wood preservatives, pesticides, solvents, and other chemicals. The Last Job Held section asked questions about current employment status and, if the participant was disabled, the nature of the disability.

The Occupational and Environmental History Questionnaire was administered in a face to face interview by a trained interviewer who was blinded to the participant's health status.

3. Chloracne as a Marker of Exposure

In the present study incident cases of chloracne were identified through a review of the plant medical records using the method developed by Dr. Michael O'Malley of NIOSH and by participants' self-reporting of a medical diagnosis of chloracne. Prevalent cases of chloracne were

diagnosed by a board-certified dermatologist's physical examination of the participants.

D. Ascertainment of Health Status

Each participant was asked to respond to a Worker Medical Questionnaire prepared and administered by the University of Illinois Survey Research Laboratory. The purpose of this questionnaire was to obtain information on the medical health history (including reproductive history) independent of the medical exams that workers received. The Medical Health Questionnaire was administered in a face to face interview by a trained interviewer who was blinded to the participant's exposure status.

An attempt was made to contact each spouse or partner of every participating worker and each was asked to respond to a Spouse/Partner Medical Questionnaire prepared and administered by the University of Illinois Survey Research Laboratory. Again, the purpose of this questionnaire was to obtain information on the medical and reproductive health histories of these individuals independent of the questionnaire responses of their worker spouse/partner. Like the worker Medical Health Questionnaire, the Spouse/Partner Medical Questionnaire was administered in a face to face interview by a trained interviewer who was blinded to the exposure status of the participant's spouse/partner. In addition, birth certificates were obtained, where possible, for children born to cohort workers for the purpose of supplementing missing birth dates for children and for evaluating the accuracy of worker and spouse/partner recall. Unfortunately, the mandatory practice of registering births with birth certificates was not universally adopted by states until the 1950s. Consequently, birth certificates could be obtained for only about two-thirds of the live births.

E. Selection of the Thesis Study Population

1. Creation of a PC-Based Worker Data File

A master worker raw data file for the thesis analysis was maintained on an IBM-compatible PC. This file consisted of 857 variables for each of 835 plant workers (776 workers examined as part of the main study and 59 others). The variables captured in this file included worker employment history (start and end dates), medical examination data, birth certificate data, worker responses to the Occupational and Environmental History Questionnaire, and worker and some spouse/partner responses to the Medical History Questionnaires. A separate spouse/partner master raw data file was created, which included selected data from the Spouse/Partner Medical History Questionnaire, but only limited analysis of this data file was undertaken.

2. Creation of the Worker Data Files for Analysis

The raw data file was used to create four study groups to fulfill two purposes. First, it was felt that differentiating between any effects observed in workers ever exposed to pentachlorophenol versus unexposed workers, and those observed among workers only exposed to pentachlorophenol *versus* unexposed workers would help in the attempt to discriminate between any measured effects caused by pentachlorophenol exposure and those due to its PCDD/PCDF contaminants. These analytical distinctions were felt to be important to avoid confounding from the significantly different pattern of PCDD/PCDF contamination in penta *versus* the phenoxyherbicide esters and lower chlorinated phenols to which ever-exposed penta workers were also exposed. Based on a review of the pharmacokinetic literature, it was expected that any adverse effects on fertility resulting from pentachlorophenol exposure would be

of much shorter duration than those of PCDDs/PCDFs because of the much longer biological half-life of the latter compounds (weeks *versus* approximately 7 years).

Second, it was felt that running the fertility analyses using both subjective worker recalled data on reproductive events as well as objective data from birth certificates on these events would yield more useful information on the reliability of male worker recall for such events. The Air Force Ranch Hand project found that early evidence of adverse reproductive effects based on data supplied by military personnel and their spouses was not substantiated when the analyses were run with objective medical data acquired on these subjects. (Wolfe, Michalek, Miner & Rahe, 1992; Wolfe et al., 1995)

The first pair of study groups focused on data regarding the live births fathered by workers who ever worked in the penta production area, Department 236 (so-called "236E" workers), some of whom had been employed in other departments with potential PCDD/PCDF exposures, and the live births fathered by the unexposed workers. The only difference between these files was that the one of the study groups ("236EB") primarily relied on birth certificate data for the outcomes and dates of birth of the (live born) children, whereas the other study group ("236ER") relied almost exclusively on worker recall for the outcomes and dates of birth of the (live born) children. The sex ratio analysis relied on worker recall of the sex of their children.

The second pair of study groups focused on data concerning the live births fathered by workers whose only PCDD/PCDF exposure came from work in Department 236 (so-called "236O" workers), and the live births fathered by the unexposed workers. As for the 236E workers, the two files differed only by the source of information concerning birth outcomes and dates of birth of live born children. These two study

groups are referred to as "2360B" and "2360R". Again, The sex ratio analysis relied on worker recall of the sex of their children.

The creation of the four final study group datasets for the fertility analyses began with the development of a series of inclusionary criteria. Starting with the PC-based master raw dataset, the first criterion included only those workers who were ever examined as part of the main study, since they were the only workers from whom medical examine data and personal questionnaire information were collected that would support the proposed analyses. This yielded 776 workers: 303 unexposed workers and 473 exposed workers.

The second criterion included only male workers who ever had exposure in a penta production department and male unexposed workers. The reason for this criterion is evident. As seen in Table X, this inclusionary criterion reduced the number of candidate workers for the thesis cohort to 635: 280 unexposed male workers and 355 men who ever worked in penta departments. For the "2360" study groups, the criterion was changed slightly to include male workers who were only exposed in a penta production department and male unexposed workers. The sample size for these study groups was 530: 280 unexposed male workers and 250 men whose only exposure to the substances of interest came from a penta department.

The third criterion included only workers who stated they had been married and who stated that they had never had a sexual partner outside of marriage. The decision to include only married workers was made to relieve methodological and data quality concerns. Data on fertility rates and reproductive performance of males does not exist in the United States, the SFR methodology adopted for the primary hypothesis relies on an indirect assessment of spouses' fertility for whom high quality data exists. Second, single men and married men who admitted to having had

TABLE X

SEX COMPOSITION OF THE EXAMINED COHORT STUDY GROUPS

Exposure History / Sex	UNEXPOSED		EVER-EXPOSED IN DEPT. 236		ONLY-EXPOSED IN DEPT. 236	
	No		Yes		Yes	
	Count	%	Count	%	Count	%
Female	23	7.6%	11	3.0%	10	3.8%
Male	280	92.4%	355	97.0%	250	96.2%
Column Total	303	100.0%	366	100.0%	260	100.0%

an extramarital affairs were not included in the study because it was felt such men may supply unreliable or incomplete data about reproductive events outside of marriage, data that could not be substantiated independently by either their past sexual partners or by birth certificate records. It is recognized that there may have been some underreporting of extramarital behavior, but worker responses were taken at face value. Third, from a computational standpoint, determination of a period at risk of pregnancy is straight-forward for married couples, but becomes problematic for never married men or men in extramarital relationships.

The fourth criterion included only workers who stated they had been married once. A limitation of the fertility analysis program (described in Section F below) used for the primary hypothesis is that worker marital and reproductive history data input to the program is restricted to that experience shared with the last spouse. For instance, consider a worker married three times who had children only by his first and second spouses. This reproductive information would not be captured by the fertility analysis program. In light of concerns regarding this partial data loss, it was decided to simply remove these workers from the final study groups. As can be seen in Table XI, the prospective male worker study cohort sizes are now 198 unexposed workers and 247 ever-exposed workers, and 198 unexposed workers and 177 workers only exposed to penta.

Finally, the study groups were limited to only those workers whose spouse had never been pregnant by a man other than her worker husband. This criterion was introduced to avoid including births that may have been fathered by a man not part of the study cohort. As one can see in Table XII, this selection criterion eliminated another 35 workers (19 unexposed and 16 exposed) from the Department 236 "ever" study groups,

TABLE XI

MARITAL HISTORY OF THE EXAMINED MALE COHORT STUDY GROUPS

Exposure History / Worker Marital History	UNEXPOSED		EVER-EXPOSED IN DEPT. 236		ONLY-EXPOSED IN DEPT. 236	
	Count	%	Count	%	Count	%
Examined Male Study Group Total	280	100.0%	355	100.0%	250	100.0%
Never Married	11	3.9%	7	2.0%	5	2.0%
Ever Married:	269	96.1%	348	98.0%	245	98.0%
Married Once	206	73.6%	255	71.8%	183	73.2%
Married Once & No Extramarital Affairs	198	70.7%	247	69.6%	177	70.8%

TABLE XII

MARITAL HISTORY OF THE SPOUSES' OF EXAMINED MALES WHO WERE MARRIED ONLY ONCE WITH NO HISTORY OF AN EXTRAMARITAL AFFAIR

Exposure History / Spouse Marital History	UNEXPOSED		EVER-EXPOSED IN DEPT. 236		ONLY-EXPOSED IN DEPT. 236	
	Count	%	Count	%	Count	%
Married	198	100.0%	247	100.0%	177	100.0%
Ever Pregnant by Another Man	19	9.6%	16	6.5%	11	6.2%
Pregnant Only by Worker Husband	179	90.4%	231	93.5%	166	93.8%

leaving 410 workers (179 unexposed worker and 231 ever exposed). This selection criterion eliminated another 30 workers (19 unexposed and 11 exposed) from the Department 236 "only" study groups, leaving 345 workers (179 unexposed worker and 166 only exposed).

At this point, the Department 236 "ever" study group of 410 total workers included worker recall of the birth outcome and dates of birth for 1,137 "live" births, 480 children born to 179 unexposed workers and 657 children born to 231 ever-exposed workers. In fact, 16 of the "live" births (10 incorrectly recalled by unexposed workers and 6 by exposed workers) were stillbirths incorrectly recalled as live births. These "live births" were maintained in the worker recall version of this study group to be compared later with the results from the birth certificate augmented outcome and date of birth version of this study group.

The worker recalled data on children's' date of birth was used except for the 36 missing years of birth (from 10 workers) and 50 missing months of birth (from 19 workers, eight of whom had also failed to recall the year of birth for one or more their children). Birth certificate data were available and used for 33 of the 36 missing year of birth values, and for 42 of the 50 missing month of birth values. The three missing year values were derived synthetically by computing the average number of months from marriage to a birth of the missing parity order. Otherwise, missing month and day of birth values were arbitrarily coded to "06" (i.e., June) and "15" (i.e., the middle of the month). Twelve recalled or synthetic birth dates from eight workers were recoded to correct for apparent recall errors or problems that would have resulted in chronologically impossible birth date values (e.g., the birth of a second child occurring after the birth of a third child). Otherwise, in keeping with the original analytical strategy to evaluate the influence of worker recall on reproductive events, worker

recalled dates were used as they existed in the original data files. Discrepancies between recalled and birth certificate dates of birth were not corrected in this file. For instance, if a worker recalled the month of a birth as September, and the birth certificate date indicated it was March, the September value was allowed to remain in the worker recall-based study cohort file.

At this same time, the Department 236 "ever" study group, based primarily on birth certificate data, included birth outcome and dates of birth for 1,121 live births, 470 children born to 179 unexposed workers and 651 children born to 231 ever-exposed workers. The 16 stillbirths incorrectly recalled as live births in the worker recalled 236E file, were removed from this data file. Of the 1,121 live births, 766 of the birth dates (67.37%) were obtained from birth certificates. The remaining birth dates were obtained from worker recall except for three dates corrected for apparent worker recall or coding errors, and two birth years for which there were no birth certificate data and the recalled years conflicted with the preceding and successive births, so synthetic birth year values were created.

The Department 236 "only" study group based primarily on birth certificate data for children's birth outcomes and dates of birth contained 945 live births, 470 children born to 179 unexposed workers and 475 children born to 166 workers only exposed to penta. The corresponding figures for the companion study group based on worker recall of childrens' date of birth contained 959 "live" births, 480 children born to 179 unexposed workers and 479 children born to 166 workers only exposed to penta.. The difference in the number of live births was again due to the Department 236 "only" workers recalling 14 live births that birth certificate data revealed were stillbirths.

These stillbirths were retained in the worker recalled file for the reasons enumerated above.

As a result of a program written for the secondary hypothesis regarding the sex ratio of workers' children, a flaw was discovered in the fertility analysis program that incorrectly classified all pre-marital conceptions leading to live births as pre-employment conceptions, regardless of the fact that some occurred after employment. These individual cases were then identified and deleted from each of the four study groups. Table XIII presents the final number of births among the unexposed and exposed workers for the final four study groups. It should be noted that these final study group data file changes did not materially affect the results of the fertility analyses run with the uncorrected study group data, nor did it alter any of the conclusions for any of the study groups. Nonetheless, these changes did provide the correct values for the number of pre- and post-employment conceptions leading to live births that were consistent with the sex ratio analysis conducted for the secondary hypothesis.

Another limitation for these data files was the fertility analysis program's restriction that dates of employment for no more than 30 consecutive jobs could be included for each case. In these data files, where most workers ever employed in Department 236 were rotated in and then rotated out after a relatively short duration of exposure and where there were up to 51 periods of employment in an exposed Department, this restriction could have meant a serious loss of data.

However, only four workers, all exposed, had more than 15 different periods of exposure or employment at the plant, and only two had more than 22 different exposure periods. Thus, the loss of this information should not have had a profound effect on the fertility

TABLE XIII

CONSTRUCTION OF THE FINAL STUDY GROUPS

Study Group / Study Group Cohort Composition	EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS		EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS		ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS		ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS	
	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed
Examined Males, Married Once with no Affairs, & Spouse Married Once	179	231	179	231	179	166	179	166
Deletions to Correct for SFRCAL Error	4	6	3	5	4	4	3	3
Final Study Group Column Total	175	225	176	226	175	162	176	163
Final Study Group Total	400		402		337		339	

analyses since all post-employment exposed births are grouped together for the fertility analyses.

3. Description of the Four Final Study Groups

Table XIV presents the breakdown of the racial composition of the four final thesis study groups. The racial composition in the group comprising all examined males (data not shown) is similar between the unexposed worker (85 percent white, 15 percent black) and exposed workers (88 percent white, 12 percent black). For the final thesis study groups, the racial composition is even more similar between unexposed worker and exposed workers (about 92 percent white). No statistically significant differences were evident in the racial composition between unexposed worker and exposed workers in any of the four study groups.

Table XV presents the age distribution of the four final thesis study groups. It is apparent that the age imbalance noted earlier still persists. The unexposed worker population is younger than the exposed populations. These age distribution differences were highly statistically significant ($p<0.0001$) for each study group, according to chi-square analysis. The mean and standard deviation of exposed workers' ages in the four final thesis study groups, and among all examined penta-exposed males (N=355, Mean=58.75 years; Std. Dev.=10.44 years) are similar to one another. Likewise, the mean and standard deviation of unexposed workers' ages in the four final thesis study groups, and among all examined unexposed males (N=280, Mean=51.93 years; Std. Dev.=14.76 years) are similar. Clearly, though there are age differences between the unexposed and exposed workers.

TABLE XIV

RACIAL COMPOSITION OF THE FINAL COHORT STUDY GROUPS

Study Group / Race	EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS			
	Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
White	161	92.0%	209	92.9%	161	91.5%	210	92.9%	161	92.0%	150	92.6%	161	91.5%	151	92.6%
Black	14	8.0%	15	6.7%	15	8.5%	15	6.6%	14	8.0%	12	7.4%	15	8.5%	12	7.4%
Other	0	0.0%	1	0.4%	0	0.0%	1	0.4%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Column Total	175	100.0%	225	100.0%	176	100.0%	226	100.0%	175	100.0%	162	100.0%	176	100.0%	163	100.0%
Study Group Total	400				402				337				339			

TABLE XV

AGE DISTRIBUTION AT EXAMINATION OF THE FINAL COHORT STUDY GROUPS

Study Group / Age at Exam	EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS			
	Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
Unknown	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
<20	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
20-24	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
25-29	12	6.9%	0	0.0%	12	6.8%	0	0.0%	12	6.9%	0	0.0%	12	6.8%	0	0.0%
30-34	21	12.0%	6	2.7%	21	11.9%	6	2.7%	21	12.0%	4	2.5%	21	11.9%	4	2.5%
35-39	8	4.6%	3	1.3%	8	4.5%	3	1.3%	8	4.6%	2	1.2%	8	4.5%	2	1.2%
40-44	19	10.9%	9	4.0%	19	10.8%	9	4.0%	19	10.9%	7	4.3%	19	10.8%	7	4.3%
45-49	15	8.6%	28	12.4%	15	8.5%	29	12.8%	15	8.6%	15	9.3%	15	8.5%	16	9.8%
50-54	14	8.0%	20	8.9%	14	8.0%	20	8.8%	14	8.0%	15	9.3%	14	8.0%	15	9.2%
55-59	21	12.0%	47	20.9%	22	12.5%	47	20.8%	21	12.0%	31	19.1%	22	12.5%	31	19.0%
60-64	25	14.3%	48	21.3%	25	14.2%	48	21.2%	25	14.3%	35	21.6%	25	14.2%	35	21.5%
65-69	20	11.4%	36	16.0%	20	11.4%	36	15.9%	20	11.4%	30	18.5%	20	11.4%	30	18.4%
70-74	17	9.7%	16	7.1%	17	9.7%	16	7.1%	17	9.7%	13	8.0%	17	9.7%	13	8.0%
75-79	1	0.6%	11	4.9%	1	0.6%	11	4.9%	1	0.6%	10	6.2%	1	0.6%	10	6.1%
80 and Over	2	1.1%	1	0.4%	2	1.1%	1	0.4%	2	1.1%	0	0.0%	2	1.1%	0	0.0%
Column Total	175	100.0%	225	100.0%	176	100.0%	226	100.0%	175	100.0%	162	100.0%	176	100.0%	163	100.0%
Mean Age	51.95		58.74		51.97		58.69		51.95		59.56		51.97		59.49	
Standard Deviation	14.40		10.08		14.36		10.10		14.40		10.04		14.36		10.07	
Study Group Total	400				402				337				339			

Table XVI presents the distribution of the level of educational achievement for the four final thesis study groups. The unexposed worker population is better educated than the exposed populations. This difference between the cohorts may reflect a secular trend toward hiring better educated employees over time, or that better educated employees were not placed in high hazard production operations. Regardless of the cause, the proportion of men in the higher education categories among the unexposed workers exceeded the proportion for the corresponding exposed workers. Approximately 45 percent of the unexposed workers in each study cohort completed more than a high school education versus only about 25 percent of the exposed workers. These education distribution differences were highly statistically significant ($p<0.0001$) for each study group, according to chi-square analysis. The distribution of educational achievement among all examined penta-exposed males was nearly identical to that of exposed workers in the four final thesis study groups, and that of all examined unexposed males was nearly the same as that of the unexposed workers in the four final thesis study groups (data not shown).

Table XVII presents the income distribution in 1985 for the four final thesis study groups. The unexposed workers reported higher income than the exposed workers. This difference may simply reflect the fact that the younger, better educated, unexposed workers were more likely to still be gainfully employed somewhere while the older, more poorly educated, exposed workers were more likely to be retired and living on a fixed income. In each study group, the proportion of men in the higher income categories ($30,000 or more) among the unexposed workers exceeded the proportion for the corresponding exposed workers. The income distribution among all examined penta-exposed males was nearly identical to that of exposed workers in the four final thesis study groups, and

TABLE XVI

EDUCATION LEVEL DISTRIBUTION OF THE FINAL COHORT STUDY GROUPS

Study Group / Education Level	EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS			
	Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
< Grammar School	7	4.0%	21	9.3%	7	4.0%	21	9.3%	7	4.0%	19	11.7%	7	4.0%	19	11.7%
Some High School	14	8.0%	46	20.4%	15	8.5%	47	20.8%	14	8.0%	33	20.4%	15	8.5%	34	20.9%
High School Graduate/GED	75	42.9%	102	45.3%	75	42.6%	102	45.1%	75	42.9%	72	44.4%	75	42.6%	72	44.2%
Post-High School/Tech. Training	9	5.1%	9	4.0%	9	5.1%	9	4.0%	9	5.1%	9	5.6%	9	5.1%	9	5.5%
Some College	47	26.9%	41	18.2%	47	26.7%	41	18.1%	47	26.9%	23	14.2%	47	26.7%	23	14.1%
College Graduate	10	5.7%	5	2.2%	10	5.7%	5	2.2%	10	5.7%	5	3.1%	10	5.7%	5	3.1%
Post-Graduate Work/Degree	13	7.4%	1	0.4%	13	7.4%	1	0.4%	13	7.4%	1	0.6%	13	7.4%	1	0.6%
Column Total	175	100.0%	225	100.0%	176	100.0%	226	100.0%	175	100.0%	162	100.0%	176	100.0%	163	100.0%
Study Group Total	400				402				337				339			

TABLE XVII

INCOME DISTRIBUTION IN 1985 OF THE FINAL COHORT STUDY GROUPS

Study Group / Annual Income in 1985	EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS			
	Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
< $10,000	2	1.1%	7	3.1%	2	1.1%	7	3.1%	2	1.1%	4	2.5%	2	1.1%	4	2.5%
$10,000-19,999	29	16.6%	53	23.6%	29	16.5%	54	23.9%	29	16.6%	50	30.9%	29	16.5%	51	31.3%
$20,000-29,999	38	21.7%	52	23.1%	38	21.6%	51	22.6%	38	21.7%	33	20.4%	38	21.6%	32	19.6%
$30,000-39,999	50	28.6%	61	27.1%	51	29.0%	61	27.0%	50	28.6%	40	24.7%	51	29.0%	40	24.5%
$40,000-49,999	37	21.1%	28	12.4%	37	21.0%	29	12.8%	37	21.1%	18	11.1%	37	21.0%	19	11.7%
> $50,000	19	10.9%	23	10.2%	19	10.8%	23	10.2%	19	10.9%	16	9.9%	19	10.8%	16	9.8%
Refused to Answer	0	0.0%	1	0.4%	0	0.0%	1	0.4%	0	0.0%	1	0.6%	0	0.0%	1	0.6%
Column Total	175	100.0%	225	100.0%	176	100.0%	226	100.0%	175	100.0%	162	100.0%	176	100.0%	163	100.0%
Study Group Total	400				402				337				339			

that of all examined unexposed males was nearly the same as that of the unexposed workers in the four final thesis study groups. The income distribution differences between the all examined unexposed and penta-exposed males, and the exposed and unexposed workers in the Department 236 "ever" study groups were not statistically significant. However, the income distribution differences between unexposed workers and exposed workers were statistically significant for the Department 236 "only" study groups.

Table XVIII presents the age distribution of the spouses for the four final thesis study groups. As expected based on the age distribution differences noted for workers in Table XV, the spouses of unexposed workers tend to be younger than those of the exposed workers. In each study group, the proportion of spouses in the younger age categories among the unexposed workers exceeded the proportion for the corresponding exposed workers. These age distribution differences were highly statistically significant ($p<0.0001$) for each study group. The mean and standard deviation of the ages of exposed workers' spouses in the four final thesis study groups, and among all examined penta-exposed males are similar, as are the mean and standard deviation of the ages of unexposed workers' spouses in the four final thesis study groups, and among all examined unexposed males. In addition, over one-third of the wives of unexposed workers are 45 or younger, and thus potentially fertile. In contrast, only 12 percent of the wives of exposed workers are 45 or younger.

Table XIX presents the number and sex of live born children, conceived prior to or after employment at the plant, born to unexposed workers and exposed workers for the four final study groups. The proportion of male babies in the pre- and post-employment categories are used later (Tables XXXII through XXXIV) to evaluate the secondary

TABLE XVIII

AGE DISTRIBUTION OF THE WIVES' OF THE FINAL COHORT STUDY GROUP MEMBERS

Study Group / Age of Spouse*	EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				EVER-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH BIRTH CERTIFICATE DATA ANALYSIS				ONLY-EXPOSED IN DEPT. 236 & UNEXPOSED WITH WORKER RECALLED DATA ANALYSIS			
	Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
Missing	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
<20	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
20-24	1	0.6%	0	0.0%	1	0.6%	0	0.0%	1	0.6%	0	0.0%	1	0.6%	0	0.0%
25-29	16	9.1%	0	0.0%	16	9.1%	0	0.0%	16	9.1%	0	0.0%	16	9.1%	0	0.0%
30-34	18	10.3%	5	2.2%	18	10.2%	5	2.2%	18	10.3%	4	2.5%	18	10.2%	4	2.5%
35-39	11	6.3%	4	1.8%	11	6.3%	4	1.8%	11	6.3%	2	1.2%	11	6.3%	2	1.2%
40-44	13	7.4%	18	8.0%	13	7.4%	19	8.4%	13	7.4%	13	8.0%	13	7.4%	14	8.6%
45-49	22	12.6%	23	10.2%	23	13.1%	23	10.2%	22	12.6%	15	9.3%	23	13.1%	15	9.2%
50-54	9	5.1%	28	12.4%	9	5.1%	28	12.4%	9	5.1%	18	11.1%	9	5.1%	18	11.0%
55-59	24	13.7%	54	24.0%	24	13.6%	54	23.9%	24	13.7%	38	23.5%	24	13.6%	38	23.3%
60-64	29	16.6%	42	18.7%	29	16.5%	42	18.6%	29	16.6%	30	18.5%	29	16.5%	30	18.4%
65-69	17	9.7%	29	12.9%	17	9.7%	29	12.8%	17	9.7%	22	13.6%	17	9.7%	22	13.5%
70-74	10	5.7%	14	6.2%	10	5.7%	14	6.2%	10	5.7%	13	8.0%	10	5.7%	13	8.0%
75-79	4	2.3%	7	3.1%	4	2.3%	7	3.1%	4	2.3%	6	3.7%	4	2.3%	6	3.7%
80 and Over	1	0.6%	1	0.4%	1	0.6%	1	0.4%	1	0.6%	1	0.6%	1	0.6%	1	0.6%
Column Total	175	100.0%	225	100.0%	176	100.0%	226	100.0%	175	100.0%	162	100.0%	176	100.0%	163	100.0%
Mean Age	50.87		57.13		50.86		57.08		50.87		57.90		50.86		57.82	
Standard Deviation	14.38		9.92		14.34		9.93		14.38		10.26		14.34		10.28	
Study Group Total	400				402				337				339			

* Age values presented were used in the fertility analyses of the four data files. The ages were obtained from spouses' recall, calculated from birth certificate data, or derived from a substitution technique (husband's age minus two). All wives were assumed to be alive to maintain comparability. The age of the first spouse was used for the examined males who were ever married.

TABLE XIX

NUMBER AND SEX OF CHILDREN CONCEIVED PRIOR TO AND AFTER EMPLOYMENT FOR ALL FOUR STUDY GROUPS

Timing of Conceptions / Study Group	Pre-Employment				Post-Employment				Total Number of Births				Grand Total
	Boys		Girls		Boys		Girls		Boys		Girls		
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	
Study Group 1[a]													
Unexposed	106	53.8%	91	46.2%	111	42.9%	148	57.1%	217	47.6%	239	52.4%	456
Ever-Exposed Dept. 236	154	49.2%	159	50.8%	162	50.8%	157	49.2%	316	50.0%	316	50.0%	632
Total:	260	51.0%	250	49.0%	273	47.2%	305	52.8%	533	49.0%	555	51.0%	1,088
Study Group 2[b]													
Unexposed	106	53.8%	91	46.2%	119	43.3%	156	56.7%	225	47.7%	247	52.3%	472
Ever-Exposed Dept. 236	156	49.2%	161	50.8%	159	49.5%	162	50.5%	315	49.4%	323	50.6%	638
Total:	262	51.0%	252	49.0%	278	46.6%	318	53.4%	540	48.6%	570	51.4%	1,110
Study Group 3[c]													
Unexposed	106	53.8%	91	46.2%	111	42.9%	148	57.1%	217	47.6%	239	52.4%	456
Only-Exposed Dept. 236	117	51.8%	109	48.2%	121	50.8%	117	49.2%	238	51.3%	226	48.7%	464
Total:	223	52.7%	200	47.3%	232	46.7%	265	53.3%	455	49.5%	465	50.5%	920
Study Group 4[d]													
Unexposed	106	53.8%	91	46.2%	119	43.3%	156	56.7%	225	47.7%	247	52.3%	472
Only-Exposed Dept. 236	118	51.5%	111	48.5%	118	49.4%	121	50.6%	236	50.4%	232	49.6%	468
Total:	224	52.6%	202	47.4%	237	46.1%	277	53.9%	461	49.0%	479	51.0%	940

Study Group 1[a] = Ever-Exposed Dept. 236 & Unexposed Workers Using Birth Certificate Dates of Childrens' Birth for the Fertility Analysis
Study Group 2[b] = Ever-Exposed Dept. 236 & Unexposed Workers Using Worker Recalled Dates of Childrens' Birth for the Fertility Analysis
Study Group 3[c] = Only-Exposed Dept. 236 & Unexposed Workers Using Birth Certificate Dates of Childrens' Birth for the Fertility Analysis
Study Group 4[d] = Only-Exposed Dept. 236 & Unexposed Workers Using Worker Recalled Dates of Childrens' Birth for the Fertility Analysis

hypothesis concerning the live-born sex ratio of children born to workers. A noteworthy finding in Table XIX is the reduced proportion of boys conceived by unexposed workers after employment at the plant.

F. Statistical Methods of Analysis

1. Reduced Fertility among Exposed Workers

The primary study hypothesis was evaluated with a fertility analysis software package created by the Chemical Industry Institute for Toxicology (CIIT) in Research Triangle Park, North Carolina during the early 1980s. The FORTRAN source code for this software package was kindly provided by Ms. Michelle Brown and Mr. James Gerard of CIIT, along with a copy of the fertility analysis software users' manual. (Shaw & DalCorso, 1983) The CIIT software package, which was designed to run on a Digital Equipment Corporation VAX mainframe computer, consists of nine programs. The most important of these programs are SFRCAL, TABLIT, and FSTAT. These are briefly discussed below.

The SFRCAL program tallies the observed births and calculates the expected number of live births classified according to each worker's exposure and marital status, and his spouse/partner's parity, and calculates the Standardized Fertility Ratio (SFR) for these user-defined groups pursuant to the SFR methodology developed at CIIT. (Levine et al., 1980; Levine, Symons, Balogh, Milby & Whorton, 1981) The TABLIT program produces a summary table of pre- and post-employment births and fertility from SFRCAL. The FSTAT program calculates a point estimate for theta (θ), the ratio of the SFRs of the exposed workers post-employment and all workers pre-employment, the 90 percent confidence interval for θ, and the binomial probabilities of a birth

given exposure and of observing X or fewer births given two sets of observed and expected births.

The source code for the fertility software package was compiled on an IBM-compatible PC using Microsoft FORTRAN 5.1 for the PC. Only relatively minor modifications were needed to the 62 files received from CIIT in order to complete the mainframe to PC porting process.

The only other change or modification to the CIIT software package was to update the national birth probability data for U.S. women from 1981 through 1986, the year that the worker interviews were conducted. The SFRCAL program uses these maternal age-, birth year-, parity level, and race-specific birth probabilities from the National Center for Health Statistics (NCHS) to calculate the expected number of live births for each woman, and overall. The 1981-1986 birth probabilities for U.S. white women were kindly supplied by Dr. Rob Schnatter, Exxon Biomedical, East Millstone, New Jersey, in two electronic data files derived from published NCHS data. The 1981-1986 NCHS birth probabilities for U.S. nonwhite women were obtained from published NCHS annual vital statistics series data (National Center for Health Statistics, 1985; National Center for Health Statistics, 1986; National Center for Health Statistics, 1987a; National Center for Health Statistics, 1987b; National Center for Health Statistics, 1988a; National Center for Health Statistics, 1988b) and, after validation for accuracy, added to the existing birth probability values.

2. Altered Sex Ratio in Children

The proportion of liveborn boys conceived by unexposed and exposed workers, pre- and post-employment, in the four final study groups was compared to the expected proportion of live born white male babies, based on empirical vital statistics data from the United States between

1975 and 1986 (National Center for Health Statistics, 1988b, p. 40), using a test of inferences about a single proportion formulae. (Fleiss, 1981, p. 13)

A reference proportion based on the expected proportion of live born white male babies was used as a first-cut approximation to the demographics of the study groups since approximately 92 percent of all of the workers in the four study groups were white. Since the sex ratio among black Americans is slightly lower than among whites (e.g., an average of 51.4 percent boys for whites versus an average of 50.7 percent boys for blacks during the period 1975-1986), the use of this approximation would slightly overestimate the reference proportion of boys and slightly increase the likelihood that a reduction in the observed proportion of boys would be declared statistically significant. Solving the formulae given by Fleiss yields a z-score from the normal curve. The z-score can then be evaluated against some critical value. A two-tailed test of significance was used.

Also, the proportion of baby boys born during the pre-employment period to unexposed workers and exposed workers in the four final thesis study groups was compared using a test of significance between two independent sample proportions. (Fleiss, 1981, p. 23)

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III. RESULTS

A. Main Hypothesis

Tables XX through XXIII present the results of the Standardized Fertility Ratio (SFR) analysis program for the four final study groups. Collectively, the fertility of these plant workers, exposed and unexposed, exceeded the expected by 11 to 14 percent (*i.e.*, SFRs ranged from 1.11 to 1.14). In addition, after marriage the unexposed and exposed workers in each of the four study groups have higher than expected fertility based on values derived from national fertility rates. The unexposed workers' post-employment fertility universally surpassed the post-employment fertility of the exposed workers. The similar patterns of the findings and the stability of the results across the four study groups among these four Tables is noteworthy.

Tables XXIV through XXVII depict the data from Tables XX through XXIII in a different fashion. They summarize the post-marriage reproductive experience of the four study groups for all parities and for parities greater than or equal to one (parity 1+), providing sums of the number of conceptions leading to live births by employment period (i.e., pre-employment period, and post-employment period) and exposure category (i.e., unexposed and exposed workers). As described in Chapter 1, the pre-employment live births are pooled for unexposed and exposed workers in the calculation of the pre-employment SFR, while the post-employment SFRs are presented for unexposed and exposed workers separately. Tables XXVIII and XXIX summarize the post-marriage reproductive experience of the Department 236 "ever" exposed study groups with the exposed workers including only those men diagnosed with current or historical diagnoses of chloracne.

TABLE XX

FERTILITY ANALYSIS OF THE STUDY GROUP COMPOSED OF DEPT. 236 EVER-EXPOSED AND UNEXPOSED WORKERS
BASED ON BIRTH CERTIFICATE DATES OF CHILDREN'S BIRTH

RISK PARAMETERS	PARITY																	
	0			1			2			3			≥4			TOTAL		
EXPOSURE STATUS : MARITAL STATUS	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR
All Workers Pre-Employment : Pre-Marriage	50	243.3	0.21	2	2.4	0.83	0	0.0	0.00	0	0.0	0.00	0	0.0	0.0	52	245.8	0.21
All Workers Pre-Employment : Post-Marriage	202	70.8	2.85	141	130.4	1.08	61	59.7	1.02	33	23.0	1.43	21	19.3	1.09	458	303.2	1.51
Unexposed Worker after Employment : Post-Marriage	56	25.3	2.21	77	59.6	1.29	56	46.2	1.21	24	25.9	0.93	46	17.4	2.64	259	174.4	1.48
Exposed Worker after Non-Exposed Employment Period: Post-Marriage	52	22.2	2.34	84	63.8	1.32	71	76.3	0.93	36	35.3	1.02	48	39.5	1.22	291	236.9	1.23
Exposed Worker after Exposed Employment Period: Post-Marriage	6	3.7	1.62	9	5.0	1.80	6	7.1	0.85	3	1.5	2.00	4	2.7	1.48	28	20.0	1.40
TOTAL	366	365.4	1.00	313	261.3	1.20	194	189.3	1.02	96	85.6	1.12	119	78.7	1.51	1,088	980.3	1.11

	OBS	EXP	SFR
All Workers Pre-Employment : Post-Marriage	256	227.4	1.10
Unexposed Worker after Employment : Post-Marriage	203	149.1	1.36
Exposed Worker after Non-Exposed Employment Period: Post-Marriage	239	247	41
Exposed Worker after Exposed Employment Period: Post-Marriage	22	16.3	1.35

TABLE XXI

FERTILITY ANALYSIS OF THE STUDY GROUP COMPOSED OF DEPT. 236 EVER-EXPOSED AND UNEXPOSED WORKERS
BASED ON WORKER RECALLED DATES OF CHILDREN'S BIRTH

RISK PARAMETERS	PARITY																	
	0			1			2			3			≥4			TOTAL		
EXPOSURE STATUS : MARITAL STATUS	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR
All Workers Pre-Employment : Pre-Marriage	50	245.9	0.20	2	2.1	0.95	0	0.0	0.00	0	0.0	0.00	0	0.0	0.0	52	248.1	0.21
All Workers Pre-Employment : Post-Marriage	201	70.7	2.84	142	129.9	1.09	63	60.1	1.05	33	22.8	1.45	23	19.6	1.17	462	303.2	1.52
Unexposed Worker after Employment : Post-Marriage	58	25.6	2.27	77	59.6	1.29	60	48.3	1.24	31	25.3	1.23	49	20.2	2.43	275	178.9	1.54
Exposed Worker after Non-Exposed Employment Period: Post-Marriage	54	22.5	2.40	87	64.3	1.35	70	74.8	0.94	38	34.6	1.10	47	40.0	1.18	296	236.2	1.25
Exposed Worker after Exposed Employment Period: Post-Marriage	5	3.8	1.32	7	5.1	1.37	6	6.9	0.87	3	1.2	2.50	4	3.4	1.18	25	20.4	1.23
TOTAL	368	368.5	1.00	315	261.1	1.21	199	190.1	1.05	105	84.0	1.25	123	83.2	1.48	1,110	986.8	1.12

TABLE XXII

FERTILITY ANALYSIS OF THE STUDY GROUP COMPOSED OF DEPT. 236 ONLY-EXPOSED AND UNEXPOSED WORKERS
BASED ON BIRTH CERTIFICATE DATES OF CHILDREN'S BIRTH

RISK PARAMETERS	PARITY																	
	0			1			2			3			≥4			TOTAL		
EXPOSURE STATUS : MARITAL STATUS	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR
All Workers Pre-Employment : Pre-Marriage	44	201.9	0.22	2	2.4	0.83	0	0.0	0.00	0	0.0	0.00	0	0.0	0.00	46	204.4	0.23
All Workers Pre-Employment : Post-Marriage	161	57.5	2.80	118	107.7	1.10	53	50.3	1.05	27	20.9	1.29	18	15.5	1.16	377	251.9	1.50
Unexposed Worker after Employment : Post-Marriage	56	25.3	2.21	77	59.6	1.29	56	46.2	1.21	24	25.9	0.93	46	17.4	2.64	259	174.4	1.48
Exposed Worker after Non-Exposed Employment Period: Post-Marriage	40	17.8	2.25	63	45.2	1.39	49	55.8	0.88	29	23.0	1.26	44	32.3	1.36	225	174.2	1.29
Exposed Worker after Exposed Employment Period: Post-Marriage	5	1.9	2.63	3	3.4	0.88	1	3.9	0.26	1	0.3	3.33	3	1.2	2.50	13	10.7	1.22
TOTAL	306	304.5	1.00	263	218.4	1.20	159	156.3	1.02	81	70.2	1.15	111	66.3	1.67	920	815.6	1.13

TABLE XXIII

FERTILITY ANALYSIS OF THE STUDY GROUP COMPOSED OF DEPT. 236 ONLY-EXPOSED AND UNEXPOSED WORKERS
BASED ON WORKER RECALLED DATES OF CHILDREN'S BIRTH

RISK PARAMETERS	PARITY															TOTAL		
	0			1			2			3			≥4					
EXPOSURE STATUS : MARITAL STATUS	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR	OBS	EXP	SFR
All Workers Pre-Employment : Pre-Marriage	44	204.5	0.22	2	2.1	0.95	0	0.0	0.00	0	0.0	0.00	0	0.0	0.00	46	206.6	0.22
All Workers Pre-Employment : Post-Marriage	160	57.4	2.79	119	107.2	1.11	54	50.7	1.07	27	20.8	1.30	20	15.8	1.27	380	251.9	1.51
Unexposed Worker after Employment : Post-Marriage	58	25.6	2.27	77	59.6	1.29	60	48.3	1.24	31	25.3	1.23	49	20.2	2.43	275	178.9	1.54
Exposed Worker after Non-Exposed Employment Period: Post-Marriage	42	18.0	2.33	65	46.3	1.40	49	54.6	0.90	29	22.0	1.32	42	32.5	1.29	227	173.5	1.31
Exposed Worker after Exposed Employment Period: Post-Marriage	4	1.9	2.11	2	3.4	0.59	1	3.8	0.26	1	0.3	3.33	4	1.3	3.08	12	10.7	1.12
TOTAL	308	307.4	1.00	265	218.7	1.21	164	157.5	1.04	88	68.4	1.29	115	69.8	1.65	940	821.7	1.14

TABLE XXIV

POST-MARRIAGE LIVE BIRTH ANALYSIS OF THE STUDY GROUP COMPOSED OF
DEPT. 236 EVER-EXPOSED AND UNEXPOSED WORKERS
BASED ON BIRTH CERTIFICATE DATES OF CHILDREN'S BIRTH

EXPOSURE AND PARITY STATUS	TOTAL		
SFR Analysis for All Parities:	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	458	303.2	1.51
Unexposed Worker after Employment	259	174.4	1.48
Exposed Worker after Employment	319	256.9	1.24
TOTAL LIVE BIRTHS (All Parities)	1,036	734.5	
SFR Analysis for Parity ≥1	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	256	232.4	1.10
Unexposed Worker after Employment	203	149.1	1.36
Exposed Worker after Employment	261	231.0	1.13
TOTAL LIVE BIRTHS (Parity ≥1)	720	612.5	

DSW 476038.1663

TABLE XXV

POST-MARRIAGE LIVE BIRTH ANALYSIS OF THE STUDY GROUP COMPOSED OF
DEPT. 236 EVER-EXPOSED AND UNEXPOSED WORKERS
BASED ON WORKER RECALLED DATES OF CHILDREN'S BIRTH

EXPOSURE AND PARITY STATUS	TOTAL		
SFR Analysis for All Parities:	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	462	303.2	1.52
Unexposed Worker after Employment	275	178.9	1.54
Exposed Worker after Employment	321	256.6	1.25
TOTAL LIVE BIRTHS (All Parities)	1,058	738.7	
SFR Analysis for Parity ≥1	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	261	232.5	1.12
Unexposed Worker after Employment	217	153.3	1.42
Exposed Worker after Employment	262	230.3	1.14
TOTAL LIVE BIRTHS (Parity ≥1)	740	616.1	

TABLE XXVI

POST-MARRIAGE LIVE BIRTH ANALYSIS OF THE STUDY GROUP COMPOSED OF
DEPT. 236 ONLY-EXPOSED AND UNEXPOSED WORKERS
BASED ON BIRTH CERTIFICATE DATES OF CHILDREN'S BIRTH

EXPOSURE AND PARITY STATUS	TOTAL		
SFR Analysis for All Parities:	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	377	251.9	1.50
Unexposed Worker after Employment	259	174.4	1.48
Exposed Worker after Employment	238	184.9	1.29
TOTAL LIVE BIRTHS (All Parities)	874	611.2	
SFR Analysis for Parity ≥1	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	216	194.4	1.11
Unexposed Worker after Employment	203	149.1	1.36
Exposed Worker after Employment	193	165.2	1.17
TOTAL LIVE BIRTHS (Parity ≥1)	612	508.7	

TABLE XXVII

POST-MARRIAGE LIVE BIRTH ANALYSIS OF THE STUDY GROUP COMPOSED OF
DEPT. 236 ONLY-EXPOSED AND UNEXPOSED WORKERS
BASED ON WORKER RECALLED DATES OF CHILDREN'S BIRTH

EXPOSURE AND PARITY STATUS	TOTAL		
SFR Analysis for All Parities:	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	380	251.9	1.51
Unexposed Worker after Employment	275	178.9	1.54
Exposed Worker after Employment	239	184.2	1.30
TOTAL LIVE BIRTHS (All Parities)	894	821.7	
SFR Analysis for Parity ≥1	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	220	194.4	1.13
Unexposed Worker after Employment	217	153.3	1.42
Exposed Worker after Employment	193	164.3	1.17
TOTAL LIVE BIRTHS (Parity ≥1)	630	512.0	

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TABLE XXVIII

POST-MARRIAGE LIVE BIRTH ANALYSIS OF THE STUDY GROUP COMPOSED OF DEPT. 236 EVER-EXPOSED CHLORACNE AND UNEXPOSED WORKERS BASED ON BIRTH CERTIFICATE DATES OF CHILDREN'S BIRTH

EXPOSURE AND PARITY STATUS	TOTAL		
SFR Analysis for All Parities:	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	213	138.4	1.54
Unexposed Worker after Employment (N=175)	259	174.4	1.49
Exposed Worker with Chloracne after Employment (N=30)	28	25.9	1.08
TOTAL LIVE BIRTHS (All Parities)	500	338.7	
SFR Analysis for Parity ≥1	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	120	106.6	1.13
Unexposed Worker after Employment (N=154)	203	149.1	1.36
Exposed Worker after Employment (N=27)	22	23.3	0.94
TOTAL LIVE BIRTHS (Parity ≥1)	345	279.0	

TABLE XXIX

POST-MARRIAGE LIVE BIRTH ANALYSIS OF THE STUDY GROUP COMPOSED OF DEPT. 236 EVER-EXPOSED CHLORACNE AND UNEXPOSED WORKERS BASED ON WORKER RECALLED DATES OF CHILDREN'S BIRTH

EXPOSURE AND PARITY STATUS	TOTAL		
SFR Analysis for All Parities:	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	214	138.4	1.55
Unexposed Worker after Employment (N=176)	275	178.9	1.54
Exposed Worker with Chloracne after Employment (N=30)	28	25.8	1.09
TOTAL LIVE BIRTHS (All Parities)	517	343.1	
SFR Analysis for Parity ≥1	**OBS**	**EXP**	**SFR**
All Workers Pre-Employment (Post-Marriage)	121	106.6	1.14
Unexposed Worker after Employment (N=154)	217	153.3	1.42
Exposed Worker after Employment (N=27)	22	23.0	0.96
TOTAL LIVE BIRTHS (Parity ≥1)	360	282.9	

In Tables XXIV to XXVII, for all parities, the pooled post-marriage pre-employment fertility of the four final study groups was substantially greater than expected (SFRs = 1.50 to 1.52), based on national birth probabilities. The SFRs of the unexposed workers post-employment were virtually identical to the pre-employment/post-marriage SFRs (SFRs = 1.48 to 1.54). In contrast, the fertility of the exposed workers post-employment was substantially lower than those either pre-employment/post-marriage or among the unexposed post-employment (SFRs = 1.24-1.30), although their fertility rates were still greater than expected, based on national birth probabilities. The SFRs of the unexposed and exposed groups pre-employment were virtually identical, 1.55 for the unexposed group and 1.49 for the exposed group (data not shown).

For the parity 1+ experience, the pooled post-marriage pre-employment fertility of the four final study groups were only slightly greater than expected (SFRs = 1.10 to 1.13), based on national birth probabilities. Unlike the all parities fertility experience, the SFRs of the unexposed workers post-employment were substantially larger than those of the pre-employment/post-marriage SFRs (SFRs = 1.36 to 1.42). Again, unlike the all parities experience, the fertility of the exposed workers post-employment overlapped that of the pre-employment/post-marriage period (SFRs = 1.13-1.17), although their fertility rates were still greater than expected. As with the all parity fertility experience, the SFRs of the unexposed and exposed groups pre-employment were virtually identical, 1.14 for the unexposed group and 1.08 for the exposed group (data not shown).

In Tables XXVIII to XXIX, the pre-employment/post-marriage fertility of the Department 236 "ever" exposed study groups (with the exposed being defined as only those men diagnosed with current or historical diagnoses of chloracne) were again substantially greater than

expected (SFRs=1.54 and 1.55), based on national birth probabilities. Again, the SFRs of the unexposed workers were virtually identical to the pre-employment/post-marriage SFRs (SFRs=1.49 and 1.54). Again, the fertility of the exposed workers post-employment was substantially lower than those either pre-employment/post-marriage or among the unexposed, but here the exposed workers' fertility was markedly lower (SFRs=1.08-1.09) than those in the "ever" exposed final study groups, although their fertility rates were still marginally greater than expected based on national birth probabilities.

Table XXX presents the point estimates and 90 percent confidence intervals (CI) for theta (θ), the ratio of the SFRs of the exposed and pre-employment workers, for the four final study groups, and the chloracne subgroup analyses, for all parities and the parity 1+ experience. The values for θ were 0.82 for both of the "ever" exposed study groups, and 0.86 for both of the "only" exposed study groups, indicating that the exposed workers had roughly 82 or 86 percent of the fertility of the unexposed workers. The upper 90 percent confidence limit failed to include unity for any of the four study groups. In the Department 236 "ever" exposed study groups with chloracne, the values for θ were 0.70 for both of the chloracne subgroups, indicating that the exposed workers had a 30 percent reduction in fertility relative to the unexposed workers. The upper 90 percent confidence limit for these two subgroups also failed to include unity.

In contrast, for the parity 1+ fertility experience, the values for θ were 1.03 and 1.01 for the "ever" exposed study groups, and 1.04 and 1.05 for the "only" exposed study groups. These result indicate that the exposed workers were of comparable fertility to the pre-employment fertility experience. All of the 90 percent confidence intervals included unity. In the Department 236 "ever" exposed study

TABLE XXX

STATISTICAL SUMMARY OF THE STUDY GROUPS' FERTILITY

STUDY GROUP	θ = SFR (Exposed) / SFR (Pre-Employment)		90% C.I.	
	PARITY ≥0	PARITY ≥1	PARITY ≥0	PARITY ≥1
Study Group 1[a]	0.82	1.03	0.73, 0.93	0.88, 1.19
Study Group 1[b] (Exposed Chloracne Workers)	0.70	0.84	0.49, 0.99	0.55, 1.24
Study Group 2[a]	0.82	1.01	0.73, 0.93	0.87, 1.17
Study Group 2[b] (Exposed Chloracne Workers)	0.70	0.84	0.49, 0.99	0.55, 1.25
Study Group 3[c]	0.86	1.05	0.75, 0.99	0.89, 1.24
Study Group 4[d]	0.86	1.04	0.75, 0.99	0.88, 1.23

Study Group 1[a] = Ever-Exposed Dept. 236 & Unexposed Workers Using Birth Certificate Dates of Childrens' Birth for the Analysis
Study Group 1[b] = Ever-Exposed Dept. 236 Workers with Chloracne & Unexposed Workers Using BC DOBs of Children for the Analysis
Study Group 2[a] = Ever-Exposed Dept. 236 & Unexposed Workers Using Worker Recalled Dates of Childrens' Birth for the Analysis
Study Group 2[b] = Ever-Exposed Dept. 236 Workers with Chloracne & Unexposed Workers Using Worker Recalled DOBs of Children
Study Group 3[c] = Only-Exposed Dept. 236 & Unexposed Workers Using BC DOBs of Children for the Analysis
Study Group 4[d] = Only-Exposed Dept. 236 & Unexposed Workers Using Worker Recalled DOBs of Children for the Fertility Analysis

groups with chloracne, the values for θ were 0.84 for both of the chloracne subgroups, indicating that the exposed workers had a 16 percent reduction in fertility relative to the pre-employment fertility. However, the upper 90 percent confidence limit for these two subgroups also included unity.

B. Secondary Hypothesis

Table XXXI presents a tally of the number and the sex of live born white children in the United States from vital statistics records. Based on data for the 12-year period 1975-1986, constituting over 33 million births, baby boys constitute about 51.4 percent of white live births. This value, 51.4 percent, is used as the reference proportion in the test of inferences about a single proportion formulae by Fleiss. (Fleiss, 1981, p. 13)

Table XXXII presents the statistical analysis of the proportion of live born boys conceived by the unexposed and exposed groups during pre- and post-employment periods, and overall (all parities). The exposed workers with chloracne did display a lower sex ratio post-employment, but this result was not statistically significant. The only statistically significant finding ($p<0.01$) was the reduction in the proportion of boys born to unexposed workers who were conceived after employment began at the plant.

Table XXXIII examines the pre-employment proportion of male babies between the unexposed and exposed workers for the four study groups. No statistically significant differences were evident between the two groups using a test of significance between two independent sample proportions. (Fleiss, 1981, p. 23)

TABLE XXXI

U.S. VITAL STATISTICS DATA ON LIVE BIRTHS OF WHITE BABIES: 1975-1986*

SEX / YEAR	BOYS		GIRLS		TOTAL	
	Count	Proportion	Count	Proportion	Count	%
1986	1,523,914	0.51303	1,446,525	0.48697	2,970,439	8.8%
1985	1,536,646	0.51369	1,454,727	0.48631	2,991,373	8.8%
1984	1,500,326	0.51319	1,423,176	0.48681	2,923,502	8.6%
1983	1,492,385	0.51386	1,411,865	0.48614	2,904,250	8.6%
1982	1,509,704	0.51315	1,432,350	0.48685	2,942,054	8.7%
1981	1,494,437	0.51379	1,414,232	0.48621	2,908,669	8.6%
1980	1,490,140	0.51407	1,408,592	0.48593	2,898,732	8.6%
1979	1,442,981	0.51381	1,365,439	0.48619	2,808,420	8.3%
1978	1,378,222	0.51405	1,302,894	0.48595	2,681,116	7.9%
1977	1,383,440	0.51409	1,307,630	0.48591	2,691,070	8.0%
1976	1,319,717	0.51399	1,247,897	0.48601	2,567,614	7.6%
1975	1,312,308	0.51423	1,239,688	0.48577	2,551,996	7.5%
Total:	17,384,220	0.51373	16,455,015	0.48627	33,839,235	100.0%

* Reference: National Center for Health Statistics (1988). Vital Statistics of the United States, 1986, Volume 1, Natality. Washington, DC: U.S. Government Printing Office.

TABLE XXXII

STATISTICAL ANALYSIS OF THE PROPORTION OF LIVE BORN MALE CHILDREN IN THE STUDY GROUPS *VERSUS* THE EXPECTED VALUE BASED ON U.S. VITAL STATISTICS DATA

TIMING OF CONCEPTIONS / STUDY GROUP	PRE-EMPLOYMENT			POST-EMPLOYMENT			ALL BIRTHS		
	Proportion Boys	# Live Births	*p*-Value	Proportion Boys	# Live Births	*p*-Value	Proportion Boys	# Live Births	*p*-Value
Unexposed Workers									
Study Group 1[a]	0.5381	197	0.54	0.4286	259	0.0074	0.4759	456	0.12
Study Group 2[b]	0.5381	197	0.54	0.4327	275	0.0086	0.4767	472	0.12
Study Group 3[c]	0.5381	197	0.54	0.4286	259	0.0074	0.4759	456	0.12
Study Group 4[d]	0.5381	197	0.54	0.4327	275	0.0086	0.4767	472	0.12
Exposed Workers									
Study Group 1[a]	0.4920	313	0.48	0.5078	319	0.88	0.5000	632	0.52
Study Group 1[b]	0.4894	47	0.85	0.3929	28	0.28	0.4533	75	0.35
Study Group 2[a]	0.4921	317	0.48	0.4953	321	0.55	0.4937	638	0.33
Study Group 2[b]	0.4894	47	0.85	0.3929	28	0.28	0.4533	75	0.35
Study Group 3[c]	0.5177	226	0.96	0.5084	238	0.92	0.5129	464	0.97
Study Group 4[d]	0.5153	229	0.96	0.4937	239	0.58	0.5043	468	0.72

Study Group 1[a] = Ever-Exposed Dept. 236 & Unexposed Workers Using Birth Certificate Dates of Childrens' Birth for the Fertility Analysis

Study Group 1[b]= Ever-Exposed Dept. 236 Workers with Chloracne Using Birth Certificate Dates of Childrens' Birth for the Fertility Analysis

Study Group 2[a] = Ever-Exposed Dept. 236 & Unexposed Workers Using Worker Recalled Dates of Childrens' Birth for the Fertility Analysis

Study Group 2[b] = Ever-Exposed Dept. 236 Workers with Chloracne Using Worker Recalled Dates of Childrens' Birth for the Fertility Analysis

Study Group 3[c] = Only-Exposed Dept. 236 & Unexposed Workers Using Birth Certificate Dates of Childrens' Birth for the Fertility Analysis

Study Group 4[d] = Only-Exposed Dept. 236 & Unexposed Workers Using Worker Recalled Dates of Childrens' Birth for the Fertility Analysis

TABLE XXXIII

STATISTICAL ANALYSIS OF THE PROPORTION OF LIVE BORN MALE CHILDREN IN THE STUDY GROUPS PRE-EMPLOYMENT: EXPOSED *VERSUS* UNEXPOSED WORKERS

TIMING OF CONCEPTIONS / STUDY GROUP	PRE-EMPLOYMENT				
	Unexposed		Exposed		
	Proportion Boys	# Live Births	Proportion Boys	# Live Births	*p*-Value
Study Group 1[a]	0.5381	197	0.4920	313	0.36
Study Group 2[b]	0.5381	197	0.4921	317	0.36
Study Group 3[c]	0.5381	197	0.5177	226	0.75
Study Group 4[d]	0.5381	197	0.5153	229	0.71

Table XXXIV examines the post-employment anomaly in the sex ratio among unexposed workers. The births were stratified into five year time periods, according to a child's sex and the fathers age at the child's birth (≤30 and >30). When the data are rearranged, 155 of the 305 children born to fathers ≤30 (50.8 percent) are sons, while only 62 of 151 births to fathers >30 (41.1 percent) are sons.

C. Concordance Analysis for Dates of Birth

A separate analysis was conducted that compared the dates of childrens' birth recalled by workers in the Department 236 "ever" exposed study group against birth certificate data matched for the same worker and pregnancy. Table XXXV presents the results of this analysis. There was remarkably close concordance between the recalled and birth certificate dates of birth, with nearly 83 percent of the 733 recalled live births occurring within one month of the actual date of birth recorded on a birth certificate. There were a few outliers, but they were too few to substantially skew the results of these fertility analyses. The close concordance between the recalled and birth certificate dates of birth goes far in explaining the similar patterns of results among these analyses.

TABLE XXXIV

ANALYSIS OF THE BIRTHS OF CHILDREN OF UNEXPOSED WORKERS
STRATIFIED ON THE TIME PERIOD OF BIRTH, SEX OF CHILD, AND FATHER'S AGE

CHILDRENS' YEAR OF BIRTH	PRE-EMPLOYMENT				POST-EMPLOYMENT			
	Father ≤ 30		Father > 30		Father ≤ 30		Father > 30	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
1925-1929	1	1	0	0	0	0	0	0
1930-1934	2	2	0	0	0	0	0	0
1935-1339	3	3	0	0	1	0	0	0
1940-1944	8	5	2	0	6	4	1	2
1945-1949	14	9	1	3	2	9	4	4
1950-1954	9	12	2	2	13	18	5	11
1955-1959	13	15	3	5	7	7	9	16
1960-1964	16	12	0	2	7	7	15	10
1965-1969	12	3	2	2	10	7	8	11
1970-1974	3	3	0	1	4	8	4	12
1975-1979	11	10	1	0	3	1	3	4
1980-1984	3	1	0	0	7	12	2	3
1985-1989	0	0	0	0	0	1	0	1
SUBTOTALS	95	76	11	15	60	74	51	74

TABLE XXXV

ANALYSIS OF THE ACCURACY OF WORKER RECALL OF CHILDRENS' DATE OF BIRTH COMPARED WITH BIRTH CERTIFICATES

TIME DIFFERENCE IN WORKER RECALLED-BIRTH CERTIFICATE DATES OF BIRTH	EVER-EXPOSED DEPT. 236 & UNEXPOSED MALES	
	Count	%
> -2 Years	8	1.1%
> -1 Year to -2 Years	7	1.0%
> -6 Months to -1 Year	24	3.3%
> -3 Months to -6 Months	5	0.7%
> -1 Month to -3 Months	5	0.7%
> 0 to -1 Month	35	4.8%
Exact Match	528	72.0%
> 0 to +1 Month	45	6.1%
> +1 Month to +3 Months	3	0.4%
> +3 Months to +6 Months	4	0.5%
> +6 Months to +1 Year	52	7.1%
> +1 Year to +2 Years	14	1.9%
> +2 Years	3	0.4%
Total Number of Live Births with Data for the Same Birth	733	100.0%
Mean Deviation (Days)	7.95	---
Standard Deviation (Days)	295	---

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IV. DISCUSSION

A. Primary Study Hypothesis

The estimates from the fertility analyses based on all parities using the SFR methodology presented in Chapter III are in the hypothesized direction. Workers ever or only exposed to pentachlorophenol exhibited a reduction in fertility as compared to the unexposed fertility experience of all workers pre-employment. The values for theta (θ), the ratio of the SFR of the exposed workers to that of the unexposed fertility experience of all workers pre-employment, for the four final study groups were either 0.82 or 0.86, indicating that there has been an apparent 14 to 18 percent reduction in fertility among the exposed workers, depending upon the exposure type. At a significance level of 0.10, this reduction reached statistical significance in all four of the study groups evaluated. The small group of penta workers diagnosed with chloracne exhibited an even more profound reduction in fertility as compared to the fertility experience of the unexposed workers. The value for θ was 0.70 for both subgroups analyzed, indicating that the exposed workers with chloracne had a 30 percent reduction in fertility relative to the unexposed fertility experience of all workers pre-employment. At a significance level of 0.10, this reduction was statistically significant for workers with chloracne.

The results of the parity 1+ fertility experience analyses are more complex. Here, the values for theta (θ) for the four final study groups were all greater than one (θ = 1.13 - 1.17), although none of the values of θ reached statistical significance. For exposed workers with chloracne, the value of θ was diminished to 0.84, a 16 percent reduction

in fertility, but given the small numbers of workers this reduction was not statistically significant.

The parity 1+ analyses were conducted to correct for the marital status artifact introduced through use of the national fertility statistics for all women, regardless of their marital status. However, since it limits analyses to women of proven fertility, it omits women with primary infertility, and thus an exposed group of special interest.

The chloracne findings are of special interest. It is unknown whether chloracne is a biomarker of greater individual exposure or greater individual susceptibility to the effects of halogenated hydrocarbons like penta, and PCDDs/PCDFs. If these substances adversely effect fertility, then the more pronounced reduction in fertility among exposed workers with chloracne is consistent with a greater biological effect among these workers. It should be remembered that the number of workers "ever" exposed to penta with chloracne in these study groups was small (n=30). The even smaller number of workers "only" exposed to penta with chloracne (n=13) precluded further analysis and precludes a definitive conclusion. Nonetheless, fertility was consistently and more greatly reduced among the exposed workers with chloracne than among all exposed workers.

As expected, the pre-employment/post-marriage fertility of both unexposed and exposed workers in the four final study groups that included all parities exceeded the expected number of live births. This result was expected "because the U.S. national birth rates do not control for marital status, they represent weighted averages of the underlying birth rates specific to marital status, with the weights given by the proportions of women who are married and unmarried, respectively. These average rates underestimate the birth rates for married women, especially for those age and parity classes in which a substantial fraction of the women are unmarried." (Starr, Levine &

Boyle, 1986, p. 579) Starr *et al.* illustrate this point by noting that married women aged 20-24 in 1965 had a fertility rate twice the national rate. (Starr *et al.*, 1986) Elsewhere, they note that approximately 74 percent of never married women between the ages of 15 and 49 were childless. (Levine *et al.*, 1983) Since only married men were included in the all parities analyses, and they tended to marry at young ages, the fertility of their wives would be expected to be greater than that of all women in their respective age groups.

In fact, one of the vocal critics of the SBR and SFR methodology has disparged these reproductive epidemiology tools precisely because of their insensitivity to reductions in fertility. Dobbins pointed out that Wong *et al.* have acknowledged but underestimated the degree of underestimation of the expected number of live births using only the married experience of the employees compared to the national birth probability rates that include both married and unmarried women. Dobbins estimated that the expected married fertility is underestimated by "...at least 20%." (Dobbins, 1987) In part for this reason, Dobbins repeated the suggestion raised earlier by Wong *et al.* that the alpha level be increased from 5 percent to 10 percent or more to increase the power of a study to detect adverse effects of workplace exposure on fertility. He illustrated this point by stating that had this been done for three fertility studies of ethylene dibromide (EDB) plant workers (Dobbins, 1987), the conclusions on human reproductive risk would possibly have been far different.

It is important to note that the pre-employment fertility of both unexposed and exposed married workers was nearly identical in the final study groups (SFR of 1.49 for the exposed workers and 1.55 for the unexposed workers). This similarity provides reassurance that at least prior to employment the exposed and unexposed workers did not differ

greatly in the characteristics governing fertility. That is, that the value of ϕ pre-employment was comparable in the different thesis cohort members.

In addition, it is important to note that the pre- and post-employment fertility of the unexposed married workers was similar. The significance of this finding is that it contradicts one of the criticisms that Wong *et al.* made of the SFR's comparing post-exposure fertility to pre-exposure fertility. Wong *et al.* opined that such comparisons are inherently flawed and will bias the SFR towards a positive (i.e., reduced fertility) finding because pre-exposure reproductive experience is usually accompanied by younger age, lower parity and earlier calendar time, the exact opposite of the post-exposure experience. (Wong, Morgan & Whorton, 1985) Here Wong *et al.* repeated a methodological weakness originally identified by Harber. (Harber, 1981) That is, there is an artificial increase in theta greater than one for time periods closer to the time of interview. This bias stems from the selection protocol into the study. Since only married men (and their spouses) are included in the study group at the time of interview, the further one moves back in time the more the cohort resembles the national population's mixture of married and unmarried women. Since fertility is higher for married women than unmarried women a comparison of pre- versus post-exposure fertility will bias the results toward a value of theta greater than one. The fertility summary analyses limited to the parity 1+ experience appears to have eliminated much of this bias, and yet preserved the patterns of higher fertility among unexposed workers post-employment versus exposed workers post-employment. However, there was no longer a significant reduction in fertility among any of the exposed workers even though the chloracne workers still had a 16 percent decrease in fertility.

Wong *et al.* state their belief that if the pre- and post-exposure experience differ substantially "...no adjustment procedure will be able to remove the confounding effects completely. In other words, if the two distributions do not overlap substantially, there is no valid basis for comparison." (Wong *et al.*, 1985, pps. 303-304) No evidence of the bias Wong *et al.* describe is evident in these study groups.

Rigorous examination of confounder distributions among the exposed and unexposed study groups was not conducted. Thus, only a detailed analysis of the potential reproductive confounders discussed in Chapter I can provide the required assurances to rule out uncontrolled confounding as the factors responsible for the large and consistent differences observed between the exposed and unexposed workers, confounding that could not be controlled for with the SFR methodology use of national birth probabilities and an internal comparison group (as recommended by both Levine and Wong to better approximate site- or region-specific confounder distributions).

B. Secondary Hypothesis

The meaning of the sex ratio analyses are less clear. Although a reduction in the proportion of boys born to exposed workers after employment was expected, the only statistically significant finding ($p<0.01$) was the reduction in the proportion of boys who were conceived by unexposed workers after employment at the plant. However, the proportion of boys born after employment to exposed workers with chloracne was reduced (39.3 percent (11 boys of 28 children) observed overall and 29 percent (4 boys of 14 children) among fathers 30 or younger, 51.4 percent expected), but the numbers were small and again not statistically significant. Examination of the pre-employment

proportion of male babies between the unexposed and exposed workers for the four study groups revealed no statistically significant differences. Analysis of the sex ratio anomaly among unexposed workers indicates that the excess number of daughters occurred among older fathers, and appeared to occur at higher parity levels. This finding is consistent with the literature that indicates that the sex ratio varies inversely with parity and a father's age.

These results prompt two considerations. First, one might question whether the unexposed workers were in fact exposed to some toxic agent at the plant (penta, PCDDs/PCDFs or some other agent) that may have adversely affected their fertility, as manifested in the disruption in the sex ratio of their children, but not in their reproductive capacity. In line with the thoughts for the first idea are some conceptualized dose-response curves Selevan and LeMasters developed for different theorized reproductive toxins. (Selevan & Lemasters, 1987) Selevan and LeMasters theorized that different reproductive outcomes (responses) may be manifested depending on the dose. "For example, a very high exposure could result in early fetal loss, whereas a lower one might result in a congenital malformation observed at birth." (Selevan & Lemasters, 1987, p. 451) Perhaps, at high exposure levels penta, PCDDs/PCDFs affect male fertility or libido, but at lower levels of exposure above background they affect hormonal levels and alter the child sex ratio as hypothesized by William James.

The simplest explanation for the anomalous finding among unexposed workers is that the older age of these fathers and the higher parity levels of the mothers was responsible for the statistically significant finding. However, the reduction in the sex ratio among workers with chloracne is tantalizing. It may be real and thus be tentative confirmation of the effect hypothesized by William James. Larger numbers of workers and exposed births would be necessary to verify this

supposition. In the interim, it may be prudent to keep in mind the words of Schull *et al.* based on their investigation of an apparent reduction in the sex ratio in post-war Hiroshima and Nagasaki that subsequently disappeared (Schull, & Hashizume, 1966, p. 337) that:

> "We have repeatedly stressed the unsatisfactory nature of the sex ratio as a variable. It is apparently influenced by any number of factors, e.g., maternal age, paternal age, parity, etc. While the effects of these variables are generally small, adequate explanation of their origin has not been advanced despite the existence of data far larger than those pertinent to the radiation problem. Unfortunately, these unexplained perturbations are often lost sight of, and only the elegance of the genetic argument is seen."

C. Limitations of the Study

1. Primary Study Hypothesis

There are several major areas of uncertainty associated with the results of this study. The use of the CIIT fertility analysis package rightfully prompts questions.

The source code for the CIIT fertility package was developed over a period of several years in the early 1980s by a number of programmers, and had not been reviewed, updated, or used by anyone for several years. There is some question regarding the functioning of the original coding, and one of the package's programs, SLICE, that was to have allowed the creation of time windows surrounding exposure periods to help latency and persistence of toxins' effects on reproduction clearly never ran.

There is some uncertainty surrounding the porting of the CIIT fertility analysis package FORTRAN source code written for a Digital Equipment Corporation VAX mainframe to FORTRAN on an IBM-compatible personal computer. The only assurance that the programs run properly was that the output from the PC-ported package was identical to those

from the mainframe results presented in the Users Manual for the test data file provided along with the analysis package.

With respect to the study population, the inclusionary criteria required to suit the study hypothesis and the CIIT fertility analysis programs' limitations eliminated a sizable proportion (about 37 percent) of the unexposed and ever-exposed to penta workers. It is unknown what effect this may have had.

Also, it is important to note that the SFR methodology does not adjust for a number of potentially confounding factors, and it is clear that the unexposed and exposed worker populations differed significantly in a number of respects, including worker and spouse age, and income. The older age of the exposed workers and their spouses may delineate important social, secular, and demographic differences from the unexposed workers. Also, Wong suggests that family size is a function of family income. (Wong *et al.*, 1985) If correct, the greater relative wealth of the unexposed workers would have afforded them the opportunity to create and support larger families, all else being equal. A large number of potential male or female reproductive confounders discussed in Chapter II were not addressed or only partially evaluated for this study. Significant differences, other than those already articulated, may yet exist between the exposed and unexposed worker populations that may have influenced these results.

Too, it must be remembered that the study groups evaluated in these analyses represent survivors of the original unexposed and exposed plant cohorts. It is unknown to what extent the differential forces of mortality and morbidity on these other workers may have affected these results.

Finally, it is crucial to bear in mind that the SFR methodology used for the fertility analyses was developed as a a screening-level

technique. Thus, a non-traditional significace level (α=0.10) was used. If a larger confidence interval had been calculated it is doubtful any of the results would still be statistically significant, even though the point estimates of θ would still be depressed. Too, the results of the analyses of the parity 1+ experience of the workers, as suggested numerous times by Levine *et al.*, contradict the results of the all parities fertility analyses, although suggestive evidence of an effect among exposed workers with chloracne persists. The SFR methodology is not an answer unto itself. Positive findings of reduced fertility were supposed to prompt more thorough assessment of workers with other epidemiological and medical tools. No less can be expected now.

These findings are inconsistent with the limited penta and PCDD/PCDF male animal reproductive testing literature discussed in Chapter I. However, in view of the limitations of laboratory animal models of human male fertility, such nonpositive studies are hardly surprising. In view of the limited epidemiologic literature, only one of the four epidemiologic studies of penta, the study by Gilbert *et al.* alleged to have evaluated male fertility. The numerous deficiencies in that study have already been addressed and will not be repeated. As Dobbins noted, it would take a moderately potent reproductive toxin to reduce theta to unity, and only a relatively potent one could reduce theta sufficiently to achieve statistical significance according to standard protocol. This study, however, found such effects, but the effects persisted (albeit not significantly) only in the presence of chloracne.

In contrast, far larger studies of men potentially exposed to PCDDs/PCDFs during Vietnam service have yet to identify any significant

diminishment in fertility even in the presence of evidence of persistent effects on semen characteristics.

Consequently, these results must be viewed as tentative. Their interpretation must be tempered with great caution given the circumstances surrounding the CIIT SFR analysis package used to generate the results and the nature of the methodology. Nonetheless, in light of these findings and the results of the parity 1+ experience, additional analyses should be performed.

As seen in Table XXXVI, few SFR or SBR studies exist in the literature. Those that exist typically demonstrate elevated fertility among exposed workers, and with the exception of the known male reproductive toxin DBCP, none have reported a statistically significant decrease in fertility. This may in part be due to the marital status artifact discussed earlier, and part may be due to something similar to the healthy worker effect noted in many occupational mortality studies. This study is the largest SFR study conducted, and the second largest among those using the SBR or SFR methodology.

2. Secondary Study Hypothesis

James' theory of parental hormone control or influence over the sex ratio of offspring remains a theory. His postulation that dioxin-exposed men should have a deficit of sons was not clearly supported, although there was suggestive evidence among the men with chloracne. Among the 28 children conceived by exposed men with chloracne after employment, only four sons were born to men 30 or younger out of 14

TABLE XXXVI

SUMMARY OF SFR AND SBR FERTILITY STUDIES OF MALE WORKERS

Study		# Exposed Workers	# Unexposed Workers	SFR Pre-Exposure	SFR Post-Exposure	Theta	Stat. Signif?/ Conf. Int.
Wong et al. (1979)							
EDB Manufacturing Workers	(All Parities)						
	Plant A	ns[a]	---	N/A[b]	0.99	N/A	No
	Plant B	ns	---	N/A	0.95	N/A	No
	Plant C	ns	---	N/A	0.91	N/A	No
	Plant D	ns	---	N/A	0.51	N/A	Yes
	All 4 Plants	297	---	N/A	0.80	N/A	No; 95% CI = [0.61, 1.03]
Levine et al. (1980)							
Organic Chemical Workers	(All Parities)						
	Plant A	27	---	1.78	1.44	0.81	90% CI = [0.21, 2.16]
	Plant B	14	---	1.00	1.56	1.57	90% CI = [0.48, 4.10]
Levine et al. (1981)							
DBCP Workers	(All Parities)	39	---	1.88	0.75	0.40	90% CI = [0.13, 0.94]
Levine et al. (1983)							
DBCP Workers	(All Parities)	87	---	1.41	0.88	0.62	90% CI = [0.39, 0.96]
	(Parity 1+)	ns	---	1.23	0.63	0.51	90% CI = [0.28, 0.90]
Wong et al. (1985)							
Wastewater Treatment Plant Workers	(All Parities)	55	---	N/A	1.82	N/A	95% CI = [1.14, 2.76]
DBCP Workers	(All Parities)	36	---	N/A	0.64	N/A	95% CI = [0.23, 1.39]
Lauwerys et al. (1985)							
Mercury-exposed Workers	(All Parities)	103	101	1.03	0.90	1.03[c]	90% CI = [0.65, 1.16][c]
Manganese-exposed Workers	(All Parities)	85	81	1.07	0.55	0.52[c]	90% CI = [0.37, 0.71][c]
Schnatter (1990)							
Corporate Office Employees	(All Parities)	508	---	N/A	1.41	N/A	95% CI=[1.33, 1.50]
Welch et al. (1991)							
Glycol Ether-exposed Painters	(All Parities)	74	51	1.68	1.58	0.94	90% CI = [0.70, 1.26]
Lemasters et al. (1991)							
Wastewater Treatment Plant Workers	(All Parities)	133	86	2.43	2.16	0.89	90% CI = [0.72, 1.10]
	(Parity 1+)	ns	ns	1.78	1.79	1.01	90% CI = [0.74, 1.36]
Dolan (1995)							
Pentachlorophenol Workers	(All Parities)	226	176	1.52	1.25	0.82	90% CI = [0.73, 0.93]
	(Parity 1+)	208	154	1.12	1.14	1.01	90% CI = [0.87, 1.17]
PCP Workers with Chloracne	(All Parities)	30	176	1.54	1.08	0.70	90% CI = [0.49, 0.99]
	(Parity 1+)	27	154	1.14	0.96	0.84	90% CI = [0.55, 1.25]

[a] = Not Stated

[b] = Not Applicable to the SBR methodology

[c] = Calculated from data presented in the original paper

children (p=0.15). What makes this limited data more intriguing is that at these younger paternal ages one would expect a preponderance of sons. Thus, it is possible that only very high dioxin exposure or susceptibility, as manifested by exposed workers with chloracne, is required to manifest a significant reduction in the sex ratio. Nonetheless, the marked decrease in the proportion of sons born even among the chloracne workers was not statistically significant given their small numbers. An anomalous decrease in the sex ratio among unexposed workers after employment was deemed due to older paternal age.

D. Recommendations for Future Research

There are several additional steps that could be taken to refine these results. First, other statistical models, such as proportional hazards or regression models should be used with the current thesis datasets, or better still include all of the married males. These results could then be compared against the current results. The advantage of these other programs is that they would permit the testing and control of multiple confounding factors. Independent confirmation of these preliminary results with an alternative methodology would lend credence to the results of the SFR analyses. The exposure matrix NIOSH was developing for its national Dioxin Registry might be evaluated for its ability to possibly develop dose estimates for individual workers based on job codes and titles that could be incorporated into these alternative statistical models. Also, the serum hormone level data could be analyzed in conjunction with such dose estimates to independently evaluate the effect of pentachlorophenol and its PCDD/PCDF contaminants on endocrine function, similar to a recent NIOSH study. (Egeland *et al.*, 1994)

Alternatively, improvements could be made in the SFR analysis package. However, before any improvements could be made the FORTRAN code for the SFRCAL, TABLIT, and SLICE programs would have to be:

- thoroughly evaluated and updated to contemporary programming standards to ensure that each works properly;
- enhanced to eliminate the limitations on data analysis currently in the program; and
- thoroughly documented for future users.

The modified CIIT fertility analysis programs could then be run again to determine whether the programming changes had any effect on the results and whether these results can be replicated. In particular, it would be very desirable to get the SLICE program running and attempt to discern whether the putative reduction in male fertility following exposure to pentachlorophenol and its PCDD/PCDF contaminants is of short- or long-term duration. This information might help elucidate the mechanism of reproductive toxicity. That is, whether the observed reduction in fertility is due to effects of pentachlorophenol or its much longer lasting PCDD/PCDF contaminants. A speedier alternative to spending time fixing the SLICE program would be to use another program, like SPSS® to pre-process the data to mimic the intended function of the SLICE program.

E. Conclusions

The analyses of the fertility of the four study groups has provided several interesting findings. First, for all parities, workers ever or only exposed to penta suffered impaired fertility as manifested by significantly lower SFRs than unexposed workers. These findings persisted whether based on worker recalled or birth certificate dates of

childrens' birth. The decrease in fertility was most marked among workers with chloracne.

Since these results may have been due to the marital status artifact observed by both Levine and Wong, additional analyses limited to parity 1+ fertility experience was conducted. These results failed to confirm the all parities analyses, although chloracne workers still experienced a large (16 percent) nonsignificant deficit in fertility. The parity 1+ findings may be correct, or they may have given a false level of comfort due to the exclusion of nulliparous spouses of exposed workers. That is, by excluding women with primary infertility, couples potentially most affected as a consequence of their husbands occupational exposure may have been eliminated from the analysis. However, similar percentages of exposed and unexposed workers reported an inability to conceive after one year of trying (22.7 and 22.3 percent, respectively). An analysis of the proportion of nulliparous couples among the exposed and unexposed workers may be be informative.

Second, no statistically significant reduction in the sex ratio of exposed workers post-employment was observed, although a large decrease in the proportion of sons conceived by chloracne workers after employment was seen among exposed fathers post-employment. The fact that the deficit was greatest among the younger fathers (only 4 sons out of 14 births) at a time when a greater proportion of sons would be expected is intriguing. However, the number of births was small. The sex ratio of unexposed workers post-employment was significantly reduced. This finding appears to be due to the older age of these fathers when their children were conceived and probably also to the higher parity levels these births represent.

As Buffler and Aase note: "Several large studies of sex ratio in man have been frustrated by inconsistent results, and the many different factors potentially associated with changes in the sex ratio make the

detection and investigation of the cause of such changes extremely difficult." This study may be another such investigation. (Buffler & Aase, 1982, p. 309) A far larger number of exposed births, particularly among exposed workers with chloracne, would be needed to determine whether the suggestive evidence of a reduction in the sex ratio is real.

Third, workers recalled the dates of birth of their children remarkably well, 83 percent of the recalled birth dates were within 30 days of the date recorded on a birth certificate. These findings are consistent with the results of a reproductive surveillance system investigation for Exxon Biomedical Corporation. (Schnatter, 1990) His doctoral dissertation reported that husbands' and wives' recall of their childrens' birth dates matched exactly for 349 of 361 births (96.7 percent). Other studies have reported high concordance between husbands and wives for the number of births. (Niemi, Hemminki & Sallmén, 1985; Selevan, 1980) Collectively, they are indicative of the general reliability of paternal recall of birth events.

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