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161

FROM: AIHC

DATE: JULY 23, 1979

Mutagenesis, teratogenesis and cancer in the  
offspring of persons occupationally exposed to  
petroleum derived hydrocarbons

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*chrom*  
*biol.*

Report prepared for the American Petroleum Institute.  
June 1978

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AP00012615

mutatis mutandis

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## I. Introduction

This review evaluates epidemiologic evidence for carcinogenic, teratogenic and mutagenic effects of petroleum-derived hydrocarbons in the offspring of occupationally exposed populations. Human studies are considered in the light of pertinent background information from the laboratory and from animal studies. Where epidemiologic investigations are reviewed, particular attention is paid to the strengths and limitations of the research strategy employed. The last portion of this paper considers the problems which remain unsolved and suggests realistic approaches to clarify those issues.

Derived from the Greek work "repaí", meaning a marvel, prodigy or monster, the word "teratogenic" was used by 1857 to mean the "production of monstrous formations or births" (OED, 1971). Since creation of monsters interests many disciplines, the term has been refined to refer to the biological science dealing with "the causes, mechanisms, and manifestations of developmental deviations of either structural or functional nature (Wilson, 1973). Such causes may be chemical, viral, pharmacological or physical. Staples (1975) pointed out that an agent may act as a teratogen when administered in a number of ways, whether to the male or female before mating, to the female during pregnancy, or to the fetus directly. Strictly defined, agents which alter the rate of growth of the fetus or are lethal to the fetus without producing specific anatomic or functional anomalies are better termed developmental toxins than teratogens. In this discussions of potential adverse effects of environmental agents, particularly polycyclic hydrocarbons and their derivatives, evidence for teratogenicity and for developmental toxicity will be considered.

Agents in the environment may produce alterations in the genome, mutations, and by that mechanism lead to abnormalities of development in the offspring. Viral, drug, or chemical agents acting through common mutagenic pathways may produce indistinguishable results. The outcome of such mutations might be fetal defects or predisposition to neoplasia.

Cancer in the offspring of individuals exposed to a carcinogen might theoretically be the result of any one of three types of exposure: prezygotic, by alteration of parental germ cells; transplacental, by passage of carcinogenic substances across the placental barrier; postnatal, by contamination of the environment with carcinogenic substances, primarily through ingestion, including substances excreted in human breast milk, and through inhalation.

II. Relationships between mutagenic and teratogenic characteristics of an agent and potential for carcinogenicity in the offspring of exposed organisms

It has been argued that the "same chemical that causes abortion in the early stages may produce malformations during organ development and neoplasia when exposed later in pregnancy. Therefore, screening for transplacental hazards should include, whenever possible, the entire range of fetal response, including cancers that may develop sometime after birth" (Fraumeni, 1974). Mutagenesis, teratogenesis and carcinogenesis are related phenomena, but the nature of these relationships is complex and occasionally controversial.

While a relationship exists between teratogenicity and carcinogenicity when a multiplicity of systems are considered, they should not be viewed as expressions of the same phenomenon, mediated only by the stage of development of the exposed organism (Di Paolo and Kotin, 1966). Teratogenic, mutagenic and carcinogenic activities are all demonstrable for a number of compounds, but transplacental carcinogenic potential has rarely been demonstrated for known teratogens and mutagens. Teratogenic effects may reflect a variety of actions involving the mother, the placenta or the feto-placental unit, which can be indirect and may not involve immediate action of the agent on the target fetal tissue. Rice (1973) suggests that transplacental carcinogens do act directly on the fetal tissues which ultimately become neoplastic and that other effects, such as immunosuppression, play a secondary role.

To appreciate potential damage of an environmental exposure to offspring of the exposed organism, one ought well consider the outcome of all exposed pregnancies. Early fetal wastage commonly results from abnormal fetal development. At the other extreme, transplacentally induced tumors

hardly ever appear in rodent species until the animal reaches maturity. In the only documented example of chemical transplacental carcinogenesis in humans, vaginal adenocarcinoma following diethylstilbestrol (DES) exposure, the tumor occurs decades following exposure. Thus, since transplacentally induced tumors, unlike malformations, are rarely present at birth, one must also observe the offspring into adulthood to see the full spectrum of resulting tumors.

Fetal tissue sensitivity to a carcinogen may differ from adult tissue response. Although fetal mice may develop lung tumors following transplacental exposures to the water soluble anesthetic agent ethyl carbonata (Urethan, II), they are much less prone to develop tumors than are adult mice. By contrast, 1-ethyl-1-nitrosourea (ENU, III) produces neurogenic tumors in mice offspring after a single transplacental exposure, but is far less active in the adult mouse (Rice, 1973).

The nature of the tumors produced by in utero exposure to a carcinogen is determined both by the gestational age of the exposed fetus and, most importantly, by the kind of tumors produced by the same agent in the adult of the species. This species or strain specificity doubtless reflects genetic factors. At the same time some agents, which are carcinogenic in adult tissues, are not carcinogenic in fetal tissues, the latter lacking enzymes to metabolize the substance to its active derivative. In other circumstances, carcinogenic derivatives may cross the placenta and act directly on the fetus. A question of considerable interest is whether carcinogenic effects of a parental exposure may be visited on offspring subsequently conceived. Such a trans-generational effect would necessitate alteration in the parental germ cells involving one of three distinct mechanisms: chromosome breakage, point mutation, or abnormalities in gametogenesis or fertilization.

### III. Evidence for trans-generational carcinogenesis in animals

Trans-generational carcinogenic effects have been reported in second generation descendants of mice treated with 7,12-dimethylbenz(a)anthracene and of rats exposed to nitrosomethylurethan. Tomatis and Goodall (1969) exposed pregnant mice to intraperitoneal injections of 400 micrograms of 7,12-dimethylbenz(a)anthracene (DMBA) in olive oil and observed a significant excess of tumors in offspring exposed in utero, and also in the offspring of in utero exposed mice, the F<sub>2</sub> generation. Oncogenic effects in the F<sub>2</sub> generation could, however, reflect persistence of DMBA into adulthood in animals originally exposed while in utero, and its excretion in milk and feces with resultant direct exposure of the F<sub>2</sub> offspring. Germ cell alteration would be the alternative explanation, although tumor excesses in the F<sub>3</sub> generation and in other inbred generations, which would be expected, were not demonstrated.

Untreated offspring of rats which had been exposed in utero to nitrosomethylurethan (NMU) were said to have a higher incidence of tumors than expected in a strain with low spontaneous tumor incidence (Tanaka, 1973). The usual spontaneous tumors in this strain are mammary, 2.1%, and lung adenomas, 1.8%. Among the offspring of two rats whose mothers had been exposed in utero, the frequency of tumors was 7.2% in males and 9.7% in females, with tumors in four and seven animals, respectively. Control animals had tumors in two males (2.1%) and three females (2.7%). The possibility that ostensibly unexposed offspring might have had some exposure through NMU retained or excreted by the in utero exposed mothers was not specifically excluded. Among the untreated offspring of in utero exposed females crossed with untreated males there was no tumor excess, nor were such effects detected in the third generation. Trans-generational carcinogenic effects of NMU exposure require further

documentation and, as a phenomenon in general, trans-generation chemical carcinogenesis requires confirmation. Such doubts were expressed in the discussion and conclusions of the meeting on transplacental carcinogenesis at which Tanaka's work was presented (Tomatis and Mohr, 1973).

IV. Association between congenital abnormalities and childhood neoplasms

Carcinogenic and teratogenic effects of exposure to a given agent might rarely be manifest in the same individual resulting in the development of both a congenital defect and a neoplasm. For some childhood neoplasms, associations with congenital defects have, in fact, been demonstrated. Congenital hemihypertrophy occurs in excess in three childhood neoplasms: Wilms' tumor, primary liver cancer, and adrenocortical neoplasms. Wilms' tumor also occurs in association with aniridia. Diseases involving chromosomal aberrations (Down's syndrome, Bloom's syndrome, and Franconi's aplastic anemia) are associated with leukemia in early childhood. Congenitally impaired cell mediated immunity, as encountered in Wiskott-Aldrich syndrome, and ataxia-telangiectasia, is associated with a childhood excess of lymphoreticular neoplasms (Miller, 1968; Fraumeni, et al., 1968).

Chromosomal defects may be the soil for development of leukemia in Down's syndrome, in which leukemia occurs in a three to fifteen-fold excess. Review of sibship histories for 459 children with leukemia and an equal number of controls matched for age, birth order, family size and race revealed that six leukemic children had Down's syndrome, while that condition was not present in any control child. In addition to Down's syndrome, other major congenital defects, including pyloric stenosis, urinary tract malformations, neurological defects and musculo-skeletal malformations, occurred with greater than expected frequency in leukemic children. In the siblings of leukemic children, Down's syndrome and a variety of tumors (among them bladder neoplasm, acute leukemia, and mesothelioma) occurred more frequently than expected. Congenital defects other than Down's syndrome were not present in excess in the siblings of

leukemic children. Previous pregnancies which terminated in miscarriages were reported more frequently by the mothers of leukemic children than by the mothers of controls. Childhood leukemia and Down's syndrome are both related to increasing maternal age. The increase in Down's syndrome probably reflects increased frequency of nondisjunction with aging, although Down's syndrome with leukemia occurring in children of young mothers may more often reflect chromosomal damage due to translocation. One revealing case reported was that of a child with Down's syndrome who developed acute monocytic leukemia. An abnormal chromosome, reflecting a reciprocal transformation, was present in the proband as well as in the mother, and in two of three unaffected siblings (German, et al., 1962).

To summarize, children with leukemia have an excess of Down's syndrome and congenital abnormalities; their siblings have an excess of other cancers and of Down's syndrome; their mothers report an excess of miscarriages. These associations might be tied to the ability of ionizing radiation or other agents to produce persisting cytogenetic abnormalities. Chromosomal fragility syndromes also are associated with an excess of leukemia. In identical twins, where one sib develops leukemia under age seven, there exists a 20% risk of the same disease developing in the other twin, generally within months. These observations have spurred speculation linking leukemogenesis, especially in children with genetic damage.

V. Knudson's model of multistage mutations and carcinogenesis

A multistage model of carcinogenesis beginning with two or more mutations has been elaborated by Knudson (1973). Such mutations might be induced by chemical, physical or biologic agents. The proper sequence of mutations might induce neoplasia independent of the specific mutagenic stimuli.

Environmental chemical agents can, under certain situations, initiate cancer through inducing mutations. In mouse cells, stable chromosomal alterations produced by 3-methylcholanthrene, can be transmitted in vitro through 30 cell generations. The cells of such transformed colonies are able to produce fibrosarcomas in mice. The mutagenic potential of chemical carcinogens has been exploited through development of in vitro screening systems for potential carcinogenic agents (McCann and Ames, 1976). Radiation-induced carcinogenesis could readily fit the Knudson model, as would viral alteration of the host genome. In non-hereditary forms of cancer, mutation would bring a normal somatic cell one step closer to becoming a tumor cell. Other factors, namely the presence of growth promoters or failure of immune surveillance, might be required to produce a clinically evident tumor.

A related hypothesis would apply to hereditary tumor syndromes. A mutation occurring in germ cells might persist and be transmitted to offspring. Predisposition to neoplasia would be inherited since one of the mutational steps leading to carcinogenesis would have occurred prezygotically. Not only would there be a familial predisposition to the tumor, but clinical cases would be expected to occur early in life, since part of the required sequence of mutations would be present at birth. In recognized hereditary tumor syndromes, tumors characteristically do appear decades earlier than the same tumors in the

general population. For some childhood tumors it is proposed that, perhaps, as few as two mutations are required. In hereditary patterns of early childhood tumors, one of these mutations would have been inherited through the parental germ cell.

Retinoblastoma appears to fit this model. The relative responsiveness of this tumor to treatment has permitted affected individuals to reproduce, and the observed pattern of the disease in the offspring of patients with retinoblastoma is consistent with the hypothesis outlined above. Bilaterally affected individuals with retinoblastoma appear to carry a dominant gene for the disease and 50% of their offspring are carriers and are affected. Among unilaterally affected individuals, only 10% of the offspring are affected, suggesting that a substantial proportion of unilateral cases are not inheritable. Hereditary cases presumably reflect transmission of a mutation carried in a parental germ cell. Each cell in the retina of the carrier offspring bears the first mutation, predisposing the cell to malignant transformation by further insult. Undefined, but presumably widespread, environmental factors would affect some of the retinal cells and the carrier of the defect would develop one or multiple tumors in the retina. The frequency distribution of tumors per gene carrier has been enumerated and is regarded as consistent with a Poisson distribution of tumors per carrier having a mean of three events per person (Knudson, 1971). Since the susceptible embryonal cell population vanishes in early childhood, persons carrying the gene have a 95% probability of ever developing retinoblastoma. It is possible that the predisposition to neoplasia in carriers of the retinoblastoma gene is not entirely confined to the retina and that other tissues may be more prone than expected to develop

sarcomas, particularly following therapeutic irradiation (Li, 1977). Other tumors of childhood might show patterns of inheritance suggesting prezygotic mutation, if affected individuals were to survive and reproduce. Knudson and Strong (1972a, and 1972b) have proposed similar models for Wilms' tumor and for neuroblastoma.

VI. Use of peripheral blood lymphocytes for the analysis of chromosomal abnormalities

The human blood lymphocyte can be utilized as a chronicle of genetic damage. Changes in chromosome structure are readily apparent when these cells are stimulated to divide, and this method has been used to measure damage produced by environmental or occupational exposure to chemical or physical agents, including irradiation, benzene and vinyl chloride.

These techniques have a number of advantages. The cells are readily sampled, long-lived, and distributed throughout the body. Peripheral lymphocytes are synchronized in the same  $G_0$  or  $G_1$  stage of mitotic interphase and rarely proliferate in vivo in healthy persons. Stimulation by mitogens causes some cells to undergo division in a cell culture system and these dividing cells are readily scored for chromosome aberrations.

The lymphocytes are not an entirely homogeneous system, and there may be different responses to stimuli in different subpopulations. It is possible that the less damaged cells may grow better in culture than those which have been heavily damaged. Since it is the T-lymphocyte population which, in fact, multiplies and since these cells are closely concerned with the immune response, other antigenic stimuli to the organism may affect the growth of these cells in culture and alter the number of cells with chromosome aberrations.

Although scoring for chromosome-type structural aberrations (Figure 1) is generally reliable, scoring more subtle chromatid-type aberrations is much more open to inter-observer variability, and to artifacts resulting from technical processes. This must be taken into account where chromatid and isochromatid breaks and gaps are included in the total number of aberrations.

rationale for their inclusion in these counts is that certain chemical agents and viruses may cause only chromatid-type damage. An important technical factor which affects the yield of aberrations is the length of time in which cells are maintained in culture. The first division for the majority of transformed lymphocytes occurs after two days of culture at 37° C. After longer periods of culture, many cells will be in their second or subsequent division and readings at this time may reflect selective reproduction of lymphocytes with lesser degrees of damage.

These factors constitute limitations to the use of human lymphocyte culture systems for study of in vivo response to environmental mutagens. It is clear that chromosome aberration rates cannot readily be compared between studies unless procedures have been carefully coordinated and standardized between investigators. Secondly, the importance of matching study and control groups by age, sex and pertinent environmental factors is apparent. Such factors include occupational history, radiation and drug histories, exposures to toxic substances, and immunologic stimulation (viral infections, recent immunizations). The samples themselves should be processed simultaneously with processing and coding done blindly, and coding best done by someone not directly involved in the conduct of the investigation. To eliminate technical and observer bias such precautions are essential (Evans and O'Riordan, 1977).

Figure 1

|                      | NORMAL | TERMINAL<br>DELETION | INTERSTITIAL<br>DELETION | CENTRIC<br>AND ACRO-<br>TERMINAL<br>FRAGMENT | ACENTRIC<br>RING | TERMINAL<br>INVERSION |
|----------------------|--------|----------------------|--------------------------|----------------------------------------------|------------------|-----------------------|
| SYNDROMIC<br>GROUP 1 |        |                      |                          |                                              |                  |                       |
| SYNDROMIC<br>GROUP 2 |        |                      |                          |                                              |                  |                       |

Fig. 1 Seven classes of chromosome-type aberrations cytologically distinguishable at mitotic metaphase. Evans and O'Riordan, 1977.

VII. Environmental threats to pregnancy outcome

Hirschorn and Cohen (1968) have warned of the widespread use of chemicals including pharmacotherapeutic agents "capable of producing chromosome breaks and rearrangement in vitro, and in vivo in human material. Such chromosome damage could be produced by a variety of mechanisms and could result in an increased incidence of neoplasia in the exposed individual, abortions and malformations of offspring if exposed in utero and chromosomal rearrangements in future generations, again resulting in fetal wastage and damage. Which of these drugs is, in fact, capable of producing such effects will only be determined by the use of large scale epidemiologic studies." Freeman et al (1971) have shown that extracts of city smog are 60 times as potent in transforming rat and hamster embryo cell culture lines as is benzpyrene. Clearly, agents able to produce damage to genetic material are widespread in the environment.

Recognized threats to pregnancy outcome are not uncommonly encountered in the environment and must be excluded when new problems are suspected. Threats are posed by biologic, chemical, and physical agents, and may derive from medical therapeutic interventions, drug and substance abuse, and environmental or occupational exposures to chemical agents.

a. Alcohol - The most widespread documented threats to the fetus come not from the maternal physical environment but from the use of tobacco and alcohol during pregnancy. The risk of alcohol ingestion in pregnancy only recently has been evaluated and the concept of the fetal alcohol syndrome refined (Clarran and Smith, 1978). Of infants born to recognized chronic alcoholic mothers, 83.3% had birthweights under the tenth percentile, compared with 2.34% in a comparable non-alcoholic population (Ullaland, 1972). In addition to intra-uterine growth retardation, the infants had retarded post-natal growth and

intellectual development. A characteristic facies has also been described with short palpebral fissures, hypoplastic philtrum, and thin upper vermillion as well as retrognathia in infancy.

In addition to these features of fetal alcohol syndrome, congenital malformations of all types occur with excess frequency. Recognition of an associated increase in the occurrence of congenital malformations ironically rediscovers ancient observations and admonitions against alcohol use during pregnancy (Jones and Smith, 1973). The adverse effects of maternal alcohol abuse during pregnancy on the fetus were further quantified by Ouellette et al (1977), who classified women at the first prenatal visit into four categories according to alcohol consumption and independently evaluated pregnancy outcome. Heavy drinkers drank five or more drinks on occasion and had an average daily intake of more than 45 ml. of absolute alcohol. Women who drank more than once a month, but did not meet the criteria for heavy drinking, were considered moderate drinkers. Abstinent women and those drinking less than once a month were grouped together in the lowest category. The mean daily dose of absolute alcohol consumed by the heavy drinkers was 174 ml, an average of 2.2 gm per kilogram per day. The results showed that infants born to heavy drinkers had twice the risk of having an abnormality at birth compared to those born to abstinent or moderate drinkers. The frequency of congenital abnormalities in the offspring of heavy drinkers was extremely high, 32% when minor abnormalities were included, 17% if only major anomalies were considered. Multiple abnormalities were present in 20% of the offspring in heavy alcohol users. Major, minor and multiple abnormalities occurred significantly less often among abstinent or moderate drinkers. Alcohol use was not the only factor distinguishing the group of mothers. Heavy drinkers were also heavier

smokers and more likely to have a history of previous use of psychoactive drugs. Heavy drinkers as a group were older, and of higher parity than the other groups. It is unlikely that these factors entirely explain the sizable differences in pregnancy outcome which were observed. The evidence suggests that heavy regular maternal use of ethanol during pregnancy produces both retarded development and a variety of congenital anomalies in the child. Animal models, including chicks, rats and guinea pigs, have been developed for evaluating ethanol effects alone on offspring (Kronick, 1976), and support the view that ethanol itself is the harmful agent, and that its teratogenic potential is common to several species.

b. Smoking - Another factor influencing pregnancy outcome, maternal smoking habits, should be addressed in efforts to tie environmental exposures of the parents to the survival and integrity of the fetus. Kline et al (1977a) suggest that the odds of spontaneous abortion among women who smoke are 1.8 times those of nonsmokers. This association remains statistically significant after maternal age, and number and outcome of previous pregnancies are taken into account.

How smoking affects fetal survival is controversial. Smoking results in lowered birth weight (Rush and Kass, 1974; Meyer et al, 1976), which may be unrelated to the increased frequency of spontaneous abortion. Lowered birth weight might come from impaired maternal nutrition or fetal anoxia, neither of which would produce an excess of chromosomal abnormalities in the conceptus. Alternate explanations for an association between smoking and spontaneous abortion have been proposed. Since spontaneous abortion serves as a means of selectively terminating abnormal conceptions, and 95% of abnormal pregnancies

are believed to terminate this way, an association between an environmental factor and increased spontaneous abortion should always provoke suspicion of a teratogenic phenomenon (Stein et al., 1975).

Frequency of karyotypic abnormalities in spontaneously aborted products of conception is yet to be linked to maternal smoking habits (Alberman et al., 1976), although the problem is complicated by variation in the proportion of chromosomally abnormal fetuses with increasing maternal age. Higher maternal social class and a history of oral contraceptive use appeared to be associated with a higher proportion of chromosomally abnormal fetuses independent of maternal age. A slightly lower proportion of chromosomally abnormal fetuses was reported among smokers compared to nonsmokers, but this effect disappeared when maternal age was taken into account because a large proportion of smokers were younger women. Since smoking increases the risk of spontaneous abortions almost two-fold, and the proportion of chromosomally abnormal fetuses is close to that expected, the possibility persists that one mechanism by which smoking may impair fetal survival is by damage to chromosomes. An increase in the rate of chromosomally abnormal conceptions would be masked in a proportionate ratio analysis if there was also an increased loss of normal conceptions.

Evidence for increase in congenital malformations in children of smoking mothers is limited and conflicting. In the course of following the development of a cohort of births which had occurred in the first week of March, 1958, in England, Scotland and Wales, Fredrick et al. (1971) suggested that there was a 60% increased risk of congenital heart disease among children of women who had smoked at least one cigarette per day after the fourth month of pregnancy (7.3 versus 4.7 cases per 1000 live births). This effect was

statistically significant after adjustment for maternal age, parity and social class. Commenting on a study by Richards (1969), which had failed to detect any association between maternal smoking habits and congenital heart disease, Fredrick and colleagues noted the limited power of the previous study and suggested that a 1.6-fold excess risk of congenital heart disease in offspring of smokers would likely have gone undetected as a result of the sample size of only 100 cases of congenital heart disease. Richards (1969) did, however, examine a variety of congenital defects of all systems which had been registered in South Wales from 1964 to 1966 and could not associate maternal smoking habits with congenital malformations in any system, after accounting for maternal age, parity, social class, area of residence and date of delivery. Given the current status of our understanding, it is essential that in the search for exogenous causes of teratogenesis and abnormal pregnancy outcomes, the potential confounding impacts of maternal smoking, drinking, and medication intake be taken into account.

c. Drug Exposure - In utero exposure to diethylstilbestrol (DES), with the subsequent development of vaginal adenocarcinoma, is the prototype for trans-placental oncogenesis in humans. The (1971) interpretation by Herbst et al. of the causal role played in utero exposure to DES followed hard upon the report (Herbst and Scully, 1970) of seven cases of a hithertofores exceedingly rare tumor presenting in individuals much younger than usual. A carefully executed case-control study of eight cases of vaginal adenocarcinoma, each matched with four controls of the same age and sex, born in the same hospital, and on the same type of service, showed that the mothers of seven of the eight cancer cases reported having been treated during pregnancy with DES, whereas

none of the 32 control mothers had been so treated. Subsequent reports (Herbst et al., 1972; Herbst et al., 1975) served to confirm and extend these observations. The syndrome appears to involve not only cases of vaginal adenocarcinoma, but a spectrum of abnormalities of the vagina and cervix. Only when exposure to DES occurred during the first four months of gestation were neoplasms or abnormalities observed. The abnormalities ranged from inconsequential ectropions in otherwise normal genitalia, to gross distortions of the cervix or upper vagina fornices caused by mucinous epithelium substituting for normal stratified squamous cells, a condition referred to as vaginal adenosis. Ulfelder (1976) has suggested the use of the term "cervico-vaginal epithelial dysgenesis" to describe the spectrum of abnormalities. It is estimated that the total number of females exposed to DES in utero from 1960 to 1970 is most likely between 10,000 to 16,000 and substantially under 50,000 (Heinonen, 1973).

The DES vaginal adenosis-adenocarcinoma relationship is the only documented example in humans of cancer in the offspring attributable to parental environmental chemical exposure. The therapeutic agent was given in a high dose, more than 10 grams commonly being given in the first half of pregnancy. DES, a synthetic estrogen analogue, was likely to have a direct effect on the development of the target tissue at a crucial point in embryogenesis. Since the risk to the offspring appears limited to exposures which occur during the first four months of gestation, direct exposure of the fetus is likely to be essential to the pathogenic mechanism. The long latent period between exposure in utero and emergence of clinical sequelae in adolescence probably reflects the importance of pubertal endogenous estrogens as promoting factors, and is

of the need for an extended period of observation following suspect  
a rer  
s. This association, as important as it is, does not serve as a model  
expr  
fo  
ng. Teratogenic effects of a multiplicity of drugs is now documented or  
s  
pected and these will not be discussed here further except to reiterate  
the need to remove the potentially confounding effects of such agents in  
investigation of other possible causes of impaired fetal development.

d. Radiation - If preconception radiation exposure increases cancer risk in  
the offspring, it would heighten concern over other agents which produce  
chromosomal damage. Chromosomal aberrations attributed to vinyl chloride and  
benzene exposure, for example, are reminiscent of those induced by radiation.

That human populations heavily exposed to radiation develop chromosome  
aberrations has been clearly shown in the Japanese studies conducted by the  
Atomic Bomb Casualty Commission (Bloom et al., 1966; Bloom et al., 1967).  
Estimates of doses sustained by atomic bomb survivors considered to have been  
heavily exposed to radiation are in the range of 204 to 991 rad of mixed gamma  
and neutron radiation. Controls, all of whom were more than three kilometers  
from hypocenters at the time of the bombings, were estimated to have had less  
than one rad exposure. Controls were matched to cases by sex and by age within  
five years. Studies of chromosome aberrations were performed some 20 years  
after the exposure.

Heavily exposed survivors who had been under 30 years old at the time  
of the bombings had an excess of complex chromosomal abnormalities (rings,  
dicentrics, fragments and translocations). Stable aberrations, including  
chromatid breaks and gaps, and isochromatid breaks and gaps, occurred with  
equal frequency in both exposure groups.

Results of analogous investigations undertaken in individuals who had been over 30 at the time of the bombings differed from the results in the younger group in two ways. First, the overall frequencies of chromosome aberrations were higher than in the younger age groups. This was true within the heavily exposed and within the control survivor groups. The second difference was in the pattern of excess chromosomal aberrations. In the older, heavily exposed group, most of this excess was due to stable aberrations, rather than the complex types which predominated among those heavily exposed at a younger age.

It is interesting that among 38 persons exposed in utero whose mothers received more than 100 rads, 39% had complex chromosome aberrations compared to 4% of controls. All but four of the heavily exposed had been in the second or third trimester of gestation at the time of the bombing (Bloom et al., 1968). Cancers and leukemia did not occur in excess during the first decade of life among 1292 children with prenatal exposure during the atomic bombings (Jablon and Kato, 1970). No abnormality in the sex ratio of offspring of the group exposed in utero was observed, regardless of the sex of the exposed parent or the trimester of gestation during which the exposure occurred (Jablon and Kato, 1971; Schull et al., 1966).

While genetic consequences of irradiation of survivors of the atomic bombings might be expected to produce some lethal mutations in their offspring, this effect has been difficult to demonstrate. Cohort studies of offspring of survivors classified by radiation exposure level did not show excess mortality in the children of the high exposure group. Neither were excess congenital malformations, increased infant mortality, nor impaired survival during the first ten years of life detected regardless of parental exposure level or sex

of exposed parent. Thus, although animal experience strongly suggests that such effects should be manifest in human offspring of individuals exposed to ionizing irradiation, studies of Japanese atomic bomb survivors have not demonstrated a measurable effect (Kato et al., 1966).

No substantial increase in leukemia risk has been detected among offspring of survivors (Hoshino et al., 1967). Analyses having a 90% chance of detecting a four-fold increase in risk of leukemia among children of heavily exposed survivors were negative. Neither did age-at-onset of observed leukemia cases or type of leukemia in offspring differ by level of parental radiation exposure. Despite the documented persistence of chromosomal aberrations in the somatic cells of adults exposed to the atomic bomb, no measurable impact in mortality, malformation or leukemia has been detected in the following generation.

In addition to radiation exposures related to the atomic bombings, diagnostic and therapeutic medical radiation of the parents have been investigated to assess risks to the offspring. In a detailed case-control study of the relationships between radiation exposure and childhood leukemia which involved 319 childhood leukemia patients and 884 randomly selected child controls, an excess risk of leukemia in offspring was tied to maternal history of preconception radiation. The magnitude of leukemia risk associated with previous radiation was estimated, after adjustment for maternal age and pregnancy history, to be 1.73 times that of mothers who had not been x-rayed. Oddly, only the second and later pregnancies were at increased risk; the first was apparently not. Despite the suggestion of greater risk in women with higher x-ray exposure, no clear-cut dose-response gradient was demonstrated.

The relative risk of leukemia in the child associated with preconception diagnostic radiation to the father (1.31) was not statistically different from unity. When both mother and father had histories of preconception diagnostic x-rays, the order of magnitude of the relative risk, 1.49, was about the same as that for children whose mothers alone had received radiation (Graham, et al., 1966).

Exposure to medical radiation has been widespread. By the early 1960's, approximately 35% of a group of mothers of healthy children reported having received diagnostic radiation prior to conceiving. Similarly, about 22% of fathers of the same children reported having had some sort of diagnostic radiation at some time prior to the child's conception (Graham, et al., 1966). Self-reporting considerably underestimated the extent of x-ray exposures. When histories of x-ray exposure in 200 subjects were verified, it was estimated that 79.6% of the total diagnostic x-ray films documented in physicians' and dentists' records and 66.5% of those in hospital records had not been mentioned in interviews specifically designed to elicit such exposures (Graham et al., 1963). The tendency to substantially underreport x-ray exposure points up the difficulty of excluding differences in prior radiation exposure as a basis for observed differences in chromosomal aberrations. Neither is it reasonable to automatically presume that medical radiation exposures have been equally present in the study and comparison groups, especially if these groups have not been matched by age or calendar period of observation.

*Smith  
Holt (Hepko)  
reprint*

VIII. Occupational exposures and pregnancy outcome

a. Vinyl Chloride - Chromosomal abnormalities may occur more often than expected in persons exposed to vinyl chloride, especially following intense exposures of long duration. The import of such abnormalities for reproductive function remains ill-defined.

The first reports of chromosomal aberrations in vinyl chloride workers from Sweden in 1975 (Funes-Cravioto et al.) described abnormalities in cells drawn from seven males with occupational exposure histories of nine to 29 years (mean 16.6 years) at vinyl chloride concentrations which had declined to 20-30 p.p.m. by 1974. Cells from three non-exposed individuals working in the same factory provided comparison values. Analysis of chromosomal abnormalities were made from conventional lymphocyte cultures, which were read without knowledge of exposure group. The group exposed to vinyl chloride had aberrations in a total of 9.52% of all cells, compared to 1.94% for cells of the three controls. The frequency of chromatid and isochromatid breaks accounted for most of the disparity. Considerable variation was exhibited in the exposed workers, with cells considered abnormal ranging from 1.3% to 18%. There was no apparent relationship between duration of exposure to vinyl chloride and the proportion of cells which were abnormal. Age, radiation exposure or other environmental factors were not evaluated, nor was it clear on what basis individuals had been selected for study. Chromosomal damage in humans exposed to this widely used industrial product was nonetheless a possibility.

In New York State, chromosomal aberrations were reported in a group of 11 men who had worked in a polyvinyl chloride polymerization plant and who

were believed to have experienced intense intermittent exposures to vinyl chloride in concentrations of over 500 p.p.m., as well as chronic, but unquantitated exposure for four to 28 years (mean, 15 years). Control samples were drawn from four males in the same factory who were not known to have vinyl chloride exposure, and from six males from outside the factory. The mean age of the controls was 27 years, and that of the subjects, 40 years. Chromosome studies were read blindly. Stable aberrations of the kind which persist in circulating lymphocytes (monosomy, trisomy, deletions, exchanges) were not increase in frequency in the exposed group, although a small increase of borderline statistical significance in the number of chromatid and isochromatid gaps and breaks was observed. Complex chromosomal aberrations (rings, dicentrics, fragments) occurred significantly more frequently in the exposed group than the control, a result which was properly interpreted with caution. The authors pointed out the absence of age-matched controls and the substantial age difference between study group and controls. The frequency of chromosome breaks and gaps was unexpectedly high in both control and study groups (Ducatman et al., 1975). As with the Swedish report (Funes-Cravioto et al., 1975), no relationship could be defined between dose or duration of exposure and frequency of chromosomal aberrations.

Investigators reporting from the United Kingdom (Purchase et al., 1975) studied 56 men with chronic exposure to vinyl chloride monomer in the course of manufacturing polyvinyl chloride and compared them with 24 unexposed individuals. Vinyl chloride exposure to the men employed in its manufacture could not be easily quantitated. Individuals with exposures to radiation, recent viral infections, or prolonged drug treatment were excluded. Samples were coded and read blindly. A higher proportion of cells from vinyl chloride

exposed workers had chromosomal aberrations, the majority of which were breakages, compared to the comparison group. Unstable and stable chromosomal aberrations and breaks were all significantly more common in cells from exposed workers.

Not all investigators have identified such changes. Picciano et al. (1977) tested 209 employees of one vinyl chloride plant with occupational exposures lasting from 1 to 332 months (average 48.3 months). These were compared with 295 individuals undergoing preemployment physicals, not surprisingly a group considerably younger than the vinyl chloride workers. The determination of chromosomal aberrations was apparently made with foreknowledge of the exposure group from which the sample was drawn. No differences were found between the exposed and unexposed samples in frequency of all types of chromosomal aberrations or of any subgroup. No relationship was established between intensity of vinyl chloride exposure and degree of chromosomal damage. The absence of blind evaluation in this study, as well as the inclusion in the exposed group of individuals whose exposure was probably quite low, makes interpretation difficult. This difficulty is compounded by the substantial difference in mean age of study and control group members.

Infante, et al. (1976a, b) inquired whether pregnancy outcome in the wives of men exposed to vinyl chloride monomer (V.C.M.) might be less favorable than that experienced by wives of a pooled group of polymerization and polyvinyl chloride fabrication workers (P.V.C.). One comparison group, the rubber workers, were not believed to have been exposed to vinyl chloride monomer (V.C.M.), whereas the P.V.C. workers had low V.C.M. exposure. Data on pregnancies and pregnancy outcomes in the wives were derived from interviews and questionnaires administered to male workers. Participation rates for the groups queried ranged from 62% to 77%. Fetal death was defined as

any known conception not born alive and fetal death rates were adjusted by paternal age. The term "fetal death rate" was not used in the conventional vital statistics sense, but rather referred to the percentage of reported conceptions which did not result in a live birth. Maternal age was not known, but was assumed to correlate closely with paternal age. Analysis of questionnaire responses suggested that, after beginning work with vinyl chloride monomer, the number of fetal deaths per 100 conceptions was higher in the vinyl chloride exposed group than in the comparison group. No difference was reported between the two groups for the period prior to vinyl chloride exposure. Adjustments intended to remove women who were chronic aborters from the study reduced the differences to the extent that they were no longer statistically significant. While the authors felt that these observations were likely to reflect a real difference in pregnancy outcome not attributable to either interviewer or patient recall bias, it is unfortunate that the conclusions were based on such indirect sources of information and that there was no possibility of taking into account the multiplicity of maternal factors known to affect pregnancy outcome. Studies of pregnancy outcome (i.e., spontaneous abortion, late fetal death [stillbirth], low birthweight, neonatal death) in other settings have shown the profound and subtle effects of confounding variables such as race, socio-economic status, maternal age, birth order, parity, smoking and alcohol exposure during pregnancy, maternal infections, and previous pregnancy outcomes. These effects may readily be sufficient to reverse the direction of the relationship between a suspect antecedent factor and the outcome of pregnancy (Daling and Emanuel, 1977). Moreover, the study design precluded documenting in even the crudest manner, the validity of

pregnancy histories. Without such adjustments and validation, the inferences made by Infante and colleagues cannot be sustained and little light is shed on the possibility of abnormal pregnancy outcome. Future efforts must validate information on pregnancies and take into account known confounding factors which can affect pregnancy outcome.

b. Benzene - Benzene also may cause chromosomal aberrations in heavily occupationally exposed individuals. In one investigation 20 males working in a factory in which benzene had been used as a solvent underwent study for chromosomal aberrations. The exposed group had one to 20 years of benzene exposure and 14 were known to have previously been neutropenic. Control samples were drawn from five non-exposed workers in the same factory and from 38 randomly selected males 15 to 64 years of age. Chromosome analysis was performed blindly. Chromosomal abnormalities were reported in 2.5% of all cells from exposed workers, compared to 1.0 to 1.4% for controls. Most of the excess was due to chromosomal aberrations of the unstable types. In addition to the excess of abnormal cells in the exposed group, the number of unstable alterations per abnormal cell was higher in the exposed group than in the controls (Tough and Court Brown, 1965).

In a separate investigation, ten workers in an Italian rotogravure plant were studied who had had sequential exposures, first to benzene and later to toluene and xylene. These workers had been subjected to concentrations of benzene ranging from 125 p.p.m. to 532 p.p.m. or higher. The maximal allowable concentration at the time of exposure had been 25 p.p.m. Another group of 24 workers had only toluene exposures except for trace contamination of toluene with xylene. These 34 workers were matched with healthy controls of the similar age and sex, who had been drawn from the general population and who had no history of benzene or radiation exposure (Forni, Pacifico and

Limonta, 1971). Chromosome changes in lymphocytes were analyzed blindly. A significantly higher proportion of abnormal cells were found in the benzene exposed individuals as compared to their matched controls. In the group with toluene exposure only, no such excess was present. When the benzene and toluene groups were compared to each other, an excess of chromosome changes was present in the benzene group. Most of the excess abnormalities were attributable to unstable chromosomal alterations.

These studies and others (Forni et al., 1971) support the impression that exposure to benzene, particularly exposures intense enough to result in acute toxicity, can be followed by a measurable excess of chromosomal aberrations which may persist for years. If similar effects occur in germ cells of exposed individuals, the consequences in fetal wastage, congenital anomalies and even neoplasms might be manifest in their progeny. To date no excess of such abnormalities has been reported in offspring of benzene exposed individuals.

c. Anesthetic Cases - Occupational exposures of female operating room personnel to waste anesthetic gases have been held responsible for decreased fertility, increased rate of spontaneous abortion, low birth weight and possibly impaired development in the offspring. An excess of spontaneous abortions in wives of men exposed to anesthetic gases has been suggested but not confirmed.

Among operating room nurses, 29.7% of pregnancies terminated with spontaneous abortion, compared to 3.8% among control nurses and a similar excess of spontaneous abortions was observed for female physician anesthesiologists compared to female physicians in other specialties (Cohen et al., 1971). Spontaneous abortions occurred two weeks earlier on the average among the operating room groups than among controls, at an average of eight rather than ten weeks

of gestation. Another survey suggested that for female nurse-anesthetists who had worked during pregnancy, the frequency of birth defects in the offspring was 16.4% compared to 5.7% for pregnancies in which the mother had not worked (Corbett et al., 1974). A variety of birth defects occurred. Ten mothers had defects in offspring of two or more pregnancies during which they had worked. In the same study, three neoplasms occurred in two of the 434 children whose mothers had practiced during pregnancy. One child had a neuroblastoma at birth and developed a second primary, a thyroid malignancy, at puberty. A parotid tumor developed at age 22 years in another child. Among 261 pregnancies in which the mother had not worked, there was one case of acute leukemia occurring in a child three years of age.

Studies from Finland and the United Kingdom also suggest a deleterious effect on pregnancy outcome from maternal exposure to operating room environments. An increase in frequency of early spontaneous abortions, as well as low birth weight, has been reported for Finnish female operating room staff (Rosenberg and Kirves, 1975). Among female physicians in England and Wales, congenital defects, low birth weight and higher stillbirth rates, but not higher spontaneous abortion rates were reported from pregnancies of women holding anesthesiology appointments compared to other women doctors (Pharoah et al., 1977).

The effects of paternal exposure to anesthetic gases are much more in doubt. Congenital anomalies were reported to be increased by 25% in the offspring of male anesthesiologists compared to offspring of male members of the American Academy of Pediatrics. No difference was reported for spontaneous abortion rates among wives of male operating room personnel compared to controls (Ad Hoc Committee ASA, 1974). Questionnaires completed by 5119 married

male physicians in the United Kingdom suggested an increase in minor but not major congenital anomalies, and no difference in frequency of infertility, spontaneous abortion or cancer in the offspring of male anesthesiologists compared to control physicians (Knill-Jones et al., 1975).

IX. Cancer in the offspring of hydrocarbon exposed fathers

Two studies offer conflicting evidence with respect to the risk of cancer in children of men whose work might lead them to be exposed to petroleum derived hydrocarbons.

Fabia and Thuy (1974) reviewed death certificates of children dying in Quebec during 1965 to 1970, in order to identify children who had died from malignant disease while under five years of age. For 386 of 402 such children, birth certificates were found in the Quebec population register. A control group of 772 patients was selected using the birth registration record preceding and following that of each case in the official files. This effectively matched for season, calendar period, and province of birth. Occupation of the father at the time of birth was taken from the birth certificate. An industrial hygienist independently grouped the birth certificates into three levels of probable exposure to petroleum derived hydrocarbons. Occupation of the father was unknown for 30 cases and 56 controls. The distribution of occupation of the father at birth differed for cases and controls. Most of this difference was the result of an excess among cases of occupations considered to be hydrocarbon-related. The relative odds of cancer in the children of men holding hydrocarbon-related jobs at the time of the child's birth were 3.1, an increase which was unlikely to have occurred by chance. Most of the excess of exposed occupations was accounted for by motor-vehicle mechanics, machinists, miners and painters. When similar analyses were conducted by the type of cancer in the child, the excess of hydrocarbon-related occupations prevailed for the following groupings: leukemia-lymphoma, nervous system malignancy, other tumors. For four of five cases of Letterer-Siwe disease fathers were in the hydrocarbon-related work groups. No excess of exposed fathers was

present for children with Wilms' tumor. The differences observed were not attributable to differences in parental age or geographic residence at the time of birth. The investigation was based on deaths in children under five, and it is possible that social class bias may have been introduced in that fashion. If children of more affluent parents with a childhood neoplasm are more likely to survive to over age five, and therefore not be included in the study group, compared to children of poorer parents, this would lead to a higher proportion of children with parents of lower socio-economic status in the cancer death group and would bias the distribution of father's occupation. Moreover, it was not possible to confirm that fathers in the so-called "exposed" group did, in fact, have contact with hydrocarbon-derived chemicals. While not ideal, the death certificate probably is a valid source of diagnostic information for cancer deaths in young children. The cancer diagnoses employed in the analysis - "leukemias and lymphomas", "nervous system" and "others" - were quite broad and included many histopathologic entities. Occupational association was not restricted to specific entities within the broader categories. Because the epidemiologic features of the various histopathologic types of leukemias, lymphomas, and nervous system tumors are distinctive, a specific chemical exposure or class of exposures, if assumed to be of causal significance, would more likely be linked with specific histopathologic as well as organ system effects. Although an analysis taking into account the matched pairs design would have been more elegant, its use would not have altered the substance of the conclusions.

A study with a similar design but different findings was conducted in Finland (Hakulinen et al., 1976). Children under 15 years of age who developed

cancer were identified from the Finnish Cancer Registry, 1959-1968. Of 1409 cancer cases so identified, the final series consisted of 852 pairs for whom birth records which included father's occupation were available. Each case had been matched with a child whose birth date immediately preceded that of the case and who was born in the same maternity welfare district. This procedure matched effectively by calendar period, season and domicile at the time of birth. Father's occupation was drawn not from birth registration records but from records of the free, nationwide antenatal care system in operation in Finland. Father's occupation was classified by likelihood of hydrocarbon exposure in a manner which was shown to be comparable with the study done in Quebec. No excess risk for hydrocarbon exposed fathers was detected for any class of neoplasms either for children whose cancers occurred under five or under 15 years of age. The power of the study to detect a two-fold risk ratio was not determined, but appeared to be insufficient for children under five years of age.

This study differed in design from that done in Quebec primarily in the source of information on father's occupation. For the Finnish study, that was drawn from antenatal records compiled for the most part in the first trimester of gestation. This may be a better index of exposure at the time of conception than that recorded at the time of birth in the birth certificate itself. Although a large number of pairs were discarded because of unavailability of father's occupation, these came from a circumscribed calendar period, and since the matched pairs analysis was maintained throughout the analysis, should in any case not influence the result. The Finnish study used incident cases and was able to look at age groups up to five as well as up to 15 years at the time of cancer diagnosis. A further explanation for the discordant

findings in the two studies is possible variation in terminology of occupational classification. At present, the contradictory findings of the two studies are not readily reconciled.

Because of the initial Canadian report (Fabia and Thuy, 1974) of an increase in risk of cancer death in children of fathers whose occupation involves potential exposure to petroleum derived hydrocarbons, a study was undertaken in Harris County, Texas to assess the relationship of parental occupation to childhood neoplasms using a case-control approach. Data collection in this study is near completion but, since data analysis is only beginning, report of its conclusions is some time away (Heath, 1978).

Cases consist of all cases of malignant disease being followed in the Research Hematology Clinic at Texas Children's Hospital during 1975-76, approximately 350-400 cases, ages 0-14 years, with half under five years old. Somewhat more than half of these patients have leukemia and lymphoma and are residents of Harris County in Texas. It is estimated that these cases represent 80-90% of all such childhood malignancies in that county.

Three sets of controls are to be used. Clinic controls are children from the Research Hematology Clinic who have non-malignant disease. Clinic controls will not be individually matched to cases, but group characteristics are being ascertained to insure comparability. Interviews would be conducted with controls in the course of regular clinic visits.

The second set of controls are "self-matched" to the case, with the parents of the case choosing a family from their neighborhood and acquaintance to act as controls. Data would be collected from this group by interview as well as by telephone and mailed questionnaire. This unusual method was proposed by Detre and Wyschak in 1971.

The final set of controls are drawn from the case's families... Married siblings closest to the patient's parents in age are to be selected so that there would be one maternal and one paternal sibling. Family controls would be interviewed at the same time as the parents of the case.

Occupational groupings by an industrial hygienist are being established according to the 1970 U.S. census with duration of parental "exposure" estimated and with account to be taken of socio-economic class. The questionnaire itself is designed to elicit demographic information about the family, as well as a detailed job history from both of the cases' parents and the control parents. Family residence and school histories are also collected.

It is premature to comment extensively on this investigation in the absence of further information about its progress and results. A number of difficulties can be conjectured in its conduct and design -- particularly in the use of unmatched clinic controls with non-malignant diseases and in the "self-selected" control group. When Detre and Wyszak (1971) used self-selected controls, the independent variables of interest were related to menstrual history and were unlikely to be biased by the fact that the control was selected by the case. The application attempted in the Harris County study is quite different and it is likely that for the self-selected controls, and also for the family controls, there is a tendency for control parents to have an occupation similar to the case's parents. This would, however, tend to minimize any association between occupational exposure to petroleum derived hydrocarbons and childhood neoplasms.

X. Surveillance of spontaneous abortions as a strategy of environmental monitoring

As the previous discussions have suggested, the relationship between mutagenic, teratogenic and carcinogenic effects in the offspring of persons exposed to noxious environmental agents is a complex one. Surveillance of spontaneous abortions may have a number of advantages as a means of prospective monitoring for such effects since defective conceptions are aborted selectively. As a result, studies of teratogenesis in spontaneous abortions may be considerably more efficient than those conducted in newborns. Because the frequency of abnormalities is higher in spontaneous abortions, the sample size needed to demonstrate a change in risk is much smaller than for a parallel query addressed to chromosomal defects or developmental abnormalities recognized at birth. The magnitude of this difference can be dramatic. If chromosomal defects diagnosable on the appearance of the newborn are considered alone, the sample size required may be hundreds of times that of an investigation examining prevalence of chromosomal anomalies in early abortions. This is illustrated in Table 1, adapted from Kline et al. (1977). In addition to sample size considerations, study of spontaneously aborted conceptions offers a lead time of at least six months over studies of live births. The abortion specimen can be studied with care and thoroughness, permitting detection of anomalies lethal to the fetus which would not have been detected as readily in studies of live births.

[Insert Table 1 about here]

The problem of power and sample size is only one of the issues which beleaguers investigators of associations between exposures to parents and outcomes of pregnancy. Related factors are the timing of the exposure, maternal

or paternal, in relation to conception and/or gestation, and the specific measures of pregnancy outcome (Strobino et al., 1978).

Paternal exposures may act in two ways -- by contamination of the maternal environment resulting in secondary maternal exposures, or directly by affecting paternal germ cells. Since spermatogenesis is a continuous process, paternal exposures pre-conception, rather than cumulative or delayed effects, are those most likely to have been responsible for deleterious effects. By focusing on early pregnancy wastage, particularly if the frequency of chromosomal abnormalities in the aborted product of conceptions can be determined, the objectives of study are better focused and achieved with reduced sample size, and the confounding effects of the maternal in utero environmental factors are minimized.

#### XI. Further studies

Based on the evidence summarized in the preceding discussion, risks to the fetus which result from paternal exposure to hydrocarbons, if they exist at all, are likely to be small in magnitude. The conflicting scientific evidence and the social implications of an occupational exposure which may affect the fetus, however, urge further investigation.

"Are the pregnancies of women employees and wives of men who are occupationally exposed to petroleum-related hydrocarbons likely to come to a less favorable conclusion than those of unexposed petroleum industry employees?" This question can be addressed using the developing resource of the ongoing study of morbidity and mortality among petroleum industry employees. In the families of the more than 33,500 mostly male employees whose census data is now recorded, there will be some 2050 conceptions per year. The background for this estimate is recorded in Table 2, which gives the age and sex breakdown of part of that study population, along with the corresponding age-specific birth rates for females in the corresponding age group. On the assumption that the age of the workers' wives corresponds closely to that of male workers, it can be estimated that there will be approximately 1375 live births in this group annually. Since 314 induced abortions take place for every 1000 live births nation-wide, there would, in addition, be some 400 conceptions terminated by induced abortions in the same year. On the assumption that of all conceptions, approximately 15% will terminate in spontaneous abortion, the total annual number of conceptions is estimated at about 2050 (Kline et al., 1977). About 50 per 1000 recognized conceptions are chromosomally abnormal, and approximately 92% of all chromosomally abnormal conceptions abort

spontaneously. Changes in rates of chromosomal abnormality can be studied directly through examination of spontaneously aborted products of conception or indirectly through the study of frequency of spontaneously occurring abortions. The number of pregnancies which must be studied to detect a given increase in the spontaneous abortion rate was shown in Table 1. To detect a relative risk of spontaneous abortions of two ( $\alpha=0.05$   $\beta=0.2$ ), there would have to be 146 pregnancies each in the "exposed" and "unexposed" group. To generate this number of pregnancies in one year (assuming United States birth rates) would require surveillance of a total population of about 6500 workers given the age-sex distribution of employees in the MSKCC-API study.

These estimates suggest the suitability of following the reproductive experience of a portion of the population in the MSKCC-API study. This could be achieved by monitoring a sample of male employees and their wives as well as all female employees, under age 49 years. Such surveillance would require the cooperation of the employees under study, since only through voluntary reporting would all conceptions be identified and their outcome determined. It would be necessary to document previous obstetrical history, past and present smoking history and medical history, and spouse's occupational history prior to and post conception. While area-wide surveys have provided information on the expected frequency of various pregnancy outcomes (Stein et al., 1975; Creasy et al., 1976), a more appropriate comparison group of "unexposed" pregnancies can be drawn from employees (and their wives) of the petroleum industry who have little or no petroleum derived hydrocarbon exposure. Given the personal nature of such inquiries, considerable attention would have to be given to developing approaches which would permit the study to proceed successfully. The advantage of such an approach is that, if tactical problems can be

surmounted, it directly addresses the question of pregnancy outcome as it pertains to employees in the petroleum industry and the occupational exposures which they experience.

TABLE 1

Sample Sizes to Detect Increased  
Spontaneous Abortion Rates

Number of exposed pregnancies  
required to reach indicated power

| <u>Prevalence<sub>2</sub></u> | <u>.80</u> | <u>.90</u> |
|-------------------------------|------------|------------|
| .20                           | 984        | 1290       |
| .25                           | 289        | 373        |
| .30                           | 146        | 186        |
| .35                           | 91         | 116        |
| .40                           | 64         | 80         |
| .45                           | 48         | 60         |
| .50                           | 37         | 46         |
| .55                           | 30         | 37         |
| .60                           | 25         | 30         |

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Number of exposed pregnancies needed to detect an increase in spontaneous abortions to Prevalence<sub>2</sub> given Prevalence<sub>1</sub> equal to 0.15 and  $\alpha = 0.05$ . The same number of pregnancies is required in the unexposed group.

TABLE 2

Preliminary Employee Population in the MSKCC-API study  
by Age and Sex\*\*

| <u>Age</u> | <u>Male</u> | <u>Female</u> | <u>Age-specific<br/>birth rates<br/>per 1000*</u> | <u>No. of births in one year by sex<br/>and age group of employee parent</u> |               |
|------------|-------------|---------------|---------------------------------------------------|------------------------------------------------------------------------------|---------------|
|            |             |               |                                                   | <u>Male</u>                                                                  | <u>Female</u> |
| 19         | 6           | 5             | 55.5                                              | 0                                                                            | 0             |
| 20-24      | 1358        | 248           | 158.3                                             | 214                                                                          | 40            |
| 25-29      | 4202        | 543           | 141.7                                             | 595                                                                          | 77            |
| 30-34      | 4441        | 404           | 70.3                                              | 312                                                                          | 28            |
| 35-39      | 2813        | 276           | 29.5                                              | 83                                                                           | 8             |
| 40-44      | 2136        | 201           | 7.3                                               | 16                                                                           | 1             |
| 45-49      | 2960        | 178           | 0.4                                               | 1                                                                            | 0             |
| 50-54      | 4591        | 168           |                                                   |                                                                              |               |
| 55-59      | 5196        | 173           |                                                   |                                                                              |               |
| 60-64      | 3302        | 104           |                                                   |                                                                              |               |
| 65         | <u>462</u>  | <u>10</u>     |                                                   |                                                                              |               |
|            | 31,472      | 2320          |                                                   | 1221                                                                         | 154           |

Total  
 births: 1375

\* U.S. white, 1969-71.

\*\*This is a portion of the MSKCC-API study of Mortality and Morbidity  
 in Petroleum Industry Employees which represents about half of the  
 final population.

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