Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
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Quantitative Effects of Chemicals on Fertility

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ABSTRACT

Human fertility is a complex biological attribute that can be affected by exposure to chemicals. Measurement of fertility can be based on the fertilizing capacity of sperm, prevalence of ovulatory cycles, demographic assessment of time to pregnancy, and biochemical indicators of the likelihood of conception in each cycle. In theory, any of these measures could be used to quantify the effects of chemicals. In practice, the difficulties of measuring both exposure to chemicals and fertility have severely limited the estimation of dose–response relationships. The utility of animal studies in estimating human risk is discussed and new approaches to the study and measurement of human fertility are considered.

1 INTRODUCTION

Fertilization is the cytoplasmic and nuclear union of two gametes, and fertility is broadly defined as the capacity to reproduce. Chemicals that impair fertility may directly obstruct fertilization or may interfere with processes that precede or follow fertilization. Thus, effects on fertility include but are not limited to effects on fertilization.

Several common chemicals may disrupt the fertility of laboratory animals (Dixon, 1980). The fertility of humans is probably at least as susceptible, and yet there are fewer chemicals that are known to affect human fertility. One reason may be that methods for measuring damage to human fertility are not yet well established. There are the usual difficulties of measuring human exposures to chemicals. In addition, human fertility has several possible measurement endpoints, not all of which are clearly understood. Unlike cancer, which can be defined by essentially the same criteria in animals and humans, fertility can rarely be measured in animals and humans in the same way. This paper will emphasize diverse components of fertility and describe several approaches to measuring fertility. A proposed new method for measuring human fertility may avoid some common problems. Finally, what little is known about dose–response relation-

ships for chemicals affecting fertility will be discussed, including the problem of a safe dose.

2 A MODEL OF FERTILITY

In this section, the cycle of human reproduction will be outlined, and anatomical, physiological and behavioural aspects of fertility will be summarized in a model. This model provides a framework for subsequent discussion of effects of chemicals on fertility.

2.1 A Review of the Human Reproductive Cycle

The germ cell line that eventually produces sperm or eggs in the sexually mature person has its origins in prenatal life. Distinct primordial germ cells may differentiate as early as the ninth day after conception (Ozdzenski, 1967). In males, these germ cells transform after birth into spermatogonia, which are dormant until puberty. At puberty there begins a continuous proliferation of mature sperm, involving mitosis and then meiosis. This sequence of transformations is well characterized and requires about 12 weeks from stem cell to mature sperm. Up to 200 million human sperm are generated daily.

The female germ line experiences an entirely different sequence of events. The proliferation of the oogonia, including mitosis and part of meiosis, takes place before birth. Unlike the male, the female does not produce germ cells after birth. In fact, the total number of gametes in the human female steadily declines from about 7 million at 5 months' gestation to 250 000 at puberty and about 25 000 at age 30. Only a relatively few oocytes go on, under hormonal stimulation of the menstrual cycle, to resume their meiotic division and develop into ova. Normally, a single ovum is released in each cycle. Thus, while men produce hundreds of billions of mature gametes in their lifetime, women typically produce around 400.

Fertilization is a closely spaced sequence of events in which a single sperm penetrates the egg membrane, the egg completes meiosis, and the genetic material of the sperm and ovum are fused. Ordinarily this occurs in the oviduct. The new zygote proceeds down the oviduct and into the uterus where, 7–10 days later, it implants in the endometrium. Only a few days later, the developing embryo will have differentiated those primordial germ cells that eventually go on to produce the next generation.

2.2 Developing a Model of Fertility

Fertility depends not only on biological capacity but on appropriate behaviour. Figure 1 integrates biological and behavioural elements of fertility into two groups: factors that precede fertilization, and factors that occur around the time of fertilization. The third section of the model defines several fertility endpoints.

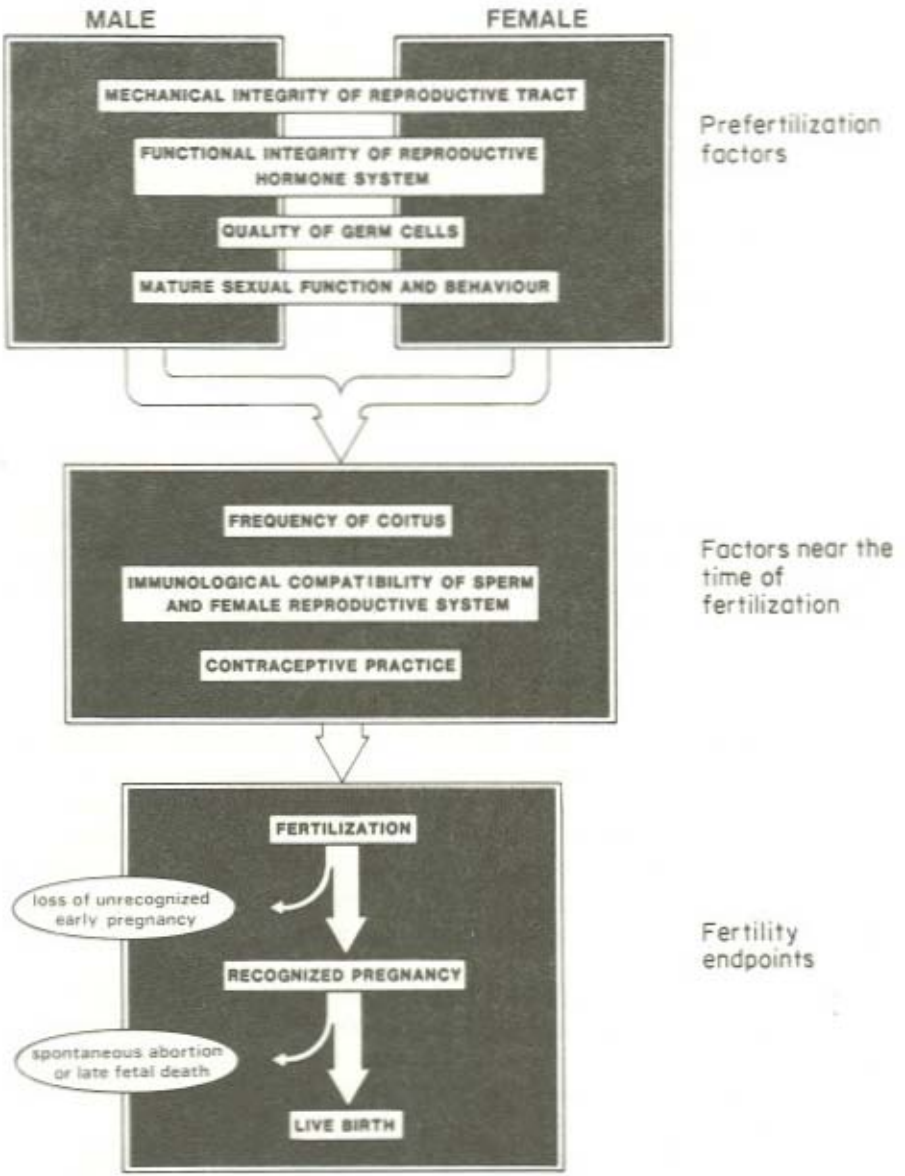

Figure 1 A model of fertility

2.2.1 Prefertilization Factors

The reproductive tract of both males and females consists of a pair of organs that contain the gametes, and structures for transporting the gametes. The mechanical integrity of this structural system is essential to fertility: the simplest

sterilization procedure for men or women is to obstruct the tract. In addition to harbouring gametes, the testes or ovaries also act as endocrine glands. Disruption of hormone production, directly or through the central nervous system, can likewise affect fertility.

Another element in fertility is the quality of the germ cells. In its profligate production of gametes, the male reproductive system produces many irregular or immature sperm. Some of these are presumably less capable of fertilization. Irregular types are detectable by semen analysis. Ova are not so directly observable, but presumably vary in quality as well.

Fertility depends on mature sexual function and behaviour. Sexual intercourse requires both involuntary and voluntary nervous system activity. In humans, cultural norms and the socialization of the individual further affect readiness and capacity for intercourse.

2.2.2 Factors Near the Time of Fertilization

Frequency of coitus has a major effect on the probability of fertilization (Barrett, 1971; Bongaarts, 1975a). There is some evidence that sperm antibodies in the female reproductive tract may interfere with fertility (Ansbacher *et al.*, 1973). Finally, the use of contraception powerfully influences fertility.

2.2.3 Fertility Endpoints

Live births provide a convenient endpoint for estimating fertility, particularly when studying the fertility of large groups of people. The advantage of using live births as an endpoint is that data are easily obtained; virtually all live births are routinely registered. The disadvantage is that live births do not represent all pregnancies. For example, about 15% of pregnancies end with a miscarriage or stillbirth (Leridon, 1977). Thus, when using live births as an outcome, one cannot separate failure to conceive from failure to carry a pregnancy, even though these two are biologically distinct.

Pregnancy would be more specific than live births as an endpoint for fertility. However, there is no routine registration of pregnancy. Specifically, pregnancies ending in spontaneous abortion are not documented except in special studies. As a result, data describing pregnancies are less available and often less reliable than live birth data.

To complicate matters, there is probably a large amount of fetal loss that occurs very early after conception, before pregnancy is even recognized. Miller *et al.* (1980) found that one-third of pregnancies diagnosed by urine assay terminated before becoming clinically apparent. In other words, the occurrence of all clinically recognized pregnancies is still an underestimate of the occurrence of fertilization.

The true incidence of human fertilization is unknown. Under natural

conditions, human fertilization is virtually an unobservable event. The most sophisticated of the proven tests for human pregnancy is the radioimmunoassay of human chorionic gonadotropin (hCG). The radioimmunoassay can detect hCG around the time of implantation, 7–10 days following conception. Other assays of hCG are being developed including enzyme-linked systems. Even earlier detection of conception may be possible using a serum assay for an 'early pregnancy factor'. This experimental assay has been reported to detect changes in the maternal immune response within hours following conception (Morton *et al.*, 1977; Shaw and Morton, 1980).

There is a spectrum, then, of fertility endpoints, ranging from live birth data which are easily accessible and lack sensitivity, to biochemical data which are less accessible and more precise. While none can pinpoint the event of fertilization, any of these endpoints could be useful in the appropriate setting.

2.3 Occurrence and Causes of Fertility Impairment

Figure 1 presents various factors which, if disrupted, can impair fertility. How common is fertility impairment in the general population? A World Health Organization's Scientific Group used the following operational definitions for epidemiological analysis:

(1) primary infertility: the woman has never conceived despite cohabitation and exposure to pregnancy for a period of 2 years. (The Scientific Group, after reviewing the subject, concluded that 'exposure to pregnancy' is difficult to define and standardize except in the context of specific local conditions);
(2) secondary infertility: the woman has previously conceived but is subsequently unable to conceive despite cohabitation and exposure to pregnancy for a period of 2 years; if the woman has breast fed a previous infant, then exposure to pregnancy should be calculated from the end of the period of lactational amenorrhoea (WHO, 1975).

According to this report, primary infertility is as high as 30% in areas of the world where gonorrhoea, genital tuberculosis and other infections are prevalent.

Using the same criteria, a study of a carefully selected sample of Danish women between the ages of 25 and 45 found that 9% met the requirement for primary infertility and 7% more were secondarily infertile (Rachootin and Olsen, 1981). A study of a national sample of US women 18–39 years old classified 21% of couples as 'involuntarily subfertile', and estimated that 6–8% US couples were involuntarily sterile for their whole lives (Whelpton *et al.*, 1966). These numbers underestimate the actual prevalence of impaired fertility in the general population in that they include only those who wish to have a child and cannot. Unrecognized secondary infertility no doubt occurs among couples who have completed their family and are using long-term contraception.

In developed countries, the causes of fertility impairment are largely unknown.

Physicians who treat infertile couples classify patients into clinical categories including men with abnormal semen analysis, women with abnormal ovulation, endometriosis or obstructed tubes, a few with defects of the uterus or cervix, and a group with no obvious problem (Dor *et al.*, 1977). However, few of these conditions have obvious aetiologies. Among known causes, infection is frequently invoked (MacLeod, 1951; WHO, 1975). Other less common causes are radiation exposure (Ash, 1980), genetic disease such as sickle cell anaemia (Osegbe *et al.*, 1981), chromosomal anomalies (Chandley, 1979), or severe malnutrition (Stein and Susser, 1975). These account for a small number of all cases. Until recently, the possibility of effects of chemicals on fertility has not been given much consideration.

3 MECHANISMS BY WHICH CHEMICALS AFFECT HUMAN FERTILITY

While it is difficult to detect actual effects on human fertility, there are chemicals known to affect most of the biological and behavioural factors that in turn affect fertility. General principles of reproductive toxicology have been discussed elsewhere (Vouk and Sheehan, 1983). Some specific examples of effects of chemicals on human reproductive function are mentioned here.

Most of the human fertility-related factors in Figure 1 are known to be affected by chemical exposures. Even the structure of the reproductive tract can be altered, for example, by prenatal exposure to diethylstilboestrol: Stenchever *et al.* (1981) found abnormalities of the reproductive organs among 13 of 17 young men exposed to diethylstilboestrol *in utero*.

Foreign chemicals can interfere with the output of reproductive hormones. Many commonly prescribed medications alter reproductive hormone production through either central nervous system action or peripheral effects (Soyka and Mattison, 1981). In their review of chemical effects on reproduction, Sullivan and Barlow (1979) cite Soviet literature in which women working with phthalate plasticizers were found to have low oestrogen levels.

The effect of chemicals on the quality of sperm production has been of particular interest because characteristics of the sperm are conveniently measurable. A decrease in numbers of sperm has been associated with lead exposure (Lancranjan *et al.*, 1975), pesticide exposure (dibromochloropropane) (Whorton *et al.*, 1977), and cottonseed meal (gossypol) (Pösö *et al.*, 1980). Increases in abnormal sperm are reported with lead exposure (Lancranjan *et al.*, 1975), cigarette smoking (Evans *et al.*, 1981) and carbon disulphide (Lancranjan, 1972). Human eggs are not so accessible, and thus the effect of chemicals on oocytes is not so easily measured. Mattison and Thorgeirsson (1978) suggest that the decreased age at menopause associated with smoking is an indirect measure of oocyte destruction.

Impotence or loss of libido can be side effects of many prescribed medications

(Soyka and Mattison, 1981), Kepone (chlordecone) (Sullivan and Barlow, 1979) and perhaps other pesticides or herbicides (Espir *et al.*, 1970).

The effects considered so far are those which would tend to impair fertility. It is not impossible that exposures to environmental agents could have the opposite effect. Low-dose radiation *in utero* has been reported to increase fertility, possibly by accelerating oocyte maturation (Meyer and Tonascia, 1981). A chemical might behave similarly, although no such effect from accidental exposures is known.

In sum, there is evidence of qualitative effects of chemicals on most reproduction-related processes in humans as well as in laboratory animals. In the following sections, quantitative effects of chemicals on fertility will be discussed.

4 QUANTITATIVE ASSESSMENT OF THE EFFECTS OF CHEMICALS ON FERTILITY

Given that chemicals can disrupt the processes leading to fertilization, how can dose-related effects be assessed? In the laboratory, this problem can be approached under controlled conditions: dose can be regulated and nearly any stage of the reproductive process observed. The limitation of this approach is that it can be applied only to non-human species. In the study of humans, chemical exposure and fertility effects are harder to quantify, and the results are less certain. Thus, neither the laboratory nor the epidemiological approach is entirely satisfactory. The pitfalls of extrapolating from laboratory species to humans may be no less than the problems of extrapolating from one human study to all exposed persons.

In this section, various approaches to infertility risk assessment in animals and humans will be considered. This section concludes with a discussion of the problems of establishing safe or acceptable levels of exposure with regard to fertility.

4.1 Quantitative Estimation of the Effects of Chemicals on Animal Fertility

Laboratory tests of fertility involve dosing one or both parent animals and observing subsequent pregnancies. In serial mating, the male is dosed and then caged with a series of previously unmated females over several weeks. The females are usually killed and fertility assessed in terms of numbers of dead and viable fetuses. This method can detect the transient male infertility that accompanies damage to a particular stage of spermatogenesis. With some chemicals, exposure may have to be extended to very young males in order to detect adverse effects on adult fertility. For example, topical exposure of infant male rats to hexachlorophene leads to decreased fertility when they become adult (Gellert *et al.*, 1978).

When females are dosed, a forced-breeding design is often used: litters are removed immediately after birth, which encourages repeated pregnancies. Fertility is assessed in terms of frequency of pregnancies and size of litters. As with males, the exposure of females may have to be extended to early life in order to detect effects on fertility. Even exposure *in utero* can have effects on subsequent fertility. Diethylstilboestrol exposure *in utero* produces a clear dose-related effect on the fertility of female mice (McLachlan and Dixon, 1976). Benzo(a)pyrene similarly causes severe impairment of fertility among both male and female mice who are exposed as fetuses (MacKenzie and Angevine, 1981).

The possible importance to fertility of very early exposures favours the use of multigeneration studies. In this study design, both parents are dosed and then mated. Their offspring (F_1) are continuously dosed and then mated with unexposed partners. In some cases, animals from the next generation (F_2) are also dosed and mated. Fertility of each generation is assessed using such measures as number of copulations per oestrous cycle, number of pregnancies per copulation, proportion of males that impregnate, proportion of females that are impregnated, and number of offspring per pregnancy.

Multigeneration studies of reproductive function are performed for regulatory purposes. Many of these have not been published (Clegg, 1979). Among published reports of fertility impairment in multigeneration studies, the effects on fertility are generally more marked among the F_1 and F_2 generations than in the F_0. This is true for polychlorinated biphenyls (Linder *et al.*, 1974), organophosphate pesticides (Ambrose *et al.*, 1970), and, less clearly, for carbaryl (Collins *et al.*, 1971). These findings underscore the importance of remote exposures for the effects of chemicals on fertility. The magnitude of an effect may depend not only on the dose and on the age at which the dose is given, but also on whether the parents and grandparents were exposed.

4.2 Quantitative Estimation of the Effects of Chemicals on Human Fertility

Accurate measurement of chemical effects on human fertility is limited by uncertainty in estimating chemical exposure, as well as by difficulties of measuring fertility endpoints.

4.2.1 Measuring Human Exposure to Chemicals

The exposure of a person to chemicals can rarely be determined exactly. When the chemical is a prescription drug, medical records may provide documentation of the prescribed dose. Estimates of exposure are also possible with non-prescription drugs and other self-administered substances, for which the dose rate may be derived from persons' reports of their own consumption. Occupational exposures are even less definite. Persons may be unaware of the

kind or amount of their exposure. Furthermore, occupational exposures can involve complex mixtures of chemicals, such that the separate effects of individual chemicals cannot be easily isolated. Where job-specific exposure data are available, individual exposure can be estimated on the basis of job type and duration. Other environmental exposures, such as exposure to pesticides, are the most elusive. This is especially true if the exposure has taken place some time in the past. Laboratory methods for estimating chemical exposures, including biological monitoring, are steadily improving but provide only partial answers to these problems (Rogan, 1981).

There are specific aspects of exposure to be taken into account when considering fertility as an outcome. To affect fertility, a chemical must gain access to germ cells or to the accessory organs that regulate reproduction. Thus, the route of exposure (gastrointestinal tract, skin, lungs) may influence the potency of effect. Timing of exposure is paramount for effects on fertility. A chemical that damages one stage of spermatogenesis or oogenesis may produce infertility only during a specific interval after exposure. Extremely long latency effects are possible, as in the impairment of fertility reported with fetal exposure to diethylstilboestrol (DES) (Mangan *et al.*, 1982). Unfortunately, remote cause-and-effect relationships are hard to recognize in humans. The damage apparently inflicted by DES on fertility would probably have gone unnoticed were it not for the attention already drawn to DES as a transplacental carcinogen.

4.2.2 Measuring Human Fertility

Every method for measuring human fertility is arbitrary in that each method takes into account some factors and disregards others. This is demonstrated in the following sections, which summarize approaches that have been or could be used to measure human fertility.

4.2.2.1 Measuring Prefertilization Factors

A chemical that totally disrupts a prefertilization factor can produce sterility. Incomplete disruption of these factors may only partially impair fertility. The advantage of prefertilization factors is that they are usually easier to measure than the fertility endpoints (Figure 1). For this reason, prefertilization factors are sometimes used as substitute endpoints for fertility in the study of the effects of chemicals on fertility. However, the degree to which chemical damage to a prefertilization factor will actually impair fertility is not easy to predict.

Semen Analysis The relation of the properties of semen to fertility is not certain. MacLeod (1950, 1955) and MacLeod and Gold (1951a,b,c, 1952, 1953) analysed ejaculate volume, sperm number, sperm motility and sperm morphology, and the relation of these properties to fertility. They concluded that ejaculate volume and sperm morphology were unrelated to the length of time required for a couple to

achieve pregnancy. Similarly, sperm number was unrelated to fertility except when the sperm count was less than 20 million/ml. The best predictor of fertility was the fraction of active sperm and the quality of sperm activity, although even those were not highly correlated with time to pregnancy. Thirty intervening years of research have not greatly advanced our understanding of these relationships (Mann and Lutwak-Mann, 1981).

In addition to their indirect relation to fertility, semen characteristics are not as simple to measure as they might seem. The results depend on methods of collection and sample preparation, and on the selection criteria for donors. To complicate matters, semen characteristics fluctuate with the time of day, season, temperature and other factors (Mann and Lutwak-Mann, 1981). The lability of semen composition and variations in analytical methods have led to disagreement over such basic questions as whether semen characteristics have changed over time (Nelson and Bunge, 1974; MacLeod and Wang, 1979). These uncertainties make it hard to know what effect a chemical that alters sperm characteristics might have on reproductive capacity.

More complex assays of sperm have been developed. Wyrobek and Bruce (1978) considered that abnormal sperm shapes would indicate exposure to mutagens. Yanagimachi *et al.* (1976) developed an assay in which human sperm were tested for their capacity to penetrate hamster ova. While this assay directly addresses the question of fertilization, it has not yet been established that it predicts fertility more reliably than other semen properties.

Anovulatory Cycles The male has millions of mature germ cells at any given time. The female typically has only one. If no egg is produced, the woman is sterile for that cycle. The occurrence of anovulatory cycles is commonly observed among couples who are infertile. Dor *et al.* (1977) attributed the infertility of 32% of couples seeking medical care to anovulatory cycles. Anovulatory cycles occur in the general population, but at a low frequency. Vollman (1977) collected basal body temperatures in a longitudinal study of menstruating women. Among women in their twenties and thirties, 4% of menstrual cycles failed to produce the temperature spike of ovulation.

Anovulatory cycles are apparently common among infertile women, but the extent to which exposure to chemicals may contribute to anovulation is not known. The relative ease with which ovulation can be monitored (using daily temperatures or urine assays of menstrual hormones), and the direct relation of ovulation to fertility, would seem to make this a promising endpoint in the study of the effects of chemicals on fertility.

4.2.2.2 Measuring Factors near the Time of Fertilization

Chemicals that affect a factor near the time of fertilization will have a more direct effect on fertility. However, these factors are less likely targets for chemical interference than prefertilization factors.

Frequency of coitus is strongly related to the probability of conception (MacLeod and Gold; 1953, Glasser and Lachenbruch, 1968; Bongaarts 1975a). The decline in fertility found with increasing age is at least partly due to a decline in frequency of intercourse. While chemicals are known to affect libido and sexual performance, many other conditions affect these also. The inherent privacy surrounding sexual behaviour makes it difficult to study such behaviour as an endpoint.

In developed countries, the use of contraceptives is the major regulator of fertility. Secondary infertility due to chemical exposures would occur to contracepting couples without being recognized. In theory, it might be possible to detect such infertility by studying rates of contraceptive failure (pregnancy) in exposed and unexposed users of contraceptives. In practice, a prohibitively large number of contracepting couples would be required to obtain the number of unintended pregnancies required to estimate fertility. The opposite hypothesis is also possible: a chemical might interfere with the mechanism of action of the oral contraceptive or intrauterine device, leading to an increase in the failure rate. While both are plausible, neither is likely to prove useful in detecting the effects of chemicals.

4.2.2.3 Measuring Fertility Endpoints

The total number of children or pregnancies is not a sensitive measure of fertility in a contracepting population. The average reproductive capacity of humans is so much greater than the average desired family size that even couples with impaired fertility are often able to achieve their desired number of children, given enough time. When measuring partial impairment of fertility, a more sensitive measure of fertility is the probability of pregnancy or birth occurring under defined conditions and within a fixed time. This probability can be estimated from data as simple as vital statistics, or as sophisticated as the radioimmunoassay of human chorionic gonadotropin. In this section, several study methods will be reviewed, and a new method proposed.

Demographic Methods Demographers define fertility in terms of live births, and rely heavily on routine vital statistics. A couple's capacity to conceive is called 'fecundability', and is distinguished from 'fertility', or actual number of children. While fecundability has not been a prime concern of demographers, there has been some effort to estimate this underlying capacity based on a population's pattern of live births and some assumptions about fetal loss. The best estimates of fecundability are obtained either from the time required for pregnancy to occur or the number of births within a certain time period (Mustafa, 1973). Lee and Lin (1976) and Pullam and Williams (1977) have proposed methods based on the intervals between live births. James (1963, 1973), Suchindran and Lachenbruch (1975) and Bongaarts (1975b) have described

methods based on the time required for conception of a live birth to occur. In general, these methods produce coarse estimates of fecundability based on large populations. The most sophisticated work in this area has been the development of models to simulate the relationships between fecundability and birth spacing or total number of live births (Potter *et al.*, 1968; Bongaarts, 1975a, 1976).

Demographic methods have recently been adapted to the purpose of estimating the effect of occupational exposures on fertility (that is, live births). Dobbins *et al.* (1978) discussed a survivorship approach to the interval between live births for use in occupational settings. Levine *et al.* (1980, 1981) proposed a method for standardizing the occurrence of live births by year of birth, race, and maternal age, to obtain an 'expected' fertility that was then compared with the observed fertility. Neither of these methods took into account possible differences in the extent of birth control. Levine assumed that the use of birth control was independent of occupational exposure, and therefore that their method was unbiased. This may be true for occupationally exposed men but is not necessarily so for occupationally exposed women whose decisions regarding pregnancy can be influenced by employment and vice versa. Furthermore, slight differences between groups in the prevalence of sterilization may lead to large (if unbiased) disturbances in these measures of fertility.

The simulation techniques described by Bongaarts (1975a, 1976) and others would be useful for evaluating the utility of these applied demographic measures of fertility. For example, if a chemical exposure reduces the probability of conception by one-third, how might this affect time to pregnancy, or spacing of live births? How many persons would have to be included in a study design such as Levine's in order to detect a fertility decline of that magnitude?

In sum, demographic methods usually assume large samples, often involve the interpretation of patterns of live births, and are more advanced in theory than in practice. They have only recently been applied in studies of the effects of chemicals on fertility, and their usefulness in this context has not yet been established.

Other Approaches The time required for pregnancy to occur can be measured in more precise ways. For instance, women can be asked the length of the non-contraceptive interval that precedes pregnancy. In one study designed for other purposes, 93% of women enrolled at pregnancy are able to specify the length of the preceding non-contraceptive interval (B. Strobino, Columbia University, personal communication). This type of information necessarily excludes women who are unable to conceive and under-represents women whose subfertility leads them to have fewer pregnancies than desired. None the less, the usefulness of this method for detecting impairment of fertility deserves to be explored.

The bias inherent in the previous approach could be avoided by enrolling women before or at the beginning of their non-contracepting interval, and following them until they are pregnant. One way to do so would be to enlist

women of reproductive ages into a history-keeping study in which menstrual histories and contraceptive use would be continuously recorded. A previous study, using menstrual cycle calendar cards, has been successful in collecting prospective data on pregnancies (Treloar *et al.*, 1967; Wilcox *et al.*, 1981). Such an approach would offer complete data on the time required for recognized pregnancy to occur, as well as on the numbers of women who fail to conceive. One disadvantage to this approach is that it requires a large number of participants over an extended period of time in order to identify enough pregnancies for analysis.

A smaller number of subjects would be required for a study that enrolled women at the time they are first vulnerable to pregnancy. Women could be followed from the time they discontinue contraception, for example, until they become pregnant. This approach could be further refined by using a biochemical assay to continuously monitor for pregnancy. Recent developments in the radioimmunoassay of human chorionic gonadotropin (hCG) make it feasible to detect very early pregnancies using assays of urine. The sensitivity and specificity of the radioimmunoassay of hCG has been steadily improving (Wehmann *et al.*, 1981) to the point that its application now seems practical for epidemiological investigations.

A case-control study design, ordinarily used for investigating rare diseases, might reasonably be applied to exposures that may affect fertility. Couples with infertility of unknown cause could be compared with fertile couples for differences in exposure to chemicals. The selection of cases depends on the definition of infertility. Cases could be chosen by broad criteria such as suggested by WHO (1975), or could be confined to particular classes of infertility, such as anovulatory women or men with abnormal semen analysis. This problem of defining a case is further complicated by the selective manner in which infertile persons present themselves for medical care (Rachootin and Olsen, 1981).

A very different approach to the measurement of fertility uses women who are being artificially inseminated by donor sperm (for example, Fédération CECOS *et al.*, 1982). These are wives of men with known fertility impairment and therefore represent a supposedly normal group of women. (In fact, this group probably includes a slight excess of subfertile women (Emperaire *et al.*, 1982).) The fertility of these women is expressed in the number of cycles requiring artificial insemination before pregnancy occurs. The quasi-experimental setting of artificial insemination makes it possible to monitor factors (such as the times of ovulation and insemination) that otherwise are not accessible. The application of this approach to the study of environmental factors on fertility has begun in at least one centre (J. Overstreet, University of California at Davis, personal communication). Such a method could explore the effect of common exposures such as smoking, coffee or tea, and alcohol on female fertility. However, it could not easily be extended to study the effects of less common exposures.

4.2.2.4 Measuring Fertility among Users of Intrauterine Devices

Most of the approaches discussed above cannot distinguish between infertility and early pregnancy loss. With this in mind, a method is proposed here which would provide a relatively specific measure of the capacity to conceive. The intrauterine device (IUD) is a widely used contraceptive that may not be contraceptive in the strictest sense. It was once thought that the IUD prevents fertilization, but more recently it has been suggested that it acts to disrupt implantation. Human chorionic gonadotropin is produced by trophoblastic tissue after implantation. In the late 1970s, there began to appear reports of hCG detected fleetingly among women using IUDs. In 1978, Saxena and Landesman reviewed positive and negative findings of hCG among IUD users. They suggested that implantation could indeed be occurring among IUD users, and that the inconsistencies in hCG assay findings might in part be due to the use of insensitive assays. Since then, two studies applying the most advanced methods of early pregnancy detection have found persuasive evidence of frequent pregnancy among IUD users. Hodgen and his colleagues (1978) simultaneously applied three urine assays to highly concentrated urines, and found brief but distinct spikes of hCG among five of 26 women using IUDs. Smart and colleagues (1982) detected the recently identified 'early pregnancy factor' in 6 of 23 cycles occurring to IUD women, but in none of 13 cycles occurring to sterile or sexually inactive women. These studies suggest that an IUD user has a 20–25% chance of a detectable pregnancy in any given cycle.

If women conceive while using the IUD, then their rates of conception offer a direct measure of their fertility. Moreover, this measure is available continuously, rather than being confined to the non-contracepting interval preceding a birth. IUDs are used by about 14% of contracepting women in the US between the ages of 20 and 40 (US Vital and Health Statistics, 1981). The proportion of users does not vary greatly by race, age, parity, educational level or family income. This suggests that a pool of IUD users could be found in most populations exposed to a suspected hazard to fertility.

4.3 Quantitative Estimation of Risk: Extrapolation from Animals to Humans

There are few if any data from which to construct a dose–response curve for an adverse effect of chemicals on human fertility. Inferences concerning quantitative effects on fertility can be drawn from animal studies. One of the best examples of animal dose–response data is the relation of *in utero* exposure to diethylstilboestrol, and subsequent fertility. In a laboratory study previously cited (McLachlan and Dixon, 1976) fertility was measured in terms of live births to force-bred female mice. A decline in fertility was directly related to prior fetal

Table 1 Fertility of female mice exposed to DES prenatally

Maternal DES dose (μg/kg)	No. of offspring	Total reproductive capacity of female offspring (% control)
0	74	100.0
0.01	55	89.4
1	54	76.0
2.5	18	49.4
5	16	22.6
10	61	8.0
100	39	4.3

dose (Table 1). The highest dose given in this study (per unit body weight) is about one-twentieth of the average dose prescribed to pregnant women in the 1950s.

There are two observations to be made regarding these data. One, the effect on fertility does not rapidly approach zero even at the lowest doses tested. This may indicate that the effect of DES on fertility has no lower threshold. The absence of an apparent threshold effect with one chemical is notable, but does not reliably predict what might be found with other chemicals, considering the variety of mechanisms by which fertility can be impaired. The second observation is that the strong effect on fertility occurs at doses roughly comparable to doses previously experienced by humans. This offers an opportunity to compare the intensity of animal and human responses to a chemical exposure. There have been several studies of pregnancies among women exposed to DES *in utero* (for example, Barnes *et al.*, 1980; Mangan *et al.*, 1982). These women produce slightly lower numbers of live births. (Specifically, DES is associated with an increase in spontaneous abortion, ectopic pregnancies, and premature births.) While DES does produce a detectable effect in humans, this effect does not approach the magnitude observed in the comparable animal study. This example suggests that human reproduction might be less sensitive than that of laboratory animals. However, other evidence suggests humans may sometimes be a more sensitive species. Three chemicals with a known dose-dependent effect on fertility in both humans and laboratory animals (ethylene dibromide, carbon disulphide, and dibromochloropropane) affect humans at lower doses than animals (CEQ, 1981). Furthermore, looking at particular reproductive processes, human fertility appears less robust than that of lower mammals. For example, human sperm production is only 20–50% as efficient as that of laboratory animals (Amann, 1981), and the proportion of abnormal sperm types is higher in humans than in non-primates. Similarly, women typically produce only one fertilizable egg at a time, and the occurrence of early fetal loss seems to be higher than

observed in animals. In conclusion, the relative sensitivity of animal and human fertility remains to be established.

5 CONCLUSIONS AND RECOMMENDATIONS

Scientific interest in chemical toxicity in relation to fertility is relatively recent. There are promising methods for studying human fertility that have not yet been applied. Suggestions for further research are summarized below.

(1) Prefertilization factors are often easier to measure than fertility endpoints, and may be suitable substitutes for assessing the impact of chemicals on reproductive functions (section 4.2.2.1). There is a need for further development of sperm assays that may predict fertility (for example, *in vitro* fertilization techniques). The monitoring of ovulation may also provide a means of detecting effects of chemicals on fertility processes.

(2) Specific chemicals have been shown to affect fertility in animals and humans. However, it is not clear whether the fertility of humans or of animals is the more sensitive to disruption (section 4.3). Some assessment of their relative sensitivity is necessary in order to extrapolate accurately from animals to humans. A thorough review and comparison of animal and human data would be helpful, although the shortage of human data may preclude sound generalizations.

(3) There are several feasible methods for quantifying human fertility that have yet to be applied (section 4.2.2.3). These include the use of time to pregnancy as an endpoint, monitoring women for pregnancies by use of menstrual record-keeping, and monitoring for pregnancies by urine assays. Artificially inseminated women and women using IUDs offer opportunities for special studies of fertility (sections 4.2.2.3 and 4.2.2.4). Computer simulations may provide a tool for making the complicated comparisons necessary to evaluate demographic methods, biological monitoring methods and other ways of measuring fertility.

(4) The estimation of a safe dose is difficult for any chemical. In the case of fertility, this problem is complicated by the apparent amplification of effects seen in multigenerational studies (section 4.1). The mechanism of this cumulative effect over several generations needs further study. This phenomenon also needs to be explored in human populations, although there are limited opportunities to do so.

(5) Wherever adverse effect of chemicals on fertility is discovered, it is necessary to identify the mechanism of action. The effect of some agents on fertility is so specific and transient (for example, the disruption of spermatogenesis by gossypol) that it may be judged innocuous, or even desirable as a method of contraception. On the other hand, an adverse effect on fertility may be accompanied by permanent effects on fertility, as well as other adverse effects (like mutagenesis) that have implications beyond fertility.

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