Editorial

Detection of agents causing genetic or reproductive damage

Reproductive effects have been called "the new frontier in occupational and environmental health research"¹ and previously, "the occupational health issue of the 1980s."² By comparison with the many potentially toxic or mutagenic occupational agents currently in use, however, little evidence has so far accumulated to incriminate or to exonerate the effects of specific exposures on reproduction. Even less evidence exists for dose effect relations.

Reproductive effects embrace outcomes such as congenital malformations, which present enormous emotional and practical problems for those affected. Furthermore, germ cell mutation has profound implications for public health, as such damage could survive the individual and be heritable. Monetary costs also exist. For example, in the United States it has been estimated that in 1987 the total cost of fertility treatment alone was one billion dollars; among congenital defects, the cost of treating neurological and communicative developmental problems was 114 billion dollars. Although only a small proportion of such problems would have been attributable to known effects of occupational or environmental agents, nearly 60% of birth defects have no known cause, and might therefore involve unknown environmental or occupational factors.³

Processes and manifestations

Both men and women may be affected. Men are at risk from agents that alter endocrine function or germ cell development. Non-pregnant women are similarly at risk from agents that affect germ cell maturation or endocrine balance, and from agents, like lead, which persist in the body and can therefore affect a subsequent pregnancy. In the case of toxicity to the germ cell in either sex, the damage may or may not involve mutation. Pregnant women are also at potential risk, firstly from agents that affect transport or implantation of the fertilised ovum (long before the pregnancy can be recognised), and subsequently from embryotoxic or fetotoxic agents. Finally, chemicals may be secreted in breast milk.

A range of possible manifest effects exists, from subfertility and menstrual disturbance, through miscarriage, to perinatal death, low birthweight, congenital malformation, and cancer or other disease

occurring in the child. A given outcome can result from different biological processes. For example, miscarriage can result from germ cell mutation or from embryotoxic or fetotoxic effects during pregnancy. Conversely, each biological mechanism can result in different manifestations. Thus damage to the developing embryo or fetus can lead to apparent non-conception manifest as reduced fertility (if embryonic death takes place before the recognition of pregnancy), miscarriage, malformation, perinatal death, or disease such as cancer in the offspring (transplacental carcinogenesis).

An increase in germ cell mutations can cause various types of adverse health effects. These can be divided into categories, each one including many different conditions: dominant effects causing embryonic or fetal death manifest as apparent non-conception or miscarriage; possibly those few congenital defects that have an important genetic component; a heterozygous (carrier) state of recessive disorders manifest as genetic disease in subsequent generations; probably cancer, especially that occurring in childhood.

In adult life men are more susceptible than women, both to point mutations and structural rearrangements.⁴ Point mutations may affect the stem cells causing permanent damage, and could therefore accumulate and lead to a paternal age effect. Conversely, structural chromosomal damage is unlikely to survive cell division and will, therefore, mostly affect post-spermatogonial cells. These are continually replaced so that the effect will be reversible.

There is reason to believe that occupational or environmental agents that increase the rate of germ cell mutation may be in current use: such increases would be difficult to detect with the conventional methods of counting affected cases because of the multiplicity of effects, each being rare. They would be difficult to link with the exposure, both at the stage of initial recognition, and in formal epidemiological research.

One possible solution to this difficulty is that apparent non-conceptions, resulting from dominant mutations with lethal effects in early pregnancy, would collectively be manifest as a decrease in biological fertility (the ability to conceive) in an exposed compared with a non-exposed population;

fertility can be measured as time to conception.

Timing is crucial in the study of reproductive and genetic damage, at least for acute toxic effects. In the case of exposed pregnant women, there are windows of sensitivity to exposure, and the type of effect depends crucially on the timing of the dose in relation to the stage of development. In the case of gametotoxicity in men, as the process of sperm production is continuous, the importance of the timing of the dose is simply that damage at a given time only shows in the ejaculate up to 90 days later, this latent interval depending on the stage of spermatogenesis or spermiogenesis affected. Later effects can also occur due to stem cell damage or to chemicals that accumulate in the body.

The current research position

A review of publications on reproductive and genetic effects shows two outstanding features. Firstly, little information is available on the presence or absence of reproductive or genetic toxicity of specific occupational or environmental agents, particularly in relation to humans, except in a few instances, such as on the soil fumigant DBCP (dibromochloropropane), ionising radiation, and lead. The epidemiological evidence that does exist largely refers to broad occupational categories or to whole groups of agents, such as work in the electronics industry or exposure to organic solvents. Unfortunately, lack of evidence cannot be taken to indicate lack of effect. The research effort is growing, and the probable outcome will be that some agents will be found to produce an adverse effect at *concentrations currently regarded as safe*. Thus the results of future research are likely to affect permitted concentrations.

Secondly, this type of research is beset with problems. Extrapolation of the results of non-human toxicological research may be suggestive of effects,⁵ but such extrapolation is unreliable because of profound differences in reproductive or toxicological mechanisms between species. Epidemiological data are therefore indispensable.

Reviews of existing epidemiological research have highlighted the widespread methodological defects of previous studies; these include problems with accuracy of data, inadequate sample size, inappropriate control groups, lack of information on confounding factors, and selection effects.^{6,7} These and other defects can be avoided if optimal measurement methods and designs comparable between studies are used.⁸ Thus measurements can be developed and tested so that they are as reliable and valid as possible. Data from different studies can be pooled, which increases statistical power while having other advantages, including generation of expected (baseline) rates. Then a systematic study of possible confounding factors can be carried out.

Studies of female workers require particular care to avoid selection effects due to the interaction of their domestic responsibilities and working lives.⁹ For example, because in many countries most women are absent from the workforce for some time after the birth of a child, any employed female population is likely to contain an increased proportion of relatively infertile women (the “infertile worker effect”).

Other methodological problems occur particular to this area of study, including the non-independence of different pregnancies that occur in the same subject and the “dose-response fallacy.”¹⁰ It is therefore a difficult area of research for the non-specialist.

Study design

Perhaps surprisingly, the assessment of exposures is more problematic than ascertainment of the reproductive outcome variables for at least three reasons. Firstly, quantitative measurements or estimates may not be available and even the accurate descriptive characterisation of agents may present difficulties. Secondly, for fluctuating or accidental exposures, the timing may not be accurately known. Thirdly, there is usually not just one, but a mixture of exposures.

Such problems are less intractable in study designs based on populations having a common exposure. Occupational categories have a particular advantage in this respect as the population at risk can be clearly specified from personnel records and the exposure(s) can be documented. A further strength of the exposure based design is that it is possible to study multiple outcomes, which, as already noted, may correspond to a single biological process.

If the population is not defined in terms of exposure but rather in clinical terms, the difficulties of assessment of exposure become more serious. This is true both of the case-control study design in which the starting point is a case series, and of national population registers of adverse outcomes—for example, of congenital malformations or miscarriages—which exist in certain countries. Furthermore in these circumstances, even more fundamental than the problems of measurement already mentioned, the distribution of exposures is likely to be adverse as the study population will include only small numbers of workers exposed to each specific agent, except in the case of exposures which are highly prevalent in the general population.

Outcome measures

Outcomes can be assessed by means of biological or questionnaire based methods. Each has its strengths and weaknesses, and they are best regarded as complementary. In evaluating each method, there

are five important considerations: (a) does it have good *measurement properties* such as accuracy and stability; (b) how well does the measure relate to disturbance of *function*; (c) how well does the measure correspond with a biological *mechanism*; (d) where the previous two do not apply, is it a good *indicator variable*; and (e) how *practicable* is it to collect?

Semen examination traditionally includes concentration or number, motility, and morphology of sperm but these measurement properties are far from ideal. Concentration and number are affected by within person variability (for example, duration of abstinence); motility is sensitive to temperature; morphology is subject to differences in criteria between laboratories.³ In recent years, a computer assisted videomicrographic technique has been developed that enables many parameters to be measured quickly and automatically. Their measurement characteristics largely remain to be determined. Whereas some parameters have been examined to determine their relation with function (fertilising capacity),¹¹ the significance of newer variables is largely unknown. In terms of mechanism, the most important is germ cell mutation, but it remains unclear which semen parameters would be affected by a mutagenic agent. In the future DNA adducts and new genetic techniques may prove valuable in this respect. A practical problem with semen studies is that they typically have a low response rate.

The menstrual cycle has been neglected as an indicator of a reproductive effect, but interest in this approach is increasing. For example, studies can be carried out on irregularity, cycle duration, dysmenorrhoea, intermenstrual blood loss, and reduced or excessive bleeding.¹² Retrospective studies do not give data of acceptable quality,¹³ and diary methods may be useful.

Hormone concentrations can be measured in both sexes, not only in blood samples, but also in urine or saliva although with some loss of sensitivity.³ Measurement of hormone concentrations, even in blood, is too insensitive for some purposes, such as the detection of direct testicular damage. Endocrine methods may be useful, however, when the toxicity primarily affects endocrine processes, as in the case of DDT, or during the manufacture of certain pharmaceuticals. In general, this is justifiable only when an endocrine effect is already suspected, either because functional disturbance such as subfertility has been established, or when toxicological findings suggest an effect.

Urinary human chorionic gonadotrophin (hCG) assay detects pregnancy within a few days after implantation of the fertilised ovum. It can therefore be used in prospective research and is applicable to studies both of male and female exposures. It is a functional measure, corresponding not to any

specific biological process, but rather to the final common path of conception. This is one way of measuring time to conception. At present, the use of this method is limited because the immunoradiometric assay (IRMA),¹⁴ although highly sensitive and specific, is too labour intensive for epidemiological field studies.³

Karyotyping of clinically recognised miscarriages has been extensively used, but the conclusion has been that there is little or no relation between environmental factors and the rate of human chromosomal aberrations at this stage of gestation.¹⁵

Mutagenicity tests are useful in that they indicate the potential of an agent to initiate a particular type of biological process, but they are not able to indicate whether this occurs in the context of human germ cells. They are thus complementary to the detection of disturbance of function by, for example, questionnaire based or hCG studies. Various systems are available, using bacterial systems, laboratory animals, and human somatic cells, and further developments are anticipated.

Questionnaire based methods aim at collecting an accurate reproductive history. As most of the variables correspond to important events in the lives of respondents, the accuracy of reporting is surprisingly high, even for recall periods as long as 20 years,¹⁶⁻¹⁸ and (for birthweight and gestational age) when there are many children per family.¹⁶ The possibility of bias in the reporting of miscarriages¹⁹ can be avoided if suitable precautions are taken.²⁰ The outcome variables correspond directly to function (such as time to conception, live births, or birthweight), rather than to biological mechanisms. The information is simple to collect, and such research is acceptable in occupational populations.

Retrospective questionnaire based studies have two important advantages. Firstly, it is not difficult to include large numbers of subjects in such research, each of whom is likely to have given birth to or fathered more than one child, although many of these may have been born before the exposure occurred. Secondly, because they deal with the past, it is possible to study a historical period when exposures may have been considerably higher than at present, and companies that have made improvements may be more motivated to take part in the research. A limitation is that exposure data may sometimes be difficult to obtain for earlier periods. For data collection, interviews are generally preferable to self completion questionnaires, as it is more feasible to achieve a satisfactory response rate. The cost of interviews, although high relative to the self completion approach, is justifiable in terms of the benefit derived from resources deployed.

The role of an occupational health service

Despite the present uncertainty surrounding genetic

and reproductive effects, it is necessary to be able to make decisions. Companies should have a genetic and reproductive health policy that applies to both men and women, and which includes lactating women. To exclude women of childbearing age from work with chemicals is a form of discrimination, and may also be illegal.

What should an occupational physician do when confronted by an apparent cluster of reproductive ill effects? In the present state of knowledge, such an event has the potential to make a contribution: a new hypothesis may be generated or an existing one may receive some support. It should not be lightly dismissed. It is well recognised, however, that from time to time, apparently impressive clusters will occur in the course of random variation.

Should a responsible employer consider surveillance? Other fields of epidemiology have gained benefit from the routine collection of data, including those in an occupational context. From the point of view of employees, however, reproductive events are different from other aspects of health. They may be unwilling to provide information on their partnership and use of contraception both because these matters are personal, and because they may believe that employment and promotion prospects could be jeopardised if the possibility of pregnancy were mooted. Miscarriages would tend to be under reported; it is probable that most women employees would wait until a pregnancy was well established before notifying their employers.

Nevertheless, consideration could be given to a surveillance programme which includes birth related variables such as sex of the child, birthweight, perinatal mortality, malformations, and time to conception (measured in a standardised way); this could be done both for male and female workers, and in the second case would need to include women who have left employment during pregnancy. For female workers, occupational medical care could include monitoring of menstrual function.

Occupational health departments, and other sections in industry, have an important role in facilitating the epidemiological research that is necessary. The research itself is likely to require the collaboration of independent outsiders, but the existence of an infrastructure of reliable basic data is of inestimable worth.

Good records on exposures are invaluable. Ideally, results of environmental and biological monitoring would be available, for the past as well as for the present. When this is not possible, clear description of agents used is an acceptable minimum. Similarly,

accurate employment records are essential, especially for tracing ex-employees; and records on work assignments are crucial for matching individual workers to departments, except in those work sites where personnel tend to remain in the same type of job throughout their time with the company.

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