SOURCES AND EFFECTS OF IONIZING RADIATION

United Nations Scientific Committee on the Effects of Atomic Radiation

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NOTE

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ANNEX G

Hereditary effects of radiation

CONTENTS

INTRODUCTION

1. Evaluation of the hereditary hazard associated with the exposure of human populations to ionizing radiation has been a major concern of the Committee since its inception. Many approaches have been used to formulate optimal predictions of the extent to which a given dose of ionizing radiation will increase the naturally occurring mutation rate of germ cells and of how such an increase will affect the health of future human populations. However, an extremely complex set of problems remains, and there are many questions that cannot be answered given the present state of knowledge.

2. Attempts at risk estimation entail uncertainties, in part because it has not been possible to directly confirm radiation-induced mutations in human populations. Genetic risk estimates have relied on a general knowledge of human genetics and on the extrapolation of results from animal experiments. Limited data from long-term studies on the children of radiation-

exposed parents, particularly those exposed to the atomic bombings of Hiroshima and Nagasaki, may be used to set outside limits on genetic risk estimates. These data indicate that the hereditary effects of moderate irradiation of a large human population are minimal, at least for acute exposure.

3. Progress is being made in several areas of human genetics. There have been major advances in the knowledge of non-traditional inheritance and so-called multifactorial disease in humans. Previous UNSCEAR Reports raised the question of whether, and how, to include estimates of genetic risk for multifactorial diseases. This category of disease represents a heterogeneous group for which the underlying mechanisms are poorly understood, although they clearly have a genetic component. Multifactorial diseases are related in a complex way to other risk factors and to population structure and living conditions. Radiation damage may affect the regulation of genes involved in multifactorial inheritance by many different mechanisms and may also affect the structure and function of single genes. These multifactorial diseases and non-traditional mechanisms of inheritance are discussed in this Annex in order to emphasize the complexity of human genetics and biology, as well as the difficulty of obtaining accurate risk estimates from studies of only a few well-defined disorders in animal models.

4. The understanding of human genetics on a molecular level is increasing extremely rapidly. As discussed in this Annex, new laboratory techniques allow a more precise analysis of the type of genetic damage caused by various agents, including radiation. Eventually, the sequencing and identification of every gene on every chromosome will provide a method of direct access to the effects of radiation on human genes at the molecular level. Knowledge of population genetics, gained from registries of birth defects, is improving estimates of the current incidence of serious birth defects, with which any estimate of risk from radiation may be compared.

5. The knowledge gained from the Japanese experience, from advances in human genetics, and from animal studies should allow uncertainties of risk estimates to be gradually reduced. However, as the difficulties inherent in the estimation procedures become even more apparent, there is increased uncertainty and/or inability to specify appropriate and realistic values. Practical solutions are needed. In this Annex, the complexities involved in making human risk estimates using animal models are pointed out, the best estimates of genetic risk that can be made at this time are discussed, and ways of obtaining information that could lead to more reliable genetic risk estimates are suggested.

I. GENETIC DISEASES IN HUMANS

6. Genetic diseases occur because of alterations to the structure or regulation of DNA in the cells of an organism. Genes are units of heredity, comprised of specific sequences of DNA and carried on the chromosomes and in mitochondria. Mutations (changes in DNA structure, arrangement or amount) occur spontaneously or are caused by physical or chemical agents.

7. Genetic disorders have traditionally been classified into three categories: (a) single-gene disorders, (b) chromosomal aberrations, (c) multifactorial disorders. Although many recent developments in molecular biology and new concepts relating to mechanisms of disease processes have made these distinctions less clear, the classification will be retained for the discussion that follows.

A. SINGLE-GENE DISORDERS

8. Single-gene disorders are usually recognized by their clinical manifestations, but research is now identifying many disorders on the molecular level. Disease traits occur because of mutations to the normal gene. Alleles are alternative forms of a gene at a particular locus, or position on the chromosome. There are two levels on which the genetic constitution of an individual may be considered: the genotype, or particular alleles of a given gene carried by an individual, and the phenotype, or the physical, bio-chemical or physiological characteristics determined by these alleles. These single gene defects are often called Mendelian, after Mendel, who first described these units of heredity.

9. Simple Mendelian traits are inherited by autosomal transmission (i.e. the genes for them are carried on one of the 22 pairs of autosomes, or non-sex chromosomes) or by X-linked transmission (i.e. the genes are carried on the X-chromosome). With autosomal genes, one copy of the gene is normally contributed by the mother and one by the father. X-linked genes will always come from the mother in a male (as he has received a Y-chromosome from his father), or from both parents in a female (since she has received an X-chromosome from each parent).

1. Dominant traits

10. Dominant disorders are those in which there is a clinically recognizable abnormality produced, even if only a single copy (allele) of the gene is abnormal, i.e. in the heterozygous state. On average, the autosomal dominant gene (i.e. the abnormal allele for the trait) will be transmitted to 50% of the offspring of an affected individual. Unaffected individuals will not usually carry or transmit the abnormal gene. Affected individuals will have an affected parent, except in the event of a new mutation of the gene.

11. Autosomal dominant traits are not always expressed to the same degree in all individuals who carry them. In complex genetic and environmental interactions, some members of a family may be severely affected and others only mildly so. There may even be transmitters of a dominant allele who appear phenotypically normal. To describe this phenomenon, the terms penetrance and expressivity have been developed [T9].

12. Heterogeneity is the term used to describe situations in which the same or nearly the same phenotype is produced by different mechanisms. There are many situations in which heterogeneity appears to exist. For instance, homocystinuria can produce a symptom complex that strongly resembles Marfan syndrome [B7].

13. Conversely, it is possible that two different mutations in the same gene will produce different phenotypes. This may occur when the mutations lie in different domains, or regions, of the protein product and thus affect different functions of the same protein. For example, osteogenesis imperfecta types I and II and some occurrences of Ehlers Danlos syndrome may be caused by mutations in the alpha 1 chain of type I collagen, but depending on which portion of the collagen molecule is altered, markedly different diseases occur [B7].

2. Recessive traits

14. Recessive disorders are those that are usually clinically expressed only when both copies of the gene are abnormal (homozygosity). If two different abnormal alleles of the gene are present, the individual is said to be a compound heterozygote. A recessive disorder will also be clinically expressed when there is one abnormal copy and no normal copy of the gene is present (hemizygosity). Hemizygosity occurs normally in males for the X-chromosome; this leads to disease when the single normal copy of a gene carried on the X-chromosome is lost or damaged. This is known as an X-linked disorder. Hemizygosity can also occur in autosomal genes if there is a deletion of part of the chromosome or if one of a pair of chromosomes is lost. When an autosomal recessive condition is present in a family, cases usually occur among siblings. The abnormal copy of the gene (the abnormal allele) may also be carried by other relatives, offspring and parents, but the trait or disease will appear only in the offspring of two individuals who carry the abnormal gene. This type of pairing is rare in the general population.

15. Frequently, homozygotes for autosomal recessive disorders do not reproduce, because the homozygous state of the deleterious gene reduces their biological fitness. For this reason, some autosomal recessive traits must have conferred a selective advantage on the individual during the course of evolution, otherwise the mutation would not be as prevalent in the general population as it is. A mutation may be maintained at relatively high frequency in a population by this kind of selective advantage or because of a founder effect, or because of genetic drift (see glossary) [V10].

16. Consanguineous marriages may also play a role in producing a homozygous individual for a rare autosomal recessive condition. In a consanguineous marriage, the parents of an affected individual are related (e.g. first cousins), which makes them more likely to have inherited the same abnormal allele [L5]. Some human populations have higher rates of consanguineous marriage than others.

17. Occasionally, each parent may carry two different alleles of a particular gene; in this event the offspring could inherit two differently abnormal copies of the gene. This might result in a disease characterized by a combination of the symptoms of two somewhat different diseases. Alternatively, each of the two defective alleles could compensate for the other, resulting in a normal phenotype. This is called complementation.

18. Mutations occur on a regular but unpredictable basis. They are most frequently observed as dominant mutations, since the dominant nature of the gene allows expression and immediate recognition. However, new mutations must be occurring in genes for recessively inherited traits as well. It is estimated that the mutation frequency for some genes is in the range of 1 in 30,000 to 1 in 50,000 live-born individuals, but most mutation rates are probably lower. Some human disorders, such as neurofibromatosis I, have far higher mutation frequencies (1 in 6,000). This difference in mutation frequencies may be influenced by the size of the gene, its vulnerability, its exact position on a chromosome (e.g. at a mutational hot spot) [V3, W3], or by other as yet undefined factors [V5].

3. X-linked traits

19. X-linked traits, as mentioned above, are those in which the gene producing the abnormal phenotype is located on the X-chromosome. Because males do not pass their X-chromosome on to their sons (i.e. the sons are male because they have inherited their father's Y-chromosome), there will be no male-to-male transmission of an X-linked trait in a pedigree. Females, with two X-chromosomes, may or may not manifest an abnormal phenotype for an X-linked trait, depending on whether the trait is dominant or recessive. In addition, the manner in which normal X-inactivation (lyonization) occurs may affect expression. Thus, if by chance the normal allele is on an X-chromosome that is inactivated in more than 50% of the cells, a carrier female may express some features of even a recessive disorder.

B. CHROMOSOMAL ABERRATIONS

20. Each species has a characteristic chromosomal constitution with respect to number and morphology. This is called the karyotype and can be visualized under the microscope at the stage of the cell cycle where condensation occurs. Normally, a human being carries 46 chromosomes in each somatic cell. These comprise 22 homologous pairs of autosomes and one pair of sex chromosomes. The members of a homologous pair are matched with respect to the genetic information that each carries, although they may contain different alleles of the same genes.

21. There are two types of cell division. Mitosis is the usual type that occurs as the body grows and replaces cells. Mitosis involves the precise duplication of each chromosome so that the daughter cells will be identical to the original cell in terms of genetic information. The second type of cell division is meiosis, the specialized process of producing gametes (ova and sperm). During meiosis, the diploid number of 46 chromosomes is reduced to the haploid number, in which only one copy of each chromosome pair will be present.

22. The union of the egg and the sperm at fertilization reestablishes the normal diploid number of chromosomes. Abnormal numbers of chromosomes, breaks, or rearrangements of chromosomes or of segments of them can produce major abnormalities in the affected individual. The presence of abnormal numbers of chromosomes is called aneuploidy (i.e. not euploid). Certain chromosomal rearrangements are not compatible with viability or are selected against by the growth of more vigorous normal cells during development. However, a wide variety of rearrangements (translocations, inversions etc.), breaks and extra or missing chromosome segments may be tolerated.

C. MULTIFACTORIAL AND POLYGENIC INHERITANCE

1. Determining factors

23. The terms polygenic and multifactorial refer to those traits, diseases or congenital anomalies whose development has a genetic component but whose inheritance does not follow standard Mendelian patterns for autosomal dominant, autosomal recessive, or sex-linked transmission, suggesting that more than one gene is involved. Rather, many genes acting in concert are thought to be responsible. If environmental factors appear to play a role in the development of the trait or disease, it is described as multifactorial. These so-called multifactorial disorders are a very heterogeneous group, including about 95% of conditions having a genetic predisposition. They are observed at all ages and in all human populations. Their aetiology is complex, heterogeneous and poorly understood. It is important that this term be used only when referring to a single condition or trait (e.g. clubfeet, not multiple contractures) and that all disorders due entirely to a defect in a single gene be excluded.

24. Observations suggest that there is a gradation of factors contributing to the development of a multifactorial trait and that a certain number of such factors are necessary to produce the trait. If an individual has a susceptible genotype and is exposed to a predisposing environment, the likelihood that he or she will manifest the trait depends upon the accumulation of both genetic and environmental factors beyond a certain threshold. The threshold is the point on a liability scale below which individuals are not affected and above which they are affected [F7].

25. Numerous environmental factors may affect the development of multifactorial traits. Environmental factors include the milieu in which the embryo and fetus develop, including both intrinsic and extrinsic factors such as drugs, airborne toxins, viral or bacterial agents and radiation; abnormalities of maternal metabolism, such as diabetes mellitus, malnutrition, or maternal hyperthermia, may also be a factor [F7, F8].

26. In calculating the recurrence risk for such multifactorial disorders, it is first essential to determine, along with the family pedigree and the preconceptional and prenatal history, whether there are other cases of such disorders in unrelated families who live in the same geographic area. A case cluster of this type would suggest that environmental factors may be particularly important in the aetiology of the defect [B8].

27. Human susceptibility to teratogenic or mutagenic agents, including radiation, may differ between individuals and between humans and mice. Likewise, there

are ethnic or strain differences within a single species. Cleft lip with or without cleft palate can be induced in 100% of offspring of A/J strain mice when pregnant females are treated with corticosteroids on days 11-14 of gestation. By contrast, only 20% of the offspring of C53B1/6 mice given the same treatment will have clefts [F7]. Direct comparisons between humans and mice are not possible; however, ethnic differences are known to exist for multifactorial conditions in humans.

28. Both genetic and environmental factors can influence the development of a given embryo relative to a particular threshold by acting at any point during development. When different strains of mice are crossed and interbred, the picture is further complicated by new combinations of genetic backgrounds. The same is most likely true for humans with different ethnic and genetic backgrounds.

29. Thus, in applying the mouse model to human studies, it must be borne in mind that the embryo's teratogenic (or mutagenic) susceptibility can be influenced by its normal developmental patterns and interactions. Thus an ethnic (strain) or regional group difference in the frequency of an induced malformation could occur even if the primary effect of the teratogen or mutagen was the same in the two strains. This is a very important aspect of the threshold model of multifactorial inheritance.

30. It should also be kept in mind that two or more mutations (or external mutagens) can act together either additively or synergistically on any developmental process. Furthermore, multifactorial systems are heterogeneous, i.e. two individuals who are similarly liabile to develop a particular disorder may be so as a result of completely different genes and/or mechanisms.

31. In humans, there are numerous examples of differences in incidence between different ethnic groups and different geographic regions, as can be observed in studies of neural tube defects. Neural tube defects are a heterogeneous group of anomalies that involve the developing brain and spinal cord. The epidemiologic characteristics of neural tube defects include a higher incidence in females, in lower socioeconomic groups, and in people of Celtic or East Indian ancestry, and prevalence rates that vary substantially in different geographic regions [H4]. While neural tube defects are generally registered as a single category of defect, their causes are heterogeneous and can be influenced by teratogenic exposure or maternal nutritional deficiency or illness. In some families recurrence is frequent, in others only sporadic. Each of these components of the overall category "neural tube defect" is likely to be differentially affected by exposure to various mutagenic agents [V12], and, as with other multifactorial disorders, nutrition and lifestyle, i.e. environment, can have a protective effect as well as a damaging effect. For example, folic acid supplementation has been shown to reduce the recurrence of neural tube defects [W4].

32. It is also important to remember that multifactorial disorders often manifest with different gradations of severity. Thus if a new mutation simply increased the severity of a disorder, it would probably not be noted as a change in the incidence of the disorder in the population.

33. In exploring the relationship between environmental factors and the occurrence of congenital anomalies, one is confronted with an extremely complex situation. In multifactorial disorders, an environmental factor or factors interacts with a susceptible genotype to produce the defect. The uniqueness of this interaction in a given family may preclude generalization to the population as a whole. As a result, it is perhaps not surprising that it is so difficult to define specific environmental factors that correlate significantly and consistently with particular congenital anomalies in humans. This difficulty is particularly relevant to any attempt to predict the effects of radiation exposure on multifactorial disorders.

2. Risk of recurrence within families

34. When determining the risk of recurrence for common multifactorial disorders, empirical data are used, as no single model explains the observed variables. But even empirical data from a large random sample will not take into account the uniqueness of each family, its environment, or the particular events of a given pregnancy that might affect the outcome. Thus, only generalizations can be used for dealing with questions of risk and recurrence. The models developed to describe multifactorial inheritance are just that, models. In no case have all of the specific genes involved and their interactions been defined. Thus it becomes very difficult to predict the effects of a particular type of mutation (e.g. radiation-induced), much less the multiple environmental agents involved [F8, F10].

35. In general, the risk of recurrence for multifactorial disorders is directly related to the severity of the disorder in the proband or index case. This is, in a sense, a dose-response phenomenon, i.e. the more genes that are involved in causing the defect and the more environmental factors that contribute, the more likely the defect is to occur with a severity proportional to both genetic and environmental contributions. Assuming that the potential gene pool of a couple

remains constant and that they continue to live in the same environment, the risk of a congenital anomaly recurring will be greater if the index case child is severely affected than if it is mildly affected [F8, F10].

36. Because in most cases many unknown factors are involved in recurrence, figures for recurrence risk represent average probabilities rather than certainties. Thus, if only one child in a family is affected, the average risk of recurrence is 3%-5% for most multifactorially inherited birth defects. If two siblings are affected, the average risk of recurrence is usually 5%-10%. After three siblings are affected, the risk for a fourth child is 10%-25%. As the number of affected children in a family increases towards a 25% recurrence rate, it becomes essential to ask whether one is perhaps dealing with a specific autosomal recessive trait having variable penetrance rather than with a multifactorial continuum [F10].

37. Mathematical models are available for the distribution and risk of recurrence of many multifactorial disorders [B8]. But it must be made clear that each disorder requires a separate model, and there is no guarantee that recurrence risks will remain the same from one family to another. The basic principles of recurrence risk assessment for a multifactorial disorder are as follows:

(a) the correlation of phenotype between relatives is proportional to the number of alleles in common;
(b) the correlation for offspring is halfway between the risk for parents and that for the general population;
(c) if a disorder is more frequent in one sex, the recurrence risk depends on the sex of subsequent offspring;
(d) if the population frequency is p, the risk among first degree relatives is the square root of p (when heritability is high);
(e) the recurrence risk is higher when more than one member of the family is affected;
(f) the more severe a malformation, the higher the recurrence risk;
(g) the risk to relatives drops off rapidly with increasing remoteness of relation;
(h) monozygous twins are several times more likely to be concordant than dizygous twins, but concordance is never complete (it is usually less than 40%) [S34];
(i) consanguinity increases the risk of polygenic and multifactorial conditions.

38. To estimate recurrence risks with the multifactorial model, two pieces of information are required: the frequency of the condition in the population and the empirical frequency in first-degree relatives of affected individuals. The correlation in liability between first-degree relatives can be estimated by the method of Bonaitie-Pellie et al. [B8]. This method takes into account differences in frequency between sexes and different severity-age classes. Computer programmes are available to derive the risk estimates (e.g. [B8]).

39. It must be reemphasized that it is at present difficult, if not impossible, to discriminate between a multifactorial model and a unifactorial model with incomplete penetrance for most multifactorial conditions in humans, since the different modes of inheritance lead to similar frequencies in relatives [F10]. Understanding of such disorders in humans is primarily empirical at this stage; cases are observed and recorded, but the underlying molecular mechanisms remain obscure. Gradually, more and more human genetic disorders are yielding to new molecular techniques.

D. NON-TRADITIONAL INHERITANCE

40. Many newly recognized mechanisms of gene regulation and genetic disease in humans were not known to classical geneticists and thus have not been considered in previous estimates of either background incidence or risk of heritable disorders following irradiation. Since they could, however, be significantly affected by radiation, they are discussed in this Section in some detail, with the caveat that any estimation of risk must include an understanding and consideration of these additional potential sources of hereditary disease.

1. Mosaicism

41. Mosaicism refers to the presence of both normal cells and cells carrying a mutation within a single individual. Somatic mosaicism results from a gene mutation or chromosomal anomaly arising in a somatic cell. Since the number of cells in the human body (approximately 10^{14}) exceeds the magnitude of the mutation rate for almost all genetic disorders thus far recognized, it seems likely that during the course of embryonic, fetal and postnatal life, virtually the entire repertoire of known mutations might occur within all normal human beings [H3].

42. Mosaicism may result from chromosomal abnormalities (missing or extra chromosomes or parts of chromosomes), from single gene mutations or from changes in gene control, such as X-inactivation, genomic imprinting or loss of imprinting (see below). It may also result from uniparental disomy (see below), gene amplification (see below) or from the incorporation of extrachromosomal DNA.

43. The expected effects of somatic mosaicism would depend on a number of factors, including (a) the type of mutation (deletion, point mutation etc.), (b) the type of gene in which the mutation occurs (housekeeping, structural, regulatory etc.), (c) the locus (or loci) at which the mutation occurs, (d) the domain involved (intron, exon, regulatory region), (e) whether the mutation has led to heterozygosity or homozygosity of the mutant or wild-type allele, (f) the specific cell type(s) involved and the tissues and organs affected, (g) the stage of development in which the mutational event occurs, and (h) the fate of the particular cell lineage in which it arose (migration, mingling, selection etc.). Very different effects would be expected if the mutation occurred in a growing and developing organism rather than in an end-stage differentiated cell.

(a) Chromosomal mosaicism

44. With the advent of chorionic villus sampling in prenatal diagnosis, some interesting and unexpected types of chromosomal mosaicism have been reported, such as confined placental mosaic aneuploidy in fetuses who have intrauterine growth retardation and a normal karyotype [K1]. In some cases, when the fetus is aneuploid, the presence of a normal cell line in the placenta may even explain why a small minority of fetuses afflicted with lethal chromosome anomalies are able to survive to term. This has also been shown in fetuses with altered chromosomes 18 and 13 that survive to term [K2].

45. It seems quite possible that there are genetic reasons why mosaicism is tolerated in some individuals and in some tissues. The mechanisms involved are unknown at this time but could be affected by radiation, making an individual more tolerant to mosaicism and thereby more likely to develop abnormal tissues.

(b) Germ-line mosaicism

46. There have been a number of reports suggesting that mosaic mutations of the germ line may be present in phenotypically normal individuals. Families with Duchenne muscular dystrophy [H3], pseudoachondroplasia [H5], Apert syndrome [A3], osteogenesis imperfecta type II [B11], tuberous sclerosis [H5] and many other disorders [H3] whose transmission is normally either dominant or X-linked have been reported in which parents are phenotypically normal by all known tests but more than one of their children are affected by the disorder.

47. Germ-line mosaicism is a mechanism of mutation that may produce transgenerational effects, as the germ cells of the carrier parent are formed in embryogenesis during the grandmother's pregnancy. Alternatively, if a parent carried mosaicism for a mutation that involved a multifactorial trait, it could be passed through the children and not manifest with visible phenotype until the grandchildren's generation or later [H3].

48. With regard to recurrence risk, the data available on single-gene "new" mutations from the disorders so far characterized on a biochemical or DNA level suggest that many (at least 5%) of what appear to be new mutations may actually represent a substantial parental germ-line mosaicism [H3]. One implication of this is that there may be a real risk for the recurrence of a new dominant mutation in subsequent offspring in what had previously been thought to represent a risk-free situation.

49. Depending on the particular tissue and mutation, some chromosomal anomalies and single-gene mutations may be lethal to the cells, others may be tolerated if they do not have a severe effect on that tissue, while still other mutations may actually have a selective advantage, as in the case of malignancies [F5]. For example, observations on patients with mosaicism for trisomy 8 and tetrasomy 12p support this concept, since mosaicism for these aneuploidies appears to be much better tolerated in fibroblasts that in lymphocytes [P2].

50. Normal cells may occasionally arise in dominant lethal disorders via back-mutation, gene conversion, mitotic crossover, suppressor mutation or double mitotic nondisjunction. They then outgrow the mutant cells, interspersing themselves throughout the body and allowing survival of what would otherwise be a lethal condition. Such may be the explanation for the occasional survival of males with X-linked incontinentia pigmenti [H10] and Melnick Needle syndrome [D6].

51. Mosaicism is a pervasive phenomenon that almost certainly affects all multicellular organisms. When expressed in somatic cells, it can be an important cause of neoplasia and possibly other aspects of the aging process [H3]. Somatic mosaicism can also be an important and sometimes dramatic cause of phenotypic variation in the expression of genetic traits [H3].

52. It should be kept in mind that if a mutation occurs in a DNA repair function, then somatic mutations, and therefore mosaicism, will occur far more frequently (as seen in DNA repair disorders such as Fanconi's anaemia etc. which predispose to cancer). Thus, a radiation-induced mutation would have a very different effect in families with a DNA repair defect than in other people. Likewise, a radiation-induced mutation in a DNA repair gene would affect many other genes as well.

53. In the past, only in the case of chromosomal abnormalities has it been possible to confirm the existence and significance of mosaicism, but the development and application of molecular genetic techniques should provide several approaches for identifying and analyzing a wider range of mosaic states in humans.

2. Genomic imprinting

54. Genomic imprinting is a newly recognized genetic phenomenon in humans. It appears to be a regulatory mechanism by which certain genes are differentially expressed, and thus convey different phenotypic effects, depending on whether they are inherited from the mother or the father. An "imprinted" gene is inactivated. Thus, a paternally imprinted gene would be expressed in a child only when it has been inherited from the mother; the paternal copy would be inactivated [H4].

55. A mechanism such as imprinting might further complicate efforts to estimate hereditary risk from radiation, as paternal vs maternal irradiation could have different influences on the phenotypes of F_1 offspring, even if damage to the genetic material was identical [V6]. In addition, imprinting effects may remain hidden for several generations. This is due to the fact that a mutation in an imprinted gene may remain silent (i.e. inactivated) through many generations before it is actually expressed in offspring.

56. As with the DNA repair genes discussed above, a mutation in a gene that regulates the imprinting process could have pleiotropic effects at multiple gene loci, causing them to be improperly expressed or improperly silenced. Imprinting appears particularly to affect early embryonic development and growth and to play a role in cancer. In other words, if a particular gene inherited from one parent is damaged, it may result in overgrowth or tumour development, while loss of the same gene from the other parent may have no effect.

57. Another line of evidence to support the existence of parent-of-origin differences in gene expression has developed from the study of human chromosome deletion syndromes. There are indications that the parental origin of the chromosome that carries the deletion or translocation may be associated with or modify the clinical manifestations of a number of observed syndromes [H6].

58. Two striking examples are the Prader-Willi and Angelman syndromes. Both are associated with deletions of the same region of the proximal long arm of chromosome 15. The clinical features of the two syndromes are remarkably different. On a cytogenetic level, it has been determined that in Prader-Willi patients with visible chromosomal deletions, the deletion is always of the paternally derived 15q11-13 region, whereas in Angelman patients with visible deletions, the deletion is always of the maternally derived 15q11-13 region [H6]. The deletions in the two syndromes thus apparently involve the critical region(s) of chromosome 15 but result in different phenotypes, depending on the parental origin of the chromosome.

59. These observations strongly suggest that this region of chromosome 15 is imprinted. Presumably there is a gene or group of genes in this region that is expressed only from the maternal chromosome, resulting in Angelman syndrome when deleted, while a second gene or group of genes in this region is expressed only from the paternal chromosome, resulting in Prader-Willi when deleted. A deletion or translocation in this region of chromosome 15 will have quite different effects in different generations, depending on the parent of origin [H6].

60. The concept of checks and balances between the parental contributions is further supported by recent studies on endogenous mouse genes. It has been found that in mice, only the paternally inherited gene for insulin-like growth factor II (Igf-2) is expressed [D1] and only the maternally derived gene for the Igf-II receptor (Igf-2r) is expressed [B6].

61. A second group of chromosomal deletions whose phenotypic effects are now recognized to have a non-random pattern of parental origin are those involved in oncogenesis [H2]. Familial cancer syndromes usually behave as dominantly inherited traits. Loss of the wild-type allele in individual cells is thought to result in loss of a suppressor gene function, which in turn allows oncogenic transformation and the development of tumours. It has recently been recognized that in a large number of sporadic Wilms' tumours there is loss of all or part of chromosome 11. Now that DNA markers allow identification of parental origin, it has been determined that those deletions or losses of chromosome 11 almost always involve the chromosome of maternal origin [S9]. The Philadelphia chromosome translocation associated with leukaemia and other haemopoietic neoplasms has recently been shown to demonstrate parent-of-origin effects, with chromosome 9 always being paternal in origin and chromosome 22 always maternal [H16].

62. Work on the retinoblastoma *(rb)* gene also suggests that there are tissue-specific, parent-of-origin differences in the expression of maternally and paternally derived genes [H2]. For instance, new mutations resulting in retinoblastoma are almost always paternal in origin, whereas sporadic sarcomas or rhabdomyo-

sarcomas with deletions of the *rb* gene almost always involve loss from the maternally derived chromosome 13.

63. Imprinting probably involves modifications of the nuclear DNA in somatic cells in order to produce these parent-of-origin differences in the phenotype. Thus the same nucleotide sequence may confer different phenotypic effects in different generations, depending upon whether it has been inherited from a male or from a female. It is not yet clear at which stage in development genomic imprinting occurs.

64. It should be noted that if the imprinting process or part of it occurs during meiosis, this may be another mechanism for which mutation would have such transgenerational effects. These effects would begin with the radiation exposure of the grandmother, because the formation of an oocyte, which when fertilized will eventually become a new individual, occurs during the *in utero* life of the mother (i.e. during the mother's own embryonic development within the grandmother). The effects of radiation thus might not become apparent for two or more generations following irradiation. Imprintable genes would be expected to be transmitted in a Mendelian manner, but their expression would be determined by the sex of the parent transmitting the gene, by way of this epigenetic form of regulation. Thus, studies of irradiation in females may produce results that are markedly different from those in males, depending on how much of the human genome is imprinted.

65. Now that molecular markers are available, the parental origin of various chromosomes and chromosome segments can easily be traced, and phenotypes dependent upon this phenomenon are being recognized in many areas of biology and medicine. Many human disorders are now being identified as imprinted [H6]. Disorders whose transmission and inheritance have been poorly understood and described as variably penetrant, multifactorial or variably expressed should now be examined for parent-of-origin differences and possible imprinting effects.

66. Imprinting has obvious implications for understanding the hereditary effects of radiation, since parent-of-origin effects would be expected and might be masked in animal experiments, where parents of only one sex are irradiated. Also, the effects of mutations involving imprinted genes may be masked for several generations.

3. Uniparental disomy

67. Uniparental disomy occurs when, in a cell with a normal number of chromosomes, both members of a chromosome pair have been inherited from a single parent. Normally, one chromosome of each pair is maternal in origin and the other paternal. Experiments using translocation chromosomes in mice have shown that at least seven segments of the mouse genome produce marked phenotypic differences in growth, behaviour and survival when uniparental disomy is present; in other segments, no difference is observed [C5, S14]. In other words, for some regions of the genome, a totally different phenotype is observed depending on whether both segments come from the mother or both from the father. Because of extensive homologies between the mouse and human genomes, it is reasonable to expect that the imprinted regions of human chromosomes will follow a similar distribution.

68. Not all cases of the Prader-Willi and Angelman syndromes carry visible cytogenetic deletions. Using molecular markers, many of these cases can be shown to have resulted from uniparental disomy [H6, H15, M2]. In these instances of Angelman syndrome, both members of the chromosome 15 pair have been inherited from the father; in the cases of Prader Willi syndrome with uniparental disomy, both have been inherited from the mother. Uniparental disomy occurs far more frequently than originally assumed [E9]. It may occur through the loss of a chromosome, in the case of a trisomy, or through the duplication or complementation of the remaining chromosome, in the case of a monosomy (i.e. salvage, since monosomy is lethal) [H6]. Irradiation is known to lead to chromosome deletion and loss and could therefore be expected to uncover imprinting and uniparental disomy effects [C17].

69. An interesting corollary of uniparental disomy is that it can, for the following reason, result in a child being affected by an autosomal recessive disorder when only one parent is a carrier for that disorder: if a parent carries a chromosome that contains an abnormal recessive gene and a son or daughter inherits two identical copies of this chromosome (uniparental isodisomy), the son or daughter will now carry two copies of the abnormal gene, i.e. will be homozygous for the mutant gene. Thus, he or she will express an autosomal recessive disorder that has been inherited from only one carrier parent. Precisely this situation has been shown to have occurred in two cases of cystic fibrosis [V11] and a collagen defect [S41]. The percentage of cases where an autosomal recessive disorder is uniparental rather than familial is unknown at this time, but clearly the question deserves further study.

70. The effects of uniparental disomy for chromosome 7 (intrauterine growth retardation) and chromosome 15 (Angelman and Prader-Willi syndromes) have been described above. In addition, a rare,

dominantly inherited human overgrowth syndrome, the Wiedeman-Beckwith syndrome, has been shown to occur in cases with paternal disomy for chromosome 11 [W2]. When familial, this syndrome has been known for some time to be nearly always transmitted through the mother. As discussed above, this again suggests a role for imprinting in growth disorders, as well as in human cancer, since patients with this condition frequently develop several types of cancer.

71. Other chromosomes for which uniparental disomy has been documented are chromosome 4 [C3], chromosome 6 [W6], chromosome 14 [T2], chromosome 16 [K17] and chromosome 21 [W1]. More information will be needed before it can be known if these chromosomes contain imprinted regions. Whether uniparental disomy occurs for other chromosomes remains to be determined. The implications for the hereditary effects of radiation are that damage to a chromosome may lead to loss of part or all of a chromosome, with complementation by the remaining chromosome producing uniparental disomy with increased frequency.

4. Cytoplasmic inheritance

72. Cytoplasmic components are present in the ova but not the sperm. Thus, the elements of the cytoplasm, such as the mitochondria (and possibly the mitotic spindles, endoplasmic reticulum etc.), are initially derived from the mother by cytoplasmic inheritance. One specific type of cytoplasmic inheritance is mitochondrial inheritance. The nucleus is not the only cellular organelle to carry genetic information. The mitochondria contain a separate genome, comprised of over 16,000 base pairs. This genome is circular in structure; both strands of mitochondrial DNA (mtDNA) are transcribed and translated, in contrast to the single coding strand of the nuclear chromosomes, which is usually transcribed in only one direction [M3].

73. Each mitochondrion contains many copies of these circular genomes, and thus each cell (with its many mitochondria) contains thousands of copies of the mitochondrial genome, as opposed to only two copies of each nuclear chromosome. In addition, the mitochondria contain essential enzyme and other protein molecules that are transcribed in the nucleus, translated in the cytoplasm and then transported to the mitochondria.

74. The mitochondrial genome is transcribed as a single messenger RNA (mRNA) that is cleaved into various genetic units. The products of the mitochondrial genes participate in a number of functions, the most important being the energy-generating synthesis of ATP via oxidative phosphorylation. (Of the 69 separate polypeptides known to be required for oxidative phosphorylation, 13 are coded for by the mitochondrial DNA [T3].)

75. As discussed above, the inheritance of mitochondrial DNA follows a strictly maternal line of transmission. Thus a clue to this cytoplasmic mode of inheritance is that a trait is passed only through females, but all (or almost all) offspring are affected or are at risk of being affected (as opposed to X-linked recessive traits, where only males are affected or X-linked dominant traits, where only 50% of the offspring are affected because there are two X-chromosomes).

76. Disorders such as Leber optic atrophy, myoclonic epilepsy with ragged red fibres and progressive external ophthalmoplegia (alone or as part of Kearns-Sayre syndrome) have been shown to be due to mutations in mitochondrial DNA. Mitochondrial disorders tend to have a more severe effect on tissues that require high levels of metabolic energy, such as muscle and brain. The mothers of these cases, and the cases themselves, are often heteroplasmic, meaning that each of their cells carries some normal and some abnormal mitochondria and thus may appear unaffected. Each offspring (and each tissue of offspring) may thus also carry varying proportions of abnormal mitochondria.

77. While the mitochondria have few genes compared to the approximately 100,000 genes in the nuclear genome, mitochondrial genes have a significantly higher rate of spontaneous mutation than nuclear genes [R2]. Thus the daughter of a heteroplasmic mother may develop additional mutations in her mitochondrial genome, and so on down the generations, increasing the likelihood that a child will carry a sufficient number of abnormal mitochondria to become symptomatic and to manifest one of the mitochondrial disorders. It is also likely that the effects of radiation may be more severe for the mitochondrial genome than for the nuclear DNA, but because of heteroplasmy, the effect may not become apparent for one or more generations [W12].

5. Anticipation and allelic expansion

78. Genetic anticipation is a phenomenon in which the phenotype of a disorder becomes progressively more severe in each subsequent generation inheriting the gene. Although until recently this phenomenon was thought not to occur, a number of studies have identified molecular mechanisms by which it can, and does, occur [H8].

79. One mechanism that can cause anticipation is found in the autosomal dominant disorder myotonic muscular dystrophy (MMD). This disorder is highly variable in its expression and is often more severe in the children than in the mildly affected parents. Part of the gene for MMD has recently been isolated and appears to be highly unstable, becoming larger in subsequent generations [H8]. The mutation responsible for fragile-X syndrome, the most common single-gene mental retardation syndrome in humans, has also been identified: it shows a similar instability in both phenotype and gene size in subsequent generations inheriting the mutation [Y3].

80. This type of increase in the size of a particular gene is called allelic expansion and can occur either somatically or during meiosis. In traditional Mendelian genetics, it has always been assumed that genes are essentially stable units of genetic information, and that either a normal allele or a mutant allele carried by the parent will be passed to offspring unchanged. When allelic expansion occurs, however, it has been found that patients with the most severe symptoms have the greatest enlargement of the defective gene, owing to an increase in the number of units of a repetitive sequence in the DNA. The number of repetitive sequence units continues to increase from generation to generation and is associated with a worsening of symptoms, i.e. anticipation [F9].

81. A similar increase in gene size has also been found in the fragile-X syndrome, with expansion of a CGG repeat [Y3], Huntington disease, with expansion of a CAG repeat [H17], and in X-linked spinal bulbar atrophy, also with an expansion of a (CAG)n repeat which occurs in the coding region of the gene for the androgen receptor [L1]. When amplification occurs at the fragile-X site, it is always when the mutation is passed by a female to her children [Y3]; when the Huntington expansion is passed from a male, it can expand rapidly. Thus it seems likely that parent-of-origin effects could play a role in the transmission and expression of the fragile-X phenotype [L2]. Other disorders are likely to involve allelic expansion, or a similar mechanism. For example, unstable sequences called microsatellite DNA have been shown to be associated with familial colorectal cancer [A8, P4, T7].

82. The implications of allelic expansion for radiation are as follows: if certain regions of the genome are more sensitive than others to allelic expansion, radiation damage might cause this sort of mutation to increase at a different rate than classical mutations. There may also be differences between the effects of irradiation of the mother and those of irradiation of the father. Many familial cancers have been demonstrated to have widespread alterations in short repeat DNA sequences, which may predispose to oncogenic events [A8, W11]. In addition, the spontaneous mutation rate in these regions may be unusually high; thus estimates of background incidence of mutations may be different than for other genetic disorders.

6. Gene amplification

83. In gene amplification, an entire gene or portions of it are duplicated. This may lead to an increased expression of gene product, either normal or defective, or to disruption and loss of gene function. Amplification can produce a selective advantage, as in cancer cells that are able to survive chemotherapy by virtue of having amplified the multi-drug resistance (MDR) gene to gain many active copies [S30]. Alternatively, it can produce a disease phenotype, such as Lesch-Nyhan syndrome, in which an internal amplification has been found to disrupt a normal allele for the enzyme hypoxanthine-phosphoribosyl transferase (HPRT), causing loss of gene function and thus leading to the disease [S32].

84. Amplification differs from allelic expansion in that the latter term refers to an increase in the length of a fragment consisting of multiple copies of a short repetitive sequence (for example, [CGG]n) [H8]. Such short repetitive sequences are found throughout the genome, usually flanking structural genes. They are also referred to as a variable length polymorphisms, as the sequences alter the restriction fragment size for a particular gene. Amplification refers to an increase in the number of copies of a longer nucleotide sequence unique to a particular gene. The molecular mechanisms for each may or may not be similar and may or may not be affected by radiation [M3].

7. Transposable elements

85. Transposable elements are sequences of DNA that integrate unstably into the genome at random (or possibly by homologous recombination at specific sequences). These include the Alu and LINE elements [L3, S33]. This integration, if it occurs in the middle of a structural gene or regulatory sequence, can disrupt gene function and produce a mutant phenotype. Transposable elements have long been observed in a number of lower organisms, including yeast, maize and drosophila [L3]. They were suspected in the human genome but until recently had not been proven.

86. However, Dombroski et al. [D5] have identified a LINE-1 transposable element disrupting the factor VIII gene in two haemophilia patients. This element also contains a full-length copy of a gene for reverse transcriptase, making it possible, after the element is

copied to RNA, for the transposable sequence to be copied back into DNA, which can then integrate back into the genome at a new site, often disrupting functional genes [M6]. It is thought that this type of element may have originated with retroviral integration into the human genome. Similar LINE-1 transposable elements have caused mutations in the neurofibromatosis type 1 gene [W10] and the cholinesterase gene [M12]. It seems likely that radiation should be able to mobilize or destabilize such elements, which lead to increased mutation or gene disruption in later generations.

E. SUMMARY

87. Genetic diseases occur because of alterations (mutations) to the structure or regulation of genes in the cell. Traditionally, genetic disorders have been classified into one of three categories: single-gene disorders, chromosomal aberrations and multifactorial disorders. Single-gene traits and disorders are either recessive (i.e. a normal copy of the gene will prevent the disease phenotype) or dominant (i.e. one abnormal copy of the gene will result in expression of the disease phenotype). A single gene can have multiple and apparently unrelated effects on many different tissues; this is called pleiotropism.

88. Phenotypic diversity within a single heritable disorder can be caused by (a) environmental factors, (b) allelic series (an individual with two alleles of a single gene with two different mutations), (c) genetic compounds (mutations in two different genes), (d) mutations in different domain coding regions of the same gene and (e) interaction with the products of other genes in that individual. Similar phenotypes can be caused by the action of any one of several different genes. This is called heterogeneity of disease. Multifactorial traits and disorders are those where a single condition (i.e. not complex disorders or multiple anomalies) is thought to have a genetic component but whose inheritance cannot be explained by single-gene inheritance.

89. Modes of non-traditional inheritance include cytoplasmic inheritance, mosaicism, imprinting and uniparental disomy. These mechanisms may prove to be increasingly important as causal factors in diseases whose inheritance does not follow standard Mendelian patterns of inheritance, and they may well be affected by radiation. Genetic disorders, particularly severe ones that interfere with reproduction, may be the result of new, as opposed to inherited, mutations. Human gene mapping using family pedigrees and restriction fragment length polymorphisms is being used to localize and isolate genes related to specific disorders.

II. MONITORING THE BACKGROUND INCIDENCE OF GENETIC DISEASE

90. The term genetic disease has been used to refer to any disorder (anatomical or metabolic) that is severe enough to interfere with a normal life and that has a genetic component, regardless of the age at which it occurs. The term congenital means present at birth. Congenital anomaly is a more precise term than birth defect and refers to structural anomalies present at birth. There are three major types of structural anomaly that may be apparent at birth [S35]:

(a) malformation, a defective or abnormal formation of a structure from its origin;
(b) deformation, an improper formation of a structure because of some physical impediment (e.g. too little amniotic fluid causes restricted fetal movement, which in turn results in joint contractures);
(c) disruption, an injury to a formed structure caused by an extrinsic or intrinsic force (e.g. amniotic bands disrupting circulation to a limb or digit).

A. REGISTRIES OF CONGENITAL ANOMALIES

1. Types and incidence of congenital anomalies

91. Congenital anomalies are not usually the consequence of Mendelian or chromosomal disorders, which they greatly outnumber. They have the advantage of being easy to document, as birth, surgery and death involve recorded events; moreover, in earlier days, when their nature was less clear, they were considered useful genetic markers. It is now possible to assess fairly accurately whether a congenital anomaly represents a significant problem and whether treatment is available. The anomalies can be classified as major (e.g. hydrocephalus, achondroplasia, amelia) or minor (e.g. skin tags, pigmented nevi, supernumerary nipples, minimal polydactyly).

92. Access to multiple registries allows the development of fairly accurate background rates for congenital anomalies. Some registries reflect concentrations of specific ethnic groups or high rates of consanguinity. Different registries have different incidences of anomalies, reflecting regional, ethnic and temporal variations. However, taken all together, they allow establishing fairly accurate background incidence of various congenital anomalies. Anomalies that are subject to environmental influences, such as neural tube defects, can be identified as varying with social class and region.

93. There is a consistent finding in all populations that 2%-3% of the serious congenital anomalies that will alter the length of life or ability to function normally without medical intervention are ascertained in neworns and that another 2%-3% of serious congenital anomalies are ascertained by 5 years of age [B2]. An additional 5%-13% of minor anomalies are found in all populations [B2, N11]. Efficacious treatment is available for at least one half of the defects, allowing an affected individual to become functional, independent and a contributing member of society. However. the remaining one half of congenital anomalies presently leave the affected individual with considerable disability in spite of therapy.

94. A study by Baird et al. [B3], conducted since publication of the UNSCEAR 1988 Report [U1], used a database of more than 1 million consecutive live births, followed from birth through the age of 25 years, obtained from the British Columbia Health Surveillance Registry through 1983. A hierarchical approach ensured that individuals were not counted more than once. Although the authors generally followed the approach of Trimble and Doughty [T6], they chose to proceed from single-gene disorders (autosomal dominant, autosomal recessive, X-linked) to chromosome disorders and then to multifactorial disorders. Congenital anomalies were considered last. Their analysis was restricted to those relatively common conditions that are generally accepted as having a major genetic component. The aetiological category "genetic unknown" was used when it was evident that the condition had a genetic basis but the inheritance pattern was not known.

95. It is recognized that this approach yields minimal estimates of incidence rates, since cases with relatively mild manifestations may not be diagnosed or may not come to the attention of the ascertainment sources. In addition, the count of multifactorial cases was assumed to be falsely low, because of an inherent bias in the counting process that could be present whenever a case had more than one diagnosis. Only those cases with a single diagnosis were counted as multifactorial. This means that the chance occurrence of a second non-multifactorial diagnosis or a second, unrelated multifactorial diagnosis in the same individual would cause that case to be omitted from the multifactorial category, occurring in the same individual. Baird et al. discussed an adjusted value for this rate that would correct this problem in the methodology.

96. The study found that before they reached the age of 25 years, more than 53 of 1,000 live-born individuals can be expected to have diseases with an important genetic component (see Table 1). The breakdown was as follows: 3.6 per 1,000 for single-gene disorders, consisting of autosomal dominant (1.4 per 1,000), autosomal recessive (1.7 per 1,000) and X-linked recessive disorders (0.5 per 1,000); 1.8 per 1,000 for chromosomal anomalies and 46 per 1,000 for multifactorial disorders, including those present at birth and those whose onset was before the age of 25 years. If all congenital anomalies are considered as part of the genetic load, the total rises to 79 per 1,000 live-born individuals.

97. It was found that if all cases of congenital anomaly (non-genetic as well as genetic) were considered, including those to which no genetic aetiology was attributed, the combined rate of all congenital anomalies was approximately twice that for genetic anomalies alone (i.e. 52,808 per 1 million live births, or 5.3%). If inguinal hernia was added, approximately 6.1% of the live-born in this population had a congenital anomaly (recent Hungarian data found 7.2% [B2]). However, inguinal hernias are easily corrected and are not considered a serious congenital anomaly. The study by Nelson et al. [N11] in Massachusetts found the same level of incidence and genetic distribution as the study by Baird et al. [B2, B3].

98. The point was made that *in utero* diagnosis of genetic abnormality has become increasingly common in recent years, and that this could bias the estimates of genetic defect in live-born children, since a positive test may lead to termination of the pregnancy. The potential impact was calculated from the records of the British Columbia Provincial Prenatal Diagnosis Programme [B2], and it was concluded that the impact of pregnancy termination on the rates in the study was extremely small, with an incidence of approximately 0.027% in the mid-1980s.

99. Baird et al. noted that the present records of the British Columbia Health Surveillance Registry make it possible to identify those cases within the congenital anomaly group that were judged to have a genetic aetiology. This is a considerable advance, because many earlier studies did not attempt to quantify the relative importance of the genetic versus the non-genetic categories within the broader "congenital anomalies" grouping. The other advantage of this study is that it provides follow-up data to age 25 years for at least a portion of the study population; the

follow-up data reveal that many congenital anomalies are not ascertained during the first few months of life.

2. Scope of monitoring

100. There are two approaches to monitoring congenital anomalies: epidemiological, i.e. the detection of outbreaks or clusters of predefined conditions by statistical methods, and teratological, which stresses the importance of clinical details in the search for unusual events, either rare malformations or unusual combinations of malformations, that may indicate the introduction of a new teratogen [W8].

101. In attempting to calculate the genetic consequences of any agent, be it a drug, a chemical or radiation, it is essential to begin with an accurate estimate of the background incidence rate for a given genetic disease or congenital anomaly. Previous reports on the genetic effects of radiation [C1, C2, U1, U2, U3, U4] raised the issue of background incidence. These reports relied heavily on data that are now almost 20 years old, and that were derived primarily from two sources [C13, C14]. A number of developments in recent years should make it possible to improve the accuracy of background estimates and to address the question of which genetic disorders and congenital malformations are most informative and worthy of inclusion in the study. There are now numerous registries of birth defects worldwide, each with unique features and each with advantages and disadvantages; these should all be taken into account when attempting to assess background incidence for human populations in general. Likewise, the accuracy of specific diagnosis has been greatly improved and refined in recent years, providing more precise definitions of significant defects.

102. There are bound to be regional, ethnic and temporal variations in the incidence of congenital malformations. For example, a study comparing congenital malformations in Aboriginal and non-Aboriginal newborns using the Western Australia Congenital Malformations Registry found that although the birth prevalence of all malformations was 3.5% for both groups, nervous system and cardiovascular defects and cleft lip and palate were significantly more prevalent in Aborigines and pyloric stenosis and urogenital defects were significantly less prevalent [B9]. In another example, Sikhs in British Columbia were found to have a significantly higher rate of neural tube defects than the general population, which is largely of northern European extraction [H4]. Many alarms generated by the International Clearinghouse for Birth Defects Monitoring Systems [W8] have, upon further investigation, yielded negative results.

103. Stochastic temporal variations in incidence may result in apparent clusters of a particular congenital anomaly or group of anomalies. While it is important to investigate such increases, it is probably not appropriate to rely on registries containing substantial deviations from the majority of registries in estimating background incidences of congenital malformations. However, if the concern is for a specific region, e.g. the Ukraine, where the reactor accident occurred, the background incidence of genetic disease for that particular population should be used, if it is available, to provide the baseline from which to calculate any increase in risk. Japan has regional monitoring programmes [K18]. It should be kept in mind, however, that any effect from a specific mutagen is likely to be obscured by the effects of social disruption, as some of the commoner malformations, e.g. neural tube defects [H4], are known to be greatly influenced by diet and standard of living [S16].

104. One other point to bear in mind is that, within a category of congenital malformation, there are often multiple subcategories with divergent causes and incidences, i.e. heterogeneity. For example, neural tube defects are generally classified as a single category, yet the cause of this defect can be single-gene mutation, chromosomal anomaly, teratogenic exposure, nutritional deficiency or ethnic predisposition. In some families recurrences are frequent, while in others the cases are sporadic; some cases can be caused by physical disruption such as amniotic bands. High lesions (anencephaly and thoracic spina bifida) differ from low lesions (lumbosacral spina bifida), and cases that occur in the presence of other birth defects differ from those that occur alone [H4]. Each of these components of the overall category "neural tube defect" could be differently affected by exposure to various mutagenic agents, and yet they are usually grouped together.

105. Registries may also differ in other aspects. It is important to consider the following sources of variation when comparing them: (a) exclusion or inclusion of stillbirths, (b) effects of prenatal diagnosis (rates of incidence may be lower owing to increased prenatal diagnosis and selective abortion for particular anomalies), (c) number of people contained in the registry (too small a sample may not yield statistically significant results), (d) ascertainment, as mentioned below and (e) duplication of reporting (multiple congenital anomalies may be reported singly as well as collectively).

106. According to Cordero [C9], if registries are to be useful in surveillance, epidemiological and other kinds of studies, they must contain four critical elements of information: who, what, when and where. Once these elements are specified, i.e. number of cases (what), divided by the population (who), in a specified area (where) and for a specific time period (when), incidence rates can be calculated [C9].

107. The collection of data can be either active or passive. With an active system, the registry has trained staff who follow a particular methodology to ascertain infants with birth defects and to collect data. With a passive system, the registry relies on reports from sources such as physicians, hospitals or vital records departments [C9].

108. Active systems have several strengths: (a) they have fairly complete ascertainment, (b) they can obtain data in a timely fashion, (c) they can include quality control measures for the data gathering process, (d) they define the type of data to be collected and (e) they usually allow for the follow-up of cases. A disadvantage of active systems is their cost, which tends to limit the sample size of the population to be studied. This, in turn, limits the registry's ability to collect sufficient data for some types of epidemiological studies [C9].

109. Passive systems have one major strength, low cost, which allows them to cover larger populations with a minimum of resources. Their disadvantages include a lack of diagnostic specificity, little control over time delays in obtaining data and measurable underreporting of data. In some passive systems, the quality of the data cannot be evaluated. Moreover, few systems provide a means to track cases, making follow-up studies impossible.

110. The process of finding persons with the disease under study is referred to as ascertainment. It is important to bear in mind that methods of ascertainment are bound to differ between registries, often resulting in the under- or overreporting of a particular birth defect in a particular population. For example, physicians in Hungary were paid for every instance of congenital hip dysplasia they reported; this malformation subsequently comprised an artificially high percentage of overall birth defects in Hungary. It is also important to bear in mind that many birth defects do not become apparent until after the first few months of life. Thus, a registry that reports only birth prevalence may be underreporting.

111. An alternative approach for complete ascertainment is to review medical records. In the United States, every baby born in a hospital has a medical record that generally indicates if birth defects are present. The strengths of this approach include nearly complete ascertainment, the ability to achieve population-based ascertainment and a lower cost than if every baby were examined independently,i.e. not by its own physician. Its disadvantages include labour intensiveness, inefficiency and cost [C9].

112. Some programmes, e.g. those in Sweden, Australia and Atlanta, Georgia in the United States, do not routinely record possible exposures during pregnancy. Such programmes are usually found in areas where it is relatively easy to go back to medical records and to contact the parents of the damaged infant if exposure information is thought to be of interest. In other programmes, such as the Central-East France programme and the large hospital-based programme in Italy, information on possible exposures during pregnancy is collected at the same time the malformed infant is reported, but no similar data are collected for normal infants. A third group of programmes, including one in Mexico, Spain and South America, has ongoing case-control data collection, in which information on possible exposure is obtained for each malformed infant and for a control (normal) infant born at the same hospital. Each technique has its advantages and disadvantages, but in areas of the world where it may not be possible to follow up an observation by interview at some time after birth, collecting exposure data on a case-control basis seems the most effective technique [C9].

B. CONSIDERATIONS ON BACKGROUND INCIDENCE

1. Sentinel Mendelian diseases

113. The concept that certain phenotypes were so obvious that they could not be missed led to the idea that their frequencies could be easily monitored for sudden increases. It is not difficult to establish the background incidence of autosomal-dominant disorders, especially those that have a strong selective disadvantage, the prevalence of which is maintained in the population by an equilibrium between constant mutation pressure and selection (see [V8, V10]).

114. Theoretically, in situations where illegitimacy can be ruled out and genetic heterogeneity can be recognized, this method should lead to reliable estimates of the spontaneous mutation rate and its increase because of a mutagenic agent. Such mutations are called sentinel mutations, because they are expected to indicate mutation rate increases caused by a new agent in the environment. Czeizel [C13, C14] enumerated 15 sentinel anomalies that are thought to be caused by dominant new mutations and that can be diagnosed at birth or shortly thereafter. However, the molecular aetiologies of these disorders, which are beginning to be defined, appear to be very heterogeneous. Thus, it may not be valid to apply one estimate to the group as a whole, as each subgroup might be expected to have a different rate of mutation. Moreover, as suggested by Strobel et al. [S39], such sentinel mutations are too rare to easily provide realistic mutation rate increases. Furthermore, continuous screening of very large populations would be required.

115. From the experience accumulated so far, it is extremely unlikely that human populations will ever be exposed to mutagenic agents that will cause an observable statistically significant increase in this type of disorder above the natural rate of mutation (see paragraph 213). The Hungarian data were used to test whether the Chernobyl accident in April 1986 had led to any increase of new mutations [C15]. No evidence was found that it had. This is, however, not surprising, because according to Strobel [S39], for a mutation incidence of 3 per 10,000, it would have been necessary to screen of a population of almost 2 million newborns distributed in two samples of equal size (before and after irradiation) to recognize an increase of 30% in 95% of instances. A smaller increase would require an even greater sample size. Down's syndrome alone (7.02 per 10,000) is more common than all the sentinel mutations taken together. But even for it, a population of approximately 1 million newborns would be necessary to recognize a statistically significant change, and Hungary has only about 150,000 live births per year [C14, C15] (see paragraphs 213 and 338).

116. Many autosomal-dominant disorders, especially many rarer ones, are thought to be maintained in the population by an equilibrium between mutation and selection. This may be true for such disorders as neurofibromatosis, Marfan syndrome and autosomal-dominant types of osteogenesis imperfecta. Other disorders, such as bilateral retinoblastoma or haemophilia, were probably maintained by such an equilibrium in the past, but successful therapy is very likely to have upset this equilibrium in recent decades. Assuming constant mutation rates, the incidence of such disorders is bound to increase until a new equilibrium has been reached unless there are counteracting circumstances, for example, artificial selection.

117. It is, however, very unlikely that the more common disorders are maintained by an equilibrium between mutation and selection. The mechanisms that have caused the present-day incidence of many dominant diseases are unknown. It follows that dominant and X-linked diseases cannot be subdivided easily into those whose incidence is maintained by an equilibrium between mutation and negative selection and those in which a selective advantage under certain living conditions has been the decisive factor. More complicated situations may occur and the equilibrium conditions may change over time, depending on living conditions.

118. There are, of course, a great number of fairly rare and, in most cases, very rare disorders that may indeed be maintained by an equilibrium between mutation and selection, but for an overall estimate of the mutational component, it is not the number of these disorders but their combined incidence that is important in calculating risk. Since they are mostly lethal at an early age, fertility is 0 and there is a 100% mutational component, i.e. all cases are caused by a new mutation, rather than inherited from the parents. These disorders comprise only about 1/30 of the entire group of dominant and X-linked disorders. In other disorders, the mutational component is smaller, and for some of the most common ones, it may not exist at all. In some earlier UNSCEAR Reports, the Committee estimated the mutational component of the entire group as 15%. In this, the medical geneticists may have been persuasive, having in mind the more severe and debilitating forms of genetic disease (this would be understandable, since these diseases are the ones most frequently seen in daily practice), but in the general case, the estimate is high.

119. To place an increase in morbidity due to autosomal dominant and X-linked radiation-induced mutations in proper perspective, it should be remembered that the natural spontaneous mutation rate, the causes of which are unknown, is not a constant. The best known factor that influences the mutation rate is paternal age. For some autosomal-dominant anomalies, such as achondroplasia, acrocephalosyndactyly (Apert syndrome), Marfan syndrome, myositis ossificans and probably many others, the mutation rate at a paternal age of 40-45 is four to six times higher than at a paternal age of 20-25 [V8]. Modell and Kulieve [M10] have calculated how much a given shift in the distribution of paternal ages in a population would change mutation rates. They compared the mutation rates expected with the present paternal age distributions with those expected if all fathers were less than 30 years old at the time of birth of their child. They found that even a relatively small shift in the distribution of paternal ages, and especially a reduction in the fraction of older fathers, could influence the mutation rate for such paternal-age-dependent mutations appreciably.

120. Not all known dominant mutations show such a strong increase with paternal age (for details see [V8]). In recent decades, and with a decrease in the average number of children per marriage, a decrease in the fraction of older fathers has been observed in many populations, and a corresponding reduction in the number of such paternal-age-dependent new mutants has to be assumed in the developed countries. Hence, even a relatively small shift in paternal age distribution, especially an increase or a reduction of older fathers, could influence the mutation rate for such paternal-age-dependent mutations appreciably, probably much more than could a change in exposure to mutagenic agents, e.g. radiation.

2. Autosomal recessive diseases

121. Searle and Edwards [S16] stressed that the degree of inbreeding influences only the immediacy of

the effects and that in the absence of a strong heterozygote disadvantage it will affect only slightly the total number of casualties. That is, in a population with a high rate of inbreeding (i.e. consanguinity), damage due to homozygosity of mutations becomes visible sooner. The authors assumed for their calculations a rate of 1% first-cousin matings.

122. Estimates of the manifestation of homozygotes in future generations depend critically on consanguinity rates. In industrialized countries, the rate of first-cousin matings has dropped to one or a few per thousand in recent decades. Since this reduction is caused on the one hand by greater mobility and on the other hand by smaller numbers of children (and, hence, a reduction in the number of available cousins), the decrease will probably continue and also occur in the populations of countries only now becoming industrialized. However, even if consanguinity can be neglected in the future, it is open to question whether effects distributed to thousands of generations should be considered at all in genetic risk estimates. It may be most reasonable to assume that civilization will develop in about the same direction as it has in recent centuries and that gene therapy and/or prenatal diagnosis at the zygote level will eventually become routine, especially for autosomal recessive diseases that involve mostly simple enzyme defects.

123. In humans, few data on phenotypic deviations in heterozygotes of autosomal-recessive diseases are available [V5], and those that are related primarily to enzyme studies. As a rule, heterozygotes have about half the activity of normal homozygotes for the product of the gene affected by the mutation, which in many cases is an enzyme. This reduced activity is, however, in most cases sufficient for normal function.

124. Finally, it should not be forgotten that the background incidence of recessive mutations, and especially of recessive diseases, in human populations is not constant from one disease or population to another. The human species is a patchwork of extremely different frequencies of recessive genes. The breaking up of isolated subpopulations and having strong intermixture between them will not lead to a reduction of frequencies of all recessive genes in a similar fashion, but it will lead to an assimilation of gene frequencies and especially to an appreciable reduction of frequencies of genes that had become common in one or a few populations owing to random drift. This, in turn, will lead to a general decrease of homozygotes of autosomal-recessive diseases, as a consequence of the Hardy-Weinberg Law [V10]. This decrease will then be followed by a very slow increase in gene frequencies, because fewer alleles will be eliminated in homozygotes. This is the complex background against which any possible effect of additional radiation should be viewed, since any attempt to derive an estimate of increased risk will depend on population structure.

3. Chromosomal diseases

125. There is general agreement that estimates of chromosomal disease incidence at birth disregard the great majority of (numerical and unbalanced, structural) chromosomal aberrations in human germ cells and early zygotes. Most embryos and fetuses with chromosomal aberrations die some time during embryonic life. It seems very unlikely that more than 1% of all conceptions with recognizable, chromosomally aberrant phenotypes survive to birth [V10]. This is a reasonable but cautious estimate, i.e. the true fraction of survivors might well be lower. The number of zygotes dying in the first days after fertilization, before implantation, cannot be estimated, but it may be appreciable. In studies of chromosomes of human sperm, the fraction of those showing chromosome aberrations is high [B12, K13, K14, K15, K16, M4, M5, S42]. It is, of course, unknown which of these chromosomally abnormal cells are still able to fertilize; but the use of this method of sperm karyotyping for mutagenicity testing, especially in men exposed to high doses of mutagenic agents, such as radiation, should be encouraged. Studies of ova used during *in vitro* fertilization suggest that human ova also have a high fraction of spontaneous chromosomal aberrations.

126. Calculations similar to those for paternal age effects in autosomal-dominant and X-linked recessive diseases have been performed for numerical chromosomal aberrations, such as Down's syndrome, to determine maternal age effects. The baseline is especially variable for trisomies, because the spontaneous mutation rate increases with the age of the mother: the risk for mothers above 40 is 10-20 times higher than that for 20-year-old mothers [H12]. The conclusion reached is therefore the same as for paternal age and dominant mutations: even a small shift in the distribution of maternal ages in a human population, and especially in the fraction of mothers above 35, will alter the incidence of trisomy syndromes at birth much more than any probable increase caused by radiation.

4. Congenital anomalies and multifactorial diseases

127. The background incidences of congenital anomalies and multifactorial diseases are not easy to establish. The UNSCEAR 1986 Report [U2] gave a figure of 60,000 per million for congenital anomalies and 600,000 per million for other multifactorial dis-

eases [U2]. BEIR V [C1] estimated congenital abnormalities as 20,000-30,000 per million and subdivided "other disorders of complex aetiology" into three categories: heart disease (600,000), cancer (300,000) and selected others (300,000). These three figures add up to more than 1 million, so they cannot be meant to be mutually exclusive. These discrepancies reflect the difficulties in attempting to establish reliable background incidence mentioned earlier in this Annex.

128. The seemingly simple task of determining the incidence of congenital malformations at birth continues to pose problems; results from one study to the next may show considerable differences, occasionally because of real differences between populations but much more often because of differences in ascertainment and classification. To predict a possible increase attributable to a specified radiation dose experienced by the germ cells of parents, the mutational component of this incidence needs to be estimated. This involves estimating the frequency and degree of genetic determination of single anomalies and their modes of inheritance, which, as discussed earlier, is very difficult to achieve and must be individualized. Furthermore, any possible selective disadvantages of such anomalies under present and earlier living conditions (and, if possible, in populations of industrialized countries and of developing countries with poor medical systems) should be known. This information is needed to estimate which part of the genetic component of a certain anomaly is lost in every generation and which is therefore replaced by new mutants. Even then, the estimate would be of the right order of magnitude only if there were an equilibrium between mutation and selection [H1].

129. In the British Columbia registry data, 4.6% of individuals were noted to have a multifactorial condition by age 25 years (Table 1) [B3]. Many congenital anomalies are consistent with the concept of multifactorial inheritance. A few are caused by environmental factors such as teratogenic drugs or, very rarely, irradiation during pregnancy. For many congenital anomalies, no cause can be identified; they are attributable to an accumulation of random processes during early embryonic development. However, data from radiation experiments in mice suggest that some may also be caused by irregularly manifesting dominant mutations. Their frequency may increase after the irradiation of fathers. For example, Ehling [E1, E8] and Selby et al. [S21, S24, S25, S28] have shown that the irradiation of mouse spermatogonia may lead to occurrence of a wide array of skeletal malformations. The genetic variation that influences some malformations may, indeed, have a strong mutational component. It is remarkable, on the other hand, that studies on the association of malformations with known genetic polymorphisms, such as the ABO blood groups (see [V4, V10]) and the major histocompatibility system [T4], have failed to point to any such association.

130. Some aspects of mammalian embryology should be mentioned here, since they are relevant to estimation of risk. Mammalian development is not entirely pre-programmed; rather it is influenced to a significant degree by the environment of the developing embryo. In the event of physical or chemical insult, the embryo has an remarkable ability to catch up and correct the damage. Many buffering effects are built into biologic systems, such that if one element is disrupted, others may be able to compensate. In addition, the evolution of gene duplication has led to a biological system of buffering, such that if one gene is knocked out, others may be able to take over its function. There appear to be thresholds during the course of development and aging, such that timing is extremely important. Mutations that upset the timing of events (heterochronic mutations) may produce unpredictable results [W7].

131. The incidence of all types of multifactorial diseases given in the UNSCEAR 1986 Report [U2], 660,000 per million, was based on the study of Czeizel et al. in Hungary [C12], which considered morbidity up to the age of 70, and some individuals had more than one disease. The Hungarian study comprised incidence estimates for 26 such multifactorial diseases, which were classified according to ICD and subdivided into three groups:

(a) very severe (schizophrenia, multiple sclerosis, epilepsy, myocardial infarction);
(b) moderately severe and/or episodic or seasonal (Graves' disease, diabetes, gout, affective psychoses, duodenal ulceration, asthma);
(c) less severe (varicose veins, atopic dermatitis etc.).

With the exception of epilepsy, none of these diseases causes death in the age group 0-19 years, but they are among the leading causes of death in advanced age. Such incidence estimates are extremely useful for many purposes, but in the context of radiation risk estimation, these prevalence estimates need to be carefully analysed to further identify subsets of conditions that may potentially respond to an increase in mutation rate. Such analysis is necessary because of the following:

(a) a major part of morbidity for many of these diseases is not the result of genetic predisposition or inescapable environmental exposure or both but is the result of voluntary and avoidable behaviour. Type 2 diabetes is one example; coronary heart disease and gout are other examples;
(b) in some of these diseases, the quality of life is not impaired decisively, providing that the individual finds a way of adapting his or her lifestyle to the disease;

(c) parameters such as the attitude of the society, the number of doctors and the quality of the health care system influence whether a disease is diagnosed and how much detriment it causes.

The relative importance of these factors may vary in different populations and may vary over time within the same population.

C. MOLECULAR AND BIOCHEMICAL STUDIES OF SPONTANEOUS AND RADIATION-INDUCED MUTATIONS

132. In addition to numerous types of DNA damage leading to stable, heritable mutations (i.e. single base deletions, base-pair modifications, strand breaks, base-pair substitutions, nondisjunction etc., that lead to nonsense, missense, frameshift, chromosomal mutations etc.), the site of a particular mutation must also be taken into account when attempting to analyse its impact on genetic risk. The relevance of various sites of mutation and their effect on cell function is of crucial importance in understanding the effect of molecular damage on phenotypic abnormality. Thus, a knowledge of molecular damage can be important in predicting genetic risk.

1. Location and effects of mutations

133. Structural mutations occur in the coding region of a gene, altering the protein product of a single gene. If the change occurs in a sequence that codes for a non-critical region of the protein, it will have little or no effect and will be well tolerated (e.g. amino acid substitution into the non-active site of an enzyme). If, however, it occurs in a critical region, it may impair the function of the protein (e.g. the disruption of the disulfide bridge in the oxygen-carrying haemoglobin molecule, which results in sickle-cell anaemia).

134. While the structure, and thus the function, of the gene product will not be affected by mutations in regulatory regions, the amount of gene product synthesized may be. Loss of promoter function will render a gene inactive even though its structural integrity remains intact, making the mutation difficult to identify, the loss of other regulatory elements, such as repressors, enhancers etc. may result in the loss of responsiveness to environmental conditions (e.g. liver cytochromes, which are upregulated in response to toxic insult).

135. If the protein serves a single, limited function, its loss or overproduction may have only a minor effect on the survival of the organism (e.g. the loss of tyrosine hydroxylase, which results in albinism). However, if the gene product is a regulatory protein involved in a pathway that amplifies its effect, the effects of the mutation may be far-reaching or even devastating to the organism (e.g. protein kinase proto-oncogenes, in which activating mutations lead to cancer). Other examples include molecules like the chaperonins, which regulate the secondary structural folding of many different proteins; *p53*, which has a dominant negative effect; the ubiquitins, which regulate the degradation of all messenger RNA molecules such that they are not transcribed into protein indefinitely; transport proteins, which carry the gene products to their proper location in the cell; genes involved in gene inactivation, such as dosage compensation resulting from X-chromosome inactivation in females [T3].

136. Likewise, if the gene product is a structural molecule essential to the development and maintenance of normal anatomy, such as connective tissue constituents, the loss of this type of gene function will have far-reaching effects for the organism as a whole (e.g. defective keratin which leads to epidermis bullosa, and defective collagen, which leads to osteogenesis imperfecta). Moreover, any gene involved in the synthetic pathway of such structural elements will have major effects (e.g. defective hydroxylase enzyme, which leads to mucopolysaccharidoses and concomitant skeletal changes).

137. In molecules where there is a repeating structure, any type of mutation in these repeating segments of the gene will destroy the structure of the whole molecule. For example, collagen contains multiple repetitive elements, such that any mutation in the third amino acid of the repeat disrupts the entire secondary structure of the glycoprotein.

138. Recent developments in cancer research suggest that somatic mutations are responsible for most, if not all, leukaemias, lymphomas and solid tumours [C6, M11, S11]. This is generally due to the loss or mutation of an oncogene suppressor gene function (as, for example, retinoblastoma or Wilms' tumour) or to an "activating" mutation in an oncogene that renders it immune to normal regulation (e.g. *RAS* in colon cancer). There are also many hot spots for mutation throughout the genome; since these regions show a higher frequency of mutation than other regions of the genome, they may be far more sensitive to mutagenic agents [V3].

139. The failure of crossover during meiosis can lead to non-disjunction, resulting in the loss or gain of large regions of chromosomal material. Thus, this single event can adversely affect many genes (e.g. trisomy 21 causes Down's syndrome, while most other trisomies and monosomies are not compatible with life.) Mutations that disrupt this genetically regulated process will have major ramifications.

140. Mutations in one region of a gene may produce a phenotype that is completely different from mutations in another region. This is particularly true for genes coding for products with multiple domains, such as connective tissue molecules (for example, dislocated lens of the eye and rupture of the aorta are both caused by mutations in the fibrillin gene) [V10].

141. It has also become apparent that alternative splicing of messenger RNA molecules occurs (e.g. the messenger RNA for several different hormones can be produced from one gene); that there are "genes within genes" (e.g. the neurofibromatosis locus); and that some genes code for precursors that are then cleaved enzymatically to yield the active product (e.g. prothrombin to active thrombin, encephalon to endorphin). Thus, one gene can, in effect, code for several products.

142. It is now clear that damage to mitochondria (including those in ova) must also be considered in genetic risk estimates. In the past it was assumed that damage to DNA was the only concern, but changes to other components of the cell structure, such as mitochondria, may affect subsequent generations [W12].

(a) Nature and origin of spontaneous mutations in human Mendelian disease

143. A large number of spontaneously arising mutations that cause disease states in humans have been described. Only a few are known at the molecular level. As of 1990, molecular data were available for some 76 Mendelian diseases in humans [S3]. For 33 of these, the predominant event is a point mutation (base-pair change), and for 39 it is a length mutation (mostly DNA deletions, but sometimes duplications or other gross changes). In the 4 remaining diseases, both point mutations and length mutations occur. These relative frequencies may be revised as more data become available, but for now, it can be assumed that point mutations and length mutations each account for about half of the Mendelian diseases [S7].

144. In spontaneously occurring mutations, point mutations (i.e. mutations in which a single base pair is altered or deleted) do not appear to be distributed at random throughout the genome. This is thought to be related to the sequence organization of the gene and its genomic context [S7]. CpG dinucleotide sequences, when present in a gene, provide hot spots for transition-type mutations (i.e. A to G or G to A and C to T and T to C). Vertebrate DNA is highly methylated at the cytosine residue, and about 90% of 5-methyl-cytosine occurs within CpG sequences. At the level of the gene, C to T transitions and the corresponding G to A transitions in the complementary DNA strand occur at a high frequency within these methylated regions; this is thought to be due to the propensity of 5-methylcytosine to undergo spontaneous deamination to form thymine [C10]. It can be anticipated that each gene will have its own susceptibility pattern, and it is not known whether these patterns will be similar in humans and in mice. Other endogenous damage to DNA is thought to come from replication errors and from oxidative attack mediated by chemical radicals [A9].

145. Examples of non-random point mutations that have been identified in human cancer biology studies include the point mutations in codons 12, 59 and 61 of the *RAS* genes involved in myeloid leukaemia, lung cancer etc. and those in codons 110-307 of the *P53* tumour suppressor gene in diverse types of cancers. Such site preferences have long been known to occur in visible chromosomal changes in neoplasias, particularly leukaemias and lymphomas, and molecular studies are now shedding light on these specificities.

146. There is good evidence that the breakpoints of length mutations are also non-randomly distributed. Of 60 small (<20 bp) deletions at the 23 loci studied, 59 had direct repeats of 2-8 bp. For large deletions, sequence homologies and repetitive sequences such as Alu located within or between genes appear to play important roles. The mechanisms involved in the generation of deletions and duplications are listed in Table 2.

147. Data from a number of well-analysed spontaneous gene deletions are consistent with mechanisms that assume base mispairing between repeat sequences and slippage during replication; homologous unequal recombination between evolutionarily related genes; homologous unequal recombination between repetitive sequences such as Alu; and non-homologous recombination. There is circumstantial evidence supporting the hypothesis that repetitive sequences may play an important role in chromosome pairing [S3]; if true, the deletions and duplications that have been found to be associated with spontaneously arising mutations in many diseases may represent the inevitable by-products of occasional mispairing.

148. Examples of deletions arising as a result of non-homologous recombination are provided by some alpha-thalassemias [N12, O1], some beta-thalassemias [A5, H11] and some complex thalassemias [J1]. In all these deletions, the 5' breakpoints were mapped either in or close to Alu sequences. The interpretation is that these deletions presumably arose during DNA replication (when sequences widely separated in the linear DNA molecule might be physically close to one another as a result of anchorage to the nuclear matrix and chromatin loop formation) as a consequence of non-homologous intrachromosomal breakage and reunion events [A5, V2]. The models proposed differ in some details.

149. Intragenic partial deletions (well over 300 are now known) appear to be the most common defect leading to Duchenne and Becker muscular dystrophies [D2, F6, G5, K8]. Less common are intragenic duplications that appear to duplicate one or a few exons by the tandem duplication of a portion of the gene, presumably by unequal crossing-over between repeat elements [H13, K8]. Data have also been published suggesting that duplications can arise as an intrachromosomal event through unequal sister-chromatid exchange [H14].

150. Thus, in spontaneously occurring mutations there occur both point mutations (base-pair changes) and length mutations, which include DNA deletions (small and large), insertions, rearrangements and duplications. The length mutations are occasionally microscopically detectable as chromosomal aberrations. Several other mechanisms of mutation are involved in spontaneously occurring mutations in humans, including gene conversion and mutation due to the insertion of mobile, or transposable, genetic elements. (Transposable elements are also discussed in Section I.D.) The term gene conversion describes the local transfer of DNA sequences from one gene to a related gene elsewhere in the genome in an event that resembles a double crossover [M1]. Gene conversion events leading to disease phenotypes are now well documented in humans. One example is congenital adrenal hyperplasia due to 21-hydroxylase deficiency [D7, H7, U12]. There are two 21-hydroxylase genes in humans, one of which is a non-functional pseudogene; if any of the inactivating sequences in the pseudogene are introduced into the functional gene by gene conversion, there will be a deficiency of the enzyme. Other examples include spontaneous mutations at the HLA-A and thymidine kinase (TK) loci in human somatic cells *in vitro*, in which gene conversion has been shown to play a significant role (see [S5]).

(b) Radiation-induced mutations in mammalian experimental systems

151. Data from mouse mutation studies (with x- or gamma- and neutron irradiation) have provided most of the information on the genetic effects of radiation. They have been reviewed from time to time [E4, F3, F4, R8, R9, R10, S1, S3, S4, S5, S6, S7, S13, S19]. Points of interest include the following:

(a) spermatogonia, post-meiotic male germ cells and mature and immature oocytes differ in their sensitivity to the induction of mutations by radiation;
(b) the yield of mutations varies between gene loci;
(c) a majority of radiation-induced mutations are lethal in the homozygous condition (i.e. two copies of the same mutant gene);
(d) the relative frequency of various molecular changes seen in the mutational event differs between spontaneous mutation and radiation-induced mutation; and
(e) in mice, the frequencies of radiation-induced recessive and dominant mutations differ.

152. Russell et al. [R3], in a detailed genetic and molecular characterization of large numbers of specific-locus mutations collected at the d, se and c loci, have shown that the simple phenotypic classification of specific-locus mutations can effectively separate mutations into the following three categories: multilocus deletions, presumed intragenic mutations and viable null mutations. Comparisons between mutations induced in different germ-cell stages, spontaneous mutations and mutations induced by low-LET radiation, neutrons and ethylnitrosourea (ENU) have shown that there are marked qualitative differences between spontaneous and induced mutations and between mutations induced by low-LET radiation, neutrons or ENU in different germ-cell types. A total of 264 radiation-induced mutations and 45 spontaneous mutations were classified in this way. Most of radiation-induced mutations studied in the mouse and in mammalian *in vitro* systems, however, are either presumed to be or actually demonstrated to be DNA deletions; however, the relative proportions of point mutations versus deletions vary with the locus and the test system under study [S7]. In one study [R13], 31 mutations were analysed by Southern blot analysis with a tyrosinase cDNA clone and with other probes, which identified 13 radiation-induced and one spontaneous mutation to be deletions or rearrangements ranging from 36 to 2,000 kb. The fact that such large viable deletions can be recovered suggests that 1 or 2 Mb of DNA including and surrounding the c-locus harbour no genes essential for viability or fertility.

153. There are also hot spots for radiation-induced breaks, as observed in the chromosomes of blood lymphocytes in human radiotherapy patients. These hot spots are in T bands, which are very rich in both GC and Alu sequences, suggesting that at least some radiation-induced deletions may arise by mechanisms similar to those inferred for naturally occurring deletions mediated to Alu sequences [R13]. (T bands, which are a subset of R bands, represent only 15% of all bands, but contain 65% of mapped genes and 42% of x-ray-induced breaks [S7]).

154. The findings in mouse studies are consistent with the view that in mouse germ cells, most radiation-induced mutations are DNA deletions. This has now been shown to be the case by molecular methods for a number of mutations [S4]. However, most work done on the effects of germ-cell irradiation have been done in males; female germ cells may have very

different susceptibility to different types of induced mutation at different stages. Surprisingly large deletions can be tolerated in viable mice [C18].

155. Data on the induction of mutations that lead to observable congenital structural abnormalities in the progeny of irradiated mice suggest that these abnormalities are not very sensitive end-points. In addition, it should be mentioned that different strains of mice may have different sensitivities to radiation [N15, N16], although the work of Favor et al. [F17, F18] suggests no strain differences when using radiation and ethylnitrosourea.

156. From available studies that have analysed the molecular nature of mutations, the following conclusions may be drawn:

(a) ionizing radiation induces very few point mutations;
(b) when ionizing radiation induces mutations in enzymatic proteins, the changes lead to altered enzyme activity or lack of enzyme activity; the molecular changes may include a mixture of events at the DNA level, ranging from point mutations to intragenic DNA deletions, multilocus deletions, or rearrangements;
(c) the limited data on radiation-induced mutations at the haemoglobin loci in mice suggest that radiation induces deletions, duplications and translocations, but not point mutations.

The fact that radiation-induced mutations are likely to differ from spontaneous mutations in the type of mutation produced, the frequency and the sites affected must also be considered, and indeed expected. The above examples offer a convincing argument that a simple, direct correlation between the number of mutations and the degree of mutation damage would be exceedingly difficult, if not impossible, to demonstrate in either animal or human studies.

2. Protein studies

157. Quantitative and qualitative protein variations in the children of atomic bomb survivors were examined, using a variety of methods. Presumed new mutations were verified by carefully excluding false paternity. No increase in comparison with the controls was observed. The number of loci screened was $6.67\ 10^5$ in the exposed group (parents within 2 km of the centre of the atomic bombings) and $4.67\ 10^5$ in the non-exposed group. Three new mutants were identified in each group. The mutation rates per locus per generation were estimated to be $6\ 10^{-6}$ (95% CI: $2\text{-}15\ 10^{-6}$; exposed group) and $6.4\ 10^{-6}$ (95% CI: $1\text{-}19\ 10^{-6}$; non-exposed group) [N7, N9].

158. For the monitoring of large population groups, another approach has been suggested that avoids the logistically most difficult part of protein studies, namely contacting the families and collecting the blood samples [V9]. In most countries, practically all newborns are screened for inherited metabolic diseases such as phenylketonuria (PKU). A few drops of blood are put on a special test card that is sent to a screening centre. A method has been developed by which haemoglobin (Hb) and other proteins can be extracted from this card and studied by electrophoretic methods [A4]. In a pilot project in Japan, blood samples from 40,003 newborns (for Hb variants) and 30,659 individuals (for other protein variants), were screened, representing altogether 722,719 gene loci. In three instances, the transmission test was negative but there was no evidence for non-paternity. These three individuals can be regarded as new mutants. From these data, the following mutation rates were estimated: $5.2\ 10^{-6}$ per locus per generation, $2.0\ 10^{-8}$ per codon and $6.0\ 10^{-9}$ per base. These data are in good agreement with other spontaneous mutation rates (see [V10]).

159. Given the number of offspring included in the study by Neel et al. [N9] and the relatively low gonadal exposure of parents in the exposed group, it would be premature to use these data to speculate on the biological mechanisms of radiation-induced mutations. But one likely conclusion can be drawn: medium- to low-dose irradiation in humans does not induce an unexpectedly large number of mutations detectable at the protein level. As discussed later, radiation does not cause significant visible genetic damage in humans in the next generation, and the result for proteins just noted seems to decrease the likelihood that radiation could enhance the long-term genetic load in the human population by producing many recessive mutations. Many radiation-induced recessive mutations in the mouse are deletions, as evidenced by studies with the seven recessive test loci [R3]. From widespread experience in medical genetics it would be expected that such deletions, if induced in human gene loci coding for known enzyme proteins, would reduce enzyme activity by about one half. Such effects have not been observed at a higher rate in children of irradiated parents in Japan.

160. The methods for assessing protein variants in children of parents exposed to the atomic bombings tend to be very time-consuming and personnel-intensive. They might be applicable to the monitoring of a limited population group that has been exposed to relatively high doses of radiation (or any other mutagenic agent), but they are not suited for the long-term screening and monitoring of large population groups.

D. SUMMARY

161. The estimation of additional genetic risk is meaningful only in relation to the spontaneous mutation rate. Thus, in attempting to calculate the genetic consequences of any agent including radiation, it is essential to begin with an accurate estimate of the background incidence rate for a given genetic disease or congenital anomaly.

162. There are now numerous registries of birth defects worldwide, each with unique features and each with advantages and disadvantages; these should all be taken into account when attempting to assess the background incidence for human populations in general. Access to multiple registries allows the development of fairly accurate background rates for congenital anomalies. Registries have different incidences of different anomalies with regional, ethnic and temporal variations in specific birth defects. However, when they are taken all together, fairly accurate background incidences for various congenital anomalies can now be established. Anomalies that are subject to environmental influences, such as neural tube defects, can be identified as varying with social class and region.

163. There is a consistent finding in all populations of 2%-3% incidence of serious congenital anomalies that will alter the length of life or ability to function normally without medical intervention that are ascertained in newborns. Another 2%-3% of serious congenital anomalies are ascertained by 5 years of age. An additional 5%-13% of minor anomalies are found in all populations.

164. Dominant mutations, together with X-linked mutations, are usually considered to provide the most important contribution to an increase in genetic disease that results from exposure to environmental mutagens. Contributions from autosomal recessive mutations would have less impact initially and would not be expected to be evident for many generations.

165. There is general agreement on the background incidence of autosomal dominant disorders. There is even less difficulty in establishing a baseline for autosomal-dominant diseases that have a strong selective disadvantage, the prevalence of which is maintained in the population by an equilibrium between constant mutation pressure and selection. These sentinel mutations are expected to indicate increases in the mutation rate caused by a new mutagenic agent in the environment. However, sentinel mutations are too rare to provide a basis for estimating realistic mutation rate increases. Continuous screening of very large populations would be required, but from the experience accumulated so far, it is extremely unlikely that human populations will ever be exposed to mutagenic agents that will cause an observable increase above the natural rate of mutation.

166. Other factors, such as paternal age, may greatly influence the rate of occurrence of certain autosomal dominant congenital anomalies; thus a shift in paternal age distribution within a population may have a greater effect on incidence than a change in exposure to radiation or other mutagenic agents. Maternal age may influence the rate of chromosomal anomalies such as Down's syndrome in a similar fashion.

167. Both heterozygote advantage and heterozygote disadvantage have been observed in autosomal recessive diseases in humans [V10]. Induced recessive mutations may cause harm in four ways:

(a) partnership with a defective allele already established in the population;
(b) partnership with another recessive mutation induced at the same locus;
(c) the formation of homozygous descendants of the induced mutation; that is, identity by descent;
(d) heterozygous effects (i.e. a carrier of the gene may have adverse effects).

Estimates of the manifestation of homozygotes in future generations depend critically on the assumptions made about consanguinity rates.

168. The incidence of visible structural anomalies or unbalanced translocations in chromosomal disease has been based on population studies on newborns; they do not include data from studies on spontaneous abortions. Most fetuses with numerical chromosomal anomalies (e.g. monosomy or trisomy) do not survive to birth. Thus, any radiation-induced increase of nondisjunction and/or early chromosome loss is likely to lead to an increase in the rate of spontaneous abortion rather than to an increase in chromosomally abnormal newborns. Thus, any increase in structural and numerical chromosomal aberration among newborns attributable to additional radiation of the parents would very probably be small and would depend on the age structure of the population.

169. It is important to estimate the mutational component of the incidence of congenital malformations. This involves estimating the frequency and degree of genetic determination of single anomalies and their modes of inheritance, which is very difficult to do, and indeed must be individualized. The complex genetic basis of most malformations makes it almost impossible to be sure of the effects of specific chemical or physical agents such as radiation.

170. In addition to multiple types of DNA damage leading to heritable mutations (i.e. single base dele-

tions, base-pair modifications, strand breaks, base-pair substitutions, nondisjunction etc., leading to nonsense, missense, frameshift, chromosomal mutations etc.), the site of a particular mutation must be taken into account when attempting to analyse its impact on genetic risk. Structural mutations occur in the coding region of a gene, altering the protein product of a single gene. Mutations in regulatory regions may affect the amount of gene product expressed or the time at which it is expressed. The type of gene in which a mutation occurs, e.g. a gene with limited function or a gene coding for a regulatory factor, will influence the extent of the mutation's harm to the organism. Failure of crossover during meiosis can lead to nondisjunction and may have a serious detrimental effect on the organism (chromosomal effects). Mutations in one region of a gene may produce a phenotype that is completely different from that caused by a mutation within another region. Mutations that upset the timing of events may produce unpredictable results (heterochronic effects).

171. It had been assumed that DNA damage was the only concern in radiation damage, but it now appears that changes in other components of the cell structure, such as the mitochondria, may have effects that are transmitted to subsequent generations. Mutations that affect the non-traditional mechanisms of inheritance (e.g. the methylation that might be involved in genomic imprinting) could affect numerous genes and have consequences that might not become visible for several generations.

III. GENETIC RISK ESTIMATION

172. Radiation exposure of the germ cells of animals, and therefore presumably also of humans, causes mutations and chromosomal aberrations that in turn may lead to genetic defects or diseases in the offspring and in later generations. Studies of atomic bomb survivors, industrial accidents and occupational and medical exposures have allowed some rough estimates to be made of genetic effects following human exposure to radiation. However, the paucity of direct observations regarding the genetic effects of radiation in humans has led to considerable uncertainty in the estimates of overall genetic risk and of the relative proportions of the various types of mutations that may occur from radiation. Animal experiments, most of them in mice, have been undertaken in an attempt to further quantify these effects. Although mice or other animals and humans differ in many ways that are biologically important, it has been necessary to assume similar responses in extrapolating the results of mouse and other animal studies to human populations.

173. In previous UNSCEAR Reports, the Committee described the various methods used to make genetic risk estimates and applied them to the available data. The history of these and other attempts to estimate the genetic risk of radiation has been described by Sankaranarayanan [S2, S3, S4, S5, S8]. Much has been learned about the limitations of these methods and about the molecular nature of the mutations that must be taken into account in estimating risk.

174. In the early 1970s, the following conclusions were derived from studies of animals, predominantly mice, and they were thought also to apply to human beings [S13, V6]:

(a) even at a relatively low dose, radiation leads to a sterile phase in males because it kills most of the spermatogonia; the testicular tissue is later repopulated by repeated division of a few especially resistant A spermatogonia;
(b) in the male mouse, a majority of visible chromosomal aberrations that are present in the F_1 offspring are induced in the pre-sterile phase, i.e. in postmeiotically irradiated male germ cells. Thus, if a human male is irradiated, chromosomal aberrations are not likely in the next generation unless conception occurs less than about 6-8 weeks after irradiation;
(c) in the female mouse, chromosomal anomalies may be induced, most of which lead to the death of the zygote at a very early stage of development (equivalent to early abortions in humans). The same is true for chromosomal aberrations that are induced in male germ cells and that are present in the zygote;
(d) in the hours around fertilization there is increased susceptibility to the induction of aneuploidies, especially the loss of single chromosomes, e.g. the X-chromosome;
(e) acute irradiation at relatively high dose leads to a considerable increase in recessive mutations in both sexes;
(f) a strong dose-rate effect has been observed in spermatogonia and in oocytes: chronic irradiation induces only about one third of the recessive mutations that are induced by acute irradiation. A dose-rate effect is also present for predisposition to dominant mutations;
(g) many radiation-induced mutations, especially those induced in postspermatogonial cell stages,

have been identified as deletions. Many induced recessive mutations were found to be lethal in the homozygous state;

(h) dominant mutations with clear-cut phenotypic effects and full penetrance are induced relatively rarely; dominant effects within multifactorial genetic systems, e.g. mutations affecting the skeleton, appear to be more common. However, before extrapolating this result to humans, the much easier and more detailed assessment of human anomalies should be considered;

(i) most translocations induced in spermatogonia are unable to pass through meiosis; they do not lead to abortions or to malformed offspring.

These conclusions have since been supplemented on the basis of data from further studies in animals and humans. The results of these studies will be discussed in the following Sections.

A. HUMAN STUDIES

1. Genetic follow-up studies on the survivors of the atomic bombings

175. While people who were exposed to radiation have been shown to suffer direct effects from that exposure, such as increased cancer rates, the data on the survivors of the atomic bombings of Hiroshima and Nagasaki indicate that acute irradiation at moderate doses has a negligible adverse effect on the health of the subsequent generation. Any minor effects that may be produced are so small that they are submerged in the background noise of naturally occurring mutational effects; they have not been demonstrated even by the refined epidemiological methods that have been employed over the last five decades [N7, N8, N9].

176. The first steps towards organizing a genetic follow-up study at Hiroshima and Nagasaki were taken in 1946, and the full programme was initiated in 1948. Now, after 45 years, that programme, conducted by the Radiation Effects Research Foundation (formerly the Atomic Bomb Casualty Commission), has become the largest and longest-running exercise in genetic epidemiology ever carried out. In recent years, the significance of the study has been greatly enhanced by the revised radiation dose estimates for survivors that became available in 1986. All of the accumulated data have now been analysed on the basis of the revised dose system.

177. The study has had two phases. In the early years (1947-1954), because of the Japanese ration system, it was possible to register virtually all pregnant women in the two cities at the end of the fifth month of their pregnancy and to arrange that once the child was born, it would be examined by a Japanese physician especially instructed in the diagnosis of congenital malformations. The first phase of the study collected the following data on each child: presence of congenital defect, viability at birth, survival through the first two weeks of life, birth weight and sex. What made it unusual was that because of the pre-birth registration, it was a prospective study embracing a total newborn population; studies of this type are less prone to bias than retrospective studies, in which at some fixed date one attempts to reconstruct pregnancy outcomes over a preceding period. About one third of the infants who were examined under this study were reexamined at the age of 9 months. All children born before May 1946 were excluded from the study of heritable genetic effects, since they may have been conceived prior to the bombings and received *in utero* exposures [N3].

178. In the second phase of the study (1954 to the present), the births continued to be registered until 1985, but the clinical programme was terminated. The data now collected are on the survival of liveborn infants, the occurrence of cancer in the children and, for selected subsets of the registered children, physical development, the presence of chromosome abnormalities and the occurrence of mutations that alter certain characteristics of the proteins of blood serum and red blood cells. The birth registry was also extended backwards in time, to include all infants born between 1 May 1946 (i.e. conceived after the bombings) and December 1947 (after the latter date infants would have been registered in the earlier programme). By now, the cohort of all children born to survivors of the atomic bombings who received significant exposures to radiation at the time of the bombings and who still live in Hiroshima and Nagasaki is thought to be complete. Significant exposure to radiation is defined as having been within 2,000 meters of the hypocentre of either bomb; individuals in this category are spoken of as proximally exposed, while those more distant from the hypocentre are referred to as distally exposed. The cohort of children born to a parent or parents who were proximally exposed consists of 31,150 individuals. An age- and sex-matched control group of 41,066 children was established by selection from the much larger group of children in the two cities born to parents in the distally exposed category. The number of children drawn from these cohorts for the second phase of the study varied according to the indicator. The average gonadal dose (parents combined) for the proximally exposed category was about 0.4 Sv, the actual dose varying somewhat from study to study depending on which children were included. The dose curve is quite asymmetrical, skewed to the right, with some parents having received combined gonadal doses as high as 2.5 Sv [N7, N8, N9]. The results of the various end-points studied are summarized in the paragraphs below.

179. *Untoward pregnancy outcome.* Because major congenital defect, stillbirth and neonatal death are inter-related, these end-points have been treated as a single entity, termed "untoward pregnancy outcome" and defined as an outcome resulting in a child with major congenital defect and/or a stillbirth and/or death within the first two weeks of life. Between 1948 and 1954, data were collected on these outcomes for a total of 76,617 births in Hiroshima and Nagasaki; data on 69,706 of them were sufficiently complete in all respects to permit inclusion in the analysis [N3, O2]. In later analysis the category of untoward pregnancy outcome is separated into stillbirths and congenital anomalies, giving eight end-points in all for analysis.

180. *Pre-reproductive deaths among liveborn children (exclusive of those resulting from a malignant tumour).* The frequency of death of live-born children has been analysed through 1985, when the mean age of the members of the study groups, if still surviving, would have been 26.2 years. In the two cohorts assembled for the second phase of the study, there are 67,202 individuals, among whom there had been 2,584 deaths by 1985 [K3, N3, Y2].

181. *Cancer incidence.* Data on malignancies occurring before age 20 have been collected on all children born at Hiroshima and Nagasaki after May 1946, i.e. on all children conceived following the exposure of their parents and on a suitable set of control children in the two cities. There were 43 malignant tumours in the 31,150 children of proximally exposed parents and 49 such tumours in 41,066 children of distally exposed or unexposed parents. The incidence of leukaemia (a malignancy of particular interest because of the study of Gardner et al. [G2], see Section III.A.2) is essentially the same in the children of parents one or both of whom were proximally exposed as it is in the children of parents who were not significantly exposed [Y1].

182. *Frequency of certain types of chromosomal abnormalities (balanced structural rearrangements of chromosomes and abnormalities in sex chromosome number).* Among the 8,322 children of the proximally exposed who were studied, 19 showed sex-chromosome abnormalities and 23, chromosomal rearrangements; among the control group of 7,976 children, 24 showed sex-chromosome abnormalities and 27, chromosomal rearrangements. Since there is no known instance of a parent with a sex-chromosome abnormality transmitting it to a child, all children with sex-chromosome aneuploidy were assumed to result from a mutation in the germ cells of the preceding generation. With respect to the chromosomal rearrangements, only one child in each group was shown to result from a mutation in the preceding generation [A6]. It must be noted, however, that the youngest children in the chromosome study were 13 years of age at the start of the study, and the study thus could not be expected to yield adequate data on the frequency of cytogenetic anomalies associated with increased mortality rates, such as unbalanced autosomal structural rearrangements and autosomal trisomies. Most patients with these aberrations will already have died by the age of 13. A possible exception is Down's syndrome (i.e. trisomy 21), but even in these cases, a high percentage of the patients may have died during childhood and early youth, mostly from recurrent infections but also from congenital malformations of the heart and other organs, given the living conditions prevalent in post-war Japan. The data on sex chromosomal abnormalities and balanced autosomal structural rearrangements should, however, be relatively unbiased.

183. *Frequency of mutations affecting certain characteristics of proteins.* A total of 667,404 tests were performed for protein mutations that alter electrophoretic mobility or enzyme activity in the children of the proximally exposed parents, and 466,881 tests were performed in the children of parents who did not receive significant exposures. The appropriate family studies on the 747 rare protein variants detected revealed that there were four mutations in the children of the proximally exposed and three in the control children [N4].

184. *Sex ratio.* The most pertinent data on the effect of parental radiation on sex of the child derives from the situation where the mother was exposed and the father unexposed. In this case, the sons would be expected to manifest the deleterious effects of radiation-induced X-linked dominant and X-linked recessive mutations as well as loss of the Y-chromosome, whereas the daughters would experience the effect of only the dominant mutations. A radiation effect should manifest itself as a relative decrease in male offspring, i.e. the sex ratio (male births/female births) should decrease. In fact, at the time the data were last analysed, there was an insignificant increase in the sex ratio, i.e. the data were counter-hypothesis [S10].

185. *Physical development of child.* The physical development of a subset of the children of exposed and control parents was studied at birth and at age 8-10 months [N3] and during the school years [F11, F12, F13, F14, F15]. The data for the most part pertain to height, weight, and chest circumference.

186. A variety of analyses of these seven data sets (and in later publications presented as eight data sets) has failed to reveal a statistically significant effect of parental radiation on the indicator. The average combined gonadal dose of acute ionizing radiation received by the proximally exposed parents (0.4 Sv) approximates that which in the past had been estimated to be a genetic doubling dose for mice. The

statistical power of these studies is such that the absence of an effect of parental exposure to the bombings on any of the indicators suggests that humans may not be as sensitive to the genetic effects of radiation as had for some years been projected on the basis of the murine doubling dose data.

187. The argument can be, and was, carried a step further. The investigators of the genetic effects of the atomic bombings accepted the proposition that some mutations did indeed result from the exposures to the atomic bombings and that this corpus of data should reflect the first-generation impact of these mutations on the population. The proposition was bolstered by the increase in chromosomal damage and somatic cell mutations observed in lymphocytes and red blood cells of the atomic bomb survivors [A7, N1], as well as by the increase in leukaemia and other malignant neoplasms in survivors [P3]. Accordingly, an effort has been made to estimate from these data the doubling dose of radiation for humans [N7, N9]. The doubling dose (relative) approach was felt to be imperative in this setting, as it would confer a perspective on the relative risks of radiation that would be lacking with the direct (absolute) approach. But although simple in principle, the doubling dose concept has been difficult to implement: for the estimate to have maximum accuracy, it requires the widest possible spectrum of genetic end-points, each weighted as to its importance in the total phenotypic burden imposed on a population by spontaneous mutation.

188. The doubling dose approach requires estimating the contribution of spontaneous mutation in the parental generation to each indicator anomaly or mutation. For technical reasons, this is not feasible for the sex ratio and the physical measurements. The approach also requires deriving a simple linear regression of each indicator on dose. This was judged to be not justified for balanced chromosomal rearrangements, where only a single mutation was observed in the children of both exposed and of unexposed parents. There remained five indicators from which to derive the regressions. (Since the data for the three indicators that were eliminated from the calculation did not suggest a radiation effect, their elimination should not bias the calculation.)

189. To derive a doubling dose, the impact of spontaneous mutation on the indicator in the parental generation must be estimated for each indicator. The value for sex-chromosome aneuploids and for loci encoding for proteins may be directly determined from the appropriate family studies. The value for the other three indicators has been estimated from the genetic literature. These three estimates are relatively uncertain and should improve with time. The background incidence for these indicators and the parental mutational component in the indicator, expressed in absolute terms and as a per cent, are given in Table 4 [N5]. The range given for three of the indicators reflects uncertainty as to the exact magnitude of the mutational component.

190. The data can be used for two different types of calculations. In Table 4, the doubling dose of radiation in relation to the contribution of spontaneous mutation to the indicator has been calculated for lower confidence limits of 99%, 95% and 90%. In principle, such estimates may be combined if it is assumed that the true doubling dose is the same for all the phenomena under study. Such an assumption is not warranted in this situation, the radiation literature suggesting that the doubling dose for the genetic phenomena resulting in untoward pregnancy outcomes, F_1 (first generation) mortality and F_1 cancer may be lower than the doubling dose for sex chromosome aneuploids and the nucleotide substitutions, which were the predominant end-points of the protein studies. The minimal doubling dose at the 95% probability level for the first three indicators combined was estimated to be between 0.63 and 1.04 Sv; for the last two, it was estimated to be 2.71 Sv.

191. Since these five end-points are essentially independent of one another, the most probable doubling dose for the totality of phenomena measured by these end-points can be obtained by summing these five regressions, summing the estimated contribution of spontaneous mutation to the various indicators and dividing the latter by the former [N9].

192. The resulting estimate was between 1.7 and 2.2 Sv, with the range again reflecting some of the uncertainty in estimating the contribution of the parental mutation to the end-point. The investigators considered this estimate conservative, for two reasons: (a) as noted, certain indicators for which there is no evidence for a radiation effect could not, for technical reasons, be included in the estimate, and (b) the data on socio-economic status, which were routinely collected on a subset of the population, suggested a slightly lower status for the proximally exposed survivors [K3]. This fact might increase the frequency of untoward pregnancy outcomes and death among live-born infants in this group and so give an upward bias to the estimate of the genetic effect of radiation.

193. As the investigators pointed out, the extrapolation of these results to the effects of chronic (or small and intermittent) exposures to ionizing radiation requires the selection of an appropriate dose-rate reduction factor. For the murine data, which was for the most part collected at doses of 3 and 6 Gy, a dose rate reduction factor of 3 has been employed. In the light of the much lower gonadal exposures experienced by proximally exposed survivors at Hiroshima and Nagasaki, the investigators elected to employ a

dose rate reduction factor of 2 [N6]. This resulted in a minimal estimate of the doubling dose for humans for chronic exposure to ionizing radiation, approximately 4.0 Sv.

194. The error in this estimate is indeterminate but must be considerable. First, there is the statistical error inherent in the estimation procedures, which is relatively large in relation to the regressions. Next, there is the error inherent in the present uncertainty concerning the contribution of parental mutation to such indicators as untoward pregnancy outcomes and early death. In addition, there is a potential error in the use of a dose-rate reduction factor of 2 in extrapolating from the effects of acute to chronic radiation. The fact that three additional indicators could not, for technical reasons, be incorporated into the estimate renders it conservative, as was already mentioned, but does not reduce its error. Finally, the human controls are not as accurately defined as would be the controls in a similar mouse study. Although the control parents were taken from the distally exposed group (they had been 2.5 km or more from the hypocentre of the bomb), it is not possible to ascertain that they were not exposed to radiation from other sources.

195. Some further guidance as to the lower limit of this estimate may be derived from the fact that although the average conjoint parental dose of acute radiation experienced by the proximal survivors was 0.4 Sv (a dose which in past UNSCEAR Reports was thought on the basis of murine experiments to approximate a doubling dose for acute exposures), it was not observed to have a significant effect on any of the eight indicators (see paragraph 186). This would be very unlikely if the true doubling dose for acute radiation is as low as 0.4 Sv. From the lower 95% confidence limits cited earlier, it seems unlikely that the human doubling dose for acute ionizing radiation under these circumstances is less than 1.0 Sv, and for chronic ionizing radiation, less than 2.0 Sv. The estimation of the doubling dose for humans must be regarded as a dynamic and continuing process, subject to revision as further data become available. Furthermore, it must be recalled that any estimate of the doubling dose is time- and place-specific, reflecting the genotype-environment interaction at a particular time. This is certainly true of the estimate based on the Hiroshima-Nagasaki experience.

196. In evaluating the importance to be accorded to the results of this study vis-a-vis the results of the more controlled murine experiment (see following Sections), four considerations stand out: (a) it is based directly on human data, (b) the indicators (congenital malformation, early death, cancer and syndromes attributable to sex-chromosome aneuploidy) are highly relevant to human affairs, (c) since the study includes virtually all the children ever to be born to exposed parents at Hiroshima and Nagasaki, it provides a total appraisal of the genetic effects of exposures to the atomic bombings rather than a snapshot in time, (d) the findings are of necessity obtained at doses compatible with human survival and hence do not require the degree of extrapolation from the much higher (for humans, unrealistically higher) doses employed in the murine experiments.

2. Epidemiological study of leukaemia cases at Sellafield

197. Many epidemiological studies have been carried out on populations exposed to radiation [A2, B5, C7, C11, C15, D3, K7, L4, S8]. One in particular raised concern about the harmful hereditary effects of chronic exposure. In 1990, Gardner et al. [G1, G2] reported the results of a case-control study of leukaemia and lymphoma among young people near the Sellafield nuclear plant in the United Kingdom. The study involved 52 cases of leukaemia and 22 cases of non-Hodgkin's lymphoma. The increased incidence of leukaemia among children near Sellafield was associated with recorded whole-body penetrating radiation to the fathers who worked at the Sellafield plant before conception. The authors suggested that the radiation exposures of the fathers caused mutations in their germ cells that, when transmitted to their children, caused those children to develop leukaemia. The authors pointed out that since low doses were involved, there were important potential implications for radiobiology and for the protection of radiation workers and their children. Those fathers who were employed at the Sellafield nuclear plant and whose children developed leukaemia had received total doses of ≥100 mSv before conception and doses of ≥10 mSv in the 6 months before conception.

198. The implications of Gardner's conclusions, if correct, would be far-reaching, since they suggest that as small a dose as 10 mSv, delivered at a low dose rate to the fathers, is sufficient to cause a large increase in the incidence of leukaemia among their children. The authors point out that their results on leukaemia conflict with the results available for children of parents exposed to radiation at Hiroshima and Nagasaki; they suggested that the difference might be explained by the fact that the exposures in Japan were acute, with a high dose rate, and those in Sellafield were chronic, with a low dose rate.

199. From extensive studies in mice it has been known for some time that chronic exposure over a prolonged period has an important effect on mutational response to radiation. However, that effect is in the opposite direction from that hypothesized by Gardner, since protracted radiation induces only one third as many mutations in spermatogonia as high-dose-rate

radiation [R6, R12]. Recent results on other populations provide no support for the conclusions of Gardner et al. [G1, G2]. Indeed, when Yoshimoto et al. [Y1] calculated the slope of the dose-response curve for the incidence of cancer below the age of 20 years versus conjoint parental dose, using linear multiple regression, they found negative slopes both for leukaemia and for all cancers combined. (The standard error of the slope, in each case, was larger than the absolute value of the slope.) The average radiation exposures of the survivors of the atomic bombings were much higher than those of the fathers employed at the Sellafield nuclear plant.

200. In addition, the statistically significant increase in relative risk of leukaemia found for fathers employed at the Sellafield nuclear plant was due almost entirely to only four affected children. Most of the other affected children had fathers employed in other industries. The conclusions of Gardner et al. are inconsistent with expectations from the risk estimates presented in this Annex, both from the doubling dose method and the direct method. Although the Gardner study used accepted procedures and appears to have been carefully done, it should be kept in mind that its conclusion is based on a correlation and is not consistent with other observations of exposed parents, and it may be a chance observation. A correlation alone cannot show causation. Furthermore, this particular correlation is heavily dependent on a very small number of cases and is contradicted by all of the studies that have been done in other populations with similar exposures (see paragraph 208).

201. The chromosomal translocations resulting in proto-oncogene activation that characterize the early phases of many leukaemias and lymphomas would tend to produce phenotypic effects incompatible with embryogenesis [E7]. For this reason such events are unlikely to be genetic determinants of leukaemia that could account for the Gardner et al. [G1, G2] findings. However, specific gene losses are also believed to characterize the early phases of some leukaemias [W11]. Since predisposition to some solid tumours of childhood (retinoblastoma and Wilms' tumour) is known to involve suppressor gene loss, a germ-line origin for some fraction of human leukaemias related to loss of specific genes or loss of suppressor gene function cannot be excluded as having a possible causal relationship in childhood cancer. Even in the case of leukaemia associated with proto-oncogene translocation, recent observations imply that germ-line-mediated epigenetic events (imprinting) can affect the subsequent formation of the translocation in somatic cells of the offspring [H16]. In the mouse there is more direct molecular evidence of germ-line mutations resulting in a predisposition to leukaemia/lymphoma. In one case this predisposition centres on the loss of the *p53* tumour suppressor gene [D8]; in the other it centres on changes to the structure of an anonymous telomere-like repeat sequence that may represent a heritable chromosomal fragile site [S43]. None of these observations serve, however, to account for the extraordinarily high induced mutation frequency that is necessary to explain the epidemiological findings at Sellafield. Modern molecular genetic techniques allow the identification of the parent from whom a particular chromosome has been inherited. If it could be demonstrated that in every case of leukaemia, the defective chromosome(s) was(were) inherited from the father, this would at least lend support to Gardner's hypothesis. However, no such cytogenetic or molecular studies have been done on the Sellafield cases [E7]. Had there been such a biological follow-up, it is likely that some of the children with leukaemia would have been found to have chromosome rearrangements in only a fraction of their somatic cells. Such a finding would have been more consistent with a mutation of somatic origin rather than with the hypothesis that the mutation came from the father.

202. A large number of individually rare genetic diseases are known to increase the risk of cancer in both the homozygous and heterozygous states. Some of these predispose to leukaemia and lymphoma and others to other types of cancer. Leukaemias would be expected to make up only a small fraction of the induced genetic disorders predicted by the risk estimates developed in this Annex. Thus, if a dose of radiation as low as 10 mSv induced a cluster of leukaemias, as suggested by Gardner, that same dose would be expected to induce an increase in other diseases in the same population. Since no such epidemic of genetic diseases has been reported at Sellafield [E7] or in long-term follow-up in the Russian Federation [K12, P5] or around other nuclear plants [A2], it seems highly unlikely that the conclusions of Gardner et al. are correct.

203. There are some data from mice that could support Gardner's conclusion. Studies by Nomura [N13, N14] found that there were significant increases in tumour incidence in the progeny of x-irradiated male and female mice in some strains, suggesting that the different genetic backgrounds of different human ethnic groups could influence radiation susceptibility. The dose-effect relationship was clear-cut for male post-meiotic stages and less clear-cut for spermatogonial stages. Oocytes at late follicular stages were resistant to x rays in the range 0.36-1.08 Gy but highly sensitive to higher doses. At the highest dose, 5.04 Gy, the tumour incidence in the offspring was around 30% following irradiation of spermatids in the male or oocytes in the female. This incidence was six times higher than the tumour incidence observed in untreated controls. Matings to the F_3 generation

demonstrated that the tumours were heritable and dominant, with about 40% penetrance on average. Some strains of mice had different sensitivities to these heritable effects of radiation. Other strains, however, showed no increase in heritable tumours with equivalent doses of radiation. While the dose used in Nomura's studies, 5.04 Gy was far higher than that calculated for the Sellafield workers and well above the doubling dose used in estimating genetic risks, the studies do indicate that heritable tumours can be induced by radiation.

204. Nomura [N17] has proposed three possible reasons for the differences between the Sellafield studies and other studies on human populations:

(a) different germ-cell susceptibility to leukaemia-causing mutations in Japanese and English people (similar to different susceptibility in different strains of mice);
(b) different germ-cell stages exposed in the two populations;
(c) different postnatal tumour-promoting environments.

Nomura noted that lung cancer, for example, had a seven times higher incidence in white uranium miners than in non-white miners who had received equal doses, although this was not a hereditary effect. He cited mouse studies demonstrating that the offspring of mice irradiated before conception show persistent hypersensitivity to tumorigenesis when exposed postnatally to tumour-promoting agents, developing clusters of tumours.

205. However, there are difficulties with Nomura's conclusions as well. The UNSCEAR 1986 Report [U2] reviewed the results of Nomura on the induction of dominant mutations causing tumours, and Selby [S21] and Sankaranarayanan [S4] also reviewed those results in detail. Most of Nomura's results are on pulmonary adenomas. A major difficulty in applying Nomura's results to risk estimation is the high control frequency found for each end-point studied. Attempts to extrapolate from mice to humans become especially uncertain if only a few different abnormalities are studied and if each of them occurs at much higher frequencies in mice than in humans. In addition, the high control frequencies make it much more difficult to evaluate the strength of the evidence on transmission and penetrance.

206. While Nomura treated his data as if each offspring with a tumour represented a dominant mutation, it seems much more likely that the tumours were threshold traits and that most of the affected offspring were non-mutational variants [S21]. Although it is probable that radiation can induce mutations that predispose individuals to develop tumours, the extent to which it does so is unclear from Nomura's work. There is no indication that parents were randomized or that offspring were coded in Nomura's experiments, and without such precautions it becomes especially difficult to interpret experiments for end-points having such high control frequencies (for example, 4.7% for pulmonary adenomas). According to a report of the International Agency for Research on Cancer [I2], positive results in lung tumour bioassay in susceptible strains of mice "may be strongly suggestive of carcinogenicity but are not conclusive by themselves". The report advises that when positive results are found in this assay, replicating them in another laboratory would greatly increase confidence in them, and a long-term, full-fledged bioassay might be necessary to remove all doubt.

207. Nomura's results on pulmonary adenomas and leukaemia suggest that the offspring of heavily irradiated male mice should have a high frequency of induced tumours. A recent small experiment by Takahashi et al. [T8] on liver tumours supports this view. Although it would seem, from this work, that it should be easy to find increased numbers of tumours in progeny carefully autopsied at time of natural death, no effect was found by Kohn et al. [K9] or by Cosgrove et al. [C19] in mice exposed to 5.3-7.2 Gy or 6.0 Gy of acute x rays, respectively. In both studies the offspring of both experimental and control groups developed many tumours. The Cosgrove et al. study also found no difference in longevity between experimental and control groups.

3. Epidemiological studies on other human populations

208. The Sellafield report stimulated other studies on radiation exposure in human populations as well as a reevaluation of previous studies. In addition to studies on the Japanese atomic bomb survivors, studies have been conducted on patients receiving radiation treatment for diseases such as rheumatoid spondylitis [L4, S8]; on populations living in areas with elevated background radiation in India [K7], Brazil [B5], Canada [A2], the Russian Federation [B4, K12, P5, T5] and China [C7, C11, D3]; on the population in Hungary exposed to fallout from the Chernobyl accident [C15, C16]; and on x-ray technicians [T1].

209. The only effect found in children of therapeutically irradiated patients and in x-ray technicians was a sex ratio shift in the direction expected if additional X-linked recessive lethal mutations are induced in the X-chromosomes of irradiated females and X-linked dominant mutations in the X-chromosomes of irradiated males. However, results from sex ratio studies are not easily interpreted and have not yet been found suitable for the estimation of genetic risk.

210. Two areas in southern China (near Yangjiang) with especially high levels of natural background radiation were studied. The radiation arises from radionuclides in fine particles of monazite that are washed down year after year from nearby mountains. These areas have been inhabited for about 800 years; the families of about 80,000 present-day inhabitants have been living there for more than two generations. The annual gamma-ray exposure is about three times that of the control population, which is similar to that of average population groups in other parts of the world. In this population, parameters such as cancer mortality, incidence of congenital malformations and other health impairments were studied, generally with negative results [C7, C11, D3]. One result, however, is of special interest: Down's syndrome (trisomy 21) was found to be far more common in the exposed than in the control group. To put this result into proper perspective, a number of factors must be considered. Incidence in the control group is much lower than in all other populations for which fairly reliable incidence figures are available. An incidence of only 1.8 cases per 10,000 births is without parallel in the international literature. Even the incidence of 8.7 cases per 10,000 births found in the exposed group is lower than the incidences reported in most population groups outside of China, which have been found to be 13-14 per 10,000. The most obvious explanation is that the diagnosis and/or reporting of Down's syndrome cases has been incomplete in both series and less complete in the control than in the exposed sample. The incidence of Down's syndrome in six other Chinese populations was compared with that of these two population groups. It ranged between 2.7 per 10,000 and 6.2 per 10,000, i.e. between the figures for the irradiated and the control groups. Again comparison with international figures suggests underreporting of varying degrees.

211. It is common knowledge that the incidence of meiotic non-disjunction, and therefore of Down's syndrome, increases sharply with advancing maternal age. Thus, first hypothesis of the Chinese scientists was that the difference between the two groups was caused by a difference in the age distribution of the mothers. And indeed, 12.02% of mothers in the exposed group, as compared with only 4.44% in the control group, were older than 35 years at the birth of the children included in the study. But even in the younger age group (below 35 years), the difference between irradiated and control populations remains significant (3.6 per 10,000 versus 0.5 per 10,000).

212. The observed differences between exposed and unexposed population groups is very probably caused by a combination of two factors: different age distribution of mothers and underreporting, especially in the control population. In the light of these two biases, the study results cannot demonstrate an additional radiation effect; indeed they cannot even be regarded as hinting at such an effect. This outcome highlights some of the problems encountered in assessing large populations for induced effects. As is described in detail in Section II.A, the accurate ascertainment of index cases is both difficult and crucial.

213. No change in the incidence of genetic disease in Hungary was detected by Czeizel after the Chernobyl accident [C15]. The lack of effects in studies of populations in areas of varying background is only to be expected, given the low statistical power of the investigations. Although some studies have included large population groups, the dose differences have been too small to observe statistically low risk effects (see paragraph 115). The most useful human data are still those collected at Hiroshima and Nagasaki.

B. METHODS OF RISK ESTIMATION: ANIMAL STUDIES

214. Animal data, especially those collected in mouse studies, still provide a basis for genetic risk estimation in humans. Extensive work has been done to try to develop accurate animal models for estimating human genetic risk following radiation exposure. The data on survivors of the atomic bombings that are becoming available suggest many additional animal studies that would be useful. Thus, despite the criticisms and limitations of each of the methods outlined below, animal studies are still invaluable and should be continued and refined as greater understanding of the complexity of the problem is gained.

215. It has been assumed, based on general principles of radiation genetics, that mutations induced by radiation are more likely to be neutral or harmful than beneficial and that the frequency of mutations will increase linearly with increasing dose. Two main methods are used in animal experiments (primarily mouse) that attempt to quantify genetic risk: the doubling dose (or indirect) method and the direct method. Since they have been described and used with varying emphasis in previous UNSCEAR Reports [U1, U2, U3, U4], as well as in other reports on radiation effects, such as those of the Committee on the Biological Effects of Radiation (BEIR) of the United States National Research Council [C1], they are discussed only briefly here, with a mention of their respective advantages and disadvantages.

216. Both methods extrapolate from animal data on induced mutations to risk of genetic disease in humans, although in different ways, as discussed below. The terms direct and indirect refer to whether the estimate of damage in the first generation is based directly on phenotypic damage found in mice in first-

generation progeny (direct method) or whether it is based on extrapolation back to the first generation from a prediction at genetic equilibrium (indirect method). Although the frequency of harmful effects from induced mutations (from the exposure of a single generation) would be expected to decrease beyond the first generation, owing to negative selection, it should be kept in mind that some of the genetic effects of radiation, e.g. aneuploid segregants from certain translocations or mutations leading to abnormal imprinting in the gametes of first-generation offspring, might not manifest until the second or subsequent generations.

1. The doubling dose (indirect) method

(a) Concept

217. The doubling dose method is used to estimate expected risk to a population under conditions of continuous irradiation, expressed in terms of the natural prevalence of genetic diseases. It is based on the concept that with stable population structure and living conditions, there is a balance between mutations that arise spontaneously and those that are eliminated by selection every generation. When an additional mutation source (such as radiation exposure) is introduced, the population will eventually (over a number of generations, depending again on mutation rate and selection) reach a new equilibrium between mutation and selection. It is the additional risk at this new equilibrium that is estimated with this method. Estimates of risk for the first or subsequent generations are then obtained from that at equilibrium using assumptions on the persistence of mutations in the population.

218. The method involves the estimation of the doubling dose, which thus is used to designate the method. The doubling dose is the dose of radiation required to produce as many mutations in a generation as those arising spontaneously. It is obtained by dividing the spontaneous rate at a set of gene loci by the rate of induction by radiation at the same set of loci. The doubling dose currently used in risk estimation is 1 Gy for low-LET, low-dose-rate irradiation conditions and is based on mouse data. The choice of this value has been discussed in previous UNSCEAR Reports.

219. The risk is estimated from three quantities used in the following equation:

$$\text{Risk per unit dose} = P F_m / D_d \qquad (1)$$

where P denotes the natural prevalence of the disease class under consideration, F_m, the mutational component and D_d is the doubling dose.

220. The doubling dose in experimental animals, especially mice, is defined as the radiation dose that doubles the spontaneous mutation rate (not the incidence or prevalence of a certain condition). Lüning and Searle [L6] have reviewed doubling doses for various genetic parameters. From their report, two aspects are obvious:

(a) there is an appreciable difference between estimates of doubling doses for various end-points. In mice, these end-points are defined as dominant and recessive mutations leading to defined phenotypes; dominant mutations affecting the skeleton; recessive lethals; and chromosomal translocations leading to semi-sterility. It should be noted that in humans, there may be other more meaningful end-points, and they may not be so well defined or consistent;

(b) the confidence intervals of these estimates are very large. Earlier UNSCEAR Reports [U1, U2, U3, U4] used a doubling dose of 1 Sv for irradiation at low dose rates. This estimate, based mainly on data from studies of seven recessive mutations in mice, is probably at least of the correct order of magnitude. The doubling dose might, however, be quite different for other end-points (i.e. other types of genetic effects), such as predisposition to cancer. This limitation or possibility should be kept in mind.

221. Regarding attempts to estimate doubling doses for some of the end-points examined in the offspring of atomic bomb survivors, Ehling [E8] concluded that the doubling doses based on data available from the atomic bomb survivors were not significantly different from estimates of doubling doses based on data from mice (see direct method, below). The absolute effects in humans are much too small to permit such a conclusion, and the end-points are too different to allow direct comparison. This does not, however, mean that no attempt should be made to estimate the doubling dose from human data or to compare it with that from the mouse data. In view of the overwhelming difficulties in arriving at rationally founded risk estimates, all reasonable approaches should be tried and optimized as far as possible, and the results compared. The application by Neel et al. [N8, N9] of the doubling dose method to studies of human populations was discussed earlier in this Chapter.

222. Since the doubling dose method uses the concept of mutation-selection equilibrium, the method can be applied to conditions whose prevalence can be attributed to this mechanism. All autosomal dominant and X-linked conditions (combined prevalence of 10,000 per million) have been traditionally considered to belong to this group; they are also the conditions for which the relationship between mutation and disease can be considered straightforward.

223. Autosomal recessive diseases require the combination of two mutant alleles to manifest the condition and are strongly dependent on population structure, in addition to selective factors. For these the relationship between mutation and disease is less direct. For multifactorial conditions, as mentioned earlier, the complexities of interactions between genes and with the environment preclude any direct relationship between mutation and disease, and this makes the application of the doubling dose method for these diseases very uncertain.

224. In order to circumvent at least some of the difficulties in risk estimation for the rather large group of multifactorial diseases, the concept of the mutational component was first introduced in the BEIR 1972 report and was subsequently elaborated upon by Crow et al. [C20]. Estimates of the mutational component are based on equilibrium theory. Although this approach was used in the BEIR V report to estimate the mutational components for congenital abnormalities and with these to obtain tentative risk estimates for this class of disorders, the Committee [U1, U2] refrained from doing so.

225. There has been no new empirical data that would warrant revision of the risk estimates presented in the UNSCEAR 1988 Report [U1]. However, arguments questioning the validity of some of the assumptions used and suggesting that these risk estimates for Mendelian diseases may be conservative have been advanced [S6, S7]. Nonetheless, for the present, the Committee favours the view that it is prudent to retain the 1988 estimates, if only because it is better to err on the side of caution. These estimates are presented in Table 5.

(b) Strengths and weaknesses

226. The major strength of the doubling dose method is that the risks are expressed in terms of the background load of genetic diseases, so that some tangible perspective can be derived to indicate whether the projected increases are trivial, small or large. Furthermore, it has the apparent advantage that whole classes of genetic diseases can be handled as units. However, many of the assumptions used seem open to doubt and have recently been discussed [S6, S7, V6].

227. The existence of a balance between mutation and selection (and its corollary, the mutational component) are among the central assumptions of the method. As discussed in Chapter II.B, even for Mendelian conditions, the assumption of mutation-selection equilibrium may be applicable to only a small fraction of these. This means that the prevalence value (for these and X-linked conditions) of 10,000 per million used in the risk equation may be high. For multifactorial conditions, the estimation of mutational components is still fraught with considerable uncertainties.

228. Another significant difficulty in application of the doubling dose method for genetic risk estimation lies in establishing the baseline of spontaneous mutations. (It should be mentioned that use of the term spontaneous does not mean that these mutations occur without cause. It simply means that in this context they are not caused by the agent of interest, namely additional radiation.) The disorders caused by spontaneous mutations may be very different and may occur in proportions very different from those caused by radiation. The doubling dose method assumes that spontaneous mutations have the same rate relative to mechanisms as do radiation-induced mutations. As pointed out above, however, there is evidence that this is not the case.

229. The use of the doubling dose method to analyse human populations assumes a stable population and environment. However, human populations are constantly shifting, and there are times, particularly during war, when there are shortages of food and health care, with increased infection and illness. Such conditions may well have an adverse effect on the radiation sensitivity of humans and their susceptibility to the mutagenic effects of radiation. Furthermore, human populations (including isolated, inbred groups) are genetically heterogeneous in comparison with mouse laboratory strains, which have been bred through multiple generations to be highly homogeneous.

230. Further discussions on the doubling dose method centre on the data on the molecular nature, specificities and mechanisms of origin of mutations underlying human Mendelian diseases and on the nature of radiation-induced mutations in mammalian experimental systems [S4, S5, S6, S7]. These suggest that (a) the doubling dose method may be applicable only to a small proportion of Mendelian diseases, (b) the doubling dose for autosomal dominant diseases may be higher than 1 Gy (i.e. lower relative risk) and (c) risk estimates for these presented in the 1988 Report are conservative, but provide a margin of safety in radiological protection.

2. The direct method

(a) Concept

231. The so-called direct method for genetic risk estimation was suggested by Ehling in 1974 [E8], with the intention of circumventing many of the difficulties encountered in the practical application of the doubling dose method. This has allowed the Committee to make

alternative estimates of genetic risks [U1, U2, U3]. The direct method of genetic risk estimation has as its basis a measure of the extent of induced phenotypic damage found in the offspring of mice exposed to radiation. Damage in the first generation would be expected to result almost entirely from induced dominant mutations, including deletions, that can have a range of penetrance from complete to low. The procedure for estimating genetic risk by this method is shown mathematically below:

$$\text{Risk per unit dose} = F_d M N \qquad (2)$$

where the risk per unit dose applies to the expected number of significant, radiation-induced dominant diseases in humans per million live-born in the first-generation progeny of irradiated parents; F_d is the frequency of radiation-induced dominant mutations per unit dose; M is the multiplication factor, i.e. the reciprocal of the fraction of total mutations thought to affect the body system(s) under study; and N is the number of children born in the population for which risk is being estimated, which is usually 1 million.

232. The multiplication factor has been assumed by the Committee in the past to be 10 for skeletal damage [U4] and 36.8 for cataracts [U3] (see Table 7). (The derivation factor and some qualifications concerning its use are discussed below.) The direct method has been used to estimate risk in only the first generation, although assumptions about the persistence of mutations in the population could permit the extrapolation of first-generation estimates to later generations. Dominant mutations are those that show clear-cut phenotypic effects in heterozygotes of the F_1 generation. Experiments for inducing and registering such mutations in the mouse have been performed almost since the beginning of radiation genetic studies in the 1930s and have often been reviewed (see, for example, [R10, S21]). End-points studied include mutations affecting the skeleton, the eye lens (leading to cataracts); dominant visible mutations, i.e. those in the living mouse recognizable with the naked eye; litter size reduction; congenital malformations; tumours; and effects on behaviour (see [S22]).

233. The UNSCEAR 1986 Report [U2] included, in consideration of the direct method, an estimate of the frequency of induction of sublethal effects that kill between birth and early life. Infant mortality is undoubtedly much higher in mice than in humans, because human mothers make great efforts to keep their young alive. Thus, it is important to realize that a large fraction of the sublethal effects in mice probably correspond to serious disorders in humans that may not be lethal early in life. An estimate of the amount of induced damage expected from unbalanced products of induced balanced reciprocal translocations has also been presented in evaluating genetic risk according to the direct method [U1, U2]. This estimate is based on the frequencies of induction of translocations in several species, including primates, and it uses various assumptions to determine the risk that a live-born child will be genetically abnormal. Details of such calculations were presented in the UNSCEAR 1986 and 1988 Reports [U1, U2].

234. A fundamental assumption of the direct method is that the genetic damage observed in mice can be related to genetic damage observed in humans. Critics of the method doubt that this can be done. Mutations can range from those with minimal effect to those with devastating effects on a particular organism, and the opportunity to observe the phenotypic consequences of induced dominant mutations in mice provides a sense of the seriousness of the induced mutations. For example, the largest data set used in the direct method was a sample of 37 dominant skeletal mutations [S23, S24, S25]. The two clinical geneticists involved in the first application of the direct method, McKusick and Carter, both concluded that approximately half of the 37 mutations would represent a serious health problem in humans [U4]. The many structural similarities between the skeletons and lenses of mice and humans make it much simpler to address the question of whether effects seen in mice relate to serious genetic diseases in humans. For example, almost all of the bones in the feet are similar in the two species. As a result, it is much easier to compare foot malformations between humans and mice than, for example, between humans and mammals with hooves. Molecular studies of mice and humans have shown marked similarities at the DNA level and have even proved to be useful in predicting the location of genes in chromosomes in humans based on their location in mice [S17]. Such similarities increase the chances that extrapolations of induced dominant damage from mice to humans may be reasonably valid.

235. Some induced dominant skeletal and cataract mutations in mice appear to be homologous to mutations known in humans. Other mutations seem to have different effects in the two species. These differences between the effects of mutations in mice and humans would be an especially serious complication if the direct method depended on recognizing the same disorders in mice and humans. It does not, however. Instead, the basis for comparison between the species comes from thinking of the malformations caused by many dominant mutations as threshold traits, according to the threshold concept advanced by Wright [W9]. Some dominant mutations shift the distribution entirely over the threshold, in which case they are simple dominants with full penetrance. Other dominant mutations shift the distribution only partially over the threshold, in which case they have incomplete pene-

trance. Many dominant mutations affect several developmental pathways and can thus influence several distributions of underlying factors relative to thresholds. Many mutations that cause genetic diseases, including those of complex aetiology, can be thought of as acting in this way in all mammals.

236. In the direct method, an attempt is made to determine the effects of induced mutations on the entire range of underlying factors and distributions that must exist in normal development. The genetic background of the mouse provides a very large number of different threshold traits on which the effects of induced mutations can be tested. Mutations can be detected that occur anywhere in the genome and that involve any type of molecular damage, providing that the mutation is capable of shifting a distribution over a threshold, such that a phenotypic effect is revealed in a first-generation offspring. The mouse, or other experimental mammal, used in the direct method thus becomes a tool for revealing the effects of induced mutations on the vast array of threshold traits present. It is hoped that by carefully looking for effects on threshold traits in first-generation offspring of mice, and by evaluating them for severity, some idea may be obtained of the likely effects of radiation in inducing serious genetic diseases in humans.

(b) Application and qualifications

237. The most recent application by the Committee [U1] of the direct method is summarized as follows. The expected approximate frequencies of induction of genetically abnormal children per million live-born, following 0.01 Gy of exposure of males, are 10-20 for mutations having dominant effects, 0 for recessive mutations and 1-15 for unbalanced products of reciprocal translocations. A footnote indicated that risk from dominant sublethal mutations is estimated to be 5-10. For exposure of the female, the same categories, reported in the same order, have risks of 0-9, 0 and 0-5. No estimate is available for dominant sublethal mutations in the female.

238. A difficulty with the direct method is that risk is estimated for only a small part of the total damage of concern to humans. Skeletal malformations and cataracts have been used because they can be examined in detail in the mouse. A few other end-points, for example adenomas in the lungs [N13], have been investigated, but it is not yet clear how to apply such end-points in the direct method. Much discussion and several lines of argument went into the choice of the multiplier of 10 to extrapolate from induced serious skeletal damage to induced serious total damage.

239. Several of the geneticists most familiar with genetic diseases in mice and humans felt that the number 10 was a reasonable figure to use in making such an extrapolation. Russell had suggested using 10, and Selby [S23] had suggested a range of 5 to 20. The Committee examined McKusick's catalogue of Mendelian inheritance in man [M7] and concluded that about one fifth of the diseases listed involved the skeleton [U4]. Although McKusick's catalogue gives no indication of the relative incidences of the diseases listed, in a rough way this analysis suggested that it might be appropriate to multiply the skeletal effects by 5 to derive total serious damage. However, the Committee noted that because there is a bias of ascertainment for skeletal effects, the true multiplier must be larger than 5. Since pleiotropy is common for genetic diseases in both species, it seemed that the multiplier should not be too much larger than 5, and 10 seemed to be a useful round number that would suggest little precision. McKusick [M8] agreed that the factor of 10 was reasonable, recognizing that the catalogue does not reflect the human genome in reality.

240. In view of these and other considerations, the Committee adopted 10 as the multiplication factor, and the estimate of risk, calculated as it was from data on mice, was assumed to be approximately correct for humans. Another limitation in using the direct method is that both skeletal and cataract mutations are connective tissue disorders, and it is anticipated that the genes involved in other tissues may respond differently to DNA damage. As more is learned about the relationships between DNA damage and phenotypic changes, it is expected that the multiplication factor will require adjustment, perhaps for each tissue type.

241. The Committee estimated that paternal exposure to 0.01 Gy per generation would lead to 20 serious dominant disorders per million live-born. According to the BEIR V report [C1], this figure was estimated to be between 5 and 15. Risk from exposure of the mother was estimated to be 0%-44% of that from paternal exposure (see Table 3).

242. Starting with the UNSCEAR 1982 Report [U3], the Committee included, in addition to skeletal malformation, dominant eye cataracts in its direct estimates. The radiation-induced mutation rate for such cataracts was established by mouse experiments mainly by Ehling et al. [E5, E6]. Dominant cataracts of various types are known to occur in humans and can be diagnosed in mice relatively easily. Again, the problem arises of finding a way of extrapolating from a very limited selection of mutations observed in mice to all loci expressing dominant mutations of clinical relevance in humans.

243. The multiplication factor suggested by Ehling to convert the dominant cataract rate to an overall mutation rate was again based on McKusick's catalogue of autosomal phenotypes [M7]. The number of well-established dominant mutations, 42, was assumed to be the same in man and mouse. This ratio is then used to convert the induced mutation rate of dominant cataracts to the estimate of the overall dominant mutation rate. As new knowledge of the human situation is gained, it should be possible to readjust the multiplication factor.

244. Ehling et al. [E8] have recovered more than 85 independent dominant cataracts in the mouse. About one third were observed in radiation genetic experiments. Combined experiments with the well-known specific-locus test (the seven specific recessive mutations) [E3] showed that the yield of dominant cataract mutations was one fourth that of specific-locus mutations.

245. Using an argument similar to that mentioned above for skeletal mutations, the dominant cataract mutation frequency was estimated to be 0.45-0.55 10^{-6} mutations per 0.01 Gy per gamete for high-dose-rate exposure. For low-dose-rate exposure, this estimate was divided by 3, yielding an estimate of 0.15- 0.18 10^{-6} mutations per gamete. Again using the McKusick catalogue listings [M7] and a multiplier of 36.8, a risk estimate of 6-7 10^{-6} serious dominant disorders per 0.01 Gy of paternal exposure was derived. Both estimates, that based on skeletal mutations and that based on cataract mutations, are similar, considering the multiple assumptions they require. The Committee decided in favor of a rough estimate of 10-20 serious dominant disorders per million live-born [U3].

246. Several correction factors are needed to estimate risk by the direct method. All estimates of phenotypic damage are based on high-dose-rate exposures, even though risk is estimated for low-dose-rate exposures. It is necessary to assume that the dose-rate effect for serious dominant damage is the same as that seen for specific-locus mutations, which are recessive mutations at one of seven genes in the mouse. (The predictive value of the specific-locus data seems stronger in this regard because many of the mutations at the *s* locus, which is one of the seven genes studied in the specific-locus test, are associated with reduced size, suggesting an associated dominant effect [R5].) Most data used in the direct method also come from experiments using fractionated exposures, which were administered with the expectation of increasing the mutation frequency. These experiments require correction factors, again based on specific-locus data, for the fractionation effect.

247. The estimate of genetic risk to the female depends on the assumption that the relationship for induced dominant damage and specific-locus mutations will be the same as that for the male. No experiments have been conducted studying the induction of skeletal anomalies or cataracts following the irradiation of female mice, and qualitative differences in the mutations in the two sexes could affect the level of induced damage to an important extent.

248. Even though many large experiments have been conducted to learn about the induction of mutations in mammals, the direct method still rests upon relatively few experiments including few mutations. The total includes only 42 dominant skeletal mutations and 12 dominant cataract mutations [S8, U3, U4], a small number on which to base such an important application, particularly when the mouse and human disorders may be quite different. It would be reassuring to have much more data collected on different strains or species; this would provide some indication of whether the frequency of induced dominant damage is highly dependent on the genetic background of the animals being examined. It would also be helpful to confirm the conceptual model on a molecular level. The direct method has only been used to estimate the genetic risk of serious genetic diseases, but since this is the information about genetic risk that is most needed, this is not a serious limitation of the method.

249. Although the direct method requires no information on the incidence of genetic diseases in a particular human population, it is important to have such an estimate to help put the mouse estimates into perspective. The incidences presently used in the doubling dose method (see above) are probably not valid for this purpose, however, for two reasons: (a) they include many conditions that are probably far less serious than those used in the direct method and (b) they are based on the incidences of disorders instead of the incidences of individuals with disorders. Concerning the latter point, it is relevant to note that many of the dominant skeletal mutations cause numerous effects, sometimes on other body systems as well [S24]. In spite of this, when estimating risk by the direct method, the number of affected individuals (which corresponds to the number of induced mutations) is used instead of the total number of malformations. This would seem to be a reasonable approach, since only one individual is disabled as the result of each mutation.

(c) Strengths and weaknesses

250. The principal advantage of the direct method of risk estimation is that it makes no assumptions regarding spontaneous mutations with similar genetic mechanisms or phenotypic effects, and no assumptions are necessary regarding the mutational component in complex diseases or malformations. The method relies,

however, on extrapolations from mice to humans and from a small fraction of dominant mutations, for example those affecting the skeleton or the lens, to all dominant mutations. In these repects, the so-called doubling dose (or indirect) method is much more direct: doubling doses have been estimated not only in mice, with subsequent extrapolation to humans, but also in humans, from the results of studies on atomic bomb survivors. In addition, estimates of the spontaneous incidence of dominant mutations and their increase in relation to a certain radiation dose were based not only on certain categories but on all such mutations.

251. The indirect method requires two main steps. In the first, the absolute number of mutations in relation to a certain dose and quality of radiation is estimated. This estimate is compared in the second step with the spontaneous mutation rate (or the assumed mutational component of complex conditions). The direct method requires only the first of these steps. The logical relationship between the two approaches should be kept in mind when the so-called direct approach is discussed in the following paragraphs. Selby [S22] correctly distinguishes between the indirect method as a method of relative risk estimation and the direct method as a method of absolute risk estimation. Moreover, an absolute risk estimate should always be put into perspective by relating it to spontaneous mutations, i.e. by transforming it into a relative estimate.

252. Many of the criticisms of the doubling dose method also apply to the direct method. In addition, there are a few points of criticism from the viewpoint of medical genetics. It is certainly too simplistic to calculate the multiplication factor from the number of different dominant skeletal malformations and cataracts in humans in comparison with the total number of known, dominantly inherited human phenotypes. The phenotypes enumerated in McKusick's catalogue of 1975 [M7] are a mixture of moderately common, relatively rare and extremely rare phenotypes. There have been many changes and additions to the catalogue since 1975. Many new entries in the category of confirmed human skeletal and cataract disorders, as well as many additional multiple anomaly disorders involving skeleton and cataracts should be included in such a calculation. One purpose of McKusick's catalogue is to permit the medical geneticist a quick orientation in the vast area of genetically determined human phenotypes. It has never been McKusick's intention to say anything about the incidence or prevalence of such phenotypes or their mutational origin.

253. The life expectancy of a mouse (approximately two to three years) is very much shorter than that of a human being. Certain genetic defects that lead to hereditary cataract in humans later in life simply have no time to manifest themselves in mice. Thus, only cataracts manifesting at birth or a short time afterwards can be assumed to be genetically homologous to the mouse cataracts recorded for use with the direct method. It must also be kept in mind that all experiments considered in the direct method have been done on male mice. The genetic risk for the offspring of irradiated female mice could be considerably different.

254. The estimate from skeletal anomalies may be criticized on similar grounds. For example, it is well known to anatomists that minor, and sometimes not so minor, variations of the human skeleton are widespread. Many, if not most of these variants have no clinical significance at all. In this context it should be remembered that human populations are mixed genetically, whereas the mouse populations used in experiments are often inbred strains. The best way to arrive at reasonable extrapolations to relevant mutation rates in humans would be to compare the patterns of manifestation of skeletal mutants and cataracts in the mouse with homologous dominantly inherited diseases in the human skeleton and eye, as documented and described in the medical genetic literature. For example, as Selby suggests [S22], it would be particularly reassuring if mouse mutant [S31] involving cleidocranial dysplasia could be identified for which there is a homologous human syndrome .

255. Selby [S22] suggested that clinical geneticists could help to classify animals as to clinical severity. In his opinion, the biggest advantage of the direct method is that it includes within its scope the irregularly inherited disorders, which constitute 85% of the serious genetic disorders in humans. The direct method requires no assumption that spontaneous and induced mutations have approximately the same likelihood of causing harm, no knowledge of the current incidence of serious genetic disorders in the human population, no estimate of how many of the irregularly inherited disorders are multifactorial or dominant disorders with low penetrance and no knowledge of the persistence of mutations in the population. However, these advantages are offset by the fact that it does not allow correlating a possible increase in mutations of known incidence with the spontaneous occurrence rates of relevant traits in human populations.

256. Another difficulty with the direct method is that risk is measured for only a small portion of the total damage of concern to humans. Skeletal malformations and cataracts are used because they can be examined in detail in the mouse. Much discussion, and several lines of argument, went into the choice of the multiplier of 10 to extrapolate from induced serious skeletal damage to induced serious total damage. However, both skeletal and cataract mutations are disorders of connective tissue, and it is likely that the DNA

damage in these tissues will be somewhat different from that in other tissues. As more is learned about the relationships between DNA damage and phenotypic damage, it would not be surprising for the multiplication factor to require some adjustment because of tissue differences. Multifactorial disorders and disorders of complex aetiology are probably far more common than is accounted for by a multiplier of 10.

257. A major shortcoming of the direct method has been the uncertainty about the inclusion of risk from serious disorders of multifactorial (complex) aetiology, which make up the great majority of the human genetic load. Because radiation-induced mutations often have incomplete penetrance [S23, S24], it would not be surprising if many disorders of complex aetiology involve mutations with incomplete penetrance. Other important classes of induced damage for which the aetiology is not presently understood might be overlooked if only those mutations are included that have proven transmissibility or meet particular presumed mutation criteria, as has been done thus far in applying the direct method. In addition, generations beyond the F_1 could express visible effects of induced mutations that involve non-traditional mechanisms of inheritance, such as genomic imprinting, not all of which will be visible in the F_1 generation.

258. In conclusion, the direct method, at least in its present form, does not allow estimating the expected increase of dominant phenotypes overall in humans. However, it has provided an enormous amount of information in carefully controlled conditions. In addition, it does not contradict the doubling dose estimates from data on the atomic bomb survivors and seems to be a useful alternative approach to the problem of estimating genetic damage in humans. If molecular analyses are used, the method could probably be developed to provide additional interesting information on the genetic basis of some irregularly inherited anomalies and on genetic effects in humans.

C. EXPERIMENTAL STUDIES

1. New experimental data relevant to risk estimation

259. Basic data on the genetic effects of radiation continue to be derived from earlier animal experiments. A major study in progress, assessing dominant damage in mice, is intended to have direct relevance to the estimation of genetic risk in humans. This study and others on the genetic effects of radiation are reviewed in this Section.

260. The results of studies of dominant skeletal and cataract mutations in mice remain the foundation of the direct method of genetic risk estimation. Ehling [E2] reported that three of his presumed dominant mutations were indeed shown to transmit their effects in a dominant manner. Selby and Selby [S23, S24, S25] conducted a large breeding-test experiment whose main purpose was to determine conclusively that the dominant skeletal mutations induced by exposure of stem-cell spermatogonia to low-LET radiation were indeed dominant mutations. It is noteworthy that many of the dominant mutations were shown to have incomplete penetrance and variable expressivity (and thus might or might not be truly autosomal dominant). Selby [S20] later demonstrated the statistically significant induction of dominant skeletal mutations in stem-cell spermatogonia following acute x-irradiation with 6 Gy or 1 Gy + 5 Gy (24-hour interval) or exposure to ethylnitrosourea [S26].

261. Ehling et al. [E3, K10] demonstrated that acute gamma radiation induces dominant cataract mutations in mouse stem-cell spermatogonia and in post-spermatogonial stages. This fractionated experiment and two later ones that yielded induced mutation frequencies that were not significantly higher than in controls were used in the UNSCEAR 1982 Report [U3] to apply the direct method to cataracts. The resulting risk estimate, based on cataract data, was 10 genetically abnormal children per 0.01 Gy of paternal exposure to low-dose-rate, low-LET radiation. It was used in the UNSCEAR 1982 Report [U3] and in the UNSCEAR 1986 and 1988 Reports [U1, U2] as the lower bound of the risk estimate made by the direct method. Ethylnitrosourea has also been shown to induce dominant cataract mutations [F1]. Many dominant cataract mutations exhibit incomplete penetrance and variable expressivity [F2, G6].

262. Selby et al. [S20] are using the assessment of dominant damage approach [S22] to investigate the induction of dominant mutations in stem-cell spermatogonia, with emphasis on skeletal and cataract mutations. Results to date [S29] are presented for 6 Gy of low-LET ionizing radiation, delivered either as 0.04 mGy min^{-1} ^{137}Cs gamma radiation (chronic) or 0.89 Gy min^{-1} x radiation (acute), and for a matched control. The frequencies of mice with possibly serious cataracts in the different groups were as follows: chronic irradiation, 5/1,502 (0.33%); acute irradiation, 4/1,290 (0.31%); and matched control, 5/1,693 (0.30%). The frequencies of mice with severe skeletal malformations were as follows: chronic irradiation, 11/1,291 (0.85%); acute irradiation, 20/1,193 (1.68%); and matched control, 23/1,457 (1.58%). When less severe skeletal malformations were included, the frequencies were chronic irradiation, 25/1,291 (1.94%); acute irradiation, 37/1,193 (3.10%); and matched control, 41/1,457 (2.81%). No significant differences were observed.

263. Graw et al. [G6] conducted experiments on the induction of dominant cataract mutations in stem-cell spermatogonia in mice, as detected in first-generation offspring produced in matings with untreated females, and found no significant effect of radiation up to 5.1 Gy + 5.1 Gy (24-hour interval). The reason for this lack of effect is not known, but at least the results are consistent with a low frequency of induction of cataract mutations. Specific-locus data collected in the same experiments showed that both treatments were clearly effective in inducing other types of mutations.

264. Selby [S21] reanalysed the published data of Graw et al. to assess whether there had been a significant increase over control in the frequency of cataracts. The findings led Selby to suggest that many of the cataracts found in such experiments may result from induced mutations with such low penetrance that proof of transmission is unlikely.

2. Studies of mutations in mouse oocytes

265. For many years estimates of the genetic risk to offspring of exposed women have been more uncertain than those of the risk to offspring of exposed men. Large-scale experiments using neutrons or x rays do not show that specific-locus mutations are induced in the immature arrested primary oocytes in the mouse (i.e. oocytes ovulated more than six weeks after irradiation). Russell [R11] presented a series of arguments suggesting that it might be reasonable to apply the apparently negligible risk for this germ-cell stage in the mouse to immature arrested primary oocytes in women, even though the arrested oocytes of mice are in diffuse diplotene (dictyate stage) and those in women are in a more condensed state (typical diplotene). Russell [R11] also argued, however, that for the sake of caution one might want to consider the possibility that the human arrested oocyte could be as mutationally sensitive as the most sensitive oocyte stages in the mouse, namely, the maturing and mature oocytes. To provide a basis for such extrapolation, he provided four different fits for the low-level radiation, specific-locus experiments in female mice. Only the highest frequency (0.44 times that in spermatogonia) was significantly above the control value. Russell concluded that genetic risk in the female is probably less than that in the male, and the Committee has applied the value of 0.44 in the direct method when calculating the upper limit for risk following maternal exposures [U1, U2].

266. Dobson and Straume [D4] suggested that it is inappropriate to base risk estimates for human arrested primary oocytes on the specific-locus data collected for arrested primary oocytes in the mouse. They demonstrated [S37] that the target for cell death in arrested primary oocytes of mice is not the DNA but rather the plasma membrane or something similar to it in terms of geometry and location in the cells. They have suggested that in Russell's large 0.5 Gy specific-locus experiment using x rays [R11], which provided most of the basis for concluding that risk for this stage might be negligible, the oocytes that survived the membrane damage would have received doses substantially lower than 0.5 Gy. They suggested that the estimate of risk based on a 0.5 Gy exposure might be much lower than it should be. In the UNSCEAR 1986 Report [U2] it was noted that current concepts of microdosimetry agreed with Russell's view that the distribution of ionizations from x rays would be so diffuse that the dose to the large nucleus of an oocyte could not differ appreciably from the exposure of 0.5 Gy administered.

267. Many more details of the Monte Carlo calculations by Straume et al. have now been published [S38], however, and those calculations suggest that there is a very small fraction of oocytes in the 0.5 Gy x-ray experiment that received as little as about 0.2 Gy to their DNA and there is a very small fraction of the oocytes that received as little as about 0.1 Gy to their plasma membrane. Furthermore, their calculations indicated significant coupling between the plasma membrane and nuclear doses, such that when the dose to the membrane was lower than average, the dose to the nucleus tended also to be low, and vice versa. In the view of Straume et al. [S38], only oocytes with the lowest dose to DNA (presumably about 0.2 Gy) survived, so Russell's finding of no mutations in 92,059 offspring [R11] was for a dose of only about 0.2 Gy instead of the 0.5 Gy administered. In their view, Russell's data do not suggest as low a mutation frequency as he estimated. Even if the interpretation of Straume et al. is accepted, however, it should be noted that the data from Russell's large 0.5 Gy experiment provide no basis for concluding that arrested primary oocytes are highly mutable, or even mutable, because no specific-locus mutations were found.

268. Besides providing evidence that the arrested primary oocytes in Russell's 0.5 Gy experiment may have received a lower dose than had been thought, Straume et al. [S38] demonstrated the induction of genetic damage in those oocytes by exposure to monoenergetic 0.43 MeV neutrons. They selected this form of radiation because of its short track lengths, which they say permits the recoil protons to deposit energy in the nucleus without traversing the plasma membrane. As a result, there would presumably be little or no correlation between energy deposition in the plasma membrane and the DNA, and oocytes receiving higher doses could survive. The two types of induced genetic damage detected in mice that had been superovulated using hormonal injections 8-12

weeks after irradiation were (a) chromosome aberrations, detected in oocytes arrested at metaphase I, and (b) dominant lethals, detected by the success with which cultured two-cell embryos survived to the morula or the blastocyst stage or hatched from the zona pellucida and formed a sheet of trophectoderm with a proliferated inner cell mass. The induction of both types of genetic damage was reported to be significant, with 6.0% chromosome aberrations and 16.9% dominant lethality at 0.25 Gy.

269. Although the authors demonstrated the induction of genetic damage in arrested primary mouse oocytes, it is not clear whether these types of genetic damage, detected following superovulation, are relevant to the genetic damage that would be seen in the offspring of irradiated females. They referred to the work of Griffin and Tease (next paragraph) and to much earlier work by Brewen et al. [B10] on the induction of chromosome aberrations in maturing mouse oocytes, and they state that the intrinsic mutational sensitivity of mouse immature oocytes is not very different from that of maturing oocytes. They claimed that their results make it possible to estimate genetic risk for women more confidently using the substantial amount of genetic data previously available for maturing oocytes in the mouse. The risk estimates presented in the UNSCEAR 1986 Report [U2] ranged from the lower limit of zero, based on the assumption that the mutational sensitivity of immature human oocytes is similar to that of immature mouse oocytes, to the upper limit of about 9, based on the assumption that the sensitivity of the human oocytes is similar to that of mature and maturing mouse oocytes. The latter is 0.44 times that for spermatogonia. Thus, the Committee took the position that risk in the female could be as high as Straume et al. now suggest that it should be.

270. Griffin and Tease [G7] provided the first clear evidence of the induction of genetic damage in arrested primary mouse oocytes by radiation (or by any agent, for that matter). Young (4-5 weeks-old) female mice were exposed to whole-body gamma radiation at a mean dose rate of 0.1 mGy min^{-1} until they received a total of 1, 2 or 3 Gy. Eight weeks after the end of the treatment, they were induced to superovulate by hormonal injections, and metaphase II oocytes were screened for numerical and structural chromosome anomalies. Hyperhaploidy (i.e. the presence of an extra chromosome) was used to assess the effect of the radiation on chromosome segregation. Several kinds of structural anomalies could also be detected in these cells. Again, the extent to which this genetic damage seen in superovulated oocytes would affect the health of the progeny of such females is unknown. The chromosome aberrations reported are probably not compatible with survival, but they may indicate a low level of induction of smaller types of rearrangements that might be viable.

271. Returning to studies on immature oocytes, Selby et al. [S28] found that females acutely irradiated near birth later produced as many as four or five litters, in sharp contrast to adult females, which became sterile after one or two litters following such an acute exposure [R7]. Based on the known radiosensitivities to cell killing of the different oocyte stages present near the time of birth, it seems likely that this experiment provided the mutation frequencies for pachytene and perhaps diplotene oocytes. Unlike in the adult female, the mutations found following an acute exposure were not restricted to the first six weeks after treatment; in fact, all mutations were found later or much later than this. No evidence was found for induction of mutations when females were exposed to 3.0 Gy at a moderately low dose rate near the time of birth.

272. As discussed above, it is uncertain whether the finding of no induction of specific-locus mutations in arrested primary oocytes of adult female mice can be extrapolated to women. If the degree of condensation of the chromosomes has an important bearing on the mutational response, those immature oocytes with condensed chromosomes that are present near the time of birth, which were studied by Selby et al. [S28], may be more comparable to the vast majority of oocytes in women than any other oocyte stage studied in the mouse. The finding of no mutation induction in those oocytes at moderately low dose rates suggests there may be no reason to abandon the view that mutation induction in female mice could be negligible compared to that in male mice.

3. Induction of translocations in primates

273. Van Buul [V1] and Adler and Erbelding [A1] conducted studies on the induction of reciprocal translocations in various strains of monkeys. Based on these results, there appear to be significant strain differences in monkeys. The frequency for stump-tailed macaques represents the lowest induction rate per gray ever recorded for an experimental mammal [V1]. This may raise questions about differences between primates and mice and about the validity of using mice to study radiation risk for humans. Generoso et al. [G3, G4] also found strain differences in mice for the induction of reciprocal translocations.

4. Induction of dominant mutations causing congenital malformations

274. Mutation studies aimed at detecting anomalies present at birth in mice and rats often examine fetuses

late in pregnancy instead of soon after birth, to avoid the problem of mothers eating grossly abnormal offspring. Earlier work was discussed in previous UNSCEAR Reports [U1, U2, U3], and recently there has been a detailed review of these studies [S21]. While some experiments have shown clear-cut effects of mutagens, others have shown weak effects or no effect at all for strong mutagens. Because of this inconsistency and the high levels sometimes found in controls, Lyon and Renshaw [L10] concluded that, in humans, the incidence of malformations is likely to be a relatively insensitive indicator of an increased mutation rate. Many of the congenital malformations are probably threshold traits (see Section I.C), and some of them are fairly common in the strains used. There is also reason to believe that some of the anomalies might result from spontaneous mutations with low penetrance that are segregating in the stocks [S21]. Selby pointed out that the randomization of parents in such studies, which does not appear to be standard practice, would help to guard against misinterpretations and improve the usefulness of the results [S21].

275. Nomura [N16] has almost doubled the sizes of his samples for studies of the induction of congenital malformations in mice by x-rays. The statistically significant induction of congenital malformations was shown for acute irradiation of post-spermatogonial stages, stem-cell spermatogonia, oocytes in adult females that were ovulated within 6 weeks after irradiation and oocytes present in 21-day-old mice. Nomura reported that frequencies of congenital malformations increased with dose for spermatozoa, stem-cell spermatogonia and mature and maturing oocytes of the adult. However, the slope of the simple regression line of dose versus frequency was statistically significantly above zero only in the mature and maturing oocytes. While much importance was given to the clear linear relationship between dose and mutation frequency found in spermatogonia between 0 and 2.2 Gy, the much lower mutation frequency reported for 5.0 Gy was ignored. In the total data reported, 42% of the anomalies found by Nomura were open eyelid, 25% were dwarfism and the rest were tail anomalies or cleft palate. Most of the mice with open eyelid showed only a small unilateral gap, and it was felt that had they been born, most would have been indistinguishable from normal mice by a few days after birth.

5. Results of the direct method to estimate risk

276. The experiments of Ehling [E1] and of Selby et al. [S23] both yielded estimates of an induced frequency of about 4 10^{-6} dominant skeletal mutations per gamete per 0.01 Gy for low-dose-rate irradiation, after applying correction factors derived from specific-locus experiments. As explained in describing the concept of the direct method, this frequency was multiplied by 0.5 (for severity), by 10 (multiplication factor for total damage) and by 1 million, to yield the estimate that 0.01 Gy of paternal exposure would result in 20 genetically abnormal children per million live births. In the UNSCEAR 1982 Report [U3], risk in the female was considered possibly negligible or, at most, 44% of that in the male, yielding the range 0-9 for 0.01 Gy of maternal exposure. The correction factors used to estimate maternal risk came from the suggestion of Russell [R11], based on specific-locus data in mice. Data on the induction of dominant cataract mutations were used in that same report to derive an estimate of 10 genetically abnormal children per 0.01 Gy of paternal exposure to low-dose-rate, low-LET radiation as the lower bound of the risk estimate made by the direct method.

277. Selby et al. [S29] point out that the preliminary results of assessment of dominant damage experiments in progress show no large error of underestimation in the direct estimate of genetic risk following paternal irradiation, even if that estimate is applied to all genetic disorders causing serious handicaps. They suggest that the data from an assessment of dominant damage experiment using protracted exposure [S29] could be used to derive an estimate of genetic risk as follows: subtraction of control results from experimental results will yield the frequency of induced serious genetic disorders. The estimate of genetic risk per 0.01 Gy could be calculated by dividing the induced frequency by 600 and then by multiplying it both by 10 (to expand to all body systems) and by 1 million to obtain an estimate of genetic risk expressed per million live births. Many of the correction factors and assumptions used before in applying the direct method would thus no longer be needed. Multiplication by the correction factors of 0 and 0.44, derived from specific-locus results, would yield an estimate of maternal risk, as was done previously.

278. If a mouse dies during the first few weeks of life, most of the types of phenotypic damage discussed above would not be detected. The omission of early deaths from induced dominant mutations would be a serious deficiency in a risk estimate. The assessment of dominant damage experiments [S29] are specifically designed to circumvent this problem by including extraordinary efforts to examine the skeleton of every mouse living beyond three weeks of age. This eliminates overlooking serious mutations because of early deaths. In the UNSCEAR 1986 Report [U2] the Committee made an estimate for protracted gamma radiation of the frequency of induction of dominant mutations causing death between birth and early life.

That estimate was based in part on an analysis by Selby and Russell [S27] of first-generation litter-size reduction in 14 radiation experiments involving 158,490 litters. That experiment yielded the estimate that for 0.01 Gy of low-LET, low-dose-rate paternal irradiation, there would be 19 deaths caused by dominant mutations between conception and three weeks of age for every million F_1 mice that would have lived to that age in the absence of irradiation.

279. The data of Lüning [L7], from one large experiment, yielded a similar estimate, 24 induced deaths per million. While the data of Selby and Russell could not be used to partition the total mortality rate into that occurring before and after birth, the data of Lüning [L7] and of Searle and Papworth [S15] could be used for this purpose. Based on these three data sets, the Committee estimated that the induction rate of dominant genetic changes causing death between birth and weaning would be 5-10 cases per million births per 0.01 Gy [U2]. This estimate has since been referred to as the "frequency of dominant sublethal effects". Although no such estimate has yet been made for maternal risk, it would seem reasonable to make a rough estimate by assuming that risk in the female is possibly negligible and, at most, 44% of that in the male, as has been done for other end-points. Risk in the female would thus be about 0-5 for dominant sublethal effects.

280. It should be noted that the chances that children with any one of many different serious birth defects will survive is heavily dependent on the sophistication of the medical technology available. The risk estimate for dominant sublethal effects based on the mouse is thus probably especially relevant when considering genetic risk from radiation in countries where advanced medical technology is less readily available. Baseline risk estimates are very difficult to obtain in such countries.

281. Table 3 shows the estimate of genetic risk based on the mouse model. It is noteworthy that there is no longer a separate listing for unbalanced products of reciprocal translocations. Those effects are presumably already included in the new risk estimate. It is important to note that research specifically aimed at understanding the induction of translocations in mice or other species, especially primates, must be carefully followed to see whether the mouse model, as applied in Table 3, could lead to a large underestimation of risk. Appropriate additions could be made to the Table if needed.

282. All modes of inheritance that could lead to first-generation effects are presumably included in the estimates given in Table 3, regardless of whether the aetiology is understood. Earlier work [S23] showed that an important part of the total consists of dominant mutations with full or incomplete penetrance.

283. Studies on skeletal and cataract mutations have shown that many of these mutations are recessive lethal mutations that have effects in heterozygotes [K11, S18]. Roughly 10% of the earlier direct estimate of risk based on the skeleton was thought to result from balanced translocations that acted like dominant mutations [S18].

284. The equilibrium estimate is the frequency of induced damage expected in each generation if the hypothetically increased mutation frequency stays constant until an equilibrium is reached. The estimate of genetic risk for the first generation could be extrapolated to an estimate at equilibrium if the persistence of mutations were well enough known. This is the reverse of the procedure that is necessary to estimate first-generation risk from equilibrium risk by the indirect method. Persistence, however, is rather well known only for the better understood diseases, such as those caused by simple dominants. For these, it is thought to be about 5 generations, although this is very dependent on the specific disorder. In the past, the Committee assumed that mutations responsible for disorders of complex aetiology persist for about 10 generations [U3], but this is an especially uncertain estimate. The discussion of multifactorial disease and non-traditional inheritance in this Annex underlines the difficulty of understanding the persistence of mutations that cause serious genetic diseases in humans.

285. In view of these uncertainties, no attempt is made to estimate risk at genetic equilibrium using the direct method. An equilibrium estimate is probably much less necessary for reaching decisions than a first-generation estimate, since as knowledge of genetics and medicine advances, ways may be found to reduce future impacts. As noted elsewhere, it would be useful to extend the direct method to at least a few later generations by measuring induced dominant damage following radiation exposure of successive generations.

6. Mouse versus human genes

286. Comparisons between humans and mice have been extremely useful in understanding development and genetic disease. The homology and sequence conservation between mouse and human genes is extensive. However, there are also notable differences in the expression of disease phenotypes. Several of the most frequent human disease mutations (e.g. neurofibromatosis) are not observed in mice. The most frequent mouse mutations (e.g. W locus) are rarely seen in humans. Furthermore, a number of human disease genes have been isolated, the abnormal mouse homologues of which do not produce any alteration in phenotype (e.g. Duchenne muscular dystrophy and Lesch Nyhan disease). Thus, the same mutation may

lead to disease in one species but not in another. There also appear to be marked strain differences in mice with regard to the severity of the phenotypic defect produced by a particular gene mutation. This type of strain variation in mice is probably comparable to the ethnic differences observed in humans [L11].

287. In addition to differences in disease phenotypes, there are also significant differences between mice and humans in early developmental processes, placentation and types of congenital anomalies. For instance, monozygous twinning is rare in most strains of mice but occurs quite frequently in humans [N2].

D. GENETIC RISK ESTIMATES

288. Some new information relevant to estimating the genetic effects of radiation has been presented in this Annex. There has, however, been no reason to revise the risk estimates, although the uncertainties could well be widened in view of the many complexities that are emerging. The genetic risk estimates of the Committee are summarized here and compared with those of other groups, national and international.

1. Estimates of UNSCEAR

(a) Dominant and X-linked diseases

289. The Committee has not changed its estimate of the incidence of dominant and X-linked diseases in the population since the UNSCEAR 1972 Report [U5]. This value of 10,000 cases per million live births divided by the doubling dose of 1 Gy, multiplied by a mutational component of 100% for these diseases and by a continuing dose of 0.01 Gy per generation gives an equilibrium estimate of 100 cases per million live births. The first generation increment (15 cases per million live births) is assumed to be 15% of that at equilibrium and the second generation increment (13 cases per million live births) is 15% of the equilibrium less the first generation cases (100-15 cases per million live births). These results are listed in Table 5.

(b) Recessive diseases

290. The Committee has estimated that autosomal recessive diseases occur at a rate of 2,500 per million live births. Calculations based on a combination of data from observations on human populations and from mouse experiments suggested that an extra dose of 0.01 Gy of low-LET radiation to each parent in a stable population with a million live-born offspring would induce up to 1,200 extra recessive mutations [S16]. From these data it was calculated that partnership with an established or newly induced recessive allele in the population would produce about one extra child with a recessive disorder in the following 10 generations (per million born in each generation). About 10 extra cases of recessive diseases would be expected from this dose by the tenth generation, assuming about 1% of first-cousin matings.

(c) Chromosomal diseases

291. Based on extensive cytogenic data from human populations, the Committee estimated that visible structural anomalies or unbalanced translocations occur at the rate of 400 per million live births [U1, U2, U3]. The indirect method of estimation (doubling dose of 1 Gy) thus gives an incidence rate at equilibrium of 4 cases per million live births from a continuing dose rate of 0.01 Gy per generation. The first-generation increment has been assumed to be three fifths of the equilibrium value [U3], giving 2.4 cases per million live births. The second-generation increment is three fifths of the remainder: $3/5 \times (4 - 2.4) = 1$ case per million live births.

292. The estimate of genetic risk was based on evidence that about 9% of individuals with unbalanced chromosome rearrangements survive to birth. The question of how many such rearrangements can be induced by radiation is not easily answered. Comparative studies in mice, rhesus monkeys, marmoset monkeys, crab-eating monkeys and human males have revealed striking differences (see the UNSCEAR 1986 Report [U2], Table 21, page 128). One interesting point should be noted: many spontaneously occurring translocations in humans are Robertsonian in type, but it has been concluded from mouse data that radiation does not induce such Robertsonian translocations [F16].

293. For numerical chromosomal diseases (mainly trisomies), the Committee has used a figure for the current incidence of 3,400 per million live births [U2]. The increase following radiation exposure could not be calculated but was assumed to be very small, based on mouse data. The human data from the Japanese studies also support a small risk.

294. Studies in the mouse have shown that the rates of aneuploidy induction in male and female germ cells by radiation were of the same order of magnitude ($2\text{-}7\ 10^{-2}$ per Gy) and were not very different from those reported by various authors for translocation induction in spermatogonia [P1]. The oocyte, when irradiated at the time of fertilization, is especially susceptible to chromosome loss, especially loss of the X-chromosome. However, most monosomic human zygotes do not survive early pregnancy; even the great majority of surviving 45 X-zygotes are thought to be attributable not to nondisjunction or chromosome loss during meiosis but to mitotic events during early

pregnancy. Moreover, most trisomics do not survive to birth [H9]. Hence, in view of the well-known high incidence of chromosomal anomalies among spontaneous abortions, any radiation-induced increase in nondisjunction and/or early chromosome loss is likely to lead to an increase in the rate of spontaneous abortion rather than to more chromosomally abnormal newborns, as explained in greater detail in earlier UNSCEAR Reports. This conclusion is corroborated by a study in the mouse [R1], in which the frequency of chromosomal radiation effects was followed from zygotes to early and late embryos. The fraction of chromosomally disturbed germ cells, which was very high at the beginning, was found to be practically zero in embryos surviving to birth.

295. In the UNSCEAR 1982 Report [U3] (Table 8, page 525), 12 studies were listed that addressed the question of whether pre-conceptual irradiation of mothers increases the incidence of Down's syndrome. Four studies described a significant increase in this syndrome; eight studies failed to show such an effect. The studies included women who had been exposed at some time in their lives to small doses of radiation for medical reasons. Obviously, they were not an unbiased population sample; there is ample opportunity for the action of confounding variables. On the other hand, the data do not allow dismissing the possibility that low radiation doses enhance the risk for autosomal nondisjunction in female human meiosis, at least under certain conditions (see also the studies of populations living in areas of high natural background radiation in India and China, discussed in Section III.A.2). The Japanese data do not support an increased risk for live-borns with trisomics.

(d) Congenital anomalies and multifactorial diseases

296. In the UNSCEAR 1988 Report [U1], the Committee estimated the incidence of congenital anomalies and multifactorial diseases to be 60,000 and 600,000 per million live births, respectively, but did not estimate the increase caused by radiation [U1]. In the UNSCEAR 1977 and 1982 Reports [U4, U3] the Committee had used a doubling dose of 1 Gy and a mutational component of 5%, but the uncertainties did not justify continuing this procedure, given the higher estimated incidence of these conditions of varying seriousness that can arise throughout a lifetime. The study of the survivors of the atomic bombings in Japan indicates no increase in congenital anomalies or multifactorial disorders subsequent to parental irradiation [N8, N9]. In theory, a system of continuous registration of these conditions that is complemented by ad hoc studies on special problems, such as genetic data (for example empirical risks in families) or environmental factors (radiation, drugs and environmental chemicals), is the best system for answering questions of incidence and causation. However, in view of the complex genetic basis of most malformations and of multifactorial diseases, it is impossible to estimate increases caused by radiation, and no such estimate was attempted in the UNSCEAR 1988 Report [U1].

2. Estimates of BEIR, ICRP AND NUREG

297. While the BEIR III Committee [C2] relied mostly on the direct method to estimate first-generation risk for genetic disorders and traits that cause a serious handicap at some time during a lifetime, the BEIR V Committee [C1] described the direct method but stated that the "Committee had little confidence in the reliability of the individual assumptions required by the direct method let alone the product of a long chain of uncertain estimates that follow from these assumptions. Therefore, they did not place heavy reliance on the direct method in making their risk estimates, but used it only as a test of consistency". The BEIR V Committee discussed relatively few of the many assumptions used in the indirect method, which it did apply. In contrast, the United States Nuclear Regulatory Commission report [N10], in discussing the BEIR V report, noted that the direct method involved fewer uncertainties than the indirect and was thus preferable.

298. The BEIR V report presented a much higher current incidence for genetic disorders than the UNSCEAR 1988 Report [U1] (Table 5). It concluded that the current incidence is 1,247,300 genetic effects per million live-born offspring. Risk was not estimated for the 1,200,000 of these effects that consisted of heart disease, cancer and selected other diseases. Regarding these three categories, they concluded as follows: "The magnitude of the genetic component in susceptibility to heart disease and other disorders with complex aetiologies is unknown. Because of great uncertainties in the mutational component of these traits and other complexities, the committee has not made quantitative risk estimates for them. The risks may be negligibly small, or they may be as large or larger than the risks for all other traits combined". For the remaining genetic disorders (current incidence of 47,300 per million), the estimate of first-generation risk of radiation-induced genetic effects per 0.01 Sv of exposure was between 16 to 53 per million live births (Table 5). Of that total, between 1 and 15 were estimated to be clinically mild.

299. The risk of congenital anomalies was given in the BEIR V report [C1] as about 10 in the first generation and 10-100 at equilibrium for 0.01 Sv of additional radiation. These estimates assumed that the

relevant malformations consist of those caused in part by multifactorial inheritance in combination with a threshold and in part by irregularly manifesting dominant mutations. The overall mutational component was estimated to be between 5% and 35%. The upper limit (35%) was then used to estimate the increase (Table 5). The result is quite uncertain. Moreover, the argument is based in part on results of twin studies, which are misleading for congenital malformations since the background incidence of most congenital abnormalities is increased in monozygous twinning, apparently because of the special conditions that produce monozygotic twin pregnancies [V10].

300. The ICRP estimated the component of risk for multifactorial diseases from radiation exposure to be 0.5 10^{-2} Sv^{-1} and the total for severe hereditary effects for all generations to be 1 10^{-2} Sv^{-1} (see Annex B in [I1]). The ICRP risk coefficients [I1, S44] relied heavily on the UNSCEAR 1988 risk estimates [U1], with some important additions. The effect of 0.01 Gy per generation per million live births on the incidence of Mendelian and chromosomal diseases was taken to be 120 at equilibrium [S44], as in Table 5. The natural prevalence of congenital abnormalities was taken to be 6%, as in Table 5, and of other multifactorial disorders 65%. Assuming a doubling dose of 1 Gy of low-dose-rate, low-LET radiation, an average mutational component of 5% and a weighting factor of one third for severity of effects, the risk coefficient for all multifactorial diseases, including congenital anomalies, becomes 120 per million live births at equilibrium for exposure to 0.01 Gy per generation [I1, S44], or 1.2 10^{-2} Sv^{-1}. The total risk coefficient for all Mendelian, chromosomal and multifactorial diseases is thus 2.4 10^{-2} Sv^{-1} at equilibrium. However, when the total population is considered, the genetically significant dose will be markedly lower than the total dose received over a lifetime. If it is assumed that the mean age at reproduction is 30 years and the average life expectancy at birth is 70-75 years, the dose received by 30 years is about 40% of the total dose. The risk coefficient for the total population is thus 0.4 × 2.4 10^{-2} Sv^{-1}, or 1 10^{-2} Sv^{-1} at equilibrium, i.e. when the effects are summed over all generations [I1, S44]. The corresponding risk coefficient summed over the first two generations only would be 0.2 10^{-2} Sv^{-1}.

301. The NUREG Committee [N10] expressed genetic risk per 480,000 live births instead of per million live births. This figure is an estimate of first generation offspring in the United States, predicted from the 1978 demographic data of 1 million persons of all ages (i.e. 16,000 live births per year for 30 years). Its combined first-generation risk estimate for single-gene disorders, chromosome aberrations (including aneuploidy) and congenital abnormalities was 30 radiation-induced genetic disorders, and its first-generation estimate for selected irregularly inherited diseases (having a normal incidence of 576,000 per 480,000 live births, which is equivalent to the current incidences used by the BEIR V Committee), was 35 radiation-induced genetic disorders. The Committee stressed the "extremely tenuous nature of these numerical estimates for diseases of complex aetiology in light of the very large uncertainties involved" [N10].

302. The NUREG Committee pointed out that its estimate of risk from unbalanced translocations, as well as the estimate in the UNSCEAR 1988 Report [U1], was based on an estimate of the frequency of balanced translocations at least an order of magnitude higher than that actually observed cytologically in the offspring of the atomic bomb survivors [A7]. It thus may be that the risk estimates in the UNSCEAR 1988 Report [U1] for this category of genetic disease are too high.

3. Re-evaluation of doubling dose estimates in mice and humans

303. It has been suggested that, for humans, the doubling dose of acute low-LET ionizing radiation of the gonads is 0.3-0.4 Sv, with limits of 0.1-1 Sv [C1, U2]. That estimate appears to be based primarily on the data summarized by Lüning and Searle [L6], namely, data on semi-sterility (i.e. reciprocal translocations), the seven-locus (or specific locus) system of Russell [R4], dominant visible mutations recovered in the course of specific-locus studies, dominant skeletal mutations and recessive lethals. The average of these values was 0.31 Sv. When a dose-rate reduction factor of 3 was employed for conversion to the effect of chronic radiation, the doubling dose for chronic and/or intermittent radiation was about 1 Sv, with lower and upper limits of 0.3 and 3 Sv. It has been suggested that for chronic radiation the doubling dose is not less than 1 Sv [C1]. This estimate was based on a wider variety of end-points of genetic damage than those used by Lüning and Searle, and the confidence limits for some of the additional end-points were very wide.

304. In view of the apparent discrepancy between these estimates and those resulting from the follow-up studies on the children of atomic bomb survivors, Neel and Lewis [N7] compared the findings on mice and humans point-by-point. They concluded that because of biological differences between the two species, a precise comparison was impossible for many end-points. For instance, the newborn mouse corresponds roughly, in terms of development, to a human fetus at 100 days of gestation [N7]. The data on congenital malformations in mouse fetuses following paternal irradiation were obtained by sacrificing pregnant mice at day 18 or 19 of gestation, equivalent to less than

100 days of human fetal development [K5, K6]. Because in humans some fraction of the corresponding defects would be lost through early miscarriage and in most studies go unrecorded, and because the very immature mouse fetus cannot be subjected to the same type of physical examination as a newborn infant, the mouse and human data are not comparable. Furthermore, the polytocous nature of mouse reproduction results in pre- and postnatal competition between litter mates, which does not exist in humans. This competition renders a comparison of the two species with respect to postnatal mortality following radiation uncertain and complicates any extrapolation. Finally, since several of the mouse strains employed in radiation research were originally developed for research on cancer, it may not be entirely valid to compare the results of Nomura [N14, N15] on mice with the results obtained in Hiroshima and Nagasaki, particularly since the tumour types that dominate Nomura's data are not those associated with germ-line mutations in humans.

305. Given the manner in which the recent estimate of the human genetic doubling dose resulting from acute radiation (the Japanese studies) was derived, the mouse estimate that would seem to be most comparable to the human would be that based on locus-specific phenotype studies. In Table 6, the findings for eight different types of locus-specific phenotype studies in the mouse are compared. These studies are of rather uneven informational content, and some not quite congruous studies are combined, particularly those in which the results had not previously been incorporated into doubling dose estimates. The simple, unweighted average of the results of these eight types of tests is 1.35 Gy. Applying the dose rate factor of 3 customarily applied to such data in the light of the results of Russell et al. [R6], the estimate of the murine genetic doubling dose of chronic ionizing radiation becomes about 4 Gy. As for the human data, it is difficult to develop a precise error term for these data.

306. The potential difference between acute and chronic exposure and between different types of radiation needs further study. Searle and Edwards [S16] in particular have shown an increase in translocations with protracted exposure to low levels of high-LET radiation (i.e. alpha particles and fission neutrons).

307. It will be noted that the results obtained with different test systems appear to vary widely. Since for several of these estimates (e.g. electrophoretic variants and recessive visible mutations), only a single mutation has been encountered in the controls, the error of the estimate is large. There may also be real differences between systems. The data of Russell et al. [R5] indicate significant differences among the seven loci in their system with reference to radiation-induced rates. Similar data on locus differences in spontaneous locus mutability are beginning to emerge for humans [N7]. Thus, it should not be regarded as surprising if different systems yield different estimates, and there is no objective basis for preferring the results from one system over those derived from another.

308. This summary of the mouse data indicates the need to consider the results from many systems in arriving at a balanced view of the genetic effects of radiation. It should be noted that the studies in Japan (Section III.A.1) involve end-points that reflect the input of many loci. There may, however, be an additional reason for differences between the results of the different mouse systems: inadvertent selection in some of the murine systems of the more mutable loci for study. For example, the valuable and much used seven-locus test system developed by Russell [R5] was developed on the basis of known, contrasting phenotypes associated with each of the loci in question. This was the key to developing the test crosses, which permitted rapid locus scoring for mutation. It now seems possible that this derivation of the system may have introduced inadvertent selection for loci with relatively high spontaneous and induced mutation rates. This and the possible strain differences in mice again raises the question of ethnic differences in humans: could different genetic backgrounds result in loci with differing susceptibility to mutation?

309. Although the error in both the mouse and human estimates is, for various reasons, indeterminate but presumably large, these considerations, in the view of Neel and Lewis, may bring the mouse and human results into much better congruence than appeared to be the case in the past. There is, of course, no reason why two species differing in as many respects as mice and humans should have identical doubling doses, but there is also no reason to think they should be unequal. Given the difference in the end-points used for the two species, this congruence is somewhat surprising and should be regarded with caution.

4. Molecular biological developments

310. Sankaranarayanan [S3, S4, S5, S6, S7] considered at length the impact of molecular biology on the estimation of genetic risk from ionizing radiation and made a number of points. In genetic risk estimation, adverse genetic consequences have generally been viewed through the prism of naturally occurring genetic diseases. The concept of radiation-induced genetic disease relies on the premise that the types of events induced are similar to those arising spontaneously; this was supported by the recovery in experimental systems of induced mutations at selected gene loci, the phenotypes of which were similar to those of spontaneous mutations. This idea continues to catalyse

the search for increases in the frequencies of known dominant or X-linked genetic diseases in human populations exposed to radiation (e.g. as a result of the Chernobyl accident [C15] and the atomic bombings of Hiroshima and Nagasaki).

311. However, as discussed in Section II.C, molecular data for Mendelian diseases and for radiation-induced mutations in experimental systems now demonstrate that (a) while the types of events are indeed similar, those that lead to naturally occurring Mendelian diseases show specificities both in their distribution and their mechanisms of origin and (b) the overlap in mechanisms between spontaneous and induced mutations may be small. Thus, the probability that ionizing radiation will induce the specific mutations that result in known Mendelian diseases is likely to be small. It is not to be implied that gonadal radiation exposures have no adverse genetic effects. Rather, the message is that the frame of reference used, namely naturally occurring Mendelian diseases, may not be entirely adequate [S7].

312. With regard to the two standard methods of risk estimation using animal models the following may be noted. The size of the multiplication factor used in the direct method is partly based on an understanding of the relative damage to different body systems from spontaneous mutations. As noted earlier, the estimate of this is uncertain. It would be subject to additional uncertainty if the very large numbers of genes that can mutate to cause damage to different body systems respond to radiation damage to very different extents.

313. With the doubling dose method, the risk is estimated by multiplying the natural prevalence of autosomal dominant and X-linked diseases by the relative mutation risk. A number of arguments suggest that the value of the natural prevalence may need to be revised downwards and that of the doubling dose upwards; as a consequence, the estimate of risk will be lower than at present. These arguments are as follows:

(a) since radiation generally induces deletions, and assuming that only about 50% of naturally occurring Mendelian diseases are due to deletions, clearly the value of the natural prevalence used in the risk estimation should be lower, pertaining only to the subset of genes that are responsive to induced deletions;
(b) the estimate for a doubling dose of 1 Sv is based primarily on mouse data for recessive visible mutations at seven loci, which may be more mutable than most genes. Genes that mutate to recessives have been observed to do so at a higher rate than those that mutate to dominants. This suggests that if a doubling dose based on recessives is used for estimating the risk of dominant genetic disease, the risk will be overestimated;
(c) the risk estimation used in the doubling dose method assumes that genes that mutate at high rates spontaneously will also mutate at high frequencies after irradiation. This assumption is open to doubt. A high spontaneous rate depends, among other factors, on the size and sequence of the gene and on the types of mutational mechanisms involved. A high induction rate is more dependent on whether a random change in the gene can give rise to the phenotype being measured [S7].

314. Again, as emphasized above, the growing understanding of non-traditional mechanisms by which genetic diseases may arise (see Section I.D) has introduced another dimension of complexity and may influence estimations of genetic risk, depending on how these mechanisms respond to radiation.

E. SUMMARY

315. Two main methods have been used in animal experiments (primarily mouse) that attempt to quantify genetic risk from exposure to radiation: the doubling dose (also referred to as the indirect) method and the so-called direct method. Both methods involve collecting similar or identical data on radiation exposures and abnormalities in offspring. However, they use different approaches in extrapolating animal data to humans. The terms direct and indirect refer to whether the estimate of damage in the first generation is based directly on phenotypic damage found in mice in first-generation progeny (direct method) or whether it is based on extrapolation back to the first generation from a prediction at genetic equilibrium (indirect method). Although the frequency of harmful effects from induced mutations (from the exposure of a single generation) would be expected to decrease beyond the first generation, owing to negative selection, it should be kept in mind that some of the genetic effects of radiation, e.g. aneuploid segregants from certain translocations, or mutations leading to abnormal imprinting in gametes of first generation offspring, might not manifest until the second or subsequent generations.

316. The doubling dose (or indirect) method of genetic risk estimation expresses risk in relation to the natural incidence of mutations and genetic diseases that are evident at birth in the general population. The doubling dose is the amount of radiation necessary to produce twice as many mutations as would occur spontaneously in the population in a generation. It is obtained by dividing the average rate of spontaneous

mutations at a given set of gene loci by the rate of induction of mutations at the same set of loci. The reciprocal of the doubling dose is the relative mutation risk. A low doubling dose means a high relative mutation risk, and vice versa. This method is generally used to estimate risks under equilibrium conditions. The currently used doubling dose estimate, 1 Sv, for low-dose-rate or chronic exposures to sparsely ionizing radiation such as x rays or gamma rays is based primarily on mouse data on autosomal recessive mutations at seven specific loci and is expected to be at least of the correct order of magnitude. One difficulty of the doubling dose method in estimating the risk of autosomal dominant and X-linked diseases in man is to specify correctly the mutational component, or the component of a given disorder that is due to genetic mutation.

317. In the direct method, the estimated rates of induction of dominant mutations affecting the skeleton or causing cataracts in the eye of the mouse are used to derive estimates of the total risk of dominant genetic disease to the first-generation (F_1) progeny of an exposed human population. Assumptions about the persistence of mutations in the population, however, could permit extrapolating first-generation estimates to later generations. The direct method and the doubling dose method each has its inherent advantages and disadvantages in using the results of animal experiments to calculate the risks in humans of induction of hereditary disorders caused by radiation. It is thus recommended that both methods continue to be used and compared. Caution should be exercised, however, when extrapolating mouse studies to humans: there are notable differences between mice and humans in disease expression, early development, placentation and types of congenital anomalies that are most frequent. There are also strain differences in mice, which are probably comparable to ethnic differences in humans.

318. While people who have been exposed to radiation have been shown to suffer direct effects from exposure, such as increased cancer rates, the data on survivors of the atomic bombings indicate that acute irradiation with moderate doses of ionizing radiation has a negligible adverse effect on the health of the subsequent generation. A number of different indicators, such as untoward pregnancy outcome, cancer in the children of exposed parents and mutations affecting certain protein characteristics, have been used to infer doubling doses. Several types of analyses of seven data sets failed to reveal a statistically significant effect of parental radiation for various indicators. The average combined gonadal dose of acute ionizing radiation received by the proximally exposed parents (0.4 Sv) approximates that which in the past had been estimated to be a genetic doubling dose for mice. The statistical power of these studies is such that the absence of an effect of parental exposure to the atomic bombings on any of the indicators suggests humans may not be as sensitive to the genetic effects of radiation as has for some years been projected on the basis of murine doubling-dose data.

319. The estimate obtained from the studies on the atomic bomb survivors suggests that a doubling dose estimate for humans of between 1.7 and 2.2 Sv for acute irradiation and 4.0 Sv for chronic exposure, should be used. It also suggests that the genes used in the specific locus studies in mice may be more radiosensitive than most genes in humans. Studies of populations living in areas of high background radiation, or exposed to radiation through accidents, support the conclusions from the Japanese studies, i.e. that humans have little risk of hereditary damage from moderately low exposures to ionizing radiation. The potential differences between acute and chronic exposures and between different types of radiation require further study.

IV. FUTURE PERSPECTIVES

A. MOLECULAR INVESTIGATIONS

320. So far, the hereditary effects of radiation have been discussed and assessed mainly at the level of phenotypes. Indeed, changes in phenotypes are of primary interest to society. There are, however, complex problems of methodology in estimating such effects, as discussed in the previous Chapters. Many uncertainties have to be bridged by assumptions and extrapolations. On the other hand, basic research in genetics, and especially in human genetics, is concentrating more and more on the structure of genes themselves and their products. To allow using these new technologies in testing for radiation effects, new approaches have been, or are being, developed at the levels of gene DNA and proteins.

321. In view of the difficulties in assessing mutational effects at the phenotypic and gene-product levels, on the one hand, and recent progress in studying DNA sequences directly, on the other, it is not surprising that the possibility of studying possible radiation effects directly in the DNA has been explored. A number of approaches have been suggested, including the following:

(a) sequence analysis;
(b) analysis of restriction fragment length polymorphisms;
(c) RNase A and gradient denaturation electrophoresis analysis of mutational change;
(d) detection of deletions, insertions and rearrangements by single restriction enzyme digest;
(e) determination of mini-satellites to allow unique identification of particular segments of DNA;
(f) amplification of small amounts of DNA by the polymerase chain reaction;
(g) refined cytogenetic analysis by chromosome *in situ* hybridization (chromosome painting).

322. At first glance, a comparison of the DNA sequences in parents and children would be the most promising approach. Assuming a spontaneous base-pair mutation rate of 10^{-8} and about 6-7 10^9 base pairs in the diploid human genome, there would be 60-70 spontaneous new mutants in a child's genome [T3]. Any statistically significant increase should be attributed to induced mutations. At present, however, sequencing would not be sufficiently precise and would be too time-consuming and expensive to allow carrying out the large number of tests needed to obtain statistically significant results. Further progress in techniques is awaited. The analysis of restriction fragment length polymorphisms is still too time-consuming and expensive to be used for tracing mutations in population studies, although it can be applied to individual cases. Many, if not most, radiation-induced changes of the genetic material are structural chromosomal aberrations, such as deletions, insertions and reciprocal translocations. Larger changes of these kinds can be ascertained by conventional cytogenetic techniques. More recently, however, techniques have been developed in which cytogenetics has been combined with hybridization. The resolution power of such methods, while not approaching that of sequencing DNA base pair by base pair, is much better than that of conventional cytogenetics [N8]. Such methods could also be used for studying human and animal germ cells.

323. Methods of DNA analysis are not yet contributing to risk assessment. Nevertheless, some of the approaches mentioned above, or others, may help one day to improve appreciably the understanding of genetic radiation risks. Even now, however, it may be asked whether and to what degree such studies will further knowledge of possible health hazards. Some of these hazards could adversely affect the health of the current generation, or that of their children and grandchildren. In conjunction with studies of the children of atomic bomb survivors, work is proceeding on such methods of hazard assessment, and lymphoblastoid cell lines are being established for further study.

B. THE HUMAN GENOME PROJECT

324. The human genome (the gene complement of all of the chromosomes) is estimated to comprise approximately 3 10^9 base pairs of DNA and to contain 50,000-100,000 functionally expressed genes [M9]. The Human Genome Project, now in progress, is an international cooperative effort to map and eventually sequence the entire DNA content of the human genome. It is expected that the results will offer clues to the molecular causes and possible treatment of more than 4,000 known genetic diseases, as well as many others with a suspected genetic link [C4]. The project may well revolutionize the approach to human disease and mutation, bringing a shift from estimates based on indirect methods to estimates based on molecular analysis.

325. Both short- and long-term goals have been established for the Human Genome Project. Short-term (five-year) goals include mapping (as opposed to immediately sequencing) the human genome, and mapping and sequencing the genomes of non-human model organisms, including those of bacteria (Escherichia coli), yeast (Saccharomyces cerevisiae), nematode (Caenorhabditis elegans), fruit fly (Drosophila melanogaster) and laboratory mouse (Mus musculus) [U11]. It is felt that mapping will allow scientists to search for specific genes of medical and biological importance, as nearly 95% of the human genome comprises what are presumed to be non-functional sequences [C4]. In the meantime, new sequencing technologies will be developed that will facilitate the eventual sequencing of the entire human genome.

326. For the physical mapping (cytogenetically or molecularly based), one of the initial goals is to assemble a map of sequence tagged sites. These are short sequences of DNA that occur only once in the genome and thus uniquely identify a mapped gene or other marker. The order and spacing of these sequences will allow them to be used as signposts for assigning positions to subsequently mapped genes and will provide a uniform system for reporting data, regardless of the mapping strategy or technique used. Sequence tagged sites will be reported as pairs of oligonucleotide primers that have been tested and shown to produce a polymerase chain reaction product that identifies a single band in a Southern blot with total DNA from a human male [C4].

327. The short-term goal for genetic linkage mapping is to enhance the resolution of present maps that are based on DNA polymorphisms. Over 2,000 of these polymorphisms are already known, and eventually the map should provide an average interval size between adjacent loci of about 2 centimorgans [S36]. The mapping will allow maps of overlapping clones and

panels of genetic markers, along with sequence tagged sites, to be used in positioning a genetic defect, greatly facilitating the identification of genes for specific diseases and malformations.

328. The mapping and sequencing of non-human genomes will allow scientists to work with simpler systems (i.e. smaller genomes) and to manipulate and study the structure and function of mapped genes in the intact organism, which is extremely difficult to do in humans. Because many essential genes are largely conserved over species boundaries, insights gained from animal models can often be applied to the study of human genes [C4]. According to researchers who support the project, the traditional manner of searching for an abnormal gene is unnecessarily costly and wasteful of the scientist's time; it is, moreover, practically untenable for genetically complex defects such as Alzheimer's disease, cancer or schizophrenia [C8]. A positional approach (as opposed to a functional approach) makes it possible to isolate the abnormal genes when the structure and function of the gene product are unknown. It was the positional method that led to the cloning of the genes for cystic fibrosis and neurofibromatosis. It has an even greater advantage for polygenic and multifactorial disorders [C8].

329. The long-term goal of the project is to sequence the entire human genome. The undertaking should be completed in 15 years [W5]. Because the information gained from the mapping and sequencing of genes for human disease, as well as for traits such as sex and intelligence, will have profound social implications, 3%-7% of the budgets for most genome projects have been allocated for studying the ethical, legal and social implications of the information. The purpose of these studies is to anticipate and address implications for individuals and society in areas such as health insurance and prenatal testing and will develop policy options, e.g. in areas where there might be conflict of interest, to ensure that the information is used for the benefit of individuals and society [U11].

330. The Human Genome Project is expected to have at least four corollary benefits [W5]:

(a) it will provide a direct method for analyzing mutation rates, as well as the effects of particular mutagens (including radiation), using human DNA. Thus, risk estimates will be far more accurate than at present, and the various complicating factors will be able to be defined and analysed individually;
(b) new laboratory technologies will be developed to achieve the goals of the project;
(c) new computer technology will be applied to molecular biology, with a concomitant development of new hardware, software and database designs to support and facilitate the massive scale of the undertaking;
(d) the identification and cloning of medically and biologically important genes will, in turn, furnish scientists with material for research on their identity, structure and function for many years to come. From the standpoint of the hereditary effects of radiation, it is expected that once the human genome has been sequenced, a much better understanding of radiation biology, susceptible areas of the human genome and the tracing of suspected damage will be possible.

CONCLUSIONS

331. The present situation in radiation genetic risk estimation can be summarized as follows: (a) current risk estimates for Mendelian diseases appear to be conservative and to provide an adequate margin of safety in radiation protection; and (b) while none of the methods used for risk estimation is free of uncertainties, in the absence of more reliable methods it would not be prudent to abandon any of the approaches or to alter the risk estimates presented in the UNSCEAR 1986 and 1988 Reports [U2, U1]. New data and understanding have served primarily to increase the complexity of the task of risk estimation.

332. Some relatively reliable data are available on the incidence of chromosomal aberrations and on a few rare dominant or X-linked conditions (sentinel mutations) in humans. It is, in principle, possible to determine the background spontaneous incidence of congenital anomalies at birth in a human population. However, to do so requires a sophisticated logistical network that includes, among other things, a precise definition of end-points. To allow comparing data from various countries and different time periods within the same country, international coordination is necessary. The Hungarian registry is a good example of a national screening system.

333. Even with data from such a system at hand, it is not yet possible to predict an increase in the incidence of congenital anomalies attributable to a specific exposure of the gonads to radiation, since such a prediction would require estimating the mutational component of such an increase. An estimation of this com-

ponent would in turn require much more thorough knowledge of the genetic basis of these anomalies and the involvement of environmental factors.

334. Observations on human populations and experiments with animals are improving risk estimation; however, both of these methods require considerable extrapolation: from a very few sentinel mutations to all genetic disease, and from animal to human. It must, therefore, be kept firmly in mind that both the direct and the doubling dose method still provide only rough estimates of risk. Because both methods require extrapolation and estimation, the terms direct and indirect (or doubling dose) are not entirely accurate. It might be more appropriate to refer to the two methods as the absolute method and the relative method, or perhaps as the first-generation method and the equilibrium method.

335. The direct and doubling dose methods have yielded risk estimates that are of a similar order of magnitude; because each method has its advantages and drawbacks, it would seem prudent to continue using both to derive information that is as complete as possible. The estimates obtained should be regarded as lower limits, with the caveat that radiation effects on multifactorial disease, gene regulation and non-traditional forms of inheritance are not well understood and may require different methods of estimation.

336. One purpose of this Annex has been to point out the difficulties that are inherent in any attempt to quantitatively predict the health hazards to future generations caused by exposures to ionizing radiation. Such predictions, based on extrapolations from animal experiments and on the direct observation of exposed population groups, are possible in genetically simple and straightforward situations, such as for cytogenetically visible chromosomal aberrations or rare dominant and X-linked diseases. For all other groups of diseases, such an estimate is not yet possible and will probably remain impossible for some time to come. The studies on the children of atomic bomb survivors at Hiroshima and Nagasaki suggest that the adverse effects on the first generation of progeny from a single moderate radiation dose will probably be only minor. A more specific statement cannot be made at this time.

337. When considering mutations on the molecular level, several points should be kept in mind. The relative frequency of the various molecular changes seen in spontaneous mutation differs from that seen in radiation-induced mutation. It has been noted that most spontaneous mutations tend to be small point mutations, while a majority of radiation-induced mutations are larger DNA deletions. The fact that radiation-induced mutations are likely to differ from spontaneous mutations in the type of mutation produced, frequency and sites affected, and in the disease phenotypes produced, must also be considered and, in fact, expected in any attempt at risk estimation. It should also be kept in mind that almost all work done on the effects of germ-cell irradiation on phenotypic damage in progeny has been done in males; female germ cells may have very different susceptibility to different types of induced mutation at different stages, particularly during *in utero* development of the female. These points offer convincing arguments that a simple, direct correlation between the number of mutations and the degree of mutation damage would be exceedingly difficult, if not impossible, in both animal and human studies.

338. Many newly recognized mechanisms of gene regulation and genetic disease in humans were not known by classical geneticists and thus have not been considered in previous estimates of genetic risk. They could, however, be significantly affected by radiation. Any estimation of risk must understand and consider these additional potential sources of hereditary disease; however the current state of knowledge of non-traditional mechanisms such as genomic imprinting and allelic expansion (see Section I.D) is limited. It may be assumed that these mechanisms could have transgenerational effects and would not necessarily manifest in the F_1 or even the F_2 generation, but there are few data with which to quantify risk.

339. From a purely scientific point of view, then, the problem cannot be defined more precisely at this time, and numerous questions require further examination. Thus, risk estimates can be made only with great uncertainty. It remains impossible to make any responsible statement on the morbidity of genetic effects in middle and advanced age in humans, but a preliminary statement regarding morbidity and mortality in young age might be attempted. Almost the only empirical data available for such an estimate are the data on children of survivors of the atomic bombings at Hiroshima and Nagasaki, since the extensive experimental animal work can only approximate effects in humans.

340. The study of children of the atomic bomb survivors has shown that the long-term monitoring of certain risk groups is possible. However, despite a relatively large sample size and exposure to relatively high radiation doses, the outcome of this study has been largely negative so far; i.e. it has found little or no convincing evidence that radiation influences the incidence of congenital malformations (or other multifactorial conditions). In view of this result, it can hardly be imagined that an even larger risk group would lead to positive findings. Studies on populations living in areas of high natural background radiation in various parts of the world, such as China, Brazil and southern India, have also demonstrated no clear evidence of any genetic risk from exposure to radiation.

341. The situation for multifactorial diseases, which include not only congenital disorders but also disorders with onset at all ages, is still more difficult. Complex systems of intertwined genetic polymorphisms in combination with a great variety of mutations leading to disease in human populations are becoming evident to medical genetic researchers. Estimates of incidence and even prevalence in a population critically depend on parameters such as the definition of disease in general and the delineation of diseases from the range of normal variability of conditions. Moreover, epidemiological research has shown that the incidence and prevalence of many such diseases differ from one population to another, and even within the same population from one time period to another, mainly owing to environmental factors.

342. A series of recommendations can be formulated to assist future attempts at risk estimation. It is first of all recommended that studies in both mice and humans of genetic damage due to radiation should be carried to the molecular level. Information from the Human Genome Project will be especially helpful in this regard. Since it is now possible to determine which chromosomes were inherited from which parent, it can be determined on a molecular level whether a mutation in a child was inherited from the parent with higher exposure to radiation.

343. However, it is likely to be a long time before the findings of the Human Genome Project provide definitive ways of estimating genetic risk from radiation. In the meantime, information is needed on the possibility that radiation induces mutations that cause specific genetic disorders with non-traditional inheritance. If it can be shown that some mechanisms leading to abnormal development differ considerably between mice and humans, experiments must be designed to indicate whether such differences are likely to lead to substantially different radiation risks in the two species. Taking mouse studies to a molecular level would help to make this comparison.

344. In addition, a formal protocol should be established for follow-up studies on heritable effects in the event of accidents involving ionizing radiation; such investigations may yield a great deal of information on human genetic risk that could not be obtained in any other way.

345. Additional information is needed on the induction of phenotypic damage observed in the progeny of irradiated experimental mammals. It would be useful to have data on body systems other than those involving skeletal and cataract mutations. It would be especially valuable if data could be developed that would, in some straightforward way, permit estimates of the risk of inducing dominant mutations that affect predispositions to cancer. More animal experiments are also needed to determine the risk of nondisjunction, Robertsonian translocations, autosomal recessive mutations manifesting in subsequent generations and the role of radiation in upsetting nontraditional mechanisms of disease inheritance, such as genomic imprinting and transposable elements.

346. Several obvious gaps in knowledge exist regarding the induction of dominant phenotypic damage. Until such damage is measured in offspring following the irradiation of oocytes (probably mature and maturing oocytes in mice), the direct method can be applied to women only by assuming that the relationship between the sexes is the same as predicted from specific-locus results. Estimates are needed for the phenotypic damage that results when both males and females are exposed to high-LET radiation.

347. Additional experiments using different strains of mice or other small experimental mammals could indicate the extent to which genetic background determines the overall level of induced dominant genetic damage. It would also be useful to determine the extent of induced phenotypic damage following several successive generations of radiation exposure. Such results could validate the assumptions used in extrapolating from the first-generation direct estimate to later generations.

348. It is known that Mendelian diseases in mammals can be induced by radiation. It seems likely that risk estimation can become more precise as more human genes are sequenced, mutation spectra analysed and mechanisms unravelled. It is therefore essential that scientists making genetic risk estimates keep abreast of progress in molecular biology.

349. New mouse *in vivo* test systems should be designed, including tests for dominant mutations, which take into account the homologies between the human and mouse genomes. Molecular studies with somatic cell mutations, both spontaneous and induced, will extend the knowledge of mutation spectra and mechanisms.

350. In well-studied experimental systems, most radiation-induced mutations are recessive and are due to DNA deletions. If human germ cell responses are similar, such mutations will accumulate in the gene pool. Given the very low levels of inbreeding in present-day human populations, homozygosity for induced recessives may occur only rarely. Thus it would be useful to have assessments, instead, of the overall adverse health effects in heterozygote carriers of induced recessive mutations. In humans, a useful starting point would be to compare the health of normal individuals with that of obligate heterozygotes for known recessive mutations.

Table 1
Incidence of genetic or partially genetic diseases having serious health consequences before the age of 25 years [a]
[B3]

Category	*Rate per million live births*	*Percentage of total births*
Part A: Serious diseases with known genetic aetiology		
Dominant	1,395	0.14
Recessive	1,655	0.17
X-linked	532	0.05
Chromosomal	1,845	0.18
Multifactorial	46,583	4.64
Genetic unknown	1,164	0.12
Total	53,175	5.32
Part B: Serious congenital anomalies		
All congenital anomalies (ICDA codes 740-759)	52,808	5.28
Congenital anomalies with genetic aetiology (included in Part A above)	26,584	2.66
Part C: Serious genetic diseases and congenital anomalies		
Disorders in Part A (above) and congenital anomalies not included	79,392	7.94

[a] Based on the British Columbia Health Surveillance Registry in a study of 1,169,873 births from 1952 to 1983.

Table 2
Examples of molecular mechanisms that cause spontaneous deletions and duplications
[S7]

Mechanism	*Example*
Replication slippage in tandem repeats	Some beta-globin structural variants Some mutations in factor VII and factor IX genes
Unequal homologous recombination-related genes	Most alpha-thalassemias, anomalous trichromacy
Unequal homologous recombination between Alu repeats	Some alpha-globin gene deletions Low-density lipoprotein receptor gene deletions and duplications Fabry disease, steroid sulphatase deficiency
Nonhomologous recombination with one breakpoint in or near Alu repeats	Some deletions in alpha, beta and complex thalassemias Lipoprotein lipase deficiency
Unequal sister chromatid exchange	Some dystrophin gene duplications
Gene conversion involving a functional gene and a pseudogene	21-hydroxylase deficiency (adrenal hyperplasia)

Table 3
Genetic risk estimates for serious effects in humans from 0.01 Gy of low-LET radiation from application of the direct method to mouse data

Basis of estimate	*Expected frequency in the first generation (Number per million live births)*	
	From exposure of males	*From exposure of females*
Phenotypic changes in mice living 3 weeks or longer dying between birth and 3 weeks of age	 10-20 [a] 5-10 [b]	 0-9 [c] 0-5 [c]
Total, each sex	15-30	0-14
Total, both sexes	15-44 [d]	

[a] Lower estimate based on induced cataracts; multiplication factor 36.8. The data, collected at high dose rates, have been corrected based on specific locus results.
[b] Based on many experiments on the amount of induced death and on two experiments that indicate that part of total occurring after birth.
[c] Data unavailable. Estimate assumed to be 44% of result with irradiated males, an upper bound suggested by specific-locus experiments. Because of qualitative differences in mutations between males and females, there is additional uncertainty in this risk estimate.
[d] Includes risks for all types of inheritance, including translocations, unbalanced products of reciprocal translocations and reciprocal translocations that act like dominant mutations.

Table 4
Estimates of minimal gametic doubling doses from analysis of end-points of genetic effects in survivors of the atomic bombings
[N5]

Genetic effect	*Observed total background incidence*	*Estimated mutational contribution to background incidence* [a]	*Mutational component* [b] *(%)*	*Regression parameters*	*Doubling dose (Sv)* [c] *at lower confidence limit of*		
					99%	*95%*	*90%*
Untoward pregnancy outcome	0.0502	0.0017-0.0027	3.4-5.4	β: 0.0026±0.0028 α: 0.039±0.0058	0.14-0.23	0.18-0.29	0.21-0.33
F_1 mortality	0.0458	0.0016-0.0026	3.5-5.7	β: 0.00076±0.0015 α: 0.063±0.0018	0.51-0.83	0.68-1.10	0.81-1.32
F_1 cancer	0.0012	0.00002-0.00005	2.0-4.0	β:-0.00008±0.00028 α: 0.0010±0.00033	0.04-0.07	0.05-0.11	0.07-0.15
Sex-chromosome aneuploids	0.0030 [d]	0.0030	100	β: 0.00044±0.00069 α: 0.0025±0.00043	1.23	1.60	1.91
Loci encoding for proteins	0.000013 [d]	0.000013	100	β:-0.00001±0.00001 α: 0.00001±0.00001	0.99	2.27	7.41

[a] Per diploid locus.
[b] Equal to mutational contribution divided by observed total background incidence (× 100 for %).
[c] The doubling dose is equal to α/β (equivalent to the reciprocal of the excess relative risk per sievert). The minimal doubling dose is the reciprocal of : β/α + the normal derivate at the desired probability level times the square root of the variance of β/α.
[d] Observed zygotic mutation rates.

Table 5
Incidence of genetic disease and risk estimates in humans from 0.01 Gy of low-LET radiation from application of the indirect method

Genetic disease	*Incidence per million live births*		*Effect of 0.01 Gy per generation per million live births*				
			UNSCEAR [U1]			*BEIR V [C1]*	
	UNSCEAR [U1]	*BEIR V [C1]*	*First generation*	*Second generation*	*Equilibrium*	*First generation*	*Equilibrium*
Autosomal dominant	10,000		15	13	100		
Clinically severe		2,500				5-20	25
Clinically mild		7,500				1-15	75
X-linked		400				<1	<5
Autosomal recessive	2,500	2,500	0.05	0.05	15	<1	Very slow increase
Chromosomal							
Structural anomalies	400	600	2.4 [a]	1 [a]	4 [a]	<5	Very little increase
Numerical anomalies	3,400	3,800				<1	<1
Congenital anomalies	60,000	20,000-30,000	Not estimated			10	10-100
Multifactorial diseases	600,000		Not estimated				
Heart disease		600,000				Not estimated	
Cancer		300,000				Not estimated	
Selected other		300,000				Not estimated	
Total			17	14	120		

[a] Probably very small.

Table 6
Estimates of gametic doubling doses for acute, high-dose irradiation of spermatogonia derived from specific-locus, specific-phenotype systems in the mouse
[N7]

System	*Origin of treated males*	*Doubling dose (Gy)*	*Reference*	
			Data summarized in	*Calculated by*
Russell seven-locus	101 x C3H	0.44	[E5, S12]	[N7]
Dominant visibles	Various	0.16	[L6]	[L6]
Dominant cataract	101/E1 x C3H/E1	1.57	[F3]	[F3]
Skeletal malformations	101	0.26	[E1]	[L6]
Histocompatibility loci	C57B1/6JN	>2.60	[B1]	[B1]
Recessive lethals	DBA	0.51	[S40]	[L6]
	C3H/HeH x 101/H	0.80, 1.77	[L8]	[B1]
	DBA, C3H	4.00	[L9]	[B1]
Loci encoding for proteins	Various	0.11	[N7]	[N7]
Recessive visibles	C3H/HeH x 101/H	3.89	[L8]	[N7]

Table 7
Correction factors used with the direct method to obtain estimates of risk of dominant genetic disease in humans [S6]

Step	*Quantity or correction factor*	*Correction procedure*	*Result*
	Skeletal mutations		
1A	Mutation frequency (1 + 5 Gy; 24-h fractionation, γ rays)	37 + 2646	$1.4\ 10^{-2}$
1B	Mutation rate (per 0.01 Gy)	Divide (1A) by 600	$2.3\ 10^{-5}$
1C	Correction for dose fractionation and dose-rate effects [a]	Multiply (1B) by 1/1.9 and 1/3	$4.0\ 10^{-6}$
1D	Extrapolation from skeletal effects to all dominants (proportionality correction factor) [b]	Multiply (1C) by 10	$40\ 10^{-6}$
1E	Correction for severity	Divide (1D) by 2	$20\ 10^{-6}$
	Risk of dominant genetic disease to the first-generation progeny per 0.01 Gy of paternal exposure		$20\ 10^{-6}$
	Cataract mutations		
2A	Mutation frequency (4.55 + 4.55 Gy; 24-h fractionation; γ rays)	6 + 5,231	$1.15\ 10^{-3}$
2B	Mutation rate (per 0.01 Gy)	Divide (2A) by 910	$1.26\ 10^{-6}$
2C	Correction for dose fractionation and dose-rate effects [c]	Multiply (2B) by 1/1.2 and 1/3	$3.5\ 10^{-7}$
3A	Mutation frequency (5.34 Gy acute γ rays)	3 + 10,212	$0.29\ 10^{-3}$
3B	Mutation rate (per 0.01 Gy)	Divide (3A) by 534	$5.5\ 10^{-7}$
3C	Correction for dose-rate effect	Multiply (3B) by 1/3	$1.8\ 10^{-7}$
4A	Mutation frequency (6 Gy, acute γ rays)	3 + 11,095	$0.27\ 10^{-3}$
4B	Mutation rate (per 0.01 Gy)	Divide (4A) by 600	$4.5\ 10^{-7}$
4C	Correction for dose-rate effect	Multiply (4B) by 1/3	$1.5\ 10^{-7}$
5	Average of (2C), (3C) and (4C) weighted by the number of mutants		$2.6\ 10^{-7}$
6	Extrapolation from dominant cataracts to all dominants [d]	Multiply (5) by 36.8	$-10\ 10^{-6}$
	Risk of dominant genetic disease to the first-generation progeny per 0.01 Gy of paternal exposure		$-10\ 10^{-6}$

[a] The correction factors are based on specific-locus experiments carried out at Oak Ridge.

[b] Based on the McKusick catalogue of autosomal phenotypes, 1975 edition [M7]; at that time, it was estimated that about 74 out of 328 clinically important autosomal dominant conditions in man involved one or more parts of the skeleton (about 20%); however, since skeletal defects are more easily diagnosed than those of other organ systems, the true figure was assumed to be about 10%.

[c] The correction for dose-fractionation effects (1/1.2) is based on concurrent specific-locus studies in Neuherberg.

[d] Based on the McKusick catalogue of autosomal phenotypes, 1978 edition [M7]; at that time, it was estimated that 20 out of 736 of all known and proven dominant mutations (2.7%) were associated with one or another form of cataract in man; recent analysis by Favor [F3], based on the McKusick catalogue of autosomal phenotypes, 1986 edition [M7], shows that these numbers are, respectively, 28 and 1,172, i.e. 2.4% of known dominant mutations are associated with cataracts. The multiplication factor is therefore 41.

Glossary

allele	an alternative form of a gene at a given locus. Being diploid organisms, humans may have two alleles at a given locus, i.e. a normal and a mutant allele. Abbreviation of allelomorph
allelic association	the association of two alleles at distinct loci beyond chance expectation. Normally a consequence of close linkage: loci within a megabase usually show some allelic association
allelic disorders	disorders, which may be phenotypically different, that are due to mutations in the same gene
alu repetitive sequence	repetitive sequence found about 500,000 times in human genome. The sequence contains a recognition site for the restriction enzyme AluI and is around 300 base pairs in length.
amplification	an increase in the number of copies of a particular DNA fragment. Can occur under natural circumstances, e.g. amplification of a repeat sequence, as in fragile-X syndrome, or during laboratory procedures such as cloning or polymerase chain reaction
aneuploid	a chromosome number that is not an exact multiple of the haploid number; an individual with an aneuploid chromosome number. Usually refers to an absence (monosomy) or an extra copy (trisomy) of a single chromosome
annealing	see hybridization
anticipation	phenomenon in which the severity of a genetic condition appears to become more severe and/or arise at an earlier age with subsequent generations
antisense strand (of DNA)	the non-coding strand of the DNA double helix that serves as the template for mRNA synthesis
association	the occurrence of an allele with a disease more often than chance should allow
autosome	any chromosome other than a sex chromosome. Men have 22 pairs of autosomes and an X- and a Y-chromosome; women have the same autosome pairs and two X-chromosomes. In the Paris convention, written 46XY and 46XX
bacteriophage	see phage. Bacterial virus used as a vector for cloning segments of DNA
band	a chromosomal segment defined by distinct staining. Both lighter and darker segments are called bands and are numbered from the centromere outwards, with smaller bands classified by a second number. Bands 11, 12, 13, 21, 22, 31 could be a continuous series.
base pair (bp)	in the DNA double helix, a purine and pyrimidine base on each strand that interact with each other through hydrogen bonding. The number of base pairs is often used as a measure of the length of a DNA segment, e.g. 500 bp.
base sequence	the order of nucleotide bases in a DNA molecule. Length is usually defined in base pairs.
blastomere	one of the cells produced by cleavage of a fertilized ovum, forming the blastoderm
breakpoint	refers to sites of breakage when chromosomes break (and recombine)
carrier	an unaffected individual who is heterozygous at a particular locus for a normal gene and an abnormal gene which, although it may be detectable by laboratory tests, is not expressed phenotypically. Variously used to cover both permanent non-expression in recessives and X-linked recessives and temporary non-expression in dominants (e.g. Huntington's chorea). More recently used to describe unaffected individuals who carry unstable or dynamic mutations that can expand and cause a genetic condition in offspring
cDNA	complementary DNA. The synthetic DNA equivalent of messenger RNA (mRNA) with a sequence complementary to the DNA strand from which it is derived
cDNA library	a collection of clones containing inserts of overlapping cDNA fragments representing expressed sequences (mRNA). cDNA libraries differ from one tissue or cell type to another.

centimorgan (cM)	the unit of genetic distance defined as the length of a segment of chromosome which has a 1% chance of recombining at meiosis. See also recombination percentage. Equivalent segments of chromosomes usually recombine more frequently at oogenesis than at spermatogenesis. Because even numbers of recombinant events between two strands cancel out, the recombination percentage is always less than the genetic distance and can never exceed 50%. The percentage recombination and the genetic distance in centimorgans are very similar when linkage is close (i.e. less than 10%).
centromere	the part of the chromosome by which it is moved at cell division and which separates it into two arms, appearing as a distinct "waist" on microscopy. Point of spindle attachment to the chromosome during meiosis and mitosis
chimaera	an organism compounded from two or more zygotes. A mosaic is formed from variant cells derived from the same zygote.
chiasma	the crossing of chromatid strands of homologous chromosomes during meiosis
chorionic villus sampling	procedure used to obtain fetal cells for prenatal diagnosis; involves biopsy of the placental membranes. Now usually done transabdominally from 8 weeks of pregnancy
chromatid	during mitosis each chromosome replicates into two DNA strands called chromatids. At meiosis recombination is due to chiasmata between non-identical pairs of chromatids.
chromatin	the composite of DNA and proteins that comprises chromosomes
chromosome	thread-like, deep-staining bodies situated in the nucleus. They are composed of DNA and protein and carry the genetic information.
cis	on the same chromosome, usually quite close. The opposite of trans, which relates to the other homologue. Cis effects are due to physical action between segments of the same DNA strand; trans effects are due to diffusion. Historically implies on the same chromosome. In molecular biology refers to an effect on a gene directed by the sequence of that gene or very close to it on the same chromosome (in contrast to trans effects, which are produced by other factors, such as the transcription factors encoded by other genes). The terms are commonly used to describe factors that influence gene expression.
cleavage	mitotic segmentation of the fertilized ovum, the size of the zygote remaining unchanged and the cleavage cells, or blastomeres, becoming smaller and smaller with each division
clone	a group of individual organisms or cells derived from a single individual by asexual reproduction
cloning	production of genetically identical cells (clones) from a single ancestral cell; cloning is utilized in molecular biology to propagate single or discrete DNA fragments of interest.
coding sequence	those parts of the gene from which the genetic code is "translated" into amino acid sequences of a protein
co-dominant	when both alleles are expressed in the heterozygote
codon	a group of three adjacent nucleotides that codes for particular amino acids or for the initiation or termination of the amino acid chain
codon usage	given the degeneracy of the genetic code, refers to the preference of codons used to specify particular amino acids. Often differs among species and among different genes and proteins
complementary	two nucleotide sequences are complementary when they can form a perfect double helix because they have a mirror-image relationship
compound heterozygote	an individual who has different mutant alleles at a given locus
congenital	existing at, and usually before, birth; referring to conditions present at birth, regardless of their causation
consanguinity	relationship by descent from a common ancestor; a consanguineous mating is between individuals who have one or more common ancestors. As all individuals have common ancestors it is usually restricted to couples with a common pair of grandparents, e.g. first cousins.

consensus sequence	a minimum nucleotide sequence found to be common (although not necessarily identical) in different genes and in genes from different organisms that is associated with a specific function. Examples include binding sites for transcription factors and splicing machinery.
conserved sequence	base sequence in a DNA molecule (or an amino acid sequence in a protein) that has remained essentially unchanged throughout evolution
contiguous gene syndrome	syndrome due to abnormalities of two or more genes that map next to each other on a chromosome; most often caused by a deletion that involves several contiguous genes
contig map	genetic map showing the order of (contiguous) DNA fragments in the genome
cosmid	a cloning vector derived from a natural bacterial parasite capable of accommodating up to 40 Kb of DNA (see plasmid)
coupling	when alleles from two loci are known to be on the same chromosome; the opposite of repulsion. Also, all alleles derived from one parent
crossing-over	the exchange of segment of a chromosome in meiosis. Small chromosomes usually have a single chiasma, so that of the four chromosomes entering gametes two are hybrid and two unchanged, e.g. if the parental chromosomes are ABCDE and abcde, the gametes could be ABCDE, ABcde, abCDE and abcde. The middle two are recombinant chromosomes with a crossover between loci B and C.
DNA	deoxyribonucleic acid. The long double-stranded molecule whose sequence of the four possible nucleotide bases provides the genetic information. The strands are held together by hydrogen bonds between nitrogenous bases that constitute the code: adenine (A) and thymine (T) which pair with each other, and guanine (G) and cytosine (C), which pair with each other.
DNA marker	a DNA sequence variation that is easily detectable; examples include restriction fragment length polymorphisms and dinucleotide and trinucleotide repeat polymorphisms.
DNA methylation	attachment of methyl groups to DNA, most commonly at cytosine residues. May be involved in regulation of gene expression
DNA polymerase	enzyme responsible for replication of DNA
DNA sequence	the relative order of base pairs
degeneracy	(of the genetic code) different codons code for the same amino acid
deletion	loss of a portion of a gene or chromosome; a type of mutation; a synonym of deficiency
diploid	containing two chromosome sets. The normal condition of most human cells except gametes; megakaryocytes, Purkinje cells and a few others have multiple sets.
dizygotic	twins derived from two distinct zygotes
domain	a discrete portion of a protein (and corresponding segment of gene) with its own function. A protein may have several different domains and the same domain may be found in different proteins.
dominant	a trait that is expressed in the heterozygote, sometimes only late in life
dominant mutations	mutations that produce an abnormal clinical phenotype (disorder or trait) when present in the heterozygous state
dominant negative mutations	heterozygous mutations in which the product of the mutant allele interferes with the function of the product normal allele
doubling dose	the dose of radiation that, under a given set of conditions, will lead to an overall mutation frequency that is double the spontaneous frequency
downstream	a DNA sequence is written from the left, or 5', direction or to the right, or 3' direction. Downstream refers to the 3' direction, i.e. the stop codon for a gene is downstream (3') of the coding sequences of that gene.
dysmorphology	study of abnormalities of morphologic development
electrophoresis	an analytical method used to separate nucleic acid, peptide or protein fragments based on size and charge of the molecule; typically smaller fragments travel further through the media (gel) in which separation is carried out.

enchromatin	darkly stained chromatin
enhancers	DNA sequences that increase transcription of a nearby gene; they can act in either orientation, may be either 5' or 3' to the gene or within an intron.
euchromatin	the chromatin that is thought to contain active or potentially active genes. Light (vs. dark) bands on G-banding
exon	a region of a gene containing a coding sequence. Most genes have several exons separated by introns, which are usually longer.
expressivity	the extent to which a genetic defect is expressed
F_1, F_2 etc.	the first (F_1) or second (F_2) generation of progeny of a mating
founder effect	a genetic effect due to the establishment of a new population by a few original founders who carry only a small fraction of the total genetic variation of the original population, with the consequence that some mutant alleles may reach unusually high frequencies in the new population. [Examples: the 2,000 Dutch settlers in South Africa in the 17th and 18th centuries, who did not marry outside the small ethnic group, eventually giving rise to a population of about 3 million. Frequency of familial hypercholesterolemia (FH) heterozygotes: 1/85 to 1/100 and 95% of mutations accounted for by only three alleles. Likewise, the current population of French Canadians of 5.8 million descended from 7,000 French settlers between 1608 and 1763. One familial hypercholesterolemia mutation accounts for about 60% of the heterozygotes in this group.]
frameshift mutation	a mutation that alters the normal triplet reading frame so that codons downstream from the mutation are out of register and not read properly
fragile site	gap or defect noted in the continuity of a chromosome when stained, e.g. fragile-X site. Many are apparent only when cells are cultured under special conditions.
gamete	mature reproductive cell (sperm or ovum); contains a haploid set of chromosomes (23 for humans)
gene	the unit responsible for transmitting an inherited character; the region of DNA that specifies the synthesis of a protein
gene targeting	artificial modification of a gene in a specific and directed fashion. Typically refers to substituting one DNA sequence for another to inactivate a gene or introduce or correct a mutation in a gene
genetic locus	a specific position or location in the genome
genetic fingerprint	a pattern of restriction fragments detected by probes that recognizes alleles at highly polymorphic loci; this is effectively unique to all individuals except identical twins.
genetic marker	an allele used in following the inheritance pattern of loci in cell lines, pedigrees or populations
genetic distance	the functional distance between two loci defined through recombination; it is measured in centimorgans; for small values (<10%) it is approximately equal to the recombination percentage.
genetic drift	the tendency for variations to occur in the genetic composition of small isolated inbreeding populations by chance. Such populations become genetically different from the original population from which they were derived.
genome	the complete genetic composition of an individual's chromosome; the complete set of genes characteristic of a species
genome DNA	DNA from a genome containing all coding (exon) and non-coding (intron and other) sequences, in contrast to cDNA, which contains only coding sequences
genomic library	a collection of clones containing DNA inserts of overlapping DNA fragments representing the entire genome of an organism
genotype	the alleles present in an individual at a locus or loci under consideration
germ cell	see gamete
germ-line mosaicism	presence of two or more cell lines in the gonadal cells. Implies risk of transmission of mutations present in the gonads to offspring

gonadal mosaicism	see germ-line mosaicism
haploid	containing one chromosome set as found in gametes after meiosis. The normal condition for gametes. The human haploid number is 23, half the diploid number of 46.
hemizygous	the condition of cells with respect to genes when only one set is present, as for genes on the X-chromosome in the male
heterochromatin	chromatin composed of repetitive DNA; stains as dark (versus light) bands in G-banding
heterozygote	an individual with two different alleles at a particular locus (adj. heterozygous)
histones	proteins associated with DNA in chromosomes
homeobox domain	a short DNA sequence common to a group of DNA binding proteins involved in pattern formation in early embryogenesis
homologies	similarities found in DNA or protein sequences when individuals of the same or different species are compared
homologous	matched. The other of a pair of chromosomes
homologous chromosomes	chromosomes containing the same linear gene sequences. In a normal mating, 1 of a pair of homologous chromosomes is derived from each parent. Humans normally have 22 pairs of homologous chromosomes and 2 X-chromosomes or 1 X- and 1 Y-chromosome.
homologous recombination	substitution of a segment of DNA by another that is identical (homologous) or nearly so. Occurs naturally during meiotic recombination; also used in the laboratory for gene targeting to modify the sequence of a gene
housekeeping genes	genes that encode proteins necessary for basic cellular functions. They are expressed in virtually all cells.
human gene therapy	insertion of normal DNA directly into cells to correct a genetic defect
hybridization (annealing)	the artificial conjunction of two complementary DNA strands, one of which usually carries a radioactive marker. Also used for the production of cells containing chromosomes from more than one species
imprinting	phenomenon in which an allele at a given locus is altered or inactivated depending on whether it is inherited from the mother or the father. Implies a functional difference in genetic information depending on whether it is inherited from the father or the mother
in situ hybridization	use of a nucleic acid probe to detect the presence of a DNA sequence in chromosome spreads or in interphase nuclei or of an RNA sequence in cells. It is used to map gene sequences to chromosomal sites and to detect gene expression.
insert	in molecular genetics, refers to DNA sequence of interest that has been inserted into a cloning vector such as a plasmid or bacteriophage
insertion	type of mutation in which a DNA sequence of variable length is inserted into a gene disrupting the normal structure of that gene
intron (intervening sequences)	the DNA sequences that interrupt the protein-coding sequences of a gene. The region of a gene that separates exons or coding sequences. They are removed during processing of mRNA. Introns may contain sequences involved in regulating expression of a gene.
karyotype	the chromosome set; the number, size and shape of the chromosomes of a somatic cell may be displayed diagrammatically as an idiogram.
kilobase (kb)	a thousand bases. A common unit for specifying the size of genes and physical distances along a DNA region
library	collection of clones in which overlapping genomic or cDNA fragments have been inserted into a particular cloning vector
linkage	the non-independent meiotic segregation of alleles at different loci, which is usually because the loci concerned are all on the same chromosome, and only separable by recombination. Linked loci are within measurable genetic distance of one another on the same chromosome, or are members of the same linkage group, e.g. on the same chromosome. Distant loci on the same chromosome may show independent segregation and now show linkage. They are then described as syntonic.

linkage disequilibrium	see allelic association
locus	the position on a chromosome. Usually that of a gene, but may refer to a DNA marker
lod score	a statistical method used to determine if a set of linkage data indicates two loci are linked or unlinked. A lod (log of odds ratio) score of +3 (1,000:1 odds) is commonly accepted to indicate that linkage exists, and a score of -2 (100:1 odds against) excludes linkage.
mapping	the process of determining the location of a gene by either direct observation or family study
marker	a detectable physical location on a chromosome. It can be a restriction enzyme cutting site, a gene, or a di- or trinucleotide repeat polymorphism whose presence and inheritance can be monitored.
maternal inheritance	inheritance pattern displayed by mitochondrial genes that are propagated from one generation to the next through the mothers; the mitochondria of the zygote comes almost entirely from the ovum.
megabase (Mb)	one million base pairs of DNA sequence roughly equal to 1 cM of genetic distance
Mendelian	a trait obeying Mendel's first law of independent segregation of the alleles at the same locus conveyed by each parent
meiosis	the type of cell division that occurs during gamete formation and results in the halving of the diploid somatic number of chromosomes so that each gamete is haploid and contains one of each chromosome pair. These post-meiotic chromosomes are usually partly paternal and partly maternal in origin.
messenger RNA (mRNA)	processed RNA that serves as a template for protein synthesis or for synthesis of cDNA
microsatellite	highly polymorphic DNA marker comprised of mononucleotides, dinucleotides, trinucleotides or tetranucleotides that are repeated in tandem arrays and distributed throughout the genomes. The best studies are the CA (alternatively GT) dinucleotide repeats. They are used for genetic mapping.
minisatellites	highly polymorphic DNA markers comprised of a variable number of tandem repeats that tend to cluster near the telomeric ends of chromosomes. The repeats often contain a repeat of 10 nucleotides. They are used for genetic mapping.
missense mutation	mutation that causes one amino acid to be substituted for another
mitochondrial (mt) DNA	DNA distinct from nuclear DNA in that it is mostly unique sequence DNA and codes for proteins that reside in mitochondria
mitosis	the type of cell division that occurs in somatic cells
monogenic	a synonym of Mendelian, i.e. governed by only one gene
monozygotic	twins derived from a single zygote
morphogenesis	evolution and development of form, as the development of the shape of a particular organ or part of the body
mosaicism	an individual with substantial proportions of two or more cell lines derived from a single zygote
motif	three-dimensional structure of gene product (protein) with known or implied function, i.e. DNA binding, traverse membrane etc. Often inferred from cDNA sequence
multifactorial	refers to the type of inheritance determined by many factors including both genes and the environment. If these are assumed additive, estimates of heritability may be made. In Mendelian and infective disorders a single factor will have a deciding role in manifestation, although not necessarily in severity or the potential for prevention or treatment. See also polygenic
mutation	a permanent and heritable change in genetic material (includes point mutations, deletions and changes in number or structure of chromosomes)
mutation frequency	number of mutations observed divided by number of progeny or cells examined
non-disjunction	failure of two members of a chromosome pair to disjoin (separate) during cell division
nonsense mutation	mutation that changes a codon for an amino acid to a termination or stop codon and leads to premature termination of translation

nucleosome	the basic structural unit of chromatin, in which DNA is wrapped around a core of histone molecules
nucleotide	a purine or pyrimidine base to which a sugar (ribose or deoxyribose) and 1, 2 or 3 phosphate groups are attached
nucleus	the organelle in eukaryotic cells that contains the genetic material
oligonucleotide	a short piece of DNA, typically 5-50 nucleotides
oncogene	a gene, one or more forms of which is associated with cancer. Many oncogenes are involved, directly or indirectly, in controlling the rate of cell growth.
open reading frame	a stretch of DNA following an initiation codon that does not contain a stop codon. Open reading frames in a nucleotide sequence suggest an exon and therefore a gene.
Paris convention	the notation system in which the karyotype is defined by the number of chromosomes followed by the sex chromosomes and information, if any, on an abnormality, e.g. 46XY, 47XHY (+21); 45XO; 47XXY. The position on a chromosome is defined by p and q (petit and queue) for the short and long arm and then by numbers defining bands and sub-bands, which are numbered outwards from the centromere. Usually there are 2-4 major bands and 2-5 minor bands, the term band covering both deeply and lightly staining segments.
pedigree	a diagrammatic representation of a family history
penetrance	the frequency of expression of a trait or genotype. The proportion of individuals observed to show a particular phenotypic effect of a mutant gene compared with the number expected on the basis of Mendelian inheritance
phage	a virus that infects bacteria and is a useful cloning vector for medium size pieces of DNA between 5 and 25 kb
phenocopy	an environmentally induced mimic of a genetic disorder
phenotype	the appearance (physical, biochemical and physiological) of an individual that results from the interaction of environment and genotype. Often used to define the consequences of a particular mutation
physical map	a map of physical landmarks on a DNA fragment or chromosome measured in base pairs. Landmarks include restriction endocnucleose recognition sites, DNA sequence and chromosomal bands.
plasmid	extrachromosomal small circular DNA molecule capable of autonomous replication within a bacterium. Commonly used as a cloning vector for small pieces of DNA, typically 50-5,000 bases
poly A RNA	RNA transcript that contains a tail of poly A residues at its 3' end; implies that an RNA sequence is mRNA. The poly A residues serve as stop signals to terminate transcription.
polyamines	compounds with many amino groups that are associated in the cell with nucleic acids
polygenic	inheritance determined by many genes at different loci, each with small additive effects. A simple example is height within either sex. See also multifactorial
polymerase	see DNA polymerase, RNA polymerase
polymerase chain reaction (PCR)	a method to amplify a DNA sequence using a heat-stable polymerase and two sets of primers that define the sequence to be amplified. Several variations have been developed for specific needs. May be combined with reverse transcription of mRNA to cDNA to amplify an mRNA, so-called RT-PCR
polymorphism	the occurrence in a population of two or more genetically determined forms in such frequencies that the rarest of them could not be maintained by mutation alone. Used in various distinct senses, especially in RFLPs where it is used to imply alternative forms. Usually implies commonest allele is less than 99% so that over 2% of individuals are heterozygous.
polyploid	an abnormal chromosomal complement that exceeds the diploid number and is an exact multiple of the haploid number

positional cloning	strategy for identifying and cloning a gene based on its location in the genome rather than on the biologic function of its product. Usually involves linking the gene locus of interest to one that has already been mapped
pre-mutation	a permanent and heritable change in a gene that does not have phenotypic consequences (does not cause disease) but predisposes to a "full" mutation that may
primary transcript	the initial RNA transcript of a gene, before processing to mRNA; it contains introns as well as exons.
primer	short polynucleotide chain that anneals to a nucleic acid template and promotes copying of the template from the primer site
proband	a synonym of propositus or proposita. The affected individual who brings the family to medical attention
probe	single-stranded DNA or RNA molecule of specific base sequence, labelled either radioactively or by other means, that is used to detect a complementary base sequence by hybridization. A labelled fragment of DNA (usually labelled with a radioactive isotope) used to identify a complementary sequence
promoter	a sequence on a gene that is upstream (5') to coding sequences to which RNA polymerase binds and initiates transcription of a gene
protein	a large molecule composed of one or more chains of amino acids in a specific sequence; the sequence is determined by the sequences of nucleotides in the gene coding for the protein. Proteins are required for the structure, function and regulation of the body's cells, tissues and organs, and each protein has unique functions. Examples are hormones, enzymes and antibodies.
pseudogene	sequence of DNA that is very similar to a normal gene but has been altered slightly so that it is not expressed
RNA	ribonucleic acid, the nucleic acid found mainly in cytoplasm. Messenger RNA (mRNA) transfers genetic information from the nucleus to the ribosomes in the cytoplasm and acts as a template for the synthesis of polypeptides; transfer RNA (tRNA) transfers activated amino acids from the cytoplasm to messenger RNA; ribosomal RNA (rRNA) is a component of the ribosomes that function as the site of polypeptide synthesis.
reading frame	register in which translation machinery reads the genetic triplicate code
recessive	a trait that is expressed in individuals who are homozygous for a particular allele
recessive mutations	mutations that produce an abnormal clinical phenotype when present in the homozygous or hemizygous state. Heterozygosity for the mutation, i.e. carrier state, may often be detected in persons whose clinical phenotype is normal.
recombinant DNA	DNA that is artificially transferred from the genome of one organism to that of another
recombinant DNA molecules	DNA molecules of different origins that are combined and manipulated in the laboratory
recombinant DNA technologies	laboratory procedures used to manipulate DNA fragments, e.g. cut, modify and ligate, and introduce them into an organism so that their number can be amplified as the organism replicates, i.e. cloning
recombination	the formation of a new combinations of linked genes by crossing-over between their loci during meiosis
recombination percentage	equivalent segments usually recombine more frequently at oogenesis than at spermatogenesis. Because even numbers of cut-and-join events between two strands cancel out, the recombination percentage, often termed theta, is always less than the genetic distance and can never exceed 50%. They are almost the same at less than 10%, which is just over 10 cM.
repulsion	when specific alleles at two different loci are derived from different parents. The opposite of coupling
restriction enzyme	bacterial-derived enzyme that recognizes a specific, short nucleotide sequence and cuts DNA at that site
restriction fragments	DNA fragments that result from digestion of DNA with restriction enzymes

restriction endonuclease	a group of enzymes each of which cleaves DNA at specific base sequences (recognition site)
restriction map	a map of a DNA sequence with restriction enzyme recognition sites serving as landmarks
restriction site	shortened term for restriction endonuclease recognition sequence
retrovirus	RNA viruses that encode the enzyme reverse transcriptase so that their RNA can be transcribed into DNA in the host cell; modified retroviruses are used as vectors to introduce genes (or portions thereof) of interest into eukaryotic cells.
reverse transcriptase	an enzyme that catalyses the synthesis of DNA from an RNA template (and thus can also make cDNA from mRNA)
RFLP	restriction fragment length polymorphism. The occurrence of two or more alleles in a population differing in the lengths of fragments produced by a restriction endonuclease
RNA polymerase	enzyme that synthesizes (transcribes) RNA from a DNA template
RNA splicing	process by which introns are removed from primary RNA transcripts, leaving only exons that encode the amino acid sequence of a protein
segregation	separation of alleles at meiosis
sequencing	determination of the order of nucleotides in a DNA or RNA fragment, or the order of amino acids in a protein
sequencing gel analysis	electrophoretic technique by which nucleotide size differences as little as a single base pair can be discerned
sequence-tagged sites (STSs)	short sequences of genomic DNA for which the base sequence is known. Polymerase chain reaction can be used to amplify the known sequences, which can serve as physical landmarks for mapping.
sex chromosome	the chromosomes that primarily govern sex determination (XX in women and XY in men). The other chromosomes are autosomes.
somatic cells	all cells in the body except gametes and their precursors
somatic cell hybrid	a hybrid cell line derived from fusion of cells from different sources. Human/rodent hybrids containing a small amount of human genetic material, such as a single chromosome, are used in human gene mapping.
somatic mosaicism	the presence of two or more cell lines in somatic (non-germinal) cells
Southern blotting	a technique, developed by E.M. Southern in 1975, for transferring DNA to a backing sheet prior to hybridization. Northern and Western blots are non-eponymous variations relating to RNA and protein analyses. DNA is fractionated by electrophoresis, transferred to a membrane (blotted) and detected by a complementary labelled probe that hybridizes to the DNA, revealing information about its identity, size and abundance.
splicing	removal of introns during the processing of mRNA
stop codon	one of the three codons (UAG, UAA or UGA) that cause termination of protein synthesis
synteny	loci on the same chromosome which may or may not be within range of detection through cosegregation
tandem repeat sequences	multiple copies of the same base sequence on a chromosome. When the number of repeats varies in the population, they are useful as DNA markers.
telomeres	refers to the ends of chromosomes that contain characteristic repetitive DNA sequences
termination codon	see stop codon
transfection	transfer of a DNA fragment into prokaryotic or eukaryotic cells
trans	(a) historically implies on a different chromosome; (b) in molecular biology, refers to an effect on a gene caused by a factor distinct from the sequence of that gene, in contrast to cis effects, which are encoded in the sequence of the gene. Cis and trans are commonly used to describe factors that influence gene expression. On different chromosomes, usually quite close. The opposite of cis
transcript	refers to an mRNA molecule that encodes a protein
transcription	the synthesis of an RNA molecule (transcript) from a DNA template in the cell nucleus catalyzed by RNA polymerase

transcription start site	site within a gene where transcription of RNA begins
transgenic	containing foreign DNA. For example, transgenic mice contain foreign DNA sequences in addition to the complete mouse genome
translation	assembly of amino acids into peptides based on information encoded in mRNA, i.e. mRNA sequence of bases is translated into sequence of amino acids in a peptide or protein. Occurs on ribosomes
translocation	the transfer of genetic material from one chromosome to another non-homologous chromosome, usually through a reciprocal event at meiosis
trisomy	the state of having three homologous chromosomes instead of the usual pair, as in trisomy 21 (Down's syndrome)
triploid	a cell with three times the haploid number of chromosomes, i.e. three copies of all chromosome types
uniparental disomy	situation in which an individual has two homologous chromosomes (or chromosomal segments) from one parent and none from the other. May be heterodisomy if both chromosomes from the single parent are present orisodisomy if two copies of the same parental chromosome are present
unique sequence DNA	non-repetitive DNA that potentially codes for mRNA and protein
upstream	a DNA sequence is written from the left, or 5', direction to the right, or 3' direction. Upstream refers to the 5' direction, i.e. regulatory elements of a gene are typically located upstream (5') of the coding sequences of that gene.
vector	the vehicle into which DNA is inserted prior to cloning in bacteria. Includes plasmids, phage and cosmids
X-inactivation	the random turning off of all the genes on one of the X-chromosomes in somatic cells during early embryonic development
X-linked	genes carried on the X-chromosome. The term sex-linked should only be used on the very rare occasions both X- and Y-chromosomes are involved.
zygote	the diploid cell resulting from the union of the haploid male (sperm) and female (ovum) gametes

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