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GENETIC RISK ASSESSMENT

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INTRODUCTION

All organisms are the product of a long evolutionary history during which favorable genes have been preserved and deleterious genes were eliminated by natural selection. Beneficial mutations of the past are part of the present population. A random change now is much more likely to be deleterious than to improve the fitness. Therefore, the general harmfulness of mutations is both an empirical fact and an expectation based on evolutionary reasoning.

Crow (17) emphasized that "if any way of reducing the spontaneous mutation rate can be found, it could have enormous humanitarian benefits."

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Based on this reasoning, we must avoid an increase of the human mutation rate due to exposure to ionizing radiations and chemical mutagens.

The proof that X-rays induce germ-cell mutations in the fruit fly, *Drosophila melanogaster,* came in 1927 from Muller (85). He emphasized that "many . . . of the changes produced by X-rays are of just the same kind as the 'gene mutations' which are obtained, with so much greater rarity, without such treatment, and which we believe furnish the building blocks of evolution." Based on his observations Muller (80) suggested improvements for medical X-ray treatments.

Methods to detect mutations in mammals have been discussed by Hertwig (58, 59), Snell (118, 119), Catcheside (10), and Falconer (47). The pioneering studies of Hertwig (59), Brenneke (7), and Schaefer (110) indicated that male mice are fertile after X-ray treatment, then go through a sterile phase and recover their fertility later. The litters sired during the presterile period of irradiated male mice were of reduced size. Since there was no effect on sperm mobility and since the number of fertilized eggs was normal, it was concluded that the reduced litter size was due to death of embryos after fertilization. The observation of various nuclear and chromosomal abnormalities in fertilized ova led to the conclusion that embryonic death was caused by chromosomal abnormalities, induced by irradiation in spermatozoa. These earlier studies on dominant lethal mutations and translocations by Hertwig, Snell, and Strandskov have been reviewed by Russell (96).

A systematic effort to investigate dominant mutations was conducted from late 1943 until about 1950, at the University of Rochester School of Medicine, under the auspices of the wartime Manhattan Project and, later, its successor, the US Atomic Energy Commission. It was concluded that the incidence of mortality, rare morphological anomalies, visible mutations and mutations affecting fertility taken together are definitely increased by radiation at the rate of at least 1.16×10^{-4} per R (15, 16).

The first description of irradiation-induced recessive mutations in mice was published by Hertwig (60). A systematic attempt to study the induction of recessive mutations was initially made by Russell (95) in what is now called the specific-locus test (113). With this method the effect of various biological and physical factors on the radiation-induced mutation frequency at a sample of seven loci has been explored. The investigation of mutation induction by chemical mutagens was initiated by Cattanach (11).

Based on the induction of germ cell mutations in mammals international and national committees developed concepts for quantifying radiation-induced genetic risk in humans. Genetic effects dominated the thinking of the UNSCEAR (United Nations Scientific Committee on the Effect of Atomic Radiation) Report in 1958 (124), the BEAR (Biological Effects of Atomic Radiations) Report from the National Academy of Sciences, the National

Research Council in 1956 (3), and the British counterpart, the Medical Research Council, in 1956 (77). An interesting personal account of the development of the work of the BEIR (Biological Effects of Ionizing Radiations) and UNSCEAR Committee was published recently by Russell (102). The quality of risk estimation depends on the data base and on the concepts used. The current status of both aspects for quantifying genetic risk is reviewed below.

METHODS FOR DETECTING GERM CELL MUTATIONS IN MAMMALS

Estimates of the risk from radiation-induced damage of a human population are based on results extrapolated from animal experiments. There are two main approaches in making genetic risk estimates. The first, termed the doubling-dose or indirect method, is based on experimental results of the specific-locus method. The second, referred to as the direct method, is based on the induction of dominant mutations. A clear comprehension of the methods used for detecting recessive and dominant mutations in mice is vital if the problems and limitations of the quantification of genetic risk are to be understood.

Recessive Specific-Locus Mutations in Mice

The principles of the specific locus method developed by Russell (95) are summarized in Figure 1. A specific-locus test is conducted by mating treated wild-type mice with animals homozygous for seven autosomal recessive visibles. The offspring are expected to be heterozygous at the marker loci. In the event of a mutation at one of the marker loci in a germ cell of the treated wild-type animal, the offspring will express the recessive phenotype characteristic for the locus. The specific-locus stock extensively used has the following markers: *a* (non-agouti), *b* (brown), c^{ch} (chinchilla), *d* (dilute), *p* (pink-eyed dilution), *s* (piebald spotting), and *se* (short-ear). Pictures of these phenotypes were published by Ehling (28, 31). These seven loci are distributed among five autosomes. The *c* and *p* loci are linked on chromosome 7, with an average recombination frequency of 15%, and the *d* and *se* loci are closely linked, being only 0.13 cM apart on chromosome 9 (18). A double *d-se* mutation may represent a deletion involving both loci. The length of a deficiency that is compatible with viability of heterozygotes probably depends on the content of specific chromosome regions and may be as great as 3–11 centimorgans (92, 94).

The specific-locus method was used to evaluate the physical and biological factors that affect mutagenesis: radiation dose, dose rate, dose fractionation,

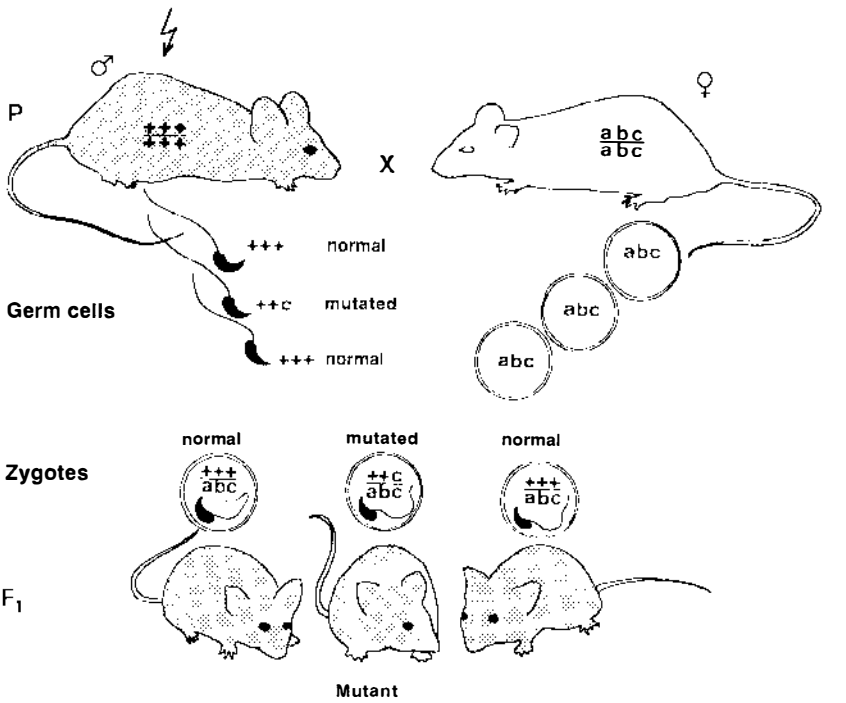

Figure 1 Mating scheme for the detection of recessive mutations in the F_1-generation. The test-stock is homozygous for seven recessive genes (*a*/*a*; *b*/*b*; $p,c^{ch}/p,c^{ch}$; d,se/d,se; s/s). The treated mouse has the corresponding wild-type alleles.

age at exposure, and the interval between irradiation and conception (98, 100).

Of special importance for risk estimation is the detection of the dose-rate effect by Russell and coworkers (105). The dose rate (dose per unit time) at which a total dose is delivered affects the induced mutation rate observed. In A_s-spermatogonia the induced mutation rate is reduced when the total dose of irradiation is delivered at a low dose rate as compared to the same total dose delivered at a high dose rate. The observed mutation rate following low dose rate irradiation (10R/week) is approximately one third of that observed after high dose rate irradiation (80–90R/min). The interpretation of such results has been controversial. Russell (99) and Lyon et al. (72) considered a limited DNA repair capability to be the preferred explanation. In germ cells capable of DNA repair, a dose rate effect is observed because at a lower dose rate more premutational DNA lesions may be repaired than when the same total dose is delivered at a high dose rate.

Abrahamson & Wolff (1), Brewen & Payne (8), and Wolff (131), on the other hand, argued that a portion of the recovered specific-locus mutations result from two-hit events. A dose rate effect is observed because at a lower dose rate, DNA premutational lesions are induced over a longer period of time, during which they may dissipate or be repaired, such that the probability of interaction of two premutational lesions is reduced from that when the premutational lesions are induced essentially simultaneously (high dose rate). To interpret the dose-rate effect, the frequency of lethal induced specific-locus mutations in the homozygous condition is important. The frequency of mutations in the control which are lethal in homozygous condition is 1 in 13 or 8%. Following chemical mutagen treatment of spermatogonia, the frequency of specific-locus mutations lethal in homozygous condition is 51 in 198 or 26% (38). In contrast, the frequency of lethal mutations after irradiation of spermatogonia is 165 in 251 or 66% (31). Additional data on the viability of radiation-induced specific-locus mutations in mice were reported by Lyon et al (73) and Russell & Russell (104). The high frequency of recessive lethal mutations induced by radiation suggests that small deficiencies are the main cause for mutations. The relatively small proportion of recessive lethal mutations induced by chemical mutagens indicates that the majority of mutations may be due to intragenic changes.

In the female mouse, germ cells enter meiosis on or soon after the 12th day of embryonic life and reach the dictyate stage shortly after birth. They are arrested in this stage until 12 h before ovulation. Therefore, this female germ-cell stage is of special importance in evaluating radiation-induced genetic hazard. In the mouse, the immature, arrested oocyte has a low sensitivity to mutation induction by radiation (101).

In adult male mice, no effect of the interval between irradiation and fertilization has ever been observed in the induced specific-locus mutation frequency in spermatogonia (100, 103). In mouse oocytes, on the other hand, the interval between irradiation and conception is one of the most important factors affecting the mutation rate (101).

The factors affecting the mutation rate have to be taken into account when estimating radiation-induced genetic risk. (For a detailed review of these factors, see refs. 41, 106, 112, 115).

Dominant Mutations in Mice

Dominant mutations can be measured by comparing first generation descendants from treated and untreated populations. The difficulty of such a comparison is in distinguishing between variants and mutants. A variant can be characterized by a deviation from a normal phenotype; mutations cannot be definitively characterized by morphological criteria. The critical test is the

transmission of the observed phenotype. The estimation of genetic risk must be based on the observation of mutants, not on the observation of variants (32). (For detailed accounts of methods and the results obtained in these studies, see refs. 41, 116.)

DOMINANT MUTATIONS AFFECTING THE SKELETONS OF MICE Dominant skeletal mutation experiments represent the earliest attempt to screen systematically for mutations affecting one body system. In the initial experiments, inbred 101 male mice were exposed to X-radiation or neutrons at various doses. F_1 offspring from matings with C3H females were killed at 26–28 d of age for skeletal preparation, and the Alizarin Red S-stained and cleared intact preparations were coded before examination. The skeleton was chosen because it is formed over an extended period of development and is presumably, therefore, subject to modification by gene action falling within a wide range of time (45). It would have been desirable to use another combination, namely $(101 \times C3H)F_1$ hybrid male mice and test-stock females. In this case, the induction of specific-locus mutations could have been used as a positive control of the effectiveness of the exposure.

The classification used to separate, as far as possible, the existing natural variation from that caused by newly occurring genetic changes was based on the following considerations. If the frequencies of mutation for specific loci are known one can assume that in an experiment where the sample size is such that not more than one mutation would be expected for any particular gene locus, mutations can best be screened for by dividing the abnormalities according to whether they occur only once in the whole experiment (Class 1) or more frequently (Class 2). The most sensitive indication of a mutation is probably the statistically significant increase over the control value of Class-1 multiple abnormalities, and Class-1 abnormalities of the bilateral type in the appendicular skeleton (23, 24). The frequency of these two subgroups of Class-1 abnormalities are summarized in Table 1 as presumed mutations.

It is noteworthy that two features characteristic of radiation-induced specific-locus mutations—the augmenting effect of dose fractionation (97), and the quantitative differences between spermatogonial and postspermatogonial stages (114)—can also be seen in the 1966 experiments shown in Table 1. Therefore, a pilot experiment was specifically designed to permit breeding tests on a sample of presumed mutations. Three dominant mutations affecting the skeleton were found to be transmitted to the second and later generations (25). In addition, the frequency of Class-1 abnormalities in mouse embryos after irradiation of the sire was determined by Bartsch-Sandhoff (2).

In a subsequent experiment the inheritance of the radiation-induced presumed mutations was systematically tested. The classification devised by

Table 1 Frequency of dominant mutations affecting the skeleton of mice (31)

Classification	Dose (R)	Interval between dose fractions	Germ cell stage treated	No. of F_1 skeletons examined	Events		Reference
					n	(%)	
Presumed mutations (1966)	0			1739	1	0.06	
	600	0	pg	569	10	1.8	
	600	0	g	754	5	0.7	24
	100 + 500	24 h	g	277	5	1.8	
	500 + 500	10 weeks	g	131	2	1.5	
Mutations (1977)	100 + 500	24 h	g	2646	31–37	1.2–1.4	117

pg: Postspermatogonial stages
g: Spermatogonia

Ehling (24) was used to determine the presumed mutations. Offspring of presumed mutants were inspected for the presence of the respective skeletal defects (117). The mutation frequency determined by statistical considerations is in good agreement with the breeding test (Table 1). Pictures of Alizarin Red-S preparation of normal and mutant mice were published (27, 28).

The difficulty with the skeletal system is in proving that the variant is due to a mutation. To test the transmission of the variant, F_2 offspring must be obtained before the skeletal preparation of the F_1 generation. Because this procedure is time- and space-consuming, we developed another method by screening for dominant cataracts. When compared with screening for dominant skeletal mutations, screening for phenotypic variants of the lens has the advantage of a more rapid examination that can be performed on living animals.

DOMINANT CATARACT MUTATIONS IN MICE A cataract is an opacity of the lens causing a reduction of visual function. The organogenesis of the lens is similar in various mammals. Therefore, a gene that disturbs the same process in the normal development of the lens in different species allows the same cataract type to be seen in these different species. Ehling (21) pointed out that morphologically comparable cataracts in humans and other mammals frequently have the same mode of inheritance. Based on these observations it is surprising that Sankaranarayanan (108) doubts the induction by radiation of dominant genetic diseases in man. His notion is contradicted by the results of investigations described below. In fact, the comparability of the genetic endpoint in mice and man is one advantage of this system. A detailed description of radiation-induced dominant cataracts in mice (64, 67), drawings (64), and photographs of these dominant lens-opacity mutants (29, 54,

64) were published. Similar types of cataracts were described in man (130). A genetic homology between the human X-linked cataract-dental syndrome, Nance-Horan Syndrome, and the mouse X-linked cataract was reported by Favor & Pretsch (54).

The systematic investigation of induced dominant cataracts in mice was initiated by Ehling in 1977 and subsequently reported in a series of papers (28, 30, 34, 42, 52, 56, 66). Table 8 lists the observed mutation frequencies to dominant cataract alleles following single, high-dose rate spermatogonial exposure in mice along with the estimated doubling dose. Additional results are summarized in Table 3.

The detection of dominant cataract mutations can be combined with the scoring of specific-locus mutations (42). This combination is the basis for the multiple endpoint approach.

The Multiple Endpoint Approach

The problem with the specific-locus experiments is that only one class of mutations (recessive visibles) can be detected. This disadvantage can be overcome by combining the scoring of specific-locus alleles at 7 loci with the screening of approximately 30 loci coding for dominant cataract mutations, 23 loci controlling protein-charge changes (89), and 12 loci for enzyme-activity alterations (14). The multiple endpoint approach was developed in Neuherberg (40). It allows the comparison of induced mutation rates to recessive visible alleles and to dominant cataract alleles, as well as mutation rates to protein-charge or enzyme-activity alterations in the same animals.

Experiments to screen for the approximately 70 loci in the same offspring of treated male mice were performed with ethylnitrosourea (ENU), procarbazine, and X-ray exposure. Mutations were recovered for each genetic endpoint in all treatment groups where a sufficient number of offspring was scored. The mutations were confirmed by breeding tests (Table 2). The mutation rates calculated per locus for the four different genetic endpoints following spermatogonial treatment suggest two groups that differ in their mutation rate per locus (Table 3). The frequencies of the specific-locus mutations were greater than those of the enzyme-activity mutations. However, the mutation rates to specific-locus alleles and enzyme-activity alleles were both much higher than the mutation rates to either dominant-cataract alleles or protein-charge mutations. Reasons for the lower mutation rate per locus to dominant-cataract alleles as compared to specific-locus alleles have been discussed (48–50). The explanation includes the possibility that the specific loci employed have an unusually high mutation rate to recessive alleles. Further, a mutation leading to a simple loss or increase of the normal gene function should be recessive, whereas a mutation leading to an alteration of the gene product that interferes with the normal gene product would be

Table 2 Mutation frequencies of different genetic endpoints following spermatogonial treatment of (101/ElxC3H/E1)F_1-hybrid male mice (40)

Treatment	Specific-locus mutations[a]	Dominant-cataract mutations	Protein-charge mutations	Enzyme-activity mutations
Historical control	19(13)/227805	1/22594	0/5812	0/3610
ENU				
160 mg/kg	35(32)/8658	14/6435	nt	nt
	52(50/13018	nt	1/1892	14/3093
250 mg/kg	64(58)/9766	17/9352	3/4254	4(3)[b]/505
Procarbazine				
600 mg/kg	4/13071	1/12056	1/7506[c]	1/2974
X-Irradiation	18/10054	3(2)[b]/8889	nt	nt
3 + 3 Gy	11/8085	0/6662	0/1003	1/3388

[a] Number of independent mutational events in parentheses.
[b] Presumed cluster (2 mutations were recovered in the same litter and express the same phenotype. However, a test for allelism has not yet been conducted).
[c] Includes results of the present multiple endpoint experiment (0/5725) as well as results of an independent experiment (1/1781).
nt, not tested.

dominantly expressed. A limited spectrum of DNA alterations may result in a dominant allele whereas a broader spectrum of DNA lesions may lead to loss of gene function that is expressed recessively (61). Thus, the mutational event leading to a recessive or a dominant allele may be qualitatively different. It would be inadvisable to base all characterizations of the mutational process in germ cells of mammals on results with recessive alleles given that the mutational events leading to recessive or dominant alleles may differ (51).

A comparison between species has not been possible until recently because the specific-locus method requires special genetic stocks of the tester loci,

Table 3 Observed and presumed per locus mutation rate $\times 10^{-5}$ for different genetic endpoints following spermatogonial treatment of (101/ElxC3H/El)F_1-hybrid mice (40)

Treatment	Specific-locus mutations	Dominant-cataract mutations	Protein-charge mutations	Enzyme-activity mutations
Historical control	1.2	0.1		
ENU				
160 mg/kg	57.8	7.3	nt	nt
	57.1	nt	2.3	37.7
250 mg/kg	93.6	6.1	3.1	66.0
Procarbazine				
600 mg/kg	4.4	0.3	0.6	2.8
X-Irradiation				
3 + 3 Gy	22.8	0.6		2.5

nt, not tested.

which are only available in mice. Multiple endpoint approach experiments are in progress to compare dominant-cataract (65) and enzyme-activity mutants with results in other species, for example, the golden hamster *(Mesocricetus auratus)*.

Estimation of the genetic hazard from radiation has been based almost exclusively on results with the specific-locus method, for example, the dose-rate effect and the response of treated female mice (101, 105). It is now possible to compare the results for the recessive loci in the same experimental group with those for other genetic endpoints.

Until recently, the frequency of radiation-induced recessive mutations could not be quantified (6). Systematic comparison of dominant and recessive mutations made quantification possible by using the ratio between the induced dominant and recessive mutations (35). Another advantage of the multiple endpoint approach is that the data from the same experiments with mice can be used for both methods to estimate the radiation-induced genetic risk in humans. Screening results for specific-locus mutations can be used to estimate indirect risk (doubling-dose method) and screening results for dominant cataracts can be used to estimate direct genetic risk.

QUANTIFICATION OF GENETIC RISK

In using the murine data to arrive at quantitative estimates of the genetic risks from radiation for humans, the following assumptions have been made: (*a*) The various biological and exposure factors affect the magnitude of the induced mutation frequency in a similar way and to a similar extent in mice and in humans. (*b*) There is a non-thresholded linear or linear quadratic dose-response. In the simplest case, response depends linearly on dose: $y - y_o = bD$, where y is the frequency of observed mutations per gamete, y_o is the background or spontaneous frequency of mutations per gamete, D is the dose and b is the slope of the dose-response line.

Risk estimation is based on the results of induced mutations in stem-cell spermatogonia of mice (6, 126). The reason is that only A_s (stem-cell) spermatogonia continue to divide throughout reproductive life. Cells that go through meiotic divisions and through successive differentiation stages are known as spermatocytes, spermatids, and spermatozoa. These postspermatogonial germ-cell stages have only a transitory existence, approximately five weeks in mice (87) and almost twice as long in man (57).

Indirect Risk Estimation (Doubling-Dose Method)

The doubling dose can be defined as the dose necessary to induce as many mutations as occur spontaneously in one generation. One underlying assumption in calculating the doubling dose is a *linear dose-response relationship*.

Another assumption is that the damage to genetic material resulting from exposure to mutagenic agents is similar in nature to mutations arising spontaneously (120). If these conditions are fulfilled the doubling dose can be calculated. The calculation of the doubling dose by the UNSCEAR and BEIR Committees is based on the results of specific-locus experiments in mice.

In their estimate of risk to the population, the UNSCEAR (126, 128) and BEIR (6) Reports used a doubling dose of 1 Gy for low dose rate, low LET (Linear Energy Transfer, J/m) irradiation. The estimation of the doubling dose is discussed in the corresponding reports, by Lüning & Searle (70) and Denniston (20). An overview of the different doubling doses used by BEIR and UNSCEAR between 1956 to 1986 was given by Sankaranarayanan (107).

A crucial assumption of the indirect method is that the doubling dose calculated for selected recessive mutations is also used to quantify dominant mutations, chromosomal diseases, and for mutations with irregularly inherited diseases. Based on these assumptions, estimates of genetic risk by the UNSCEAR Committee are summarized in Table 4 and by the BEIR Committee in Table 5.

Table 4 Estimated effect of 1 Gy per generation of low dose or low dose rate, low-LET irradiation on a population of one million live-born according to the doubling dose method. Assumed doubling dose: 1 Gy (126)

Disease classification[a]	Current incidence[b]	Effect of 1 Gy per generation	
		First generation[c]	Equilibrium
Autosomal dominant and X-linked diseases	10,000[d]	1,500	10,000
Recessive diseases	2,500[e]	Slight	Slow increase
Chromosomal diseases			
Structural	400[f]	240	400
Numerical	3,000[g]	Probably very small	Probably very small
Congenital anomalies anomalies expressed later and constitutional and degenerative diseases	90,000[h]	450	4,500[i]
Total	105,900	2,190	14,900

[a] Follows that given in the BEIR Report (4), except that chromosomal diseases are divided into those with a structural and those with a numerical basis.

[b] Based on the results of Trimble & Doughty (123) and other studies.

[c] The first generation incidence is assumed to be about 15% of the equilibrium incidence for autosomal dominant and X-linked diseases, about 60% of the equilibrium incidence for structural anomalies and about 10% of the equilibrium incidence for diseases of complex inheritance.

[d] Includes disease with both early and late onset.

[e] Also includes diseases maintained by heterozygous advantage.

[f] Based on the pooled values of Table 2 of UNSCEAR Report (126) but excluding euploid structural rearrangements, Robertsonian translocations and "others" (mainly mosaics).

[g] Excluding mosaics.

[h] Includes an unknown proportion of numerical (other than Down syndrome) and structural chromosomal anomalies.

[i] Based on the assumption of a 5% mutational component.

Table 5 Estimated genetic effects of 1 rem per generation[a] (6)

Type of disorder	Current incidence per million live-born offspring	Additional cases/10^6 live-born offspring/rem/generation	
		first generation	equilibrium
Autosomal dominant			
Clinically severe[b]	2,500[c]	5–20[d]	25[e]
Clinically mild[f]	7,500[g]	1–15[d]	75[e]
X-linked	400	<1	<5
Recessive	2,500	<1	Very slow increase
Chromosomal			
Unbalanced translocations	600[h]	<5	Very little increase
Trisomies	3,800[i]	<1	<1
Congenital abnormalities	20,000–30,000	10[j]	10–100[k]
Other disorders of complex etiology[l]			
Heart disease[m]	600,000		
Cancer	300,000	Not estimated	Not estimated
Selected others	300,000		

[a] Risks pertain to average population exposure of 1 rem per generation to a population with the spontaneous genetic burden of humans and a doubling dose for chronic exposure of 100 rem (1 Sv).

[b] Assumes that survival and reproduction are reduced by 20–80% relative to normal (s = 0.2–0.8).

[c] Approximates incidence of severe dominant traits.

[d,e] The equation for the calculation is given in the report.

[f] Assumes that survival and reproduction are reduced by 1–20% relative to normal (s = 0.01–0.2).

[g] Obtained by subtracting an estimated 2,500 clinically severe dominant traits from an estimated total incidence of dominant traits of 10,000.

[h] Estimated frequency from UNSCEAR (126, 127).

[i] Most frequent result of chromosomal nondisjunction among liveborn children. Estimated frequency from UNSCEAR (126, 127).

[j] Based on worst-case assumption that mutational component results from dominant genes with an average selection coefficient of 0.1; the equation is given in the report.

[k] Calculated with the mutational component 5–35%.

[l] Lifetime prevalence estimates may vary according to diagnostic criteria and other factors.

[m] No implication is made that any form of heart disease is caused by radiation among exposed individuals. The effect, if any, results from mutations that may be induced by radiation and expressed in later generations, which contribute, along with other genes, to the genetic component of susceptibility. This is analogous to environmental risk factors that contribute to the environmental component of susceptibility. The magnitude of the genetic component in susceptibility to heart disease and other disorders with complex etiologies is unknown. Most genes affecting the traits are thought to have small effects, and new mutations would each contribute a virtually insignificant amount to the total susceptibility of the individuals who carry them. However, a slight increase in genetic susceptibility among many individuals in the population may produce, in the aggregate, a significant effect overall. Because of great uncertainties in the mutational component of these traits and other complexities, the committee has not made quantitative risk estimates. The risks may be negligibly small, or they may be as large or larger than the risks for all other traits combined.

In a randomly mating natural population newly induced recessive mutations will result in a mutant phenotype only when they occur as a homozygote. This is a function of the square of the allele frequency, and in the generations following the exposure a negligible increase could be expected in the frequency of mutant individuals. Dominant mutations, by definition, express phenotypic effects as a heterozygote. Thus, newly induced dominant muta-

tions would be detected in the F_1 population following radiation exposure of the parental generation.

If the current incidence of autosomal dominant and X-linked diseases is 10,000 per 1 million live-born then an exposure of 1 Gy per generation would induce the same number of diseases per 1 million live-born at equilibrium. The number of generations required to reach equilibrium will depend on the rate of elimination of the induced mutations from the population. For all practical purposes, this would be achieved in some 5–20 generations in the example chosen. If the persistence is five generations, then the amount of first-generation expression would be one-fifth of the equilibrium expression; if it were 20 generations, the first-generation expression would be one twentieth of the equilibrium expression (5). The UNSCEAR Committee estimated that the incidence of autosomal dominant and X-linked hereditary diseases is 1,500 in the first generation (Table 4). This estimate is consistent with the results of animal studies (53). The estimate of the incidence of the autosomal dominant and X-linked diseases is based on a survey in Northern Ireland by Stevenson (121). Trimble & Doughty (123) estimated the same incidence in the population in British Columbia of less than 1,000 per million live-born.

The quality of risk estimation depends on assumptions of the persistence of the induced mutations and the ability to determine the current incidence of genetic diseases.

The difficulty in estimating the chromosomal diseases in Table 4 and Table 5 is fully discussed in the corresponding reports. This category includes three types: numerical aberrations, rearrangements, and deletions. The first includes extra and missing chromosomes or chromosome sets; the second, translocations and inversions. Very small deletions are often indistinguishable from Mendelian mutants. As a class, chromosome aberrations contribute heavily to human prenatal mortality. Approximately one in two spontaneous abortions is due to chromosome aberrations. Such aberrations also contribute to the postnatal genetic disease burden, e.g. Down syndrome, Turner's syndrome, and Klinefelter's syndrome. It is estimated that approximately 0.6% of live-born children possess a chromosome abnormality leading to impairment. Undoubtedly, a large proportion of chromosomal damage is lethal so early in development that it is not detected as a health burden (20).

For aneuploidy, the human epidemiological data are equivocal, and mouse studies on the induction of aneuploidy have been negative (69, 91, 93). For this reason no attempt was made by UNSCEAR (127) to estimate the possible genetic risk of this category.

The risks of unbalanced translocations can be estimated using the combined marmoset and human cytogenetic data (9) and the data from rhesus monkeys (129). UNSCEAR (126) estimated that there will be between approximately 30 and 1,000 abnormal children in the first generation per million progeny

per Gy of paternal low-level irradiation, stemming from unbalanced products of radiation-induced reciprocal translocations and 0 to 300 abnormal children after exposure of females. Several assumptions are necessary for this estimate, including the transmission component, the ratios of chromosomally imbalanced to balanced gametes, and the chance of the unbalanced translocation products to contribute to live-born children. The validity of these assumptions is discussed in detail by UNSCEAR (126).

To improve the estimation of risk in this category, it is proposed that the data base of radiation-induced translocations in mice be extended and that the method suggested by Rhomberg et al (90) to quantify the genetic risk of ethylene oxide be used. These authors suggested the multistage and the Weibull model to quantify risk. These models fit the translocations induced by ethylene oxide in mice better than the linear extrapolation.

For the more complex situation involving irregularly inherited diseases (congenital anomalies, anomalies expressed later, and constitutional and degenerative diseases) the authors of the UNSCEAR Report (126) assumed a mutational component of 5%, and in the BEIR Report (5), a mutational component of 5–50%. The increased number of irregularly inherited diseases at equilibrium was estimated in the UNSCEAR Report (126) after population exposure of 1 Gy per generation per 1 million liveborn to be 4,500 cases and in the BEIR Report (5) between 2,000 to 90,000 cases. More recently, the authors of the UNSCEAR Report (128) estimated the incidence of irregularly inherited diseases at 660,000 per 10^6 live births and BEIR (6) at 1,200,000 per 10^6 live births (Table 6). Neither report gives an estimate of the genetic component of these diseases. The genetic component must be greater than zero if there is to be any meaning to the estimation. The BEIR V-Report emphasized the importance of the category of irregularly inherited diseases: "The Committee's estimates of the total genetic damage are highly uncertain, however they include no allowance for diseases of complex genetic origin" [irregular inherited diseases], "which are thought to comprise the largest category of genetically-related diseases. To enable estimates to be made for

Table 6 Estimated incidence of irregularly inherited diseases

Report	Incidence per 10^6 live-births (in '000)	References
BEIR	40	4
UNSCEAR	90	125
BEIR	90	5
NUREG	90	86
UNSCEAR	660	128
BEIR	1200	6

the latter category, further research on the genetic contribution to such diseases is required."

The difficulties described in estimating the current incidence of genetic diseases or the persistence of the genes in the population emphasize the problems of risk estimation. For a specific disease with irregular inheritance, such as hypertony, it is possible to estimate the genetic component, but for the whole group, such an estimate is almost impossible. These are only some of the problems we face using human data. An alternative is the direct risk estimation based mainly on radiation-induced dominant mutations in mice.

Direct Risk Estimation

The direct estimation of genetic risk in man is based on experiments to screen for dominant mutations in mice. Two methods have been developed for this purpose: dominant skeletal and dominant cataract mutations. Using these data, a concept was developed by Ehling (26) for the direct estimation of genetic risk. It has the advantage of using dominant mutations to estimate genetic damage in the first generation and avoids the problem of basing the estimates of induced mutations for one genetic endpoint upon experimental data from a different genetic endpoint. Direct quantification of genetic risk is based on the following assumptions:

(*a*) The dose-effect-curve for the induction of dominant cataract mutations is linear;
(*b*) The sensitivity of dominant cataract genes is representative for all dominant genes;
(*c*) The ratio of dominant cataract mutations [31] to the total number of well-established dominant mutations [1,443] in man (76) is the same as in the mouse. The ratio of the total number of dominant loci to the number of dominant indicator loci (e.g. dominant cataract mutations) gives the multiplication factor. The multiplication factor is used to convert the induced mutation rate of dominant cataracts to estimate the overall dominant mutation rate.

Based on these assumptions one can use the following equation by Ehling (36) to quantify the genetic risk for dominant traits in the first generation:

Expected number of induced dominant **mutations** in man	=	**Induced** mutation **frequency** in mice	×	**Multiplication factor** for the overall dominant mutation frequency in man	×	Genetically **significant dose** in man

1.

Using this equation one can calculate the number of dominant **mutations expected** to occur in the offspring of survivors of the atomic bombings at Hiroshima and Nagasaki (Table 7).

The **induced** mutation **frequency** of dominant cataracts in mice for single exposure with a dose rate of 0.5 Gy/min is $0.45-0.55\times10^{-4}$ mutations/gamete/Gy. The mutation rates are based on two independent experiments.

The change in the **multiplication factor** reflects our increasing knowledge about dominant genes in man according to McKusick (75, 76). In 1983, the ratio of all dominant mutations to dominant cataracts was 934 to 23 (= 41). In 1988, this ratio is 1,443 to 31 (= 47).

The genetically **significant dose** to the survivors of the atomic bombings at Hiroshima and Nagasaki was taken from an estimate by Oftedal (88). Since then the dosimetry has been reevaluated (83) with a consequent reduction in the estimated neutron yield coupled with a less marked increase, in parts of the city, in the estimated γ-yield.

These corrections did not change the total number of **expected** dominant **mutations** of 20 in Table 7. Using the skeletal data, we would now expect less than 50 mutations (33). Because of the difficulties in separating mutants and variants for skeletal defects, the cataract mutation data are more accurate. The important message is not the actual number of the estimates, but the order of magnitude.

These estimations can be compared with the data of Schull, Otake & Neel (111), for four indicators of genetic effects from studies of children born to survivors of atomic bombings at Hiroshima and Nagasaki. These indicators are:

frequency of untoward pregnancy outcomes (stillbirth, major congenital defects, death during first postnatal week);
occurrence of death in live children through an average life expectancy of 17 years;
frequency of children with sex chromosome aneuploidy; and
frequency of children with mutation resulting in an electrophoretic variant.

Table 7 Direct estimation of the genetic risk in 19,000 offspring after parental exposure in Hiroshima and Nagasaki based on dominant cataract mutations in mice (33, 39)

	1984	1991
Mutations/gamete/Gy	$0.45-0.55\times10^{-4}$	
Multiplication factor for the overall dominant mutation rate	41[a]	47[c]
Sv (genetically significant population dose)	1.1×10^{4}[b]	0.9×10^{4}[d]
Total number of expected dominant mutations	20–25	19–23

References: [a]75; [b]88; [c]76; [d]83

In a recent publication Neel and coworkers (84) extended their observation and included four more indicators:

malignancies in the F_1;
frequency of balanced structural rearrangements of chromosomes;
sex ratio among children of exposed mothers;
growth and development of the F_1.

Using these indicators one would expect to observe only a fraction of the 20–50 dominant mutations predicted by our equation. Therefore, the conclusion of the authors, "in no instance is there a statistically significant effect of parental exposure; but for all indicators the observed effect is in the direction suggested by the hypothesis that genetic damage resulted from the exposure", is in agreement with the direct estimation of genetic risk for children of Hiroshima and Nagasaki based on mouse experiments (111).

In 1989, Neel and coworkers (85) reemphasized that the observations in Hiroshima and Nagasaki are consistent with the hypothesis of a genetic effect due to exposure. In contrast to the conclusion that these observations are in agreement with our predictions (Table 7), Neel and coworkers (84) emphasize the following points: "An effort has also been made to calculate the most probable doubling dose for the indicators combined. The latter value is between 1.7 and 2.2 Sv. It is suggested the appropriate figure for chronic radiation would be between 3.4 and 4.5 Sv. These estimates suggest humans are less sensitive to the genetic effects of radiation than has been assumed on the basis of past extrapolations from experiments with mice", a view also supported by Kondo (63).

The estimation of the doubling dose was criticized on several points. The main problem is that only for a genetic component of 100% is the calculation straightforward. This assumption for all eight indicators is very likely not correct (107). Another major problem is the assumption that recessive and dominant mutations in mice or man have the same doubling dose. Table 8 gives the doubling dose estimates for dominant cataracts. These estimates range from 0.96 to 2.14 Gy with a weighted mean of 1.6 Gy (50). Since these estimates are based on a single cataract mutation in the controls, they have wide confidence intervals.

Independent of the control frequency, the data indicate that the use of an overall doubling dose is questionable. The extrapolation must be based on experimental results using the same genetic endpoint in man and mouse. If we take into account that for some indicators of the estimates made by Neel and coworkers (84) the genetic component is less than 100% then the doubling dose is probably lower than the given range between 1.7 to 2.2 Sv. If we compare this estimate with Table 8, we have to conclude that the doubling dose for dominant mutations in man and mouse is similar. An interesting detailed comparison of the doubling doses of different endpoints in man and

Table 8 Dominant cataract mutations in mice induced by single, high dose-rate exposure of spermatogonia (50)

Dose (Gy)	Dominant cataract	Mutation rate* $\times 10^{-5}$	Doubling dose (Gy)
0	1/22,594	0.15	
1.5	2/23,157	0.29	1.61
3.0	3/22,712	0.44	1.55
6.0	3/17,599	0.57	2.14
5.3	3/10,212	0.98	0.96
6.0	3/11,095	0.90	1.20

*A total of 30 mutable loci was assumed for the calculation of the per locus mutation rate (34)

mouse was recently published by Neel & Lewis (82). They emphasized the differences of the genetic endpoints in mouse and man. In addition, they raised the very important question of whether the female mouse is an acceptable model for human risk estimation.

Critical Evaluation of Risk Estimation

The uncertainties of the assumptions and the limited data base require that quantification be critically evaluated. Crow (17) discussed these uncertainties in a very interesting lecture entitled "How well can we assess genetic risks? Not very". However, we must use our knowledge to make the best possible risk estimate. To cope with these problems, Denniston (20) suggested a method for stating the uncertainties. He wrote: "A difficulty with the general approach of multiplying a number of factors together to obtain a risk is that committees generally do not explicitly state how much confidence they have in either the individual factors or the resulting products . . . A possible method for making statements of uncertainty more precise is as follows. Treat each factor in the product as a random variable. The distribution of this random variable may be thought of as a quantitative measure of belief regarding the true value of the factor." This is an interesting approach. However, how much confidence can we have that the selected genes in these experiments are representative for the whole genome? What is the quality of the dose-effect-relationship? There is no proof in radiation genetics for the general assumption that the sensitivity of the mouse genome equals the sensitivity of the human genome. Is the female mouse a good indicator for estimating the induced mutation rate in the human female, given that the X-ray dose to induce sterility in both species differs by an order of magnitude (22, 74)? These problems are difficult to assess. Therefore, a range of our risk estimation can also give a false sense of security. Refinements in mutation recognition at the DNA level will very likely permit analysis of new mutational endpoints in the Hiroshima and Nagasaki population (19, 78, 79, 109). This

analysis must be combined with controlled experimentation in animals. Improving the experimental and the human data base and developing better concepts to estimate risk should provide the best guarantee for the protection of the genetic pool of future generations.

One lesson we can learn from Hiroshima and Nagasaki is that the proper study of the genetic risk of man is not man alone, but both, man and mouse. The observation in man indicates that it would be preferable to use a separate doubling dose for dominant and recessive mutations. In this regard it is interesting to note that H. J. Muller (81) reported that the ratio of radiation-induced recessive visibles to dominant mutations in spermatogonia of *Drosophila* is 5:1. We estimated that the per locus ratio of radiation-induced recessive specific-locus to dominant cataract mutations in spermatogonia of the mouse is approximately 10:1 (34).

A related question is how representative are the seven recessive specific-locus mutations for the whole class of recessive genes. Lyon & Morris (71) stated, "One limitation of all this work, however, is that the same seven specific loci, those of *a, b, c, d, p, s* and *se*, have been used throughout, and there is no means of knowing how representative these are of mouse loci as a whole. The seven loci vary considerably among themselves, Russell and Russell (104) having found a 35-fold range of differences in sensitivity from the most to the least sensitive locus after a dose of 600r of X-rays to spermatogonia. The specific locus mutation rates usually quoted are the average rates for the seven loci, but in view of their wide range of variation there is room for doubt whether any other group of loci would give a similar figure." An experiment by the authors was performed with the same mouse stock (C3H×101) and the same radiation dose (600 rad) to spermatogonia as had been used previously, but employing a new group of six loci, *a, bp, fz, ln, pa* and *pe*. The observed mutation rate, 5.0×10^{-8} per locus per rad, was significantly lower than that for the original seven loci (20.9×10^{-8}).

In view of the question, how representative is the doubling dose of specific-locus mutations in mice for the whole genome, and in light of the uncertainties of the human incidence data (Tables 5 and 6), the conclusion of the BEIR V Report is surprising, "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." A comparison of the estimations for autosomal dominant mutations in Table 5 and 7 clearly indicates that fewer and better-founded assumptions are made for the direct estimation. In addition, the experimental data base for the direct estimation can be improved. Furthermore, the statement of the BEIR Committee (6) ignored the difficulties with the human data base, which was

discussed in detail in the chapter of the Indirect Risk Estimation (Doubling Dose Method).

The problem with the "Gene Number Method" discussed by the BEIR V Report (6) is twofold: (*a*) It is difficult or impossible to estimate the total number of human genes; (*b*) It is meaningless to determine an average mutation rate for all genes.

However, one could use the gene number approach for one category of mutations, e.g. dominant mutations. In this case, one would make assumptions similar to those discussed for the direct estimation of genetic risk. The theoretical aspects of this method were discussed by Denniston (20).

One important aspect of risk estimation that has not been discussed until now in the literature concerns the possible interaction of chemical mutagens and ionizing radiation. For example, cyclophosphamide alone does not induce specific-locus mutations in spermatogonia of mice. In contrast, X-rays or γ-ray-induce specific-locus mutations in spermatogonial germ cell stages. The highest mutation rate after γ-ray-exposure in spermatogonia was 11.8×10^{-5} mutations/locus/gamete in the 6 Gy group (44). However, pretreatment with cyclophosphamide followed 24 h later by irradiation with 3 or 6 Gy more than doubles the X-ray-induced mutation rate in spermatogonia. The distribution of the observed mutations among the seven loci and their viability supports the hypothesis that these mutations were induced by radiation rather than by cyclophosphamide. The compound causes an immediate inhibition of DNA and RNA synthesis in spermatogonia (68). The inhibition very likely interferes with the repair process. Disturbances of the repair process probably cause the synergistic effect in inducing specific-locus mutations in spermatogonia of mice after pretreatment with cyclophosphamide 24 h before irradiation (44).

The assumption that the inhibition of the repair process enhanced the yield of radiation-induced mutations after pretreatment with cyclophosphamide can be tested. If correct, other compounds that inhibit the repair process in spermatogonia should also enhance the yield of radiation-induced specific-locus mutations.

A review of genetic effects of combined chemical-X-ray treatment in male mouse germ cells was recently published by Cattanach (12, 13). A general overview was given by Glubrecht et al (55) and Kada et al (62).

PERSPECTIVE

According to Chemical Abstracts, approximately 10 million chemicals were registered by 1984. About 53,500 distinct substances (65,725 if duplicates are counted) are regulated or inventoried by the United States Environmental Protection Agency and the Food and Drug Administration. Most have not

been tested for toxicity (122). An unknown fraction of these compounds is mutagenic and indispensible. Quantitative risk estimates are needed for these agents to calculate the possible genetic burden. In September 1986, the Environmental Protection Agency (EPA) published *Guidelines for Mutagenicity Risk Assessment* (46) in the Federal Register. These guidelines contained the following suggestions: "Any risk assessment should clearly delineate the strengths and weaknesses of the data, the assumptions made, the uncertainties in the methodology, and the rationale used in reaching the conclusions, e.g., similar or different routes of exposure and metabolic differences between humans and test animals. When possible, quantitative risk assessments should be expressed in terms of the estimated increase of genetic disease per generation, or the fractional increase in the assumed background spontaneous mutation rate in humans (4). Examples of quantitative risk estimates have been published (4, 43, 126); these examples may be of use in performing quantitative risk assessments for mutagens". The references given for quantifying genetic risk clearly indicate that the EPA has tried to establish the same high standards for chemical mutagens as we use for radiation protection. These problems must be addressed. Strict safeguards should be imposed on the controlled use of chemical mutagens, similar to the regulations that already exist for radiation protection (37).

In the future we should aim for an integrated quantification of radiation- and chemically induced genetic risk to the human population. Protection of the human genome is like an equation with two unknowns. Only if we solve both unknowns, the extent of genetic damage from radiation and from chemicals will we be able to reach our goal of minimizing the detrimental effects to future generations.

CONCLUSIONS

From a practical point of view, at present the direct method for the estimation of the first generation effects, supplemented by the doubling dose method, are the best-founded concepts for estimating risk. Regulatory decisions concerning risk to the individual or the population at large must be based on one of these concepts.

To improve our estimates, we cannot rely on an overall doubling dose. Different doubling doses must be determined for distinct genetic endpoints. Furthermore, a better experimental data base must be compiled for the direct estimation of genetic risk. Our model systems for risk estimation of chromosomal diseases need improving and experimental approaches must be developed to estimate risk for the complex constitutional and degenerative diseases. Refinements in mutation recognition at the DNA level should permit the analysis of new mutational endpoints in Hiroshima and Nagasaki. This

analysis must be combined with controlled experimentation in animals. Last, but not least, estimation of the incidence of genetic disorders in man must be improved.

We have the knowledge to protect the human genome against mutagenic agents, but our resources, especially for animal experimentation, are insufficient. The hazard from chemical mutagens and the interaction of ionizing radiation and chemical mutagens are probably more important for the genetic health of future generations than the hazard from radiation alone.

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