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Approaches to Assessing Genetic Risks from Exposure to Chemicals

by F. H. Sobels

An effort to assess and quantify genetic risks from human exposure to mutagenic chemicals is urgently needed; otherwise genetic toxicology may well lose its credibility. Genetic biomonitoring provides us with an indication of mutagenic effectiveness in human somatic cells. The populations and chemicals selected for such studies form a useful database for genetic risk-assessment studies. Extrapolation to what can be expected in germ cells of exposed individuals should be possible by using good dosimetry (adducts) and a parallelogram approach. The principle is that genetic damage in the inaccessible human germ cells can be estimated by determining the effects on lymphocytes (or other somatic cells) from humans and mice and in germ cells of mice. Worldwide, opportunities for the costly mouse germ cell studies are limited. Knowledge of type of DNA adducts, their persistence and/or removal and dominant lethal studies, will be helpful in predicting stage sensitivity. Extrapolation from a lowest effective dose level is proposed. The available data for ethylene oxide and benzene are reviewed. The risk of heritable translocations in progeny of populations exposed to ethylene oxide is so high that more precise estimates seem desirable. In discussing the expression of the induced mutations, the importance of dominant mutations and of heterozygous effects of deletions and other recessives is pointed out. The molecular changes underlying dominant mutations in man are more limited than is the case for recessive mutations. This raises the question whether mutagenic agents can produce the specific changes leading to recoverable, dominant mutations. Extrapolation from increased mutation rates to predictable increases of human disease, whether by doubling dose or direct methods, have been criticized.

Introduction

It is a well-known fact that about 10.5% of all live-born children are burdened with a disease or malformation of genetic etiology; this high natural burden will undoubtedly increase from exposure to mutagenic agents. Consequently, hereditary damage should play an important part in our concern regarding the genetic effects of chemicals. In contrast to chemically induced cancer in man, genetic diseases or abnormalities resulting from mutations induced in human germ cells have not yet been detected. An effort to assess and quantify genetic risks resulting from human exposure to mutagenic chemicals is urgently called for; otherwise genetic toxicologists may well lose credibility.

Genetic Biomonitoring and the Parallelogram

Biomonitoring provides us with a first indication of mutagenic effectiveness of a particular chemical exposure in human somatic cells. The chemicals and populations selected for genetic biomonitoring should receive priority in studies on the assessment of genetic risk. In populations exposed to complex mixtures, molecular analysis of the induced mutations may help identify the most mutagenic components. Extrapolation to what can be expected in germ cells of the exposed individuals should be possible by using good dosimetry and a parallelogram approach [Fig. 1 (*1–3*)]. Briefly, this approach is based on the principle that genetic damage which cannot be measured directly, such as that in human germ cells, can be estimated by measuring the same kind of damage in both germ cells and somatic cells of the mouse. If we have data on the induction of mutations or chromosome aberrations in both germ cells and somatic cells of the mouse, one would hope that this relationship would make it possible to estimate germ cell mutation frequencies in man on the basis of what has been measured in monitoring genetic damage in human somatic cells. The underlying assumption is, of course, that the quantitative relationship between damage induced in germ cells and somatic cells and their capacity for repair are not vastly different between mouse and man.

For those chemicals for which data on genetic effects in human somatic cells are available, I would favor research to obtain data on the induction of mutations and/or chromosomal aberrations in mouse germ cells in association with studies to measure the same kind of damage in

Department of Radiation Genetics and Chemical Mutagenesis, Sylvius Laboratory, Leiden University, P.O. Box 9503, Wassenaarseweg 72, 2300 RA Leiden, The Netherlands.

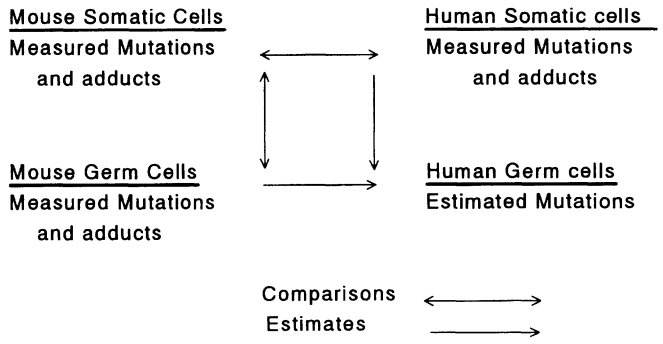

FIGURE 1. The principle of this parallelogram is that genetic damage in human germ cells can be estimated by measuring a common end point in humans and mice, such as mutations or chromosomal aberrations in lymphocytes, and a corresponding genetic end point in germ cells of mice, the desired target tissue inaccessible in man. Appropriate dosimetry should be carried out by the concomitant measurement of adducts (*2,3*).

somatic cells of the mouse. First, one would need to ascertain whether the selected chemical reaches the germ cells and interacts by the formation of adducts with germ cell DNA. If the kinds of DNA adducts are known, certain predictions for germ cell mutagenesis in the mouse can be made (*4,5*). In short, genotoxins with preference for alkylation of nitrogen atoms (S_N2 mechanism) are effectively repaired in cells with efficient error-free repair, such as mouse spermatogonia. This appears to be the reason genotoxins such as diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, ethylene oxide, and acrylamide failed to produce mutations in early-stage male germ cells of the mouse (*6,7*). In the absence of repair, such agents are highly effective genotoxins, particularly by producing chromosome breakage (*8,9*). In the mouse, all these agents are effective mutagens in late spermatids and in spermatozoa that have lost the capacity for repair. On the basis of Drosophila data, one would expect that the mutations induced would mainly consist of transversions and deletions. Mutagens with low *s* (Swain-Scott constant) like ethylnitrosourea and diethylnitrosamine predominantly alkylate oxygen atoms (S_N1 mechanism), such as the O^6 position of guanine and the O^4 of thymine. These lesions are slowly repaired, and that is why they have high mutagenic and carcinogenic potency. They produce base GC to AT and AT to GC transitions, respectively.

Dominant lethal studies may help define experimentally the most sensitive germ cell stage (*10*). In view of costs and labor involved with specific locus and heritable translocation studies, dose setting merits some consideration. Realistic conditions of human exposure most probably involve doses too low to measure genetic effects in the mouse. Linear extrapolation from the lowest effective dose level, assuming absence of repair, is the most conservative assumption. End points that can conveniently be measured in somatic cells, like micronuclei or *hprt* mutations, should be useful for selecting the appropriate dose levels (*11*). The determination of dose–effect curves at dose levels higher than the lowest effective dose in mouse germ cells may be less desirable if considered on a cost/benefit basis. It may be noted that end points representing chromosome breakage phenomena, such as dominant lethals in germ cells and micronuclei in somatic cells, are probably most convenient for defining the lowest effective dose. This raises the question of whether such data are representative for point mutations. From the pioneering studies by Vogel (*8,9*) in Drosophila, we know that agents preferentially alkylating nitrogen atoms are far more effective in breaking chromosomes than those preferentially alkylating oxygen. Comparative studies in mammals for various classes of agents on the capacity to induce mutations, say, in the spot test, and chromosome breakage seem of considerable interest. It would seem that measurement of genotoxic adducts in the mouse and in the exposed human populations may be more helpful in defining margins of safety. Data that have been explicitly collected to achieve a parallelogram kind of comparison are at present not yet available. For some chemicals like ethylene oxide and benzene, there are fairly extensive data, which I now briefly review.

Ethylene Oxide

In a large collaborative study, Tates et al. (*11*) recently reported results on hospital and factory workers occupationally exposed to ethylene oxide in the former German Democratic Republic. Dosimetry was carried out by determining hemoglobin adducts [N-(2-hydroxyethyl)valine-(HOEtVal)]. Factory workers received an estimated daily exposure of 13.2 nmole HOEtVal per gram hemoglobin (Hb). This value corresponds to a 40-hr time-weighted average (TWA) of 5 ppm ethylene oxide. With an average exposure for 12 years at 240 days per year and 8 hr per day, the total exposure time is $12 \times 240 \times 8 = 23{,}040$ hr to 5 ppm and the dose is 115,200 ppm•hour. Frequencies of cells with high frequencies of sister chromatid exchanges (SCEs) were enhanced by almost 2000%; chromosomal aberrations by 260%, micronuclei by 217%, and *hprt* mutants by 60%. The relative sensitivity of the various end points for the detection of ethylene oxide exposure is in the following order: HOEtVal adducts > SCEs > chromosomal aberrations > micronuclei > HPRT mutants. In view of the fact that ethylene oxide is a typical S_N2 type of alkylating agent, the prevalence of protein alkylation is in line with the expectation (*13*).

The higher sensitivity of the valine adducts and SCEs has been confirmed in a large, recent study by Schulte et al. (*14*) on U.S. and Mexican workers exposed to lower doses of ethylene oxide (EtO) within the U.S. Occupational Safety and Health Administration standard of 1 ppm 8-hr TWA. According to an estimate of the U.S. Department of Labor (*14*), in the United States alone, 224,000 workers are exposed to EtO.

The literature on the genetic effects of EtO has recently been reviewed by Dellarco et al. (*15*), and genetic risk assessments were reported by Dellarco and Farmlandt (*16*), Rhomberg et al. (*17*), and Dellarco et al. (*18*). The data

of Generoso et al. (*19*) for the induction of heritable translocation in the mouse were used. Translocations increased with a higher power of the dose than simple dose-squared (*17,19*). Here I assume that at low doses, the two breaks required for the formation of a translocation are formed by one event, and thus use linear extrapolation from the lowest dose point. Generoso et al. (*10*) were able to show that the response in males is restricted to late spermatids and early spermatozoa, in line with other S_N2-type genotoxins. The period of 10 days in male mice would correspond to approximately 21 days in humans (*17*). Different species of mammals show the same number of adducts at corresponding exposure levels. This species equivalence is of obvious importance, though we do not yet know whether the same frequencies of genetic changes are induced at corresponding adduct levels. If the human window of susceptibility is chosen to be 21 days, 120 hr of exposure are to be counted (3 weeks × 5 days per week × 8 hr per day). With an exposure of 10 ppm, the excess risk of fathering a translocation carrier would be about 5 per 1000 live-births (*17*). Gas chromatography data obtained in East Germany suggest exposure levels of 33 ppm in the past and of 17 ppm from 1989 onwards. The associated translocation risks would have been about 1.5% of births before 1989 and of 0.75% since then. With a background incidence of 19 translocation carriers per 10,000 live births (*17*), EtO exposure before 1989 has increased the risk by 750% and half of that after 1989.

Benzene

To integrate mutagenicity data into risk assessment procedures, Legator and Ward (*20*) advocate a multiple end point approach *in vivo* at realistic exposure levels. Benzene is cited as a case study. Cytogenetic monitoring of benzene-exposed workers established a lowest observed effect level in the range of 1–10 ppm (*21*). These exposure levels induce both micronuclei and SCEs in hematopoietic cells of rodents. Recent studies likewise show an increase in the frequency of *hprt* mutants in spleen lymphocytes of CD-1 mice (*20,22*), and this was paralleled by an increase in chromosome aberrations. A nearly linear response was reported for micronuclei in rats and mice, mutation induction, and chromosome aberrations in lymphocytes. It is of interest that *hprt* mutations are a more sensitive end point than chromosomal damage. There are some indications of genotoxic effects of benzene in germ cells, though the present data do not yet permit a parallelogram approach to quantify the expected damage.

Extrapolation from Increased Mutation Rates to Adverse Health Effects

The final aim of any genetic risk assessment consists of converting an estimated increase in mutation rate in adverse effects on human health. Two kinds of methods are usually employed: the doubling dose method and the direct method. With the doubling dose method, the damage is expressed as an increase relative to the natural load of genetic disease and malformations. It has the advantage that mutational damage is expressed as excess cases of known genetic disease, i.e., tangible human suffering. The validity of the use of the doubling dose method rests on various assumptions, such as *a*) the damage to the genetic material resulting from exposure to mutagenic agents is similar in nature to mutations arising spontaneously, and *b*) there is a proportionality between spontaneous and induced mutations. These assumptions become unlikely in the light of recent findings on the molecular nature of spontaneous and induced mutations (*23,24*).

With the direct method, dominant mutations affecting the skeleton and the opacity of the eye (cataracts) are measured in mice. Using a number of assumptions, these induced frequencies of dominant mutations are translated into an expected increase of the total load of dominant genetic disease in man. The idea that the probability of inducing a dominant disease affecting any bodily system is a simple multiple of the rate of induction of either skeletal or cataract mutations in the model system has been criticized (*24,25*). Sankaranarayanan's (*24*) argument is that the selected genes with dominant expression are flexible; that is, they have less functional constraints (see below), and it is not known whether they are representative of all genes at which dominant mutations arise.

In estimating the impact on human health of an increased frequency of translocations, a direct approach is also possible. In mice, heritable translocations induced by chemical mutagens are sometimes associated with neurological disorders, blindness, and skeletal abnormalities (*26,27*). In humans, cases are known where translocations are associated with skeletal abnormalities and various other conditions. Thus, translocation carriers have an increased risk of having a serious medical disorder, and one should realize that these are transmitted as simple Mendelian dominants. In addition, half of the gametes of a translocation carrier contain unbalanced, aneuploid segregants. Rutledge et al. (*27*) found a high incidence of developmental anomalies in mice, as exencephaly and cleft palate among descendants of translocation carriers. Although the majority of these unbalanced zygotes will lead to stillbirths in man, 6% of these are assumed to result in congenitally malformed children (*28*). We have seen before that the exposure to 33 ppm EtO will increase the incidence of translocation carriers, from a background of 19 per 10,000, to 150 per 10,000. Apart from the possible damage expressed in the carriers, these are expected to produce 300 per 10,000 unbalanced products, of which 6%, or 18 per 10,000, live births will be congenitally malformed.

In the populations exposed to EtO in East Germany, exposures have lasted 12 years on average. It is obvious that the risk of heritable translocation in the progeny of these populations is far from negligible. Consequently, more precise estimates based on a parallelogram approach would seem desirable. These could be obtained by comparing genetic end points in germ cells and somatic cells of mice to measurements of EtO adducts.

Problems in Mammalian Mutation Studies

An assessment of risks associated with the induction of mutations is not yet possible on the basis of the available mouse data. The difficulties encountered in obtaining appropriate mutation data from mouse germ cells can be illustrated by the available observations with EtO. Russell et al. (*29*) carried out a large-scale, specific locus study in which a total of 71,387 progeny were scored after exposure of male mice to high doses of EtO. No significant differences with historical control frequencies were observed. Lewis et al. (*26*) reported more positive results for electrophoretic and dominant mutations. It is assumed that these mutations are primarily derived from post-stem cells because of the negative findings for stem cells in the study by Russell et al. (*29*).

The paucity of data, the expenses involved, and the fact that there are only a few laboratories in the world where such studies can be carried out pose serious problems in genetic risk assessment studies. Future work with transgenic mice may be helpful in this respect. A database in which the nature and frequency of mutations induced in the germ cells and in somatic cells of normal mice are compared with what can be measured from the same cells and organs of transgenic mice may make it possible, in the future, to use transgenic mice directly for risk assessment studies, thereby, at least in part, circumventing the time-consuming and costly mouse mutation studies.

Expression of Mutations

The question of how mutations are expressed is of obvious importance. Mutations with a purely recessive mode of inheritance are not a matter of primary concern because it takes many generations before they are expressed through homozygosity. This is also the reason estimation of genetic risks either directly or by the doubling dose method is focused on simple Mendelian dominants. Recessive mutations are induced at considerably higher frequency than dominant ones; thus, even small effects in heterozygous condition of mutations with an apparent recessive mode of inheritance are important when estimating adverse effects on human health. In terms of deleterious effects in the heterozygous condition, multilocus deletions are of greater significance than single-site mutations. This observation has recently been demonstrated in the mouse, Neurospora, and Drosophila (*30–34*).

Well-characterized, multilocus deletions at different sites on the X-chromosome of Drosophila almost all lower viability in heterozygous condition [Fig. 2 (*34*)]. Genes essential for heterozygous viability are obviously missing in the deleted regions. Consequently, in terms of comparing genetic risks resulting from exposure to different kinds of mutagenic agents, those producing multilocus deletions should receive priority (*35,36*), such as cross-linking agents like hexamethyl phosphoramide (HMPA) and hexamethyl melamide (HEMEL), which in Drosophila

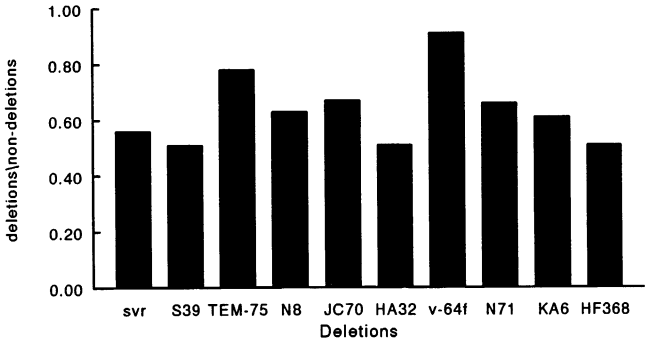

FIGURE 2. The viability in females heterozygous for deletions of the same size at different sites on the X-chromosome of Drosophila. It can be seen that almost all deletions lower viability in comparison to the controls (1.00) (*34*).

only produce deletions (Aquirrezabalaga et al., personal communication). In this respect, ionizing radiation is the prime example of an agent predominantly inducing deletions. In fact, most of the gene loci at which such mutations have been studied are nonessential for survival (*24,37,38*). Deletions in heterozygous condition lower viability, both in Drosophila and Neurospora. How this kind of damage is expressed in man and could possibly be quantified, we do not know. More than 40 years ago, Muller (*39*) stated that these deleterious changes in heterozygous condition will decrease the overall viability and fitness of an individual, will reduce life span, and will make the individual more susceptible to infectious disease.

What can one now say about recessive and dominant mutations in man? A recent review by Sankaranarayanan (*40*) has revealed a number of interesting facts. In general, it was noted that mutations in genes that code for enzymic proteins are mostly recessive, as, for example, in various inborn errors of metabolism. When a recessive mutation first arises, it is sheltered by the dominant wild-type allele in the heterozygote; that is, half the enzymic activity is on the whole sufficient for normal functioning. Therefore, a wide variety of molecular changes can be recovered; they range from base-pair substitution to rearrangements, duplications, and deletions.

For dominant mutations, however, the variety of observed molecular changes is more limited. They occur predominantly in genes that code for nonenzymic proteins, such as collagen. Here, half of the required product is not sufficient for normal functioning. Moreover, drastic alterations will be incompatible with survival, and thus the mutations will not come to light.

In view of these functional constraints, only a limited number of molecular changes leading to dominant mutations are compatible with survival. The crucial question, thus, is whether mutagenic agents are capable of producing the specific changes leading to recoverable dominant mutations (i.e., dominant mutations known to be involved in the causation of human disease). The coming years will see spectacular progress in the molecular analysis of human genetic disease, in particular through the coordinated

effort in the Human Genome Project. Comparison to the molecular changes leading to genetic disease with specific patterns of molecular change induced by the mutagens under study may in the future help to circumvent the problems with extrapolating from induced mutation to human disease.

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