CHEMICAL MUTAGENS

Principles and Methods for Their Detection Volume 8

Edited by Frederick J. de Serres

Sponsored by the Environmental Mutagen Society

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National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina

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Preface

Volume 8 of *Chemical Mutagens* covers a wide range of topics in this continuously changing field. This volume includes chapters on the detection of genetic damage in mammalian sperm both at specific loci and over the entire genome. The discussion of *in vitro* techniques for working with mammalian cells covers not only specific locus assays but also cellular activation systems. Another chapter extensively discusses the need for a revised protocol for the micronucleus assay. Structure–activity relationships are investigated in a chapter dealing with hair dye constituents. One of the most comprehensive chapters deals with problems associated with the detection of mutagenic effects in defined human populations. Finally, there is a detailed presentation of a comprehensive study tabulating the genetic bioassay data on some known or suspected human carcinogens. In keeping with our policy of publishing important legislation in the area of chemical mutagens, we have also included the Council of the European Communities Directive of 18 September 1979.

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CHAPTER 1

Detection of Effects of Mutagens in Human Populations

George R. Hoffmann

1. Introduction

The central issue in genetic toxicology is whether environmental agents are inducing mutations in people and thereby pose a threat to human health. The concern about health effects of mutagenesis encompasses both the transmission of mutations that are induced in germ cells to future generations and the consequences of genetic damage in somatic cells of exposed individuals. Among possible effects of somatic cell mutagenesis, the most notable is an increase in the incidence of cancer. The conceptual and experimental bases for associating cancer causation with somatic cell mutagenesis have recently been reviewed by Straus.[124]

Because of the possibility that mutagens can cause adverse health effects, much effort has been devoted to the identification of mutagenic agents in the environment. Test systems have been developed for the detection of mutagens in experimental organisms ranging from bacteria to mice. Although testing chemicals in experimental organisms is important throughout toxicology, genetic toxicology is especially dependent on experimental test systems because of the paucity of information on mutagenic effects in humans. In fact, there is essentially no

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data base on the induction of mutations in human germ cells and that on mutagenesis in human somatic cells *in vivo* is quite limited.

Although the mutagenic properties of chemicals should ideally be identified in experimental organisms rather than in people, there is nevertheless good reason for strengthening our capacity to study human populations for evidence of mutagenic exposure. One cannot preclude the possibility of human exposure to some potent mutagen that evades detection in experimental organisms; the ability to detect mutational effects in exposed people could be valuable in reducing further exposure. Human monitoring systems would also permit the screening of populations that have known or suspected contact with mutagenic substances. Such monitoring could be useful in determining whether there are actual biological effects of presumed exposures, in assessing mutagenic risks, and in identifying situations in which protective measures are inadequate.

Despite technical difficulties, significant progress has been made in the capacity to study human populations for evidence of mutagen exposure. The classical approach to population monitoring involves monitoring the phenotypes of offspring of the study population for evidence of mutational events. Monitoring progeny, however, requires large populations, is expensive, and would tend to be an insensitive indicator of mutagenesis. Consequently, effort is being directed toward the development of alternative methods of detecting and measuring mutagenic exposure; the indicators that are used include gene mutations and chromosomal alterations in blood cells, morphological or cytochemical alterations in sperm, nongenetic evidence of mutagen exposure, and the presence of mutagens in body fluids.

It is important to recognize the problems that have plagued the development of effective methods for detecting mutational effects in human populations. A major difficulty is the requirement that large numbers of individuals be studied to detect germinal mutations. In addition, genetic effects are often not manifest in the population until long after exposure. This chapter emphasizes methods that minimize these problems. However, even if ideal methods for monitoring human populations were available, complexities in epidemiological design must still be considered. Chemical exposures are often multiple, variable, and hard to measure; the patterns of exposure are typically complex. In order for increases in frequencies of genetic events to be related to specific environmental conditions, any population of interest must be compared with a carefully selected control population; without the controls, results are almost uninterpretable. In defining control and

exposed populations, careful attention must be paid to the existence of confounding variables.

This chapter briefly summarizes classical approaches for detecting increases in mutation rates in people and reviews new developments in the field. Cytogenetic methods are not covered in this chapter; however, the detection of chromosome aberrations,[35–37,40,55,56] micronuclei,[45,109] and sister chromatid exchanges[23,40,41,63,101] have been reviewed elsewhere. The possibility of monitoring populations for the presence of individuals with high sensitivity to mutagenesis[99,100] also falls outside the scope of this chapter.

2. Monitoring Progeny for Evidence of Germ-Cell Mutations

Means of monitoring progeny for evidence of increased gene-mutation rates in germ cells include monitoring for conspicuous phenotypes, such as genetic diseases, and biochemical monitoring for altered gene products. Monitoring for evidence of mutations in human germ cells is difficult because of the low frequency of individual genetic events, the complex etiology of many of the disorders that can be measured, the requirement that large populations be monitored, and epidemiological complications.

2.1. The Classical Approach: Phenotypic Monitoring

Classical approaches to monitoring emphasize the detection of increases in the frequency of certain "sentinel phenotypes" that are a reflection of the mutation rate. Unambiguously detected dominant mutations are the most obvious basis for monitoring, because they appear in the generation immediately after the mutational event.[32,131] To be suitable for use in screening,[32] a mutant phenotype should be dominant, uniformly expressed, without phenocopies, conspicuous and easy to diagnose, and present at early age. It is also desirable to select a characteristic that causes early sterility or death; dominant characteristics of this type could not have been inherited and therefore can be ascribed to mutation without confusion due to possible illegitimacy.[32] The ideal sentinel phenotype should not, however, cause early embryonic deaths that would be poorly detected and lead to underestimations of frequencies of occurrence.

Few disorders meet all criteria to be suitable for use as indicators of recent mutations. Apert's syndrome, achondroplasia, and aniridia

seem to be among the best candidates for serving as sentinel phenotypes.[51] These conditions, however, occur infrequently, and changes in their rates of occurrence could not be detected in small populations. Many characteristics, like cleft palate, congenital dislocated hip, diabetes, and epilepsy, are not suitable for monitoring, because of the complexity of their inheritance. A difficulty in selecting appropriate sentinel phenotypes is that a substantial proportion of genetic diseases, including some that are generally regarded as examples of simple dominant inheritance, have multifactorial etiology.[92–94] The selection of sentinel phenotypes is further complicated by the specificity of diagnosis of rare genetic diseases (e.g., epiloia) sometimes being inadequate.

The primary disadvantage of monitoring for sentinel phenotypes is that large populations would have to be screened to detect an increase in mutation rate. This disadvantage is compounded by the unavailability of ideal sentinel phenotypes and epidemiological factors. The sentinel phenotypes approach is therefore impractical for most purposes.[53] For example, it could not be applied in occupational or clinical settings, where the number of individuals with defined exposures is often small. When applied to large populations, moreover, as in collecting health statistics for many births, any increases that are detected in disease incidence may not be clearly ascribable to a known cause.

Monitoring for the occurrence of sex-linked recessive mutations has also been suggested, but would be somewhat less efficient than detecting autosomal dominants. Autosomal recessive mutations are not suitable for use in phenotypic monitoring, because they are not expressed for many generations.[32] Conditions of multifactorial origin are also not particularly useful for genetic screening, in that they would be relatively insensitive to changes in mutation rate. Monitoring for changes in sex ratio has been proposed,[51] but it is not clear that the sex ratio would provide a sensitive or specific indicator of genetic damage.[110]

The detection of embryonic, fetal, and neonatal deaths,[50,51] changes in birth weight, altered growth and development, and frequency of congenital defects[51,86] have also been suggested as characteristics for monitoring. These characteristics have the distinct advantage that they contribute to the burden of human disease and disability and are therefore important in their own right. The primary disadvantage in the use of these characteristics is that their causes are not necessarily genetic, and a variety of developmental, physiological, or even social factors can contribute to their reported rates of occurrence.[53] Loss of fertility, which has been reported in some cases of chemical exposure,[146,147] may similarly have physiological rather than genetic causes

and is therefore of limited value as a characteristic in mutational monitoring.[53] Several of these characteristics have the additional disadvantage that their identification and classification are somewhat subjective and thereby subject to appreciable variation.

Despite the disadvantages, there is some inherent merit in measuring the incidence of disorders, rather than relying on indicators of mutagenesis that do not have clinical significance. Newcombe[92–94] has emphasized the importance of not only being able to detect increases in mutation rate, but also in assessing their significance for human health and has stressed the value of collecting information on all genetic diseases that occur in a population. Methods of data collection, difficulties in obtaining reliable data, and possible applications of health statistics have been discussed in detail elsewhere.[25,51,60,92–94,123,131,141]

The mutagen for which health risks have been most thoroughly studied is ionizing radiation. The National Academy of Sciences' Committee on Biological Effects of Ionizing Radiations[28] has projected human risks for carcinogenesis largely on the basis of data from people and for germ-cell mutagenesis on the basis of tests in mice and data on the incidence of genetic diseases in human populations. Assessing risks posed by chemical mutagens is even more complicated than risk assessment for radiation, and our capability to do so is still quite primitive. Although quantification of the impact of mutagenesis on disease burden is difficult, the qualitative conclusion that no increase in the mutation rate in human germ cells is desirable is justified. Since it is likely that "any increase in the mutation rate will be harmful to future generations,"[28] prudence would dictate that mutagenic exposures be minimized.

Despite its limitations, surveillance for evidence of mutagenesis in human germ cells should continue. One should not derive great confidence, however, from the absence of detected effects. As pointed out by Sutton,[131] increases in the incidence of genetic disease are unlikely to be sensitive indicators of mutagen exposure.

Although monitoring for health effects may not alone give a clear indication of induced genetic damage, it can nevertheless be useful when combined with other approaches, in that it adds to the weight of evidence for mutational effects in a given population. For example, Bridges *et al.*[21] have compiled evidence from several sources on the occurrence of adverse genetic effects in smokers relative to nonsmokers. The study includes frequencies of perinatal mortality and congenital malformations, as well as analyses for chromosome aberrations, sister chromatid exchanges, the presence of mutagens in urine, and the occurrence of morphological sperm abnormalities.