Application of Integrated Genetic Monitoring: The Optimal Approach for Detecting Environmental Carcinogens

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Short-term in vitro genetic toxicity assays have not fulfilled their anticipated role in predicting the carcinogenicity of environmental agents reliably and economically. A redirection in emphasis from nonanimal systems to relevant animal assays and population monitoring will help to reestablish the credibility of this field. An analysis of the various steps in the carcinogenic process indicates the biological responses occurring during these stages can be utilized for early detection of environmental carcinogens. Emphasis should be placed on using the earliest significant response that indicates genetic damage (e.g., gene mutations and chromosome alterations). Assays that detect pregenomic damage (e.g., adduct formation), without evidence of subsequent heritable genetic alterations, may produce misleading results for risk assessment and should not be considered as stand-alone monitoring procedures. Late biological responses may occur in tissues or organs where genetic damage may be difficult to measure, and the opportunity for intervention diminishes as we approach the clinical outcome. For example, analyzing localized cells that contain activated protooncogenes and inactivated tumor suppressor genes, although they further document adverse response from exposure to carcinogens, may be of greater value for indicating clinical outcome than for genetic monitoring. With few notable exceptions, the window of opportunity for genetic monitoring is the period after exposure where genetic damage is evident and where circulating lymphocytes can faithfully record this damage. An ongoing study of butadiene-exposed workers illustrates an optimum protocol, where multiple assays can be carried out and correlated with both external and internal measurements of exposure. Population monitoring studies integrating the total effects of multiple chemical exposure are especially suited for monitoring communities near waste disposal sites or industrial plants. Studies to date indicate that these procedures are sufficiently sensitive to detect low-level chronic exposure to genotoxic chemicals.—Environ Health Perspect 102(Suppl 9):125-132 (1994)

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Introduction

A requisite for the survival of living forms is the ability to continually adapt and change. Since the beginning of the industrial revolution, organisms, including humans, have been required to adapt to a large number of new man-made chemicals in their environments. We are challenged to cope, not with discreet pure chemicals, but with various mixtures of chemicals and the products of their interactions. Our ability to respond and change is far from perfect, and even the very process of adaptation may lead, in some individuals, to adverse health outcomes. One basic form of adaptation to xenobiotic exposure is induction of enzyme systems that are involved in metabolism and detoxification (1). The attempt to detoxify xenobiotics frequently leads to the formation of electrophiles that interact with and alter DNA, thus setting in motion the complex, multistage process that is associated with cancer (Figure 1). A more complete understanding of the roles of genetically toxic agents in the complex process of carcinogenesis requires that genetic toxicology studies address the issues of metabolism and pharmacokinetics and that a broad spectrum of genetic events be detectable. This means that in vivo studies in appropriate animal systems should be used, and that relevant exposure conditions need to be evaluated. When feasible, studies of exposed human populations should be conducted. in vitro assays are of greatest value when they are used to examine the effects of specific metabolites or when mechanisms of genotoxicity of known proximate mutagens are to be determined.

To detect, and consequently reduce or eliminate our exposure to carcinogens, a number of complementary in vivo genotoxicity test procedures should be employed. Laboratory animals can be used as surrogates for humans, but human studies should be carried out whenever there are known exposures to potential mutagens and carcinogens. The need for human genetic monitoring is underscored by recognition of the differences in response between human and experimental animals, the potential for interactive effects from exposure to multiple chemicals, and limitations of cancer epidemiologic studies. A better understanding of the various stages in the multistep process that leads to cancer underscores the importance of early detection of genetic alterations that result from exposure to carcinogens. In this paper we will describe biological monitoring techniques as they relate to the various stages in the neoplastic process. Specifically we will indicate: a) those procedures offering the maximum opportunity for identifying carcinogenic exposure, b) limitations of these procedures, c) the limitations of classical epidemiologic studies, d) an example of an integrated genetic monitoring protocol (a current study of 1,3-butadiene), and e) an appropriate application of genetic monitoring assays.

Attributes of a Complete Carcinogen

A carcinogen, by definition, can transform normal cells to cancerous cells. As depicted in Figure 1, a complete carcinogen can induce the following detectable events: a) induces the initial genetic change leading...
to an initiated cell, b) may generate reactive oxygen species that cause further genetic alterations, c) produces further genetic alterations in the initiated cell leading to additional genetic damage, including oncogene activation and/or tumor suppressor gene inactivation, d) enhances clonal expansion of the initiated cell (promotion) and/or accelerates cell proliferation, and e) may modify immune responses. Some chemicals are active during some of the stages in this process that occur after cell initiation, thus increasing cell proliferation and enhancing the effect of the initial genetic lesion; but it is only a complete carcinogen that can influence all of the steps that lead to cancer. Given the biological attributes required of a complete carcinogen, it is understandable why we have found very few chemicals that are complete carcinogens. Since a complete carcinogen influences the various steps in the continuum that lead from initial genetic lesion to cancer, it follows that biological monitoring could be performed at any stage in this process. Before examining the various techniques available for monitoring the effects of a complete carcinogen, it may be worthwhile to discuss the concept of non-genotoxic carcinogens.

**Nongenotoxic Carcinogens**

Weisburger and Williams (2–4), have proposed the existence of a novel class of atypical, epigenetic or nongenotoxic carcinogens. Nongenotoxic carcinogens have been defined operationally as those chemicals that lack genotoxicity as their primary biological action, but yield genotoxic events as a secondary result of other types of activity, such as forced or accelerated cell proliferation. These chemicals do not react directly with DNA (5). Since non-genotoxic chemicals may be an important category of cancer-causing agents and they require attention different from genotoxic carcinogens, compounds should not be placed in this category until their complete mechanism of action is clearly established. Every attempt should be made to minimize describing chemicals as non-genotoxic simply because of incomplete, insufficient, or inappropriate testing. Ashby has correctly stated, “It is important to emphasize that nongenotoxic carcinogenic mechanisms will probably have a definable genetic basis when they are understood” (J. Ashby, personal communication) At the present time, dioxin (TCDD) is considered a prime example of a nongenotoxic carcinogen (5). TCDD is among the most potent chemical carcinogens that have been identified. This chemical is carcinogenic in many species at many sites. Its tumor-promoting, hormonally related activity has been well studied and characterized. However, this proposed epigenetic mechanism of TCDD-induced carcinogenesis does not account for the many relatively rare types of TCDD-induced tumors and other phenomena exhibited by this chemical (6,7). It is interesting to note that although this chemical has been extensively studied, an in vivo gene mutation assay after subacute or chronic exposure has yet to be reported. It may well be that just as benzene was initially classified as a nongenotoxic carcinogen due to incomplete testing, so we may find that TCDD is a genotoxic carcinogen. TCDD is a chemical that acts through many mechanisms, and trying to explain the overall biological activity of a chemical by a single mechanistic model can be misleading. If in vivo genetic monitoring tests are to serve as accurate procedures for detecting the effects of complete carcinogens, a battery of relevant assays must be carried out concurrently in an exposed population. There are several complementary procedures now available for human genotoxicity monitoring. Greater use of these tests would provide a means to correctly identify exposure to mutagenic carcinogens, thus minimizing the erroneous classification of a carcinogen as nongenotoxic.

**Biological Markers and Genetic Monitoring**

Biological markers are divided into three major categories: exposure, effect, and susceptibility (8). Genetic monitoring is mainly concerned with markers of exposure and effect, while genetic screening is concerned with susceptibility. Markers of exposure can include external exposure, which is an environmental measurement of chemicals or other agents presented to an organism, as well as internal exposure, a measurement of chemicals or their metabolites that are present within the organism. Measures of internal exposure include pharmacokinetic data, such as biological half-life, circulating peak, or cumulative dose. A biologically effective dose is the amount of material that can produce detectable effects in critical subcellular, cellular, and tissue targets. Markers of effect, such as genotoxicity, are responses of an organism that can be related to health impairment or the probability of health impairment (9). Genotoxic effects can be detected by using genetic monitoring procedures. Results of these tests can be correlated with, and are predictive of, cancer when analyzed in the context of an exposed population. In the evaluation of a population potentially exposed to a carcinogen, it is best to use both external and internal measurements of exposure and markers of effects.

Peripheral lymphocytes are the cell population of choice for most genetic monitoring studies because they are readily obtainable from a blood sample. One of the major advantages in using circulating lymphocytes is their contact with many body tissues, thus providing a more integrated measure of biological response to a chemical exposure (10). After exposure to a potential mutagen, reactive metabolites can be derived either from the inherent metabolic capacity of the lymphocytes, or, more

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**Figure 1.** Processes and factors involved in the transformation of normal cells to cancerous cells.
probably, from reactive metabolites picked up from other tissues such as the liver.

**Adduct Formation**

Many, if not most, carcinogens are electrophilic chemicals that react with nucleophilic sites of DNA resulting in covalent reaction products (adducts). In addition to the fact that most carcinogens form DNA adducts, there are several characteristics of adduct formation that would suggest that the determination of adducts would be an ideal procedure to use in genetic monitoring. Studies in experimental animals have shown a linear relationship between exposure to an electrophile and the rate of both DNA and protein adduct formation (11). This has also been demonstrated in humans exposed to ethylene oxide, and propylene oxide, and individuals exposed to ethylene from cigarette smoke (12). Even trace amounts of adducts formed from exogenous chemicals can be detected with present methods. However, there are several problems with adduct formation studies that must be considered when utilizing these procedures.

Although a linear relationship with adduct formation has been observed with many chemicals, nonlinear responses have also been documented. One example is the ethylating agent 4-(n-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone. When O6-methylguanine adducts were measured in rat lung after chronic exposure to this chemical, a nonlinear dose-response was observed (13). It should be emphasized that the adduct level found in DNA at any point following exposure is not determined by the rate of adduct formation alone, but also involves adduct elimination and cell turnover. Elimination is a function of the stability of the adduct and the type and nature of DNA repair processes (14). Adduct frequency can also be reduced by cell death or cells undergoing mitosis. In the case of the N-nitrosoamines, alkylated adducts are formed at the N and O atoms of the purine and pyrimidine bases, with the O6-methylguanine adducts being a major source for the development of mutations. The efficiency of the repair enzymes (O6-alkylguanine–DNA alkyltransferases) in replacing or selectively removing the O6 lesion is probably an important factor in determining the cancer risk of individuals exposed to N-nitrosoamines (15).

A single chemical may form a variety of different adducts, but not all of them will lead to mutations (15,16). Since the determination of adducts alone will not indicate the fixation of a genetic lesion, it should not be considered as a stand-alone procedure in the context of a genetic monitoring protocol. Studies with the potent hepatocarcinogen methapyrilene further underscores the advisability of determining adduct formation only in conjunction with assays that are further down the continuum that leads to cancer. Methapyrilene failed to induce the formation of detectable adducts in L5178Y cells at doses that induced mutations at the thymidine kinase locus (17).

Although the ability to measure trace amounts of an adduct can be considered an advantage, the extreme sensitivity of these procedures has often outstripped our ability to interpret what is measured. Workers who are not knowingly exposed to ethylene oxide have measurable amounts of hydroxethyl adducts in their hemoglobin (18). The presence of these adducts in non-exposed workers is an example of the difficulty in interpreting results having to do with adduct formation. The determination of adducts therefore should be viewed as a possible measure of exposure and potential genomic damage. The sum total of all adducts in a particular cell or cell population does not necessarily indicate the risk of tumor formation. The formation of adducts is, however, of value when viewed in conjunction with assays that represent events further along on the road to cancer.

**Initial Genetic Damage in Exposed Cells**

The greatest opportunity for genetic monitoring is in the early stage in the neoplastic process following the initial fixation of the genetic lesion in exposed cells. At this stage, we can employ a variety of procedures that identify the ability of the chemical to induce genetic lesions that can survive repair processes. A fundamental event in the carcinogenesis process, induction of heritable genetic damage, has been satisfied. Genetic lesions can be detected in easily available lymphocytes, but the lesions are not necessarily tissue-specific. In later stages in the neoplastic process, genetic alterations in the progeny of initiated cells tend to be increasingly localized in specific tissues and organs. Obtaining cells from potentially affected tissues or organs would be difficult and sometimes not possible. By using available lymphocytes, several of the most valuable procedures for genetic monitoring can be applied during or soon after exposure at a very early stage in the neoplastic process. Both point mutations and cytogenetic damage can be determined. The hprt mutant lymphocyte assay and gycophorin A assay are two tests available for determining somatic cell mutations and cytogenetic assays that can be used to include chromosome aberration studies and the micronucleus test, as well as the sister chromatid exchange (SCE) assay.

**The Hprt Assay**

The hypoxanthine-phosphoribosyl-transferase (hprt) assay (19) is one of the most fully developed tests for somatic cell mutations in humans. This test identifies and selects mutant cells in a single step by taking advantage of the biochemical pathway by which cells synthesize DNA. DNA is synthesized in two ways: either from nucleotide bases de novo or from bases recycled from degraded DNA (the salvage pathway). HPRT is one of the enzymes involved in the salvage pathway. The assay selects for mutant cells having a nonfunctioning hprt gene. The selective agent used in the hprt assay is 6-thioguanine, a toxic purine analog. When incorporated into DNA, 6-thioguanine causes cell death. Mutant cells, lacking HPRT, are unable to incorporate the 6-thioguanine. Thus, they survive in short-term culture and can be efficiently detected by either an autoradiographic or clonal assay. Mutants can be detected since the hprt locus is on the X chromosome. Only one functional copy of the hprt gene is present in the cells of subjects of either sex. Several studies have demonstrated the feasibility of this assay for determining effects of exposure to mutagens–carcinogens, including the ability to discriminate between cigarette smokers and nonsmokers (20,21).

**Sister Chromatid Exchanges**

SCEs occur through mechanisms that involve DNA breakage and rejoicing. Sister chromatids can exchange seemingly identical segments of DNA without known alterations of cell viability or function (22). SCE studies have been used in a variety of in vitro and in vivo tests to determine exposure to mutagenic–carcinogenic agents. The exact mechanism of the formation of SCEs is not known, although replication or recombination models have been proposed (23). The recombinant model is based on chromatin exchange as part of a post-replication repair process (24,25). The replication model involves recombination during DNA replication (26,27). The processes that lead to the formation of SCEs are believed to be distinct from those that lead to chromosome aberrations (28).

Chromosome aberrations can result from exposure to chemicals that can induce either single- or double-strand breaks. In
contrast, SCEs can best detect chemicals that have covalent interactions with DNA. Therefore, ionizing radiation and carcinogens such as bleomycin, which are readily detected by chromosome aberration studies, do not produce SCE. Although SCE may be a sensitive indicator for the presence of certain carcinogenic agents, the lack of understanding of the basis of the SCE induction, its failure to detect some known carcinogens, and the lack of relationship with specific health effects limits its utility. If only a single cytogenetic procedure is selected for genetic monitoring, it should not be the SCE assay.

The Micronucleus Test

Micronuclei consist of small fragments of DNA that can be detected in the cytoplasm of a daughter cell after cell division. Micronuclei are formed by the action of chemicals that induce chromosome breaks or agents that cause damage to the spindle apparatus (29). The types of genetic damage that contribute to the formation of micronuclei include: damage to kinetochore proteins that affect the centromere and spindle apparatus and lead to unequal chromosome distribution at anaphase, and unrepaired DNA strand breaks that result in acentric chromosome fragments. Studies using kinetochore antibodies to identify whole chromosomes suggest that approximately 50% of spontaneously occurring micronuclei are the consequence of whole chromosome loss and the rest presumably are derived from acentric chromosome fragments (30–32). Micronucleus assays in humans can be conducted using peripheral blood lymphocytes (33), erythrocytes from splenectomized individuals (34), and exfoliated cells from the buccal mucosa or the urinary tract (35). A relatively new technique for use with lymphocytes is the cytokinesis-block micronucleus assay. In this procedure, cytochalasin-B is employed to stop dividing cells from performing cytokinesis; thus allowing cells that have completed one nuclear division to be recognized by their binucleate appearance. This assay is more accurate and more sensitive than the conventional lymphocyte micronucleus assay which does not distinguish between dividing and non-dividing cells (36). The micronucleus test is a valuable complement to the chromosome aberration procedure and is comparatively easy and inexpensive to perform. However, the specific location of the chromosome damage cannot be determined, and rearrangements will not be detected.

Chromosome Aberration Assay

This assay is frequently used to document the induction of chromosome breakage and rearrangement resulting from exposure to mutagens and carcinogens. A variety of studies have been performed to indicate that chromosome aberrations are the consequence of DNA damage and abnormal repair and replication. For example, Natarajan et al. (37) used Neurospora endonuclease to convert radiation-induced single-strand DNA breaks into double-strand breaks in irradiated cells and observed a significant increase in chromosome aberrations. They concluded that DNA double-strand breaks are the ultimate lesions for the formation of chromosome aberrations. By incubating damaged cells in the presence of a DNA repair inhibitor, histone, and a radiosensitive, Preston (38) was able to demonstrate that chromosome aberrations can be caused by abnormal DNA repair processes. Using the premature chromosome condensation technique to reveal chromosome aberrations in different phases of the cell cycle, Sognier and Hittelman (39) showed that mitomycin-C-induced chromatid breaks are the consequence of incomplete DNA replication. Chromosome aberrations can, therefore, be caused by many different types of hazardous agents, and by different mechanisms. Ionizing radiation and radiomimetic chemicals (e.g., bleomycin and neocarcinostatin) cause the formation of chromosome-type aberrations, while the majority of chemicals induce chromatid-type aberrations. Thus, the chromosome aberration assay can be used as a general biological marker for documentation of exposure to potentially hazardous agents. Since chromosome abnormalities are frequently observed in cancer cells, in spontaneously aborted fetuses, and in abnormal newborns (40–42); the detection of chromosome aberrations in an exposed population is viewed as a pathobiological response to exposure, and as an indication of potential long-term health consequences.

It is well known that most of the detectable chromosome aberrations in the standard cytogenetic assay are lesions that are incompatible with cell survival. The long-term health consequences are, therefore, caused by the damaged cells that survive. For a precise determination of health risk, it is often necessary to identify abnormal cells that are capable of surviving and replicating. These abnormal cells may be identified by the use of chromosome banding analysis to detect viable cells having balanced chromosome translocations. In addition to the use of banding procedures, chromosome rearrangements can also be detected by using fluorescent antibodies that can recognize specific chromosomal DNA sequences (43–45).

Chromosome/Genetic Instability

Although conventional cytogenetic and gene mutation studies are established procedures for detecting exposure to a number of carcinogenic agents, other relevant biological effects may not be documented by these procedures. The interaction of carcinogenic metabolites with cellular macromolecules may alter the normal cell processes and may not be detected by the standard genetic assays.

Evidence suggests that carcinogen-exposed, potentially committed cells are unable to stabilize (or normalize) their genomes (46,47). One of the mechanisms to explain this phenomenon is that these cells are unable to repair additional DNA damage correctly. In other words, these cells may be much more susceptible to the induction of damage from exogenous and endogenous sources than nonexposed and noncommitted cells. We have developed a DNA repair assay (challenge assay) to elucidate this phenomenon (48). In this assay, lymphocytes from subjects exposed in vivo to carcinogens are subsequently administered gamma-rays in vitro in the G0 or G1 phase of the cell cycle. These cells are, therefore, challenged to repair the radiation-induced DNA damage. Cells previously exposed to toxic pollutants may have exposure-induced DNA repair deficiency and may have trouble repairing the radiation-induced (challenge) DNA damage. They will therefore have more chromosomal abnormalities than nonexposed cells in our challenge assay. In a study of cigarette smokers compared to nonsmokers, we showed that smokers had a significantly higher frequency of chromosome translocations than nonsmokers in the challenge assay (49). We have also shown that mitomycin-C (a DNA cross-linking agent) and nickel (a nongenotoxic carcinogen) can significantly enhance the frequency of radiation-induced chromosome aberrations (48). This phenomenon is observed even when nonclastogenic doses of mitomycin-C or nickel were used. The cytogenetic fidelity repair assay (challenge assay) is a useful addition to the battery of tests that are available for detecting biological effects that are relevant to the development of cancer and other long-term health problems.
Initiated or Predisposed Cell; Diminishing Opportunity for Genetic Monitoring

The initiated cell and the immediate progeny of the initiated cell exhibit both an altered responsiveness to their microenvironment and a selective clonal advantage when compared to surrounding cells \((50, 51)\). These changes are believed to occur more likely in cells having genetic instability. Exposure to agents that are tumor promoters results in proliferation and/or survival of the initiated cell to a greater extent than normal cells. Continuing cell proliferation enhances the probability of additional genetic errors including endogenous mutations to accumulate in the expanding population of these initiated cells. Although clinical cancer can theoretically result from a genetic alteration in a single cell (the clonal origin of cancer) and from exposure to any amount of a carcinogen \(\text{i.e.},\) no exposure threshold, the probability of a subpopulation of initiated cells converting to detectable malignancy can be substantially increased by the further exposure of initiated cells to DNA-damaging agents. The emerging cell population may consist of cells with activated protooncogenes and/or inactivated tumor suppressor genes that affect regulation of growth and differentiation pathways. Cells having these specific genetic alterations are increasingly localized in specific organs and tissues. This localization increases the difficulty of obtaining suitable materials for genetic monitoring.

Molecular analysis of mutationally activated protooncogenes \(\text{e.g.},\) ras in animal models of carcinogenesis supports the concept of a mutational spectrum that is characteristic of specific types of mutagenic agents. For example, the mutations found in activated ras oncogenes that are associated with tumors of rodents exposed to the methylating \(N\)-nitroso compounds are mainly caused by methylation of deoxyguanine at the \(O^{6}\) position followed by mispairing with thymine during DNA synthesis. The type of mutation leading to activation of protooncogenes and/or inactivation of tumor suppressor genes may also be indicative of the tissue in which the malignancy is found \((52)\). Following a period of genetic instability \(\text{gene amplification and altered expression, change in chromosome number and structure,}\) the resultant cell population may contain cells with specific activated oncogenes or inactivated suppressor genes. However, at this stage, the transformed cells may be localized in specific tissues, and positive assays may be mostly indicative of clinical outcome rather than being useful in preventive genetic monitoring.

The Narrow Window of Opportunity for Genetic Monitoring

In Figure 2, we have indicated the specific stage in the neoplastic process that offers the greatest opportunity for genetic monitoring. The earliest significant biological response that indicates unrepaired genetic damage occurs after adduct formation. At this stage cytogenetic studies as well as assays for point mutations can be conducted. As we move further along the continuum to cancer, clonal expansion of mutated cells occurs in the tissue in which the tumor will form. This poses difficulties for obtaining samples for analysis. Analyzing tissue to detect cells that contain activated protooncogenes or inactivated suppressor genes would further document an adverse response from exposure to carcinogens; but, as previously discussed, this may be of greater value for indicating clinical outcome rather than for genetic monitoring. With a few notable exceptions \((53)\), the window of opportunity for genetic monitoring is the period shortly after exposure \(\text{or during chronic exposure}\) when genetic damage has occurred and where circulating lymphocytes can be used to detect this damage.

**Figure 2.** Specific stages in the neoplastic process that offers the greatest opportunity for genetic monitoring.

Limitations of Cancer Epidemiological Studies Necessitates Genetic Monitoring

In characterizing the need and value of genetic monitoring, one must also consider the alternatives presently available for determining the biological consequences of exposure to carcinogenic-mutagenic agents. The most definitive procedure for detecting adverse health effects is the conventional epidemiologic study, a procedure focused on the final disease outcome. In the area of chronic diseases, however, traditional epidemiologic studies often lack sensitivity and thus should not be considered our primary method for detecting harmful exposures to hazardous substances. Furthermore, the prediction made in 1982 \((2)\), that few human epidemiologic studies of exposure to known animal carcinogens would be carried out, seems to have been accurate. A survey of industry concerning epidemiologic studies on 73 animal carcinogens identified by the International Agency for Research on Cancer \(\text{IARC}\) found that epidemiologic data were lacking for approximately 90% of the animal carcinogens and that industry anticipated few further studies \((54)\). In addition to financial considerations, the following were some of the reasons for not considering epidemiological studies: a) insufficient time
lapse since beginning of exposure, b) small work force, c) mixed exposure, and d) short exposure period for each chemical (52).

In examining the reasons given for not initiating epidemiologic studies, one is struck by the fact that biological monitoring methods, such as cytogenetic and somatic cell mutation studies, would overcome the difficulties cited. The cost of biological monitoring is a fraction of the cost of typical epidemiologic studies. The outcome is seen concurrently with exposure, thus overcoming the need to wait for the final disease outcome (which frequently results in death). The effect of mixed exposures is integrated into the overall response that is detected, and the number of subjects needed for a conclusive genotoxicity monitoring study is minimal (approximately 50 to 100) in comparison to epidemiologic studies that may involve thousands of subjects.

**Studies with 1,3 Butadiene: Example of an Integrated Genetic Monitoring Program**

1,3-Butadiene is a high-volume chemical used in the manufacture of synthetic rubber (styrene-butadiene rubber or polybutadiene rubber) and of thermoplastic resin. The annual production volume of this chemical is approximately 12 billion pounds worldwide with 3 billion pounds produced in the United States (55, 56). Approximately 65,000 workers are potentially exposed to 1,3-butadiene (57). Limited initial toxicologic studies indicated a low order of toxicity (58). Subsequent studies, however, indicate that this chemical is a potent multispecies, multiorgan carcinogen (59). It is found to be carcinogenic in B6F1 mice with the lowest concentration tested (6.25 ppm) (59). The conversion of 1,3-butadiene to the reactive metabolites 1,2-epoxy-3-butenne and diepoxidebutane have been identified in rats and mice (60).

Although metabolic and pharmacokinetic differences have been identified in different species after exposure to the chemical, the interpretation of the data as related to human exposure is questionable. An analysis of available epidemiologic studies, however, support the conclusion that 1,3-butadiene is a human carcinogen (59).

Genetic monitoring of workers exposed to this chemical would determine if current exposure levels produce detectable genetic damage and further indicate the sensitivity of humans to this chemical. At the present time several laboratories are carrying out an investigation with a population of workers exposed on average to 1-3 ppm of butadiene. The protocol includes determining adduct formation, induction of hprt variants, chromosomal aberrations, DNA repair problems using a challenge assay, sister chromatid exchanges, and urinary metabolites. The different assays are conducted using the same population, thus increasing the sensitivity of the genetic monitoring. Furthermore, the biological data can be correlated with the metabolic information. The initial results of the pilot study (61) indicate that present exposure levels produced an increase in hprt variants and that this increase can be correlated with a butadiene derived urinary metabolite.

The present genetic monitoring studies with 1,3-butadiene may serve as a model for future studies. A high-volume industrial chemical was selected and workplace exposure measurements were available. Critical indices of exposure and biological effects will be determined. The initial results of the pilot study have already answered critical questions as to the potential hazard of this chemical under current exposure conditions.

**Conclusion**

With better understanding of the multi-stage carcinogenic process, we see a window of opportunity for genetic monitoring. Since populations at risk are exposed to many hazardous agents, which may operate via different mechanisms, it is necessary to use assays that can detect the sum total of genetic damage. Evidence is presented to emphasize that the most critical stages in the neoplastic process for genetic monitoring are at the stages in the process that occur after formation of the genetic lesion leading to gene and chromosomal mutations. Pregenomic damage studies (adduct formation) will not indicate the extent of repair or subsequent genetic damage. On the other hand, as we proceed on the path to cancer, later events may occur in non-accessible target organs. Another criteria for selecting the best mix of assays is to utilize only those procedures where the result can be readily interpreted. The SCE assay is an example of an assay where results are not readily understood, therefore, it cannot be used as a stand alone assay. The ideal approach for population monitoring and subsequent risk assessment, therefore, would utilize a battery of relevant biological procedures carried out concurrently utilizing readily available peripheral lymphocytes. The challenge assay, cytogenetic studies, and gene mutation assays, when used on the same population, form the basis of an optimum study. In addition to biological indicators, it is desirable to determine critical metabolites, which can then be correlated with the biological assays. Adduct formation and SCE studies, integrated into a comprehensive protocol, may offer additional supporting information for risk determination.

An example of a model genetic monitoring study is the present ongoing international collaborative study with workers exposed to low levels of butadiene. In this comprehensive study, critical biological endpoints including gene mutations, and chromosome abnormalities are being studied. In addition, the challenge assay as well as ancillary studies including SCE and adduct formation are being conducted with the same population. The biological studies are also being correlated with urinary metabolites. The present butadiene study illustrates what can be accomplished with these techniques in evaluating low dose chronic exposure to a chemical or chemical mixtures.

Properly conducted genetic monitoring studies are the ideal approach for monitoring population exposed to suspected carcinogenic agents. These studies will produce relevant information in our target species—man—and can be used for risk assessment. The need to utilize genetic monitoring in both the occupational and nonoccupational setting is readily understood with the realization that the alternative to genetic monitoring is the costly, time consuming and insensitive traditional

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