UNDERESTIMATING RISK FOR THREE IMPORTANT HUMAN CARCINOGENS: VINYL CHLORIDE, BENZENE, AND BUTADIENE

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Contrary to frequently expressed opinions, the extrapolation from high-dose animal experiments to low-dose chronic human exposure may underestimate rather than overestimate the true risk of exposure to carcinogens. Benzene, vinyl chloride, and butadiene are examples where animal risk extrapolation may significantly underestimate the actual risk. Since a variety of different enzymes or metabolic systems are involved in bioactivation reactions, which include both phase 1 and phase 2 reactions, any concentration dependent alteration in either activation or detoxification would have a significant effect on mutation and cancer. Limited evidence would indicate that proportionately less active metabolite is formed at high concentration where phase 2 enzymes predominate, while at lower concentrations pathways leading to active metabolites are favored. The overall effect would lead to an underestimate of risk from high-dose animal experiments when extrapolating to low-level, chronic human exposure. Data with three potent carcinogens, benzene, vinyl chloride, and butadiene indicate the possible error in risk estimation from animal cancer bioassay studies. This may be a general pattern with chemicals that require enzymatic conversion to active metabolites. The public health significance of this underestimation could be considerable and underscore the urgency of reexamining our present risk procedures. New procedures, including biomarkers of effect such as chromosome painting, DNA repair evaluation, and somatic cell mutation studies can be applied to monitoring individuals exposed to toxic substances. Known animal carcinogens as delineated by the International Agency for Research on Cancer, where there is a distinct possibility of underestimation of risk based on animal studies, should be evaluated by multiple biomarker studies in exposed populations.

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2. Abbreviations: DNA, deoxyribonucleic acid, GST, glutathione S-transferase, IARC, International Agency for Research on Cancer, MTD, maximum tolerated doses.

3. Key words: benzene, butadiene, risk estimation, vinyl chloride.

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INTRODUCTION

The last ten years have seen an unprecedented development of procedures, including biomarkers of effect such as chromosome painting, deoxyribonucleic acid (DNA) repair evaluations, and somatic cell mutation assays, that can be applied to monitoring individuals exposed to toxic substances (Au, 1993). These new procedures, as well as older conventional ones, are being underutilized in human monitoring studies (Legator, 1994).

In establishing priorities for testing chemicals, one has to question the wisdom to continuing to allocate sparse resources to test chemicals that are well characterized as human carcinogens. Spending time and money conducting further studies on well-characterized human chemical carcinogens will be of little value to the victims of chemical exposure. The pressing need is to clean up the known hazardous chemical pollutants.

Where significant human exposure exists, chemicals already identified as animal carcinogens (IARC 2A or 2B) should receive our immediate attention for application of these new and relevant genetic screening procedures. The need to reevaluate this group of chemicals is especially critical given the existing data which suggest that current risk assessment, based on extrapolation from high-dose animal studies to low-dose chronic exposure in humans, underestimates the true risk of chemical exposure.

In this paper I will (a) review data which indicate that extrapolation from high-dose animal experiments to low-dose human exposure may underestimate the true genotoxic (carcinogenic) risk for human exposure to vinyl chloride, benzene, and butadiene; (b) indicate how in vitro and in vivo mechanistic and toxicokinetic studies with butadiene were misinterpreted and led to faulty conclusions as to the true risk of human exposure; and (c) discuss the need to utilize existing biomarkers of effect to characterize populations exposed to known animal carcinogens.

EXTRAPOLATING FROM HIGH-LEVEL ANIMAL EXPERIMENTS TO LOW-LEVEL HUMAN EXPOSURE UNDERESTIMATES TRUE RISK

In traditional lifetime cancer bioassays in laboratory animals, chemicals are usually tested at the Maximum Tolerated Doses (MTD) and fractions of the MTD. The rational for using high levels, upon which to base human exposure at low chronic levels, is the belief that this approach maximizes the detection of carcinogenic chemicals. This procedure is usually viewed as, and in theory should provide, a means to prevent human exposure to even weak mutagens (carcinogens). Several investigators have criticized this approach as being overly conservative, unrealistic, and capable of producing false positive results (McClain, 1994). There are several examples with important industrial chemicals, however, which indicate that extrapolation from high-dose animal experiments to the more typical low-level human exposure is not conservative but significantly underestimates the true risk to humans.

Vinyl Chloride

Bailar III et al. (1988), evaluating a series of chemicals, reported on the incidence of liver angiosarcoma in female rats after exposure to vinyl chloride. The measured dose-response curve for angiosarcoma induced by vinyl chloride indicated a tenfold underestimate of risk when high-level exposure (3000–6000 ppm) was compared to doses in the 1–50 ppm range. The authors concluded that "... in a high percentage of animal bioassays, the one-hit formula is not conservative when applied in the usual way to animal data. It remains possible that the one-hit formula may indeed be conservative at sufficiently low doses (below the observational range), but the usual procedure applied to the usual dose range can be nonconservative in estimating the slope of the formula at such low doses. Risk assessments for regulation of carcinogens should incorporate some measure of additional uncertainty." The authors suggest a number of reasons for this underestimation including saturation of enzymes at high doses.

Vinyl chloride can be considered a prototype of a group of carcinogens where there is a degree of similarity in regard to the drug-metabolizing enzymes including phase 1 and phase 2 enzymes responsible for both activation and detoxification. If we address those chemicals where P450 is one of the major phase 1 biotransforming enzymes, we could include a wide number of xenobiotics (Halpert et al., 1994). Since a variety of different enzymes or metabolic systems are involved in bioactivation reactions, which include both phase 1 and phase 2 enzymatic systems, any concentration-dependent alteration in either activation or detoxification would have a significant effect on mutation and cancer formation. These concentration-dependent differences would then be translated into different quantitative conclusions in estimating risk from animals to man.

Benzene

Benzene is known to interact with pluripotent stem cells to induce hematopoietic neoplasms (Kalf, 1987). In mice exposed to benzene for 22 hours per day seven days per week for six weeks at 40 100 and 1000 ppb, an increase in somatic cell mutations was detected with the autoradiographic *hprt* mutant lymphocyte assay (Ward Jr. et al., 1988). At all three doses, a significant increase in mutant frequency over the controls was observed. At the highest dose, however, there was a significant decrease in mutant frequency from the values obtained at the medium-dose level. Additionally, in a study to determine chromosome damage in lymphocytes (mixed chemical exposure including benzene), mice exposed for six weeks were evaluated. Chromosome breakage increased at the low and medium concentrations when compared to controls, but not at the high concentration. This lack of response at the high concentration appeared to correlate with a significant induction of glutathione S-transferase (GST) enzymes in the liver. The authors concluded that, since GST is part of a detoxification pathway, induction of this enzyme system by the higher doses of benzene may have detoxified the benzene to less mutagenic or non-mutagenic metabolites (Au et al., 1988).

In a series of papers (Medinsky et al., 1989a, 1989b; and Henderson 1989, 1992) using data from in vivo studies with mice, rats, and monkeys, and also a physiological model, both activation and detoxification pathways were investigated. At high benzene concentration in mice (600 ppm), phenyl conjugates are the predominate urinary metabolites. At low concentrations (25 ppm and

lower), the level to which humans might be exposed, the predominate toxic metabolites are hydroquinone conjugates and muconic acid. A major conclusion of these studies is that in three different animal species (mice, rats, and monkeys), the metabolic pathways leading to production of the putative toxic metabolites appear to be low-capacity, high-affinity pathways that are saturated at relatively low-exposure concentrations. The detoxification pathways are predominately high-capacity, low-affinity pathways, and they increase at higher concentrations. The authors conclude, "If the total formation of the putative toxic metabolites is predictive of the toxicity of benzene, then the animal studies suggest that calculations of the risk associated with low-dose exposures based on the results of animal studies conducted at [high] doses would underestimate the toxicity of benzene."

1,3-Butadiene

In a review article that suffered from unfortunate timing, Bond et al. concluded that butadiene is not genotoxic in occupational exposed workers, and that the preponderance of evidence suggests that butadiene will not be carcinogenic to humans at current occupational or environmental exposure levels (Bond et al., 1995). Shortly after this report, Delzell and Cole confirmed that butadiene should be classified as a human carcinogen (Delzell et al., 1995). Just prior to the Bond et al. report, Legator et al. (1993) and Ward Jr. et al. (1994) reported that in workers exposed to only 1–3 ppm of butadiene, there was a significant increase in mutant frequencies when evaluated by the autoradiographic *hprt* mutant lymphocyte assay (Delzell et al., 1995). Additionally, Au et al. (1995) reported increased levels of chromosome damage in a DNA repair assay in the same group of workers.

Major reasons for misinterpreting and indeed being misled by mechanistic and pharmacokinetic studies with butadiene may be due to the fact that non-relevant in vitro data and acute high-level exposure animal data formed the basis for many of the inferences on potential butadiene effects with chronic low-level human exposure. In the study by Himmelstein (Himmelstein et al., 1994) inferences about the presence of the butadiene mono or dioepoxide in blood were made only after a 6-hour exposure using 62.5 ppm of butadiene as the lowest concentration, with the maximum concentration being 8000 ppm. In vitro data formed the bases leading to the conclusion concerning differences in tissue metabolism, activation/detoxification kinetics, and potency of both the mono and dieposxide. Valid conclusions should be base primarily on chronic, low-level animal exposure and human monitoring data.

An example of the proper use of animal studies is the analysis of hemoglobin adducts in rats and mice (Osterman-Golkar et al., 1993). In the adduct formation studies similar frequencies in adduct formation were found at low concentrations. Species divergence only occurred at high concentrations. It is also significant that hemoglobin adducts from butadiene exposure were found in a population of workers exposed to only 1–3 ppm. One of the major conclusions by Bond, that any cancer risk assessment for butadiene should use in vitro human tissue metabolic data, has proven to be incorrect.

The overall findings with butadiene indicate that species may vary quantitatively but not qualitatively as to butadiene metabolites, and that extrapolation from high-level acute studies

may not be significant in terms of low-dose, chronic human exposure. Given the nature of the metabolic activation pathways and the detoxification pathways, it is probable that a shift in metabolism and detoxification also occurs at high doses. This suggests that, like benzene and vinyl chloride, extrapolation from high-dose animal studies to low-dose human exposure may underestimate the risk of this chemical. Butadiene is a prime example of how one can be misled by inappropriate mechanistic and pharmacokinetic studies.

FUTURE DIRECTIONS AND MONITORING NEEDS

In the future there will be numerous opportunities for genetic monitoring in the workplace and in communities exposed to toxic substances. Given the difficulties in identifying populations and scarce resources for conducting genetic monitoring, we must set priorities as to the types of chemical exposures and the nature of the exposed populations to be studied. The lowest priority should be given to those populations that are known to be exposed to high concentrations of mutagens and carcinogens. Clearly, if a population is exposed to high levels of known toxins (such as carcinogenic polyaromatic hydrocarbons, benzene, and vinyl chloride) there is little benefit to be derived for doing yet another study. Exposure to known toxins should require reduction or eliminating exposure. The highest priority should be given to those chemicals where there is substantial exposure, and where animal, or perhaps limited human studies indicate that the chemical is a carcinogen, IARC 2A or 2B chemicals. It is in this category of chemicals that we have most likely incorrectly estimated risk by extrapolating from high-dose animal studies to low-level human exposure. If vinyl chloride, benzene, and butadiene represent a more general phenomena, we have probably underestimated the risk of several carcinogens. Human monitoring will allow us to more accurately determine the risk for individuals exposed to these agents.

In addition, we must set priorities with IARC 2 chemicals receiving the highest priority. We should also consider occupational and population exposure to low levels of known toxins. These studies may permit more accurate regulatory decisions.

The argument for continually testing high levels of substances known to be genotoxic is usually based on the need for method development and validation. Unfortunately, the history of the field of genetic toxicology is one of continually evaluating methods that are seldom applied to what should be our immediate goal–eliminating or modifying human exposure to carcinogenic and mutagenic substances. Clearly, we have underutilized the methods that are already validated and are currently available for human monitoring studies. Method development and validation are critical, but we should keep in mind that the reason for developing and validating methods is to apply these techniques to identifying and characterizing genotoxic substances. Given the fact that we are dealing with our target species, humans, and that we are usually evaluating critical genetic events, we should be able to move quickly in the application of these techniques. There is every reason for using our newly developed cytogenetic techniques (such as chromosome painting), new DNA repair evaluations, and somatic cell mutation assays for evaluating populations exposed to known carcinogenic solvents such as trichloroethylene or perchloroethylene. Similarly,

we should also move out of the workplace and look at communities exposed chronically to low levels of carcinogenic and mutagenic agents. Although we can be proud of the contributions to human welfare made by genetic toxicologists, these accomplishments are minor compared to what can be accomplished in the near future. Major contributions will be made if we immediately utilize human monitoring to fill in the large information gaps that are present for most of the chemicals in our environment. The potential underestimation of risk when using animal data, as in the cancer bioassay program, makes it even more urgent to utilize genetic monitoring in industrial and community populations exposed to carcinogens.

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