Risk Assessment in Environmental Carcinogenesis\textsuperscript{1}

An American Association for Cancer Research Special Conference in Cancer Research Cosponsored by the Environmental Mutagen Society

This American Association for Cancer Research Special Conference in Cancer Research was organized by Philip C. Hanawalt of Stanford University and James A. Swenberg of the University of North Carolina. The Program Committee included John Ashby, William H. Farland, Barry W. Glickman, Carol J. Henry, B. Singer, and Thomas R. Skopek.

The purpose of this international conference was to present and discuss cutting-edge genetic toxicology, the scientific basis for extrapolation from model systems to humans, and issues relevant to the assessment of cancer risks in the workplace and in the broader environment. The meeting was designed to promote better communication and understanding between basic scientists, those who carry out risk assessment testing, and those who formulate the governmental regulations.

The conference was held at the Whistler Conference Centre, Whistler, British Columbia, Canada, January 17-22, 1994. Over 160 speakers and participants attended. James Swenberg opened the meeting with a keynote address on "The potential of science for reducing the uncertainty in cancer risk assessment." This was followed on successive days by plenary and poster sessions on topics including: Critical Events in Human Carcinogenesis (Hollstein, Bartsch, and Taylor); Molecular Epidemiology and Biomarkers of Exposure (Glickman, Phillips, Perera, Groopman, and Kadlubar); Genetic Predisposition to Cancer (Wiseman, Guengerich, Skolnick, and Drinkwater); Mutational Spectra for Environmental Carcinogenesis (Skopek, Sarasin, O'Neill, and Heddle); Endogenous Factors (Hanawalt, Sedgwick, Tannenbaum, and Chung); Scientific Basis of Extrapolation (Ashby, Beland, DeAngelo, Schulte-Hermann, Singer, Cohen, Lucier, Marnett, and Strauss); and Biologically Based Risk Assessment and Public Policy (Farland, Stratton, Moolgavkar, and Conolly). A special lecture was presented by Paul H. Kleihues, Director of the International Agency for Research on Cancer (Lyon, France) entitled "Towards a Reduction of the Human Cancer Burden."

Risk assessment based upon our increasing mechanistic understanding of carcinogenesis may be more reliable than present methods in protecting the public health, while possibly being less burdensome. In brief, the components of the current standard procedure for risk assessment include: (a) identification of hazard; (b) assessment of the dose-response relationship; (c) evaluation of the human exposure; and (d) characterization of the risk involved. The outcome of this formal process has been the assignment of a number or a probability, e.g. one-in-a-million, of an individual developing cancer as a result of a particular hazard. However, we should be concerned that the present methods can either overestimate or underestimate actual risk. Such a process does not incorporate any information on mechanisms of action of the test compound, data on high versus low dose exposure, or species differences in metabolism. Instead, the risk determination is usually based upon high-dose bioassays that may elicit mechanisms that are not applicable to low-dose exposures. It is assumed that humans are as sensitive as the most sensitive animal species. Default assumptions are made that are then extrapolated to human exposures. The upper 95\% confidence limit of this extrapolation is taken, and it is assumed that the risk will not be greater. This results in a single-point estimate of the risk that is then frequently misused to generate statements about the likely number of cancers generated by the test compound. Although the process is primarily mathematical, it is used in a manner that gives the impression that it is based upon scientific evidence. It is hoped that the process will become more accurate as new data become available. It is likely that different estimates of risk will be generated and that a range of estimates that involve statements on the most likely scenarios will be included.

The communication of risk to the public is a major challenge. In the past, the degree of uncertainty associated with traditional risk assessments had not been communicated well. Instead, the worst-case scenarios have been promulgated as fact. The Alar case is an excellent example of this. If the public equates eating an Alar-contaminated apple with smoking a cigarette, we have not protected human health. That is why it is so important to communicate the most likely extent of risk rather than the worst case. It is indeed helpful when possible to present risks in the perspective of better established risks such as smoking.

There is a difference between risk assessment and risk management in which public concern, and occasional "outrage," is balanced against a perceived "actual risk" and (governmental) spending priorities. The legal requirements that regulation not be "arbitrary and capricious" and the statement later in the meeting that "risk assessment must go forward whether or not data are available" indicate a reality and an approach to problems that basic scientists need to learn to appreciate and deal with. The incredulity with which some of the participants at the meeting reacted to the refusal of a United States Court of Appeals (subsequently confirmed by the United States Supreme Court) to permit modification of the administration of the Delaney clause, even for an additive that poses only an inconsequential risk, is to be contrasted with the reasoning of the court that decided that "the legislative history . . . reflect that Congress intended the very rigidity that the language it chose commands" (2). These are two very different points of view, and some informal discussions at this meeting attempted to reconcile them. It should be appreciated that, as the science evolves, reevaluation and revision of the regulations become necessary. The legal framework must somehow become sensitive to the advances in our understanding of chemical carcinogenicity (3).

The major component of risk assessment in the past has been derived from an extrapolation of risk, from high-exposure animal studies in the most sensitive species, to the anticipated human exposure. This extrapolation results in an estimate of the maximum number of individuals that might develop cancer. How should new methods of quantitating critical steps in the carcinogenic process such as the dose-response of absorption, distribution, metabolic activation, detoxification, receptor occupancy, DNA damage and repair, ability to induce mutations, and modulation of cell proliferation and apoptosis be incorporated into the risk assessment process? Is the assumption that presumptive carcinogens (after documentation in only one of the

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animal test systems) are intrinsically toxic and act to produce cancer by a nonthreshold mechanism that is incremental, prudent, and the best way to protect the public health (4)? We must conclude that we don’t yet have sufficient answers to these questions. But at a more basic level, we can also worry about the validity of the testing approach that is based upon acute, high-dose exposure to single compounds. Known carcinogens, such as tobacco smoke, constitute complex mixtures of many different compounds that may interact synergistically or that at least have additive effects. Smokers are exposed to such complex mixtures over many decades. Furthermore, the exposures to some other potential environmental carcinogens are also at chronic low levels over a long period rather than as a single massive dose.

It is now clear that the DNA from most human tumors contains numerous point mutations (5). Analysis of the kinds of mutations, particularly in the p53 gene, provides some evidence as to the possible mutagenic agents involved. Insofar as the altered function of the mutant proteins is a factor in the carcinogenic process, the kinds of mutations observed in a population of tumors of a particular type can provide information on the agent responsible. One quarter of the p53 mutations involve CG—>TA changes at CG sequences and are most likely due to spontaneous deamination. G—>T transversions in lung cancer and in hepatocellular carcinoma have been associated with heavy smoking and with aflatoxin ingestion, respectively. CC—>TT tandem changes are found in carcinomas of the skin, implicating solar radiation (and UV-induced dipyrimidine photoproducts, in particular) as an etiological agent. The increasing availability of sensitive methods of analysis such as the 32P postlabeling technique makes it possible to detect adducts in the cells of individuals. There is a correlation between the levels of aromatic adducts in lung cells and cigarette consumption, although not as yet a definite correlation between adduct levels and cancer (6). Several problems need to be considered in establishing a biomarker linking a measured determinant of exposure to a genotoxic agent and disease: (a) one needs an accessible source of DNA. WBC are often chosen for this purpose because of their availability, notwithstanding the heterogeneity of this population; (b) as reported again and again at this meeting in a variety of contexts, there is an enormous amount of interindividual human variation, regardless of the parameter being measured in the response to a particular environmental exposure. Risk assessment can deal with these variations if the biomonitoring studies can identify the range of susceptibilities in an exposed population; and (c) there needs to be an objective method for determining exposure. Modern analytical technology makes it possible to check on the accuracy of self-reporting. Thus, the finding of nicotine metabolites in the urine of self-reported nonsmokers reveals the difficulty inherent in any such data, whether relating to smoking status or even to past dietary intake (7). The availability of a “biomarker” that reflects “the events from carcinogen exposure through clinical cancer” would make it possible to evaluate the feasibility of intervention strategies to prevent cancer. An example of such an intervention tactic, now under trial, involves the use of the drug Olitipraz as a preventive of liver cancer due to aflatoxins (8). Olitipraz was originally developed as an antischistosomal agent and was then found to be an inducer of enzymes of carcinogen detoxification. In this case, the biomarker is the level of aflatoxin-N7 guanine adducts detected in urine.

The reaction of many carcinogens with DNA requires an activation step that is catalyzed specifically by different members of the cytochrome P-450 group of enzymes (e.g., CYP1A1, CYP2C, CYP2E1, etc.). Different members of the group are inducible by different agents; e.g., tobacco smokes, ethanol, and barbiturates. The total level of DNA adducts in the larynx correlates strongly with the levels of CYP2C, CYP1A1, and CYP3A4 activity (9). Notwithstanding the measured correlation, it can not be definitely stated that there is an association of P 450 enzyme levels with cancer susceptibility. The variability is great enough that “interhuman variation should be factored into models of interspecies risk assessment.” It is also very important to appreciate that some of the differences have no impact on low-dose exposure since the activation enzymes are not saturated.

This variability is, at least in part, genetic. Genetic predisposition is not restricted to certain rare types of cancer. Analysis of the genealogy of 1.5 million Utah descendants of ~10,000 pioneers has led to the identification of genes contributing to the susceptibility to breast cancer, melanoma, and prostate cancer (10); there has been good progress in cloning the BRCA1 gene predisposing families to breast and ovarian cancer. Of course, the experimental identification of susceptibility factors is much more convenient in mice, particularly because of the availability of genetically homogeneous strains. Given this genetic homogeneity, it is possible to demonstrate that superimposed on genetic differences are major sexual differences in hepatocarcinogenesis, with females being much less sensitive than males (11).

The initiating events in carcinogenesis are likely to be mutations. The observation of mutations at dipyrimidine sites in skin tumors and the finding that in XP patients these dipyrimidine hotspots occur on the nontranscribed strand implies that the residual repair activity in XP cells is directed toward the transcribed DNA strand (12); this has, in fact, been established for complementation group C of XP. It is convenient in experimental mutation studies to use particular reporter genes, such as hypoxanthine-guanine phosphoribosyl transferase, about which there is much information. However, one needs to be cautious about the relevance of the study of the mutation of conventional reporter genes in surrogate cells. It appears that, in addition to frequency, the determination of the sequence context of the mutations provides important information. For example, such studies have permitted investigators to follow the clonal and sequential production of mutations in a susceptible individual who seems to have an altered mismatch repair gene similar to the bacterial mut S (13); similar changes have been reported in colon carcinomas of individuals carrying the HNPCC gene (14). Determination of nucleotide sequence has also been used to suggest a role for aberrant V(D)J recombinase action in the production of deletions in newborns (15).

An implicit assumption in the interpretation of dose-response curves is that the DNA in untreated animals is not damaged and that any response is solely a result of the exogenous treatment, even though “background” responses may be subtracted. The recent emphasis on the variety of endogenous insults to which DNA is subject makes it important to question the validity of such an assumption. Lesions can be detected in DNA resulting from hydrolytic and deamination reactions, as well as from natural methylating agents (16), and from activated nitric oxide synthetase leading to cytosine deamination (17, 18). Persistent exocyclic adducts can be found in untreated animals, presumably resulting from acrolein-like compounds produced by fatty acid oxidation (19). The mutagenic potency of these endogenous adducts could, in fact, be equivalent to those produced by exogenous materials—the steady state level of the major malondialdehyde DNA adduct is about 1—10 per 107 bases in normal human liver (20). That such adducts should persist could imply that the repair processes for these lesions are already operating near saturation under the conditions of normal animal metabolism. Alternatively, the measured levels may simply represent steady-state concentrations, reflecting the balance between formation and repair. What significance do such adducts have for the evaluation of exogenous dose effects? Even

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2 The abbreviation used is: XP, xeroderma pigmentosum.
if there is some threshold, does each lesion have its own threshold, or do lesions from exogenous sources simply add to an endogenous burden, thereby shifting the dose-response toward a zero threshold?

Different questions with both health and economic aspects were raised in connection with the assessment of the hazards of so-called nongenotoxic carcinogens, defined as those that do not react with DNA or those that do not produce mutation in short-term tests; about 40% of the compounds reported as carcinogenic fall into this category (21). Dichloroacetic acid, a by-product of drinking water chlorination, is not genotoxic, and a threshold does appear in the dose-response curve. The Safe Drinking Water Act of 1974, as amended in 1977, requires the Environmental Protection Agency to investigate the health effects of disinfection by-products. The costs of treating water are borne by local communities, and the choice of model of carcinogenesis adopted as a basis for defining risk (i.e., whether a threshold dose is assumed) can make a $16 billion difference in the cost to communities. The new guidelines for judging safety “place an emphasis on mechanisms of carcinogenesis.” High doses of chloroacetic acid produce hyperplastic nodules in both rats and mice, but the mechanism of this response is not clear. What is clear is that enhanced rodent hepatocarcinogenesis at high doses of chloroacetic acid is a complex physiological process (22).

Major attention was directed to considerations of the mechanisms of so-called nongenotoxic carcinogenesis. There are at least two ways by which a nongenotoxic exposure could increase cancer risk: (a) effectively increase the rate of DNA damage induction by some other agent, for example by changing the levels of metabolic activating enzymes; and (b) increase the rate of proliferation of stem cells. For some genotoxic compounds, which may damage DNA directly but also induce cell division at higher concentrations, the shape of the dose-response curve will be greatly affected by the effect of compound toxicity on cell proliferation. Nongenotoxic materials may be either directly or indirectly mitogenic. For nongenotoxic chemicals, including those which produce urine calculi or amorphous precipitates that induce necrosis and regenerative hyperplasia, there may be definite thresholds based on physical properties (e.g., solubility) which can be defined (23). An additional parameter in the consideration of a mechanism is the control of cell death or apoptosis, since promoters may inhibit programmed death, thereby increasing the pool of stem cells (24). Many nongenotoxic substances combine at normal hormonal receptors in what may be called receptor-mediated carcinogenesis. Dioxin is an example of such a substance, and its receptor acts like a steroid receptor system. The curve for combination of agonists with their receptors is sigmoidal. If physiological factors are taken into account, vastly different values for the risk of low concentrations of carcinogen are obtained. Thus, the permissible level of such compounds in Canada, where the mechanistic factors have been taken into account, is 1600 times higher than that allowed in the United States (25).

The major or standard models now used for risk assessment are linearized multistage models which extrapolate risk from the results of high-dose experiments and assume no threshold for carcinogenesis. Such models are not specific for particular substances, but require only limited data and are the easiest to manipulate. Their implementation permits legislatively mandated risk assessment, even in the absence of extensive amounts of data. The opportunities and difficulties in the field resulting in large part from the scientific advances described at the meeting is suggested by the following two statements, from the last session of the meeting. “A major disadvantage of the default approaches is that, because they are not chemical-specific, they are inherently uncertain. Only knowledge of the shape of the exposure-response curve, based on understanding of pharmaco-kinetics, mechanisms of short-term responses, and linkages of short-term responses to cancer can lead to a reduction in uncertainty.” This needs to be contrasted with “In practice, inherently cautious regulators are reluctant to apply new risk assessment techniques to important decisions, and those who pay for the research are generally reluctant to fund studies unless they know whether and how the results will be used.” Risk assessment is only a component leading to risk management, an overlapping but distinct activity, which further interacts seriously with “risk communication.” The interplay between these activities is complex, and it seems clear that any attempt to change the way in which risk management is carried out requires major changes in the perspectives of the professionals, as well as in those of the lay public. Although this meeting was but an early step in the process, some of the players are now on better speaking terms.

References


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