Cancer Risk Assessment: Should New Science Be Applied?

Workgroup Summary

Crowne Plaza Washington National Airport Hotel
Arlington, VA
July 17-19, 2002

Organizers
Richard J. Bull, Antone L. Brooks
Washington State University Tri-Cities Campus
2710 University Drive
Richland, WA 99352

Speakers

Mary Helen Barcellos-Hoff, Rory B. Conolly, Norman Drinkwater, Richard Fishel, Eric Hall, David Hoel, Daniel Krewski, Michael Luster, Roger O. McClellan, William Morgan, J. Leslie Redpath, Douglas D. Spitz,

Corresponding Author:
Richard J. Bull, Ph.D.
Washington State University Tri-Cities Campus
2710 University Drive
Richland, WA 99352
T: 509-372-7551
F: 509-372-7552
e-mail: dbull@tricity.wsu.edu

Running Title: New Science need in Cancer Risk Assessment?

Keywords:

cancer risk assessment
bystander effects
adaptive response
genomic instability
susceptibility
research needs

Abreviations:

high-LET – high linear energy transfer
low-LET – low linear energy transfer
CDKN1A – cyclin dependent kinase 1A, also known as CIP1/WAF1
TGF-β – transforming growth factor beta
MVK – Moolgavkar, Venson & Knudsen
NIEHS – National Institute for Environmental Health Sciences
NIH – National Institutes of Health
DOE – U.S. Department of Energy

Section Headers

I. Introduction
    A. The Workshop
    B. Evolution of cancer risk assessment
    C. Technological advances leading to identification of bystander effects
II. Examples of phenomena induced in irradiated and/or bystander cells
    A. Adaptive responses
        1. Classical adaptive response
        2. Complexities of dose-response curves
    B. Genomic instability
        1. Low dose effects
        2. High dose effects
        3. Other causes of genomic instability
    C. Other cellular fates
    D. Tissue and cell-specific responses
    E. Dose-dependence of responses
    F. Potential mechanisms underlying bystander effects
    H. Genetically-determined susceptibility
III. The need for an integrated, quantitative description of phenomena that contribute to or modulate carcinogenic responses.
    A. Develop a means for organizing information
    B. Dose-dependence
    C. Integration
IV. Generalization of principles of cancer risk assessment
V. Conclusions and recommendations

References
Figure 1

Abstract

In July of 2002 a workshop was convened that explored some of the intercellular phenomena that appear to condition responses to carcinogen exposure. Effects that result from communication between cells that appear to either increase the sphere of damage or to modify the sensitivity of cells to further damage were of particular interest. Much of the discussion focused on the effects of ionizing radiation that were transmitted from cells directly hit to cells not receiving direct exposure to radiation (bystander cells). In cell culture, increased rates of mutation, chromosomal aberration, apoptosis, genomic instability, and decreased clonogenic survival have all been observed in cells that have experienced no direct radiation. In addition, there is evidence that low doses of radiation or certain chemicals give rise to adaptive responses in which the treated cells develop resistance to the effects of high doses given in subsequent exposures. Data were presented at the workshop indicating that low dose exposure of animals to radiation and some chemicals frequently reduces the spontaneous rate of mutation *in vitro* and tumor responses *in vivo*. Finally, it was concluded that considerable improvement in understanding of how genetic variation may modify the impact of these phenomena is necessary before the risk implications can be fully appreciated. The workshop participants discussed the substantive challenge that these data present with respect to simple linear methodologies that are currently used in cancer risk assessment and attempted to identify broad strategies by which these phenomena may start to be used to refine cancer risk assessment methods in the future.

I. Introduction

A. The workshop

A workshop was conducted on July 17-19, 2002 to discuss the implications of recent findings in radiation biology for assessment of cancer risks from low doses of chemical and physical agents. The objective of the workshop was to bring together basic scientists who are responsible for observations made in radiobiology, their counterparts from the field of toxicology, scientists whose main interests are risk assessment, and scientists working on incorporation of concepts of mode of action into cancer risk assessments made by various federal agencies (Wiltse and Dellarco, 1996). The regulatory agencies represented at the workshop were Occupational Safety and Health Administration, National Institute of Occupational Safety and Health, Agency for Toxic Substances and Disease Registry, U.S. Environmental Protection Agency, Food and Drug Administration, the Nuclear Regulatory Commission, the Department of Energy and the National Radiological Protection Board (UK).

This workgroup summary has been prepared by reference to the presentations that were made and materials developed from summaries and notes that were made at the workshop. The summary has been circulated to the participants and the resulting comments have been utilized in developing the final draft of the manuscript. Every attempt was made to capture any important differences of opinion that were articulated at the workshop and the subsequent comment period.

B. Evolution of cancer risk assessment

Risk assessment of potentially harmful physical and chemical agents developed gradually over the past half century (McClellan, 1999; 2002). The major impetus for this science has been the utilization of its research products in the development of regulatory policy related to allowable human exposures. However, the approach taken has depended in large part on whether the data available come from observational or experimental data.

In the case of radiation, risk estimates for cancer have been based on data from human experience, in particular the atomic bomb survivors. Data from animal experiments, and to a lesser degree cellular studies, have been used to assess the influence of dose rate, dose distribution, and the type of radiation.

In the period of 1945-50, the concept of "tolerance doses", based upon the assumption that the effects of radiation had threshold doses, was gradually replaced by the "maximum permissable limit", as the possibility that some responses might not have thresholds was recognized.

Dose-response assessments for non-cancer endpoints for chemicals have continued to evolve based upon the threshold concept. A reference dose is developed that is thought to be below the threshold. It is derived from a no-observed-adverse-effect-level or a lowest-observed-adverse-effect-level (or from a benchmark dose in more recent years) that is adjusted by uncertainty factors (related to the interspecies and low-dose extrapolation of data) and modifying factors based upon the completeness of the data that are available (NRC, 1983).

In the early 1970s a variety of developments occurred that brought greater focus to the assessment of cancer risks. The enabling legislation that formed the U.S. Environmental Protection Agency required a closer look at the regulation of chemicals in the environment. Cancer risk policy first made use of the one-hit model. This was highly criticized because it

frequently did not fit the data available and the basic assumption did not seem to apply generally. This gave way to the formalization of the linearized multistage model (Crump et al., 1976). This model had the advantage of being both biologically plausible and relatively simple in its application and has underpinned much of cancer policy in the past two decades.

Unlike radiation, chemicals that cause cancer have been largely identified by animal studies. For this reason there has been greater reliance on experimental data in risk assessment. These studies have provided evidence that many different types of mechanisms contribute to the development of cancer. The draft guidelines for cancer risk assessment (U.S. EPA, 1996) open up risk assessment to modes or mechanisms of action that would allow either the low dose or across species extrapolation of data to depart from defaults. When these data have been treated formally in models, such as that developed by Moolgavkar, Venzon & Knudson, rates of cell division and cell death, as well as changes in mutation rate, have been utilized to estimate risk at low doses (Moolgavkar and Venzon, 1979). It is recognized that even these more elaborate models remain as oversimplifications.

The main focus of the present workshop was on observations related to phenomena that suggest that a number of indirect effects (e.g. bystander effects) may be determinants of the dose-response curve in low-dose ranges. These phenomena may well introduce non-linear behavior into the dose-response curves for carcinogenic effects. Although the clearest examples are with ionizing radiation, cross-tolerance with chemicals and radiation demonstrate at least one parallel phenomenon exists with chemical carcinogens (Padovani et al., 1995; Tedeschi et al., 1995). The workshop represented one of the first attempts to assemble a group of experts to identify the implications these new findings have for cancer risk assessment in general.

C. Technological advances leading to identification of bystander effects.

Bystander effects are defined broadly as responses that occur in cells not directly impacted by the chemical or physical agent in question. The term arose in radiation biology from the observation that evidence of genetic damage occurred in many more cells at low doses of high-LET radiation (or fluences) than could have been "hit" by a particle of ionizing radiation. It is important to note that to many radiation biologists, the unmodified use of the term bystander directly refers to the genetic damage in non-hit cells. However, more recent work has demonstrated that a variety of other effects can be triggered in cells not directly hit by ionizing radiation. The more recent development of microbeams has allowed the targeting of individual cells with defined numbers and types of particles. Investigators developed single particle irradiation systems at Columbia, Gray Laboratory, and Texas A&M that allowed targeting of single cells *in vitro* to more finely study the effects of very low doses on clastogenic and mutagenic responses (Hall, 2002; Nelson et al., 1996; Zhou et al., 2000). This technology has now been expanded to the study of bystander effects on cell transformation (Sawant et al. 2001a) and has allowed earlier indirect observations to be confirmed.

Although presaged by earlier observations, the first formal recognition that bystander effects occurred for radiation came with the publication by Nagasawa and Little (1992) where sister chromatid exchanges were noted in far greater numbers of cells than could have been hit by alpha particles at the low fluences examined. Subsequent work from this same laboratory demonstrated that gene activation in non-hit cells was communicated from those hit by alpha particles via gap junctions (Azzam et al., 1998). Mothersill and Seymour (1997) found that media taken from cells that had been irradiated decreased the clonogenic survival of unirradiated

cells, indicating that all bystander effects are not mediated through gap junctions. In his presentation, Eric Hall pointed out that such communication can be one-way, two-way, or non-existent, depending upon the type of cells involved.

II. Examples of phenomena induced in irradiated and/or bystander cells

A. Adaptive responses

A number of responses are induced by radiation that could be termed adaptive responses. The term is often narrowly used in radiation biology and applied to experiments where treatment with a low priming dose of radiation reduces the response of cells to a higher dose delivered a few hours later. Apparently parallel phenomena, which could be viewed as protective adaptations, have been identified without the priming dose in dose-response curves that deviate from simple linear or log-linear functions and have been described at both the cellular and whole animal levels of biological organization. The linkage between these low dose protective (or even beneficial) effects and the classical adaptive response is tenuous at this time. Since there seems to be an intuitive relationship, they will be discussed under the broad heading of adaptive response, but the discussion will focus on modification of adverse outcomes at low doses.

1. Classical adaptive response

Adaptive responses to radiation can be observed as a decreased induction of chromatid aberrations when a small priming dose was followed by high doses of X-rays: Early work used a

prior incubation of cells with low levels of tritiated thymidine prior to a large X-ray dose (Olivieri et al., 1984). Subsequent work demonstrated that low priming doses of X-rays also resulted in fewer chromatid breaks being observed following the higher challenge dose (Wolff, 1992).

Adaptive responses to subsequent α irradiation that were induced by low doses of γ-irradiation (2 cGy) have been elegantly demonstrated when only 10% of the cells present were irradiated using a single particle microbeam (Sawant et al., 2001b). This study was of particular interest in that the adaptive response was found to reduce the bystander effect (measured as clonogenic survival) by 50%.

Experiments utilizing this same exposure paradigm have extended these observations to include a wide variety of endpoints. In the case of cell killing, relatively large priming doses confer protection to subsequent doses of similar magnitude (Raaphorst and Boyden, 1999). However, the induction of the adaptive response varies widely depending upon cell type and genetic susceptibility among individuals.

Adaptive responses have been demonstrated in mice by administering a low total dose of 10 cGy γ-irradiation delivered at two different dose-rates 24 hours before administering a 1 Gy dose (Mitchel et al., 1999). Mice receiving the pretreatment were found to have a significantly prolonged latent period for development of myeloid leukemia. Similar adaptive responses were induced by 60 min of whole body hyperthermia and injection of 1500 U of interleukin-1, intraperitoneally. These studies provide an experimental case that demonstrates adaptive responses can provide protection against radiation-induced disease *in vivo.*

2. Complexities of dose-response curves

A variety of experiments have shown that many of the classical responses to irradiation are more complex than originally thought. The experimental paradigms used for these experiments vary from that used to demonstrate the adaptive response, but they have been inferred to be related to adaptive responses (Joiner et al., 1996).

Studies of the dose-response relationships for cell killing have revealed responses that are very non-linear in the low dose range (Joiner et al., 1996). At radiation doses between 0 and 0.3 Gy, cell killing is significantly greater than would be predicted by extrapolating the dose-response observed at doses > 1 Gy. While the hypersensitive set of cells may come from populations in different arts of the cell cycle in part, the low-dose hypersensitivity is displayed in all phases of the cell cycle (Short et al., 1999). The interpretation offered of these results is that there is an induction of DNA repair mechanisms that protect the cells from killing in the high dose range.

Cells that display bystander effects (measured as reduced clonogenic survival) do not display the low dose hypersensitivity (Mothersill et al., 2002). Consequently, they are viewed as separable phenomena.

Low doses of γ-irradiation (0.1 to 10 cGy) have been shown to reduce the spontaneous transformation of cells *in vitro* (Redpath et al. 2001). Data on the reduction of background levels of damage by low dose radiation were reviewed and related to some cancer responses to radiation that have been observed in humans and experimental animals (Redpath, 2002). Thus, the experience with low-LET radiation appears to be somewhat different from the increased rates of cell transformation induced in cells that are neighbors of cells hit by high-LET α-particles (Sawant et al., 2001a).

Qualitative differences in responses in different dose ranges are reflected at the molecular level. *In vitro* studies of changes in gene expression following varying doses of γ-irradiation reveals 30 genes that were activated at all doses. However, at low doses (0.2 Gy) and low dose rates, there were 137 genes activated that were not activated at 10 Gy and 16 that were activated at 10 Gy, but not activated at the lower doses (Amundson et al., 2001).

At the workshop, a preliminary analysis on the shape of the dose response in the low dose region was presented. Radiation studies in experimental animals that have been published in the open scientific literature were reviewed and subjected to statistical analyses for J-shaped dose response curves (Krewski, 2002). It was found that the number of studies displaying J-shaped dose-response curves was significantly greater than could be expected by chance. The J-shape appeared to occur with both high and low-LET radiation. This finding is remarkable, because these studies had not been designed to demonstrate such behavior in low doses. Thus, such effects can occur. Nevertheless, the generality of the low-dose protective effect has yet to be established.

Some treatments of data on cancer mortality in atomic bomb survivors support, at least in part, the hypothesis that low doses of radiation are protective against certain types of cancer. Some analyses of these data and those in the literature were discussed (Hoel, 2002). Depending upon the selection of the dose cutpoints, mortality from leukemia can appear to be less in survivors receiving low doses of radiation relative to the control population (Chomentowski et al. 2000). However, solid tumor mortality displays no evidence of low dose protection. However, data involving chronic exposures to radiation (Miller et al., 1989; Hrubec et al., 1989) indicate that the dose response for some tumors appears linear within statistical confidence intervals, to the lowest exposures that have been characterized.

B. Genomic instability

Genomic instability induced by radiation was reviewed (Morgan, 2002). There are differences in the processes responsible for induction of genomic instability that are produced by low and high doses of radiation that may be critical to understanding the implications of these observations for the development of disease.

1. Low dose effects

Low doses of radiation to cells in culture increases the numbers of cells that display genomic instability. Genomic instability is observed as increased non-clonal mutations or chromosomal aberrations in either cells that have been subject to direct irradiation or that are bystander cells. The phenomenom has also been described when medium is transferred from irradiated cells to naïve cells.

Genomic instability is induced *in vitro* by very low doses of radiation. In media transfer experiments; evidence of clonogenic cell death in response to γ-irradiation appears to approach a maximum between 1-5 cGy (Seymour and Mothersill, 2000). Genomic instability induced in bystander cells by α-irradiation generally occurs when cells are cultured under conditions of close cell-to-cell contact and to be largely dependent upon gap junction communication (Azzam et al., 1998; Azzam et al., 2001). Responses are also seen at low doses, but with less evidence of saturation in the dose-response curve. This may be related to heterogeniety of dose/cell that is observed relative to that observed with equivalent energies of γ-irradiation in these dose ranges. Genomic instability has also been observed to be induced in bystander cells by α-irradiation in

heamopoietic cells, which do not have gap junctions, both in tissue culture experiments (Lorimore et al., 1998) and in mice (Watson et al., 2001). Collectively, these data indicate, by virtue of the very high frequencies of the induced changes, that genomic instability is not directly the result of simple mutation.

2. High dose effects

As doses of radiation increase, the influence of genomic instability on cancer outcomes may increase. The factors affecting the dose-dependent increase in genomic instability may not be simple. In experiments presented at the workshop, the differences in the dose-response curves for the induction of chromosomal instability by acute exposures to x-rays, iron ions and ^{125}IdUrd were presented (Morgan, 2002). ^{125}I seeding was used to construct an exposure system to obtain long-term, low dose rate, irradiation (48 hours) at 50 cGy/day. The medium from these experiments was transferred to non-irradiated cells and was found to contain a death-inducing factor that selectively affected non-irradiated cells. Thus, it appears that the effects of higher doses of radiation delivered at low dose rates can result in the development of selection mechanisms that favor cells with unstable genomes.

3. Other causes of genomic instability

A recent paper (Li et al., 2001), not discussed at the workshop, provided evidence that genetic instability can be induced by stresses that do not directly damage DNA. These were observed in a spontaneously arising mammary carcinoma line from Balb/c mice. Heat treatment

(45^{o} C for 30 min), serum starvation, or *in vivo* growth in a syngeneic host all increased the yield of genomically unstable cells. In the same study, genetic instability was also demonstrated with γ-rays and hydrogen peroxide treatments that damaged DNA.

C. Other cellular fates.

It has become increasingly clear that the responses of cells from exposure to radiation or carcinogenic chemicals vary widely. Cells that become transformed directly or as a result of induced genomic instability represent only two of several possible cellular fates. The diversity of these fates following exposure to radiation is sometimes difficult to recognize in the primary literature because most studies tend to focus on one aspect of the responses and try to identify the mechanisms that are responsible for the selected endpoint.

Figure 1 displays a number of these alternative fates of cells exposed to cancer-inducing agents and hints at some of the processes that might influence the fate of individual cells in a culture dish or *in vivo*. At the molecular level, damage induced in DNA directly or through the activation of indirect processes stimulates repair of lesions in DNA. Such cells survive and continue to divide, usually after a short delay in the cell cycle to allow for repair. Some cells are killed either directly from DNA damage or secondarily from apoptosis. Other cells become resistant to killing and in some cases this resistance has been associated with the increased ability to repair DNA damage. Some cells, however, apparently become permanently arrested by mechanisms that are separable from the cyclin-dependent kinase activity of CDKN1A (Savell et al., 2001). This permanent arrest appears to be secondary to DNA damage and can involve increased expression of p53 and p21 (te Poele et al., 2002). The important point to be made is

that we are just beginning to understand the signaling processes that are triggered by the effects of chemicals or ionizing radiation. They are likely to occur in both directly damaged and bystander cells. Undoubtedly there are other factors that condition the relative proportion of cells having a given fate at different doses of the agent in different cell types and tissues.

D. Tissue and cell-specific responses.

Low dose hypersensitivity, bystander effects, adaptive responses, and genomic instability are not seen uniformly in cells of differing origins (see for example, Sorensen et al. 2002). Moreover, the details of the responses to radiation appear to vary between species and strain.

These differences make it difficult to generalize the data taken from model systems. If these phenomena are to be incorporated into risk assessment models, the inability to generalize will seriously complicate the simple linear extrapolations that have been made up to now. At the very minimum, the variation in the type and magnitude of responses in different tissues suggest that low dose extrapolation will have to be tailored to the tumor site.

E. Dose-dependence of responses

An important consideration of the implications of phenomena that occur at low doses of ionizing radiation may be differences in the effects of high and low-LET radiation. Alpha particles have a high relative mass and a single track can deposit large amounts of energy resulting in catastrophic impacts upon the cell that has been hit. This correlates with the ability of a single hit by a high-LET particle to generate genomic instability in the hit cell and in

bystander cells, whereas it takes multiple hits by low-LET radiation to demonstrate such responses. The traditional view in radiation protection is that quality factors make simple and accurate adjustments for differences in energy deposition, the spatial differences involved, the background experience with repairing such damage, the parallel damage induced by metabolism, and the localized intensity of the damage. That view has been questioned in recent years, but remains controversial.

As suggested in the model of damage done to the genome in bystander cells by α-particles, the simplest models that try to take these phenomena into account can result in complex relationships between dose and response (Brenner and Sachs, 2002). The inability of low-LET radiation to induce immediate alterations in bystander cells may result in less complex responses at low doses.

F. Potential mechanisms underlying bystander effects.

Understanding of how cells generate, transmit and interpret signals as a result of an event that occurs in the hit cell is in its infancy. In the context of ionizing radiation, there are systems that are obvious candidates based upon some of the chemistry that is generated, such as reactive oxygen and nitrogen species. Information from studies of mechanisms that effect or modify chemical carcinogenesis adds more complexity to the overall picture. Presentations at the workshop dealt with examples of mechanisms that were involved in intracellular communication, intercellular communication, and influences that are exerted at the whole tissue level.

The sliding clamp model whereby mismatch repair proteins accumulate at the sites of mismatches in DNA and appear to serve as a signal for recruiting other repair proteins to these

sites was a mechanism that was discussed as an example (Fishel, 2001; 2002). Products of two of the mismatch repair genes, hMSH2 and hMLH1, have the additional property of promoting apoptosis, whereas the remaining homologues do not. Cells containing mutations in these genes are resistant to apoptosis induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Thus, mutation in either one of these two genes will protect cells from apoptosis and provide a selective advantage to an unstable genotype. The question raised was whether loss of signaling functions of other DNA damage processing proteins could contribute to the selection of genetically unstable cells that could lead to more rapid development of cancer.

The ability of hit cells to contribute to instability in non-hit cells increases the complexity of carcinogenesis. The role of free radicals that are formed as the result of cells being hit by ionizing radiation or from exposures to other chemical and physical agents was explored as a set of mechanisms that might contribute to this phenomena (Spitz, 2002). The mitochondrial electron transport chain, NADPH oxidase enzymes, xanthine oxidase, and nitric oxide synthases were identified as the most likely sources of prooxidants that could contribute to bystander effects and discussed the antioxidant systems that limit the influence of these sources. Recent studies have demonstrated that ionizing radiation does result in production of reactive oxygen/nitrogen species. It is postulated that this signal can be transmitted to other mitochondria by permeability change releasing calcium, providing a means of amplifying the signal (Leach et al., 2001). More recently, ionizing radiation was shown to increase nitric oxide synthase activity (Leach et al., 2002). NO is an attractive means of transmitting signals between cells because its relative stability allows migration over several cell diameters. To date, these studies have been limited to relatively high doses of radiation (e.g. 2-5 Gy). However, there is ample evidence that low doses of low-LET radiation (<0.02 Gy) do result in measurable changes in

gene expression in cells in culture (Amundson et al., 1999). Although these latter results certainly have implications for intracellular responses, the extent to which they contribute to intercellular communication remains to be established.

Experiments were described that demonstrated that irradiation of mammary gland stroma promoted the growth of tumors from transplanted cells that received no direct exposure to radiation (Barcellos-Hoff, 2002; Barcellos-Hoff and Ravani, 2000). This effect was associated with activation of latent TGF-β, although the precise mechanisms by which tumor cells are selected have not yet been worked out.

These selected topics illustrate that carcinogenic responses in intact organisms involve complex mechanisms that may well arise indirectly, rather than simply being set in motion by mutations induced by a carcinogenic agent.

H. Genetically determined susceptibility

The role genetic susceptibility plays in carcinogenic responses was discussed. It was pointed out that a relatively small fraction of human cancers are accounted for by dominant genes leaving much of the differences in susceptibility to be accounted for by interactions among multiple genes. Work that has focused on susceptibility to liver tumors in different strains of mice and the linkage of susceptibility to specific genes was discussed as an example (Drinkwater, 2002). Many genes modify susceptibility to cancer and they influence different stages in the carcinogenic process. In the case of liver cancer in mice, the growth rate of preneoplastic foci appears to be an important differentiator between susceptible and non-susceptible strains (Hanigan et al., 1988). However, the relative importance of a particular locus

was shown to vary among strains (6-20 fold) and accounts for only a portion of the 100-fold difference between strains. These data imply that multiple loci influence carcinogenesis and that they modify different stages. It is probable that similar mechanisms contribute to variation in susceptibility among people.

III. The need for an integrated, quantitative description of phenomena that contribute to or modulate carcinogenic responses.

Workshop participants expressed varying opinions about how the phenomena discussed should influence risk assessment. It was apparent from the discussions that there is now evidence of many effects of radiation that do not arise directly from damage to DNA in the hit cells, either by a direct hit to DNA or indirectly through the local generation of reactive oxygen. These "secondary" phenomena are clearly important and may be major determinants of whether exposure to low doses of radiation will have a cancer outcome. Discussions of topics led to varying degrees of consensus:

- **The implications of damage produced by high-LET and low-LET radiation could be quite different.** The amount of energy deposited in an individual cell decreases uniformly with low-LET radiation to a point where the total energy in any one cell fades into the background level and may not be able to produce physiological responses such as the adaptive or bystander effects. Research is needed to determine if the clustered DNA damage produced at very low doses of low-LET radiation has consequences in terms of an increased risk of cancer. The relatively low-levels of such damage with low doses of

low-LET radiation compared to that seen with high-LET radiation may be more readily repaired by molecular, cellular, or tissue processes. .

- **Many, if not all, of the phenomena that were largely identified for ionizing radiation in the presentations were also likely to play a role in cancer induced by chemicals.** There are clear examples where complex and indirectly mediated modes of action play an important role in the induction of cancer. Data from studies on the mode of action of arsenic were identified as an example of comparable complexity with a chemical carcinogen (Luster, 2002; also see Miller et al., 2001).
- **Low dose extrapolation is even more complex than has been previously appreciated.** Empirical data support a variety of shapes in the dose-response curve, depending largely upon the radiation quality, the tumor type, and the tissue environment. Some carcinogenic responses to ionizing radiation appear linear to doses as low as can reasonably be characterized (e.g. carcinomas) whereas others clearly have non-linear relationships with low doses (e.g. sarcomas). The main point is that in the former case, the empirical data are not sufficient to distinguish among linear and a variety of non-linear alternatives. However, on conceptual grounds and within the realm of experimental data, rational arguments can be constructed for more complex dose-response relationships (Brenner and Sachs, 2002; Krewski, 2002).
- **Responses of tissues and whole organisms will be qualitatively as well as quantitatively different in different dose ranges.** The data that are available clearly show that different genes are expressed in response to different dose ranges of ionizing radiation. This is reflected in different cellular behaviors as well. The probability of a

predominance of adaptive responses at low doses perhaps reflects the importance of a living system to be subject to some level of stress.

- **Intracellular and intercellular communication play important roles in determining whether there is a carcinogenic outcome to a given dose of an agent.** At this time there is not an integrated understanding of the signaling pathways involved, the interplay between them, and what damage may be induced or the nature of protection that may result.
- **Alternative cellular fates and the determinants of these fates are extremely important to new quantitative approaches to risk assessment.** Some risk assessment methods now explicitly incorporate cellular dynamics (e.g. MVK , see Hazelton et al., 2001). Clearly, bystander effects, genomic instability, and adaptive responses have important impacts on cellular dynamics within a tissue, but that it is too early to fully comprehend how they should be considered in risk assessment. A key consideration is whether and how these phenomena are involved at different dose ranges.

A. Develop a means for organizing information.

A critical need identified at the workshop was the need to have a structured means for identifying the important biological responses that collectively determine whether a cancer will develop. Hanahan and Weinberg (2000) provided an excellent classification of the traits that cells must acquire to become cancerous. These traits include self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, sustained angiogenesis, limitless replicative potential, and the ability to invade and metastasize. Carcinogens would necessarily

contribute to one or more of these properties. Both qualitative and quantitative data should be organized into a 'repository' that would provide risk assessors a means to organize the information. In turn the collection of the information into a structured package would enable researchers to more clearly identify those data gaps that impede the development of more accurate approaches.

Beyond the development of a general understanding of carcinogenesis, account must be taken of critical differences in the behavior of experimental models and what occurs in intact humans. Many *in vitro* systems utilize immortalized cells that no longer possess all the controls on growth present *in vivo*. Interspecies differences have also confounded extrapolation issues in the past. However, it is important to realize that some of the mechanisms that account for such differences are coming to be understood. For example, at least part of the basis for understanding the different numbers of mutations required for transformation of human and rodent cells have been identified (Hahn and Weinberg, 2002). It appears that adjustments for differences in target organ specificity of carcinogens may become tractable. Such issues are extremely important for translating results from experimental systems to assessing risks to humans.

B. Dose-dependence

Those phenomena that both contribute to the development of cancer and are modified by exogenous agents need to have established dose-response relationships to be useful in developing new approaches to risk assessment. It is frequently not appreciated that the dose-response relationships contribute qualitative as well as quantitative information. As was emphasized in

the conference, behaviors in one dose range frequently do not carry over to another dose range. Non-linear dose-response ultimately provides information about the underlying biology of the response, something that must be understood to address low dose issues.

Few of the dose-response data obtained in isolated systems are likely to be quantitatively utilized in risk assessment. The few data that will be transferred to the *in vivo* system (ultimately in humans) will be used only to the extent they can be shown to be consistent with *in vivo* observations. Data taken from the radiation biology literature continually reinforce the fact that phenomena initiated by radiation vary by cell of origin, are modified by the context of the tissue within which they reside, are affected by differences in physiological state, and are subject to variations in genetic background.

C. Integration

Concerns of risk necessarily focus on the response of intact humans. Responses of complex organisms are always conditioned by the physiological state. The physiological state of an individual will be modified by his/her genetic makeup, lifestyle, and by other factors that occur in the surrounding environment. Clearly molecular and biochemical effects which are triggered by an environmental agent can be important in the approach to improvement of risk assessments.

The complexity that is introduced when all the factors that can condition the development of a chronic disease such as cancer are considered will have to be simplified in several ways to be developed into a useful model or set of models to describe the process. Attempting to develop a model that includes all variables is likely to be cumbersome. An approach that is likely to be more profitable is to build dose-response relationships that are reasonable for a primary event

and determine how secondary factors are likely to modify the response. This was demonstrated at the workshop by illustrating how a response depending upon DNA damage might be modified if the responsible agent also induces DNA repair (Conolly, 2002). An example that differed in its outcome was recently published by Brenner and Sachs (2002) to describe potential influences of bystander effects.

IV. Generalization of principles to cancer risk assessment

An important point made in the workshop was that even with a single type of ionizing radiation the dose response relationships differ in different tumor sites. This probably reflects a different level of efficiency (e.g. very different levels of inducing apoptotic cell death in different tissues) and importance for the processes that are activated by radiation in different tissues. In the genesis of some tumors the effects of radiation could overcome the homeostatic mechanisms that protect against cancer in that tissue (e.g. mammary gland), whereas tumors do not develop at other sites until the cell killing effects of radiation begin to play a role (e.g. in the development of sarcomas).

Nevertheless, it is probable that intercellular communication (i.e. bystander effects), genomic instability, and adaptive responses, as well as direct consequences of DNA damage, play some role in the control and development of tumors at all sites. What is likely to differ are 1) the relative importance of these and other variables in the contribution to a carcinogenic response and 2) the details of the signaling processes that control these cellular responses.

Finally, there is the issue of risk within a population vs. the probable risk to any given individual in the population. The probability that an exogenous agent will produce cancer in a

population is a direct function of the distribution of sensitivities within that population. If there is a broad range of sensitivities in the population and if every individual in a population has some threshold to a given agent, it becomes difficult to determine whether a population threshold exists (Lutz, 1999). In a relatively homogeneous population, thresholds or protective effects may be more easily demonstrable. Similar arguments could be made for adaptive responses. They may be demonstrable for some tumors in a species, but very difficult for other tumor sites, even when examining the effects of a single agent.

V. Conclusions and recommendations

The workshop made apparent that there are diverse uses made of cancer risk assessments. Issues varied by federal agency and each agency's mission. In some cases, the issues varied among multiple missions within a single agency. Fundamentally, each agency has statutory mandates that determine its risk assessment policies and the mandates do not completely overlap. It was not possible for the workshop to go more deeply into this very important area. In part because there was little social-legal expertise among the participants and little time could be devoted to discussion of these complicated issues. A key point differentiating agencies depends upon the question of who gains the benefits and who assumes the risk. Obviously, each agency must continue to develop its own risk management policies in the context of its own mission, the interest groups it must serve, its responsibilities for protection of the general public health, and the legal requirements of its enabling legislation.

The most wide-ranging question raised in the workshop was whether these scientific observations had reached a point where they could be used to develop new approaches to risk

assessment. The draft cancer risk assessment guidelines do provide for incorporation of data related to varying modes of action (Wiltse and Dellarco, 1996). There is no reason why the diverse phenomena discussed in the workshop could not be incorporated in such a construct. However, the consensus of the group was that to insert these phenomena independently into a risk assessment paradigm at this time would not be wise. It was pointed out that the observations should be used to develop a structure that would make apparent the data that are needed to develop and validate new methodologies.

At several stages in the workshop, the availability of new research tools was cited as being responsible for being able to detect complex phenomena that contribute to carcinogenesis. The contribution of various genome projects has been not only to provide a basis for understanding how variations in genetic background affect susceptibility to disease, but also to provide research tools in the biological sciences not even dreamed of a decade ago. The ability to expose and observe responses of single cells is a reality today. The ability to detect and quantify changes in the expression of large numbers of genes simultaneously and the availability of computational tools to aid in the interpretation of these data has exploded. The time is approaching where much better experiments can be planned that can help develop an integrated picture of how a wide variety of biological phenomena can contribute to the development of cancer. The major difficulty will be to design a set of experiments that can provide the basis for integrating the information generated into a model(s) that can improve the accuracy of risk assessments. Such a model could substantially improve the ability to make cost-effective decisions in the risk policy arena.

There can be no doubt of the importance of research spawned through request for proposal approaches to identify phenomena that are important for risk assessment. The advances that

have already been made as a result of programs conducted by several government agencies illustrate this. However, many of the participants were concerned that the complexity and magnitude of the data collection and management problem is so great that some more highly coordinated efforts may also be necessary to make more specific use of the new knowledge that has been gained through traditional granting mechanisms.

The recommendations of the workshop are as follows:

- Federal and international agencies should continue to fund basic research aimed at identifying those phenomena that modify carcinogenic responses to various agents in the environment, whether they be chemical or physical agents. Currently funded programs that will make contributions to these areas are NIEHS's Toxicogenomics Program and DOE's Low Dose Radiation Program. It is important to recognize that progress in these two areas is heavily leveraged off the highly mission-oriented activities of the various genome projects supported by NIH and DOE.
- Some effort, perhaps through an interagency initiative, needs to be directed at studies that can integrate this information by the development of:
    - Consensus teams to identify important variables and data gaps that limit the utility of current information.
    - A repository of qualitative and quantitative data that documents the nature of the phenomena thought to affect carcinogenic responses. It would appear that information should be species (strain) and target organ specific.

- Quantitative *in vivo* studies that are inclusive of parameters that can impact the carcinogenic response at low doses. These studies must be designed to identify non-linear dose-responses, if they are present, in the development of pathology as well as the behavior of molecular and cellular responses.

- Develop and refine computational approaches for carcinogenesis that not only integrate the different molecular and cellular processes that contribute to carcinogenesis in a quantitative way, but to efficiently identify deficiencies in the available data to increase the utility of such models in risk assessment.

Acknowledgements:

This workshop was sponsored by the following federal agencies: U. S. Food and Drug Administration, National Institute of Occupational Safety and Health, Agency for Toxic Substances and Disease Registry, U. S. Environmental Protection Agency, U.S. Department of Energy, National Institute of Environmental Health Sciences, and National Cancer Institute.

Participants in the workshop included: Session chairs; Steven Bayard, James Cogliano, Rory Conolly, Elaine Faustman, Annie Jarabek, Michael Joiner, Carmel Mothersill, R. Julian Preston, Bernard Schwetz, Bobby Scott, and Andrew Wallo III. Invited discussants; Isaf Al-Nabulsi, Sally Amundson, David Boothman, Douglas Boreham, David Brenner, Aloke Chatterjee, Weishueh Chiu, Matthew Coleman, David Dankovic, Evan Douple, R.J. Michael Fry, Dudley Goodhead, William Griffith, Vincent Holohan, Rick Jostes, Jocelyn Kaiser, Arthur Katz, Brian Marples, Noelle Metting, Martha Moore, James B. Muckerheide, Moiz Mumtaz, Neal S. Nelson,

Hal Peterson, Jerome Puskin, Lowell Ralston, Robert Stewart, Daniel Stram, Allan S. Susten, David Thomassen, Robert Ullrich, Bruce Wachholz, Robin Wagner, Dale Walker, Vernon Walker

References:

Amundson SA, Do, KT, Fornace AJ Jr. 1999. Induction of stress genes by low doses of gamma rays. Rad Res 152:225-231.

Amundson SA, Bittner M, Meltzer P, Trent J, Fornace AJ Jr. 2001. Induction of gene expression as a monitor of exposure to ionizing radiation. Rad Res 156:657-661.

Azzam EI, de Toledo SM, Gooding T, Little JB. 1998. Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. Radiat Res 15:497-504.

Azzam EI, de Toledo SM, Little JB. 2001. Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from α-particle irradiated to nonirradiated cells. Proc National Acad Sci (USA) 98:473-478.

Brenner DJ, Sachs RK. 2002. Do low dose-rate bystander effects influence radon risks? Int J Radiat Biol 78:593-604.

Barcellos-Hoff MH. 2002. Presentation at the workshop.

Barcellos-Hoff MH, Ravani SA. 2000. Irradiated mammary gland stoma promotes the expression of tumorigenic potential by unirradiated epithelial cells. Cancer Res. 60:1254-1260

Chomentowski M, Kellerer AM and Pierce DA. 2000. Radiation dose dependencies in the atom bomb survivor cancer mortality data: A model free visualization. Rad. Res. 153:289-294.

Conolly RB. 2002. Presentation at the workshop.

Crump KS, Hoel DG, Langley CH, Peto R. 1976. Fundamental carcinogenic processes and their implications for low dose risk assessment. Cancer Res 36:2973-2979.

Drinkwater NR. 2002. Presentation at the workshop.

Fishel R. 2001. The selection for mismatch repair defects in hereditary nonpolyposis colorectal cancer: Revising the mutator hypothesis. Cancer Res 61:7369-7474.

Fishel R. 2002. Presentation at the workshop.

Hall EJ. 2002. Presentation at the workshop.

Hahn WC, Weinberg RA. 2002. Modeling the molecular circuitry of cancer. Nature Rev 2:331-341.

Hanhahn D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57-70.

Hanigan MH, Kemp CJ, Ginsler JJ, Drinkwater NR. 1988. Rapid growth of preneoplastic lesions in hepatocarcinogen-sensitive C3H/HeJ male mice relative to C57BL/6J male mice. Carcinogenesis 9:885-890.

Hazelton WD, Luebeck EG, Heidenreich WF, Moolgavkar SH. 2001. Analysis of a historical cohort of Chinese tin miners with arsenic, radon, cigarette smoke, and pipe smoke exposures using the biologically based two-stage clonal expansion model. Radiat Res 156:78-94.

Henshaw DL, Eatough JP, Richardson RB. 1990. Is radon a causative factor in inducing myeloid leukemia and other cancers in adults and children? In: 29th Hanford Symposium on Health and the Environment, Indoor Radon and Lung Cancer: Reality or Myth, Ed: FT Cross Battelle Press, Columbus, Richland pp. 935-959.

Hoel DG. 2002. Presentation at the workshop.

Hrubec Z, Boice JD Jr, Monson RR, Rosenstein M. 1989. Breast cancer after multiple chest fluoroscopies: Second follow-up of Massachusetts's women with tuberculosis. Cancer Res 49:229-234.

Joiner MC, Johns H. 1988. Renal damage in the mouse: the response to very small doses per fraction. Radiat Res 114:385-398.

Joiner MC, Lambin P, Malaise EP, Robson T, Arrand JE, Skov KA, Marples B. 1996. Hypersensitivity to very-low single radiation doses: its relationship to the adaptive response and induced radioresistance. Mutat Res 358:171-183.

Krewski, D 2002. Presentation at the workshop.

Leach JK, Van Tuyle G, Lin P-S, Schmidt-Ullrich R, Mikkelsen RB. 2001. Ionizing radiation-induced, mitochondria-dependent generation of reaction oxygen/nitrogen. Cancer Res 61:3894-3901.

Leach JK, Black SM, Schmidt-Ullrich RK, Mikkelsen RB 2002. Activation of constitutive nitric oxide synthase activity is an early signaling event induced by ionizing radiation. J Biol Chem 277:15400-15406.

Li C-Y, Little JB, Hu K, Zhang W, Zhang L, Dewhirst MW, Huang Q. 2001. Persistent genetic instability in cancer cells induced by non-DNA-damaging stress exposures. Cancer Res 61:428-432.

Lorimore SA, Kadhim MA, Pocock DA, Papworth D, Stevens L, Goodhead DT, Wright EG. 1998. Chromosomal instability in the descendents of unirradiated surviving cells after α-particle irradiation. Proc Nat Acad Sci 95:5730-5733.

Luster MI. 2002. Presentation at the workshop.

Lutz WK. 1999. Dose-response relationships in chemical carcinogenesis reflect differences in individual susceptibility. Consequences for cancer risk assessment, extrapolation, and prevention. Human & Exp. Toxicol. 18:707-712.

McClellan RO. 1999. Human health risk assessment. A historical overview and alternative paths forward. Inhalation Toxicol 11:477-418.

Miller AB, Howe GR, Sherman GJ, Lindsay JP, Yaffe MJ, Dinner PJ, Risch HA, Preston DL. 1989. Mortality from breast cancer after irradiation during fluoroscopic examinations in patients being treated for tuberculosis. New Engl J Med 321:1285-1289.

Miller WH Jr, Schipper HM, Lee JS, Singer J, Waxman S. 2002. Mechanisms of action of arsenic trioxide. Cancer Res 62:3893-3903.

Mitchell REJ, Jackson JS, McCann RA, Boreham DR 1999. The adaptive response modifies latency for radiation-induced myeloid leukemia in CBA/H mice. Rad Res 152:273-279.

Moolgavkar SH, Venzon DJ. 1979. Two-event models for carcinogenesis: Incidence curves for childhood and adult tumors. Math Biosci 47:55-77.

Morgan WF. 2002. Presentation at the workshop.

Mothersill C, Seymour CB. 1997. Medium from irradiated human epithelial cells, but not human fibroblasts reduces the clonogenic survival of unirradiated cells. Int J Radiat Biol 71:421-427.

Mothersill C, Seymour CB, Joiner MC. 2002. Relationship between radiation-induced low-dose hypersensitivity and the bystander effect. Radiat Res 17:526-532.

Nagasawa H, Little JB. 1992. Induction of sister chromatid exchanges by extremely low doses of alpha particles. Cancer Res 52:6394-6396.

Nelson JM, Brooks AL, Metting NF, Khan MA, Buschbom A, Duncan A, Miick R, Braby LA. 1996. Clastogenic effects of defined numbers of 3.2 MeV alpha particles on individual CHO-K1 cells. Radiat Res 145:568-574.

NRC. 1983. Risk Assessment in the Federal Government. Managing the Process. Washington DC. National Academy Press.

Olivieri G, Bodycote J, Wolff S. 1984. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 223:594-597.

Padovani L, Apolloni M, Anzidei P, Tedeschi B, Caporossi D, Vernole P, Mauro F. 1995. Do human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? 1. Challenge with ionizing radiation. Mutat Res 332:33-38.

Raaphorst GP, Boyden S. 1999. Adaptive response and its variation in human normal and tumour cells. Int J Radiat Biol 75:865-873.

Redpath JL. 2002. Presentation at the workshop.

Redpath JL, Liang D, Taylor TH, Christie C, Elmore E. 2001. The shape of the dose-response curve for radiation-induced neoplastic transformation in vitro. Evidence for an adaptive response against neoplastic transformation at low doses of low-LET radiation. Radiat Res 156:700-707.

Savell J, Rao S, Pledger WJ, Wharton W. 2001. Permanent growth arrest in irradiated human fibroblasts. Radiat Res 155:554-563.

Sawant SG, Randers-Pehrson G, Geard CR, Brenner DJ, Hall EJ. 2001a. The bystander effect in radiation oncogenesis: I. Transformation in C3H 10T1/2 cells in vitro can be initiated in the unirradiated neighbors of irradiated cells. Radiat Res 155:397-401.

Sawant SG, Randers-Pehrson G., Metting NF, Hall EJ. 2001b. Adaptive response and the bystander effect induced by radiation in C3H 10T1/2 cells in culture. Radiat Res 156:177-188.

Seymour CB, Mothersill C. 1997. Relative contribution of bystander and targeted cell killing to the low dose region of the radiation dose-response curve. Radiat Res 153:508-511.

Short S, Mayes C, Woodcock M, Johns H, Joiner, MC. 1999. Low dose hypersensitivity in the T98G glioblastoma cell line. Int J Radiat Biol 7:847-855.

Simmons PT, Portier CJ. 2002. Toxicogenomics: the new frontier in risk analysis. Carcinogenesis 23:903-905.

Sorensen KJ, Attix CM, Christian AT, Wyrobeck AJ, Tucker JD. 2001. Adaptive response induction and variation in human lymphoblastoid cell lines. Mutat Res 519:15-24.

Spitz DD. 2002. Presentation at the workshop.

Tedeshi B, Caporossi D, Vernole P, Padovani L, Appoloni M, Anzidei P, Mauro F. 1995. Do human lymphocytes exposed to fallout of the Chernobyl accident exhibit an adaptive response? 2. Challenge with bleomycin. Mutat Res 332:39-44.

te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP. 2002. DNA damage is able to induce senescence in tumor cells in vitro and in vivo. Cancer Res 62:1876-1883.

Watson GE, Lorimore SA, Macdonald DA, Wright EG. 2001. *In vivo* chromosomal instability and transmissable aberrations in haemopoietic stem cells induced by high- and low-LET radiations. Int J Radiat Biol 77:409-417.

Wiltse J, Dellarco VL. 1996. U.S. Environmental Protection Agency guidelines for carcinogen risk assessment: Past and future. Mutat Res 365:3-15.

Wolff S. 1992. Is radiation all bad? The search for adaptation. Radiat Res 131:117-123.

Zhou H, Randers-Pehrson G, Waldren CA, Vannis D, Hall EJ, Hei TK. 2000. Induction of a bystander mutagenic effect of alpha particles in mammalian cells. Proc. Natl. Acad Sci. (USA) 97:2099-2104.

Figure 1. A schematic rendering of key pathways involved in chemical and radiation-induced carcinogenesis. Classical approaches to risk assessment have focused on the generation of stable mutations or aberrations by direct action of a carcinogen. New research has identified diverse responses that occur as a result of low-level carcinogen exposure. These processes have the potential of magnifying or limiting the probability that these mutations will be expressed as a carcinogenic response.