1   **Cancer Risk Assessment: Should New Science Be Applied?**
2   Workgroup Summary
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1   Abstract

2

3   In July of 2002 a workshop was convened that explored some of the intercellular phenomena that

4   appear to condition responses to carcinogen exposure. Effects that result from communication

5   between cells that appear to either increase the sphere of damage or to modify the sensitivity of

6   cells to further damage were of particular interest. Much of the discussion focused on the effects

7   of ionizing radiation that were transmitted from cells directly hit to cells not receiving direct

8   exposure to radiation (bystander cells). In cell culture, increased rates of mutation, chromosomal

9   aberration, apoptosis, genomic instability, and decreased clonogenic survival have all been

10   observed in cells that have experienced no direct radiation. In addition, there is evidence that

11   low doses of radiation or certain chemicals give rise to adaptive responses in which the treated

12   cells develop resistance to the effects of high doses given in subsequent exposures. Data were

13   presented at the workshop indicating that low dose exposure of animals to radiation and some

14   chemicals frequently reduces the spontaneous rate of mutation *in vitro* and tumor responses *in*

15   *vivo*. Finally, it was concluded that considerable improvement in understanding of how genetic

16   variation may modify the impact of these phenomena is necessary before the risk implications

17   can be fully appreciated. The workshop participants discussed the substantive challenge that

18   these data present with respect to simple linear methodologies that are currently used in cancer

19   risk assessment and attempted to identify broad strategies by which these phenomena may start

20   to be used to refine cancer risk assessment methods in the future.

21

22

1   I. Introduction

2

3   A. The workshop

4

5   A workshop was conducted on July 17-19, 2002 to discuss the implications of recent findings

6   in radiation biology for assessment of cancer risks from low doses of chemical and physical

7   agents. The objective of the workshop was to bring together basic scientists who are responsible

8   for observations made in radiobiology, their counterparts from the field of toxicology, scientists

9   whose main interests are risk assessment, and scientists working on incorporation of concepts of

10 mode of action into cancer risk assessments made by various federal agencies (Wiltse and

11 Dellarco, 1996). The regulatory agencies represented at the workshop were Occupational Safety

12 and Health Administration, National Institute of Occupational Safety and Health, Agency for

13 Toxic Substances and Disease Registry, U.S. Environmental Protection Agency, Food and Drug

14 Administration, the Nuclear Regulatory Commission, the Department of Energy and the National

15 Radiological Protection Board (UK).

16   This workgroup summary has been prepared by reference to the presentations that were made

17 and materials developed from summaries and notes that were made at the workshop. The

18 summary has been circulated to the participants and the resulting comments have been utilized in

19 developing the final draft of the manuscript. Every attempt was made to capture any important

20 differences of opinion that were articulated at the workshop and the subsequent comment period.

21

22 B. Evolution of cancer risk assessment

23

1   Risk assessment of potentially harmful physical and chemical agents developed gradually

2   over the past half century (McClellan, 1999; 2002). The major impetus for this science has been

3   the utilization of its research products in the development of regulatory policy related to

4   allowable human exposures. However, the approach taken has depended in large part on

5   whether the data available come from observational or experimental data.

6   In the case of radiation, risk estimates for cancer have been based on data from human

7   experience, in particular the atomic bomb survivors. Data from animal experiments, and to a

8   lesser degree cellular studies, have been used to assess the influence of dose rate, dose

9   distribution, and the type of radiation.

10   In the period of 1945-50, the concept of "tolerance doses", based upon the assumption that

11   the effects of radiation had threshold doses, was gradually replaced by the "maximum

12   permissable limit", as the possibility that some responses might not have thresholds was

13   recognized.

14   Dose-response assessments for non-cancer endpoints for chemicals have continued to evolve

15   based upon the threshold concept. A reference dose is developed that is thought to be below the

16   threshold. It is derived from a no-observed-adverse-effect-level or a lowest-observed-adverse-

17   effect-level (or from a benchmark dose in more recent years) that is adjusted by uncertainty

18   factors (related to the interspecies and low-dose extrapolation of data) and modifying factors

19   based upon the completeness of the data that are available (NRC, 1983).

20   In the early 1970s a variety of developments occurred that brought greater focus to the

21   assessment of cancer risks. The enabling legislation that formed the U.S. Environmental

22   Protection Agency required a closer look at the regulation of chemicals in the environment.

23   Cancer risk policy first made use of the one-hit model. This was highly criticized because it

1 frequently did not fit the data available and the basic assumption did not seem to apply generally.

2 This gave way to the formalization of the linearized multistage model (Crump et al., 1976). This

3 model had the advantage of being both biologically plausible and relatively simple in its

4 application and has underpinned much of cancer policy in the past two decades.

5   Unlike radiation, chemicals that cause cancer have been largely identified by animal studies.

6 For this reason there has been greater reliance on experimental data in risk assessment. These

7 studies have provided evidence that many different types of mechanisms contribute to the

8 development of cancer. The draft guidelines for cancer risk assessment (U.S. EPA, 1996) open

9 up risk assessment to modes or mechanisms of action that would allow either the low dose or

10 across species extrapolation of data to depart from defaults. When these data have been treated

11 formally in models, such as that developed by Moolgavkar, Venzon & Knudson, rates of cell

12 division and cell death, as well as changes in mutation rate, have been utilized to estimate risk at

13 low doses (Moolgavkar and Venzon, 1979). It is recognized that even these more elaborate

14 models remain as oversimplifications.

15   The main focus of the present workshop was on observations related to phenomena that

16 suggest that a number of indirect effects (e.g. bystander effects) may be determinants of the

17 dose-response curve <u>in low-dose ranges.</u> These phenomena may well introduce non-linear

18 behavior into the dose-response curves for carcinogenic effects. Although the clearest examples

19 are with ionizing radiation, cross-tolerance with chemicals and radiation demonstrate at least one

20 parallel phenomenon exists with chemical carcinogens (Padovani et al., 1995; Tedeschi et al.,

21 1995). The workshop represented one of the first attempts to assemble a group of experts to

22 identify the implications these new findings have for cancer risk assessment in general.

23

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

1   prior incubation of cells with low levels of tritiated thymidine prior to a large X-ray dose

2   (Olivieri et al., 1984).   Subsequent work demonstrated that low priming doses of X-rays also

3   resulted in fewer chromatid breaks being observed following the higher challenge dose (Wolff,

4   1992).

5   Adaptive responses to subsequent α irradiation that were induced by low doses of γ-

6   irradiation (2 cGy) have been elegantly demonstrated when only 10% of the cells present were

7   irradiated using a single particle microbeam (Sawant et al., 2001b). This study was of particular

8   interest in that the adaptive response was found to reduce the bystander effect (measured as

9   clonogenic survival) by 50%.

10   Experiments utilizing this same exposure paradigm have extended these observations to

11   include a wide variety of endpoints. In the case of cell killing, relatively large priming doses

12   confer protection to subsequent doses of similar magnitude (Raaphorst and Boyden, 1999).

13   However, the induction of the adaptive response varies widely depending upon cell type and

14   genetic susceptibility among individuals.

15   Adaptive responses have been demonstrated in mice by administering a low total dose of 10

16   cGy γ-irradiation delivered at two different dose-rates 24 hours before administering a 1 Gy dose

17   (Mitchel et al., 1999). Mice receiving the pretreatment were found to have a significantly

18   prolonged latent period for development of myeloid leukemia. Similar adaptive responses were

19   induced by 60 min of whole body hyperthermia and injection of 1500 U of interleukin-1,

20   intraperitoneally. These studies provide an experimental case that demonstrates adaptive

21   responses can provide protection against radiation-induced disease *in vivo.*

22

23   2.   Complexities of dose-response curves

1

2 A variety of experiments have shown that many of the classical responses to irradiation are

3 more complex than originally thought. The experimental paradigms used for these experiments

4 vary from that used to demonstrate the adaptive response, but they have been inferred to be

5 related to adaptive responses (Joiner et al., 1996).

6 Studies of the dose-response relationships for cell killing have revealed responses that are

7 very non-linear in the low dose range (Joiner et al., 1996). At radiation doses between 0 and 0.3

8 Gy, cell killing is significantly greater than would be predicted by extrapolating the dose-

9 response observed at doses > 1 Gy. While the hypersensitive set of cells may come from

10 populations in different arts of the cell cycle in part, the low-dose hypersensitivity is displayed in

11 all phases of the cell cycle (Short et al., 1999). The interpretation offered of these results is that

12 there is an induction of DNA repair mechanisms that protect the cells from killing in the high

13 dose range.

14 Cells that display bystander effects (measured as reduced clonogenic survival) do not display

15 the low dose hypersensitivity (Mothersill et al., 2002). Consequently, they are viewed as

16 separable phenomena.

17 Low doses of γ-irradiation (0.1 to 10 cGy) have been shown to reduce the spontaneous

18 transformation of cells *in vitro* (Redpath et al. 2001). Data on the reduction of background levels

19 of damage by low dose radiation were reviewed and related to some cancer responses to

20 radiation that have been observed in humans and experimental animals (Redpath, 2002). Thus,

21 the experience with low-LET radiation appears to be somewhat different from the increased rates

22 of cell transformation induced in cells that are neighbors of cells hit by high-LET α-particles

23 (Sawant et al., 2001a).

1   Qualitative differences in responses in different dose ranges are reflected at the molecular

2   level. *In vitro* studies of changes in gene expression following varying doses of γ-irradiation

3   reveals 30 genes that were activated at all doses. However, at low doses (0.2 Gy) and low dose

4   rates, there were 137 genes activated that were not activated at 10 Gy and 16 that were activated

5   at 10 Gy, but not activated at the lower doses (Amundson et al., 2001).

6   At the workshop, a preliminary analysis on the shape of the dose response in the low dose

7   region was presented. Radiation studies in experimental animals that have been published in the

8   open scientific literature were reviewed and subjected to statistical analyses for J-shaped dose

9   response curves (Krewski, 2002). It was found that the number of studies displaying J-shaped

10   dose-response curves was significantly greater than could be expected by chance. The J-shape

11   appeared to occur with both high and low-LET radiation. This finding is remarkable, because

12   these studies had not been designed to demonstrate such behavior in low doses. Thus, such

13   effects can occur. Nevertheless, the generality of the low-dose protective effect has yet to be

14   established.

15   Some treatments of data on cancer mortality in atomic bomb survivors support, at least in

16   part, the hypothesis that low doses of radiation are protective against certain types of cancer.

17   Some analyses of these data and those in the literature were discussed (Hoel, 2002). Depending

18   upon the selection of the dose cutpoints, mortality from leukemia can appear to be less in

19   survivors receiving low doses of radiation relative to the control population (Chomentowski et al.

20   2000). However, solid tumor mortality displays no evidence of low dose protection. However,

21   data involving chronic exposures to radiation (Miller et al., 1989; Hrubec et al., 1989) indicate

22   that the dose response for some tumors appears linear within statistical confidence intervals, to

23   the lowest exposures that have been characterized.

1   B. Genomic instability

2

3   Genomic instability induced by radiation was reviewed (Morgan, 2002). There are

4   differences in the processes responsible for induction of genomic instability that are produced by

5   low and high doses of radiation that may be critical to understanding the implications of these

6   observations for the development of disease.

7

8   1. Low dose effects

9

10   Low doses of radiation to cells in culture increases the numbers of cells that display genomic

11 instability. Genomic instability is observed as increased non-clonal mutations or chromosomal

12 aberrations in either cells that have been subject to direct irradiation or that are bystander cells.

13 The phenomenom has also been described when medium is transferred from irradiated cells to

14 naïve cells.

15   Genomic instability is induced *in vitro* by very low doses of radiation. In media transfer

16 experiments; evidence of clonogenic cell death in response to γ-irradiation appears to approach a

17 maximum between 1-5 cGy (Seymour and Mothersill, 2000). Genomic instability induced in

18 bystander cells by α-irradiation generally occurs when cells are cultured under conditions of

19 close cell-to-cell contact and to be largely dependent upon gap junction communication (Azzam

20 et al., 1998; Azzam et al., 2001). Responses are also seen at low doses, but with less evidence of

21 saturation in the dose-response curve. This may be related to heterogeniety of dose/cell that is

22 observed relative to that observed with equivalent energies of γ-irradiation in these dose ranges.

23 Genomic instability has also been observed to be induced in bystander cells by α-irradiation in

1   heamopoietic cells, which do not have gap junctions, both in tissue culture experiments

2   (Lorimore et al., 1998) and in mice (Watson et al., 2001). Collectively, these data indicate, by

3   virtue of the very high frequencies of the induced changes, that genomic instability is not directly

4   the result of simple mutation.

5

6   2. High dose effects

7

8   As doses of radiation increase, the influence of genomic instability on cancer outcomes may

9   increase. The factors affecting the dose-dependent increase in genomic instability may not be

10   simple. In experiments presented at the workshop, the differences in the dose-response curves

11   for the induction of chromosomal instability by acute exposures to x-rays, iron ions and [125]IdUrd

12   were presented (Morgan, 2002). [125]I seeding was used to construct an exposure system to obtain

13   long-term, low dose rate, irradiation (48 hours) at 50 cGy/day. The medium from these

14   experiments was transferred to non-irradiated cells and was found to contain a death-inducing

15   factor that selectively affected non-irradiated cells. Thus, it appears that the effects of higher

16   doses of radiation delivered at low dose rates can result in the development of selection

17   mechanisms that favor cells with unstable genomes.

18

19   3. Other causes of genomic instability

20

21   A recent paper (Li et al., 2001), not discussed at the workshop, provided evidence that

22   genetic instability can be induced by stresses that do not directly damage DNA. These were

23   observed in a spontaneously arising mammary carcinoma line from Balb/c mice. Heat treatment

1 (45° C for 30 min), serum starvation, or *in vivo* growth in a syngeneic host all increased the yield

2 of genomically unstable cells. In the same study, genetic instability was also demonstrated with

3 γ-rays and hydrogen peroxide treatments that damaged DNA.

4

5 C. Other cellular fates.

6

7 It has become increasingly clear that the responses of cells from exposure to radiation or

8 carcinogenic chemicals vary widely. Cells that become transformed directly or as a result of

9 induced genomic instability represent only two of several possible cellular fates. The diversity

10 of these fates following exposure to radiation is sometimes difficult to recognize in the primary

11 literature because most studies tend to focus on one aspect of the responses and try to identify the

12 mechanisms that are responsible for the selected endpoint.

13 Figure 1 displays a number of these alternative fates of cells exposed to cancer-inducing

14 agents and hints at some of the processes that might influence the fate of individual cells in a

15 culture dish or *in vivo*. At the molecular level, damage induced in DNA directly or through the

16 activation of indirect processes stimulates repair of lesions in DNA. Such cells survive and

17 continue to divide, usually after a short delay in the cell cycle to allow for repair. Some cells are

18 killed either directly from DNA damage or secondarily from apoptosis. Other cells become

19 resistant to killing and in some cases this resistance has been associated with the increased ability

20 to repair DNA damage. Some cells, however, apparently become permanently arrested by

21 mechanisms that are separable from the cyclin-dependent kinase activity of CDKN1A (Savell et

22 al., 2001). This permanent arrest appears to be secondary to DNA damage and can involve

23 increased expression of p53 and p21 (te Poele et al., 2002). The important point to be made is

1   that we are just beginning to understand the signaling processes that are triggered by the effects

2   of chemicals or ionizing radiation. They are likely to occur in both directly damaged and

3   bystander cells. Undoubtedly there are other factors that condition the relative proportion of

4   cells having a given fate at different doses of the agent in different cell types and tissues.

5

6   D. Tissue and cell-specific responses.

7

8   Low dose hypersensitivity, bystander effects, adaptive responses, and genomic instability are

9   not seen uniformly in cells of differing origins (see for example, Sorensen et al. 2002).

10   Moreover, the details of the responses to radiation appear to vary between species and strain.

11   These differences make it difficult to generalize the data taken from model systems. If these

12   phenomena are to be incorporated into risk assessment models, the inability to generalize will

13   seriously complicate the simple linear extrapolations that have been made up to now. At the

14   very minimum, the variation in the type and magnitude of responses in different tissues suggest

15   that low dose extrapolation will have to be tailored to the tumor site.

16

17   E. Dose-dependence of responses

18

19   An important consideration of the implications of phenomena that occur at low doses of

20   ionizing radiation may be differences in the effects of high and low-LET radiation. Alpha

21   particles have a high relative mass and a single track can deposit large amounts of energy

22   resulting in catastrophic impacts upon the cell that has been hit. This correlates with the ability

23   of a single hit by a high-LET particle to generate genomic instability in the hit cell and in

1 bystander cells, whereas it takes multiple hits by low-LET radiation to demonstrate such

2 responses. The traditional view in radiation protection is that quality factors make simple and

3 accurate adjustments for differences in energy deposition, the spatial differences involved, the

4 background experience with repairing such damage, the parallel damage induced by metabolism,

5 and the localized intensity of the damage. That view has been questioned in recent years, but

6 remains controversial.

7 As suggested in the model of damage done to the genome in bystander cells by α-particles,

8 the simplest models that try to take these phenomena into account can result in complex

9 relationships between dose and response (Brenner and Sachs, 2002). The inability of low-LET

10 radiation to induce immediate alterations in bystander cells may result in less complex responses

11 at low doses.

12

13 F. Potential mechanisms underlying bystander effects.

14

15 Understanding of how cells generate, transmit and interpret signals as a result of an event that

16 occurs in the hit cell is in its infancy. In the context of ionizing radiation, there are systems that

17 are obvious candidates based upon some of the chemistry that is generated, such as reactive

18 oxygen and nitrogen species. Information from studies of mechanisms that effect or modify

19 chemical carcinogenesis adds more complexity to the overall picture. Presentations at the

20 workshop dealt with examples of mechanisms that were involved in intracellular communication,

21 intercellular communication, and influences that are exerted at the whole tissue level.

22 The sliding clamp model whereby mismatch repair proteins accumulate at the sites of

23 mismatches in DNA and appear to serve as a signal for recruiting other repair proteins to these

1   sites was a mechanism that was discussed as an example (Fishel, 2001; 2002). Products of two

2   of the mismatch repair genes, hMSH2 and hMLH1, have the additional property of promoting

3   apoptosis, whereas the remaining homologues do not. Cells containing mutations in these genes

4   are resistant to apoptosis induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Thus,

5   mutation in either one of these two genes will protect cells from apoptosis and provide a

6   selective advantage to an unstable genotype. The question raised was whether loss of signaling

7   functions of other DNA damage processing proteins could contribute to the selection of

8   genetically unstable cells that could lead to more rapid development of cancer.

9   The ability of hit cells to contribute to instability in non-hit cells increases the complexity of

10   carcinogenesis. The role of free radicals that are formed as the result of cells being hit by

11   ionizing radiation or from exposures to other chemical and physical agents was explored as a set

12   of mechanisms that might contribute to this phenomena (Spitz, 2002). The mitochondrial

13   electron transport chain, NADPH oxidase enzymes, xanthine oxidase, and nitric oxide synthases

14   were identified as the most likely sources of prooxidants that could contribute to bystander

15   effects and discussed the antioxidant systems that limit the influence of these sources. Recent

16   studies have demonstrated that ionizing radiation does result in production of reactive

17   oxygen/nitrogen species. It is postulated that this signal can be transmitted to other mitochondria

18   by permeability change releasing calcium, providing a means of amplifying the signal (Leach et

19   al., 2001). More recently, ionizing radiation was shown to increase nitric oxide synthase

20   activity (Leach et al., 2002). NO is an attractive means of transmitting signals between cells

21   because its relative stability allows migration over several cell diameters. To date, these studies

22   have been limited to relatively high doses of radiation (e.g. 2-5 Gy). However, there is ample

23   evidence that low doses of low-LET radiation (<0.02 Gy) do result in measurable changes in

1   gene expression in cells in culture (Amundson et al., 1999). Although these latter results

2   certainly have implications for intracellular responses, the extent to which they contribute to

3   intercellular communication remains to be established.

4   Experiments were described that demonstrated that irradiation of mammary gland stroma

5   promoted the growth of tumors from transplanted cells that received no direct exposure to

6   radiation (Barcellos-Hoff, 2002; Barcellos-Hoff and Ravani, 2000). This effect was associated

7   with activation of latent TGF-β, although the precise mechanisms by which tumor cells are

8   selected have not yet been worked out.

9   These selected topics illustrate that carcinogenic responses in intact organisms involve

10   complex mechanisms that may well arise indirectly, rather than simply being set in motion by

11   mutations induced by a carcinogenic agent.

12

13   H. Genetically determined susceptibility

14

15   The role genetic susceptibility plays in carcinogenic responses was discussed. It was pointed

16   out that a relatively small fraction of human cancers are accounted for by dominant genes

17   leaving much of the differences in susceptibility to be accounted for by interactions among

18   multiple genes. Work that has focused on susceptibility to liver tumors in different strains of

19   mice and the linkage of susceptibility to specific genes was discussed as an example

20   (Drinkwater, 2002). Many genes modify susceptibility to cancer and they influence different

21   stages in the carcinogenic process. In the case of liver cancer in mice, the growth rate of

22   preneoplastic foci appears to be an important differentiator between susceptible and non-

23   susceptible strains (Hanigan et al., 1988). However, the relative importance of a particular locus

1   was shown to vary among strains (6-20 fold) and accounts for only a portion of the 100-fold

2   difference between strains. These data imply that multiple loci influence carcinogenesis and that

3   they modify different stages. It is probable that similar mechanisms contribute to variation in

4   susceptibility among people.

5

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

7   modulate carcinogenic responses.

8

9   Workshop participants expressed varying opinions about how the phenomena discussed

10 should influence risk assessment. It was apparent from the discussions that there is now

11 evidence of many effects of radiation that do not arise directly from damage to DNA in the hit

12 cells, either by a direct hit to DNA or indirectly through the local generation of reactive oxygen.

13 These "secondary" phenomena are clearly important and may be major determinants of whether

14 exposure to low doses of radiation will have a cancer outcome. Discussions of topics led to

15 varying degrees of consensus:

16 • **The implications of damage produced by high-LET and low-LET radiation could be**

17 **quite different.**   The amount of energy deposited in an individual cell decreases

18   uniformly with low-LET radiation to a point where the total energy in any one cell fades

19   into the background level and may not be able to produce physiological responses such as

20   the adaptive or bystander effects. Research is needed to determine if the clustered DNA

21   damage produced at very low doses of low-LET radiation has consequences in terms of

22   an increased risk of cancer. The relatively low-levels of such damage with low doses of

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

1   predominance of adaptive responses at low doses perhaps reflects the importance of a

2   living system to be subject to some level of stress.

3   • **Intracellular and intercellular communication play important roles in determining**

4   **whether there is a carcinogenic outcome to a given dose of an agent.** At this time

5   there is not an integrated understanding of the signaling pathways involved, the interplay

6   between them, and what damage may be induced or the nature of protection that may

7   result.

8   • **Alternative cellular fates and the determinants of these fates are extremely**

9   **important to new quantitative approaches to risk assessment.** Some risk assessment

10   methods now explicitly incorporate cellular dynamics (e.g. MVK , see Hazelton et al.,

11   2001). Clearly, bystander effects, genomic instability, and adaptive responses have

12   important impacts on cellular dynamics within a tissue, but that it is too early to fully

13   comprehend how they should be considered in risk assessment. A key consideration is

14   whether and how these phenomena are involved at different dose ranges.

15

16   A. Develop a means for organizing information.

17

18   A critical need identified at the workshop was the need to have a structured means for

19   identifying the important biological responses that collectively determine whether a cancer will

20   develop. Hanahan and Weinberg (2000) provided an excellent classification of the traits that

21   cells must acquire to become cancerous. These traits include self-sufficiency in growth signals,

22   insensitivity to antigrowth signals, evasion of apoptosis, sustained angiogenesis, limitless

23   replicative potential, and the ability to invade and metastasize. Carcinogens would necessarily

1   contribute to one or more of these properties. Both qualitative and quantitative data should be

2   organized into a 'repository' that would provide risk assessors a means to organize the

3   information. In turn the collection of the information into a structured package would enable

4   researchers to more clearly identify those data gaps that impede the development of more

5   accurate approaches.

6   Beyond the development of a general understanding of carcinogenesis, account must be

7   taken of critical differences in the behavior of experimental models and what occurs in intact

8   humans. Many *in vitro* systems utilize immortalized cells that no longer possess all the controls

9   on growth present *in vivo*. Interspecies differences have also confounded extrapolation issues in

10   the past. However, it is important to realize that some of the mechanisms that account for such

11   differences are coming to be understood. For example, at least part of the basis for

12   understanding the different numbers of mutations required for transformation of human and

13   rodent cells have been identified (Hahn and Weinberg, 2002). It appears that adjustments for

14   differences in target organ specificity of carcinogens may become tractable. Such issues are

15   extremely important for translating results from experimental systems to assessing risks to

16   humans.

17

18   B. Dose-dependence

19

20   Those phenomena that both contribute to the development of cancer and are modified by

21   exogenous agents need to have established dose-response relationships to be useful in developing

22   new approaches to risk assessment. It is frequently not appreciated that the dose-response

23   relationships contribute qualitative as well as quantitative information. As was emphasized in

1   the conference, behaviors in one dose range frequently do not carry over to another dose range.

2   Non-linear dose-response ultimately provides information about the underlying biology of the

3   response, something that must be understood to address low dose issues.

4   Few of the dose-response data obtained in isolated systems are likely to be quantitatively

5   utilized in risk assessment. The few data that will be transferred to the *in vivo* system (ultimately

6   in humans) will be used only to the extent they can be shown to be consistent with *in vivo*

7   observations. Data taken from the radiation biology literature continually reinforce the fact that

8   phenomena initiated by radiation vary by cell of origin, are modified by the context of the tissue

9   within which they reside, are affected by differences in physiological state, and are subject to

10   variations in genetic background.

11

12   C.   Integration

13

14   Concerns of risk necessarily focus on the response of intact humans. Responses of complex

15   organisms are always conditioned by the physiological state. The physiological state of an

16   individual will be modified by his/her genetic makeup, lifestyle, and by other factors that occur

17   in the surrounding environment. Clearly molecular and biochemical effects which are triggered

18   by an environmental agent can be important in the approach to improvement of risk assessments.

19   The complexity that is introduced when all the factors that can condition the development of

20   a chronic disease such as cancer are considered will have to be simplified in several ways to be

21   developed into a useful model or set of models to describe the process. Attempting to develop a

22   model that includes all variables is likely to be cumbersome. An approach that is likely to be

23   more profitable is to build dose-response relationships that are reasonable for a primary event

1   and determine how secondary factors are likely to modify the response. This was demonstrated

2   at the workshop by illustrating how a response depending upon DNA damage might be modified

3   if the responsible agent also induces DNA repair (Conolly, 2002). An example that differed in

4   its outcome was recently published by Brenner and Sachs (2002) to describe potential influences

5   of bystander effects.

6

7   IV. Generalization of principles to cancer risk assessment

8

9   An important point made in the workshop was that even with a single type of ionizing

10   radiation the dose response relationships differ in different tumor sites. This probably reflects a

11   different level of efficiency (e.g. very different levels of inducing apoptotic cell death in different

12   tissues) and importance for the processes that are activated by radiation in different tissues. In

13   the genesis of some tumors the effects of radiation could overcome the homeostatic mechanisms

14   that protect against cancer in that tissue (e.g. mammary gland), whereas tumors do not develop at

15   other sites until the cell killing effects of radiation begin to play a role (e.g. in the development of

16   sarcomas).

17   Nevertheless, it is probable that intercellular communication (i.e. bystander effects), genomic

18   instability, and adaptive responses, as well as direct consequences of DNA damage, play some

19   role in the control and development of tumors at all sites. What is likely to differ are 1) the

20   relative importance of these and other variables in the contribution to a carcinogenic response

21   and 2) the details of the signaling processes that control these cellular responses.

22   Finally, there is the issue of risk within a population vs. the probable risk to any given

23   individual in the population. The probability that an exogenous agent will produce cancer in a

1   population is a direct function of the distribution of sensitivities within that population. If there

2   is a broad range of sensitivities in the population and if every individual in a population has some

3   threshold to a given agent, it becomes difficult to determine whether a population threshold

4   exists (Lutz, 1999). In a relatively homogeneous population, thresholds or protective effects may

5   be more easily demonstrable. Similar arguments could be made for adaptive responses. They

6   may be demonstrable for some tumors in a species, but very difficult for other tumor sites, even

7   when examining the effects of a single agent.

8

9   V. Conclusions and recommendations

10

11   The workshop made apparent that there are diverse uses made of cancer risk assessments.

12 Issues varied by federal agency and each agency's mission. In some cases, the issues varied

13 among multiple missions within a single agency. Fundamentally, each agency has statutory

14 mandates that determine its risk assessment policies and the mandates do not completely overlap.

15 It was not possible for the workshop to go more deeply into this very important area. In part

16 because there was little social-legal expertise among the participants and little time could be

17 devoted to discussion of these complicated issues. A key point differentiating agencies depends

18 upon the question of who gains the benefits and who assumes the risk. Obviously, each agency

19 must continue to develop its own risk management policies in the context of its own mission, the

20 interest groups it must serve, its responsibilities for protection of the general public health, and

21 the legal requirements of its enabling legislation.

22   The most wide-ranging question raised in the workshop was whether these scientific

23 observations had reached a point where they could be used to develop new approaches to risk

1 assessment. The draft cancer risk assessment guidelines do provide for incorporation of data

2 related to varying modes of action (Wiltse and Dellarco, 1996). There is no reason why the

3 diverse phenomena discussed in the workshop could not be incorporated in such a construct.

4 However, the consensus of the group was that to insert these phenomena independently into a

5 risk assessment paradigm at this time would not be wise. It was pointed out that the observations

6 should be used to develop a structure that would make apparent the data that are needed to

7 develop and validate new methodologies.

8 At several stages in the workshop, the availability of new research tools was cited as being

9 responsible for being able to detect complex phenomena that contribute to carcinogenesis. The

10 contribution of various genome projects has been not only to provide a basis for understanding

11 how variations in genetic background affect susceptibility to disease, but also to provide research

12 tools in the biological sciences not even dreamed of a decade ago. The ability to expose and

13 observe responses of single cells is a reality today. The ability to detect and quantify changes in

14 the expression of large numbers of genes simultaneously and the availability of computational

15 tools to aid in the interpretation of these data has exploded. The time is approaching where much

16 better experiments can be planned that can help develop an integrated picture of how a wide

17 variety of biological phenomena can contribute to the development of cancer. The major

18 difficulty will be to design a set of experiments that can provide the basis for integrating the

19 information generated into a model(s) that can improve the accuracy of risk assessments. Such a

20 model could substantially improve the ability to make cost-effective decisions in the risk policy

21 arena.

22 There can be no doubt of the importance of research spawned through request for proposal

23 approaches to identify phenomena that are important for risk assessment. The advances that

1   have already been made as a result of programs conducted by several government agencies

2   illustrate this. However, many of the participants were concerned that the complexity and

3   magnitude of the data collection and management problem is so great that some more highly

4   coordinated efforts may also be necessary to make more specific use of the new knowledge that

5   has been gained through traditional granting mechanisms.

6

7   The recommendations of the workshop are as follows:

8

9   • Federal and international agencies should continue to fund basic research aimed at

10   identifying those phenomena that modify carcinogenic responses to various agents in the

11   environment, whether they be chemical or physical agents. Currently funded programs that

12   will make contributions to these areas are NIEHS's Toxicogenomics Program and DOE's

13   Low Dose Radiation Program. It is important to recognize that progress in these two areas is

14   heavily leveraged off the highly mission-oriented activities of the various genome projects

15   supported by NIH and DOE.

16   • Some effort, perhaps through an interagency initiative, needs to be directed at studies that can

17   integrate this information by the development of:

18   ○ Consensus teams to identify important variables and data gaps that limit the utility of

19   current information.

20   ○ A repository of qualitative and quantitative data that documents the nature of the

21   phenomena thought to affect carcinogenic responses. It would appear that

22   information should be species (strain) and target organ specific.

1   o Quantitative *in vivo* studies that are inclusive of parameters that can impact the

2   carcinogenic response at low doses. These studies must be designed to identify non-

3   linear dose-responses, if they are present, in the development of pathology as well as

4   the behavior of molecular and cellular responses.

5 • Develop and refine computational approaches for carcinogenesis that not only integrate the

6   different molecular and cellular processes that contribute to carcinogenesis in a quantitative

7   way, but to efficiently identify deficiencies in the available data to increase the utility of such

8   models in risk assessment.

9

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4
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9

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