Prepublication Source Material for *Talk on "Modeling Multiple Causes of Carcinogenesis." AAAS 1999 Meeting, Jan. 21-26, Aneheim, CA Prepublication Draft of July 7, 1999.*

MODELING MULTIPLE CAUSES OF CARCINOGENESIS *

Troyce D. Jones, Research Staff, Life Sciences Division (tdj @oml.gov)
Oak Ridge National Laboratory, P.O. 2008, Oak Ridge, TN 37831-6101

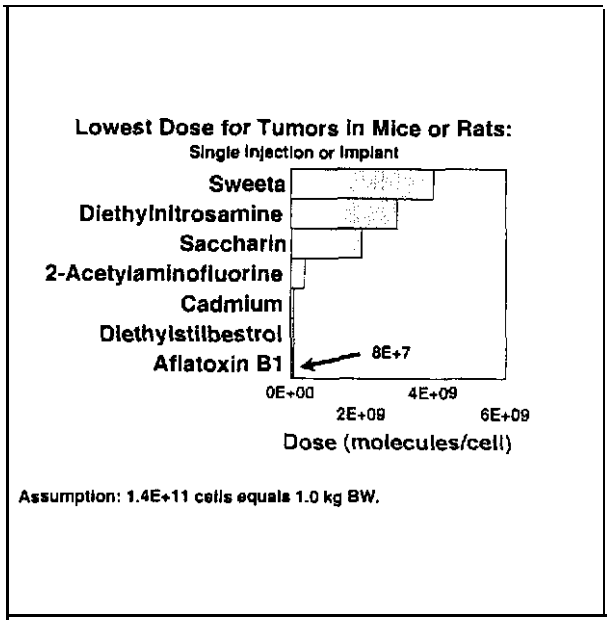
An array of epidemiological results and databases on test animals indicate that risk of cancer and atherosclerosis can be up- or down-regulated by diet through a range of 200%. Other factors contribute incrementally and include the natural terrestrial environment and various human activities that jointly produce complex exposures to microorganisms, ionizing radiations, and natural and synthetic chemicals. Ordinary personal habits and simple physical irritiants have been demonstrated to affect the immune response and risk of disease. There tends to be poor statistical correlation of long-term risk with single-agent exposures incurred throughout working careers. However, Agency recommendations for control of hazardous exposures to humans has been substance-specific and usually several orders of magnitude in the direction of safety instead of contextually and economically realistic even though there is consistent evidence for common mechanisms of toxicological and carcinogenic action by most agents that contribute to the complex exposures incurred by any individual (Jones 1995). That joint toxicological action seems to be best explained by molecular stresses from cellular oxygen metabolism, phagocytosis of antigenic invasion, and breakdown of normal metabolic compounds associated with homeostatic- and injury-related renewal of cells.

There is continually mounting evidence that marrow stroma, comprised largely of monocyte-macrophages and fibroblasts, is important to phagocytic and cytokinetic response (Jones, Morris, and Young 1993), but the complex action of the immune process is difficult to infer from first-principle logic or biomarkers of toxic injury. The many diverse database studies all seem to implicate two important processes, i.e., the univalent reduction of molecular oxygen and breakdown of aginuine, an ammo acid, by hydrolysis or digestion of protein which is attendant to normal antigen-antibody action.

This behavior indicates that protection guidelines and risk coefficients should be context

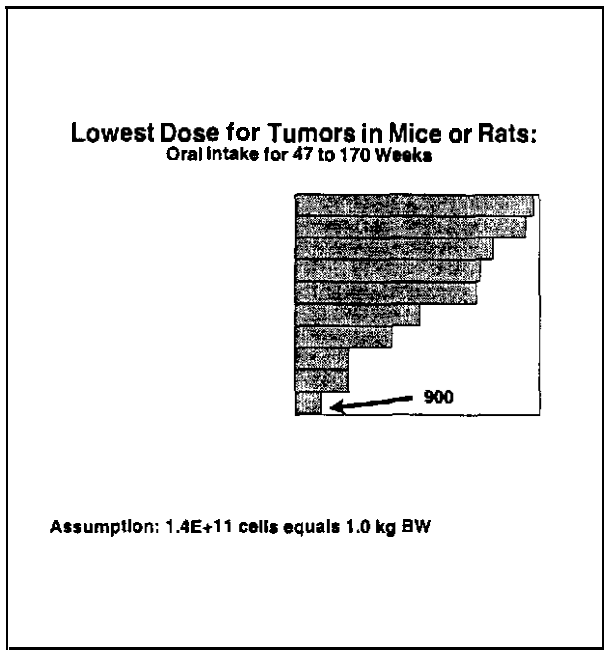
dependent to include reference considerations of the composite action of parameters that mediate oxygen metabolism (Beckman and Ames 1997). A logic of this type permists the realistic common-scale modeling of multiple causes of carcinogenesis and shifts the risk-assessment logic to considerations of 'what *dose does?'* in contrast to the current process of the substance-specific question of '*what dose* is?' Whether reactive oxygen radicals are the proximate or contributing cause of disease or simply a better, realistic, and/or practical estimate of biologically effective dose, that metric has enormous advantages for improved risk- and policy-based decisions. The following discussion proposes that: (1) simple fitting of equations to limited response data are usually incorrect, (2) immunity and risk of disease involve multiple and variable mechanisms and cannot be modeled realistically in a conventional predictive mathematical dose-response sense, (3) many agent-specific modifying factors from hazardous exposures, personal choices, daily routine, heredity, and occupation have strong capacities to either up- or down-regulate risk probably on a day to day basis (Ames and Gold 1997), and (3) values of the agent-specific modifying factors can be estimated from relevant data on test animals and in vitro bioassays and applied as simple multiplying factors to readily-available dose-response risk coefficients or estimates of relative risk, RR (or standardized mortality ratio, SMR) that Agencies have derived directly from epidemiological risk from exposures to substances such as ionizing radiation, benzene, and asbestos. Furthermore, the metabolic oxygen explanation, if practical *as* a risk and/or exposure metric, would permit practical estimates or risk for other diseases caused by similar, if not identical mechanisms, including atheroscleosis (Basavarju and Jones 1998).

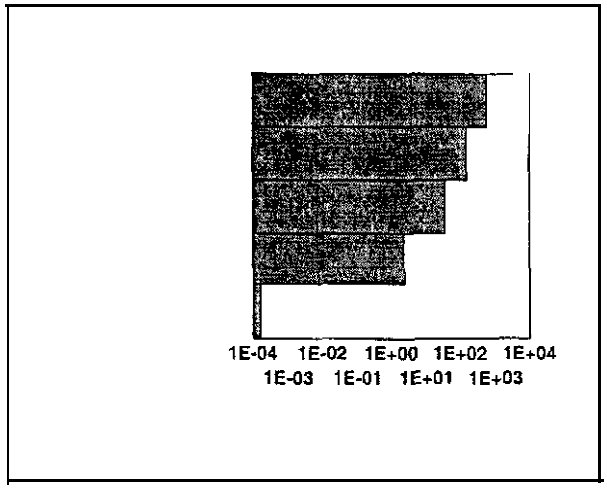
An interesting question is: what amount of dose does it take to cause cancer in test animals? Libraries have been written on this topic, but I will illustrate the barriers to first-principle modeling by a few quick visuals. For purposes of illustration, I elect to use the unconventional method of expressing average number of molecules of a carcinogen per cell averaged over the whole body of the animal instead of the traditional units of milligrams of carcinogen per kilogram bodyweight.



Panel 1 is based on the lowest doses that have been reported for increased carcinogenic response in mice or rats (in the *Registry of Toxic Effects of Chemical Substances* for sweeta, diethylnitrosamine, saccharin, 2-acetylaminofluorine, cadmiun, diethylstilbestrol, and aflatoxin B1) following a single injection or implant. Such data, in some instances, are based risk to the animal injected and in other instances are based cancer in their off-spring. For most situations, this algebra is expected to provide a reasonable lower bound of the dose of a substance that can cause cancer in any practical situation. From Panel 1, it is clear that for reasonably potent carcinogens it takes about a billion molecules of a carcinogen per average whole-body cell. This estimate is typically an extreme lower-bound to even the cells at greatest risk because certain target organs have greatest risk than the rest of the body and often receive far higher doses on either a molecular or mass basis. Even for the strong carcinogens of 2-acetylaminofluorine, cadmium, diethylstilbestrol, and aflatoxin B1, aproximately 10,000,000 molecules per cell are required.

The lower bounds suggested by Panel 1 are useful for single exposures, but most human





occur. The biology is enormously complex at any level of biological structure and the task amounts to an attempt to develop a deterministic or probabilistic relationship between an extremely rare event (monoclonal cancer) and enormous numbers of important involved target molecules, organelles, cells, and response mechanisms.

A typical cancer, either in a test animal or a human, ustually grows from only a single, original parent cell (Jones 1983). The growth has been observed to be in homeostatic equilibrium with surrounding normal (non-neoplastic) cells of the same lineage and autonomous growth may not commence in the aberrant clone until the focus exceeds about 100,000 cells so that cell to cell control is lost between normal and carcinogenic cells. Modeling of cancer involves regressing dose from one or many agents with cellular, molecular, and/or probabilistic processes. In essence modeling the association of only one cell, perhaps even one molecule of a carcinogen, with a fatal cancer. But, as shown previously, billions of molecules per cell seem to be necessary to initiate cancer even though the cancer may have only a single parent cell. To further demonstrate the impossibility of modeling such rare-event processes, Panel 4 summarizes other large number, rare-event conditions involved with targets and mechanisms.

The nature of the exact carcinogenic preciptating event is further obscured because the effective molecular dose of the primary substance is supplemented by natural background radiation and large numbers of induced free electrons. Natural sources of radiation have been

---

Panel 4: Modeling Cancer from Single/Multiple Causes.

A typical malignancy (either in a test animal or human) detives from a single parent cell after about 30 cell-doubling periods. Cancer is ~10^9 cells (or, 2^{30}) at diagnsis and ~10^{12} at death. Modeling single or multiple causes of cancer involves linking dose(es) with cellular, molecular, and/or probabilistic processes:

Carcinogenic Chemical	**10^7 to 10^{10} molecules/ceil**
Plus	**and**
Background Radiation	**30 e^- per cell-yr**
Cells at time t_0	**10^{13} human**
Cells in lifetime	**10^{16} human**

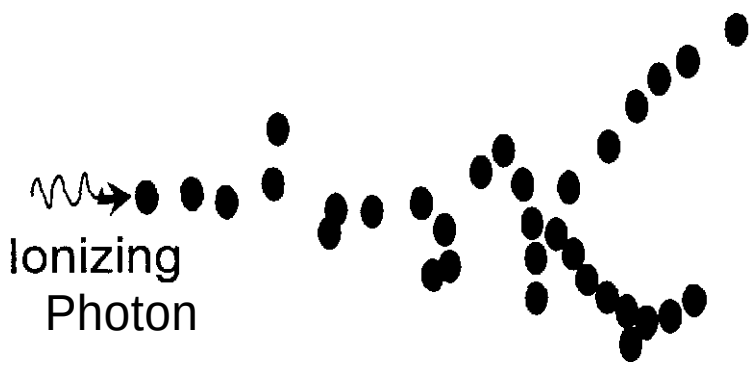
Receptors per cell	**Variable (10^2 to 10^4 typical)**
DNA base pairs	**10^9 per cell**
Immune reactions	**See Panel 6!**

sumrnarized by the National Radiological Protection Board (1989) as shown in Panel 5. Also, illustrated in Panel 5 is the electron track structure in tissue for a low-energy photon. For such conditions the energy transfer is about 3.5 keV per micrometer of biological tissue and less than 30 eV is required per ionization so that dozens or hundreds of ionizations may occur per cell--for alpha particles of a few MeV from radon and other natural isotopes, the number of track ionizations may be on the order of 10^4 and registered in only 3 or 4 cells. There, are about 10^{13} cells at any point in time in a 70 kg human (Panel 4) and it has been estimated that there are about 10^{16} cells per lifetime, with lineage-specific turnover ranging from minutes to decades. Commonly there are variable number of cellular receptors and numbers may range from dozens to thousands per cell; nuclear DNA is important with a few billion base pairs per cell; and immune system response is strongly interactive with the carcinogenic response in any target tissues whether the target cells are in hematolymphatopoietic or remote to bone-marrow related cells.

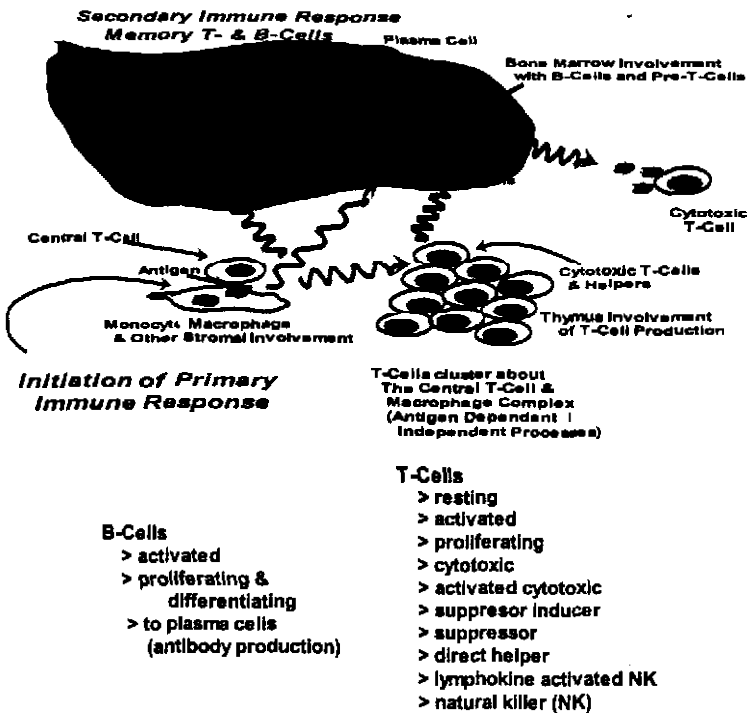
Panel 5: Natural sources of radiation (NRPB 1989):
For an average person in an average day.

4.8×10^9	gamma-photons from soil
3.6×10^8	atoms of ^{40}K decay in the body
9.6×10^6	cosmic rays
2.4×10^6	neutrons
7.2×10^5	radionuclides decay in lungs
1.7×10^5	atoms of uranium decay in the body

National Radiological Protection Board
Radiation doses-maps and magnitudes
Chilton, Didcot, Oxon OX11 ORQ, UK, 1989.


Ionizing
Photon

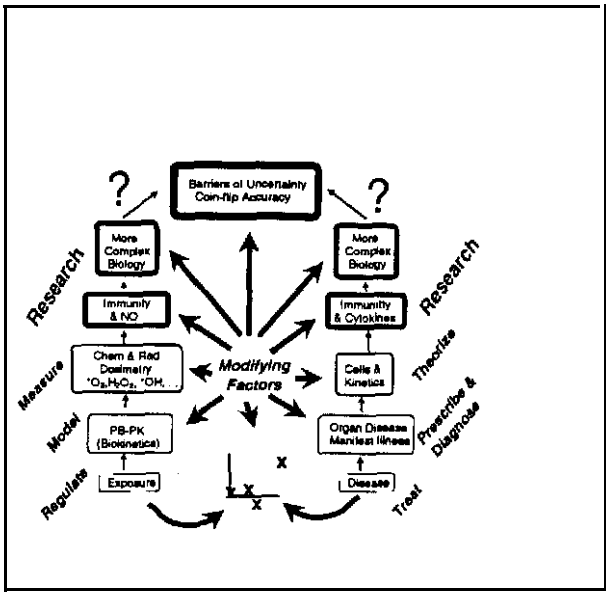
Many experiments can be cited which have demonstrated that mutated DNA is necessary for carcinogenesis, but usally is is far from being *sufficient* for conditions of frank cancer (Jones 1983). Early publications included (a) in *vitro* procedures where malignant nuclei were infused into normal embryos, (b) and in *vivo* transfusions of up to 10^5 carcinoma cells into normal and immune-deficient strains of test animals, Typically two conditions were required for malignant growth: (1) at least one viable cancer cell in the host tissue matrix, and (2) infusion of greater than about 100,000 malignant cells and/or sustained cellular proliferation from toxicity and/or homeostatic repopulation or administration of mitogens or hyperplastic agents. Throughout the accumulation of a malignant clone of about 2^{30} surviving cells (typical size at diagnosis in humans) there is continuing interaction with immunological lineages from the initiated single cell to death of the host.



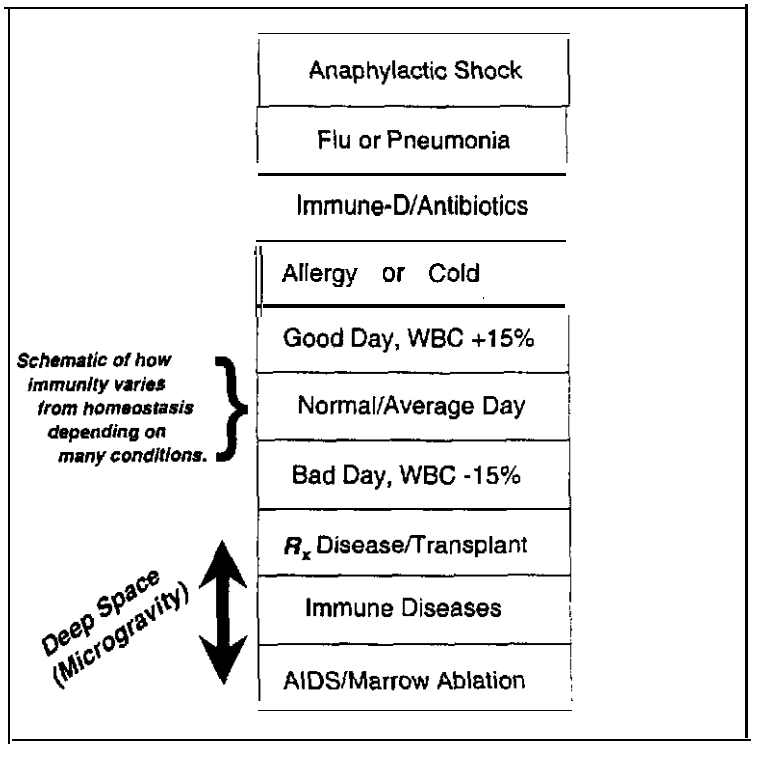
Greatly simplifed, Panel 6 illustrates prcesses of humoral and cellular imunity that interact with foreign substance whether it be antigenic matter, mutated biomolecules, infused cells, on spontaneously mutated cells, Even in this much simplified illustration, the number of required modeling parameters prohibits meaningful predictive models of risk. To further increase the complexity of the needed models , it should be noted that cells are in kinetic states depending on their cellular and biochemical environments. Some conditions for T- and B-Cells are shown at the bottom of Panel 6--and indicate enormous complexity to the apparent simplicity shown in the top of the panel.

Panel 7: Summary of bio-activity associated with selected hematopoietic

assumptions usually daisy-chained together. Usually available experimental data are not adequate to uniquely estimate or validate parameters of the models. Such models are used widely for interpolation, but when extrapolated beyond the range of available data, usually deviate from reality and even similarly developed mathematical models by several orders of environmental concentrations.

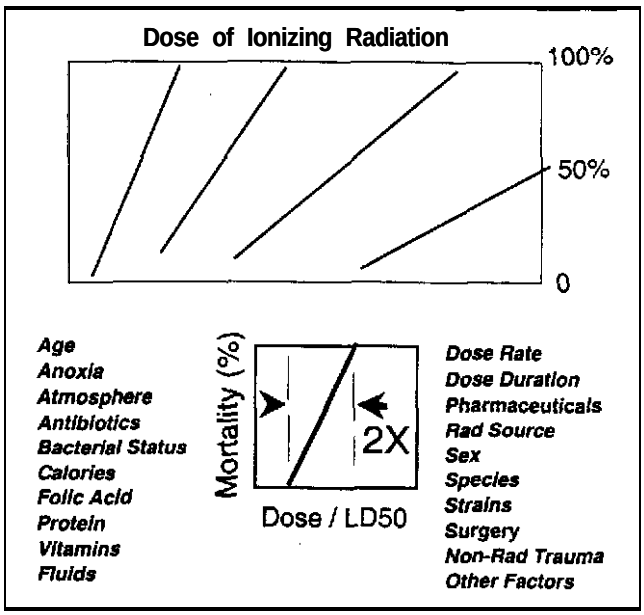


But, the grasp for practical knowledge can be enhanced by available data, modem computers, and new methods of logic that are optimized to analyze multiple and diverse databases instead of the classical single experiment (Jones, Morris, and Young 1993, Ehrenberg 1991). With respect to dose-response of cancer from multiple causes, there are vast quantaties of data that indicate personal, ethnic, cultural, occupational, environmental, and economic variables may act in similar manner to modify the biologically effective dose, mitigate the risk of disease, bind to DNA, affect sequence and/or concentrations of RNA, or structure and concentration of protein. The remainder of the talk will focus on information that can be extracted from valuable databases and how to estimate immunological susceptibility in a dynamic, changing environment.



Individual immunity within a population can be considered as having a statistical mean and variance. But, each person may have considerable day to day variability and

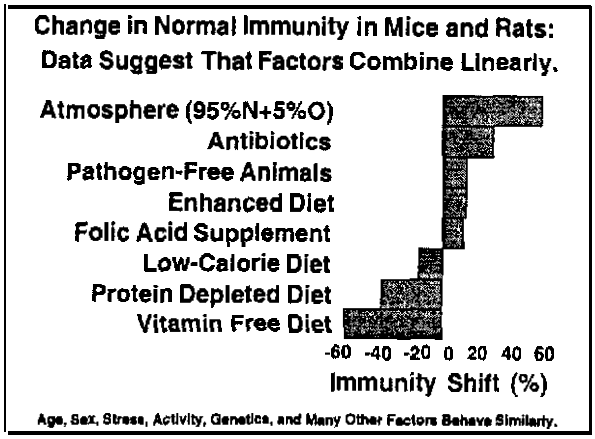
for a large population, the range may be extreme as illustrated in Panel 9. Because, cytokine concentrations vary exponentially in response to toxological or homeostatic needs, it seems reasonable to profile the genetically- and disease-diverse human population for susceptibility, and some regulatory deliberations have already considered exposure of immune-impaired individuals to ambient concentrations in air and water. Early experiments in radiation biology demonstrated a variable leukocyte count in animals that could change by 30% within 48 hours for no observable reason. In these studies, experimental conditions were controlled carefully and the effect was attributed to *'diurnal variation.'* The white blood count in humans often shows the same variability and indicates large variations in cytokine concentrations that underlie cell proliferation and differentiation. Because the range of normality (usually the central 90% of the population distribution) is considerable, estimates will be made for a few representative conditions, e.g., diet, that may *up-*, or *down-regulate* risk to a mean reference individual exposed to a particular toxic agent such as radiation or benzene. For the population, individuals of all intrinsic sensitivies should be considered to be subject to the forces of the dose- and response-modifing factors as described, If all confounding factors that act either to decrease or increase risk are considered in combination, the effect would be expected to broaden the normal response distribution by a very wide range from extreme sensitivity to hardy resistance. There are several databases that may be used, they potentially include both medical and animal archives. I will describe one such database comprised of animal research from about 1945 to 1975.



During that period, little was known about the biological effects of ionizing radiations, and there was grave threat of pending nuclear war. Many species of test animals were irradiated and important contributions were made to current practices of nuclear medicine, radiotherapy, and marrow transplantation. One imporant finding was that that at high radiation doses, near those now used in for therapy and marrow ablation, animals died in a dose-dependent manner between about 5 and 30 days post irradiation as illustrated in the top figure of Panel 10. Animals were irradiated at graded radiation doses and the midlethal estimate of dose was determined. This led to the common index referred to as $LD_{50/30}$ (or LD_{50}) associated with 50% mortality. The mortality curve was eventually found to be very steep (Morris and Jones 1989) and, in fact, 100% survival and 100% lethality values were not very far from the LD_{50} in magnitude, viz, $3/4LD_{50}$ to $4/3LD_{50}$ (Jones 1981) so the point-value estimate of mortality became a very important estimate for a wide array of medical, cancer, and policy decisions. (Note: Most statistically positive tests in the carcinogenic test protocols are within about a factor of 1/4 of the LD_{50}. Today, we know that deaths in this time interval are determined predominantly by infection resulting from pancytopenia in general and neutropenia in particular. The shift in the mortality curve between a specific LD_{50} experiment and an indentical one in which a factor such as diet varied is now understood to be a direct quantitative estimate of the shift in immunological function.

If all of the graded doses to different groups of animals (within a particular LD_{50} experiment for a unique set of animal and physical conditions) are divided by the estimated LD_{50} for that array of conditions, a univeral curve is obtained for all such normalizations. That effect is illustrated in the bottom of Panel 10. That behavior seems to hold accurately for nearly all experimental dose-response data ever taken (e.g., excluding only unusual situations such as when

the animal colony experienced competeting causes of death as from *pseudomonas* and did not necessarily die in proportion to radiation dose received).



The simple standardization for effects of confounding variables holds for age, anoxia, atmosphere, antibiotics, calories, folic acid, protein, vitamins, fluids, dose rate, duration of time over which the dose was delivered, pharmaceuticals used to enhance or reduce radiotoxicity, characteristics of the radiation source or type, sex, species, strains, *surgery or* mechanical wounds, non-radiation trauma, and a diverse array of other conditions (Lindop 1960, Krebs 1961, Dowdy 1950, Hammomd 1955, McLaughlin 1971, Wilson 1963, Oldfield 1965, Hasegawa 1970, Carroll 1961, Jennings 1949, Johnson 1946, Waldburg 1966, Corp 1959, Hanks 1966, Hagan 1956, Frolen 1961, Upton 1956, Delihas 1958, McChesney 1990, Chapman 1955, Dacquisto 1960, MacVittie 1991, Smith 1970, Ainsworth/Taylor 1983).

According to the standardization described above, it is easily demonstrated that immunity towards infection (as tested by graded doses of ionizing radiation ¡ is strongly affected by factors of diet, lifestyle, and hazardous exposure. For example, with all other parameters controlled identically, Panel 11 shows immunity decreasing to only 60% of normal on a vitamin free diet. These factors combine linearly. For example, a protein and vitamin depleted diet would correspond to an immune function of (100%-60%)x(100%-40%) = 24% of normal. Likewise, if a protein depleted diet, folic acid enriched diet, and antibiotics are combined, the immune function would be estimated to be effective at a state of (100%+30%)x(100%+10%)x(100%-40%) = 86%.

From only what I have described above, I should, by conventional scientific caution, limit my conclusions to ‘risk of death as restricted to conditions: (1) from infection in rats and mice, (2) with fragmentary data to support the same conclusions in about 2 dozen species of test animals, (3) from ionizing radiations, and (4) in ranges of dose that induce 1% to 99% mortality

within 30 days.' But, as a modeler interested in diversed data base evaluations, I exceed the traditional scientific caution and combine information (Morris, Jones, and Young 1991 and 1993, Ehrenberg 1991) from (1) individual experiments within a broad study design, (2) sets of closely related experiments from similar experimental designs, (3) sets of similarly designed experiments that all provide numerical insight into mechanistic biological function, (4) sets of diversely designed experiments from *in* vitro and *in vivo* studies into how the relevant biological mechanisms change under dynamic conditions, and (5) cellular and immunological production of reactive oxygen species. From all of this and other related experiences, I generalize the empirical findings, use fragmentary data to include humans, and offer the hypotethesis for testing and comparisons with observations from published and ongoing epidemiological studies. All models, including those used by Agencies to promulgate statutory values, depend strongly on manifold assumptions and limited data. In fact, some of the simplier models used to estimate statutory values are weakened by data and mechanisms that they ignore more than they are strengthened by their attractive simplicity.

The methods described above use far more data of higher relevance than most models on which most current protection criteria have been based. For about 50 years, reactive oxygen has been perceived as the primary basis of benzene toxicity and for about 45 years as the basis of radiotoxicity. Because, oxygen-related metabolic processes were readily found to up- or down-regulate radiotoxicity and therapeutic gain through a range of several hundred percent, radiology of cancer has researched this behavior for therapeutic gain over several decades. Now, in some sectors of science, especially radiation biology and radiology, the effect is almost accepted as dogma, because production of oxygen free radicals is fundamental to toxic response through two processes (1) the univalent reduction of molecular oxygen and (2) arginine, an amino acid, produced by hydrolysis or digestion of protein which is attendant to normal immune response (Dreher and Junod 1996):

$$O_2 \xrightarrow{e^-} {}^{\bullet}O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} {}^{\bullet}OH \xrightarrow{e^-} H_2O \text{ , and}$$

$$\text{L-Arginine} \longrightarrow NO^{\bullet} \longrightarrow ONOO^{\bullet} \longrightarrow {}^{\bullet}OH$$

‘0;

The hydroxy radical reacts with all components of DNA: deoxyribose backbone, purine bases, and pyrimidine bases.

From the aggretation of items (1) to (5) as described above, the response of a complex biological system (even a human) can be modeled under a wide range of practical conditions. We have used these methods previously to model radiation induced-kinetics of hematopoietic and marrow stromal cells (Jones, Morris, and Young 1993 and Morris, Jones, and Young 1993), and to predict carcinogenic potency of untested chemical compounds and mixtures (Jones and Easterly 1991 and 1996). Our methods are similar in logic, but distinct in application, to the adroit descriptions by Ehrenberg (1990) and Lindsay and Ehrenberg (1993) who has independently used similarly based assumption-sparce, data-rich methods to model complex situations involving finance and marketing based on “many sets of data” instead of the conventional “the analysis of single sets of data.”

Thus, it *can be proposed* **as a rather** *strong hypothesis--with a fragmentary array of validating data (Ames and Gold 1997)--that humans may be at significantly increased risk of infection and cancer when subjected to unfavorable conditions associated with floods and hurricanes, family illness and/or stress from death* **of** *a family member, famine, war, space missions, international travel, or even spring break for college students unless the individuals at risk can maintain a nearly normal cytokine environment within their own hematopoietic systems. Conversely, control ofprotective confounding factors could reduce risk* **of** *infection or secondary disease during times of adversity or therapy.*

* Research sponsored by the Defense Special Weapons Agency under Lockheed Martin

toxicological observations and mechanisms of atherosclerosis. Arch Environ Contam Toxicol 35: 152-164 and Part II. A RASH analysis of invitro and in vivo bioassay data to evaluate 45 potentially hasardous compounds. Arch Environ Contam Toxicol 35:165-177.

Beckman KB and Ames BN (1997). Oxidants, antioxidants, and aging. In: oxidative stress and the molecular biology of antioxidant defenses. J. Scandalios, Ed. Cold Springs Harbor Laboratory Press, Cold Springs Press. NY pp.201-246.

Carroll HW and Brauer RW (1961). Hypocaloric feeding and radiation tolerance. Radiat Res 15:236-243.

Chapman WH (1955). The weight and mortality response of male and female mice in the lethal x-ray dose range. Radiation Research XX:502-5 11.

Corp MJ and Neal FE (1959). The modification of acute mortality in mice by variation of the dose-rate and the overall time of irradiation. Int J Rad Biol 3:256-265.
Dacquisto MP and Blackburn EW (1960). The influence of delivery rate of whole body 250 kv roentgen irradiation (30 or 3 roentgens per minute) on mice, rats, and guinea pigs. The Am J of Roent, Rad Ther and Nucl Med. 84:699-704.

Delihas N and Curtis HJ (1958). The relative biological effectiveness of fission neutrons for the production of acute mortality in mice. Radiat Res 8:166-180.

Dowdy AH, Bennett LR, and Chastain SM (1950). Protective action of anoxic anoxia against total body roentgen irradiation of mammals. Radiology 55:879-885.

Dreher D and Junod AF (1996). European J Cancer 32A, 30-38.

Ehrenberg ASC (1990). A hope for the future of Statistics: MSOD. The Am Statistician, 44:1955-196.

Frolen H, Luning KG, and Ronnack C (1961). The effect of x-irradiation on various mouse strains due to their genetic background. Radiat Res 14:381-393.

Hagan CW and Simmons EL (1956). Effects of total-body x irradiation on rats. 1. Lethal action of single, paired, and periodic exposures. In Biological effects of external x and gamma radiation, Part 2. Ed by RE Zirkle. pp. 281-299.

Hammond CW, Vogel Jr. HH, Clark JW, Cooper DB, and Miller CP (1954). The effect of streptomycin therapy on mice irradiated with fast neutrons. Radiat Res 2:354-360.

Hanks GE, Ainsworth EJ, Leong

Hasegawa AT and Landahl HD (1970). Dose reduction factor for radiation lethality in mice as a function of the dose of Mercaptoethylamine. Radiat Res 44:738-747.

Jennings FL (1949). Effect of protein depletion upon susceptibility of rats to total body irradiation. Proc Expl Biol Med 72:487-491.

Johnson CG, Vilter CF, and Spies MD (1946). Irradiation sickness in rats. Am J Roent Rad Therapy 56:63 1-639.

Jones TD (1981). Hematopoietic syndrome in man modeled from mammalian lethality. Health Phys 41:83-103.

Jones TD (1995). Toxicological potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin relative to 100 other compounds: a relative potency analysis of in vitro and in vivo test data. Arch Environ Contam Toxicol 29:77-85.

Jones TD and Easterly (1991). On the rodent bioassays currently being conducted on 44 chemicals: A RASH analysis to predict test results from the national toxicology program. Mutagenesis

Lindsay RM and Ehrenberg ASC (1993). The design of replicated studies. The Am Statistician, 47:217-228.

Lindop PJ. and Rotblat J (1960). Protection against acute effects of radiation by hypoxia. Nature 185: 593-594.

MacVittie TJ, Vigneulle RM, Zeman GH, and Jackson WE (1991). The relative biological effectiveness of mixed fission-neutron-y radition on the hematopoietic syndrome in the canine: effect of therapy on survival. Radiat Res 128:s29-s36.

McChesney DG, Ledney GD, and Madonna GS (1990). Trehalose dimycolate enhances survival of fission neutron-irradiated mice and klebsiella pneumoniae-challenged irradiated mice. Radiat Res 121:71-75.

McLaughlin MM, Woodward KT, and Stromberg LR (1971). Effects of the germfree state on the response of mice exposed to neutron-gamma radiation. Radiat Res 46:519-532.
Morris MD and Jones TD (1989). Hematopoietic death of unprotected man from photon irradiations: statistical modeling from animal experiments. Int J Radiat Biol 55:445-461.

Morris MD, Jones TD, and Young RW (1993). A cell kinetics model of radiation-induced myelopoiesis: rate coefficient estimates for mouse, rat, sheep, swine, dog, and burro irradiated by photons. Radiat Res 135:320-331.

Morris MD, Jones TD, and Young RW (1991). Estimation of coefficients in a model of radiation-induced myelopoiesis from mortality data for mice following x-ray exposure. Radiat Res 128:267-275.

Oldfield DG, Koull J,

Lowed Dose for Tumors in Mice or Rats:

Oral Intake for 47 to 170 Weeks

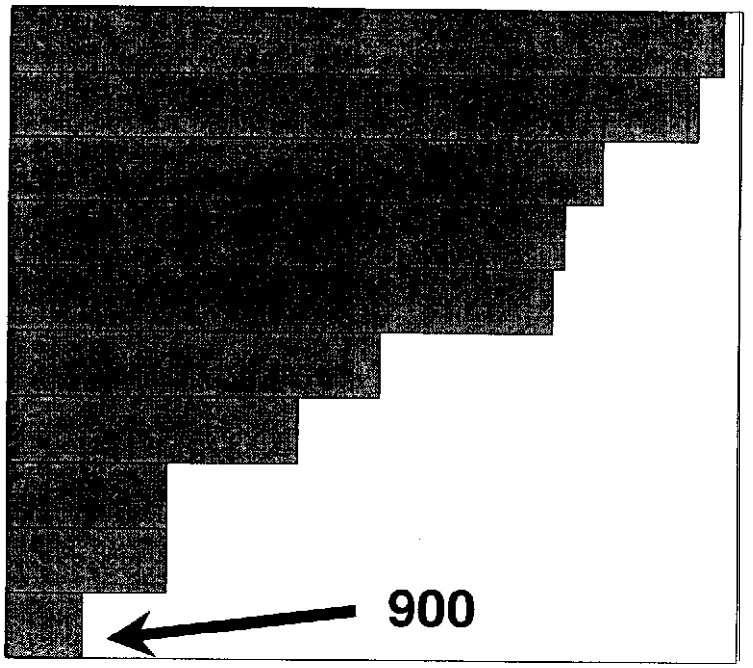
Sweeta

Saccharin

Benzene

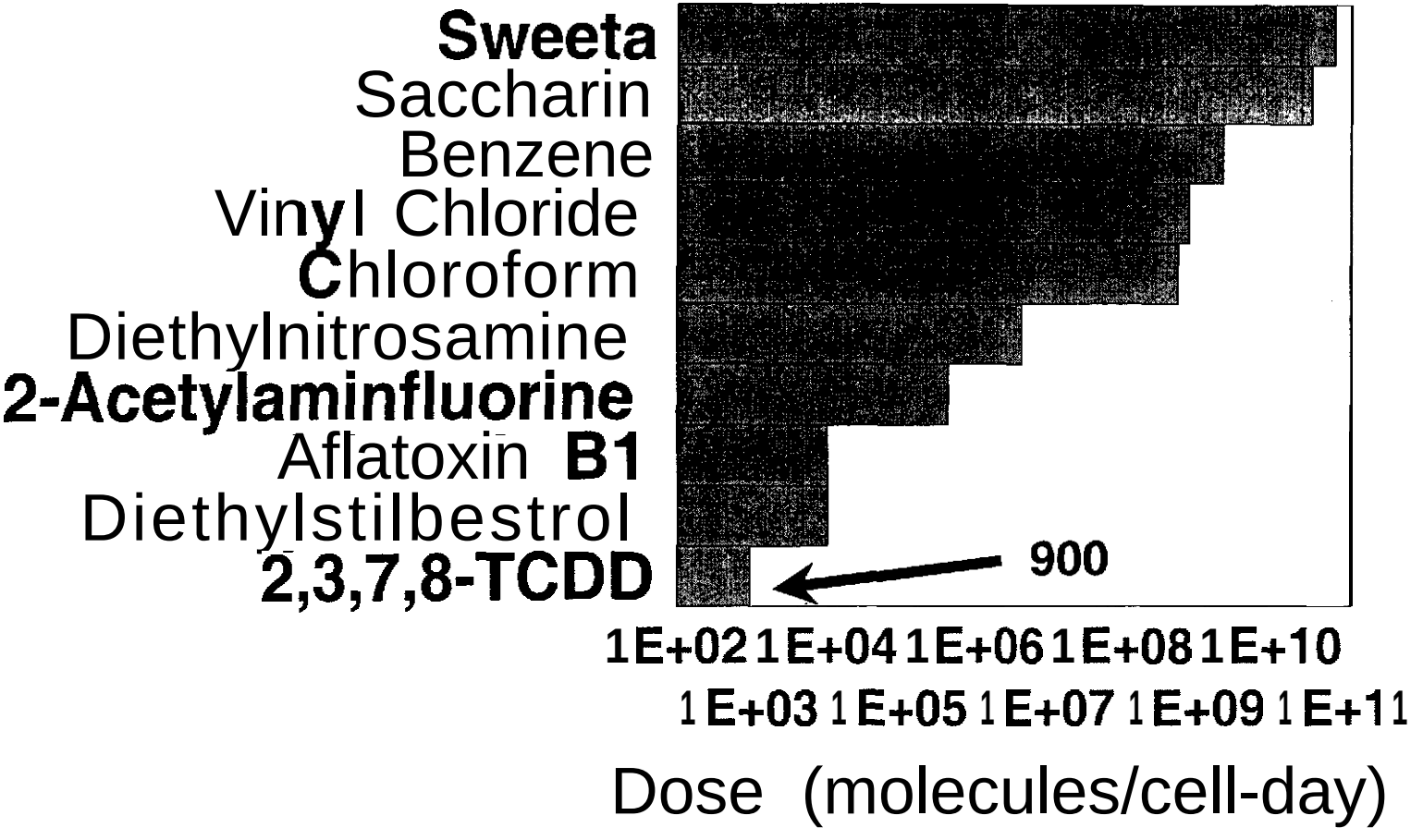
Viny

C



Lowest Dose for Tumors in Mice or Rats:

Oral Intake for 47 to 170 Weeks



Assumption: 1.4E+11 cells equals 1 .O kg BW.