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MODELING MARROW DAMAGE FROM RESPONSE DATA: MORPHALLAXIS FROM RADIATION BIOLOGY TO BENZENE TOXICITY[1]

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Running Title: *Modeling Marrow Damage*

Key Words: benzene, radiation, marrow, stroma, stem cell, CFU-S

Abbreviations:

CFC = colony forming cell
CFU-F = colony forming unit of marrow fibroblast
CFU-S = colony forming unit in the spleen
D = dose; D' = dose rate
EPD = equivalent prompt dose of a radiation given in a pulse
G_2 = phase of the mitotic cycle
GI = gastrointestinal
Gy = unit of ionizing radiation
LD_{50} = dose that is toxic to 50% of the test population
M = median of the cumulative normal distribution (e.g., LD_{50})
MLE = maximum likelihood estimation
MSOD = many sets of data
SSOD = single set of data
S9 = enzyme extract used to activate compounds in test models
S.D. = standard deviation
σ = standard deviation of the normal distribution function
T_D = doubling time
X = value of abscissa

Abstract:

Consensus principles from radiation biology were used to describe a generic set of nonlinear, first-order differential equations for modeling of toxicity-induced compensatory cell kinetics in terms of sublethal injury, repair, direct killing, killing of cells with unrepaired sublethal injury, and repopulation. This cellular model was linked to a probit model of hematopoietic mortality that describes death from infection and/or hemorrhage between ~5 and 30 days. Mortality data from 27 experiments with 851 dose-response groups, in which doses were protracted by rate and/or fractionation, were used to simultaneously estimate all rate constants by maximum-likelihood methods. Data used represented 18,940 test animals distributed according to: (mice, 12,827); (rats, 2,925); (sheep, 1,676); (swine, 829); (dogs, 479); and (burros, 204). Although a long-term, repopulating hematopoietic stem cell is ancestral to all lineages needed to restore normal homeostasis, the dose-response data from the protracted irradiations indicate clearly that the particular lineage that is "critical" to hematopoietic recovery does not resemble stem-like cells with regard to radiosensitivity and repopulation rates. Instead, the weakest link in the chain of hematopoiesis was found to have an intrinsic radioresistance equal to or greater than stromal cells and to repopulate at the same rates. Model validation has been achieved by predicting the LD_{50} and/or fractional group mortality in 38 protracted-dose experiments (rats and mice) that were not used in the fitting of model coefficients.

Introduction:

An editorial in *The American Statistician* by A.S.C. Ehrenberg (1), derived from experiences with business and marketing, insightfully describes a belief that analysis of many sets of data (MSOD) "*seems to be the only way in which we can produce results that are generalizable, lawlike, and predictable--which in fact hold for many sets of data . . . our concern will be with deciding what the main effect is quantitatively, how to model it, how consistent it is, under what different conditions it does or does not occur, why it arises, how it links up with other findings, and how it can be used in practical applications and/or in the development of theory.*" Although we have used such practices for nearly 20 years--in carcinogenic risk assessments, mathematical models of acute lethality, and marrow cell kinetics underlying radiation-induced hematopoiesis--we did not attempt to communicate those generalized ideas outside our particular areas of interest nor have we stated the essential ideas so compactly.

For mathematical models of dose-response effects, historically there has been a near-total reliance upon finding a simple equation that will approximate a single set of experimental data (SSOD) when the numerical constants are fitted appropriately. Fits to other data sets, from similar experimental protocols require additional statistical justification that the model is acceptable and new fitted parameters. Although continued use of the same functional form usually produces some attempt to establish a biological interpretation of the underlying effects (i.e., a conceptual model), in general, such interpretations usually have no fundamental validity and ignore far more important biological factors than the few they are hypothesized to approximate--even for those few factors, there is a pronounced lack of generality for protracted-, fractionated-, or variable-rate exposure protocols. Results from such exercises are without substantial validity outside the ranges of experimental conditions used and have no basis in reality when extrapolated in terms of dose, dose rate, or test species/strain used.

The general domain of biologically-based or conceptual models bifurcates into additional basic approaches. One pathway involves assumptions, either direct or indirect, that the important processes are known in terms of specific molecular/cellular effects and simple factors and descriptive models can be written accordingly. When indirect assumptions are involved, it is often overlooked that the conclusions obtained from experiment-by-experiment evaluations of the models are mandated either by the constraints of the model or by limitations of the particular experiment used for estimation of parameters. Subtle, indirect assumptions have the hazard of going unrecognized, perhaps even to the experimentalists themselves.

Our approach formulates generalized dose-response models in terms of generic processes. Those generic processes may be: in terms of molecular effects; from a cell kinetics perspective; and descriptive

of local and systemic reactions that may act through cell-to-cell and/or humoral mediated effects involving cytokines. The dosing schedules used for benzene experiments do not reflect adequate protocol-dependent variability to permit execution of the MSOD approach to a degree that provides informative insight into underlying biological mechanisms. In contrast, historical data from radiation biology do reflect those needed variations in experimental design. Those variations can be found at the molecular, cellular, organ, and organism levels, and all of those structural tiers have been considered to various degrees in model conceptualization, coefficient estimation, and model validation in our previous publications on radiation-induced hematopoiesis (2,3). Because our maximum likelihood estimations (MLE) have relied only upon lethality data from both prompt and protracted irradiations, those experiments, as summarized in Fig. 1, serve as the data base used to evaluate the generic model in terms of cells "critical" to hematopoietic recovery (4,5).

Following is a brief description of how we have formulated a generic model for cell kinetics associated with radiation-induced hematopoiesis and how MSODs can be used to generalize the model and provide strong insight into the fundamental underlying mechanisms. Specifically, our intent was to use dogmatic terms and factors (or, as a minimum condition of acceptance those common to expert consensus) to approximate generic processes associated with marrow cell kinetics underlying acute lethality. Next, maximum-likelihood estimation methods were used to evaluate the numerical parameters of the models and their associated confidence bounds. This approach provides no direct cause-effect proof that the biologically-based model is indeed correct in all details but--because enormous sets of data, reflecting wide ranges of variability, can be fitted by a common set of evaluated parameters that are consistent with specific biological rate constants--it is obvious that the model is substantially correct in behavior and provides hypotheses that in turn may be validated or modified by further refinement of experimental design considerations. In addition, we found it desirable to evaluate and test a cell kinetics model formulated in terms of those same non-specific damage, repair, and repopulation processes as derived from CFU-S experiments in contrast to the parallel evaluation made from the generic model and animal lethality data--, i.e., the underlying dependence on "critical" cells is not restricted to stem or CFU-S types of cells. As indicated by data in Table 1, the conceptual and mathematical models, used for ionizing radiations, should also be relevant to considerations of benzene toxicity.

Method and Materials:

Assumption: Acute Lethality Derives from Cytopenia of a "Critical" Bone-Marrow Cell: When animals are irradiated by acute protocols, death from infection and/or hemorrhage may occur between about 5 and 30 days post-irradiation. The frequency of death can be described by a probit distribution function with fitted parameters comprised of the LD_{50} and slope (i.e., slope = σ^{-1} which is the inverse standard deviation of the frequency distribution). The LD_{50} and σ may be for the particular radiation field of interest or for a standard or reference radiation if there is a realistic way of modeling the underlying degree of cytopenia from the exposure of interest and converting that level of effect back to an equivalent reference dose of the standard radiation associated with the LD_{50} and σ estimates. Depression of neutrophils and platelets are accepted as the proximate cause of death, but the contributing cause of death could be either the terminally differentiated cells themselves; ancestral cells; or ancestral-dependent lineages upstream in the direction of the undifferentiated pluripotent stem cells. For generality, the weakest link (i.e., lineage) was treated generically and guided by MLE evaluations in contrast to more restrictive assumptions. One major advantage of this approach is that only one (LD_{50}, σ) combination was required for a complex experiment involving different: dose rates, exposure protocols, radiation sources, etc. (4,5). In short, only changes with respect to the strain, species, cage care, and conditions of observation required additional LD_{50} and σ values. One experiment in the analysis was comprised of 26 different LD_{50} protocols but all were consistent with a common LD_{50} and σ associated with an "equivalent prompt dose."

Generic Cellular Model Evaluated from Animal Lethality Data: Theory underlying the model and likelihood analysis have been described in the cited publications. In the mathematical model, cells are compartmentalized into normal (N), injured (I), and killed (K) populations. Processes by which cells move among those populations are modeled by first-order, nonlinear equations. In an arbitrary volume of marrow, we call the numbers of normal, injured and killed cells n_N, n_I and n_K, respectively. Initial conditions are $n_N = n_O$ (normal before exposure), $n_I = 0$ (no injury before exposure) and $n_K = 0$ (no killing before exposure). The n_O need not be estimated because only ratios of n_N, n_I and n_K relative to n_O are used. The cellular component of the model is

$$n'_N = -\lambda_{NI} D' n_N - \lambda_{NK} D' n_N + \lambda_{IN} F_{IN} n_I + \lambda_{NN} M F_{NN} n_N \quad [1]$$

$$n'_I = -\lambda_{IK} D' n_I - \lambda_{IN} F_{IN} n_I + \lambda_{NI} D' n_N \quad [2]$$

$$n'_K = \lambda_{NK} D' n_N + \lambda_{IK} D' n_I. \quad [3]$$

λs are rate constants that mediate movements of cells from normal or injured states as indicated by the first subscript to the state indicated by the second subscript. D is dose given uniformly to marrow, and prime (′) denotes the derivative of a cell count or dose (i.e., dose rate) with respect to time. Factors and terms of equations [1] to [3] are given in Table 2.

Hematopoiesis Model Evaluated from CFU-S Data: The same functional form based on cellular damage, repair, and repopulation was evaluated from experimental studies on CFU-S cells as described in a previous reference (3). Damage constants were estimated from dose-rate data of Puro and Clark (6). The proliferation constant was estimated from an analysis of published values obtained from an extensive literature review. The repair constant was taken from the evaluation described above for the lethality data base, but an additional normalization was required to adjust for the shorter cycle time of stem/CFU-S cells in contrast to the longer cycle for the "critical" cells.

Results:

The two models of marrow cell kinetics involve (a) cells that are "critical" to compensatory hematopoiesis with parameters estimated from MLE analysis of animal mortality data and (b) CFU-S type stem cells with parameters fitted from in vivo and in vitro cell-survival studies. As described in previous publications (3,7), both models seem to preform remarkably well according to the foundations of their evaluations. Clearly the point estimates and confidence intervals on estimated coefficients indicate that the two cellular models are distinct and do not merely provide dual estimates for a common lineage.

Model validation: Collection of data base used in the model validation comparisons: Thirty four experiments have been analyzed previously by maximum likelihood methods in order to estimate cellular rate constants within the model and are not considered suitable for model validation considerations--27 were used to evaluate the photon models (5) and 7 experiments were used to evaluate the neutron models. The lot, 72 experiments have result from an exhaustive literature review and selection of the 38 experiments for model validation was based on: [1] dose protraction by rate or fractionation in mice or rats, [2] mortality within 30 days from the end of the radiation treatments (studies were used if a few animals died from GI damage because it was assumed that those same animals would have died from marrow depression at a later time)--in contrast, studies were rejected if even a small number of animals died of marrow depression before the irradiation schedules were completed because the minimum effective dose could not be determined, [3] no more than 60 days between successive dose fractions, [4] equivalent handling of different phases of a particular experiment (e.g., uniform marrow doses and consistency in positioning the animals--confinement was needed to be sure animals actually received the planned dosage, [5] adequate specifications of times or dose rates, and [6] the effort had to be reasonably successful at irradiating an adequate number of animals between the LD_{10} and the LD_{90}. Overall, only about a half dozen studies were actually rejected.

The 38 experiments used to validate the model (selected as described above) typically reported only LD_{50} values without giving the actual dose-response data. Although these studies were not useful for un-biased estimation of model constants, they do provide independent tests for model validation. The 12 doses rate studies ranged from 0.08 to 474 r/min and the 26 fractionation studies contained fractionations from 154 to 700 r given over periods ranging from hours to 8 weeks. Although the conversion of a protracted protocol to its prompt dose equivalence is cell-lineage dependent, that conversion for very simple fractionated protocols will generally produce numerically similar estimated of the EPD and it is not clear which lineage better explains the biology underlying acute mortality. In

contrast, complex fractionation experiments and low dose-rate studies are sensitive to lineage-specific effects and result in different estimates for the EPD. These lineage-dependent EPD estimates clearly favor either a stem or a stromal cell type model. As seen in Fig. 2, the results overwhelmingly indicate that a radioresistant, slowly repopulating cell is far more consistent with the biological processes underlying acute mortality, otherwise at least 50% of the distribution should be below the abscissa value of 1.0.

The recovery to normal tissue homeostasis in the model is not dependent upon the insult, either physical or chemical, that caused the injury. Instead, the recovery associated with repair of sublethal cellular injury and repopulation are formulated completely in terms of biologically related concepts involving populations of cells, length of the mitotic cycles, mitotic delay in G_2, etc. Thus, although the injury used to stimulate the recovery shown in Fig. 3 was due to ionizing radiations, other insults such as chemical and/or surgical ablation of the marrow used to create similar injury may, in principle, be compensated for according to recovery aspects of Fig. 4. Of course, insults that have a long biological half-life, activate different mechanisms, or are associated with toxicity to non-hematopoietic organs may not necessarily act in the manner shown.

Benzene is highly mobile inside the body and for simplicity may--like ionizing radiations--be expected to act primarily upon cells present in the body at the time of exposure. For example, Rickert et al. (8) found the benzene half-times in different organs of male Fischer-344 rats to be 48 minutes over the first 9 hours of exposure to 500 ppm by inhalation. A plot of amount expired in air was biphasic with $t_{1/2}$ times of 42 minutes and 13.1 hours. The fraction retained with the longer half-time is less than 5% of the exposure, and one or two half times associated of 13.1 hours is still shorter than the typical cell cycle for most multipotent cells and their supportive stroma (3).

Benzene-Induced Neoplasia in Animals: Nine experiments comprised: 6 different routes of administration, rats and mice as test species, treatment times in the general intervals of 2, 4, 12, and 24 months, plus variations in biological endpoint, dose, and dose rate. Obviously, the data grid is much too sparse to permit estimation of numerical coefficients even if the appropriate functional form of a biologically based dose-response model were known.

Acute Mortality from Benzene Toxicity: Fifteen experiments reflected: 6 different routes of administration, 7 test species, and exposure times ranging from minutes to 7 hours. In some regards, this data grid is more sparse than the neoplasia data, and in addition these data provide nothing useful to view/model the effects of dose protraction.

Cytotoxicity of CFC and CFU-S Cells is Often Linked to Benzene Toxicity: Seven publications described a rather limited variety of measurements for: CFC and CFU-S cells, treated by inhalation and subcutaneous injection, at different total doses and treatment concentrations, for various periods of time, and a wide range of post-exposure assay times. Those data are summarized in Table 3. The benzene experiments currently available are inadequate for development of biologically-based models, except for drawing of some very fragmentary conclusions such as those listed in Table 4.

From Table 2, compensatory repopulation by a particular cell is modeled by $\lambda_{NN}MF_{NN}$. The doubling time T_D associated with a particular surviving fraction can be estimated by $T_D = ln(2) /(\lambda_{NN}MF_{NN})$ and is shown in Fig. 4 for a λ of 0.00022 min^{-1}. The vectors shown in Fig. 4 are estimated doubling times from experimental data of Uyeki et al. (24) and Cronkite et al. (27,28).

Discussion

In this paper, benzene-induced hematopoietic toxicity is viewed in the broader context of the spectrum of exposures that: (a) are pancytotoxic and (b) induce compensatory hematopoiesis during or as a consequence of injury. Chlorambucil, chloramphenicol, chloroquine, cyclophosphamide, diethylamide, griseofulvin, ethylene oxide, ionizing radiations, lysergic acid, melphalan, methoxypsoralen, phenylbutazone, procarbazine, phosphorothioic acid triethylenetriamide, 7,12-dimethylbenz(a)anthracene, 2-acetylaminophenanthrene, N,N'-2,7-fluorenylenebisacetamide, N-2-fluorenylacetamide, 1-methyl-1-nitrosourea, and N-isopropyl-α-(2-methylhydrazine)-ρ-toluamide hydrochloride have been associated with leukemia in humans or animals. Several publications have concluded that injury to both hematopoietic stem cells and their cellular/cytokine mediated environment can be important to acute mortality and leukemogenesis. A number of experimental studies have found that all marrow-derived lineages can be regenerated from only one surviving pluripotent stem cell; whereas, a stroma of strong functional integrity is required to support that regeneration from a single surviving stem cell. For additional insight into the relative importances of stem and stromal lineages, especially as potentially related to benzene toxicity see publications by Metcalf (9), Dorschkind (10), Harigay et al. (11), Gill et al. (12), Lemischke et al. (13), Abkowitz et al. (14), Turhan et al. (15), Irons (16), Golbe et al. (17), Frash et al. (18), Laskin et al. (19), and Roberts (20).

In 1961, Cronkite (21) concluded that any agent which produces marrow aplasia is a "putative leukemogen." Later, Adamson and Seiber (22) noted that "It is possible that a given proportion of individuals who develop bone marrow depression as a consequence of chemical exposure may ultimately develop ANLL (sic., acute non-lymphatic leukemia) regardless of which agent produced the marrow toxicity, and indeed all of the chemicals which have been implicated as leukemogens can be myelosuppressive. Nevertheless, there are also chemicals which are potent depressants of bone marrow function but that have not been associated with human ANLL." Harigaya et al. (11) have proposed that the role of benzene may be more of a promoter by forcing the pluripotent stem cells (that have been exposed to leukemogenic initiating agents prior to benzene exposure) to undergo compensatory hematopoiesis. Because of existing data and simple, well-established dosimetry models, the quantitative considerations, as described in this paper, have been limited to exposures involving ionizing radiations and the relevance to benzene toxicity is implied by analogy of molecular-, cellular-, and organ-based processes.

As illustrated in Fig. 5 (a), our generic model of radiation-induced compensatory hematopoiesis has led to a strongly supported hypothesis that cell-to-cell contact and/or cytokine mediated processes between stomal and stem cells establish both the radiosensitivity and proliferation kinetics of the cells that are "critical" to hematopoietic recovery (23). Although that hypothesis is well-supported by a large array of stromal cell experiments, it is still contested by some who may have a completely justifiable but (in the context of the analysis presented herein) pre-Copernican-like perspective based on a belief that survival of hematopoietic stem cells is both necessary and sufficient for rescue from hematopoietic syndrome. In contrast, the model evaluations described in this paper indicate that even though stem cell survival is necessary, the rate limiting considerations seem to be associated with a more radioresistant and more slowly repopulating "critical" cell that is consistent with characteristics measured for marrow stroma and CFU-F type lineages. Findings that have produced Fig. 5 (a) seem to be remarkably consistent with the benzene toxicity model as described by Laskin, MacEachern, and Snyder (19), and it would provide useful perspective it they chose to consider the morphallaxis from benzene to radiation biology.

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LIST OF FIGURE CAPTIONS

Figure 1. Summary of data used from acute lethality experiments with protracted doses of ionizing radiations to determine the rate constants by maximum likelihood estimation techniques in the generic cell kinetics model of radiation induced hematopoiesis.

Figure 2. Journal publications have described LD_{50} estimates for protracted irradiations of mice and rats. The dose protractions were achieved by using low dose rates and/or dose fractionations. Because the dose response mortality data for these studies were not published, these experiments have not been used in our modeling efforts, hence these 38 experiments provide 343 LD_{50} values and serve as a good data base for model validations. The two cell-kinetics models, i.e, one for the "critical cells" (i.e., rate constants determined by MLE methods) and two, the CFU-S based rate constants were used to predict the equivalent prompt dose associated with each protracted LD_{50} estimate. For each experiment, a number of protracted irradiations were studied as part of the experimental design, i.i., an average of 343/38 = 9 per experiment. For the 'perfect' model all different dose protractions will yield the same estimated for the EPD. But because the EPD is lineage-specific, the two models will make contrasting predictions for protrated protocols. For very simple protracted irradiations, either model should model the EPD accurately and there may not be enough complexity in the experimental design to demonstrate the difference in the two models. However, for very low dose rates and/or complex fractions, the two models will predict strikingly different EPDs and clearly one will have a smaller variance within a particular experiment. If the two are statistically equal, then 50% of the cumulative distributions shown should be below 1.0. As seen, the MLE based model reduces the variance of the experiment-specific EPD distributions by factors typically ranging from 1.5 to 5. These comparisons were based on the 50% level of response and the gain is usually larger if data on the tails of the distribution function are available. Clearly, this exercise supports the idea that the critical cell for radiation-induced hematopoiesis is radioresistant and repopulates slowly--perhaps like the experimental data for marrow stroma or CFU-F cells.

Figure 3. The models based on CFU-S data and the MLE analysis of mortality data (labeled "stroma") were used to predict the time required for repopulation to 95% of normal tissue homeostasis from cytopenia ranging from surviving fractions of about 0.001 to 0.95. These estimates are driven by the injury to the lineages indicated and are not linked to the specific factors of the insults that resulted in cytopenia below normal tissue homeostasis.

Figure 4. Doubling times for CFU-S cells in mice were computed for compensatory repopulation as described in Table 2 and the text. The vectors are used to indicate ranges as estimated from the experimental data described in Table 4.

Figure 5. Panel (a) is based on consenus principles from radiation biology and from the results of our many model evaluations and validations. Clearly, the supporting stromal tissues and their cytokine mediated control of compensatory hematopoiesis are obligatory to recovery from toxic injury. Panel 4 (b) is used with the kind permission of Laskin et al. (19) and seems consistent with the concept shown in panel (a). In panel (b), toxic doses of benzene induce activation of macrophages and granulocytes which release reactive oxygen intermediates (ROI) that kill stem cells and stromal cells. The activated phagocytes can also produce cytokines and immune mediators (IM) that alter proliferation and differentiation of normal hematopoietic cells.

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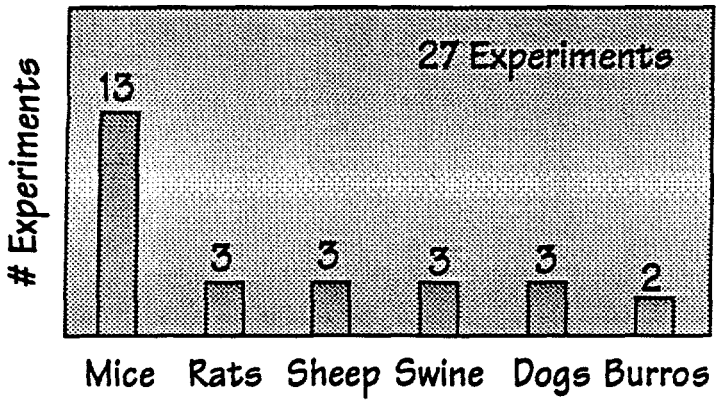
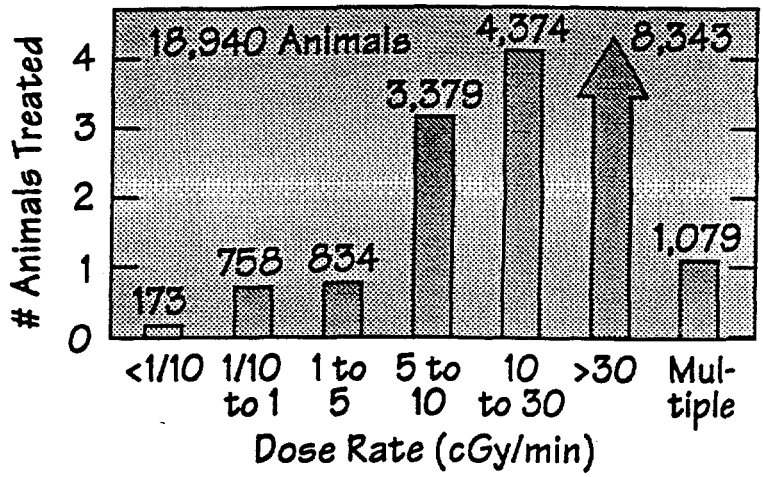
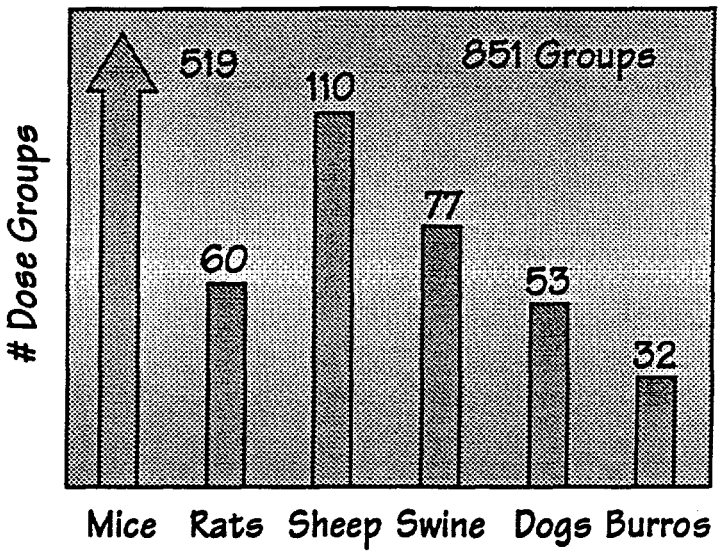
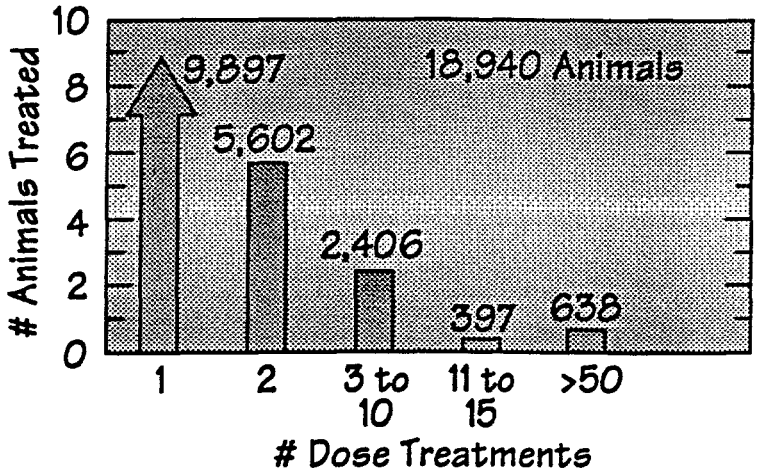
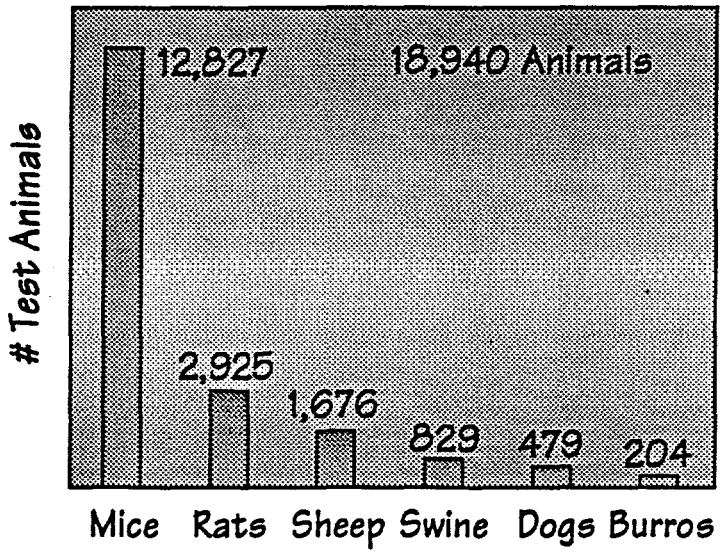
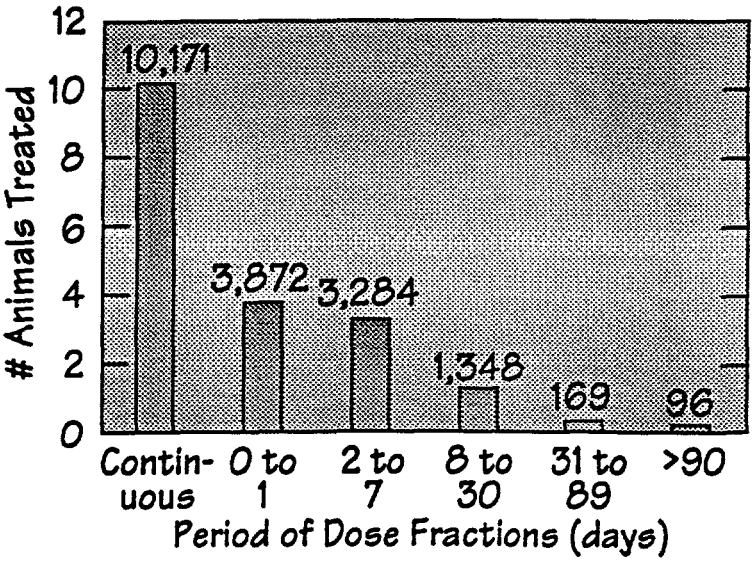


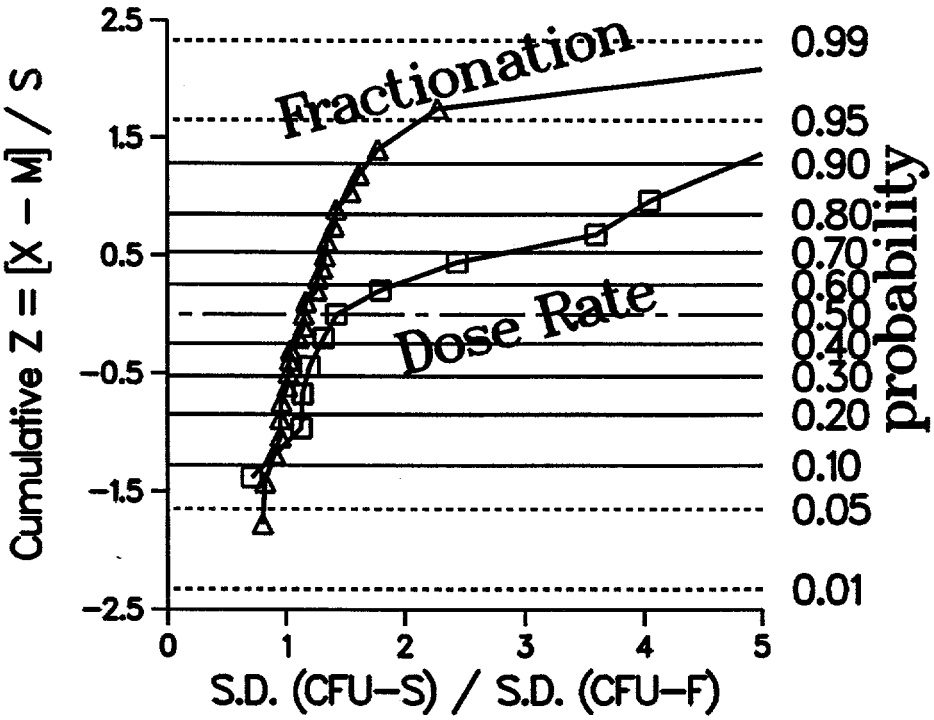


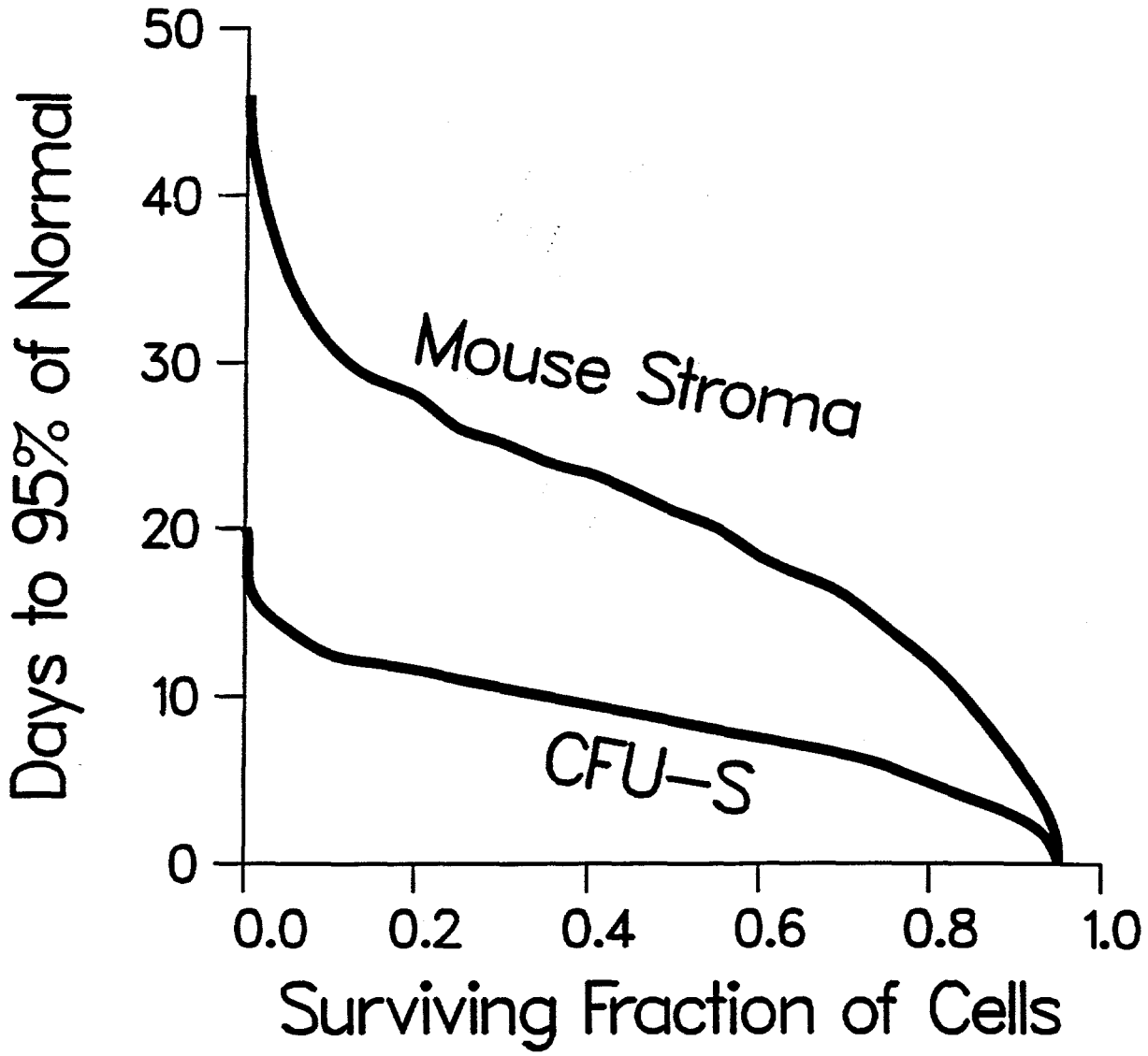


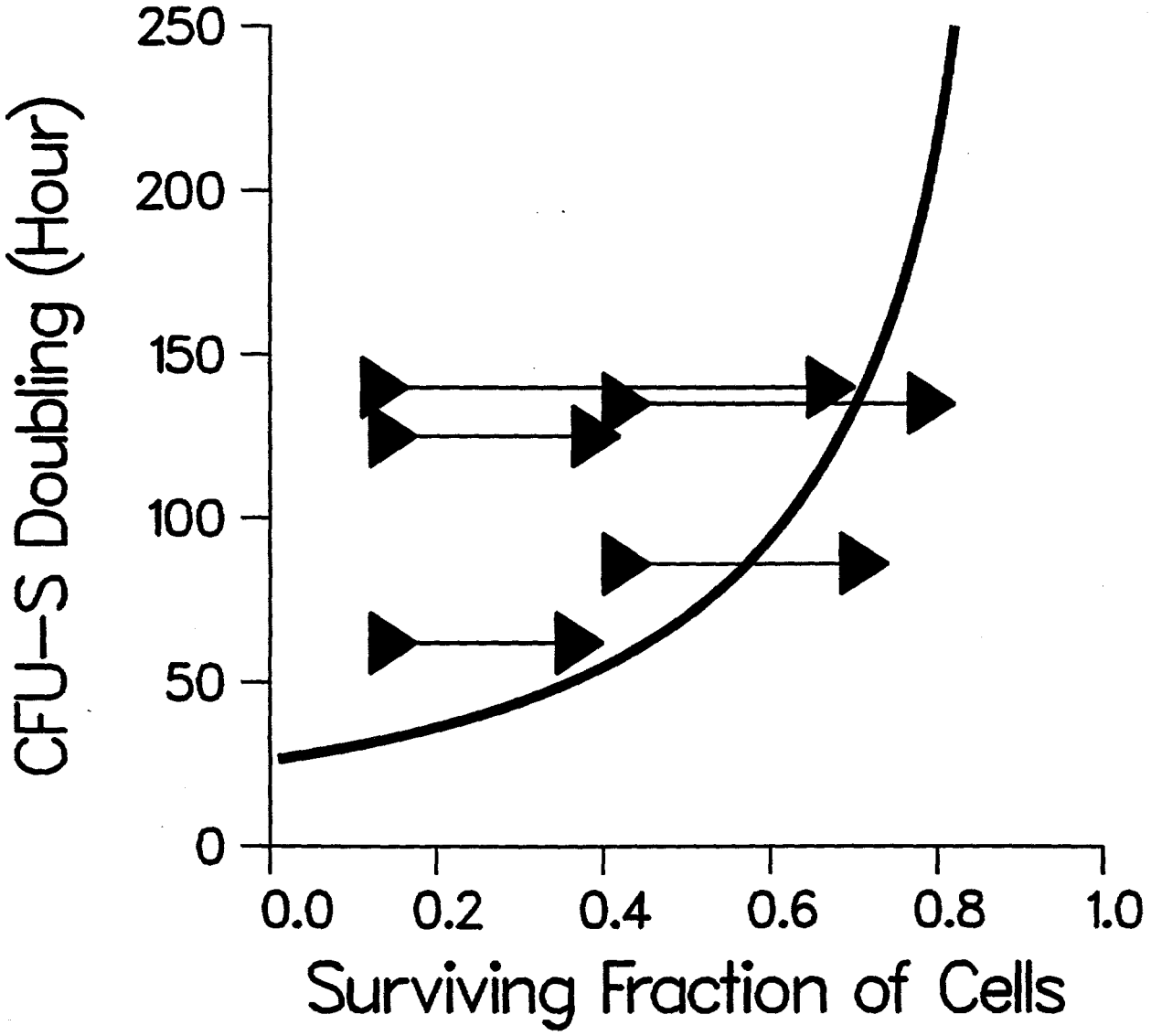


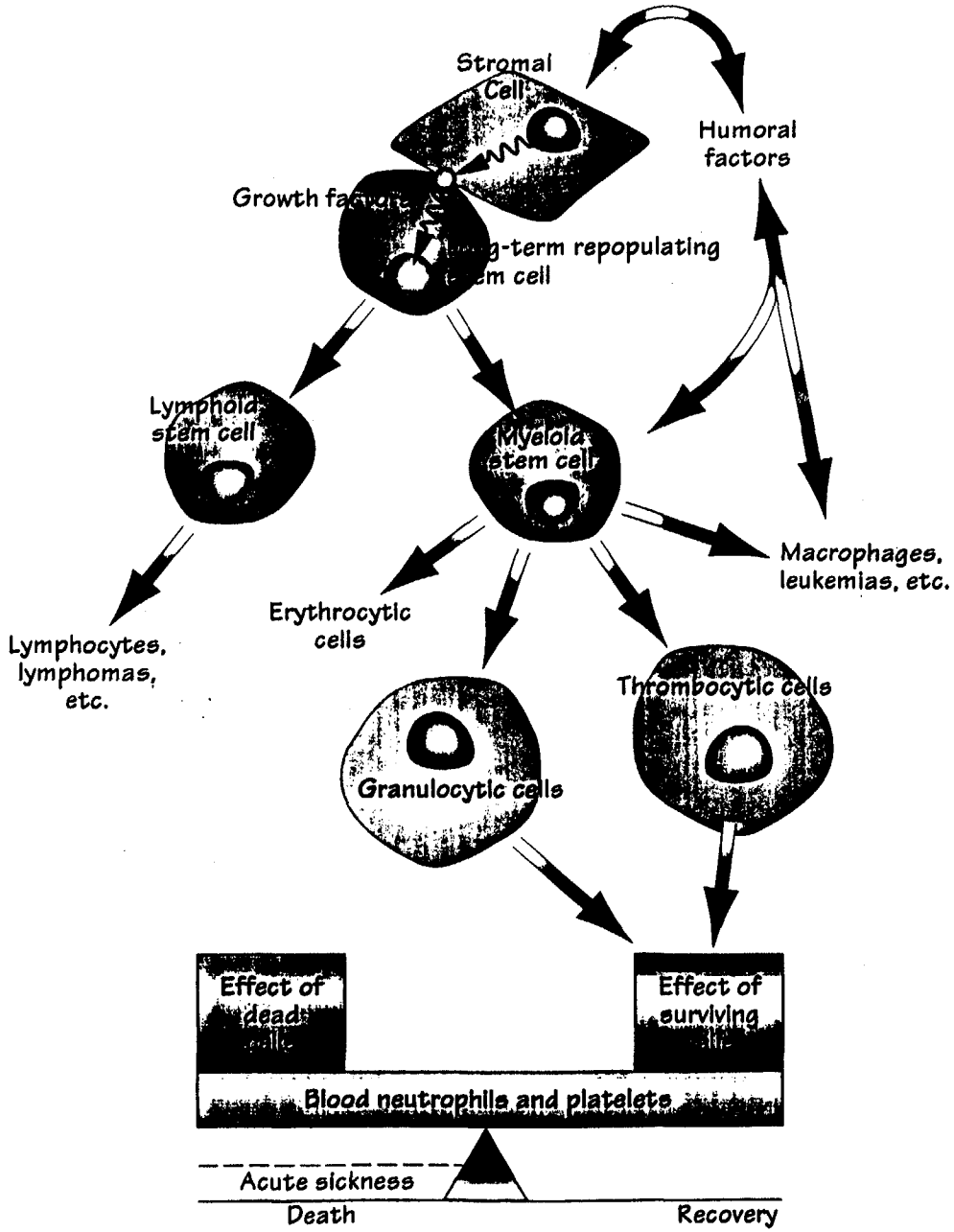





Fractionation
Dose Rate
Cumulative Z = [X − M] / S
S.D. (CFU−S) / S.D. (CFU−F)
probability
2.5
1.5
0.5
−0.5
−1.5
−2.5
0
1
2
3
4
5
0.99
0.95
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.05
0.01


Days to 95% of Normal
50
40
30
20
10
0
Mouse Stroma
CFU-S
0.0
0.2
0.4
0.6
0.8
1.0
Surviving Fraction of Cells


250
200
150
100
50
0
CFU-S Doubling (Hour)
0.0
0.2
0.4
0.6
0.8
1.0
Surviving Fraction of Cells


Stromal Cell
Humoral factors
Growth fa
g-term repopulating
m cell
Lymphoid stem cell
Myeloid stem cell
Macrophages, leukemias, etc.
Lymphocytes, lymphomas, etc.
Erythrocytic cells
Thrombocytic cells
Granulocytic cells
Effect of dead
Effect of surviving
Blood neutrophils and platelets
Acute sickness
Death
Recovery

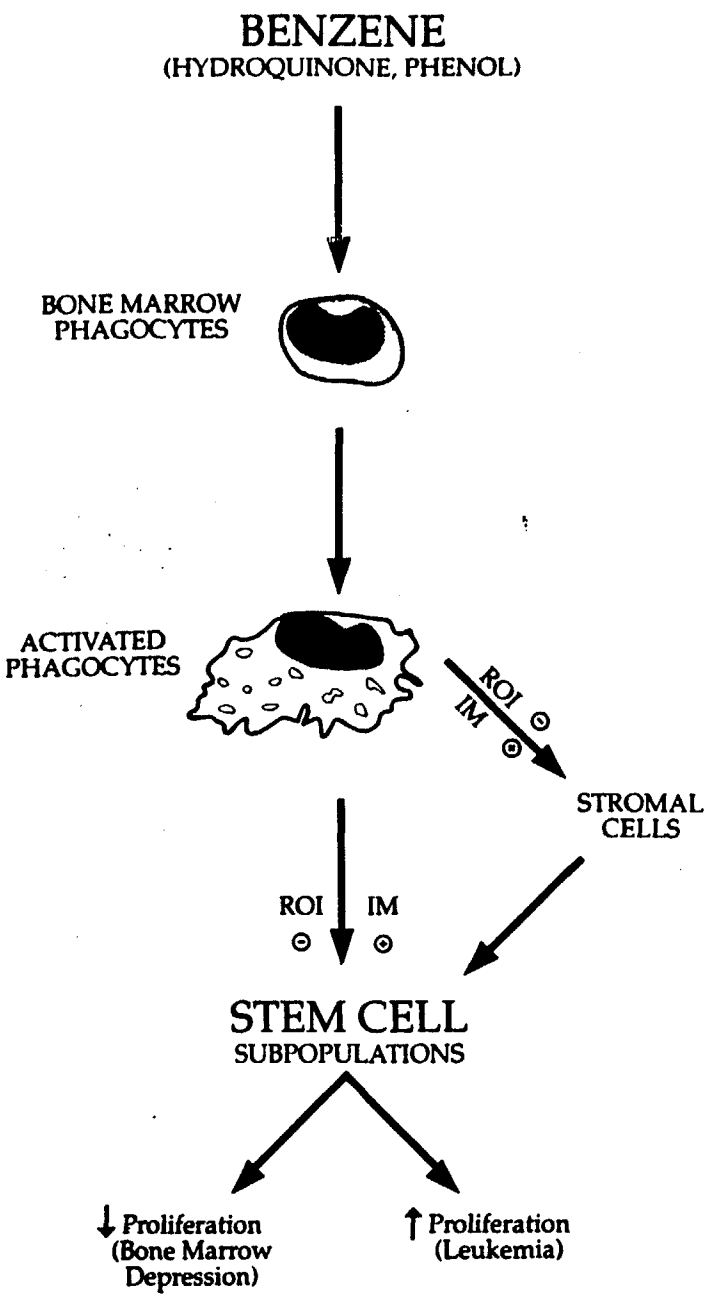

BENZENE
(HYDROQUINONE, PHENOL)
BONE MARROW PHAGOCYTES
ACTIVATED PHAGOCYTES
ROI
IM
STROMAL CELLS
ROI
IM
STEM CELL
SUBPOPULATIONS
Proliferation (Bone Marrow Depression)
Proliferation (Leukemia)

Table 1. Summary of bioassays and test conditions that have been used to measure the toxicity of benzene.

Tests	Organisms	Routes	Cell Types
Chromosome aberrations	bacteria	eye	bone marrow
DNA damage	cat	inhalation	embryo
DNA inhibition	dog	intraperitoneal	fibroblast
DNA unscheduled synthesis	drosophila	intravenous	Hela
Dominant lethal	frog	oral	leukocyte
Gene conversion & mitotic recombination	grasshopper	parenteral	liver
Micronucleus	guinea pig	skin	lung
Microsomal mutagenicity	hamster	subcutaneous	lymphocyte
Mutation in somatic mammalian cells	human		ovary
Mutation in microorganisms (w/o S9)	molds		
Mutation in microorganisms (S9)	mouse		
Oncogenic transformation	non-mammals		
Sex chromosome loss and disjunction	rabbit		
Specific locus	rat		
Sister chromatid exchanges	yeast		

Table 2. Summary of non-specific processes used to write a generic cell-kinetics model for damage, repair, and repopulation as a consequence of protracted exposure to ionizing radiations. Animal lethality studies were analyzed by maximum likelihood estimation (MLE) techniques in order to estimate the values for cellular rate constants for processes of sublethal injury, repair, direct killing, killing of cells having unrepaired sublethal injury, and compensatory repopulation. Subscripts are: I = sublethal injury; N = normal; K = kill; 0 = time at zero condition where all cells are phenotypically normal ($n_N = n_0 = 1$), the population of killed cells $N_K = 0$; and the population of injured cells $n_I = 0$. Because the model permits a cell to be in one of three phenotypically distinct states, the subscripts on the cellular rate constants (λ) indicate movement of cells from one compartment to another, e.g., λ_{IN} indicates repair to phenotypically normal function from an injured state.

Process:	Term:	Definition:
Sublethal injury:	$\lambda_{NI}D'n_N$	λ_{NI} = MLE constant (cells/Gy) $D'(t)$ = dose rate (Gy/min) n_N = cells at risk of sublethal injury
Repair of Sublethal Injury:	$\lambda_{IN}F_{IN}n_I$	λ_{IN} = MLE constant (cells/min) n_I = cells that can undergo repair of sublethal injury F_{IN} = rate modifying factor taken to be in the range of [1,2] and set at $1 + (n_0 - n_N - n_I)/n_0$ from fits to experimental data on the mitotic cycle
Direct Killing of Cells:	$\lambda_{NK}D'(t)n_N$	λ_{NK} = MLE constant (cells/Gy)
Indirect Killing of Cells:	$\lambda_{IK}D'(t)n_I$	λ_{IK} = MLE constant (cells/Gy) n_I = cells that have sublethal injury and can be killed by indirect processes
Compensatory Repopulation:	$\lambda_{NN}MF_{NN}n_N$	λ_{NN} = MLE constant (cells/min) n_N = phenotypically normal cells that can undergo mitosis $F_{NN} = (n_0 - n_N - n_I)/n_0 \bullet F_{IN}$ $(n_0 - n_N - n_I)/n_0$ controls proliferation rate; increases with cytotoxicity and stops at homeostasis M = Dirac delta function to turn on/off mitotic delay = 0, when time (hr) > accumulated dose (Gy), and = 1, when accumulated dose (Gy) ≥ time (hr)

****

MLE Values for Numerical Constants[a]:
λ_{NI} = 0.38 to 0.77 Gy^{-1}; λ_{NK} = 0.12 to 0.24 Gy^{-1}; λ_{IK} = 0.32 to 0.50 Gy^{-1}; and λ_{IN} = 0.022 min^{-1}.

Experimental studies have typically found that visible chromosomal aberrations may occur with a frequency of about 0.1 CA/Gy, which seems to be reasonably consistent with the composite action of λ_{NI}, λ_{NK}, λ_{IK}, and λ_{IN}. For λ_{IN} the repair half-times would range from 15 to 31 minutes depending upon the length of the mitotic cycle. For comparison, published half-times for repair of DNA-DSBs typically range from about 10 min to 2 hours which includes estimates for both fast and slow repair in various lineages of cells from different species and tissues.
λ_{NN} (in units of min^{-1}) values of 8.26×10^{-5} (mouse); 4.54×10^{-5} (rat); 4.23×10^{-5} (sheep); 1.65×10^{-4} (swine); and 8.89×10^{-5} (dog) give estimates of the doubling time (in units of hour) for compensatory repopulation as follows:

Species:	T_D (for LD_{10} to LD_{90})	T_D (for Therapeutic Fractions)	T_D (for 0.25 Gy[b])
Swine	35	70	9 wk
Dog	65	130	14 wk
Mouse	70	140	14 wk
Rat	130	260	26 wk
Sheep	140	280	36 wk

[a] Ranges listed include variations assigned for species-specific DNA and radiation quality for 100 kVP X, 250 kVp X, ^{137}Cs, and ^{60}Co photons. Variations due to randomness and/or analysis are not estimated in this paper.
[b] 0.25 Gy has been used historically as a maximum for radiation workers responding to a criticality accident. In addition, the estimates given in column 4 would approximate the final degree of healing following more serious, even near fatal injury.

Table 3. Summary of experimental data on CFU-S and CFC cells following treatments with benzene.

Test	<Dosing h/d	Schedule> d/w	w	# F_x's	Route	Max. Conc. (ppm)	Dose Rate (mg/kg-d)	Dose (mg/kg)	S(Femur) (%)	Assay Time (d post)
Uyeki et al. (24)										
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	45	1
CFC	8	3.5	1	3.5	Inhal.	4,680	10,700	37,600	13	0
CFU-S	8	3	1	3	Inhal.	4,680	10,700	32,200	41	1
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	39	1
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	50	1
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	40	1
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	74	4
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	82	7
Gill et al. (12)										
CFU-S	6	5	1	5	Inhal.	4,000	6,890	34,400	39	1
CFU-S	6	5	4	20	Inhal.	4,000	6,890	138,000	47	1
CFU-S	6	5	6	30	Inhal.	4,000	6,980	207,000	27	1
Green et al. (25)										
CFU-S	6	5	1	5	Inhal.	1	2	9	100	0
CFU-S	6	5	1	5	Inhal.	10	17	85	100	0
CFU-S	6	5	1	5	Inhal.	103	177	887	53	0
CFU-S	6	5	1	5	Inhal.	306	527	2,630	19	0
CFU-S	6	5	1	5	Inhal.	603	1,040	5,190	39	0
CFU-S	6	5	1	5	Inhal.	1,280	2,200	11,000	45	0
CFU-S	6	5	1	5	Inhal.	2,420	4,160	20,800	37	0
CFU-S	6	5	1	5	Inhal.	4,860	8,370	41,900	32	0
CFU-S	6	5	10	50	Inhal.	10	17	827	97	0
CFU-S	6	5	26	130	Inhal.	302	520	67,600	7.7	0
CFU-C	6	5	26	130	Inhal.	302	520	67,600	7.3	0
CFU-S	6	5	1	5	Inhal.	103	177	887	55	0
CFU-S	6	5	1	5	Inhal.	306	527	2,630	18	0
CFU-C	6	5	1	5	Inhal.	103	177	887	82	0
Cronkite et al. (27)										
CFU-S	6	5	1	1	Inhal.	400	689	689	85	1
CFU-S	6	5	1	2	Inhal.	400	689	1,380	52	1
CFU-S	6	5	1	4	Inhal.	400	689	2,760	35	1
CFU-S	6	5	1	5	Inhal.	400	689	3,440	22	1
CFU-S	6	5	1.5	8	Inhal.	400	689	5,510	20	1
CFU-S	6	5	4	20	Inhal.	400	689	13,800	40	1
CFU-S	6	5	4	20	Inhal.	400	689	13,800	12	2
CFU-S	6	5	7	35	Inhal.	400	689	24,100	42	2
CFU-S	6	5	8	40	Inhal.	400	689	27,600	43	1
CFU-S	6	5	9.5	48	Inhal.	400	689	32,700	12	1
CFU-S	6	5	11	48	Inhal.	400	689	32,700	40	7
CFU-S	6	5	12	48	Inhal.	400	689	32,700	42	14

(Cont'd on next page)

Table 3. Cont'd.

Cronkite et al. (28)										
CFU-S	6	5	2	10	Inhal.	10	17	172	98	1
CFU-S	6	5	2	10	Inhal.	25	43	431	109	1
CFU-S	6	5	2	10	Inhal.	100	172	1,720	67	1
CFU-S	6	5	2	10	Inhal.	400	689	6,890	45	1
CFU-S	6	5	2	10	Inhal.	300	517	5,170	93	14
CFU-S	6	5	2	10	Inhal.	300	517	5,170	105	28
CFU-S	6	5	4	20	Inhal.	300	517	10,300	90	14
CFU-S	6	5	4	20	Inhal.	300	517	10,300	94	28
CFU-S	6	5	8	40	Inhal.	300	517	20,700	53	28
CFU-S	6	5	8	40	Inhal.	300	517	20,700	61	56
CFU-S	6	5	8	40	Inhal.	300	517	20,700	113	112
CFU-S	6	5	16	80	Inhal.	300	517	41,300	24	3
CFU-S	6	5	16	80	Inhal.	300	517	41,300	30	14
CFU-S	6	5	16	80	Inhal.	300	517	41,300	46	28
CFU-S	6	5	16	80	Inhal.	300	517	41,300	57	56
CFU-S	6	5	16	80	Inhal.	300	517	41,300	60	112
CFU-S	6	5	16	80	Inhal.	300	517	41,300	98	175
Cronkite et al. (29)										
CFU-S	6	5	1	2	Inhal.	3,000	5,170	10,300	11	1
CFU-S	6	5	1	2	Inhal.	3,000	5,170	10,300	70	32
CFU-S	6	5	1	2	Inhal.	3,000	5,170	10,300	67	67
CFU-S	6	5	1	2	Inhal.	3,000	5,170	10,300	85	214
CFU-S	6	5	4	19	Inhal.	316	544	10,300	39	1
CFU-S	6	5	4	19	Inhal.	316	544	10,300	112	32
CFU-S	6	5	4	19	Inhal.	316	544	10,300	99	66
CFU-S	6	5	4	19	Inhal.	316	544	10,300	116	214
Tunek et al. (26)										
CFU-C	-	6	1	6	Subcut.	0.7	1	4	94	1
CFU-C	-	6	1	6	Subcut.	3.5	4	21	56	1
CFU-C	-	6	1	6	Subcut.	18	18	108	60	1
CFU-C	-	6	1	6	Subcut.	88	88	528	33	1
CFU-C	-	6	1	6	Subcut.	440	440	2,640	5	1
CFU-C	-	1	1	1	Subcut.	440	440	440	60	1
CFU-C	-	2	1	2	Subcut.	440	440	880	26	1
CFU-C	-	3	1	3	Subcut.	440	440	1,320	18	1
CFU-C	-	4	1	4	Subcut.	440	440	1,760	4.3	1
CFU-C	-	5	1	5	Subcut.	440	440	2,200	8.7	1

Table 4. Summary of experimental results on benzene toxicity to hematopoietic cells.

Uyeki et al. (24)	Dose Response--If the dose-rate is held constant, there seems to be only a weak dose response for exposures of 8 h/d because of the limited range of data. However, if one of the experimental points is corrected for the delay in assay time, then the dose response becomes more consistent with other studies. Doubling Time/Assay time: If dose and dose-rate are held constant, the time delay used in the assay appears to be very important. The doubling time seems to range from about 3.6 to 5.7 days for compensatory proliferation associated with a survival of about 40%.
Gill et al. (12)	Dose Response: Only three points are available for evaluation of a dose response, but Gill's CFU-S cells could be twice as resistant as Uyeki's CFC cells.
Green et al. (25)	Assays were made on the day that the dosing ended. These data contain both dose and dose rate variations in experimental design. Dose Response: Assays were conducted on day 0. If corrected to day 1 these results seem to be comparable to the data of Uyeki et al. Because of a wide range of doses, Green's data show a strong dose response. Dose Rate: A very strong effect of dose-rate for 6 h/d protocol is demonstrated. Dose Rate may be more biological significant than the number of days exposed (when the exposure is for 4 to 6 hours per day).
Tunek et al. (26)	Route of Intake: These data are for subcutaneous injection--toxicity appears greater than for inhalation. Dose Response: Seems to have the same functional shape as data of Green. Dose Rate: Shapes of the response vs dose rate plots are similar to those of Green but the magnitudes are different. From the literature, we have found absorption coefficients ranging from 0.28 to 0.60 (median = 0.47) for inhalation of benzene. It seems that a rigorous analysis of absorbed fraction coupled with the dosing protocol differences used with inhalation studies are adequate to bring the Tunek data in line with the inhalation data.
Cronkite et al. (27)	Dose Response: Seems very consistent with that of Green and Tunek but all doses were given at concentrations of 400 ppm. Data are given as day of assay and proliferation according to: (day, %):(1d, 12%), (7d,40%), (14d, 42%). Therefore, the doubling time seems to range from about 2.6 to 5.2 days for repopulation from a survival of about 12%.
Cronkite et al. (28)	Dose Response: Seems consistent with previous discussions, but dose rates are mostly for 300 ppm. Doubling Time: Seems to be very long, probably about 40 days--looks inconsistent with other studies.
Cronkite et al. (29)	Doubling time: Seems to be about 5.8 days from a survival of 11%.