Modeling Marrow Damage from Response Data: Evolution from Radiation Biology to Benzene Toxicity

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Consensus principles from radiation biology were used to describe a generic set of nonlinear, first-order differential equations for modeling 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: 12,827 mice, 2925 rats, 1676 sheep, 829 swine, 479 dogs, and 204 burros. 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 stemlike cells with regard to radiosensitivity and repopulation rates. Instead, the weakest link in the chain of hematopoiesis was found to have an intrinsic radiosensitivity equal to or greater than stromal cells and to repopulate at the same rates. Model validation has been achieved by predicting the LD50 and/or fractional group mortality in 38 protracted-dose experiments (rats and mice) that were not used in the fitting of model coefficients. — Environ Health Perspect 104(Suppl 6):1293–1301 (1996)

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

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 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 single 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 require 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/stain 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 to estimate parameters. Subtle, indirect assumptions have the hazard of being unrecognized, perhaps even to the researchers themselves.

Our approach formulates generalized dose–response models in terms of generic processes: molecular effects, from a cell kinetics perspective, and descriptions of local and systemic reactions that may act through cell-to-cell and/or humoral-mediated effects involving hormones, cholamines, or cytokines. The dosing schedules used for benzene experiments do not reflect adequate protocol-dependent variability to permit use of the MSOD approach to a degree that would provide 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 organisms 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 short-term and long-term irradiations, those experiments, as summarized in Figure 1, serve as the database 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 insight into the fundamental underlying mechanisms. As indicated in Table 1, the conceptual and mathematical models used for ionizing radiations, should also be relevant to considerations of benzene toxicity. Experimental data needed to estimate model parameters are fragmentary. 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, MLE 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. In addition, we found it desirable to evaluate and test a cell kinetics model formulated in terms of those same nonspecific damage, repair, and repopulation processes as derived from colony-forming unit-spleen (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).

Materials and Methods

When animals are irradiated by acute protocols, death from infection and/or hemorrhage may occur between about 5 and 30 days postirradiation. The frequency of death can be described by a probit distribution function with fitted parameters composed of the LD50 and slope (i.e., slope = σ−1, which is the inverse standard deviation of the frequency distribution). The LD50 and σ may be for the particular radiation field of interest or for a standard reference radiation if there is a realistic way of modeling the underlying degree of lethality from the exposure of interest and converting that level of effect back to an equivalent reference dose of the standard radiation associated with the LD50 and σ estimates. Depression of neutrophils and platelets are accepted as the proximate cause of death, but the contributing cause of death could be cytopenia of either the terminally differentiated cells themselves, ancestral cells, or ancestral-dependent lineages upstream in the direction of the undifferentiated pluripotent stem cells. Cytopenia of a critical lineage would result in either a deficiency of cells or

![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.]

| 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 and mitotic recombination | Grasshopper | Parenteral | Liver |
| Micronucleus | Guinea pig | Skin | Lung |
| Micronomal mutagenicity | Hamster | Subcutaneous | Lymphocyte |
| Mutation in somatic mammalian cells | Human | Ovary | |
| Mutation in microorganisms (~59) | Molds | | |
| Mutation in microorganisms (+59) | Mouse | | |
| Oncogenic transformation | Nonmammals | | |
| Sex chromosome loss and disjunction | Rabbit | | |
| Specific locus | | | |
| Sister chromatid exchanges | Yeast | | |
cell-mediated cytokines. 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 $\text{LD}_{50}\$, $\sigma$ 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 $\text{LD}_{50}$ and $\sigma$ values. One experiment in the analysis comprised 26 different $\text{LD}_{50}$ protocols, but all were consistent with a common $\text{LD}_{50}$ and $\sigma$ associated with an “equivalent prompt dose.”

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

$$n' = -\lambda_{NI} D' n_N - \lambda_{NK} D' n_K + \lambda_{IN} F_{IN} n_I + \lambda_{NN} M_{NN} n_N$$  \hspace{1cm} [1]

$$n' = -\lambda_{IK} D' n_K - \lambda_{IN} F_{IN} n_I + \lambda_{NI} D' n_N$$  \hspace{1cm} [2]

$$n' = \lambda_{NK} D' n_K + \lambda_{IK} D' n_I.$$  \hspace{1cm} [3]

In these equations, $\lambda$ is the rate constant that mediates movement 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 Tables 2 and 3.

| Process | Term | Definition |
|---------|------|------------|
| Sublethal injury: | $\lambda_{NI} D' n_N$ | $\lambda_{NI} = \text{MLE constant (cells/Gy)}$ |
|           | $D'(t)$ | dose rate (Gy/min) |
|           | $n_N$ | cells at risk of sublethal injury |
| Repair of sublethal injury: | $\lambda_{IN} F_{IN} n_I$ | $\lambda_{IN} = \text{MLE constant (cells/min)}$ |
|           | $n_I$ | cells that can undergo repair of sublethal injury |
| Direct killing of cells: | $\lambda_{NK} D' n_K$ | $\lambda_{NK} = \text{MLE constant (cells/Gy)}$ |
| Indirect killing of cells: | $\lambda_{IK} D' n_I$ | $\lambda_{IK} = \text{MLE constant (cells/Gy)}$ |
| Compensatory repopulation: | $\lambda_{NN} M_{NN} n_N$ | $\lambda_{NN} = \text{MLE constant (cells/min)}$ |
|           | $F_{IN}$ | rate-modifying factor taken to be in the range of 0.25 to 2.0 and set at 1 + [(nO - nK - nI)/nK] from fits to experimental data on the mitotic cycle |

| Species | $T_D$ for LD$_{50}$, hr | $T_D$ for therapeutic fractions, hr | $T_D$ for 0.25 Gy$^2$, weeks |
|---------|--------------------------|-------------------------------|-----------------------------|
| Swine   | 35                        | 70                            | 9                           |
| Dog     | 65                        | 130                           | 14                          |
| Mouse   | 70                        | 140                           | 14                          |
| Rat     | 130                       | 260                           | 26                          |
| Sheep   | 140                       | 280                           | 36                          |

*25 Gy has been used historically as a maximum for radiation workers responding to a criticality accident. $\lambda_{NN}$ (in units of min$^{-1}$) values of 8.26 $\times$ 10$^{-5}$ (mouse); 4.54 $\times$ 10$^{-5}$ (rat); 4.23 $\times$ 10$^{-5}$ (sheep); 1.65 $\times$ 10$^{-4}$ (swine); and 8.89 $\times$ 10$^{-5}$ (dog). In addition, the estimates given in column 4 would approximate the final degree of healing following more serious, even near fatal injury.

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 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 database, 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.

Thirty-four experiments have been analyzed previously by MLE methods 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, resulted from an exhaustive literature review, and selection of the 38 experiments for model validation was based on a) dose protraction by rate or fractionation in mice or rats; b) mortality within 30 days from the end of the radiation treatments (studies were used if a few animals died from gastrointestinal 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); c) no more than 60 days between successive dose fractions; d) 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); e) adequate specifications of times or dose rates, and f) the experiment 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 rejected.

The 38 experiments used to validate the model typically reported only LD$_{90}$ values without giving the actual dose-response data. Although these studies were not useful for unbiased estimation of model constants, they do provide independent tests for model validation. The 12 doses rate studies ranged from 0.0008 to 4.74 Gy/min, and the 26 fractionation studies contained fractions from 1.54 to 7 Gy 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 simple fractionated protocols will generally produce numerically similar estimates of the equivalent prompt dose (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 Figure 2, the results overwhelmingly indicate that a radioreistant, 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 on the insult, either physical or chemical, that caused the injury. Instead, the recovery associated with repair of sublethal cellular injury and repopulation are formulated

![Figure 2](https://example.com/figure2.png)

*Figure 2.* Journal publications have described LD$_{90}$ 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$_{90}$ values and serve as a good database for model validations. The two cell-kinetics models, i.e., one for the critical cells [rate constants determined by maximum likelihood estimation (MLE) methods] and the CFU-S-based rate constants, were used to predict the equivalent prompt dose associated with each protracted LD$_{90}$ estimate. For each experiment, a number of protracted irradiations were studied as part of the experimental design (an average of 343/38 = 9 per experiment). For the perfect model all different dose protractions will yield the same estimate for the EPD. But because the EPD is lineage-specific, the two models will make contrasting predictions for protracted protocols. For 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 low dose rates and/or complex fractions, the two models will predict strikingly different EPDs, and 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 <1.0. As shown, 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-S cells.
completely in terms of biologically related concepts involving populations of cells, length of the mitotic cycles, mitotic delay in G2, etc. Thus, although the injury used to stimulate the recovery shown in Figure 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 Figure 4. Of course, insults that have a long biological half-life, activate different mechanisms, or are associated with toxicity to nonhematopoietic 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 min over the first 9 hr of exposure to 500 ppm by inhalation. A plot of benzene expired in air was biphasic with $t_{1/2}$ times of 42 min and 13.1 hr. The fraction retained with the longer half-time is less than 5% of the exposure and therefore is 1 or 2 times the 13.1-hr half-life (i.e., 13.1–26.2 hr) is still shorter than the typical cell cycle for most multipotent cells and their supportive stroma (3).

In regard to benzene-induced neoplasia, nine experiments comprised six 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 end point, 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.

In regard to acute mortality from benzene toxicity, 15 experiments reflected 6 different routes of administration, seven test species, and exposure times ranging from minutes to 7 hr. 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.

The cytotoxicity of colony-forming cells (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 concentrations, and concentration rates, for various periods of time, and a wide range of postexposure assay times. Those data are summarized in Table 4. The benzene experiments currently available are inadequate for development of biologically based models, except for drawing of some fragmentary conclusions such as those listed in Table 5.

From Tables 2 and 3, compensatory repopulation by a particular cell is modeled by $\lambda_{NN}M_{FNN}$. The doubling time, $T_D$, associated with a particular surviving fraction can be estimated by $T_D = \ln(2)/\lambda_{NN}M_{FNN}$ and is shown in Figure 4 for a $\lambda$ of 0.00022 min$^{-1}$. The vectors shown in Figure 4 are estimated doubling times from experimental data of Uyeki et al. (9) and Cronkite et al. (12,13).

**Discussion**

In this paper, benzene-induced hematopoietic toxicity is viewed in the broader context of the spectrum of exposures that are pancytotoxic and induce compensatory hematopoiesis during or as a consequence of injury. Chlorambucil, chloramphenicol, chloroquine, cyclophosphamide, diethyl-lamylide, griseofulvin, ethylene oxide, ionizing radiations, lysergic acid, melphalan, methoxyprorsonal, phenylbutazone, procabazine, phosphorothioic acid triethylentriamide, 7,12-dimethylbenz[a]anthracene, 2-acetylaminophenanthrene, 2-acetaminophenophanthenlamp, $N/N',2,7$-fluorenylenebisacetic acid, $N$, $N'$-2-fluorenylacetamide, 1-methyl-1-nitrosourea, $N$-2-fluorenylacetamide, 1-methyl-1-nitrosourea, and $N$-isopropyl-$\alpha$-(2-methylhydrazine)-p-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 cell alone surviving pluripotent stem cell, whereas a stroma of strong functional integrity is required to support that regeneration. The importance of stem and stromal lineages, especially as potentially related to benzene toxicity, has been discussed previously (16–25).

In 1961, Cronkite (26) concluded that any agent which produces marrow aplasia is a "putative leukemogen." Later, Adamson and Seibert (27) 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 [acute nonlymphocytic 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.

Hariyaga et al. (17) proposed that the role of benzene may be more of a promoter by forcing the pluripotent stem cells (that
Table 4. Summary of experimental data on colony-forming unit-spleen cells and colony-forming cells following treatments with benzene.

| Test | Dosing schedule | No. of exposures | Route | Maximum concentration, ppm | Dose rate, mg/kg-day | Dose, mg/kg | S (femur), % | Assay time, days after administration |
|------|-----------------|------------------|-------|-----------------------------|----------------------|------------|-------------|----------------------------------|
| Uyeki et al. (9) |                |                  |       |                             |                      |            |             |                                  |
| CFU  | 8               | 1                | 1     | Inhalation                  | 6,890                | 10,700     | 45          | 1                  |
| CFU  | 6               | 3.5              | 1     | Inhalation                  | 6,890                | 10,700     | 37,600      | 13                 |
| CFU-S| 8               | 3                | 3     | Inhalation                  | 6,890                | 10,700     | 32,200      | 41                 |
| CFU  | 8               | 1                | 1     | Inhalation                  | 6,890                | 10,700     | 10,700      | 39                 |
| CFU  | 8               | 1                | 1     | Inhalation                  | 6,890                | 10,700     | 10,700      | 50                 |
| CFU  | 8               | 1                | 1     | Inhalation                  | 6,890                | 10,700     | 10,700      | 40                 |
| CFU  | 8               | 1                | 1     | Inhalation                  | 6,890                | 10,700     | 10,700      | 74                 |
| CFU-S| 8               | 1                | 1     | Inhalation                  | 6,890                | 10,700     | 10,700      | 62                 |
| Gill et al. (10) |                  |                   |       |                             |                      |            |             |                                  |
| CFU-S| 6               | 5                | 1     | Inhalation                  | 4,000                | 6,890      | 34,400      | 39                 |
| CFU-S| 6               | 5                | 4    | Inhalation                  | 4,000                | 6,890      | 138,000     | 47                 |
| CFU-S| 6               | 5                | 30   | Inhalation                  | 4,000                | 6,890      | 207,000     | 27                 |
| Green et al. (11) |                |                  |       |                             |                      |            |             |                                  |
| CFU-S| 6               | 5                | 1     | Inhalation                  | 1,777                | 887        | 53          | 0                  |
| CFU-S| 6               | 5                | 1     | Inhalation                  | 1,280                | 2,200      | 11,000      | 45                 |
| CFU-S| 6               | 5                | 5    | Inhalation                  | 2,420                | 4,160      | 20,800      | 37                 |
| CFU-S| 6               | 5                | 5    | Inhalation                  | 6,890                | 8,370      | 41,500      | 32                 |
| CFU-S| 6               | 5                | 10   | Inhalation                  | 10                   | 17         | 827         | 97                 |
| CFU-S| 6               | 5                | 26   | Inhalation                  | 302                  | 520        | 67,600      | 7.7                |
| CFU-C| 6               | 5                | 26   | Inhalation                  | 302                  | 520        | 67,600      | 7.3                |
| CFU-S| 6               | 5                | 1    | Inhalation                  | 103                  | 177        | 887         | 55                 |
| CFU-S| 6               | 5                | 1    | Inhalation                  | 306                  | 527        | 2,630       | 18                 |
| CFU-S| 6               | 5                | 5    | Inhalation                  | 103                  | 177        | 887         | 82                 |
| Cronkite et al. (12) |            |                  |       |                             |                      |            |             |                                  |
| CFU-S| 6               | 5                | 1    | Inhalation                  | 400                  | 689        | 97          | 1                  |
| CFU-S| 6               | 5                | 1    | Inhalation                  | 400                  | 689        | 1,380       | 52                 |
| CFU-S| 6               | 5                | 4    | Inhalation                  | 400                  | 689        | 2,760       | 35                 |
| CFU-S| 6               | 5                | 5    | Inhalation                  | 400                  | 689        | 3,440       | 22                 |
| CFU-S| 6               | 5                | 8    | Inhalation                  | 400                  | 689        | 5,100       | 20                 |
| CFU-S| 6               | 5                | 7    | Inhalation                  | 400                  | 689        | 8,100       | 40                 |
| CFU-S| 6               | 5                | 4    | Inhalation                  | 400                  | 689        | 13,000      | 40                 |
| CFU-S| 6               | 5                | 4    | Inhalation                  | 400                  | 689        | 13,000      | 12                 |
| CFU-S| 6               | 5                | 35   | Inhalation                  | 400                  | 689        | 24,100      | 42                 |
| CFU-S| 6               | 5                | 40   | Inhalation                  | 400                  | 689        | 27,600      | 43                 |
| CFU-S| 6               | 5                | 8    | Inhalation                  | 400                  | 689        | 32,700      | 12                 |
| CFU-S| 6               | 5                | 8    | Inhalation                  | 400                  | 689        | 32,700      | 40                 |
| CFU-S| 6               | 5                | 11   | Inhalation                  | 400                  | 689        | 32,700      | 47                 |
| CFU-S| 6               | 5                | 12   | Inhalation                  | 400                  | 689        | 32,700      | 42                 |
| Cronkite et al. (13) |            |                  |       |                             |                      |            |             |                                  |
| CFU-S| 6               | 5                | 2    | Inhalation                  | 10                   | 17         | 172         | 98                 |
| CFU-S| 6               | 5                | 2    | Inhalation                  | 25                   | 43         | 431         | 109                |
| CFU-S| 6               | 5                | 4    | Inhalation                  | 100                  | 172        | 1,720       | 67                 |
| CFU-S| 6               | 5                | 1    | Inhalation                  | 300                  | 517        | 5,170       | 45                 |
| CFU-S| 6               | 5                | 2    | Inhalation                  | 300                  | 517        | 10,300      | 40                 |
| CFU-S| 6               | 5                | 4    | Inhalation                  | 300                  | 517        | 10,300      | 39                 |
| CFU-S| 6               | 5                | 4    | Inhalation                  | 300                  | 517        | 10,300      | 39                 |
| CFU-S| 6               | 5                | 8    | Inhalation                  | 300                  | 517        | 20,700      | 53                 |
| CFU-S| 6               | 5                | 8    | Inhalation                  | 300                  | 517        | 20,700      | 61                 |
| CFU-S| 6               | 5                | 8    | Inhalation                  | 300                  | 517        | 20,700      | 113                |
| CFU-S| 6               | 5                | 16   | Inhalation                  | 300                  | 517        | 41,300      | 24                 |
| CFU-S| 6               | 5                | 16   | Inhalation                  | 300                  | 517        | 41,300      | 30                 |
| CFU-S| 6               | 5                | 16   | Inhalation                  | 300                  | 517        | 41,300      | 46                 |
| CFU-S| 6               | 5                | 16   | Inhalation                  | 300                  | 517        | 41,300      | 46                 |
| CFU-S| 6               | 5                | 16   | Inhalation                  | 300                  | 517        | 41,300      | 57                 |
| CFU-S| 6               | 5                | 16   | Inhalation                  | 300                  | 517        | 41,300      | 60                 |
| CFU-S| 6               | 5                | 16   | Inhalation                  | 300                  | 517        | 41,300      | 98                 |

(Continued)
Table 4. Continued

| Test | Dosing schedule | No. of exposures | Route | Maximum concentration, ppm | Dose rate, mg/kg-day | Dose, mg/kg | S (femur), % | Assay time, days after administration |
|------|----------------|------------------|-------|-----------------------------|----------------------|-----------|-------------|-------------------------------------|
| Cronkite et al. (14) | | | | | | | | |
| CFU-S | 6 | 5 | 1 | 2 | Inhalation | 3,000 | 5,170 | 10,300 | 11 | 1 |
| CFU-S | 6 | 5 | 1 | 2 | Inhalation | 3,000 | 5,170 | 10,300 | 70 | 32 |
| CFU-S | 6 | 5 | 1 | 2 | Inhalation | 3,000 | 5,170 | 10,300 | 67 | 67 |
| CFU-S | 6 | 5 | 1 | 2 | Inhalation | 3,000 | 5,170 | 10,300 | 85 | 214 |
| CFU-S | 6 | 5 | 4 | 19 | Inhalation | 316 | 544 | 10,300 | 39 | 1 |
| CFU-S | 6 | 5 | 4 | 19 | Inhalation | 316 | 544 | 10,300 | 112 | 32 |
| CFU-S | 6 | 5 | 4 | 19 | Inhalation | 316 | 544 | 10,300 | 99 | 66 |
| CFU-S | 6 | 5 | 4 | 19 | Inhalation | 316 | 544 | 10,300 | 116 | 214 |
| Tunek et al. (15) | | | | | | | | |
| CFU-C | — | 6 | 1 | 6 | Subcutaneous | 0.7 | 1 | 4 | 94 | 1 |
| CFU-C | — | 6 | 1 | 6 | Subcutaneous | 3.5 | 4 | 21 | 56 | 1 |
| CFU-C | — | 6 | 1 | 6 | Subcutaneous | 18 | 18 | 108 | 60 | 1 |
| CFU-C | — | 6 | 1 | 6 | Subcutaneous | 88 | 88 | 528 | 33 | 1 |
| CFU-C | — | 6 | 1 | 6 | Subcutaneous | 440 | 440 | 2,640 | 5 | 1 |
| CFU-C | — | 1 | 1 | 1 | Subcutaneous | 440 | 440 | 440 | 60 | 1 |
| CFU-C | — | 2 | 1 | 2 | Subcutaneous | 440 | 440 | 680 | 26 | 1 |
| CFU-C | — | 3 | 1 | 3 | Subcutaneous | 440 | 440 | 1,320 | 18 | 1 |
| CFU-C | — | 4 | 1 | 4 | Subcutaneous | 440 | 440 | 1,760 | 4.3 | 1 |
| CFU-C | — | 5 | 1 | 5 | Subcutaneous | 440 | 440 | 2,200 | 8.7 | 1 |

Table 5. Summary of experimental results on benzene toxicity to hematopoietic cells.

| Study | Summary |
|-------|---------|
| 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 hr/day 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 important. The doubling time seems to be about 3 days for the early 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 the CFU-S cells of Gill et al. could be twice as resistant as Uyeki et al. 's CFC cells. |
| Green et al. (125) | 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, the data of Green et al. show a strong dose response. |
| | Dose rate: A strong effect of dose rate for 6 hr/day protocol is demonstrated. Dose rate may be more biologically significant than the number of days exposed (when the exposure is for 4 to 6 hr/day). |
| Tunek et al. (26) | Route of intake: These data are for sc injection; toxicity appears greater than for inhalation. |
| | Dose response: Seems to have the same functional shape as data of Green et al. |
| | Dose rate: Shapes of the response versus dose-rate plots are similar to those of Green et al., 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 et al. data in line with the inhalation data. |
| Cronkite et al. (27) | Dose response: Seems consistent with that of Green et al. and Tunek et al., but all doses were given at concentrations of 400 ppm. Data are given as day of assay and proliferation (day, %): 1 day, 12%; 7 days, 40%; 14 days, 42%. Therefore, the doubling time seems to be about 2 days from a survival of 10% and 4 days from a survival of 20%. |
| Cronkite et al. (28) | Dose response: Seems consistent with previous discussions, but dose rates are mostly for 300 ppm. |
| | Doubling time: Seems to be long, probably about 40 days; looks inconsistent with other studies. |
| Cronkite et al. (29) | Doubling time: Seems to be about 2 days from a survival of 10% and 4 days from a survival of 20%; times close to the previous study of Cronkite et al. (27). |
have been exposed to leukemogenic initiating agents before benzene exposure) to undergo compensatory hematopoiesis. Because of existing data and simple, well-established dosimetry models, the quantitative considerations, as described here, 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 Figure 5, 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 stromal and stem cells establish both the radiosensitivity and proliferation kinetics of the cells that are critical to hematopoietic recovery (28, 29). Although that hypothesis is well supported by a large array of stromal cell experiments, it is still contested by some, based on the 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 radioreistant and more slowly repopulating critical cell that is consistent with characteristics measured for marrow stroma and CFU-F type lineages.

Figure 5. Scheme based on consensus 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.

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