American Petroleum Institute

1220 L Street, Northwest
Washington, DC 20005-4070
Tel 202 682-8341
Fax 202 682-8270
e-mail mongillo@API.org

David A. Mongillo
Manager, Health Sciences

January 20, 1998

TO: Benzene Task Force

FROM: David Mongillo

RE: RESEARCH GROUP MEETING

The Benzene Basic Research Group will meet the morning of Friday, February 13 in Houston at the Marriott Hotel following the Benzene dinner meeting the night before. The purpose of the meeting will be to update the on-going benzene research activities and discuss plans for 1998/1999. The meeting will begin at 8 am and last no longer than 3 hours. For planning purposes, please return the attached meeting confirmation sheet.

Two work products have been received recently for review. You should have received Tony Cox's report "A Biomathmatical Model of Hematotoxicity". Included in this mailing is AHF's draft "Evidence for DNA Reactivity of Benzene and Benzene Metabolites".

Please call with any questions.

American Petroleum Institute

1220 L Street, Northwest
Washington, D.C. 20005-4070
202-682-8000

# MEETING CONFIRMATION FAX BACK FORM

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Company: _____
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YES NO Benzene Basic Research Group Meeting
Houston, Texas
February 13, 1998

Please fax back to (202) 682-8270 no later than January 30.

# A BIOMATHEMATICAL MODEL OF HEMATOTOXICITY

Louis Anthony Cox, Jr.

December, 1997

## ABSTRACT

In the past decade, toxicokinetic and physiologically-based pharmacokinetic (PBPK) models have been used extensively to help clarify the dynamic relations between administered doses and biological responses, ranging from acute toxicity to chronic cancer risks. In the 1990s, researchers and modelers have increasingly recognized the need to link PBPK models to pharmacodynamic models, representing the interactions between chemicals and cells, in order to better understand how time patterns of administered dose affect the risks of adverse health outcomes. This paper describes such a model, representing the effects on the hematopoietic (blood-forming) system of myelotoxic agents such as metabolites of cyclophosphamide (CP) or benzene. The model consists of a set of physiological compartments representing hematopoietic progenitor cell, granulocyte-macrophage (GM)-committed stem cells, and more mature blood cells. These compartments are linked by nonlinear feedback control loops and are susceptible to first-order cell-killing kinetics from cytotoxic metabolites. In contrast to earlier biomathematical models of normal hematopoiesis and hematotoxicity, this model has been validated by testing its predictions against experimental and clinical data for blood cell counts following administration of CP to mice, dogs, and humans. It successfully explains apparent anomalies and patterns in previously published data, including the fact that smaller cumulative doses can cause larger hematotoxic responses and that bone marrow toxicity may be disproportionately sensitive to exposure concentration but relatively insensitive to exposure duration. An intriguing prediction from the model is that sustained exposures to sufficiently-small concentrations of myelotoxic agents may tend to provoke a protective response, increasing rather than decreasing the numbers of CFU-GM and early hematopoietic stem cells available to sustain hematopoiesis.

KEY WORDS: Hematopoiesis, myelotoxicity, cyclophosphamide, pharmacodynamics, mathematical modeling, biologically-based risk assessment

 503 Franklin Street, Denver, Colorado, 80218. 303-388-1778. TCoxDenver@aol.com

## INTRODUCTION

Applied quantitative risk assessment seeks to answer questions such as the following. Suppose that a risk manager must choose among risk mitigation options (a)-(d) for protecting workers from a potentially hazardous airborne chemical in a manufacturing plant:

(a) Reduce daily occupational exposure time from 8 hours to 4 hours.
(b) Reduce 8-hour average workplace concentration by 50%.
(c) Reduce weekly exposure from 5 days to 2.5 days.
(d) Reduce yearly exposure from 50 to 25 weeks per year.

Which option is most health-protective? By how much would each be expected to reduce the health risk due to occupational exposures? How well can these questions be answered without further specifying the options, e.g., how the 4 hours in option (a) are distributed within the work day, how the 25 weeks in option (d) are distributed within the year, and so forth? This paper shows how biomathematical, biologically-based risk assessment (BBRA) models can address such questions. Section 1 presents empirical evidence motivating the need for explicit dynamic models of dose-response relations. Using the chemotherapeutic and immunosuppressive drug cyclophosphamide (CP) as a detailed example, Section 2 develops a dynamic dose-response model for predicting and explaining cytotoxic effects of chemicals on bone marrow and blood cell populations. Section 3 presents new results on empirical validation of the model with human clinical data and experimental animal data. Section 4 discusses potential implications for risk assessment of chemical leukemogens.

## 1. WHY DYNAMIC DOSE-RESPONSE MODELS?

The need for explicit dynamic models in quantifying dose-response relations is often implicitly denied in applied risk assessments. Instead, "dose metrics", such as cumulative exposure or cumulative dose per unit body weight or per unit surface area, are commonly used to equate the risks of very different exposure histories that map to the same value of the dose metric. This principle has been used to extrapolate risks from high to low doses and from one species

and strain to others. Uncertainties about the validity of the extrapolation are often equated to uncertainty about the most appropriate choice of dose metric. In conjunction with another widely used assumption – that excess risk at sufficiently low doses increases in approximate proportion to the dose metric – this dose metric approach provides a simple, clear method for assessing the risk consequences of risk management options such as (a) - (d). For example, these principles imply that all four options reduce health risk equally at small concentrations. Different practitioners might debate which specific parametric risk model is most appropriate, e.g., logistic regression, probit, proportional hazards, linear relative risk, linear absolute risk, and so forth. Yet, the rank-ordering of risk management options and estimates of their relative quantitative efficiencies in reducing risk do not depend on such model differences. They are fully determined by the assumptions of a dose metric and approximate low-dose linearity. This may create an apparent consistency and robustness in the results of different specific parametric risk models, including all of those just listed, that is traceable to their common underlying methodological approach.

To motivate a more complicated BBRA modeling approach, this section reviews data suggesting that the dose metric strategy of mapping time patterns of exposures to single numbers before evaluating their risks is ultimately unsatisfactory. Despite its simplicity and robustness, the framework is inconsistent with experimental data that permit it to be tested, for some chemicals of practical interest. Specifically, stop-exposure experiments, in which different groups of animals are exposed to chemical carcinogens for different amounts of time, reveal results that seem to contradict the most basic tenets of applied risk assessments based on dose metrics. For example:

- Smaller cumulative doses can produce larger toxic and carcinogenic responses (e.g., Luke *et al.,* 1988a; Cox *et al.,* 1996).

- Relatively small increases in concentration can dramatically increase tumor incidence (e.g., Williams, 1993 for hepatocarcinogenesis; Melnick *et al.,* 1990 for butadiene-induced lymphomas).

- Extending durations of exposure may have little or no impact on tumor risks (Cox *et al.,* 1996 for isoprene-induced tumors at several anatomic sites).

- Sufficiently low exposure concentrations can have disproportionately small impacts, or even appear to have beneficial effects, prompting some investigators to speculate about biological hormesis (e.g., David and Srendsgaard, 1990).

Table 1 illustrates some of these phenomena for isoprene (Cox *et al.,* 1996). The following patterns are notable.

- Doubling concentration (from 70 ppm to 140 ppm between exposure groups 3 and 5) increases liver adenomas from 0.29 to 0.44. But, doubling weeks of exposure (from 40 to 80 between exposure groups 3 and 4) does not increase risk significantly at any site, and even appears to reduce it. This observation is not explained by competing risks of death from toxicity, as animals tolerated these concentrations. Nor is it explained by saturation of carcinogenic response mechanisms, as higher concentrations produce much higher tumor yields. Thus, no *dose-response theory that predicts that risk increases with cumulative exposure provides a useful model for these data.*

- Quadrupling exposure concentration from 70 ppm to 280 ppm while quartering exposure duration from 80 weeks to 20 weeks unambiguously increases tumor risk (compare exposure groups 4 and 6), even though cumulative exposures are identical. Thus, *no risk model that uses cumulative exposure (area under curve, AUC) as a dose metric adequately describes these data.*

- At higher concentrations, doubling hours-per-day of exposure while halving weeks of exposure increases the risk of adenomas and liver carcinomas (compare exposure groups 10 and 11), even though cumulative exposures are identical.

- Liver and lung tumors (both adenomas and carcinomas) are only significantly elevated at concentrations above 70 ppm. In this data set, 140 ppm is the smallest concentration for which significant increases were observed.

Such observations challenge any dose-response model that uses a dose metric in which risk increases monotonically and/or symmetrically with exposure concentration and duration.

TABLE 1: RESULTS OF A STOP-EXPOSURE EXPERIMENT FOR ISOPRENE IN MALE B6C3F1 MICE

| Group | ppm | weeks | hr/day | Liver adenomas | Lung adenomas | Other adenomas | Liver carcinomas | Lung carcinomas | Histio-sarcomas |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 8 | 0.22 | 0.22 | 0.14 | 0.18 | 0 | 0 |
| 2 | 10 | 80 | 8 | 0.24 | 0.32 | 0.12 | 0.12 | 0.02 | 0.04 |
| 3 | 70 | 40 | 8 | 0.29 | 0.16 | **0.30*** | 0.22 | 0 | 0.04 |
| 4 | 70 | 80 | 8 | 0.30 | 0.08 | 0.18 | 0.18 | 0.04 | 0.04 |
| 5 | 140 | 40 | 8 | **0.44*** | 0.20 | **0.28*** | 0.20 | 0.02 | 0.02 |
| 6 | 280 | 20 | 8 | 0.36 | 0.32 | **0.36*** | 0.24 | 0.06 | **0.16*** |
| 7 | 2200 | 80 | 4 | **0.42*** | 0.30 | **0.56*** | 0.30 | 0.06 | **0.14*** |
| 8 | 2200 | 40 | 8 | **0.57*** | **0.59*** | **0.65*** | **0.37*** | 0.06 | **0.14*** |

*Explanation:* Columns 2-4 summarize the exposure factors defining each dose group. The remaining columns show the fraction of animals in each dose group that were found to have each tumor type at necropsy.

* Tumor incidence rates in bold and marked with an asterisk are significantly greater than in the control group ($p < 0.05$ by Fisher's Exact Test).

Stop-exposure experiments for health end-points other than cancer show similar patterns. Genotoxic, cytogenetic, and cytotoxic responses are often sensitive to the time pattern of dose administration, rather than only to the AUC of administered dose (or of metabolites formed. For example, Table 2 summarizes experimental results for benzene, a relatively well-studied human leukemogen and animal carcinogen, for endpoints other than (and perhaps causally prior to) cancer. Benzene metabolites such as phenol, hydroquinone (HQ), and catechol synergize strongly in producing both cytogenetic and cytotoxic damage. Since carcinogenesis is often postulated to arise from the combination of genotoxic and cytotoxic effects (e.g., Farris *et al.*, 1997 for benzene; Williams *et al.*, 1993, for the hepatocarcinogen DEN, Monticello and Morgan, 1994 for formaldehyde), it is not surprising that chemicals with strongly nonlinear dose-response patterns for genotoxic and cytotoxic effects may exhibit similar nonlinear patterns for tumors.

**TABLE 2: RESULTS ON BENZENE HEMATOTOXICITY AND GENOTOXICITY**

| END POINT | RESULT | REFERENCE |
|---|---|---|
| Hematotoxicity in mouse bone marrow, measured by a variety of indicators | 10 ppm for 8 weeks has no hematotoxic effect but 100 ppm for 1 week has a marked hematotoxic effect. | Farris *et al.*, 1997 |
| Hematotoxicity in mouse bone marrow, indicated by changes in CFU-GM and CFU-S cell populations | 10 ppm x 10 weeks has no myelotoxic effect in mice, but 100 ppm x 1 week greatly suppresses CFU-GM and CFU-S | Green *et al.*, 1981 |
| Hematotoxicity in mouse bone marrow , measured by reduction in CFU-GM per tibia bone marrow | 21 ppm of benzene x 6 days (= 3,024 ppm-hours) had a *smaller* hematotoxic effect than 50 ppm x 2 days (2,400 ppm-hours). | Toft *et al.*, 1982 |
| Hematotoxicity in mouse bone marrow , measured by reduction in CFU-GM per tibia bone marrow | 95 ppm of benzene x 2 hr/day, 5 days/week x 2 weeks has little hematotoxic effect. Yet, 96 hours of continuous exposure to 21 ppm produces severe hematotoxicity. (Both are about 2000 ppm-hours) | Toft *et al.*, 1982 |
| Hematotoxicity in mouse bone marrow, measured by erythropoiesis | Benzene metabolites (HQ and phenol) synergize strongly in suppressing murine erythropoiesis | Chen and Eastmond, 1994, 1995 |
| Genotoxicity in mouse bone marrow erythrocytes, measured by MN-PCE. | HQ and phenol synergize in creating micronuclei (MN) in erythrocytes | Chen and Eastmond, 1995 |
| Genotoxicity in human lymphocytes in vitro, measured by clastogenicity. | HQ and catechol synergize strongly (7 x additive effect) in creating genotoxic damage in cultured human lymphocytes | Robertson, Eastmond, and Smith, 1991 |
| Genotoxicity in mouse bone marrow erythrocytes, measured by MN-PCE. | MN-PCE response is insensitive to duration of exposure | Toft et al., 1982; Luke et al., 1988 |
| Genotoxicity in mouse bone marrow *in vivo*, measured by chromosomal aberrations | Doubling administered dose from 440 to 880 mg/kg increases chromosomal aberrations 8-fold. | Ciranni *et al.*, 1991 |

These examples demonstrate the need for dose-response models that do not satisfy the usual assumption that risk is an increasing function of a dose metric that combines exposure concentration and duration in some simple, monotonic, and perhaps symmetric way. The following sections investigate the potential of BBRA models, which attempt to simulate key dynamic aspects of adverse health effects, to obtain more realistic and useful predictions.

## 2. METHODS

To assess how well BBRA models can predict and explain phenomena such as those in Tables 1 and 2, we undertook the following three steps:

a) Construct a dynamic simulation BBRA model of pharmacokinetics and cytotoxicity / cell kinetics for a well-studied leukemogen (CP). Mathematical models of pharmacokinetics and metabolism, hematopoiesis (blood formation), and hematotoxicity and myelotoxicity (blood-poisoning and marrow-poisoning) were based on previously published data and modeling efforts for CP, as described below. They were combined and implemented in a continuous simulation model using the ITHINK™ modeling environment.

b) Validate the model's predictions with data from several species and agents. Model parameters estimated primarily from canine data were used successfully to predict observed dynamic responses in humans, dogs, and mice exposed to CP. Interspecies extrapolations were made by adjusting only two scaling parameters – a time scaling factor and a dose scaling factor – to account for differences in body weight and metabolism. Similar work has recently validated similar model predictions for effects of radiation on human and canine hematopoiesis (Fliedner *et al.*, 1996; Tibken *et al.*, 1995).

c) Apply the validated model to explain and predict the dynamic responses of specific cell populations to different time patterns of dosing with hematotoxic agents. Dynamic responses were simulated for specific hematopoietic progenitor cell populations (e.g., early CFU-GM) thought to be involved in chemically induced myeloid leukemogenesis (Irons and Stillman, 1996).

The methods used to carry out each step are described next.

### 2.1 STRUCTURE OF THE BBRA MODEL

The biomathematical model of hematotoxicity is described in Cox (1996), which gives detailed model equations in an appendix. It is based largely on the model of Steinbach *et al.* (1980) with some simplifications and improved CP

pharmacokinetics based on experimental data (Sladek, 1988). In summary, it consists of the following components:

1. A *linear compartmental flow model* (Jacquez, 1997) describing the pharmacokinetics and metabolism of CP in humans. The model structure and flow rate parameter values for CP are based on measurements in human patients (Cohen *et al.*, 1971; Sladek, 1988).

2. A *nonlinear compartmental flow model* with feedback-control loops, describing the myelotoxicity and hematotoxicity of CP. This model has the following six main sequential compartments, describing the granulocyte-macrophage (GM) lineage of the hematopoietic system:
    - Early stem cells and hematopoietic progenitor cells (HPCs)
    - Granulopoietic committed stem cells (e.g., CFU-GM)
    - Proliferative cells (myeloblasts, promyelocytes, myelocytes)
    - Maturation pool
    - Bone marrow reserve
    - Peripheral blood granulocytes

Partial differential equations (PDEs) describe the dynamics of the age-structured CFU-GM cell populations. These PDEs were approximated numerically by systems of ordinary differential equations ODEs (10 serial subcompartments), to make the mean and variance of transit times conform to experimentally observed values in dogs (Steinbach *et al.*, 1980).

Nonlinear feedback loops, implicitly modeling the regulatory effects of the cytokine network, feed back information (interpreted as strengths of regulatory signals) from blood cell population sizes to control the following rate parameters:

- Fraction of early HPCs that differentiate instead of self-renewing;
- Rate of recruitment of resting stem cells into active cycling;
- Birth rates of CFU-GM and downstream proliferative cells;
- Release rate of mature granulocytes from the bone marrow reserve to the peripheral blood.

These feedback control laws were described by smooth curves constructed to pass through experimental data points. Typically, the experimental data

consisted of extreme values (e.g., maximal and minimal cell division rates observed under a variety of experimental conditions) with some intermediate data-points. An s-shaped function would then be used to interpolate among these observed values, with its parameters being estimated from experimental data by least squares. Most of the parameter values for the CP cytotoxicity and cell kinetics component were taken from previously published estimates based on a model of granulopoiesis in dogs (Steinbach *et al.*, 1980); see Cox (1996), for details and parameter values.

The combined CP pharmacokinetics and hematotoxicity model is a continuous simulation model of the standard form (van der Bosch and van der Klauw, 1994):

$$d\mathbf{x}(t)/dt = \mathbf{f}[\mathbf{x}(t), \mathbf{b}(t)]$$
$$\mathbf{b}(t) = \mathbf{g}[\mathbf{x}(t)]$$

where $\mathbf{x}(t)$ is a state vector with components describing (a) the quantity of CP and its metabolites in each compartment of the pharmacokinetic model and (b) the number of cells in each compartment of the cell kinetics and cytotoxicity model at time t. $\mathbf{b}(t)$ is a vector of rate parameters. Its components are constants for the pharmacokinetic model and are functions of the cell population sizes for the cell kinetics and cytotoxicity model. $\mathbf{b}(t)$ also includes cytotoxic parameters describing the rate of cell-killing of cycling HPCs and CFU-GM cells as a function of the concentration of toxic CP metabolites (specifically, phosphoramide mustard) in these marrow cell populations. The feedback laws determining $\mathbf{b}(t)$ from $\mathbf{x}(t)$ are symbolized by the vector function $\mathbf{g}$ and are detailed in Cox (1996). Initial values for cell population sizes were taken from published experimental data, as in Steinbach *et al.* (1980), and starting values for CP in different compartments were assumed to be zero. Given the initial conditions $\mathbf{x}(0)$ and any specified dosing history, the above simulation model determines the resulting histories of CP and metabolite concentrations and fluctuations in hematopoietic cell populations. They may be solved for by numerical integration of the simulation model equations.

## 2.2 MODEL VALIDATION METHODS

Empirical validation of the BBRA hematotoxicity model for CP consisted of the following two major efforts:

- *Validation in humans*: Using the model formulated above for dogs and a simple adjustment for interspecies dose conversion, predict the effects of repeated CP treatments on humans and compare the model predictions to published clinical data to assess its predictive validity for humans.

- *Validation in mice*: Using the above model and the same simple adjustment for interspecies dose conversion, predict the effects on hematopoiesis (CFU-GM and peripheral granulocytes) of single- and multiple injections of CP in mice. Then, test the predictions by carrying out the single- and multiple-injection experiments in mice.

### INTERSPECIES DOSE-RESPONSE CONVERSION

Since humans have a larger body weight than dogs, their biological clocks are expected to run more slowly. The most parsimonious way of adjusting for this effect is to rescale the time axis, multiplying it by a constant greater than 1 to slow down predicted canine hematotoxic responses (e.g., decreases or increases in cell population sizes) to predict the timing of corresponding human responses. Rather than using allometric arguments to estimate the scaling factor, which would introduce uncertainties about what power of body weights to use, it is simpler and more reliable to estimate it directly by comparing the results of analogous experiments in dogs and humans. Comparing the time course of peripheral white blood cells (WBCs) in canine model simulations following a single injection of CP (Steinbach *et al.*, 1980) to the time course reported for humans (Coggins *et al.*, 1960) showed that the nadir occurs at approximately 11 days in humans compared to approximately 7.5 days in dogs. Thus, predictions from the canine model are scaled by a factor of 11/7.5 = 1.47 along the time axis to obtain predictions for humans. The pharmacokinetics submodel is already developed from human data, and hence requires no rescaling. Also, rescaling its relatively fast dynamics would make little or no difference to the slower CP-hematotoxicity submodel for most dosing scenarios.

For mice, a contraction of the time axis must be made. Comparing experimental data on murine hematotoxic responses to CP injections (DeWys *et al.*, 1970) to human clinical data (Buckner *et al.*, 1972; Nissen-Meyer and Host, 1960) suggested that the time scale for hematopoietic responses in mice should be contracted by a factor of about 0.4 compared to humans. This estimated adjustment factor was used in making all predictions for mice.

Mice have a higher ratio of surface area-to-volume than do dogs or humans. It is therefore to be expected that the number of mg/kg required to produce a given dynamic response in mice (e.g., a given depth of the nadir as a percentage of the normal level) will be different from the number of mg/kg required to produce the same response in humans. The required dose-scaling factor was estimated to be about 5, based on the observation that mouse responses to about 300 mg/kg appear to correspond roughly to human responses to 60 mg/kg, appropriately speeded up. However, responses within each species to all sufficiently high single doses are similar enough to each other so that the exact correspondence between mouse and human dose scales is hard to identify from previously published data. Similarly, although it might in principle be desirable to account for differences between dogs and humans in the hematotoxic potency of CP, such adjustments make little practical difference in predicting human hematotoxic responses to the high levels of CP used in clinical trials. The hematotoxic response is approximately saturated, making detailed adjustments unnecessary.

## MODEL VALIDATION TESTS

The complete CP model contains over 120 individual equations and formulas and over 20 parameter values (all with values fixed by experimental data). Thus, it would be impractical to attempt to validate its assumptions and implications by direct inspection. Instead, the following validation tests were performed.

*Testing face validity and internal consistency* of the model. The model was checked by introducing deliberate errors into its equations (e.g., using incorrect formulas or parameter values) and confirming that the resulting outputs became unrealistic, e.g., unstable in the absence of any external perturbation from dosing.

Such experiments revealed that the self-consistent dynamic behaviors exhibited by the model (specifically, homeostasis and a stable steady state) do not survive the introduction of obvious errors, increasing confidence that such errors have not been made.

*Descriptive validity* of the BBRA model was established by checking that its predictions for HPC stem cells in bone marrow and for WBC counts after an injection of 15 mg/kg CP agree with experimental observations on dogs (Steinbach *et al.*, 1980). These same experimental data points had been used to estimate the model parameters, however, so this check only confirms the descriptive validity of the model, i.e., its ability to explain a large number (over 70) data points with a much smaller number of parameters. It does not prove that other parameter values would not fit the same experimental data equally well but make different predictions outside the range of observed experimental conditions.

*Predictive validity* of the CP model was first assessed using human data. As described in the following section, the predictive validity of the human model was tested by simulating the outcomes of clinical experiments previously reported in the literature (Buckner *et al.*, 1972; Nissen-Meyer and Host, 1960) and comparing the model-predicted time courses of blood cell counts to corresponding clinically observed values. The human data sets and clinical trials used to test the model are quite different from the animal experiment data sets used to build it, thus providing a fair test of its ability to make useful predictions across these species and experimental conditions.

The model's predictive validity was further tested through new experiments in male B6C3F1 mice, conducted at the University of Colorado Medical School in Denver by Drs. R. Irons and W. Stillman. Table 3 summarizes the experimental design used. Five mice were sacrificed at each "x" and 50 mg/kg of CP was administered via i.p. injection into surviving mice at each "D". Rows 1-3 represent three treatment groups, receiving one, two, and three doses of CP, respectively, spaced 48 hours apart. Row 0 is the control group. This design was constructed based on simulation model results, which predicted large, testable differences in CFU-GM population sizes on different days for these three dose regimens.

TABLE 3: DESIGN OF BBRA MODEL VALIDATION EXPERIMENT IN MICE

| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | x | | x | | | x | x | x | x | x | | | x |
| 1 | D | x | x | x | | | | | x | | | | | |
| 2 | D | | D, x | x | | | x | x | | x | x | | | x |
| 3 | D | | D | | | | D, x | x | x | x | x | | | x |

D = administration of dose (50 mg/kg via i.p. injection)
X = sacrifice of animals

A pilot experiment consisting of a single injection on day 1 and a one-week follow-up with sacrifices on days 2, 4, and 7 confirmed that mice could tolerate 50 mg/kg and that CFU-GM and WBC counts looked approximately as predicted on these three days. Following sacrifice by cervical dislocation, mouse bone marrow was extracted, plated, and assayed for CFU-GM stem cells using a colony-forming assay .

## 2.3 MODEL APPLICATIONS

Following the validation tests, the BBRA hematotoxicity model was applied to several different dose scenarios to determine whether it could help to explain the phenomena in Tables 1 and 2. Scenarios examined included:

1. Stop-exposure experiments, similar to those for isoprene and benzene in Tables 1 and 2, that allocate the same total administered dose according to different time patterns to determine which create the largest predicted hematotoxic effects on CFU-GM populations.

2. Continuous infusion experiments, intended to create internal dose profiles similar to those that might arise from sustained low-level exposures to an air-borne leukemogen such as benzene.

The model-based predictions for these dosing scenarios were used to draw inferences about dose-response relations that may help to explain some of the qualitative patterns noted in Tables 1 and 2 for other chemicals and end-points.

# 3. RESULTS

This section first presents the results of the model validation experiments, since validation should be an essential prerequisite for using models to explore substantive research issues. The validation tests generally support the predictive value of the model. Next, the model is applied to various dosing scenarios to identify its implications for the dynamic properties of hematotoxicity dose-response relations.

## 3.1 RESULTS OF MODEL VALIDATIONS FOR HUMANS

Buckner *et al.*, (1972) report clinical data for nine human cancer patients administered 60 mg/kg of CP each via infusion, and for seven patients each given 120 mg/kg CP as two 60-mg/kg infusions spaced one day apart. The CP model was used to predict the time courses of WBCs for these groups of patients. Figure 1 shows the model predictions (top panel) and the observed data (bottom panel) for both groups. Figure 2 shows a similar comparison of predictions with clinical data from an earlier study (Nissen-Meyer and Host, 1960) in which patients were administered doses of 60 mg/kg of CP on four consecutive days.

Figure 1 shows that the simulation results from the model provide a useful approximation to the empirically observed results. In both the simulation and the clinical data, the WBC time courses are not significantly different between the 60 mg/kg and the 120-mg/kg groups for the first week following exposure. They begin to separate in the second week, with severe leukopenia persisting for over 4 days in the 120 mg/kg group before noticeable recovery begins, compared to an earlier recovery in the 60 mg/kg group. This basic match between predictions and observations is reassuring, especially given the high interindividual variability indicated by the range bars in the experimental data. Figure 2 similarly shows that model predictions (top panel) approximately match observed data (bottom panel) throughout the duration of observations for a clinical trial in which CP was administered on four consecutive days (Nissen-Meyer and Host, 1960). Again, given the approximations made in the CP hematotoxicity model (e.g., in scaling

the dose and time axes from dogs to humans), the match between model predictions and clinical observations is encouraging.

### 3.2 RESULTS OF BBRA MODEL-VALIDATION EXPERIMENTS IN MICE

Figures 3a and 3b present the main results of the validation experiments in mice. Figure 3a shows predicted vs. actual results for CFU-GM time courses in the single-injection pilot experiment. (Peripheral granulocyte-macrophage counts were also predicted, and provide even better matches to observed values than CFU-GM. Since the bone marrow CFU-GM responses are both more volatile and hence harder to predict, and also more relevant to leukemia induction, they are the focus of this summary.) Similar to the human data, the fit between predicted and observed values, while not perfect, is close enough to be useful in understanding the approximate magnitude and timing of changes in cell counts over time. Figure 3b shows the outcome of the multiple-injection experiment described in Table 3. The three panels, a, b, and c, show the observed (squares) and predicted (circles) average values of CFU-GM for mice sacrificed on different days in each of the three treatment groups. (Straight-line extrapolations and interpolations are sketched among the data points. In the middle panel, for dose group 2, these segments are not connected for days 5 and 6 because their locations are uncertain.) Mean values of CFU-GM counts among all animal sacrificed in each treatment group on each sacrifice day are shown. All plotted values are expressed relative to the control group values, i.e., 1 is the no-effect level.

There is enough experimental variability between replicates so that an exact match between predicted and observed values would not be expected in any single experiment. For example, the left-most panel of Figure 3b repeats the single-injection dose regimen in Figure 3a, yet produces a different time course of observed relative CFU-GM counts in bone marrow. However, the predicted time courses are similar to the observed ones in key respects of the approximate magnitudes and timings of changes in CFU-GM counts. The largest discrepancies (e.g., at the left ends of the curves in Figure 3b) can be explained by noting that the CFU-GM compartment is predicted to be highly volatile over this time interval, with population sizes changing dramatically from hour to hour. Indeed, the experiment design in Table 3 was chosen to produce just such,

large, fast variations so that they could easily be detected. The rough agreement between predicted and observed values in terms of magnitude, direction of change, and approximate timing of peaks and of recovery (starting to return to pre-exposure levels) is reassuring, despite the experimental variability.

### 3.3 RESULTS OF MODEL APPLICATIONS

The model validation results summarized in Figures 1-3 suggest that the BBRA model of hematotoxicity provides useful predictions in novel situations, e.g., for humans and for multiple-dose experiments. Confidence in its predictions is further bolstered by independent investigations reported by other researchers (Fliedner *et al.,* 1996; Tibken and Hofer, 1995), who also used a modified version of the Steinbach *et al.* (1980) model to predict CFU-GM responses in dogs and humans exposed to radiation. That model, which is similar to ours, also produced predictions that match observations fairly closely. These results and the new model validation experiments reported above provide empirical support for applying the BBRA model to predict CFU-GM and peripheral granulocyte-macrophage responses to situations for which data are not yet available.

Figures 4 through 6 show results from three sets of simulation experiments performed using the CP model for humans. The first set (Figure 4) consists of infusions of CP representing alternative concentration-duration-frequency combinations with the same product (total AUC of administered CP) but with different time patterns of administration (AUCs per unit time). The second group (Figure 5) examines the predicted effects of different durations of exposure to a constant low concentration. (The concentration for the 480-hour dosing scenario is higher in Figure 5 than in Figure 4.) The third set (Figure 6) shows simulated responses to long-term infusions of different low concentrations of CP. Such simulation experiments allow detailed examination of the predicted effects of different combinations of CP concentration and timing on stem cell dynamics. Early CFU-GM cells, i.e., those in the first of the ten subcompartments in the proliferative portion of the model, are examined in the top panel of each of Figures 4 through 6, as this is a plausible starting location for the events leading to AML (Irons and Stillman, 1996). The bottom panels show corresponding predictions for peripheral granulocyte-macrophage time courses, which are easier and less expensive to observe experimentally.

# 4. DISCUSSION AND CONCLUSIONS

## DISCUSSION OF MODEL PREDICTIONS

Figure 4 shows predicted time courses of cell population sizes when the same total dose of CP is administered over three very different time intervals: 8, 120, and 480 hours. Since administered concentrations are inversely proportional to dosing durations, the AUC per day is far higher in the 8-hour dosing regimen than in the 480-hour regimen. The top panel in Figure 4 shows the effects on the earliest of the CFU-GM compartments. The 8-hour administration leads to a profound initial depression in CFU-GM cells, followed by a rapid rebound and overshoot as a result of over-compensating proliferation. It is plausible that such a pattern may create a higher risk of leukemia than the other dosing scenarios. The reasoning is that the deep nadir is typically accompanied by a premature transition into active cycling of normally GM-CSF-nonresponsive stem cells (Irons and Stillman, 1996; Irons *et al.*, 1992). The immediately following proliferation may therefore amplify a stem cell population that has just been enriched with stem cells that are especially susceptible to malignant transformation to acute myeloid leukemia (or its predecessor, myelodysplastic syndrome, MDS) (List and Jacobs, 1992). Such cells will not have had a chance to undergo a long period of detection and repair or elimination (e.g., via apoptosis) of somatically heritable damage, thus maximizing the potential for leukemic transformation.

Figures 5 and 6 may be interpreted similarly. In Figure 5, extending the duration of dosing while keeping the dose rate constant increases the predicted magnitude of post-exposure proliferation much less than proportionally to the duration. Instead, partial compensation occurs during dosing, with the number of early CFU-GM cells approaching a stressed-equilibrium level that is somewhat higher than normal. The nadir is not very low (i.e., the normal stem cell population is not much depleted) and the recovery period is comparatively lengthy. Thus, the post-dosing proliferation may be expected to occur in a population that has not been as greatly enriched with at-risk stem cells as in the first scenario of Figure 4. Indeed, if repair or elimination of inappropriately recruited stem cells and their progeny takes place at a fast enough rate, then the

240-hour exposure (leading to a zenith of proliferation about two weeks after dosing begins) might plausibly be more risky than the 480-hour exposure.

Finally, the simulation experiments in Figure 6 predict that various characteristics of the early CFU-GM respond non-monotonically to CP concentration in simulations of sustained infusions. (Other simulation experiments confirm this result for shorter-term experiments in which two brief infusions are separated by different amounts of time.) The dynamics in Figure 6 suggest that the relation between concentration and risk may be more complex than the peripheral blood response in the lower panel suggests. Specifically, suppose that sustained depression of CFU-GM stem cells below their normal equilibrium level leads to an ongoing recruitment of less mature upstream stem cells (at least in the presence of leukemogenic metabolites). Then the qualitative response patterns in the top panel of Figure 6 suggests that higher concentrations lead to higher risks for administered concentrations above a certain threshold (as indicated by the transition from curve 4 to curve 5). Below that critical concentration, however, increases in administered concentration simply lead to a larger normal stem cell population, without recruitment of less mature stem cells.

In summary, the main lessons from Figures 4 through 6 are as follows:

1. The simulation model predicts that *the same total dose of CP administered over 8 hours instead of 120 or 480 hours creates a larger hematotoxic effect* -- and perhaps to a larger leukemia risk, if the preceding speculations are accurate.

2. *Doubling the duration of exposure (e.g., from 240 hours to 480 hours in Figure 5) less than doubles the simulated hematotoxic response* (and any associated risk of leukemia).

3. *Hematotoxic response (e.g., as indicated by height of zenith) is not a simple monotonic function of concentration* (see Figure 6).

If other organ systems and stem cell populations undergo similar feedback control processes in responding to chemical carcinogens, then similar qualitative characteristics might be expected for the dynamic dose-response relations.

Table 4 summarizes recent findings for several chemicals that suggest the following common pattern: When cytotoxicity-mediated cell proliferation plays an essential role in experimentally observed carcinogenesis, the dose-response pattern is typically non-linear at low doses and may exhibit hormesis. The BBRA model described in this paper offers a detailed explanation/prediction of this pattern in terms of the predicted dynamics of bone marrow stem cell (CFU-GM) responses to myelotoxic agents such as CP.

**TABLE 4: RECENT EVIDENCE ON CYTOTOXICITY AND DOSE-RESPONSE RELATIONS FOR SEVERAL CHEMICALS**

| CHEMICAL | MAIN RESULT | CONJECTURED MECHANISM | COMMENTS | REFERENCE |
|---|---|---|---|---|
| Benzene | "Excess risk due to benzene exposure may be nonexistent or negative at sufficiently low doses." PBPK and hematotoxic models both support this **nonlinear** pattern. | Benzene exposure may increase s-MDS/leukemia risk by cytotoxic action on HPC and normal stem cell populations followed by **compensating proliferation.** | A simulation model predicts that at low concentrations, benzene increases stem cell counts in the bone marrow, instead of reducing them. | Cox, 1996. See also Farris *et al.*, 1997 and Cronkite *et al.*, 1989. |
| Caffeic acid | Forestomach and kidney hyperplasia in rats and mice are **nonlinear** ("J-shaped or U-shaped") functions of dietary concentrations. | **Hyperplasia** is considered the probable cause of cancer in the two target organs (forestomach and kidney). | | Lutz *et al.*,1997 |
| Chloroform | "The dose-response relationship for chloroform-induced tumors in rats and mice is **nonlinear**" (J- or U-shaped) | Cytotoxicity leading to **regenerative cell proliferation** is "the likely mode of action for the carcinogenic effects of chloroform". | Chloroform lacks clear direct *in vivo* or *in vitro* genotoxicity. | Golden *et al.*, 1997. See also Butterworth *et al.*, 1994. |
| Diesel Exhaust | The dose-response relation for DE-induced lung tumors is **highly nonlinear** (with an apparent threshold or very steep J or U shape). | Lung tumors in rats occur only if exposures "overburden" the lung, causing repetitive irritation/injury and **compensating cell proliferation.** | | Valberg and Watson, 1996 |
| Formaldehyde | "Formaldehyde induces **nonlinear,** concentration-dependent... cell proliferation and DNA-protein cross-link formation." (Monticello and Morgan, 1994) | "The nonlinearity observed in formaldehyde-induced rodent nasal cancer is consistent with a high-concentration effect of **regenerative cell proliferation** ..." | "The concentration-dependent increases in cell proliferation correlated strongly with the tumor response curve" | Monticello and Morgan, 1994. See also Monticello *et al.*, 1996 and Lutz, 1991. |
| TCE | "The mechanism of carcinogenesis of TCE... is **nonlinear**: very high doses, sufficient to cause cellular necrosis, are necessary." | "Malignancy arises from repeated cycles of necrosis and **regeneration with the ultimate emergence of hyperplasia** and then neoplasia." | | Steinberg and DeSesso, 1993. See also Clewell *et al.*, 1995. |

## CONCLUSIONS

The qualitative predictions about leukemia risks suggested for Figures 4 through 6 will remain no more than informed speculations until explicit modeling and empirical validation show whether they are correct. Yet, the time patterns of hematopoietic responses generated by the CP model predict several phenomena that are inconsistent with simpler dose-response models but that occur in practice. These include

- Responses that increase less than proportionally when exposure durations are increased while holding administered concentration constant.

- Nonmonotonic relations between concentration and response, suggesting that the relation between concentration and response may be different at very low concentrations than it is at higher concentrations.

- Response patterns that depend not only on the total dose (AUC of administered dose), but also on how the it is administered over time. Specifically, reducing exposure duration while proportionally adjusting exposure concentration can lead to larger responses (deeper nadir, higher and quicker zenith) for the same total administered doses.

These simulated phenomena are consistent with some of the complexities observed in real dose-response patterns from stop-exposure experiments, as mentioned in the introduction. The results in Figures 4 through 6 suggest possible explanations for the anomalies in terms of nonlinear stem cell dynamics in response to cytotoxic stress. Although the CP model is specific to hematopoietic cell populations and leukemogenesis, it is tempting to speculate that similar nonlinear dynamics and feedback control loops may help to explain the observed anomalies in other experimental systems, such as those in Table 4.

In addition to predicting specific response patterns, the CP model provides potentially useful qualitative insights into the dynamics of hematopoietic responses. For example, simulation experiments in which two doses are separated by different amounts of time show that the hematopoietic system

response smoothes rapid transients in exposure inputs. In other words, it responds slowly enough (on a time scale of days to weeks) so that closely spaced doses (on a time scale of hours to days) create essentially the same impact as a single combined dose. Thus, for example, if it is desired to constrain dosing so that both the depth of the initial nadir of WBCs and the height of the subsequent peak are minimized, then increasing the duration of the recovery period between the two successive doses from 1 day to 4 days would make relatively little difference. Allowing a week or 10 days instead of 4 days between successive doses would make a relatively large difference.

In summary, the type of dynamic dose-response model developed here for CP may be useful in designing exposure regulations and guidelines that are more truly protective of human health than are standards based on AUC or, even more simplistically, on concentration. Guidelines that take into account the importance of exposure timing might be less burdensome than some current standards that assume (incorrectly, if the simulation experiments reported in Figure 4 are an accurate guide) that long-duration, low-concentration exposure scenarios are "equivalent" in risk to brief, high-concentration exposures. The dynamic simulation approach described in this paper can help to quantify the extent to which realistic dynamic dose-response patterns are likely to differ from those predicted by simpler dose-metric models.

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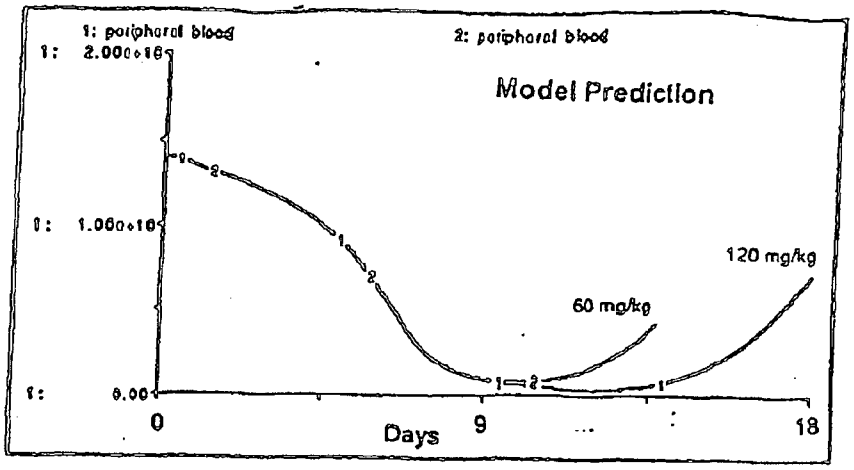
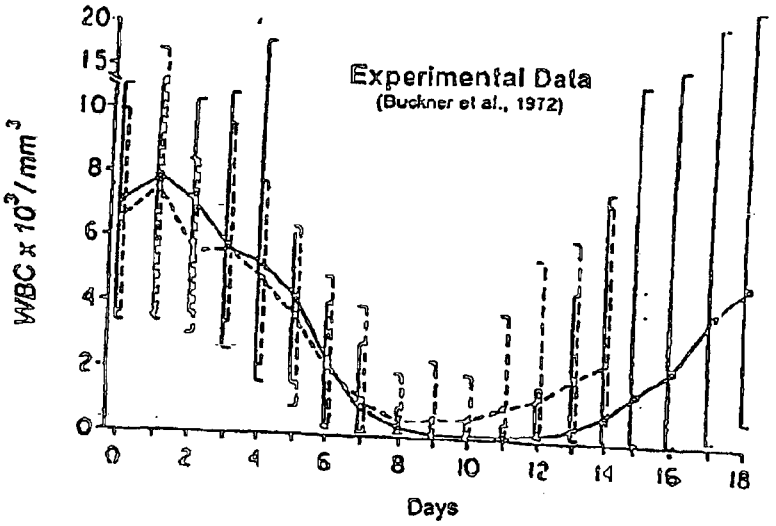
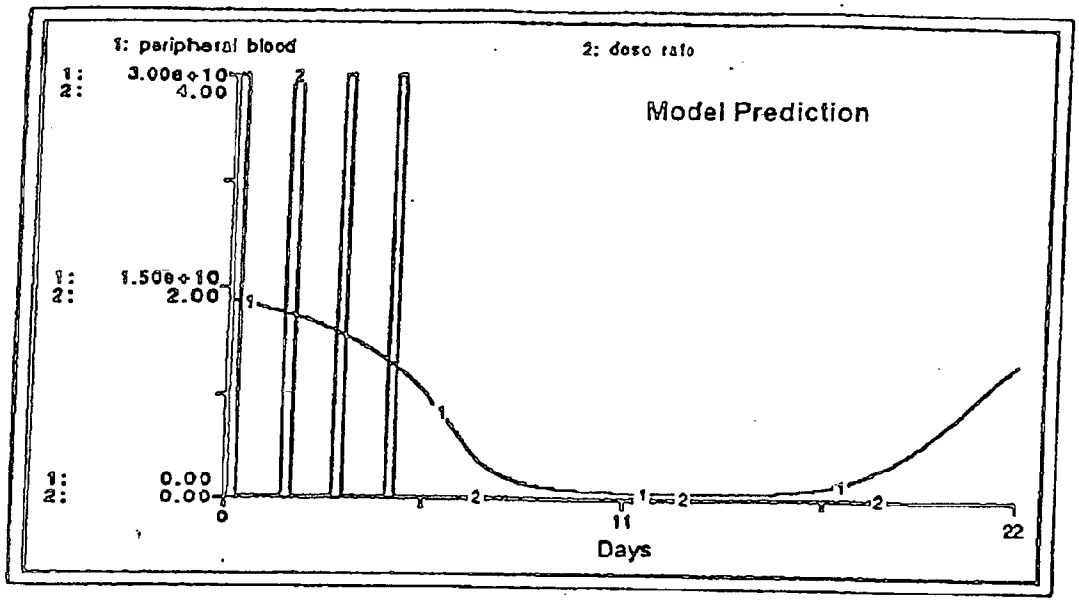
FIGURE 1: MODEL PREDICTIONS *vs.* CLINICAL OBSERVATIONS FOR TWO ADMINISTERED DOSE HISTORIES

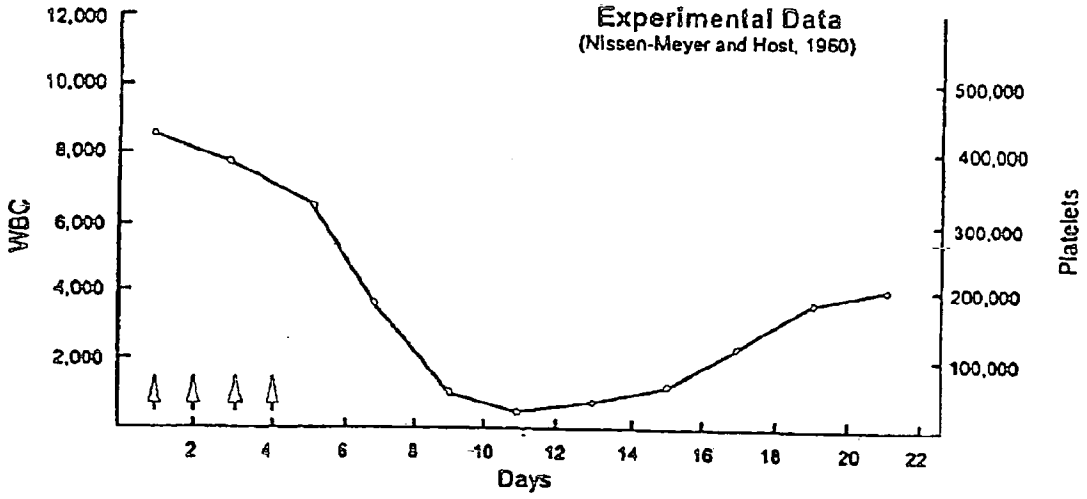



Model Predictions (top) and Experimental Data (bottom) on WBC Time Courses in Human Patients following 60 and 120 mg/kg of CP

FIGURE 2: MODEL PREDICTIONS *vs.* CLINICAL OBSERVATIONS FOR A FOUR-DAY ADMINISTERED DOSE HISTORY




Model Predictions (top) and Experimental Data (bottom) on WBC Time Courses in Human Patients following 60 mg/kg for Four (4) Days

**FIGURE 3a: Predicted vs. Actual CFU-GM Values In Mice After One CP Injection**

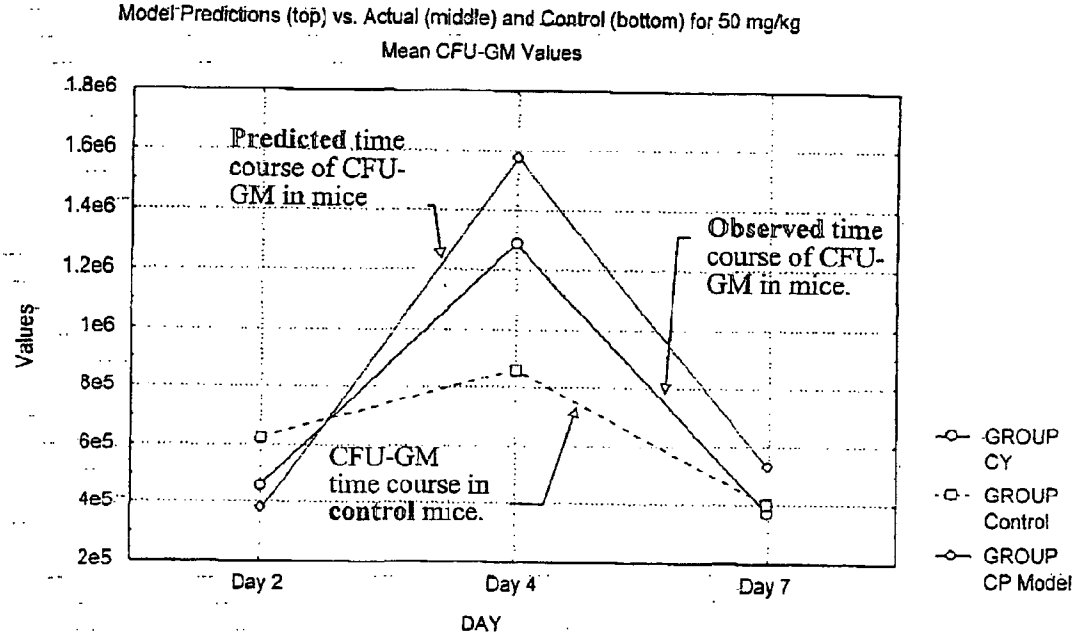



**FIGURE 3b: Predicted vs. Actual CFU-GM Values In Mice After One, Two, and Three CP Injections**

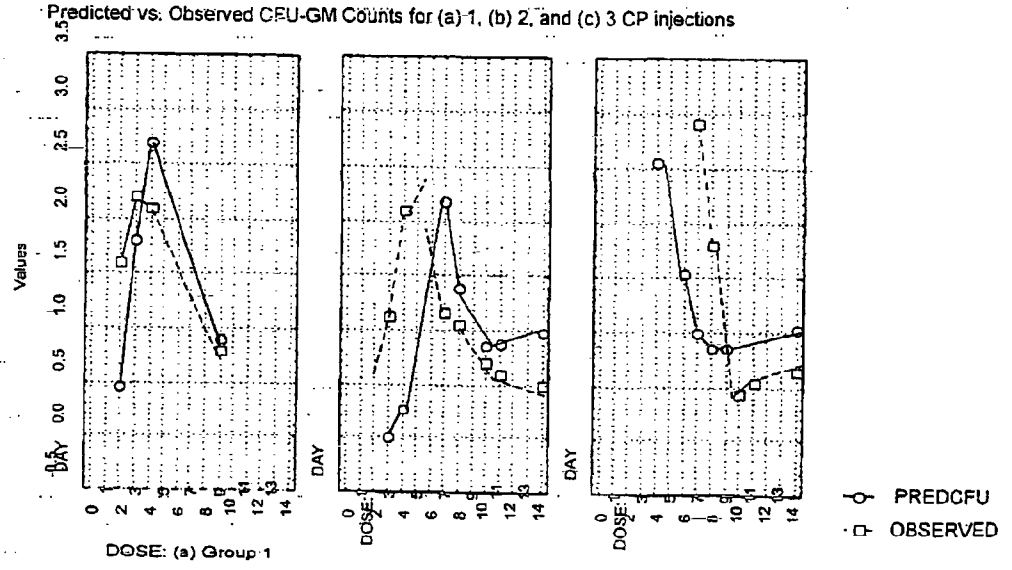
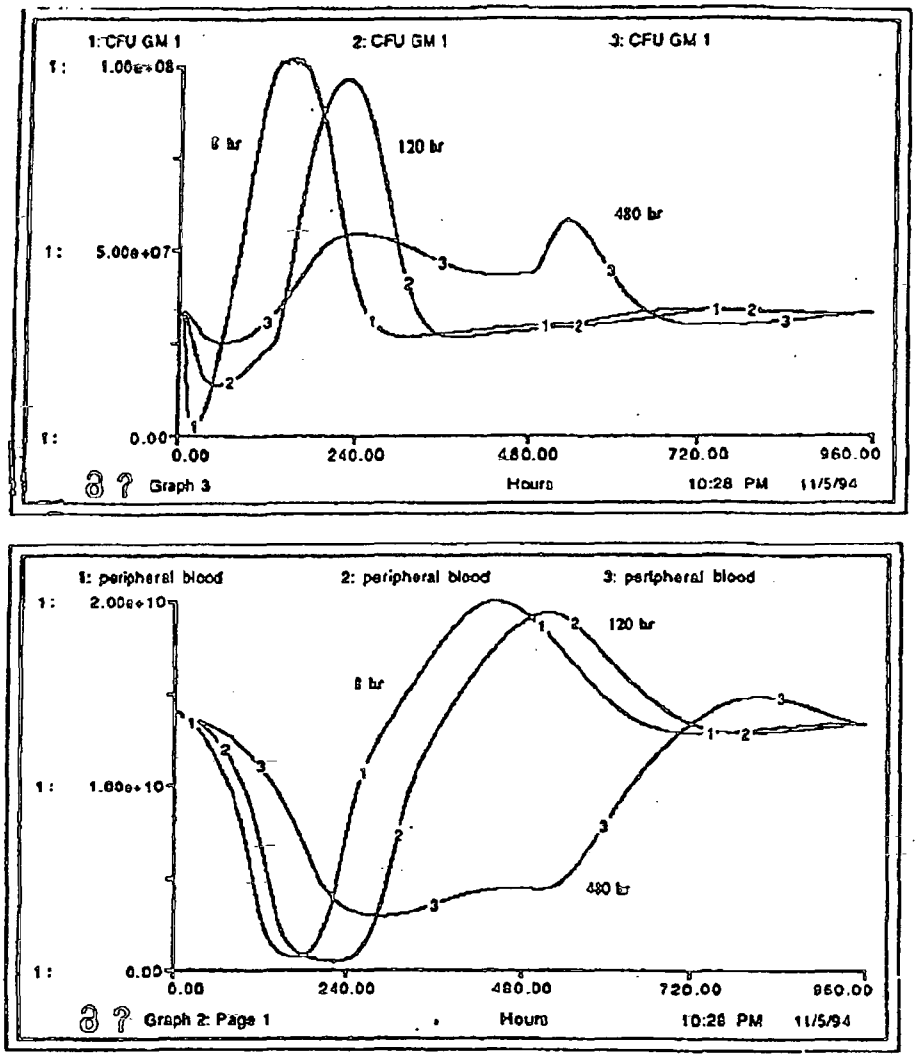
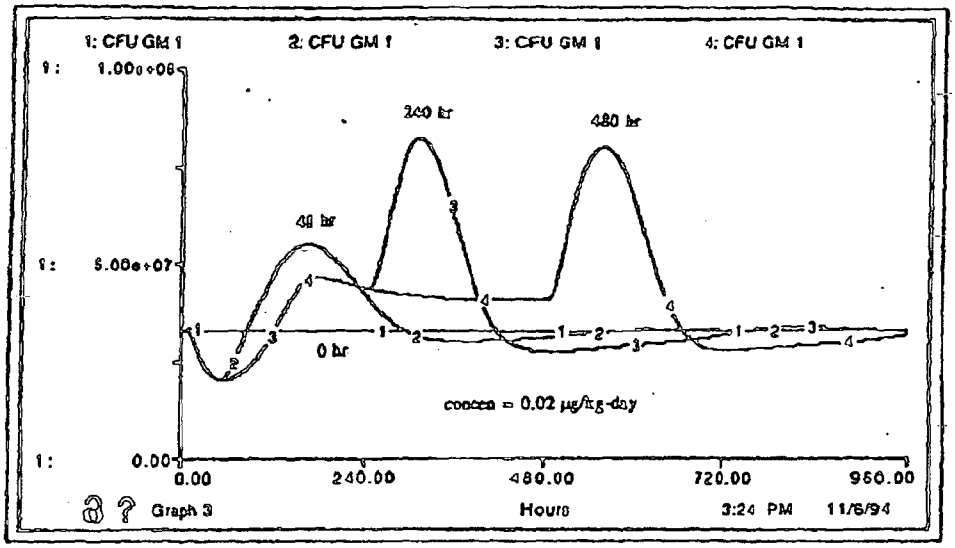


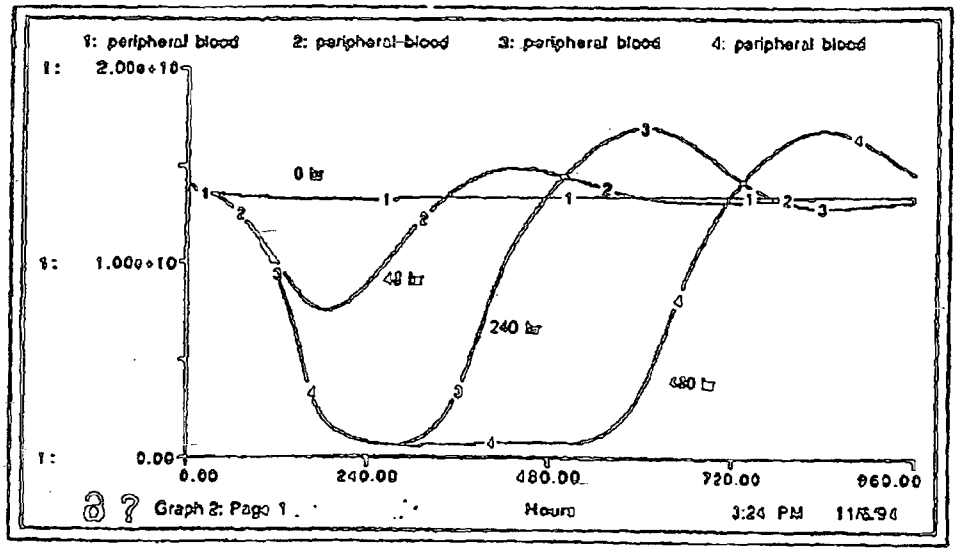
FIGURE 4: SHORT, HIGH-CONCENTRATION DOSING MAXIMIZES STEM CELL RESPONSE FOR A GIVEN TOTAL ADMINISTERED DOSE (AUC)




*Explanation:* This figure shows the effects on early CFU-GM stem cells (top panel) and peripheral WBCs (bottom panel) of different administered dose histories corresponding to the same total administered dose (AUC). Administering the dose over 8 hours instead of 480 hours leads to a deeper nadir followed more immediately by a more extensive proliferation. According to the hypothesized model of leukemogenesis described in Section 4, this response pattern maximizes leukemia risk.

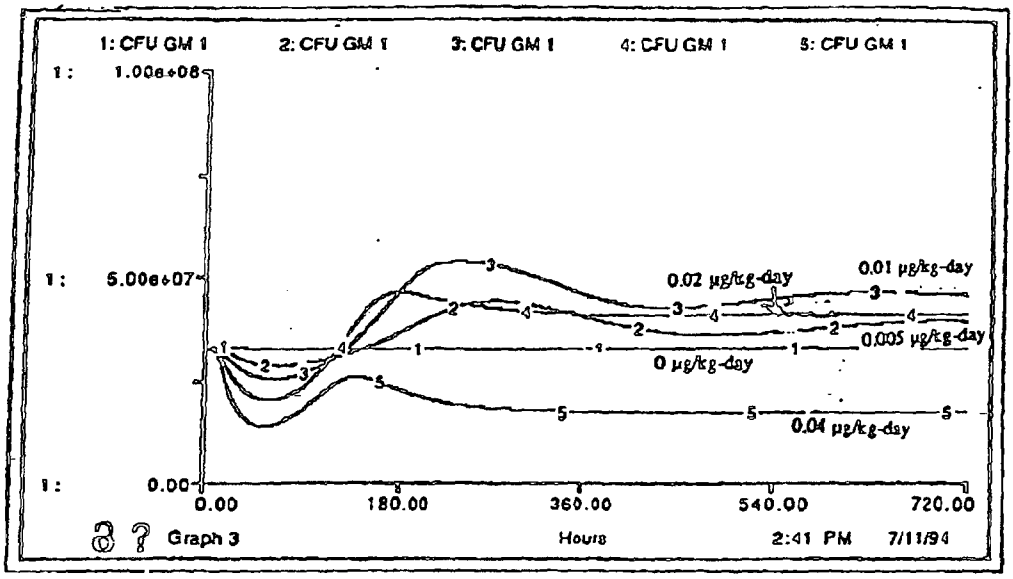
## FIGURE 5: LONGER EXPOSURE PERIODS DO NOT CREATE PROPORTIONALLY GREATER RISKS

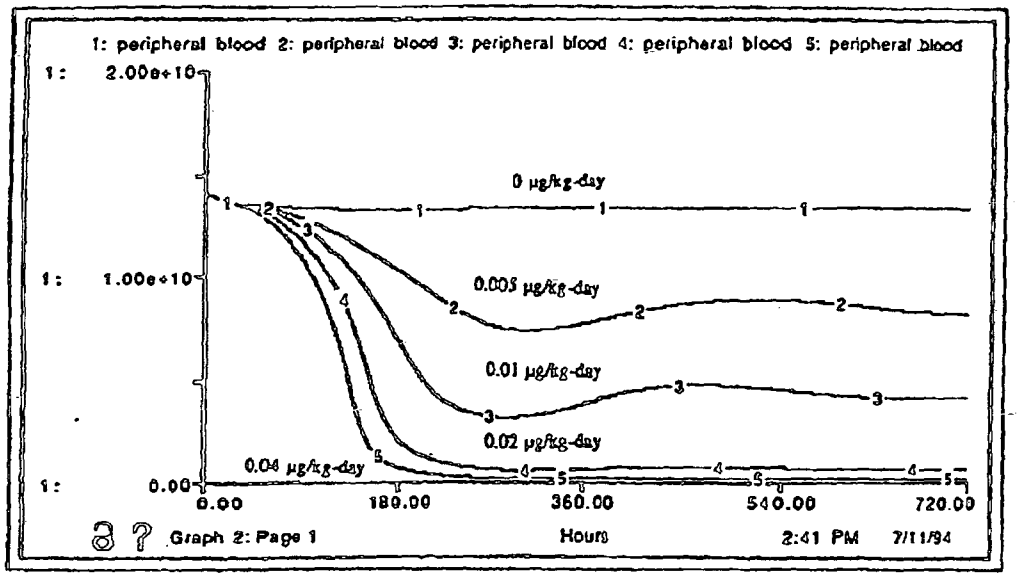



*Explanation:* This figure shows the effects on early CFU-GM stem cells (top panel) and peripheral WBCs (bottom panel) of applying the same concentration (AUC per day) for different amounts of time. Administering the dose over 480 hours instead of 48 hours leads to no deeper a nadir, but delays the post-exposure compensating proliferation. Although the proliferation is more pronounced when it occurs, the initial nadir may not be deep enough to recruit many "immature" stem cells; moreover, the increased level of (presumably predominantly normal) stem cells in the interim and the increased opportunity for DNA repair and other damage-control processes to operate may make the longer-duration exposure less hazardous. Even without these speculative hypotheses, the response of CFU-GM cells (e.g., nadir, zenith, AUC, etc.) for the 480-hour exposure are less than 10 times as great as the corresponding responses for the 48-hour exposure

FIGURE 6: HEMATOPOIETIC RESPONSE IS A NONMONOTONIC FUNCTION OF CONCENTRATION IN SUSTAINED LOW-LEVEL INFUSIONS




*Explanation:* This figure shows the effects on early CFU-GM stem cells (top panel) and peripheral WBCs (bottom panel) of different administered dose concentrations sustained throughout the simulation. Hematopoietic responses such as the zenith, AUC, and stressed equilibrium levels of CFU-GM cells first increase and then decrease as a function of concentration. (This nonmonotonic pattern also occurs in finite-duration exposures.)