American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005

memo

Date: July 3, 1996

From: Mary Paxton

To: Benzene Basic Research Work Group (BBRWG) /
Benzene Task Force (BTF)

Re: Conference Call on July 11

The BBRWG needs to address the items in the proposal present by Tony Cox (attached with memos summarizing earlier results) and to define the next steps that Rich Irons should take in validating our BBRA model for hematopoiesis with the surrogate agent, cyclophosphamide. This will also provide an opportunity to discuss our thoughts after having read Ben Thomas's revision of the "Benzene Mechanism White Paper."

A conference call at 2:00 EDT next Thursday, July 11, is proposed for these purposes. Other interested BTF members are welcome to participate. Please return the form below to 202-682-8270 indicating whether you plan to participate.

* * * * *

_____
(full name)

_____
(company)

____ will

____ will not (A better time would be:)

participate in the **BTF conference call** at **2:00 EDT** on **Thursday, July 11, 1996**. (Dial 703-736-7274 at that time to join the call.)

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To: Dr. Mary Paxton
From: Tony Cox
Re: Proposed BBRA Work for 1996-1997
Date: 6-27-1996

As we discussed, I am pleased to submit this proposed set of tasks for the Committee to consider, based on our recent meeting in Denver. As always, I look forward to working with you to refine and shape the scope of work to make it as valuable as possible to the API.

A. BACKGROUND

For several years, the API has supported development of biologically based risk assessment (BBRA) models that offer the potential to provide more realistic health risk assessments and more informative uncertainty analyses than current regulatory risk models provide, especially at low doses. This work has resulted in insights and conclusions that, if they are accepted by the regulatory risk assessment community, have strong implications for how chemicals such as benzene, 1,3-butadiene, isoprene, and styrene are regulated. Some of the key predictions and findings from the BBRA modeling and research include the following

1. *Hematotoxic responses at low concentrations are significantly over-predicted from responses at higher concentrations* using the "equivalent" dose metrics, based on area-under curve (AUC) of administered dose, adopted in regulatory risk assessments. Specifically, low concentration, long duration (measured in months or years) exposures are far less dangerous than would be predicted by AUC extrapolations from algebraically "equivalent" higher concentration, briefer duration exposures. Also, exposure episodes that occupy only a fraction of the day (e.g., less than two hours) have much smaller hematotoxic effects than would be expected based on "equivalent" doses that occupy a larger fraction of the day (e.g., 24-hour exposures).

2. When internal doses of benzene metabolites are used as a basis for risk prediction, in preference to administered doses, then *standard regulatory risk assessment models applied to animal data by the California EPA yield a best-fitting dose-response relation at low doses that is cubic*, rather than linear. As a consequence, predicted low-dose risks become vanishingly small at low doses. Projected human health risks at low concentrations (e.g., below 1 ppm), using standard regulatory extrapolation procedures, can be five or more orders of magnitude smaller than current California EPA risk estimates based on administered doses. This finding is robust to plausible changes in the specific metric of internal doses (e.g., total hepatic metabolites, marrow metabolites, AUC of HQ or MA in bone marrow, and so forth).

3. *Scientific and data uncertainties* about the PBPK model structure and parameters (and about the identities of the specific metabolites involved in carcinogenesis), when properly managed, *have little or no impact on ability to predict the relative cumulative internal doses received per unit time* at low doses from different dosing regimens. Indeed, the PBPK and hematotoxicity components of the BBRA model are only loosely coupled. From the standpoint of the hematotoxic response, only highly aggregate features of exposure (e.g., number of hours per day and approximate magnitude of exposures) are important. Detailed descriptions of exposure transients and model parameter values are not needed to predict hematotoxic effects.

4. Animal tumor data for various chemicals, including isoprene and benzene, show strong nonlinearities over a wide range of doses, suggesting that *high-concentration experimental tumor data are not useful for predicting tumor responses at lower concentrations.* For isoprene, tumor responses exhibit the same types of dose-time-response "anomalies" compared to AUC-based predictions (e.g., more weeks of exposure do not proportionally increase tumor risks, higher exposure concentrations can be disproportionately more hazardous, etc.) as hematotoxic responses. These empirical data may suggest that a BBRA-type approach, rather than AUC-based extrapolation, is needed to obtain realistic understanding of low-dose risks.

5. Preliminary statistical analysis of animal data using "model-free" methods suggest that *both hematotoxic and genotoxic dose-response relations caused by benzene exposures may have effective concentration thresholds* such that no positive dose-response relation exists unless the threshold concentration is exceeded. This appears to be generally consistent with epidemiological data for benzene cancer risks.

6. The PBPK and hematotoxicity components of the BBRA model indicate that *human epidemiological and industrial hygiene data need to be sorted and analyzed based on factors such as fraction of the day exposed and number of years exposed,* rather than being analyzed based only on AUC estimates of cumulative exposure.

API's BBRA project has permitted these and other points to be developed and presented in a number of forums to both scientific and risk analysis / regulatory audiences. Some of papers and publications growing out of this API-funded work over the past six years include the following:

Cox, L.A., Jr., "Assessing cancer risks: From statistical to biological models," *J. Energy Engineering,* 116, 3, 189-210, 1990.

"Extending biologically-based cancer risk modeling to apply to benzene-induced leukemogenesis," in B.J. Garrick and W.C. Gekler (eds), *The Analysis, Communication, and Perception of Risk.* Plenum Press, New York, 1991a.

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"Biological basis of carcinogenesis: Insights from benzene," *Risk Analysis,* 11, 3, 453-464, 1991b.

"Reassessing benzene cancer risks using internal doses," *Risk Analysis,* 12, 3, 401-410, 1992a.

"Extending the stochastic two-stage model of carcinogenesis to include self-regulation of the non-malignant cell population," *Risk Analysis,* 12, 1, 129-138, 1992b.

"A biologically-based risk assessment (BBRA) model of leukemogenesis induced by cyclophosphamide", Poster presented (by Dr. Mary Paxton) at the workshop on Biological Mechanisms and Quantitative Risk Assessment: From Experimental Design to Risk Characterization. Research Triangle Park, North Carolina, November 1-4, 1993a.

"Coping with uncertainties in a computer simulation model of cyclophosphamide-induced leukemogenesis," invited talk, 1993 Annual Meeting of the Society for Risk Analysis, Savannah, Georgia, December 5-8, 1993b.

"Machine learning for uncertainty management in complex risk models", invited talk presented at The Institute of Management Sciences (TIMS) and Operations Research Society of America (ORSA) joint meeting, Boston Marriott Copley Place, Boston, Massachusetts, April 24-27, 1994a.

"New frontiers in toxicology information: Technologies of the information superhighway." Keynote address, *21st Annual Toxicology Information Roundtable,* MICROMEDEX Toxicology, Medicine, and Environmental Series, Westin Hotel at Tabor Center, Denver, Colorado, October 13, 1994b.

"Nonlinear dose-time-response relations for chemical leukemogens: Computer simulation of the roles of cell kinetics and hematotoxicity." Invited talk presented at the Symposium on Recent Developments in Benzene Epidemiology and Toxicology. Villa Florence Hotel. San Francisco, CA. November 29-30, 1994c.

"Nonlinear cell kinetics can explain observed anomalies in dose-time-response patterns," Poster session (presented with Dr. M.B. Paxton), 1994 Annual Meeting of the Society for Risk Analysis, Hyatt Regency at the Inner Harbor, Baltimore, MD, December 4-7, 1994d.

"Simple relations between administered and internal doses in compartmental flow models," *Risk Analysis,* 15, 2, 197-204, 1995a.

"An exact analysis of the multistage model explaining dose-response concavity," *Risk Analysis,* 15, 3, 359-368, 1995b.

"Reassessing benzene risks using internal doses", paper presented at *Benzene '95,* Rutgers, New Jersey, June, 1995. Forthcoming in *Environmental Health Perspectives,* 1995c

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"More accurate estimates of dose-response functions using Monte-Carlo uncertainty analysis: The Data Cube approach." *Human and Ecological Risk Assessment,* 2, 1, 146-170, 1996a.

Schnatter, A.R., M.G. Bird, L.A. Cox, Jr., and R.F. Herrick, "Defining optimal exposure assessment methods and metrics for epidemiologic studies exposures of petroleum distribution workers to benzene." *Occupational Hygiene,* 155-160, 1996b.

In summary, the work completed to date on this project, including a substantial amount of as-yet unpublished work on BBRA model validation and comparisons to clinical data, appears to be promising in the following respects:

Reasonable approaches for overcoming scientific and data limitations and uncertainties have been developed that appear to be effective in making BBRA modeling practical with existing data.

Experimental and clinical data appear to be consistent with model predictions

The model predicts that low-dose risks (whether of tumors in animals or of myelotoxic effects in animals and humans) are significantly over-estimated by current regulatory risk assessment procedures. Thus, BBRA modeling appears to have something to offer beyond what simple AUC-based extrapolations can give.

Despite these accomplishments, our BBRA work must close some important gaps to become as influential as it potentially can be. The most important next steps, in order for the work to have its greatest possible impact with scientists and decision makers, were discussed briefly at our Denver meeting. They probably include the following:

1. Build credibility and acceptance for the BBRA model in the scientific community. Show the results and power of the BBRA approach to more of the scientific community, and achieve wide acceptance for its predictions. At present, the risk-analytic predictions and implications of the BBRA approach are vulnerable to dismissal on the grounds that they are outputs of a complicated, unfamiliar model. If non-controversial but novel predictions from the model (e.g., its analysis of CP hematotoxicity results) are more widely understood and accepted, then the risk-related predictions from the model are more likely to be accepted as well.

2. Reinforce the relevance of the model for human data. One of the outstanding potential contributions of the BBRA model of CP hematotoxicity is that it makes predictions for humans that appear to match clinical data quite well

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and that can be extended to low doses. This capability should be reinforced with more thorough examination of human data, perhaps using clinical results for other chemotherapeutic agents in addition to CP. The goal is to demonstrate that the BBRA approach is widely applicable to hematotoxic (and perhaps leukemogenic chemicals) and that it makes trustworthy predictions for humans over a wide range of data. This will position it as a more credible basis for predicting benzene effects in humans.

3. Develop the cytogenetics and genotoxic component of the BBRA model
The greatest current vulnerability of the BBRA model may be that it leaves unaddressed the causal link that regulators care most about: the genotoxic (and cytogenetic) component of health effects. This raises the possibility that all of our model approaches and predictions will be accepted as valid, interesting scientific work, but yet it will not be used to influence or replace current risk assessments, because it will be seen as too incomplete to be useful. Exploratory work in 1995 and early 1996 suggest that a BBRA simulation description of the dynamics of cytogenetic and genotoxic damage and dose-time-response is highly likely to reveal interesting and important low-dose nonlinearities, much as the hematotoxicity component did. Without this component in place, the usual "default" regulatory position of assuming low-dose linear genotoxic damage and an AUC basis for extrapolating risk is likely to continue. The BBRA framework expresses tumor hazard as a product of two factors -- namely, the number of cells at risk (output from the hematotoxicity BBRA component) and the rate at which they are affected by carcinogenic metabolites (output from the genotoxicity BBRA component). Addressing only the first component leaves open the objection that, at sufficiently low doses, the behavior of this product, and hence the age-specific and exposure-specific tumor hazard rate, is determined entirely or almost entirely by the second component. The BBRA model must be extended to cover this gap.

The scope of work proposed in the following sections undertakes to fill all three of these potential gaps, positioning our BBRA work to have strong practical impact.

In summary, the scientific and research caliber of the BBRA work to date appears to be strong. To have practical impact with decision makers and scientists in 1997 and beyond, the work should ideally be positioned as a viable, general, well-validated approach to predicting relevant biological effects in humans of several chemicals, including those of interest to regulators. To this end, I propose to extend the application of the BBRA hematotoxicity model to additional human data and to develop the genotoxicity component of the BBRA model so that it can analyze existing dose-time-response data on cytogenetic effects in humans and animals, and so that it can make quantitative predictions for these effects at low doses.

B. SCOPE OF WORK

To make our BBRA model clearly relevant and trustworthy as a basis for quantitatively predicting health effects in humans -- including health effects though to be causally relevant for chemical leukemogenesis -- I propose to undertake the following four tasks.

TASK 1: EXTEND THE HEMATOPOIESIS COMPONENT OF THE BBRA MODEL WITH ADDITIONAL HUMAN DATA AND CHEMOTHERAPEUTIC AGENTS

Description: This task will reposition our BBRA hematotoxicity model, so far developed and validated for CP, as a model of hematotoxicity (in humans and other species), rather than as a model of CP. Accordingly, published clinical data not only on CP, but also on other hematotoxic, immunosuppressive, and possibly leukemogenic chemotherapeutic agents, will be culled from the literature, and compared to the BBRA model's predictions. I will continue to rely on Rich Irons and on the Committee for recommendations and approval of the specific chemicals to be studied.

I suggest that we plan to analyze no fewer than five known hematotoxic chemicals (including CP) to provide an adequate basis for assessing and validating the general descriptive and predictive usefulness of the BBRA model. I anticipate that we can select at least one of these chemicals within the first few weeks of the contract, and agree to all of then within the first two months.

Deliverable: A technical report that

(i) Documents multiple human data sets on hematotoxic effects (dose-time-response curves for multiple periods and dosing regimens) of multiple chemicals, and

(ii) Shows the comparison between empirical observations and BBRA model predictions, including the results of any data analyses performed on data obtained from Rich's new model validation experiment for CP.

This report will provide a basis for validating and assessing the performance of the BBRA hematotoxicity model as a general approach to myelotoxicity prediction. It will be backed up by

(iii) Computer simulation software and models that can be used to run new dosing scenarios with different chemicals.

These models will take chemical-specific cytotoxicity parameter values estimated from the literature, together with user-specified dosing scenarios, as inputs. They will predict effects on the time courses of different hematopoietic cell populations as outputs.

Discussion: Only parsimonious changes in the CP model parameters to allow for different cytotoxic potencies of different chemicals, will be considered. If the BBRA model can parsimoniously predict / explain human hematotoxicity data for a wide range of myelotoxic chemicals, it will be well positioned for future use as a source of relevant predictions for benzene and other chemicals.

Estimated Resource Requirements: $26k

TASK 2: DEVELOP GENOTOXICITY COMPONENT OF THE BBRA MODEL AND ANALYZE EXISTING CYTOGENETICS / GENOTOXICITY DATA

Description: This task will create and validate an initial dynamic simulation model of the genotoxic and cytogenetic effects of the chemicals (including CP) identified in Task 1. The model will describe / predict the time courses of markers such as MN-PCE, DNA adducts, and perhaps SCE in hematopoietic populations during and following different dose regimens. It will also compare these time courses to published data.

Deliverables:

(i) A computer simulation model, probably implemented in either ITHINK or PowerSim, that implements the genotoxicity / cytogenicity component of the BBRA model.

(ii) A technical report describing the model's structure, the data used to estimate its parameters, the formulas and parameter values in the model (with their biological interpretations, where applicable), and the results of initial model validation tests based on comparisons to published data sets for several chemicals and genotoxic endpoints.

Discussion: This effort will recapitulate for the genotoxic link the methods and modeling approach already developed for the hematotoxicity link. An innovative aspect of this task will be application of appropriate methods for estimating and validating cytogenetic and genotoxic potencies from published time course data. In particular, the question of whether a low-dose nonlinear dynamic model fully explains all of the available data, or whether a significantly better description could be achieved by postulating a (perhaps small) linear component, will be addressed using accepted statistical methods for model selection and comparison. Such methods, ranging from the classical Akaike Information Criterion to modern computational methods for model selection and hypothesis testing, have already been identified in an earlier phase of this project as part of the methodology for dealing with uncertainties in BBRA modeling. They are now ready for use in this phase.

Estimated resource Requirements: $20k

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TASK 3: DOCUMENT ALL FINDINGS TO DATE IN AN API TECHNICAL REPORT

Description: All of the important findings and results achieved in the project to date should be consolidated in a technical report. This includes as-yet unpublished results on the following areas, discussed at our Denver meeting:

(i) Preliminary results of applying the BBRA hematotoxicity mode to benzene (namely, that experimental findings and apparent anomalies for CFU-GM population time courses under various benzene dosing regimens published in previous decades are well explained / described / predicted by the BBRA model).

(ii) Results of our model validation using CP (namely, that the experiments conducted by Rich Irons' lab produce data that are very consistent with the BBRA model predictions)

(iii) Results from preliminary analysis of genotoxicity and cytogenetics data (namely, that model-free statistical analysis suggests threshold-like effects for any positive dose-time-response relation).

After Tasks 1 and 2 have been completed and the results of Rich Irons' new experiment have been analyzed, so that the preliminary findings now available can be extended and tested in greater depth, it will be important to document and archive them in a form that other researchers can use. (It is also prudent project management practice to summarize all of our accomplishments to date in case the project is canceled, key personnel leave, etc.)

Deliverable: A report, suitable for publication within the API technical report series, focused solely on documenting all of the BBRA project's technical findings and results to date (including results of model validation experiments, comparisons to data in the literature, summary of the data sets used, etc.), but with minimal or no interpretation or discussion of possible implications for risk assessment. This report could furnish the material for the "Methods" and "Results" sections of scientific journal articles that API may subsequently elect to publish based on this project.

Discussion: The last API technical report produced for the BBRA modeling project was written in 1993. While numerous conference posters and presentations have reported on our progress since then, and while there has been a lot of exciting progress to report, this information is not available to others in any convenient form. Since it is premature to publish our interim findings on the results of model validation and analyses of previously published data sets, an API technical report appears too be a very appropriate vehicle for documenting the progress we have made since 1993.

Estimated Resource Requirements: $15k

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TASK 4: DEVELOP AND APPLY STATISTICAL METHODS FOR DATA ANALYSIS OF BBRA MODELS WITH A NON-STATIONARY CONTROL GROUP

Description: This task will take care of some technical issues that require attention in light of the experimental results to date. In particular, we must resolve the questions of how best to

(a) Estimate relative time courses (predicted vs. actual);

(b) Formally test hypotheses about them; and

(c) Report model validation results,

when the control population displays substantial interindividual variability and unexplained systematic variation over time. This task will develop and apply appropriate statistical methods for handling these issues, which were not anticipated in advance of the experimental data collection.

Deliverables: Analyses of new experimental data from Rich Irons' lab using the statistical methods identified and implemented in this task.

Discussion: Promising approaches to the statistical issues raised here include (i) Model reduction, in which the complex BBRA model is simplified and reexpressed in terms of a small number of quantities that support clear tests of hypotheses; and (ii) Characterization of the nonstationarities in the control group (e.g., determine whether they can be represented as the result of passing random disturbances through the nonlinear dynamic hematopoiesis system, or estimated and controlled for using time series models such as the ARIMA or ARMAX specifications). Cleaning up these statistical issues will also enable us to more nearly optimize the design of BBRA parameter estimation, hypothesis testing, and model validation experiments

Estimated Resource Requirements: $12k

Based on my previous experience with this type of modeling and analysis, I believe that the entire scope of work proposed here can be completed within 6-8 months, depending on API's needs, for a cost of approximately $73k. As always, I look forward to working with you to refine the proposed scope of work to make it as useful as possible to the API and to reflect your needs and priorities.

2: 1014

To: Dr. Mary Paxton, API
From: Tony Cox, Cox Associates
Re: Results of statistical analysis of CP experiments
Date: 6-2-1996

This memorandum summarizes the results of our 1995 BBRA Project for additional experimental validation of our CP BBRA model of hematotoxicity. The major results of this project were presented at the Benzene Workshop in Napa Valley in February and at our Denver meeting in June.

In brief, an experiment was designed in 1995 to further test our CP hematotoxicity simulation model by having it predict the time course of CFU-GM populations over a seven-day experimental period following administration of a single dose of CP. Predictions based on the BBRA simulation model were developed in August and September of 1995. Uncertainties about the dose scale and about the operational definition of the CFU-GM population were treated using sensitivity analyses, i.e., time courses were predicted for different subpopulations of the model and for different dose scale factors over about a one order of magnitude range. (This range had already been identified based on the results of literature searches and the pilot experiment conducted in the 1994-1995 project.)

PREDICTIONS

Key predictions made and documented in technical memoranda in advance of the experiment included the following:

1. *The CFU-GM level should peak around day 4* for the aggregate CFU-GM population. (This contrasts with a peak at around day 8 in the peripheral blood compartment.) Thus, if FU-GM data are collected on days 2, 4, and 7, the high value should occur on day 4. This was the design used in our experiment.

2. *Low-dose and high-dose scenarios lead to markedly different hematotoxic responses; however, very different "high" doses lead to very similar time courses, and similarly for "low doses".* A relatively small (e.g., five-fold) increase in administered concentration can make the difference between a "low dose" response that has a relatively minor depression followed by a relatively small peak, and a "high-dose" response that exhibits a clear depression in marrow CFU-GM, followed by a marked over-shoot in the rebound. This pattern, noted as a theoretical possibility based on BBRA simulations in 1994 and 1995, is consistent with what we unintentionally ended up showing in the two pilot experiments performed in 1995 - 1996. Namely, the 10 mg/kg dose showed little if any impact (the "low-dose" outcome), while the 50 mg/kg pattern conformed to the predicted "high-dose" outcome.

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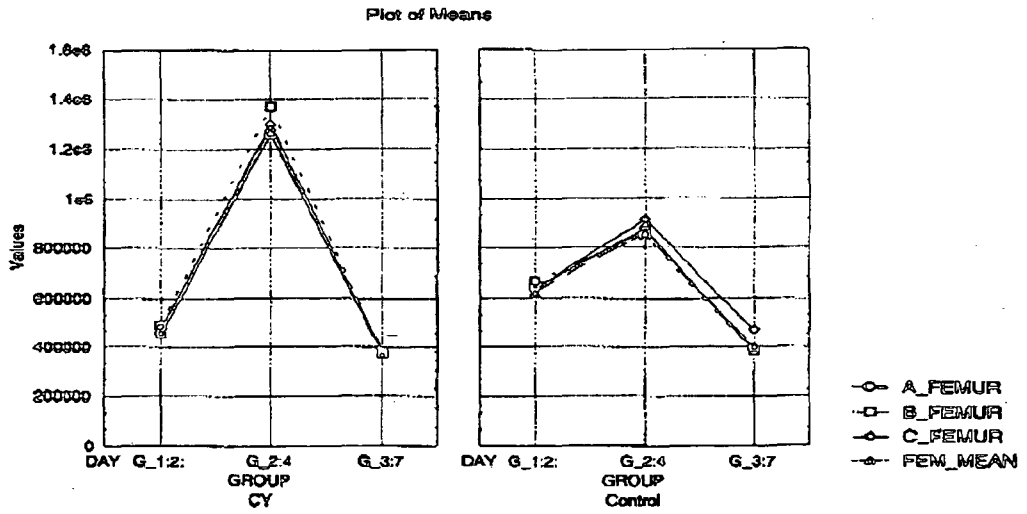
3. The value of CFU-GM on day 7 should be between its values on days 2 and 4, with the day 2 value being lowest and the day 4 value being highest. The BBRA model gives quantitative predictions, allowing the approximate ratios of the CFU-GM values on days 2, 4, and 7 to be predicted for different CFU-GM subpopulations and for the aggregate CFU-GM population as a whole.

RESULTS

The experiment was carried out in the fourth quarter of 1995, at a dose level (10 mg / kg) that was too low to give conclusive results. It was repeated at a higher dose level (50 mg/kg) in the first quarter of 1995. This dose level turned out to be high enough to create unambiguous results. The key findings were as follows.

FINDING 1. The CFU-GM time course peaked at around day 4, as predicted. This finding is illustrated in Figure 1. The left panel of Figure 1, which was one of several dozen graphs used to thoroughly examine the data, shows a clear peak at day 4 compared to days 2 and 7.

FIGURE 1: The CFU-GM Time Course Peaks at Around Day 4

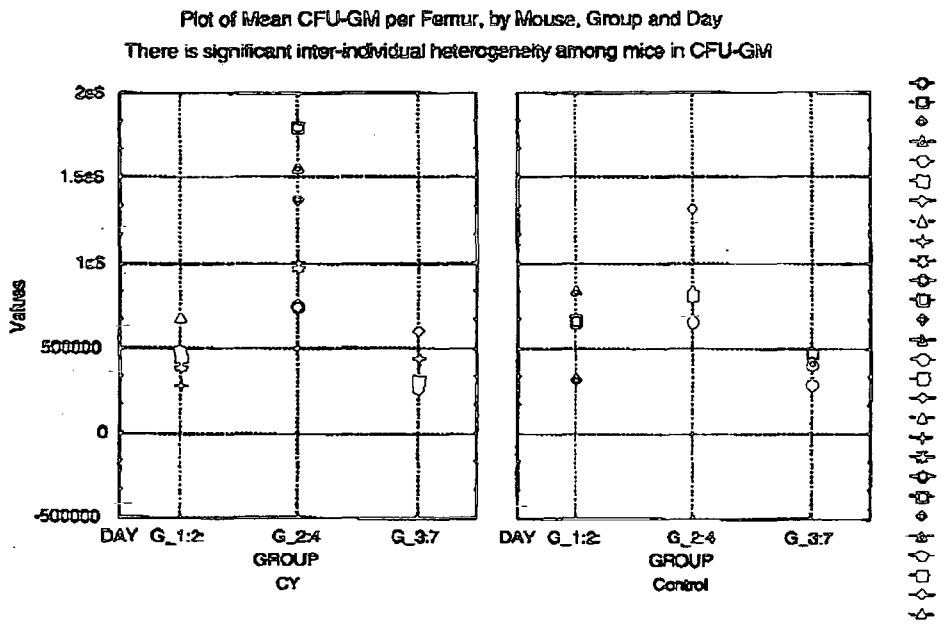


(These are time courses in which each data point is an average for five mice, with five replicates of CFU-GM counts per mouse. Average CFU-GM values are shown for three of the five replicates, to indicate that there is no significant difference across replicates. The same pattern holds when all five replicates are included, although the graphs become a little more cluttered. These and other plots are included in the handout from our recent Denver meeting.)

A surprising observation is that the control group (right panel) shows a similar pattern. For both sets of animals (control and 50 mg/kg), the peak on day 4 is consistent across replicates (animals and bone marrow samples) and is statistically significant at $p < 0.05$ based on a Kolmogorov-Smirnov test for differences in frequency distributions. There is a significant unexplained decrease in CFU-GM on day 7 compared to day 4. Thus, an unexpected finding (which held also in the 10 mg/kg treatment and control groups and held for WBCs in the pilot experiment conducted in the 19941995 project) is that *there is statistically significant variation over time in the control group hematopoietic cell populations.* This nonstationarity in the control group is not explained by the BBRA model, which predicts that the control group marrow populations should remain approximately constant over time. It is tempting to speculate that the significant changes in control group blood cell counts over time may be due to acclimatization, handling during treatment, or other experimental factors; however, the true causes are currently unknown.

FINDING 2: There is substantial interindividual heterogeneity in mouse CFU-GM counts, for the same day and treatment group (50 mg/kg or control). This aspect of the data, shown in Figure 2, can potentially be explained by interindividual variability in BBRA model parameters.

FIGURE 2: CFU-GM Data Reveal Interindividual Heterogeneity



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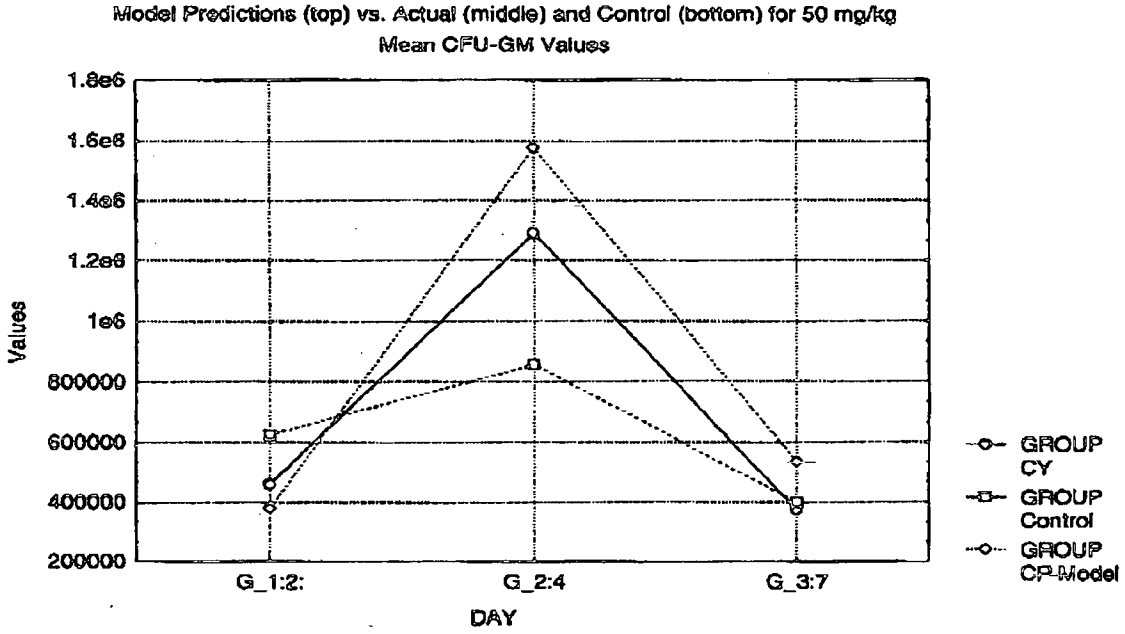
FINDING 3: The relative magnitudes of the CFU-GM counts on days 2, 4, and 7 predicted by the BBRA model are approximately correct, assuming that the aggregate CFU-GM population in the BBRA model corresponds to what the CFU-GM assay measures. Figure 3 displays this finding using the raw data, which is the way least favorable way to exhibit the BBRA model's predictive accuracy. Even so, the fit between model predictions and observed values is conspicuous, especially given that the predicted values at days 2, 4, and 7 were obtained entirely before looking at the data.

FIGURE 3: Observed and predicted values (middle and top) are close



When normalized values are used (i.e., when the ratio of treatment group to control group values is used instead of the raw data), the fit between the observed and predicted mean values improves even further.

In light of the unexplained variation in the control group data over time, and especially the unexplained decline in control group CFU-GM on day 7 (Figure 1), it is reassuring that the *ratios* of the treatment group CFU-GM to control group CFU-GM display the same pattern (i.e., peak on day 4) and approximate relative magnitudes as the BBRA model predicts. Thus, standardizing or "normalizing" the treatment group data (left panel of Figure 1) to correct for relative control group values (right panel in Figure 1) leaves the validity of this prediction unchanged. Indeed, the predictive accuracy of the BBRA model improves when

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the 50 mg/kg mouse data are expressed as ratios, relative to the control group values, in that *the day 7 normalized CFU-GM level lies between the day 2 and day 4 normalized values, as the BBRA model predicts.*

Since there is significant interindividual heterogeneity in responses (Figure 2), it is worth considering not only the ratio of the means (treatment group mean vs. control group mean, for each day) as a way of standardizing the treatment data, but also to examine the distributions of these ratios. When each treatment mouse is compared to each control mouse on each day, the values of the ratios typically range over a factor of about 2. However, the null hypothesis of no difference between treatment and control groups can be rejected using a nonparametric sign test on days 2 (when 19 out of 25 ratios are less than 1) and 4 (when 21 out of 25 ratios are greater than 1, implying that the treatment group indeed has an elevated CFU-GM count compared to the control group. More sophisticated ways of analyzing the data (e.g., using individual mice as treatment blocks, with replicates of CFU-GM measurements within blocks) and the time variation in control group counts may be developed as part of a subsequent effort.

CONCLUSIONS

The data collected and analyzed in this project generally support the conclusion that the BBRA model provides useful predictions of observed hematotoxic time courses for CFU-GM in the marrow, as well as for WBCs (previously analyzed). There is no reason to reject the BBRA hematotoxicity data based on the results of the validation pilot experiments conducted to date. However, the finding of statistically significant nonstationarity in the control group CFU-GM counts does suggest that future model validation experiments should be designed and analyzed taking into account the likelihood of unexplained variations in control group data.

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2. A potentially useful model of benzene hematotoxicity has been developed by making parsimonious changes in our CP model. (Thus, the CP model might be better regarded as a model of hematotoxicity than as a model of CP.) Specifically, cytotoxic potencies for benzene can be estimated based on previously published literature. Erythroid cytotoxicity can be modeled by increasing the rate of withdrawal from the CFU-S population in a dose-dependent way, to mimic the competition of the erythroid lineage for CFU-S cells. Finally, a dose scale for mice can be roughly estimated from previously published time course data (Green et al., 1981; Toft et al, 1982). These changes have created a benzene hematotoxicity model that can be used to make testable predictions for time courses of hematopoietic populations (e.g., WBC and CFU-GM) in response to different benzene exposures.

3. As discussed at our Denver meeting, the simple benzene hematotoxicity model has been compared to several previously published data sets, with exciting results. Several apparent "anomalies" in previously published data are well described / explained / predicted by the benzene hematotoxicity model. Specifically, there are predictable patterns of departure from a simplistic "equal AUCs create equal cytotoxic damage" model, which seems to be implicit in some previous risk assessments for benzene. Testable model predictions that agree with previously published data include

(i) A smaller cumulative dose of benzene can have a larger myelotoxic effects. (For example, 21 ppm of benzene for 144 hours is predicted to cause less CFU-GM suppression than 50 ppm for 48 hours, in agreement with the results of Toft et al., 1982.)

(ii) 10 ppm for 6 hours/day, 5 days/week for 10 weeks has almost no effect on CFU-GM, whereas 100 ppm for 6 hours/day, 5 days/week for 1 week has a marked effect.

Other comparisons to published data show similarly promising results. Although we have agreed that it is premature to publish these findings, they indicate that the benzene BBRA model may be useful in explaining past data, and potentially in predicting hematotoxic effects from other exposure scenarios (for humans) or dosing regimens (for mice). Moreover, such findings may eventually help to validate our BBRA hematotoxicity model as a useful, parsimonious approach fro predicting hematotoxic effects in humans and in mice, as well as for converting does between these species without relying on AUCs as an "equivalent" dose metric. This may help to improve aspects of risk assessment practice that are relevant for benzene and other chemicals.