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Date: October 16, 1990

To: Hugh Spitzer
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Mark Swanson
Pat Beatty
Chuck Lambert
Don Stevenson
Carol Houth

From: Paul Price

Subject: Summary of first meeting of the Biologically Based Risk Assessment (BBRA) Advisory Workgroup with Dr. Tony Cox

The first meeting of the BBRA Advisory Workgroup was held on September 24 in Denver. This group was created at the September 13 meeting of the Benzene Task Force. The purpose of the meeting was the review of Dr. Cox's work on the development of a PBPK model, as the first stage of his biologically based model. For background information, find attached a copy of Dr. Cox's contract.

The group heard a detailed presentation of the work which has been performed up to this time, see attached outline from Dr. Cox, and discussed two recent written products, "BIOLOGICAL BASIS OF CHEMICAL CARCINOGENESIS: BENZENE AS AN EXAMPLE" and "BIOLOGICAL EVIDENCE THAT THE ONE-HIT MODEL AND LOW DOSE LINEARITY ASSUMPTIONS DO NOT HOLD FOR BENZENE".

In general the group was pleased with the progress that Dr. Cox had made on the PBPK project and agreed with the general directions of his efforts. Several specific comments were made on the PBPK project.

- o PBPK in mouse, rat, and man will play an important role in the new benzene risk assessment.

- o Efforts to incorporate non-steady-state assumptions into the PBPK model should be reconsidered. Modeling of microvascular events is perhaps an unnecessary complication. Dr. Cox should perform a sensitivity analysis on the benefit of using non-steady-state equations and demonstrated that the additional complication was warranted.

- o The Work Group agreed to provide additional help in obtaining additional information for the model:

  - Peter Craig will provide a copy of a recent paper by Dr. Ross.
  - Pat Beatty will speak to Dr. Mike Gargas over recent low dose work on benzene metabolites and metabolism.

- o Dr. Cox will meet with Irons and Ross during October to discuss the PBPK modeling project.

There was considerable discussion on the paper "BIOLOGICAL BASIS OF CHEMICAL CARCINOGENESIS: BENZENE AS AN EXAMPLE". Several members expressed surprise that Tony would author a paper summarizing metabolism and pharmacokinetics of benzene since his area of expertise is Operations Research. This concern was augmented by the fact that the paper had already been submitted to the Journal of the Society of Risk Assessment and was tentatively accepted for publication. Other members found the paper to be, outside of the nomenclature and other problems of phrasing, to be an excellent summary of the current state of understanding of the biology of benzene's leukemogenic effects.

Dr. Cox explained that he had been approached to submit a paper on BBRA for benzene by the Journal's editor and after some discussion they had concluded that the draft report specified in his API contract would be appropriate basis for such a paper. He agreed that he had been in error in not notifying API of his submission and not providing opportunity for review prior to submission. However, the paper's submission was unusual in that the Editor requested an early copy of the paper and agreed to allow Dr. Cox to simultaneously distribute the paper to external reviewers and to incorporate their comments. Copies were thus sent to API and to an number of experts in benzene toxicology, including, Dr. Ross (U. of Col.), Dr. Michael Byrd (Exxon), and Dr. Ben Thomas (ENSR). Because of this simultaneous review there will be no problem in incorporating API's comments in to the document before it is published. Dr. Cox agreed that if API and he could not come to agreement over the paper then the document would not be published.

The group agreed to review the paper and prepare comments by the Santa Monica meeting of the benzene Task Force. These comments will be incorporated by Dr. Cox along with comments from other reviewers. The Task Force will review the revised document before it is resubmitted to the Journal.

The discussion on the second paper "BIOLOGICAL EVIDENCE THAT THE ONE-HIT MODEL AND LOW DOSE LINEARITY ASSUMPTIONS DO NOT HOLD FOR BENZENE" focused on whether the arguments presented actually demonstrated that non-linearities were observed in the range where the current models predict linearity. Most of the work cited in the paper clearly fell in the range where current cancer models demonstrate non-linear behavior. The group agreed that the current data was not sufficient to compellingly demonstrate that dose responses were non-linear in the nanogram/kilogram range.

The group decided that paper served a legitimate function as a summary of non-linear processes but should not be taken any further at this time.

The group agreed to meet again in Denver in November either the day before of after the meeting with Dr. Irons.

→ Paul Price, API

To: ~~Ben Thomas, Shell Oil~~

From: Tony Cox, Cox Associates

Re: Statement of Work for 1990 Research on Benzene

Date: ~~12/5/1989~~ 2/22/90

In response to your recent request, I am pleased to submit the following statement of work for applied research on quantitative risk assessment of the human health effects from benzene, to be undertaken in 1990.

A. Background

In 1989, Cox Associates developed for the American Petroleum Institute a biologically-based cancer risk assessment framework for benzene. The emphasis in that work was on integrating mathematical models of (i) benzene pharmacokinetics and metabolism (PB-PK models); (ii) cell kinetics and cytotoxic effects; (iii) leukemia induction; and (iv) leukemia progression, into a complete, computationally viable, risk model. The methodology developed involved a mixture of compartmental flow modeling and stochastic simulation for cell populations. The results of this modeling work were documented in chapter 4 of a report on *New Directions in Cancer Modeling and Risk Assessment for Benzene*, submitted jointly to the American Petroleum Institute and the Western States Petroleum Association for review and comments in August of 1989. This chapter also identified needed enhancements in current risk modeling techniques for benzene in the areas of PB-PK modeling (e.g., adding bone marrow as a separate compartment), cell kinetics (e.g., creating a disaggregated compartmental model of the hematopoietic system) and leukemia induction (e.g., representing the interaction between cytotoxic and leukemogenic effects in the bone marrow.) The remainder of the report identified methodological flaws in statistical risk assessments of benzene underlying current regulatory risk analyses, and concluded that current quantitative risk estimates for benzene health risks at low exposure concentrations are biased upwards by at least a factor of 20 (and probably much more.) This large error in current risk estimates motivated the search for more biologically realistic benzene risk models. Finally, a demonstration computer program was developed to illustrate the modeling framework and to prove its computational practicality.

The modeling work completed in 1989 established a framework for more realistic quantitative assessment of the human health risks from inhalation of benzene. However, this framework has yet to be applied. The purpose of the work proposed for 1990 is to begin

applying portions of the framework for which adequate data exist to the quantification of human health risks from benzene. The goal is to use biological knowledge, data, and models to suggest quantitative correction factors for current risk estimates.

B. Statement of Work

We propose to conduct three tasks spread over eight months. Descriptions, deliverables, and estimated resource requirements for each task follow.

Task 1: Review and incorporate existing data on benzene into the model.

Considerable experimental data have now been collected in each of the relevant areas of (i) Benzene pharmacokinetics and metabolism; (ii) Cytotoxic effects and effects of continued exposure on short-run and long-term cell kinetics; (iii) Genotoxic effects; and (iv) Possible epigenetic mechanisms of benzene-induced leukemogenesis (including protein kinase C activation as well as more traditional stem cell proliferation effects). The state of each of these areas of knowledge about benzene biology as of early 1989 is well portrayed in a special issue of *Environmental Health Perspectives* on "Benzene Metabolism, Toxicity, and Carcinogenesis," (*EHP*, 82, July, 1989.) Task 1 will be to review and integrate these data in the context of our biologically-based risk model. The purposes of this task are as follows: (i) To identify data elements required by the model that can already usefully be supplied from current knowledge; (ii) To identify critical data gaps that still need to be filled to make biologically-based risk modeling for benzene practical; and (iii) To provide a unified summary of current, practically applicable, knowledge of benzene biology as it applies to risk assessment.

Deliverable from Task 1: A technical paper, suitable for submission to a peer-reviewed journal, discussing existing quantitative data on benzene biology within the framework of the biologically-based stochastic simulation risk model of benzene developed for API in 1989.

Estimated Resource Requirements for Task 1: 150 hours, 15k, three months.

Task 2: Develop a detailed PB-PK computer model for benzene and populate it with existing data.

The most productive research strategy for developing defensible quantitative correction factors for benzene risk estimates in the short run is likely to be improved PB-PK modeling. Task 2 will build state-of-the-art computer models of benzene pharmacokinetics and metabolism in rats, mice, and to the extent now possible, man. The models will incorporate recent developments (e.g., by Bois *et al* at the University of California at Berkeley on detailed pharmacokinetics for Fischer-344 rats), including addition of bone marrow as a separate metabolizing compartment and target site. They will be validated by comparing numerical predictions with published data.

The PB-PK work described here has practical value for two reasons: (1) It can be used to interpret past animal bioassay data by more accurately quantifying the true internal doses received under different exposure regimens in different species; and (2) It can potentially yield more accurate projections of the true internal doses received by humans under assumed occupational exposure conditions. In similar modeling work on butadiene conducted for the Chemical Manufacturer's Association in 1989, we have found that regulatory risk models have underestimated animal internal doses in bioassays and overestimated projected human internal dose levels from occupational exposures, leading to an overestimate of human risks extrapolated from animal data of at least two orders of magnitude. A similar methodology applied to benzene may yield similar results. The PB-PK models developed in this task should also help to clarify some of the surprising anomalies of recent animal experiments (e.g., that increased exposure concentrations for increased exposure durations can produce dramatically *fewer* leukemia responses in mice.) Finally, the results of this task should also shed light on the relevance of different epidemiological exposure conditions and data bases (e.g., eight-hour shifts with relatively low, variable concentrations in Pliofilm workers in Ohio vs. 24-hour, high-concentration exposures for Turkish shoe workers) for occupational and public health risk assessments.

Deliverable from Task 2: Computer PB-PK models for mouse, rat, and human. The mouse and rat models shall be populated with available data from the literature. The human model will use plausible guesses for parameter values where data are not available. All models will be delivered on one or more floppy disks, and all data values used and assumptions made will be identified as part of the model documentation.

Estimated Resource Requirements for Task 2: 200 hours, 20k, six months.

Task 3: Apply the PB-PK models developed in Task 2, and other relevant biological knowledge and data summarized in Task 1, to suggest and justify quantitative correction factors for existing benzene risk estimates.

A primary reason for doing biologically based risk modeling is to obtain more accurate and more defensible risk estimates. The data to support a full biologically-based risk assessment for benzene are not yet available. Use of simple *ad hoc* "biologically motivated" modeling assumptions for benzene in place of solid data have recently drawn much criticism. But there is another way to use the considerable partial data that are available to improve benzene risk estimates. By quantifying the differences in biologically effective doses received (i) between animals and man; and (ii) between worker populations exposed to different exposure conditions, it should be possible to suggest numerical *correction factors* for previous quantitative risk estimates. The logic is as follows: using the regulatory assumption that cancer risk is approximately proportional to biologically effective dose, estimated bounds on the ratio of internal doses between alternative exposure conditions and/or species are also bounds for relative risk ratios. Thus, the available PB-PK data can be used to correct previous relative risk estimates using internal dose information alone, without requiring speculative assumptions about the biological damage mechanisms involved (except the assumption of proportionality between internal dose and health response, which is already part of the linearized multistage model endorsed by the agencies.) Making these corrections leaves open the possibility that the linearized multistage model for biological damage (via genotoxic "hits") will eventually turn out to be inappropriate for benzene. Since corrections are based only on the PB-PK portion of the complete cancer risk model, for which relevant data are comparatively available, they do not foreclose further corrections at a later date in the response portions of the model (cytotoxic, cancer induction, and cancer progression submodels) when the data for these modules become available.

This correction factor approach to revision of quantitative risk estimates based on partial biological data grows out of work performed by us for the Chemical Manufacturer's Association in 1989. It has already been successfully applied to butadiene, for which comparatively little data and primitive models are available. The goal of Task 3 is to apply it to benzene, to discover the extent to which existing knowledge of benzene biology can already support quantitative revisions in human health risk estimates. This analysis should also highlight the most critical areas for new empirical research to improve quantitative risk estimation.

Deliverable from Task 3: A technical paper, suitable for submission to a peer-reviewed journal, on proposed quantitative revisions in regulatory risk estimates for benzene based on current biological-data and on improved internal dose estimates.

Estimated Resource Requirements for Task 3: 130 hours, 13k, three months.

The resource requirements for all three tasks total to 480 hours and $48,000, spread over a proposed eight-month interval. We suggest that an additional budget not to exceed $2,000 be allocated to cover travel and preparation expenses for a meeting at the end of the project, probably in Washington, D.C., to present and discuss the results.

risk estimates as a potential benefit. The purpose of this task is to help make that potential real by providing explicit methods for using biological knowledge (represented, e.g., by partial models) to guide applied risk-research.

Task 5: Prepare technical reports and worked-out examples (including any necessary computer programs) illustrating the uncertainty-management techniques discussed and developed in Tasks 1-4.

The deliverables from this task will document the research and will provide examples (and, where necessary, simple computer programs) to help illustrate and transfer the technology being developed here. As described above, each technical task will result in a technical report; these will be revised, finalized, and submitted for publication at the end of the project.

4. Project Administration

All work outlined here will be performed on a best-effort basis by Cox Associates in a timely and cost-effective fashion. Dr. Cox will lead all technical work on behalf of Cox Associates and will be responsible for the technical content and quality of all deliverables. Dr. Paolo F. Ricci of the UCLA School of Public Health will act as designated alternate project manager should the need arise.

Task 4: Develop and illustrate methods for deriving efficient research strategies to reduce uncertainties about risk by collecting additional empirical data.

This task will create methods for using partial knowledge of a carcinogen's mechanisms of action to guide the acquisition of new empirical data, taking into account the costs, durations, and reliabilities of different tests and experiments and their relative expected contributions to reducing uncertainty about risk. We will use operations research techniques to study the problem of minimizing the resources (time and cost) required to achieve a given level of certainty about risk by achieving both greater understanding of causal mechanisms and greater confidence and precision in statistical estimates. The purely statistical issues (e.g., sample size and test selection) are well addressed in the literature on "expected value of information" in statistical decision theory. The contribution of this task will be to create analogous methods for quantifying the expected value of better knowledge of specific causal mechanisms in reducing uncertainty about health risks. The intent is to provide an analytic resource-management framework for addressing questions of research strategy such as "Which of the following applied research options would be most valuable for reducing uncertainty about the human health risks from occupational exposure to butadiene: (i) identification and isolation of the specific target cell populations affected; (ii) determination of whether the diepoxide metabolite, as well as the monoepoxide, is carcinogenic in B6C3F1 mice *in vivo*; (iii) more accurate measurement of detoxification rates in monkeys as a function of exposure concentration and duration; or (iv) more accurate modeling of the dynamic response of the human hematopoietic system to mitogens?" We do *not* propose to address the problem of evaluating competing basic research investigations that potentially provide benefits cutting across current and future applied research projects. Instead, we will focus on research strategies for efficiently reducing uncertainties about the human cancer risks from a specific chemical carcinogen. Finally, we will address the problem of selecting an applied research portfolio when different research options carry different costs and durations as well as uncertain outcomes. Throughout, current research options for benzene and butadiene will be used to provide realistic examples of risk research decision problems.

Task 4 is motivated by a belief that one of the greatest potential benefits from biologically-based risk modeling is the information that it provides about how the resolution of current scientific uncertainties can affect quantitative risk estimates. Enthusiasts of biologically-based risk assessment often mention uncertainty reduction for

Selected References

The following sources provide excellent technical background on some unconventional and non-statistical techniques of uncertainty analysis that are potentially useful for applied cancer risk analysis.

Etherington, D.W., *Reasoning with Incomplete Information.* Morgan Kaufmann, San Mateo, CA, 1988. (Requires some familiarity with contemporary mathematical logic.)

Karp, P.D., *Hypothesis Formation and Qualitative Reasoning in Molecular Biology.* Ph.D. thesis, Stanford University Department of Computer Science. Report No. STAN-CS-89-1263, Stanford University, June, 1989.

Martins, J.P. and S.C. Shapiro, "A model for belief revision," *Artificial Intelligence,* 35, 25-79, 1988.

Pearl, J., *Probabilistic Reasoning in Intelligent Systems: Networks of Plausible Inference.* Morgan Kaufmann, San Mateo, California, 1988.

Smets, P. *et al* (eds), *Non-Standard Logics for Automated Reasoning,* Academic Press, New York, 1988. (Requires some background in mathematical logic and probability.)

AGENDA Tony Cox 9/24/90

1. Point by point review of projects

   a. Detailed modelling of benzene pharmacokinetics and metabolism
   b. Low-dose nonlinearity paper
   c. Benzene biology paper

2. Other API concerns
3. Data needs for the projects
4. Future directions for the projects
5. Meeting with EPA
(6. STELLA tutorial)

Detailed PB-PK Modeling of Benzene

Short-Term ("Engineering") Project Goals:

1. Show that *humans receive smaller internal doses than rodents* from the same administered dose of benzene.

- *Empirical motivation:* Occupationally exposed workers are less at risk than current models predict
- Internal dose ∝ AUC of metabolite in tissue
- *Technical approach:* (1) Experiments with "electronic" mice, rats, and humans. (2) Improve simulation methods.

2. Build a model to *quantify the internal dose to humans* from different exposure scenarios.

- This project will transfer to API working STELLA models for rats, mice, and humans
- Models should be refined as more benzene data become available (especially on specific metabolites)

Longer-Term (Research) Goals:

3. *Extend PB-PK modeling techniques* to capture future benzene data.

- Be prepared to use forthcoming data from Rich Irons and other sources
- Improve accuracy of internal dose predictions obtained by PB-PK modeling

4. *Review current PB-PK data for benzene* within context of BBRA framework

- Prepare to integrate PB-PK data and model into the BBRA framework needed to predict cancer risk.
- Advance the BBRA framework
- Main finding to date: Interaction of metabolism and cytotoxicity models in explaining myelotoxicity.

5. Prepare journal articles, reports, etc to "socialize" the BBRA approach to risk analysis.

- Cortes Report: *Risk Analysis*, Lewis Publishers/CRC

Progress as of 9/90: Engineering Side

1. "State-of-the-art" PB-PK models have been built for mice, rats, and humans.

- Uses ORNL (Travis) work. Lovelace data to be incorporated if possible.
- Quality assurance work is on-going
- Final *STELLA* versions to be delivered to API on disks. API should plan to purchase *STELLA* separately.
- Limitations of state-of-the-art models:
  - > All metabolites are aggregated.
  - > Diffusion-limited flow is ignored.
  - > Ignores interactions with marrow macrophages, other metabolites, etc.
  - > Key metabolic parameter values are estimated, not measured.
  - > Predictions are not always accurate.
- Model implications for low-exposure internal doses and man-mouse comparisons are being developed.

Progress as of 9/90: Research Side

2. Currently experimenting with simple models of bone marrow metabolism.

- Multiple metabolites and their interactions are tracked
- Interactions with macrophages not yet incorporated.
- Data and detailed cell kinetics/cytotoxicity model are needed to obtain practical results.

3. Mathematical framework has been suggested to improve the accuracy of benzene PB-PK modeling.

- Some new data requirements created for (i) Diffusion limited flows and (ii) Metabolic rates for benzoquinone and other metabolites.
- Provides a good platform for incorporating future data.

4. Simple, aggregate models that satisfy current data constraints are still being developed.

- Theory is being developed under WSPA uncertainty research project. (Ends 4/91)
- Example: "Pasic" linear compartmental flow models to efficiently approximate a more complex PB-PK model.

STATUS OF NONLINEARITY PAPER

Main Point: Previous studies of benzene dose-response relations do not have clear implications for the *low-concentration* health effects of benzene.

Current Status and Progress:

1. Some qualitative arguments have been documented more thoroughly and included in the *Risk Analysis* review paper.

2. The most convincing quantitative arguments probably require *examining individual animal tumor data*. These arguments have yet to be made.

- Most data to support irrelevance of data at exposures above 50 ppm for predicting risks below 10 ppm are circumstantial, but good PB-PK data are available.

3. General arguments and strong evidence for nonlinearities will be presented at the SRA meeting in October.

- Saturation, overwhelming, and depletion of intracellular enzyme systems; nonlinear pharmacokinetics, etc.

DATA NEEDS

1. Metabolic rate constants for liver

- Data for "missing arrows" in Medinsky *et al* paper
- Catechol production and conversion rates
- Algebraic forms of interactions based on enzyme kinetics

2. Bone marrow data

- Hydroquinone to benzoquinone interconversion rates
- Catechol to o-benzoquinone conversion rate *in vivo*
- Some constants might be estimated from liver data if *ratios of specific enzyme activity levels* were known. (But myeloperoxidase system is unique.)
- What happens to benzoquinone?

3. Pharmacokinetic constants

- Protein binding rates and partition coefficients
  - > Competetive and other interactions *in vivo*
- Metabolite partition coefficients
- Rate constants for tissue group clearance

POSSIBLE FUTURE DIRECTIONS

"Engineering" Side

1. Incorporate new metabolism and cytotoxicity data and models from Irons' lab starting in 1991.

2. Build initial detailed compartmental flow model of bone marrow metabolism and toxicity.

- Include emerging quantitative data, qualitative understanding, and enzyme kinetics calculations

3. Develop *fast simulation techniques* using modern compartmental flow methods.

- Better approximation theory
- System identification program to automate estimation of rate constants.)

"Research" Side:

1. *Statistical analysis* of individual animal tumor data.

- Support nonlinearity arguments
- Suggests roles for different metabolites, mechanisms, and sites
- Approach: Time-to-tumor analysis for multiple tumor sites.

2. Build initial version of cytotoxicity and benzene myelotoxicity model.

- PH/CAT/HQ/macrophage dynamics
- Disaggregate model of hematopoiesis
- Interface to PB-PK component

3. Develop stochastic simulation model for cell kinetics, cytotoxicity, and cancer transitions.

- Mathematical framework developed in WSPA project.

"Socializing" BBRA:

1. Lewis Publishers/CRC interested in a book on BBRA based on SRA short course.

- Enough solid material for a good monograph or text

2. *Risk Analysis* interested in *constructive* statistical methods complementing BBRA

- Key area for making BBRA more useful is to integrate it with data analysis methods

3. Discussions with EPA (?)