To: Mary Paxton
From: Tony Cox
Re: Status update on cyclophosphamide modeling and comparison to benzene
Date: 4-6-1993

In preparation for our meeting in Denver on April 29, I am pleased to provide this summary of our current effort on biologically-based risk assessment (BBRA) modeling of cyclophosphamide (CP). This memo also offers one basis for comparison of the CP model to potential similar work that might be undertaken for benzene later in the year if the BBRA approach for CP proves useful. The following questions are addressed:

o What is the overall "conceptual flowchart" for the BBRA model of CP?

o What is the current status of development for each of its modules?

o To what extent can the different modules be reused for other chemicals, especially benzene, and to what extent must the various modules and flows be developed (or customized) separately for each new chemical?

o What data are available to populate the different modules for CP? Are comparable data available for benzene?

These questions are addressed in the following sections.

1. Background and "Conceptual Flowchart" of the CP Model

As discussed in the original proposal, a primary goal of our cyclophosphamide (CP) modeling effort is to test the feasibility of applying the full biologically-based risk assessment (BBRA) framework, developed and proposed in previous years, to a relatively well-studied human leukemogen. If the data, modeling, or computational challenges prove too great even for CP, then it would probably not be prudent to expect useful short-term (1993-1994) benefits from a similar effort directed at benzene. On the other hand, a convincing demonstration that computer simulation modeling applied to existing data for CP can lead to useful insights and quantitative results about dose-response relations in humans would raise confidence that application of the approach to benzene might also yield valuable results. Thus, as suggested at our August meeting in Denver in 1992, completion of a full BBRA model for CP may help to provide timely information about the attractiveness of the BBRA

approach (as compared to other, less knowledge-intensive techniques such as Bayesian modeling or neural nets) for achieving practical dose-response models in the short term.

Completing a BBRA model for CP remains the main goal of our current effort. June still looks realistic as a completion date. A secondary goal that has emerged during the course of the research is to show how realistic scientific knowledge gaps and data uncertainties can be dealt with during the process of preparing and validating a BBRA model. Thus, not only should this effort enable the API to make a mid-year evaluation of the feasibility of carrying out the BBRA approach (i.e., can a plausible model incorporating biological information about all of the relevant processes in CP-induced leukemogenesis actually be built in a reasonable amount of time?), but also it should shed light on some of the practical aspects of using partial, incomplete, and possibly inconsistent information to draw sound and useful conclusions while still adequately expressing model and data uncertainties.

A full BBRA model for any chemical carcinogen includes the following components or "modules":

o Pharmacokinetics and metabolism (converting time series of administered doses to corresponding time series of internal doses of reactive metabolites at target sites).

o Cell kinetics and cytotoxicity (ideally, for target cell populations and other cell populations that regulate or affect them).

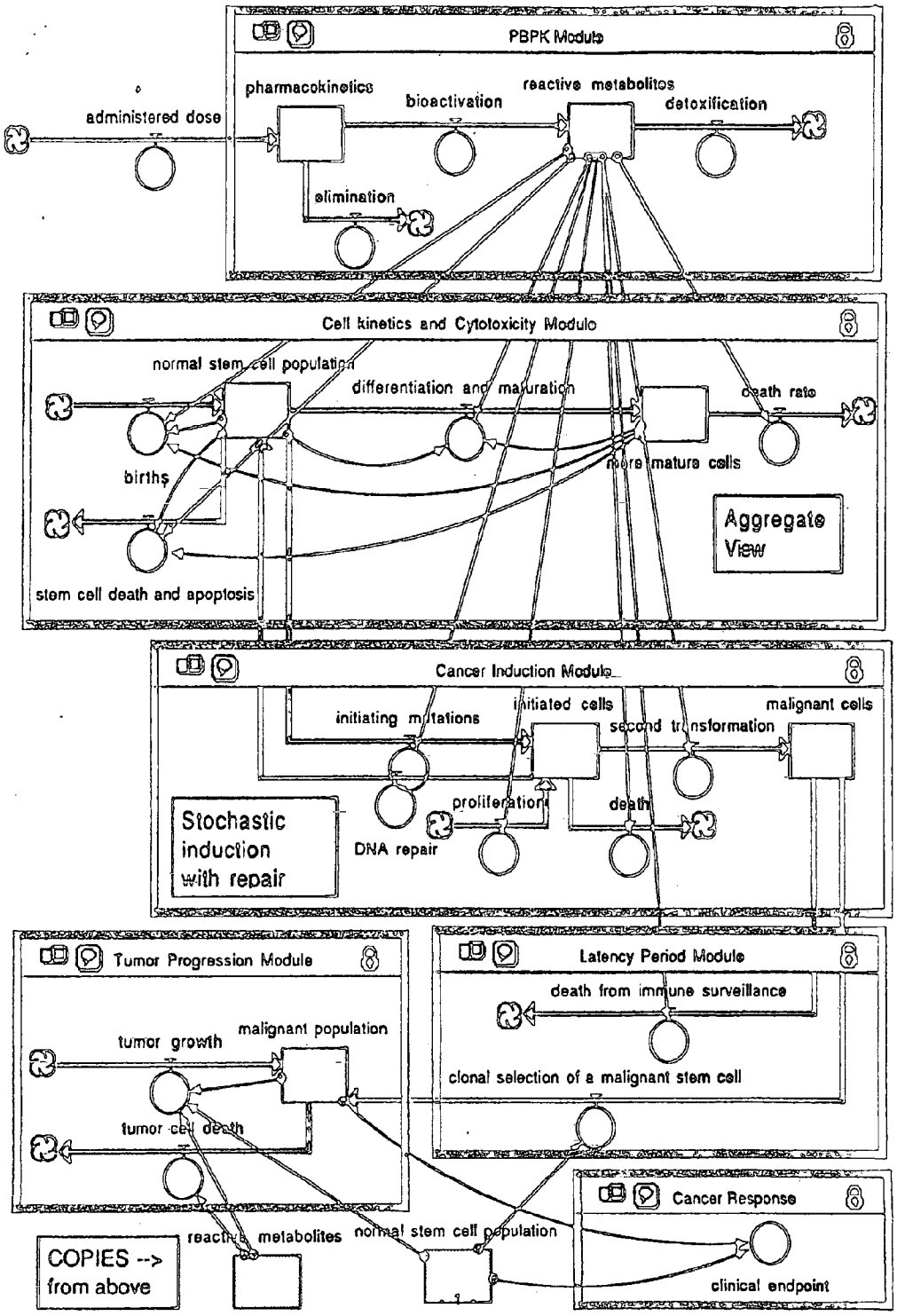
o Cancer induction (including stochastic inducing mutations and DNA repair).

o Latency period (describing the time between creation of a malignant stem cell and its clinical expression as a tumor. This module must also describe spontaneous extinction of malignant populations and the effects of continued exposure on the probability distribution of the time-to-tumor).

o Tumor progression and growth.

Figure 1 is a conceptual "flow chart" showing at a high level how these modules can be fit together to form a computational model of carcinogenesis for use in biologically-based risk assessment. The reactive metabolites (possibly also including the parent compound in some cases) resulting from the administered dose time series are shown as potentially affecting the

FIGURE 1: Conceptual Flow Chart of the BBRA Framework for Computational Dose-Response Modeling



carcinogenic process in at least the following nine ways (indicated by the thin "information arrows" running from the "Reactive Metabolites" compartment to various cell flow and transition rates, which are represented by circles attached to the thick arrows indicating flows or transitions into or out of compartments. Compartments are indicated in the diagram by rectangular boxes.)

o Carcinogens (typically, reactive metabolites of the administered compound) may affect proliferation of stem cells. This possibility is indicated in Figure 1 by the left-most information arrow directed from the "reactive metabolites" compartment to the "births" inflow arrow into the "normal stem cell-population" compartment.

o Carcinogens may alter the rate of stem cell death and apoptosis. Since the stem cell compartment helps to regulate its own birth and death rates (as shown by the feedback information arrows from the "normal stem cells" compartment to the two leftmost flow arrows in the cell kinetics/cytotoxicity module), damage to the stem cell compartment may cause very immature hematopoietic progenitor cells (HPCs) to be prematurely recruited into active proliferation. This response, recently discussed in relation to chemical-induced leukemogenesis by Irons and Stillman (1993, forthcoming), is included in the current model but can not be seen at the high level of aggregation shown in Figure 1. To show such effects, it is necessary to "zoom in" on the stem cell compartment to reveal its subcompartments (e.g., the resting and cycling subpopulations and cells in different stages of the cell cycle), as shown in Figures 2 and 3.

o Carcinogens may alter the differentiation and maturation kinetics of normal stem cells, e.g., by interacting with normal growth factors to make cells that would normally not respond differentiate and/or proliferate prematurely. Recent evidence from benzene metabolites and other secondary leukemogens suggests that such interactions with growth factors, leading to altered kinetics of hematopoietic progenitor cells (HPCs, contained in the aggregate cell compartments in Figure 1) may be a common feature in chemically-induced secondary leukemogenesis (Irons, 1993; personal correspondence).

o Carcinogens may act as alkylating agents or exert other genotoxic damage, contributing to the flow of "initiating mutations" linking the normal stem cell population compartment in the Cell Kinetics and Cytotoxicity module to the initiated cells compartment in the Cancer Induction Module. This is the classical mechanism of carcinogenesis assumed in the multistage model. It is likely to be an appropriate description of at least part of CP's

FIGURE 2: Detail of the Granulopoiesis Sub-Model in the Cell Kinetics and Cytotoxicity Module

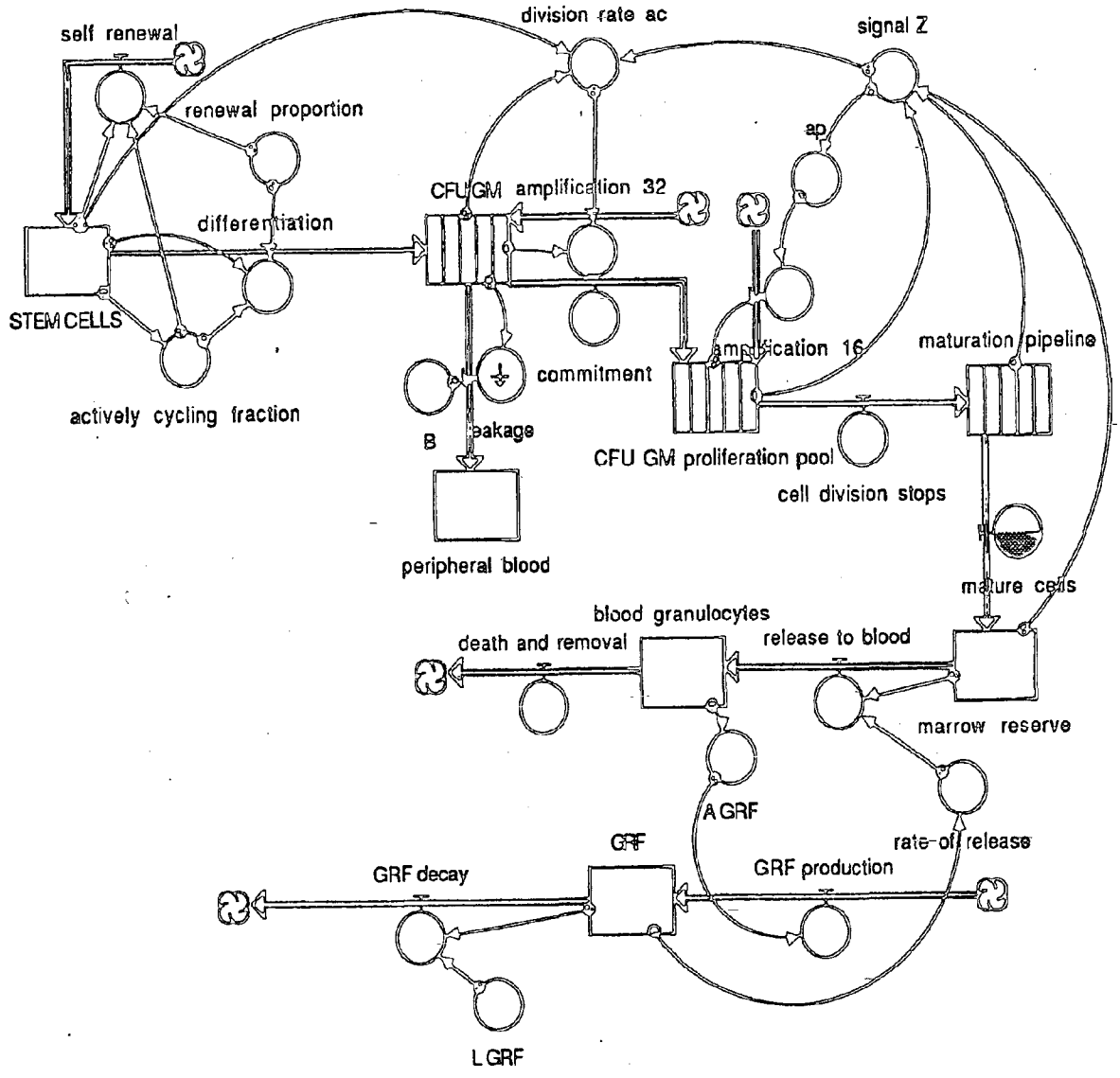
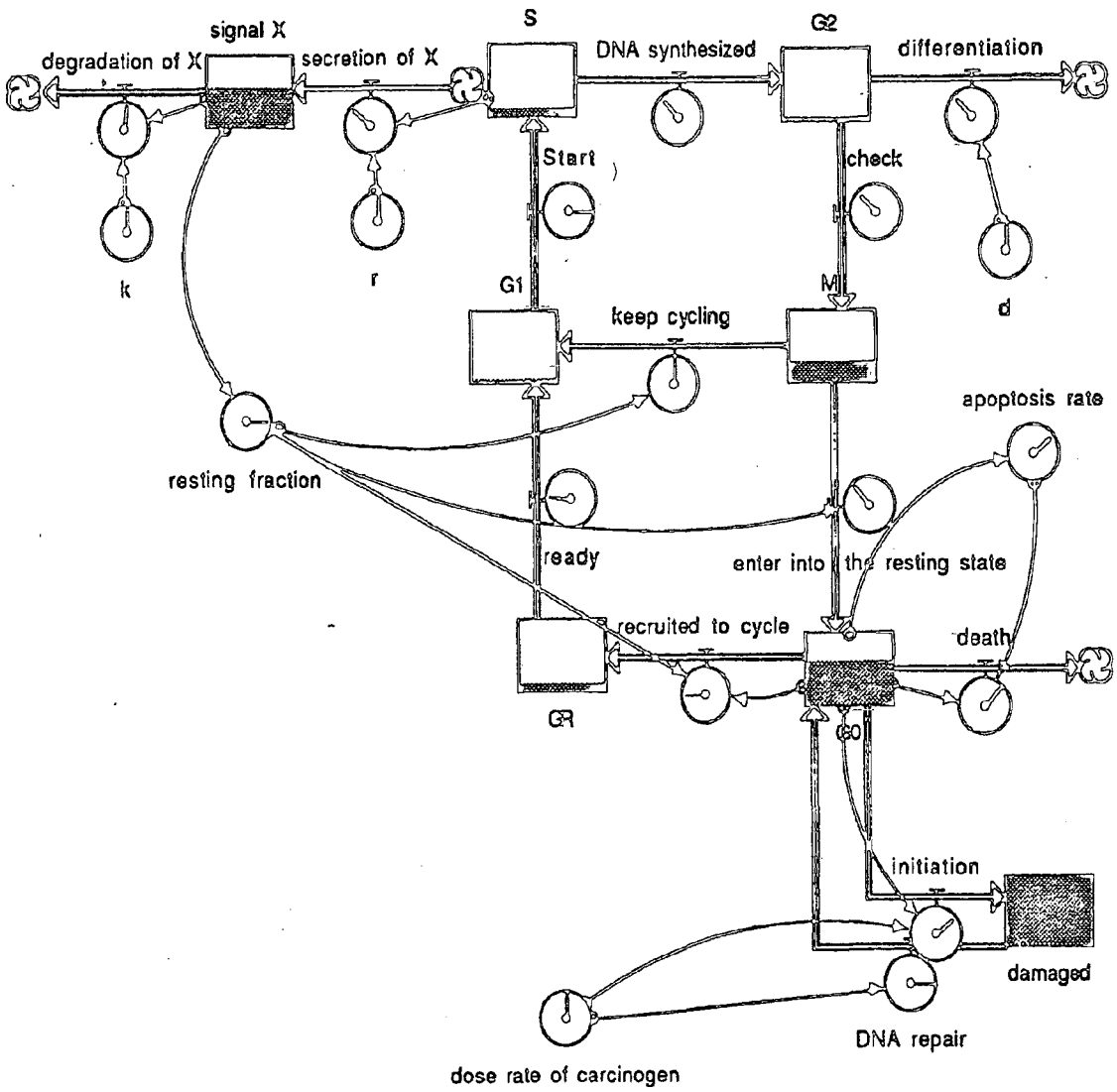


FIGURE 3: Detail of the Stem Cell Compartment in the Granulopoiesis Sub-Model



mechanism of carcinogenic action since the metabolite that is thought to be responsible for much of CP's therapeutic action (namely, the aziridinium ion of the CP metabolite phosphoramide mustard) has a bifunctional alkylating activity that allows it to cross-link guanine residues on the two strands of DNA in a cell, making the cell non-functional in most cases.

o Carcinogens may act as mitogens, not only for normal stem cells (which would increase the flow of initiating mutations, other things being equal), but also for initiated cells, i.e., cells at risk of malignant transformation. Direct mitogenic action, e.g., due to synergy between a metabolite and a growth factor in recruiting resting stem cells to cycle ("push") may add to the indirect effect of cytotoxity in down-stream compartments that becomes translated into increased stem cell activity by the feedback control loops in the hematopoietic system ("pull"). The MVK model enables such proliferative effects to be accounted for TCDD and other carcinogens.

o Carcinogens may affect the death/differentiation rates of initiated stem cells, e.g., by creating adducts that block the normal actions of tumor-suppressing or apoptosis-inducing gene products.

o "Completor" carcinogens may increase the rate of malignant transformations of previously initiated cells.

o Carcinogens may reduce the effectiveness of the immune surveillance system that normally screens for tumor cells, thus increasing the "effective" transformation rate of malignant transformation.

o Many carcinogens may also affect tumor growth rates, changing the time until a malignant cell proliferates to become a clinically detectable tumor. (Indeed, for example, this is precisely why CP has been used in cancer chemotherapy. Tumors may also affect pharmacokinetics, although that information path is not shown in Figure 1.)

These multiple mechanisms of carcinogenesis are no longer purely speculative. Within the past two years, examples and evidence supporting each as a contributor to some forms of cancer have been presented in the peer-reviewed literature. Our comprehensive model of chemical carcinogenesis makes it possible to represent and simulate all such mechanisms.

The diagram in Figure 1 represents the first stage of an iterative modeling process for creating and validating a computational dose-response model of carcinogenesis for use in BBRA. The input to the model is a time series of administered dose (upper left-hand corner). Its output is a time-to-occurrence of a clinical endpoint (e.g., time until the fraction of leukocytes in circulating blood exceeds a threshold that defines the leukemic state) (lower right-hand corner). Randomness intervenes between input and output in the Cancer Induction Module (random initiations, births, deaths, and second transformations) and in the Latency Period Model (random selection of stem cells to undergo clonal expansion). To complete the model, each module must be refined and populated with empirical data and formulas showing how its input values propagate into its output values. Model uncertainties about the structure of the diagram and the correct value-propagation formulas, as well as data uncertainties about the values of specific parameters, must be addressed by using empirical relations, assumptions, or approximations to close the essential knowledge gaps. Then, sensitivity analyses obtained by running the completed model can be used to show where improved information would be most valuable in increasing the precision and confidence of the estimated input-output (i.e., dose-response) relation.

2. Status, Data and Re-usability of the Different Modules

The general framework in Figure 1 must be specialized for any specific chemical. While the overall structure and the mass-balance flow equations relating the compartments and flows in the model may be re-used for any chemical carcinogen, the parameter values and flow rate formulas must be changed for each chemical. More importantly, the detailed structures of the modules (i.e., the detailed diagrams found by zooming in on the aggregate building blocks) mayu be very different for different chemicals. The remainder of this memo sketches our progress on each of the modules.

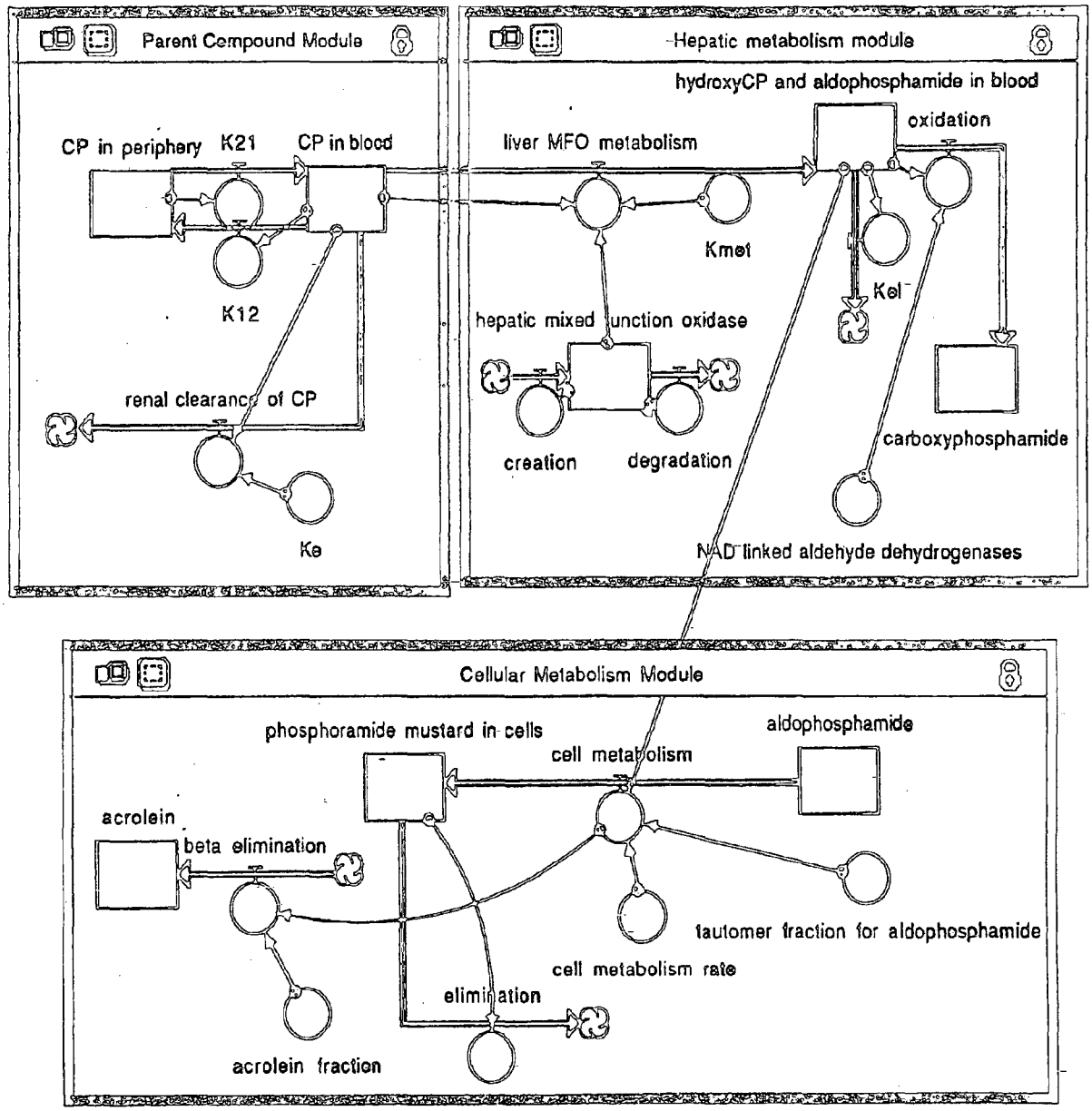
o Pharmacokinetics and metabolism. The pharmacokinetics and metablism of CP have been well studied and excellent data for building a model with circulating metabolites are available (see e.g., Sladek, 1988). For many compounds, including benzene and isophosphamide (an oxazaphosphorine closely related to CP), metabolic saturation is achieved at physiological doses, making it necessary to use nonlinear formulas based on enzyme kinetics to describe the conversion of administered doses into time courses of carcinogenic metabolites. For CP, matters are simpler: metabolism and pharmacokinetics of CP and two of its principle metabolites (4-hydroxyCP and phosphoramide mustard) in man appear to be approximately first-order (linear), making it possible to describe the time courses of blood plasma

concentrations by a simple compartmental flow model (Cohen et. al., 1971; Sladek, 1988). Figure 4 shows the structure of the model. This module is currently complete, pending any further refinement following model validation. It can be used as a stand-alone module to simulate the time course of metabolite concentrations in response to any administered dose. By contrast, for benzene, a more elaborate PBPK model (or, at best, a three-compartment model) is required to accurately simulate human pharmacokinetics of the parent compound in blood (corresponding to the Parent Compound Module in the upper left hand corner of Figure 4). Circulating metabolites of benzene have been represented for PBPK models of Fisher rats, but are not yet available for analogous models for humans.

o *Cell kinetics and cytotoxicity*. Biotransformation of CP in the liver produces 4-hydroxycyclophosphamide (a possible transport form), which may be further metabolized by target cells (via beta-elimination of the highly toxic acrolein) to form the cytostatic and cell-killing reactive metabolite phosphoramide mustard (PM). Data are presented by Sladek (1988) for rodents and humans, showing the cytotoxic potencies of acrolein and PM in different cell populations (percent cell kill per hour at different concentration levels of the parent compound, with percent of cell kill attributable to each metabolite). These data have been incorporated into a cytotoxicity and cell kinetics module customized for CP. The linkages between the Cellular Metabolism Module of the pharmacokinetics module shown in Figure 4 and the cell kinetics and cytotoxicity are being finalized based on these data. Since cell-kill in each target population is proportional to the AUC of the metabolite (Sladek, 1988), the linkages from metabolism to cytotoxicity are relatively easy to make for CP. The task is further simplified by the fact that we can concentrate on damage in the granulocytic lineage (Steinbach et. al., 1980). By contrast, benzene and its metabolites have more confusing cell-level dose-response curves and exert profound effects on the erythroid as well as the granulocytic cell populations. Thus, a more complicated cell kinetics and cytotoxicity model would be required for benzene. However, some relevant data and a useful start have been provided already by Scheding et. al. (1992) for benzene.

o Cancer induction. A stochastic cancer induction model adapted from Portier and Kopp-Schneider (1991) has been completed and is being customized for CP based on the simplifying assumption that phosphoramide mustard (PM) is the direct alkylating agent of principle interest. The potency (initiating mutations per cell per unit time) is being estimated using heavily processed (and somewhat confusing) data presented by Dedrick and Morrison (1992). Effects of CP metabolites on DNA repair rates are unknown and are assumed to be negligible (a simplification which probably won't hurt are estimates even if it is incorrect, as

FIGURE 4: Detail of the Pharmacokinetics and Metabolism Module, Customized for Cyclophosphamide



A simplified scheme of cyclophosphamide (CP) metabolism and pharmacokinetics, including its principle circulating metabolites and showing only the most important compounds.

(SOURCE: Adapted from Sladek, 1988)

this would induce a compensating error in the estimated mutation potency). This module is not yet complete, as some of the data sources are still on order; however, it should be finished (in at least preliminary form) by April 29, and may even be integrated into the rest of the model by then.

o Latency period and tumor progression. We are experimenting with a model of stress-induced clonal succession (in which a malignant stem cell becomes expressed only if selected for clonal expansion), based on empirical data modeling by Guttorp et. al. (1988). The time until a malignant cell starts to undergo clonal expansion accounts for part of the random latency period. Simple models of leukemia dynamics based on competition between normal and malignant cells are also being investigated. The progression of a secondary tumor in a patient may be strongly affected by the presence of a prior tumor, and we are not attempting to model this interaction, since it seems of limited interest in predictive cancer modeling for general populations.

The latency period and tumor progression modules will be the last ones completed. The current target date for completion and integration (which is expected to be easy, given the lack of strong linkages to previous modules) is May 20th. This will leave us on schedule for an mid-to-late June deliverable of the final model and report.

Selected References

(Of over 100 articles and papers surveyed in connection with cyclophosphamide and secondary leukemogenesis and MDS, the following few are the most useful. Of these, the single most informative one is by Sladek, 1988.)

Cohen L.L., J.Y. Jao, aand W.J. Jusko, "Pharmacokinetics of cyclophosphamide in man", *British Journal of Pharmacology*, 43, 677-680, 1971.

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Guttorp, P., et. al., "A stochastic model for hematopoiesis in the cat," *IMA Journal of Mathematics Applied in Medicine and Biology*, 7, 125-143, 1990.

Irons, R., and W.S. Stillman, "Cell proliferation and differentiation in chemical leukemogenesis," forthcoming in *Stem Cells*, 1993 or 1994.

Patel, J.M., "Metabolism and pulmonary toxicity of cyclophosphamide," *Pharmac. Ther.* 47, 137-146, 1990.

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Scheding, S., Loeffler, M., et. al., "Hematotoxic effects of benzene analyzed by mathematical modeling," *Toxicology*, 72, 265-279, 1992.

Sladek, N.E., "Metabolism of oxazaphosphorines", *Pharmac. Ther.* 37, 301-355, 1988.

Steinbach, K.H., et. al., "A mathematical model of canine granulocytopoiesis", *Journal of Mathematical Biology*, 1-12, 1980.

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April 8, 1993

Dr. Mary Burr Paxton
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Dear Mary:

Thank you for your participation in the meeting last month with EPA to discuss health effects of benzene in the context of the Agency's study on mobile source air toxics. Based on our discussions with the Human Health Assessment Group on predicting risk from low level exposure to benzene, it would seem the Agency may be receptive to concepts for incorporating biologically based mechanisms into their dose response model.

Since API has a project on the biological basis of chemical carcinogenesis using benzene as a model compound, there may be opportunities for collaboration between our associations.

At least one AAMA member company has conducted extensive experiments and analysis on metabolic transformation of benzene, cytotoxic effects, genotoxic effects of benzene metabolites and epigenetic effects of benzene. Other AAMA companies have considerable expertise in mathematical modeling techniques used to predict low level risks of exposure to chemical carcinogens. And all AAMA companies are interested in better understanding the biological basis and mechanisms of action inherent in chemical carcinogenesis.

Benzene is of particular concern because it is both a mobile and stationary source pollutant which even at very low levels of emissions is frequently calculated by regulatory agencies to represent a significant level of risk. In addition to potential public health concerns, there are very practical issues of control technology, regulatory standards, cost, etc., which make it imperative to better understand the true risk of benzene exposure. If a more scientifically credible, biologically based mechanism can be developed for understanding potential adverse health impacts and risk of benzene, both the public health concerns and regulatory challenges will be served.

Therefore, AAMA is proposing API consider a collaborative effort to develop a more biologically based, mechanistic model for predicting benzene risk at low levels of exposure. This collaboration could take the form of shared funding and/or joint efforts in research collaboration among scientists in our respective industries. A first step would be to have a meeting of interested scientists and representatives from our associations and member companies or consultants.

Please call me with your thoughts on this proposal and perhaps we can proceed to arrange a meeting of interested individuals.

Sincerely,

Richard T. Paul

Richard T. Paul
Manager, Environmental Health
Technical Affairs Division

RTP/