

From: George Woodall <Woodallg@api.org>
Sent: Friday, September 21, 2001 7:54 PM (GMT)
To: 'ralberti@zoo.uvm.edu'; 'john.cherrie@abdn.ac.uk'; 'helmut.greim@lrz.tu-muenchen.de'; 'herrick@hohp.harvard.edu'; 'rlarson@medicine.bsd.uchicago.edu'; 'guilanli' <guilan_li@sohu.com>; 'minden@uhnres.utoronto.ca'; 'nmueller@epinet.harvard.edu'; 'rice@iarc.fr'
Cc: 'Richard Irons (E-mail)' <richard.iron@uchsc.edu>; 'Robert Schnatter Ph. D. (E-mail)' <arschna@fpe.erenj.com>; 'Otto Wong (E-mail)' <OttoWong@aol.com>; 'Thomas Armstrong (E-mail)' <twarmst@erenj.com>; 'BenzConsort-TC@listserve.api.org' <BenzConsort-TC@api.org>
Subject: Additional Review Material
Attach: Albertini-1.doc; Albertini-2.doc; Li.doc; Larson.doc

Members of the SRP and Other Participants in the Protocol Review,

Attached are additional sets of comments from Richard Larson, Richard Albertini, and Guilan Li. If you are a member of the SRP and are providing a set of comments but have not done so yet, please forward them to me ASAP. I will also be posting all sets of comments and presentation materials to the web site for this project on Monday or Tuesday, so they may also be accessed via the internet. The protocols are already on this same web site, which is password-protected:

Address: | REDACTED
Username: ~ REDACTED
Password: | REDACTED

We currently have the following slate of participants:

In Chicago

Richard Larson - SRP, Hematology
Guilan Li - SRP, Genetic Toxicology
John Cherrie - SRP, Industrial Hygiene
Patrick Beatty - Technical Committee Chair
Shan Tsai - Technical Committee Vice-Chair
Richard Irons - Principal Investigator
Otto Wong - Principal Investigator
Robert Schnatter - Principal Investigator
Thomas Armstrong - Principal Investigator
George Woodall - Moderator, API
Dawn Hebron - Facilitator, API

Via Phone

Nancy Mueller - SRP, Epidemiology
Jerry Rice - SRP, General Toxicology
Richard Albertini - SRP, Genetic Toxicology
Robert Herrick - SRP, Industrial Hygiene

Contact me if you have any questions.

Thank you,

George M. Woodall, Ph.D.

Senior Toxicologist
American Petroleum Institute
1220 L Street, NW
Washington, DC 20005

Tel. (202)682-8067

Fax. (202)682-8031

Visit the New www.api.org.

Review of the API-Sponsored Benzene Health Research Project

Reviewer: Richard A. Larson, M.D.
The University of Chicago

September 26, 2001

General Comments

This is an ambitious proposal that consists of 3 separate but inter-related projects to be conducted over 5 years in Shanghai, China, an area where benzene-associated hematotoxicity is still an occupational hazard. The primary objectives are: 1) a risk assessment for developing bone marrow failure or a hematologic malignancy by the population in Shanghai using a case-control study, 2) a determination of the exposure-response relationship between these diseases and benzene and other suspected hematotoxins and carcinogens, 3) a detailed clinical and biological assessment of the progression of these bone marrow failure/neoplastic states using state-of-the-art diagnostic methods, and 4) a molecular epidemiological study of workers known to be exposed to high benzene concentrations.

This prospective, laboratory-based, clinical epidemiological study has the potential to elucidate major mechanisms of leukemogenesis in humans. The investigators and their collaborators in Shanghai have experience in similar smaller studies. The laboratory and diagnostic methods are well described in the appendices. The diagnostic laboratory to be established and equipped in Shanghai will be an important benefit to clinical investigators and physicians at Fudan University Medical Center.

Nevertheless, the proposal, as it is currently written, has major difficulties. A single overview section is sorely needed to differentiate between the various cases that are being sought and the different control groups in the 3 projects. These are described as "parallel" investigations, but some cases may opt in or out of various components of the individual studies. It is far from clear whether the cases in Dr. Wong's proposal will also be involved in Dr. Irons' proposal. It does not appear that the controls for Dr. Wong's study will be the same as the controls for Dr. Iron's disease progression study. There is no description of the coordination that will be required with regard to consent forms for a single patient to be entered onto 2 different studies, for example. Dr. Irons' proposal describes collecting information about prior infections with hepatitis virus or HIV whereas Dr. Wong's specifically excludes this information.

I suggest that the Research Project be revised using the format of an NIH Program Project with 3 (or 4) clearly demarcated projects with separate specific aims, but also, most importantly, 1 or 2 cores to provide the access to patients and control subjects and to share the laboratory methodology. The interactions

between projects and cores must be clear for the most efficient utilization of resources.

The nomenclature needs to be standardized; Dr. Wong's proposal uses the term acute nonlymphocytic leukemia (ANLL) which is no longer used to describe acute myeloid leukemia (AML). The newly published World Health Organization Classification of Tumours (Pathology and Genetics; Tumours of Haematopoietic and Lymphoid Tissues, 2001) should be used exclusively. The lack of involvement in this proposal by a clinical investigator and/or hematopathologist with expertise in leukemia is apparent in the lack of detail given about diagnosing cases of "ANLL and NHL" in the Wong project. The boundary between myelodysplastic syndromes (MDS) and AML are really quite indistinct and are subject to considerable observer variability. In addition, as indicated in the WHO Classification, the definitions of MDS and AML are dynamic and have recently changed.

Specific Comments:

1. Both case-control studies may have underestimated the accrual. The incidence of AML in the USA is 2-5/100,000 in this age group (>18 years old) and is increasingly common in patients over the age of 50 years. In Shanghai, a city of 15 million people, there should be 300-700 cases of AML per year in addition to perhaps an equal number of patients with MDS. What are the actual most recent registration data from the 15 Shanghai hospitals for AML and MDS and NHL? Most cases of NHL and MDS do not require hospitalization, but diagnosis and treatment is entirely out-patient. How will these cases be captured?

Disease Progression Project:

1. This proposal would be less confusing if there were a more clear demarcation between the case-control study with newly diagnosed cases of MDS and AML and the disease diagnosis and progression objectives that will include previously identified cases of benzene poisoning (BP) and "mild cases of aplastic anemia (AA) and MDS" that will not be enrolled until "they progress".
2. The eligibility excludes patients >75 years old based on an expected latency of 15 years. This is a potential bias since therapy-related AML is strongly age-related, some people continue to work or be exposed beyond age 60, and there is little evidence in support of a 15-year limit on late effects of hematotoxins.

3. Patients with “heritable conditions” are excluded. Does this include thalassemia, for example? There seems no obvious rationale for the various diagnoses that have been included and excluded from the cases or the control group. Why must the controls have cancer? Patients with cardiovascular, respiratory, or orthopedic diagnoses might provide a better control group. Will there be one or two controls per case? Why will there be no controls for the BP cases?
4. The comment is made that “exposure assessment will be conducted blindly.” This seems unlikely when the subject is a case getting AML therapy.
5. A strong case should be made for including hepatitis viruses and HIV in the clinical screen. A current hypothesis also implicates CMV in the etiology of MDS. Certainly, serum should be collected and frozen for later analysis should other viruses (HHV6, HHV8, animal viruses) be found to be important for the etiology of leukemia.
6. Cytogenetics should be performed on bone marrow aspirate material. The aspirate should NOT be obtained through the biopsy core hole but rather from a fresh puncture site. The core biopsy should only be used if patients are inaspirable. At least 20 metaphase cells must be examined. A consensus panel of experienced leukemia/lymphoma cytogeneticists should review abnormal findings. Cytogenetic analysis of peripheral blood cells on normal subjects is unlikely to be productive unless lymphocyte mitogens are used, and even then, their applicability to myeloid malignancies is questionable.
7. It is stated that BP, AA, and MDS cases will be followed up every 6 months, apparently with complete bone marrow exams and cytogenetic analysis. This is important, but should also include re-evaluation whenever there appears to be clinical change, e.g. 3 months after the last assessment if blast cells begin to appear in the peripheral blood. Otherwise, some cases will be lost before the next 6-month timepoint. The AML cases should also be followed, at least for clinical outcome to treatment and survival. All subjects should be followed until death or for at least 10 years.
8. It must be made more clear that the patients’ physicians will receive a full report of the diagnostic studies performed in the central laboratories so that this information can be used in treatment planning.

Molecular Epidemiology Project

1. The same comments about cytogenetic analyses apply here.

2. Buccal swabs for DNA should be obtained as back-up for those subjects where a lymphoblastoid cell line cannot be established or it fails to grow after being thawed.
3. A registry of all eligible subjects should be maintained since only "selected workers will be invited to participate". Reasons for lack of participation should be noted.

Benzene Health Research Project

Reviewer: Guilan Li, Ph.D.
Institute of Occupational Medicine, CAPM, Beijing, China
Date: 18 September 2001

General Comments:

As one of the original researchers of the China-NCI benzene study, I am very please to see that this Benzene Health Research Project is being conducted. I believe these proposed studies could have a major public impact not only to the people in Shanghai but also to the whole world. These studies include cell type specific analyses that could provide new information regarding these diseases and could solve the unsolved issues of the previous studies. In addition, I believe all three studies are feasible and practical.

Based on my experience, we should always remember that benzene is a carcinogen, although a weak carcinogen. So that the number of cases included either in the case-control study or in the disease progression project is the foundation to the success of these studies. I think it would enhance the statistical power and study validity by adding the essential cases of ANLL and NHL for the past 4 years in Shanghai as well as the essential cases of AA, MDS, AML and benzene poisoning (BP). The increased number of cases would strengthen the exposure – response analyses. We need to be sure there are enough number of cases in different benzene exposure levels for the exposure-response analysis.

I suggest that an abstract should be included in each of the protocol. The abstract would make it easier for readers to understand the main point of the protocol. Are there any relationships among these three protocols? In fact, all three projects are relatively independent and not related to each other.

Specific Comments:

1, Case-control studies of acute non-lymphocytic leukemia and non-Hodgkin's lymphoma in Shanghai

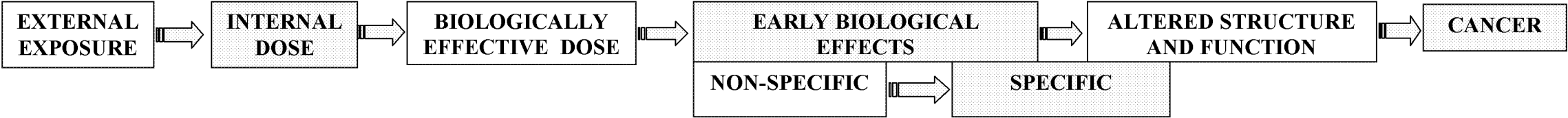
This is an important study to address whether benzene exposure causes these diseases. The study design is a prospective case-control study and some of the eligible cases will also be in the parallel disease progression project. The objectives, reasons for choosing Shanghai and, especially, the potential benefits for Shanghai are described very clearly. Shanghai is the lucky city to have been selected. It would be informative to explain why the study is designed as a prospective case-control investigation. What are the benefits of this study design as compare to the retrospective case-control study? For reaching the exposure-response objective, please describe the individualized exposure assessment as

detail as possible. Although the description the exposure assessment seems to be adequate but how do you handle those cases that the exposure assessment can not be established based on exist job title, location, material, products and so on.

2, Molecular epidemiology of benzene-exposed workers in Shanghai

First, it is a pleasing surprise that the measurement of benzene metabolites in bone marrow and the validation of possible marker between bone marrow and peripheral biomarker can be done. The possibility and feasibility of this work should be described in detail. The relationship between benzene toxicity and carcinogenicity in bone marrow is essential, especially in China. In addition, all subjects should be given a diagnostic test. One of the most important issues is that we need to make sure there are enough subjects to be included for this study.

The protocol, including objectives, background and rationale for the study, is well written. For ease of reading and understanding of the protocol, the subtitle such as Objective, Background, rationale, Health significant, Experimental design could be rearranged.



PHASE 1 (1,000 Subjects)	Work Records					WBC Hbg
PHASE 2A (1,000 Subjects)	Work Records Personal Monitoring	Bz in Inhaled Air		Chrom. Abs. (Blood)	FISH (Blood)	CBC
PHASE 2B (200 Subjects)	Work Records Personal Monitoring	Bz in Inhaled Air Hydroquinones Catechols Blood/Bone Marrow S-PMA-Urine (*Phenotypic Analysis: Chlorzoxazone Hydroxylation Genotype: CYP2E1, NQO1, GSTT1)		Chrom. Abs. (Blood/Bone Marrow)	FISH (Blood/Bone Marrow)	CBC
Smith, 1992 (Refs. 1-6)	Sample Personal Measurements (Mean = 31 ppm TWA) (Range = 1-328 ppm) (High Dose) 150 Controls 150 (0.5 <1 ppm) 100 (1 <10 ppm) 50 (≥10 ppm)	tt-MA↑ S-PMA↑ (Urine) Phenol↑ (Urine)	Quinone and Benzene Oxide Adducts - Hbs - Serum Albumin	↑ GPA NN Variants	FISH Specific Leuk. Abs. RT-PCR t(8;21) - Blood (No ↑ MN) (No ↑ Interphase FISH in Blood)	Lymphopenia
Li, 2001 (Refs. 7, 8)	Mean 11.1 ± 21.8 ppm TWA (Genotype: CYP2E1, NQO1 & MPO)	tt-MA↑ S-PMA↑ (Urine) Phenol↑ (Urine)	BO-Alb↑ 1,4-BO Alb↑	CA↑ (Blood)		RBC↓ WBC↓
SUSCEPTIBILITY						

REVIEW OF AMERICAN PETROLEUM INSTITUTE-SPONSORED BENZENE HEALTH EFFECTS PROJECT

Reviewer: **Richard J. Albertini, M.D., Ph.D.**
 Research Professor, Department of Pathology
 University of Vermont, College of Medicine
 Burlington, VT 05401 USA

Date: **19 September 2001**

OVERALL COMMENTS

The proposed research projects are all well-explained and include a wealth of biological information. The applicants clearly know the current state of the understanding of leukemogenesis. Rationales are presented for pursuing the various components of this research project. Some of these are quite persuasive, others less so. The overall research project is massive and expensive. Review, therefore, must be rigorous with attention given to comparable parallel or recent-past studies of benzene exposures on humans. The value of the information that will likely be gained and the cost effectiveness of obtaining this information through the research as proposed must be carefully weighed. Shanghai remains a place to study benzene exposure of humans because occupational exposures were in the past and probably remain widespread. It must be understood, therefore, that most investigators studying human exposures to benzene will concentrate on human populations in this region. A vast research project such as this will, in essence, take up all of the subjects for study in this region. The amount of information that can be gained from a large study such as this must be maximized so that the denial of these populations to others will be clearly balanced by the amount of information to be gained.

Specific critiques of each component and subcomponent of this overall research project follow. I have made my most thorough review of the proposed molecular epidemiology study. Most of my suggestions for change focus either on this study or on components of other studies that are related to it.

PROTOCOL I - Analysis of Disease Progression for Aplastic Anemia (AA), Myelodysplastic Syndrome (MDS), Acute Myelogenous Leukemia (AML) and Benzene Poisoning (BP) in Shanghai, China

Description

This study will extend for either five or four years (P. 15 says 4; elsewhere it is stated to be 5). Assuming a five-year study, approximately 250 new cases of AA, 300 new cases of MDS, 600 new cases of AML and 50 new cases of BP will be enrolled. This will be the total number of patients with these diseases or disorders in the Shanghai region. Extensive morphological, cytogenetic and molecular analyses will establish the correct diagnosis (in current classification) in this continuum of disorders and identify specific features (molecular, cytogenetic, etc.) that

characterize early disease. These analyses will be conducted at a joint clinical and molecular laboratory (JCML) that will be developed in Shanghai for this purpose. All cases of non-Hodgkin lymphoma (NHL) that present in any of the Shanghai study hospitals will also be enrolled, with appropriate pathological material sent to the JCML for the proper differential diagnosis. It is anticipated that approximately 500 cases of NHL will be enrolled and characterized for an accompanying case control study.

The AA, MDS and BP patients will be followed at approximately six-month intervals with complete evaluations including laboratory to identify current health status and progression of disease. Progression will be characterized as to rate of progression from these various subdisease categories and clinical cytogenetic or molecular features of the progression. It will be determined if AML secondary to benzene exposure can develop in humans in the absence of preceding AA or MDS. This will also be determined for non-benzene related AML. Any unique features of benzene related diseases will be characterized with an eye to differentiating those features that distinguish benzene related from non-benzene related hematological disease. Prognosis for all of the diseases of concern will be defined according to cytogenetic and molecular subtypes and benzene exposure. In addition to serving the purposes of this study, the diagnostic capabilities of the JCML will be used to further clinical management of these patients.

Benzene exposure assessment for all study subjects will be made by retrospective analyses of work and other records. This retrospective analysis will be supplemented by personal exposure measurements, but details are not given for this. The several diseases of interest will be characterized as benzene related or non-benzene related based on this exposure assessment. This will primarily be accomplished by a case control study that is incorporated within and will serve as a foundation for the overall disease progression study. For this case control study, each hospital-based case of BP, AA, MDS and AML will be enrolled as cases. The accrual number should be approximately the number of new cases encountered as noted above. One hospital-based control individual, matched for age and sex and other factors, will be enrolled for each study subject. Benzene exposure assessment will be as rigorous in the controls as in the cases. The purpose of the case control study is to compare dose response patterns for the different benzene related hematological disorders as well as to define those diseases related and those diseases unrelated to benzene.

The disease progression portion of this study is a descriptive analysis of cases. The case control portion is a quantitative analysis of benzene effect on producing the diseases in question. The extensive morphological cytogenetic and molecular analyses of pathological materials will determine the categories and subcategories of diseases to be related to the benzene exposure.

Critique

This is truly a massive undertaking. Virtually every case in the continuum of diseases from benzene poisoning to AML that occurs in the Shanghai region over a probable five-year period will be included in this study. The descriptive aspect of the study goes far beyond studying benzene exposure as it will attempt to define the pathogenesis of non-lymphocytic leukemia in humans. Other forms of secondary leukemia other than those due to benzene exposure are also

being studied. A good deal of descriptive and mechanistic information may result from this study. The disease progression portion of Protocol I is certainly the core of the other studies that will be pursued.

I am quite enthusiastic about the case control study that is incorporated into this protocol but am quite confused as to its relationship to an independent case control study also described as part of the overall project. The case control study presented here and that described later are overlapping but not congruent. This seems like a rather awkward way to conduct these studies and leads to many questions. I mention these below in my critique of the case control study of Wong and Hua.

The benzene poisoning (BP) patients that will be enrolled in this study constitute a unique source of materials for the study of biomarkers of genotoxic effect that may be predictive of subsequent disease outcomes. Although biomarkers are considered in an accompanying molecular epidemiological study, these BP patients, although few in number, should really be studied further with molecular epidemiological techniques. Blood and bone marrow, properly fractionated, should be archived for a later study of early and intermediate biomarkers of effect, both specific and non-specific. Archiving will entail cryopreservation. The archived materials can be kept and perhaps even added to other samples of BP obtained by others in other studies. At some later date, after the occurrence of benzene related diseases (AML, others), a nested case control study can be done whereas the biological materials, obtained and cryopreserved before the onset of disease, can be thawed and used for an entire series of biomarker analyses. These can range anywhere from current cell-based assays of mutagenesis and non-specific chromosomal changes to FISH assays to molecular genetic techniques. Individuals can also be genotyped at this time. A relationship of AML or other disease occurring in BP individuals, can then be correlated with these early biomarkers of effect in an attempt to develop truly early warning biomarkers for this disease. This is a unique use of these biological materials, designed to give a unique piece of information.

As I comment later in my review of the molecular epidemiological study, the bone marrow obtained from BP patients also should be used in any test developing assays for benzene metabolites. Developmental assays should take place in bone marrows that are being obtained anyway and not necessarily worked out in normal, albeit benzene exposed, individuals. Finally, in this and all components of the overall study, a good deal of clinical, occupational and other information will be obtained through questionnaires and medical histories. It must be emphasized that HIV status should be documented. The absence of such information will end up being a major deficiency.

PROTOCOL II - Molecular Epidemiology of Benzene Exposed Workers in Shanghai, China

The objectives of this molecular epidemiological study are:

1. To investigate dose response relationships between benzene exposure and the various biomarkers (disease or reporter) being measured, and

2. To characterize individuals' susceptibilities to benzene and the range of these susceptibilities.

The specific aims of the project are stated to be:

1. To define dose response relationships for each of the biomarkers - non-specific or disease-specific - being measured,
2. To identify the most sensitive biomarkers of effect,
3. To relate responses of biomarkers of exposure to the responses of biomarkers of effect (in blood and bone marrow), validating the latter as surrogates for benzene-induced genetic damage (see above comments re BP patients),
4. To identify biomarkers of susceptibility for benzene and to determine their influence on dose response relationships, and
5. To relate external benzene exposures to delivered doses to specific target organs, with an attempt to measure benzene metabolites in bone marrow of humans *in vivo*.

The study will progress in three phases (1, 2A and 2B).

Phase 1

Approximately 1,000 workers (800 exposed; 200 controls), primarily from shoe-making facilities, will be enrolled. Benzene exposures will be assessed by work histories with some real time monitoring as a supplement. Clinical records will be reviewed for leukopenia, which is the dependent effect variable in this phase of the study.

Phase 2A

Another 1,000 persons will be enrolled. Again, 800 will be the exposed workers and 200 the non-exposed controls. Exposure assessment will progress as in Phase 1. Blood will be collected from subjects for measurements of biomarkers of effect (see accompanying table).

Phase 2B

A nested subgroup of 200 exposed workers from Phase 2A will be selected. Biomarkers of exposure and effect will be measured in bone marrow as well as blood. Benzene metabolites will be sought in bone marrow by the development of a new assay. Biomarkers of susceptibility will be measured in lymphoblastoid cells developed from these 200 study subjects.

Critique

There are many reasons to perform molecular epidemiological studies, which are essentially invasive studies in humans. These include:

1. To validate biomarkers as measures of exposure to improve exposure assessment,
2. To help resolve the shape of the dose response curve at lower doses (for benzene <10 ppm) by using non-disease endpoints,
3. To provide comparative non-disease endpoint measurements to relate findings in test animals with those obtained from humans exposed to the toxicants in question,
4. To help elucidate mechanisms, and
5. To identify susceptible individuals and ranges of susceptibilities to the toxicants in question.

The current proposal can be critiqued in the context of these purposes. As no molecular epidemiological study stands alone, the current proposal is compared with some recently completed studies, asking what is being added and what is being compared with what is already known (see accompanying table).

Phase 1 will relate benzene-induced cytopenias to benzene exposures. That, of course, has been done in many studies, including the recent one shown in the table. Cytopenias will almost certainly be found. A justification for repeating this study on the massive scale proposed would be the ability to construct a clear-cut dose response relationship between lower levels of benzene exposures in humans and cytopenias. Will the quality of either the retrospective exposure assessment or the retrospective hematological data allow this? Unless the personal exposure monitoring of these 1,000 individuals is massive, encompassing several days of monitoring for each individual, it is quite unlikely that data to be obtained in Phase 1 will add significant information to what is already available. This should be considered.

Phase 2A is a more conventional molecular epidemiological study. This phase, however, doesn't seem to include biomarkers of benzene exposure such as benzene metabolites in blood or urine or protein adducts as surrogate measures of either acute or cumulative exposures. All have been studied in comparable recent studies. The metabolites in blood and urine have correlated with exposures. Adducts also have been found to be elevated in a dose response manner in the two studies reported in the table and new results could add to the accumulating experience with these biomarkers. In addition to moving closer to the mechanism of action (reactive metabolites), measures of protein adducts help to smooth out internal exposures over time and may relate better to subsequent genotoxic effects. This, of course, is to be determined, but both measures of acute exposure and their variability and measures of cumulative exposure give two points for comparison between internal exposure and effects. It may be very difficult to interpret the biomarkers of effect results in this study without contemporary measures of internal exposure.

Both specific and non-specific chromosomal aberrations (conventional analyses and FISH) are being determined in peripheral blood mononuclear cells (PBMC) in Phase 2A. These are certainly reasonable biomarkers of effect and have frequently been found elevated in humans exposed to benzene. Both of the recent studies have found dose related increases in these clastogenic events with FISH analyses. RT-PCR of PBMCs have also revealed increased frequencies of the t8;21 translocations in the studies by Smith et al. Again, for these biomarkers,

it must be asked what is being added to the existing knowledge base by the proposed study? How are the previous studies being extended? (In this context, there is another large study of benzene exposed workers in China in progress, with specific leukemia-associated translocations being measured in PBMCs by RT-PCR. The current study should not proceed as though the current and recent-past studies had not been performed.)

There is a lack of measures of non-specific genotoxic effects proposed for Phase 2A. A potentially very important finding in the studies of Smith et al. is an increased frequency of GPA NN homozygous mutant red cells associated with benzene exposure. This finding needs to be confirmed, of course, but, as this is a potential measure of clastogenic effect in bone marrow stem cells, it could be an important lead as to mechanisms and a surrogate measure of early genotoxic effect. Why isn't this assay being built into the current study? Performing it would be extremely cost-effective as it uses red blood cells, i.e. the fraction of the blood samples being collected that will probably be discarded anyway.

Much attention is given in these studies to specific chromosome translocations as biomarkers of effect. I could determine that these will be measured using FISH only. I am sure, however, that PCR methods will (or should) also be employed. Some thought should also be given to their interpretation. What will almost certainly be found is that young persons will show very rare or no specific translocations, especially when measured in PBMCs. However, the frequencies of cells with specific translocations will probably increase with age, being more abundant in the 50- and 60-year-olds. Such findings are emerging from the other studies of leukemia specific translocations in humans. These are the findings that are emerging. This probably means that the cells carrying these translocations are being clonally amplified *in vivo*, as a requirement for their detection. This raises two important questions. Will the investigators interpret their results as frequencies of translocations (the genetic events), or frequencies of cells carrying translocations (the cell growth component)? Will they be able to tell the difference? And what is more important - which is the more important in leukemogenesis?

It is possible that frequencies of translocations can be differentiated from frequencies of cells carrying translocations if the RT-PCR translocation products obtained are sequenced to determine breakpoint junctions. Different breakpoints equate to different genetic events.

Finally, no biomarkers of somatic mutations are being assessed in this study. The desirability of GPA measurements has been mentioned. *HPRT* mutations are clearly induced in mice exposed to benzene. One advantage of studying the *HPRT* events is that molecular mutational spectra can be characterized in an attempt to elucidate mutagenic mechanisms. Both clastogenic and point mutational events will probably enter into the full development of the leukemias secondary to benzene. A molecular epidemiological study of the scale proposed could provide, in conjunction with similar studies conducted by others, molecular information that can be compared with results in test animals with the goal of both elucidating pathogenic mechanisms and refining human risk assessment.

Although these suggested additions may seem to be a great deal of work, they are really not significant in the context of the massive study proposed with its high cost. Analysis of the

budget will reveal that most of the cost is in assembling and characterizing the population for this overall study. The studies that are added on each come at a bargain.

Phase 2B adds biomarkers of acute benzene exposure in blood and urine. It also extends the analyses of biomarkers of exposure and effect to the bone marrow in a subset of 200 individuals, adding an attempt to develop a method to measure benzene metabolites there. Also, lymphoblastoid cell lines will be developed to assess metabolic genotypes for these 200 individuals and phenotypic studies of chlorzoxazone hydroxylation will be done as a measure of susceptibility. A subproject here will be to develop an assay for measuring benzene metabolites in the bone marrow.

Most of the comments given above are also applicable here. In addition, why is the method for measuring benzene metabolites in bone marrow being done in normal human volunteers? Why aren't methods first worked out in benzene exposed animals? Then, adaption to humans should proceed using bone marrows from BP patients obtained in the disease progression portion of the overall study? These marrows are being obtained in any event and adapting new methods to humans using them will lower ethical concerns for this aspect of the study. Assaying these metabolites in the molecular epidemiological part of the study can then proceed using a well-validated assay. In similar vein, I assume that RT-PCR will be used to detect specific chromosome translocations in the bone marrow cells (even though this is not explicitly stated in the application). The sensitivities of each PCR method to be used should be well worked out and calibrated in the bone marrow samples of patients before it is used as a biomarker of effect in normals.

In summary, this molecular epidemiological study is a large and expensive undertaking. It could yield valuable results, achieving the purposes for which molecular epidemiological studies are intended. I have some concerns over the purpose of doing Phase 1. Phase 2A will be made much more valuable if some additional biomarkers are measured. Some additional thought should go into Phase 2B.

Case Control Studies of Acute Non-lymphocytic Leukemia (ANLL) and Non-Hodgkin Lymphoma (NHL) in Shanghai

Description

Approximately 500 new hospital cases of ANLL and NHL will be enrolled over a five-year period in the Shanghai region. The extent of exposures to benzene and other potentially deleterious agents will be determined by retrospective analysis of the workplace and other means as described. Morphological, immunological, cytogenetic and molecular analyses will confirm diagnostic entities. Two hospital-based controls will be assigned to each case with appropriate matching and exposure assessment. The objectives of this case control study are (1) to determine the risk of developing ANLL or NHL (and major subtypes thereof) among workers exposed to benzene in Shanghai, (2) to quantify the exposure-response relationships between exposures to benzene and ANLL or NHL among these workers, and (3) to determine other occupational and non-occupational risk factors for ANLL or NHL among these workers.

Critique

This appears to be a study of potentially great importance that should be pursued. However, there is a confusing overlap between the case control study outlined here in draft form and that proposed by Dr. Irons as part of the disease progression study. Certainly, the ANLL patients in both studies will be the same; the NHL patients will be identified and characterized in the disease progression study but entered as cases for quantitative studies only in the case control study proposed by Wong and Hua. The ALL, MDS and BP cases in the disease progression study are entered as cases only in the Irons case control study. These cases are not entered in the case control study of Wong and Hua. There is one control individual per ANLL case in the disease progression study case control component but there are two controls per case for ANLL in the study of Wong and Hua. This is very confusing. Does this mean that the ANLL cases will eventually have three controls (two in one study and one in another) or will one of the controls be the same for both case control studies? Will benzene exposure assessment be done twice for the ANLL patients - once for each case control study? Will record-keeping and blinding be duplicated with two sets of files and different security measures, two informed consents and double monetary compensation for participants?

Clearly, this is an awkward situation. Why not have one case control study? Or, if two studies are necessary, why not have the ANLL patients be considered in only one of them? Quantitative information obtained in this case control study could surely feed back to the disease progression study the same way that the molecular information originating there feeds into this study.

In another vein, it is explicitly stated in this protocol that HIV status of patients will not be determined. One wonders if it is even possible to conduct a study of NHL today without knowledge of this confounder. Lack of information on HIV status may render results of this potentially very important study uninterpretable.

A final protocol for this case control study remains to be developed. The study should proceed, but attention should be given to reconcile it with the disease progression study.

REFERENCES

1. Rothman et al. (1996) Hematotoxicity among Chinese workers heavily exposed to benzene. *Amer. J. Indust. Med.* 29:236.
2. Rothman et al. (1995) Benzene induces gene duplicating but not gene-inactivating mutations at the glycophorin A locus in humans. *Proc. Natl. Acad. Sci. USA*, 92:4069.
3. Yeowell-O'Connell et al. (1998) Hemoglobin and albumin adducts of benzene oxide among workers exposed to high levels of benzene. *Carcinogenesis* 19:1565.

4. Zhang et al. (1999) Benzene increases aneuploidy in the lymphocytes of exposed workers: A comparison of data obtained by fluorescence *in situ* hybridization in interphase and metaphase cells. *Environ. Molec. Mutagen.* 34:260.
5. Zhang et al. (1998) Increased aneusomy and long arm deletion of chromosomes 5 and 7 in the lymphocytes of Chinese workers exposed to benzene. *Carcinogenesis* 19:1955.
6. Smith et al. (1998) Increased translocations and aneusomy in chromosomes 8 and 21 among workers exposed to benzene. *Cancer Res.* 58:2176.
7. Qu et al. (2001) The validation of biomarkers in humans exposed to benzene. Annual Meeting Report, Health Effects Institute.
8. Qu et al. (2001) Genetic susceptibility to benzene induced chromosome damages. Annual Meeting Report, Health Effects Institute.