PROGRESS REPORT
July 2005

I. ANALYSIS OF DISEASE PROGRESSION FOR APLASTIC ANEMIA, MYELODYSPLASTIC SYNDROME, ACUTE MYELOGENOUS LEUKEMIA AND BENZENE POISONING IN SHANGHAI, CHINA

II. MOLECULAR EPIDEMIOLOGY OF BENZENE-EXPOSED WORKERS IN SHANGHAI, CHINA

III. EXPOSURE ASSESSMENT

A MULTICENTER INTERNATIONAL STUDY

Molecular Toxicology and Environmental Health Sciences Program
Dept. of Pharmaceutical Sciences, School of Pharmacy
Department of Pathology, School of Medicine
University of Colorado Health Sciences Center, Denver, CO.

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School of Public Health, Hua Shan Hospital, Cancer Hospital,
Fudan University Medical Center, Shanghai, China

EXECUTIVE SUMMARY

A. Overall Status of Budget

Attached is an official summary of the overall budget for the year ending June 30, 2005.

In April 2005, the consortium agreed to a revised budget (Appendix A). Based on this new budget, the overall status of the budget for the first half of this calendar year is as follows: We spent $1,787,330 compared to a projected budget of $2,018,301, resulting in savings of $230,971 in funds during this period (Table 1). This savings is largely due to a reduction in operating expenses at UCHSC.

Table 1. Budget Summary (Reporting Period)

	Budgeted[1]	Expenditures	Variance
Personnel	$227,260	$235,437	($8,177)
Operating Expenses	$247,000	$104,457	$142,543
Subcontracts	$1,420,103	$1,359,315	$60,788
Travel	$500	$0	$500
Equipment	$0	$0	$0
Indirect	$123,438	$88,121	$35,317
TOTALS	$2,018,301	$1,787,330	$230,971

[1]The budgeted amounts are the sum of each category from Jan-05 through Jun-05.

Table 2. Budget Summary (Entire Project)

	Budgeted[2]	Expenditures	Variance
Personnel	$2,176,813	$1,935,436	$241,377
Operating Expenses	$1,592,787	$1,329,463	$263,324
Subcontracts	$6,260,471	$5,873,620	$386,851
Travel	$47,576	$9,098	$38,478
Equipment	$1,560,213	$1,383,491	$176,722
Indirect	$1,018,466	$880,550	$137,916
TOTALS	$12,656,326	$11,411,658	$1,244,668

[2]The budgeted amounts are the sum of each category from Dec-01 through Jun-05.

Subcontract Summary (Entire Project)

Subcontracts	Expenditures (Dec 01 – Jun 05)
Fudan University	$3,415,417
IPHS	$167,577
SMCDC	$153,004
EMBSI	$1,873,555
Children's Hospital Cincinnati	$264,067

B. Budget Analysis

Our projected costs for the remainder of the project are outlined in Table 3. These figures are consistent with projections from the last API progress report except that we have added the EMBSI estimated additional costs.

Table 3. Projected Budget (Remainder of Project)

	July1 – Dec 31 Year 2005	Year 2006	Year 2007 **
Personnel	$236,350	$482,154	$203,265
Operating Expenses	$254,380	$396,360	$0
Subcontracts*	$1,264,180	$2,683,219	$609,595
Travel	$500	$1,000	$0
Equipment	$0	$0	$0
Indirect	$127,720	$228,677	$52,849
TOTALS	$1,883,130	$3,791,410	$1,015,709

*Subcontract figures now include EMBSI estimated additional costs.

**Post-study costs

Appendix A

	Dec-01	Jun-02	Dec-02	Jun-03	Dec-03	Jun-04	Dec-04	Jun-05	Dec-05	Jun-06	Dec-06	Post-Study thru Jun-07	**Total thru Post-Study**
Personnel													
Apr '05 Budget	$ 172,140	$ 224,883	$ 317,665	$ 314,703	$ 230,328	$ 211,016	$ 229,260	$ 227,260	$ 236,350	$ 236,350	$ 245,804	$ 203,265	$ 2,849,025
Actual	$ 172,140	$ 224,883	$ 317,665	$ 314,703	$ 230,328	$ 211,016	$ 229,260	$ 235,437					$ 1,935,432
Operating Expenses													$ -
Apr '05 Budget	$ 13,131	$ 94,401	$ 193,990	$ 158,850	$ 162,834	$ 364,542	$ 237,258	$ 247,000	$ 254,380	$ 235,580	$ 160,780	$ 150,000	$ 2,272,746
Actual	$ 13,131	$ 94,401	$ 193,990	$ 158,850	$ 162,834	$ 364,542	$ 237,258	$ 72,156					$ 1,297,162
Subcontracts													$ -
Apr '05 Budget	$ 123,629	$ 688,452	$ 213,291	$ 845,860	$ 565,870	$ 1,128,507	$ 948,696	$ 1,420,103	$ 1,264,180	$ 1,454,051	$ 1,229,168	$ 609,595	$ 10,491,402
Actual	$ 123,629	$ 688,452	$ 213,291	$ 845,860	$ 565,870	$ 1,128,507	$ 948,696	$ 1,359,315					$ 5,873,620
Travel													$ -
Apr '05 Budget	$ -	$ 8,429	$ 218	$ 346	$ -	$ -	$ -	$ 500	$ 500	$ 500	$ 500		$ 10,993
Actual	$ -	$ 8,429	$ 218	$ 346	$ 105	$ -	$ -	$ -					$ 9,098
Equipment													$ -
Apr '05 Budget	$ 291,568	$ 253,578	$ 354,496	$ 274,508	$ 17,000	$ 192,340	$ -						$ 1,383,490
Actual	$ 291,568	$ 253,578	$ 354,496	$ 274,508	$ 17,000	$ 192,340	$ -	$ -					$ 1,383,490
Indirect													$ -
Apr '05 Budget	$ 47,922	$ 111,454	$ 139,587	$ 122,902	$ 101,447	$ 148,709	$ 120,408	$ 123,438	$ 127,720	$ 122,832	$ 105,845	$ 52,849	$ 1,325,112
Actual	$ 47,922	$ 111,454	$ 139,587	$ 122,902	$ 101,447	$ 148,709	$ 120,408	$ 79,722					$ 872,151
TOTALS													$ -
Apr '05 Budget	$ 648,390	$ 1,381,197	$ 1,219,247	$ 1,717,169	$ 1,077,479	$ 2,045,114	$ 1,535,622	$ 2,018,301	$ 1,883,130	$ 2,049,313	$ 1,742,097	**$ 1,015,709**	**$ 18,332,769**
Actual	$ 648,390	$ 1,381,197	$ 1,219,247	$ 1,717,169	$ 1,077,584	$ 2,045,114	$ 1,535,622	$ 1,746,630	$ -	$ -	$ -		**$ 11,370,953**

Original Budget	$ 14,508,000
Revised Budget	$ 18,332,953
Increase	$ 3,824,953

I. JCML OPERATIONS

A. Progress

Study diagnoses for the first 18 months of case accrual (Sep 03 – Feb 05) have been completed and 100% reviewed. During the first year a total of 740 cases were diagnosed and 732 were assigned ICD codes according to WHO and ICD-10. The remaining 8 unassigned cases are follow-ups for the DP study. There were a total of 147 cases of AML (WHO), 152 cases of lymphoid (WHO) (i.e. NHL+ALL+CLL) and 113 cases of NHL+CLL (ICD-10). There were 129 cases of MDS, 48 cases of AA and 20 cases of benzene poisoning diagnosed. At 18 months a total of 1131 cases were diagnosed and reviewed with 1108 ICD codes assigned. The remaining 23 unassigned cases are follow-ups for the DP study. There were a total of 269 cases of AML (WHO), 261 cases of lymphoid neoplasms (WHO), 188 cases of NHL+CLL (ICD-10), 192 cases of MDS, 61 cases of AA and 25 cases of benzene poisoning diagnosed. Further, a total of 400 patients were diagnosed over the past 6 months, bringing total cumulative case contact to 1531. Since January, a total of 4 cases of AA, 39 cases of MDS, 56 cases of AML, 55 cases of lymphoid neoplasms and 2 additional BP cases have been diagnosed, and a total of 137 cases remain unassigned to the study database as of the time of this writing. In addition, over the course of the project to date JCML has diagnosed approximately 40 *pro bono* cases for individuals, mostly children, who do not qualify for inclusion in our studies.

B. Nutritional Deficiencies

An important observation is the number of hematologic abnormalities that can be attributed to nutritional deficiencies. As of June, 2005 we have identified 72 cases, the majority of which would be misclassified as AA, MDS or even AML in a retrospective study. Consequently, nutritional deficiencies are involved in roughly 20% of the potential case accrual for these diseases, rendering nutrition an important confounding factor in the study of AA and MDS. The frequency of diagnosis of nutritional deficiencies at JCML also suggests that this represents an important public health issue in Shanghai. Professor Fu Hua has agreed to take responsibility for preparing an analysis of the influence of nutritional deficiencies in hematopoietic disease in Shanghai.

C. HIV Serology Testing

We have encountered difficulties in implementing approved changes to the protocol for directly determining the rate of HIV seropositivity in our lymphoma cases. This is due to a number of logistic problems, primarily the fact that the majority of lymphoma cases, especially low grade lymphomas, are outpatients who are reticent to provide a blood sample for procedures not directly related to the diagnosis of their condition. Other outpatients are not willing to return to the hospital for additional blood work after diagnosis. Alternatively, screening for HIV is becoming far more commonplace in Shanghai, and participating hospitals have agreed to share HIV serology data previously obtained from IC'd subjects. Nevertheless, an effective mechanism for doing this on a routine basis has yet to be implemented. In contrast to the issues related to sample collection for NHL, all hematology cases, including ALL and CLL cases,

diagnosed at JCML have been tested for HIV. To date, no confirmed cases of HIV have been identified in these subjects (N>650). Independently, the NHL subtypes diagnosed in our patient population are not consistent with immunodeficiency-associated lymphoid disorders. Therefore, although we have only a fraction of direct measurements on NHL cases, we believe that it is very unlikely that HIV constitutes a significant confounding factor for any of our studies.

II. MOLECULAR EPIDEMIOLOGY OF BENZENE-EXPOSED WORKERS

A. Molecular Epidemiology Phase 1

The goal of the molecular epidemiology (phase 1) study is to: (a) study retrospective benzene exposure levels vs. blood counts present in worker exam records, and (b) identify candidate factories for subsequent phases (e.g. 2A, 2B) of the study.

Through the leadership of Dr. Ni, excellent progress has been made in abstracting hematology counts from physical examination data at a number of facilities. Over 1600 cases have been collected and entered into an Access database from 17 facilities. Not all of these records will qualify for the final study, since some cover only one year of readings, while the molecular epidemiology study requires at least two years (and preferably more) to be able to study potential effects on blood counts longitudinally.

A potential issue for the Phase 1 study is whether the records that have been abstracted are representative of the workforce. In some preliminary factory visits, it was noted that records for workers with 'normal' blood readings were stored at one location, while records for workers with 'abnormal' blood readings could be stored separately. Dr. Ni believes that this did not apply to the cases in the Yang-Pu hospital, where most of the records were collected. Nevertheless, frequency counts for 'normal' and 'abnormal' values of different blood parameters will be examined by factory and time period to ensure that this is the case.

Exposure assessment for the factories selected has been proceeding at a steady pace. So far, the EA team has visited 10 of the 17 factories, and 7 factories are awaiting a site visit from the team. The aim of these visits is to collect information beyond that which may be present in the IPHS database. This is needed to reliably link certain jobs/factory locations with appropriate retrospective data. In addition, the EA team has performed screening assessments with rapid-detecting instruments, consisting of over 250 measurements. The results of the initial assessments have been mixed: in some sites no records of benzene exposure can be found, while others have yielded good retrospective records. In other sites no current benzene exposure can be found, in others relatively low levels can be detected. So far, over 300 cases have useable exposure and blood data. We are actively working on exposure assessment and assessment of the hematologic data to assess how much useable data is present in these records.

While these efforts proceed, it seems reasonable to assume that less than half of the 1600 records will ultimately contain useable blood and exposure data. A

coordinated effort is underway to identify suitable factories outside of the Shanghai area that might be used to increase the number of workers in the Phase 1 study, while also identifying sites for Phase 2A.

B. Molecular Epidemiology Phase 2

A total of 42 factories have been visited in an effort to select appropriate facilities for Phase II studies. Extensive area and personal sampling has been performed in three facilities with 984 area samples analyzed with mean airborne benzene concentrations of benzene ranging between 0.01 and 326.7 ppm. A total of 231 workers have been recruited to the Phase IIa study.

Over the past year, the ME study has encountered a number of challenges which have impacted on its progress and resulted in some mid-course adjustments. First, our success in gaining access to acceptable study sites has been very limited. Out of 42 factories visited only 5 were considered to be appropriate for initial Phase IIa activities and only 3 have been extensively analyzed. Moreover, recruitment of workers to Phase IIb, as originally designed has proven to be problematic (N = 3). In addition to the obvious ramifications of documenting high benzene exposure in the workplace along with health effects, we have encountered a variety of difficulties in obtaining the cooperation of management in many facilities. The reasons for this are many and varied. Some frequently encountered problems are culturally based: First, in state-run companies market place competition has resulted in considerable financial stress, workers are at risk being laid-off and the anxiety is high. Therefore, management typically wants to avoid anything that may trigger trouble and reactions from their workers. Second, our use of informed consents and pledge to compensate workers for any problems arising from participation in the study stands in sharp contrast to similar recent or ongoing studies that do not acquire consents or offer any forms of compensation, thereby posing an indirect threat by example. Both local occupational physicians as well as Professor Xi have informed us that it is unlikely that this issue can be resolved without major changes to our ME informed consent (IC) procedures. Accordingly, I have asked a committee comprised of senior occupational physicians from local hospitals, the Vice Director of the CDC Physical Examination Center, Dr. Ye Xibiao, Lv Ling (JCML's lead occupational physician) and Professor Fu Hua to review the problem and to make suggestions for changing the IC procedure.

These issues notwithstanding, we identified factory workers with benzene poisoning, admitted them into hospital in Shanghai, and recruited them to the DP study. This strategy was inculcated in the original approved study design and has resulted in characterization of a case series of 23 individuals with persistent dysplasia following prolonged benzene exposure and for which we have robust exposure assessments. These cases also contribute quantitative exposure assessment data to the CC/DP EA matrix (see below). To date, we have identified approximately 30 such individuals using this mechanism. In addition, we have modified the original protocol by adding peripheral blood benzene metabolite and urinary sPMA analyses to Phase IIa as opposed to limiting metabolism studies to Phase IIb. To date, we have measured benzene metabolites in the blood of 103 exposed workers, conducted pre- and post- shift analyses of urinary sPMA

in 104 workers and have integrated hematology, exposure, metabolite and urinary sPMA data for 64 workers. In addition, utilizing PCR-RFLP methods, we have conducted preliminary genotyping of CYPE2E1, NQ01-465CT and –609CT, MPO-463GA, and GSST1 polymorphisms on all 231 Phase IIa subjects.

III. EXPOSURE ASSESSMENT

A. Introduction

The past 6 months have witnessed major developments in the EA process which are outlined below. This report represents the collective effort of a large group of individuals including the Fudan SHS Exposure Assessment Team under the direction of Prof. Fu Hua, Dr. Tom Armstrong and Yimei Zhou of EMBSI, with input from Dr. Schnatter and Prof. Irons.

B. Exposure assessment strategies and goals

The EA strategy originally designed for the DP study was adapted to all SHS hospital based studies upon the recommendation of the SRP in 2001. The primary goal of the CC study is to ascertain whether benzene exposures or other variables (e.g., viruses, nutritional deficiencies or other chemicals) are associated with certain diseases (e.g., NHL). Ordinal range assessments (no, low, medium, high exposure) or where feasible, quantified ranges such as <0.4, 0.4 to < 4, 4 to 40 and > 40 mg/m3 support these study objectives. This tiered approach (Figures 2 and 3) was originally outlined in the study protocol and endorsed by IH SRP members. Quantitative benzene assessment, which is the EA strategy employed for the ME study, is targeted for a fraction of the CC/DP subjects, and is dependent on feasibility. In the ME study, factories are selected based on good EA and health effects records and a willingness to participate in extensive concurrent personal and area monitoring activities. Thus, the detailed quantitative EA analysis performed in ME supplements and reinforces EA efforts in CC/DP. The timeline for EA is depicted in Figure 4. The integration of qualitative and quantitative EA processes is targeted for the second quarter of 2006 when validation and assembly of the final CC/DP EA matrix is slated to occur. The assignment of final benzene exposure will then follow.

C. Progress

1. Data collection

During the last 6 months, Questionnaire Takers interviewed 850 subjects for an average of about 40 cases per week. The EA team conducted site monitoring at 25 factories and collected 350 personal, area and bulk samples. A total of 3375 questionnaires have been collected to date with 3308 having completed preliminary exposure assessments (EA) for substances of interest, including benzene. A total of 1885 secondary questionnaires have been administered, including 484 during the last 6-month period. Data collected was immediately reviewed and telephone interviews conducted to clarify any remaining issues. Qualitative EA are now progressing at a rate consistent with subject accrual (See Figure 1). Semi-quantitative and quantitative exposure

assessment (EA) of benzene requires more detailed data assembly, and the time line on this effort naturally lags case accrual. Nevertheless, these analyses are on schedule for completion in conjunction with detailed benzene EA which is slated for third quarter 2006, for the cases accrued to that point. Initial projections for benzene-EA were based on predictions of a caseload of 500 cases per year and an expected prevalence of 10% benzene exposed in the study subjects. The caseload is approximately 50% higher than expected and the prevalence of benzene exposed subjects is about 7%.

Exposures other than benzene were assigned in over 2600 instances. The studies by design evaluate exposures to a range of risks other than benzene, since exposures to other hazards relevant to the diseases of interest may be indicated in a subject's work and life histories.

EA team members continue to close the gap on earlier shortfalls in EA assessments and progress is now meeting the expectations of the EA Coordinating Committee (EAC). Nevertheless, unanticipated challenges have required additional staffing, and we continue to experience a shortage of qualified EA personnel. Therefore, efforts are underway to recruit additional experienced staff.

Through the first half of 2005, 93 work site inspections (CC/DP and ME) have been completed. These work site inspections included extensive current exposure monitoring:

- 4300 UltraRAE screening measurements of benzene concentrations
- 2150 area samples (20 minute breathing zone by IPHS protocol)
- 1070 personal benzene exposure samples

Additional site reviews are planned.

2. Exposure analysis for the CC/DP studies

We have experienced limited success finding exact matches for factories with exposure data corresponding to time periods relevant to subject work histories. Many factories are closed and were not previously monitored by IPHS. Some subjects are from areas of China remote from Shanghai. Review of completed assessments using exact match or limited surrogate site data showed divergent results for apparently similar work histories. Therefore, the industry sector analysis previously described in the study protocol is being emphasized for providing quantitative data to support the final benzene EA. Sector analysis was further developed in April, with a pilot test conducted in May to evaluate the capabilities of performing sector analyses as part of a comprehensive EA strategy. The study focused primarily on the shoe industry and on a segment of the rubber industry. The exercise involved:

- Sorting available IPHS data for the industries of interest
- Sorting the data into sectors within each industry
- Adding supplemental task categories (not part of the IPHS database) based on sample location descriptions

- Qualitatively evaluating the frequency-concentration distributions of the sector, by task and time period
- Calculating preliminary summary statistics for the identified sector, task and time categories

As a result of this exercise for the test industry segments, sector analysis was determined to be a useful approach for evaluating other key industrial segments found in subject work histories, and is being integrated into an overall collaborative effort with IPHS. The sector analysis evaluation yielded insights that led to further research efforts that are currently underway. One main point from the pilot study is the need for additional coding based on area descriptions given for the samples in the IPHS database. The additional codes are needed to separate the data concerning where people worked, what tasks were done and by era. The additional coding will reduce heterogeneity in the data sets thereby allowing more precise exposure assignments. This additional coding and analyses will be conducted in collaboration with IPHS.

The main stages of the CC/DP process, with responsibilities and progress dates are shown in Table 4. It is important to recognize that until the EA matrix is completed, the process is dynamic and iterative, with the methods and degree of reliance on individual information sources differing for individual sectors. Several different sources of information are being used and periodically reviewed, and previous assessments are continually being compared with ongoing ones.

For approximately 13% of the CC/DP subjects qualitatively categorized as benzene exposed, quantitative analysis has been successfully completed as part of EA in the ME study. These quantitative assessments are based on current personal exposure measurements, current area concentration measurements, detailed compilations of factory changes over time that modulate exposure, use of retrospective exposure estimating techniques, and use of past IPHS monitoring data. This has resulted in a detailed exposure matrix for these subjects. Additional publications based upon this work are in preparation.

3. Changes to the data collection process

Many of the second level questionnaires were found to be of minimal value so the set has been reduced to 23. An additional summary of key task information (e.g. task description, materials used, frequency and duration) is being provided on a "key data summary" card to better prompt the staff during questionnaire interviews. Additional question-coded data recording sheets are being considered since the primary questionnaires do not always provide sufficient writing space for detailed answers.

4. Initial screening, qualitative assessment and relative ranking

The EAT and EAC have prioritized the completion of qualitative assessments and relative ranking of the subjects for benzene and the other exposures. This provides a basis for linking the subject's work history to the project exposure assessment matrix. Part of the review includes seeking current access to the work place for current

monitoring and evaluation of changes in the operation relevant to the time the subject worked.

5. Collaboration with IPHS

Collaboration with the Shanghai IPHS has been enhanced and restructured to provide an integration of database, factory site evaluation and sector information. This involves close interaction with IPHS District staff in order to conduct a comprehensive analysis of a single district, Yang Pu, that will serve as a centerpiece for the development of tools for validating individual data sets. Work with the District staff includes verifying data in the database, and completing current monitoring for factories operating in the District that coincide with study subject work histories. This collaboration provides additional resources to further code data and to develop summaries for industrial sectors important to the subject work histories.

Reviews of the CC/DP exposure assessments have been underway for the last year, and will be expanding in number and depth as the EA matrix supporting materials and their translations reach completion. It is proposed to have members of the SRP with expertise in EA participate in a review of exposure matrix in the second half of 2006.

6. Analysis of the Chinese EA literature

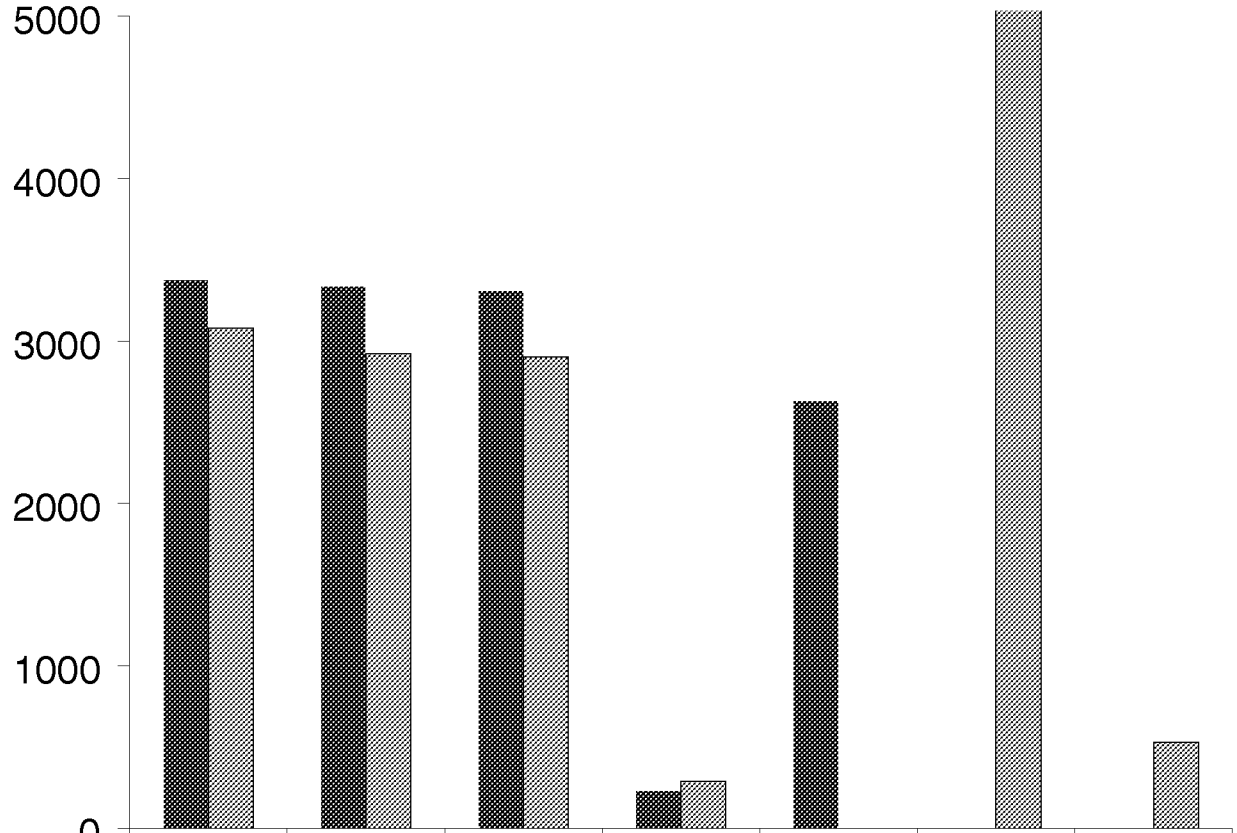
Professor Liang has been asked to complete the summary and analysis of the Chinese EA literature, including categorization of articles by quality, for use on the EA process. Another important aspect of the analysis that is underway is the preparation of summary reports that outline trends impacting exposure potential for key industries. An important part of this exercise is a summary of the benzene composition time trends for key materials (e.g., glues, solvents, paints, gasoline). This is an important and significant undertaking which is needed to complete the integration of EA processes into a final exposure matrix.

7. Management

Continued dialog and interaction among the Fudan and Western EA investigators and Consortium Committees has resulted in some streamlining of the EA committee process to improve organization and communications. The purpose of the EA Coordinating Committee (EAC) is to discuss progress and identify problems that have occurred during the past month and identify goals and approaches for the EA team (EAT) that will be followed for the next month. The EAC is under the direction of Professor Fu Hua and includes Tom Armstrong, Zhu Surong (IPHS) and Yimei Zhou (interpreter). The EAC reports directly to Dr. Irons.

The EAT, which is responsible for day-to-day EA operations, holds weekly staff meetings which are co-chaired by Ye Xibiao and Yimei Zhou. The purpose of these meetings is to manage EA operations, provide a list of weekly action items and to identify issues that need to be taken to the EAC. During the past 6 months, the team welcomed two new members who joined to fill in gaps left by a graduation and a pregnancy. Additional staffing efforts are underway to identify an experienced individual with both IH training and bilingual language skills.

Figure 1. June 2005 CC/DP EA Status



	Case recruitment	Data Entry	Prelim EA	Benzene Exposed	Other Exp	Final Subjects	Final Benzene Exposed
06/05 Actual	3375	3335	3308	233	2631		
06/05 Planned	3080	2920	2900	290		5300	530

Figure 2. CC/DP Exposure Assessment Process Overview

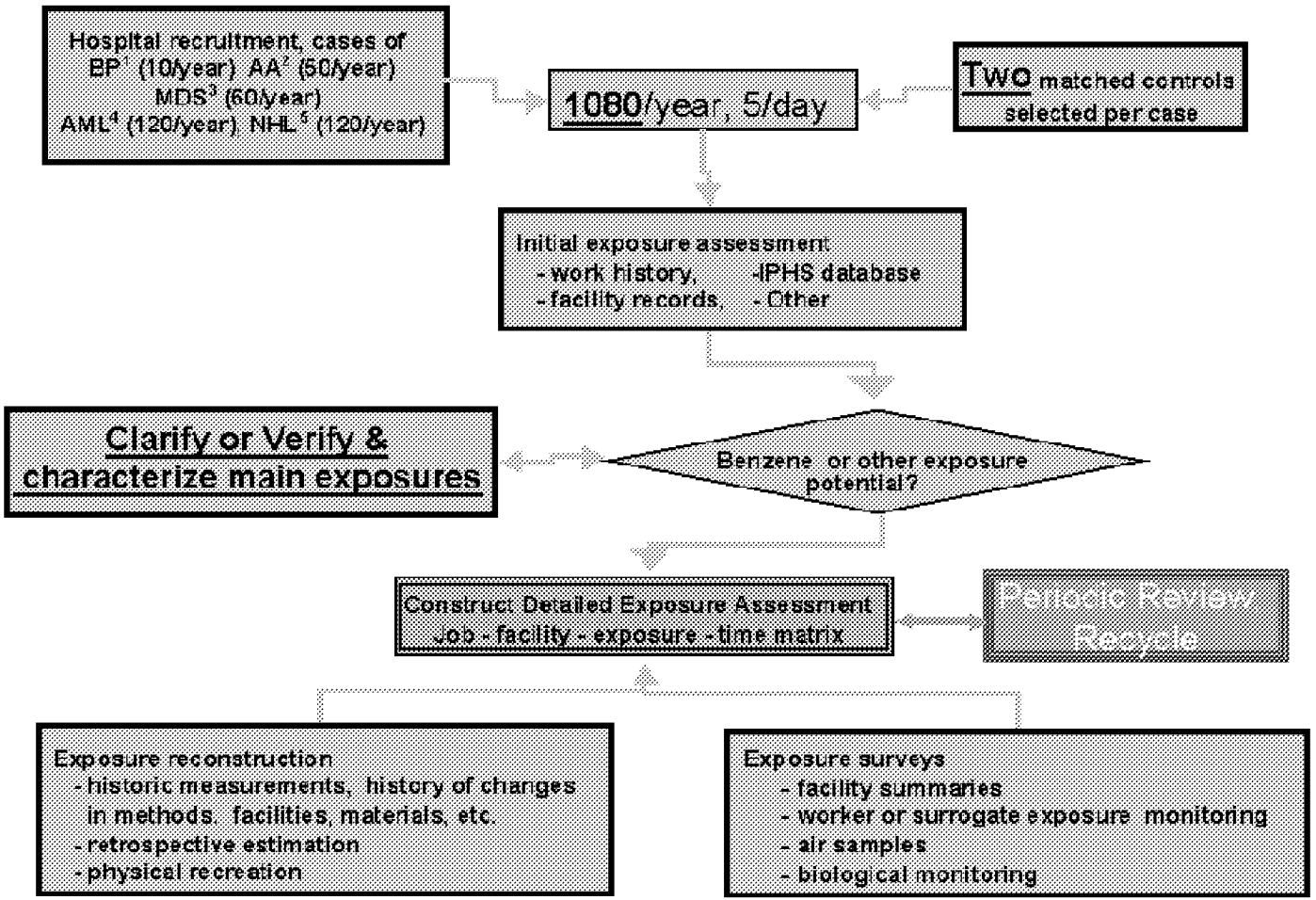


Figure 3. Case Control Studies Tiered EA Design:

Expectation of Fewer Subjects Completed at Higher Tiers

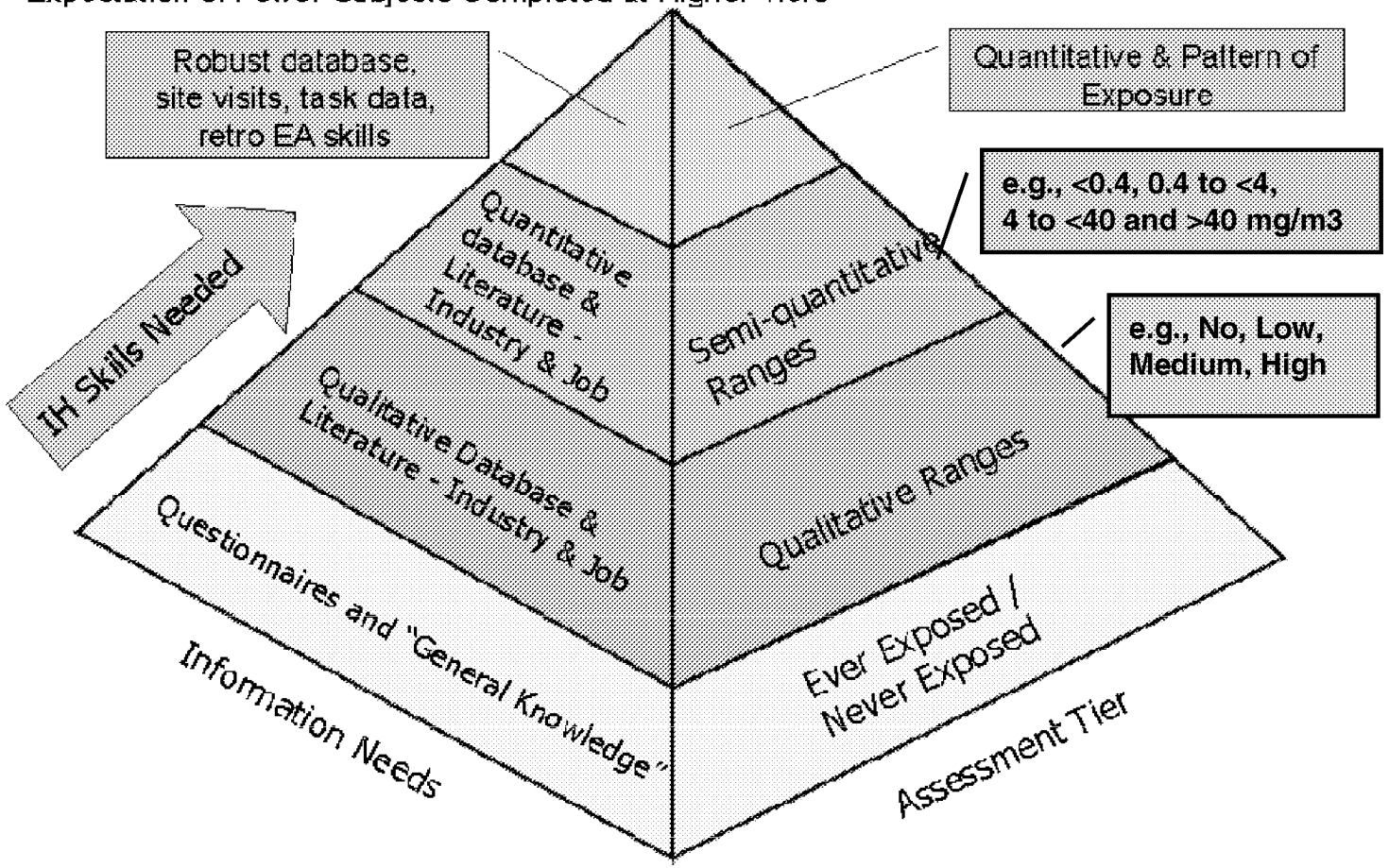


Figure 4. CC/DP EA Path Forward

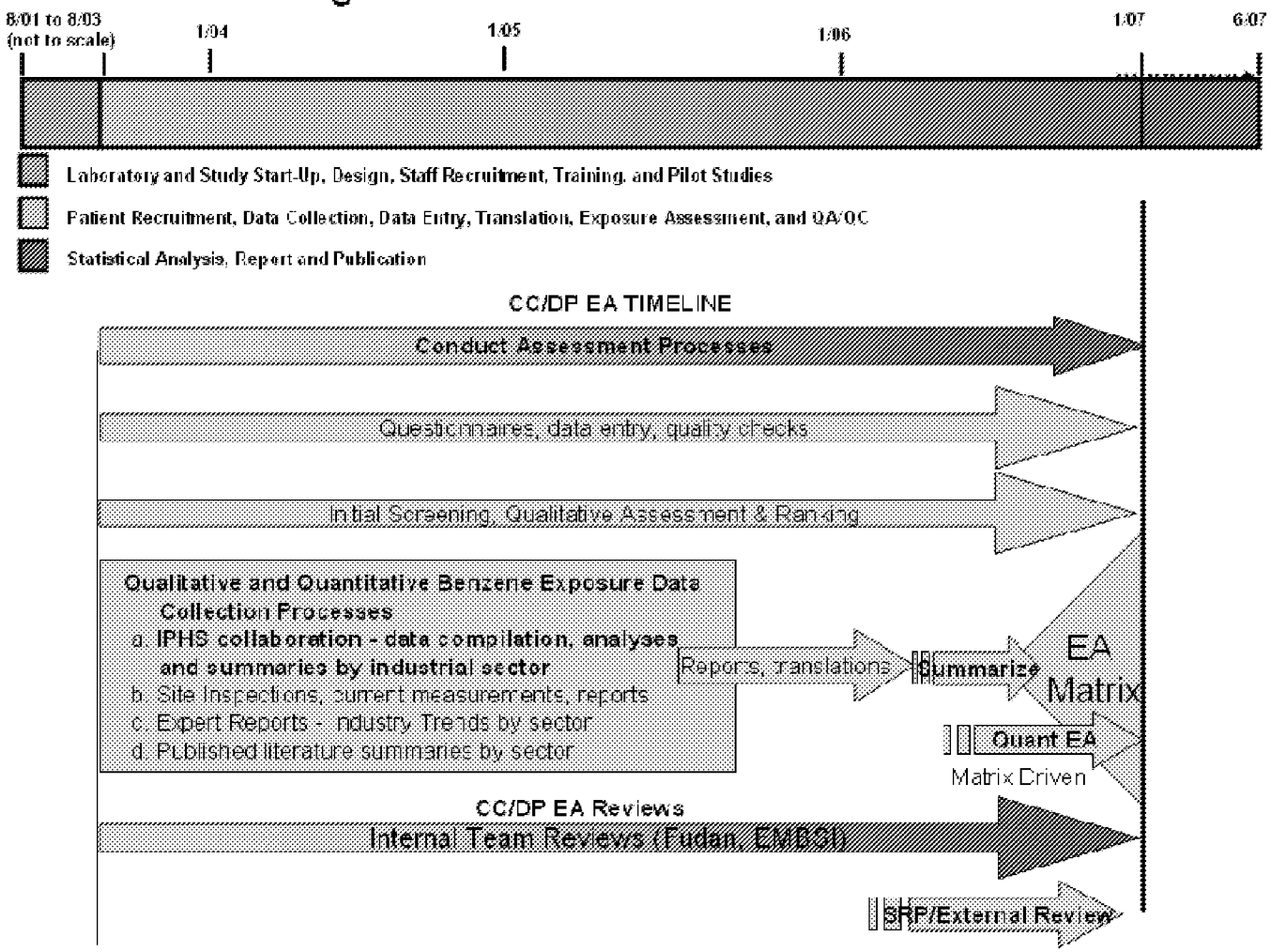


Figure 5. CC/DP Exposure Assessment for PROBABLE "BENZENE" EXPOSED Multiple Lines of Investigation and Information

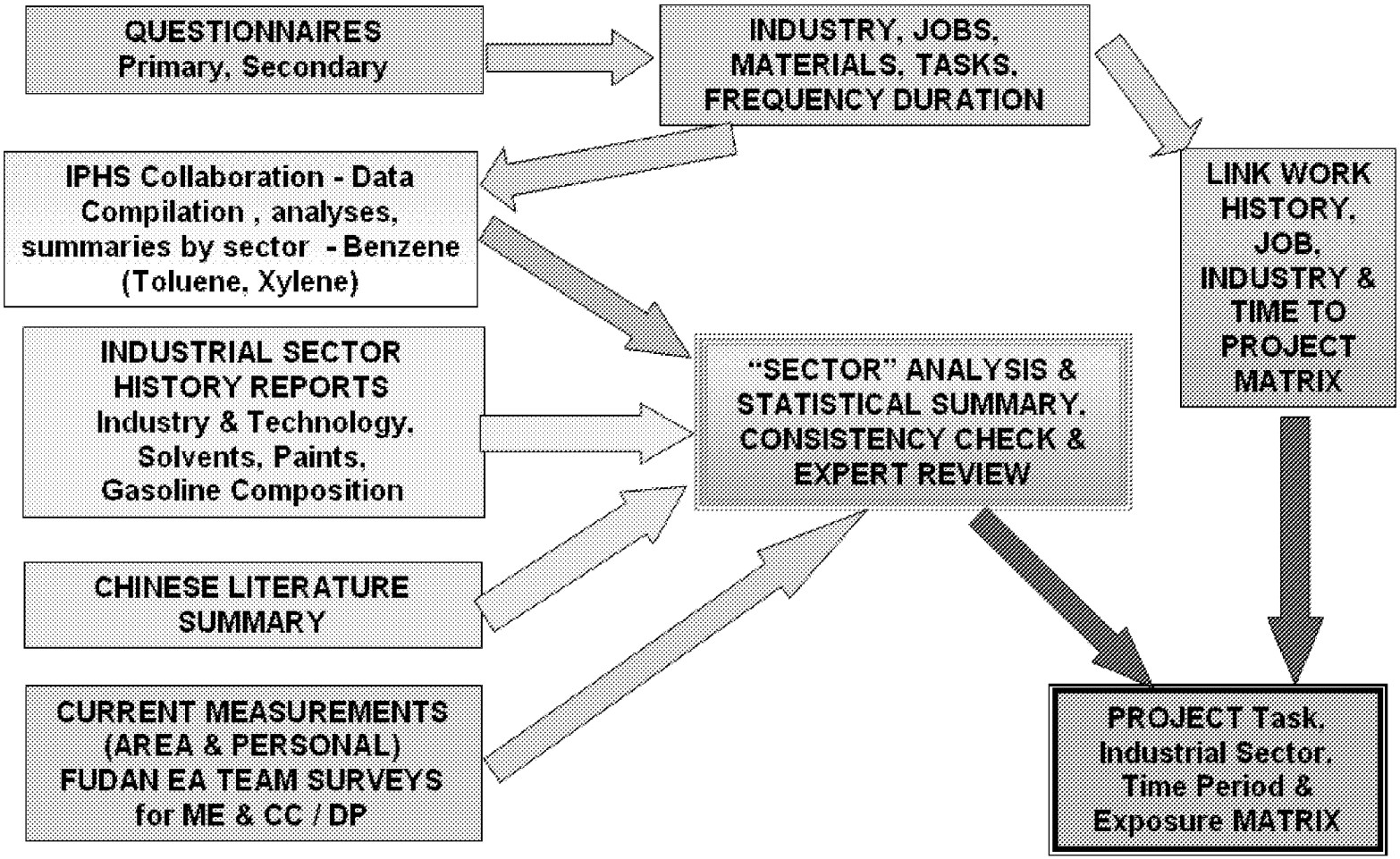


Table 4. Exposure Assessment Processes & Responsibilities for CC/DP Study

STEP	WHO	WHEN
Questionnaires – primary and secondary	Questionnaire Administration (QA)	On-going
Industry/job/materials/task/frequency/duration (key data for EA and follow-up planning)	QA/data entry/EAT	On-going
Industry codes assigned (to group work histories for consistency and follow-up planning)	EAT	On-going
Translations of key information to English	External resources	On-going
Task descriptions (essential to link to IPHS area descriptions)	EAC	On-going
Task coding (to group work histories for consistency and follow-up planning)	EAC	4Q 05'
Link work history/task/industry/time to reference data via EA matrix	EAC/EMBSI	06'
Access to IPHS data base	PIs	Continuing
Data extraction	IPHS/Fudan EAT	On-going
Industry coding (code system as for the work histories)	IPHS/Fudan EAT	Existing/verification
Key Tasks and time periods summaries	EAC	3Q 05'
Time/task coding	EAC	4Q 05'
Translation	External resources	4Q 05'
Statistical analysis of IPHS data	EMBSI	1Q 06'
Chinese literature searching/gathering (qualitative insights, quantitative information to supplement IPHS database)	Professor Liang Youxin	3Q 05'
Literature summary	Professor Liang Youxin	3Q 05'
Industry coding	Fudan EA	3Q 05'
Translation	External resources	3-4Q 05'
Exposure assessment by time/sampling objective	EAC/EMBSI	4Q 05'
Current site EA	IPHS/Fudan EA	On-going
Preliminary EA of CC/DP cases to plan follow-up actions and set investigation objectives, including site ea goals	EAC	On-going
Industry and task coding of the current data to facilitate is use including comparisons to IPHS data	EAC	4Q 05'

Translation	External resources	On-going
Expert reports – summary of industry, technology, time variations	EAC, *ad hoc* advisors (e.g, IPHS)	4Q 05'
Translation	External resources	4Q 05'
Consistency check	EMBSI	2Q 06'
Project time/industry/task exposure matrix	EMBSI/Fudan EA	3Q 06'
Final exposure assessment	EMBSI lead, Fudan support	2 – 4 Q 06'
External EA review	Fudan EA/EMBSI/External experts	4Q 06'
Final report	EMBSI/Fudan/IPHS	1Q 07'

EAT = Exposure Assessment Team. Responsible for initial screening and for field investigation work, including investigation of workplaces for current exposure sampling and historical information for the subject work history. The EAT is under the direction of the EAC.

EAC = Exposure Assessment Coordination Committee. Professor Fu Hua, Zhu Surong, Dr. Tom Armstrong, Yimei Zhou (interpreter).

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EXECUTIVE SUMMARY

I. JCML OPERATIONS

A. Progress

Study diagnoses for the first 18 months of case accrual (Sep 03 – Feb 05) have been completed and 100% reviewed. During the first year a total of 740 cases were diagnosed and 732 were assigned ICD codes according to WHO and ICD-10. The remaining 8 unassigned cases are follow-ups for the DP study. There were a total of 147 cases of AML (WHO), 152 cases of lymphoid (WHO) (i.e. NHL+ALL+CLL) and 113 cases of NHL+CLL (ICD-10). There were 129 cases of MDS, 48 cases of AA and 20 cases of benzene poisoning diagnosed. At 18 months a total of 1131 cases were diagnosed and reviewed with 1108 ICD codes assigned. The remaining 23 unassigned cases are follow-ups for the DP study. There were a total of 269 cases of AML (WHO), 261 cases of lymphoid neoplasms (WHO), 188 cases of NHL+CLL (ICD-10), 192 cases of MDS, 61 cases of AA and 25 cases of benzene poisoning diagnosed. Further, a total of 400 patients were diagnosed over the past 6 months, bringing total cumulative case contact to 1531. Since January, a total of 4 cases of AA, 39 cases of MDS, 56 cases of AML, 55 cases of lymphoid neoplasms and 2 additional BP cases have been diagnosed, and a total of 137 cases remain unassigned to the study database as of the time of this writing. In addition, over the course of the project to date JCML has diagnosed approximately 40 *pro bono* cases for individuals, mostly children, who do not qualify for inclusion in our studies.

B. Nutritional Deficiencies

An important observation is the number of hematologic abnormalities that can be attributed to nutritional deficiencies. As of June, 2005 we have identified 72 cases, the majority of which would be misclassified as AA, MDS or even AML in a retrospective study. Consequently, nutritional deficiencies are involved in roughly 20% of the potential case accrual for these diseases, rendering nutrition an important confounding factor in the study of AA and MDS. The frequency of diagnosis of nutritional deficiencies at JCML also suggests that this represents an important public health issue in Shanghai. Professor Fu Hua has agreed to take responsibility for preparing an analysis of the influence of nutritional deficiencies in hematopoietic disease in Shanghai.

C. HIV Serology Testing

We have encountered difficulties in implementing approved changes to the protocol for directly determining the rate of HIV seropositivity in our lymphoma cases. This is due to a number of logistic problems, primarily the fact that the majority of lymphoma cases, especially low grade lymphomas, are outpatients who are reticent to provide a blood sample for procedures not directly related to the diagnosis of their condition. Other outpatients are not willing to return to the hospital for additional blood work after diagnosis. Alternatively, screening for HIV is becoming far more commonplace in Shanghai, and participating hospitals have agreed to share HIV serology data previously obtained from IC'd subjects. Nevertheless, an effective mechanism for

doing this on a routine basis has yet to be implemented. In contrast to the issues related to sample collection for NHL, all hematology cases, including ALL and CLL cases, diagnosed at JCML have been tested for HIV. To date, no confirmed cases of HIV have been identified in these subjects (N>650). Independently, the NHL subtypes diagnosed in our patient population are not consistent with immunodeficiency-associated lymphoid disorders. Therefore, although we have only a fraction of direct measurements on NHL cases, we believe that it is very unlikely that HIV constitutes a significant confounding factor for any of our studies.

II. MOLECULAR EPIDEMIOLOGY OF BENZENE-EXPOSED WORKERS

A. Molecular Epidemiology Phase 1

The goal of the molecular epidemiology (phase 1) study is to: (a) study retrospective benzene exposure levels vs. blood counts present in worker exam records, and (b) identify candidate factories for subsequent phases (e.g. 2A, 2B) of the study.

Through the leadership of Dr. Ni, excellent progress has been made in abstracting hematology counts from physical examination data at a number of facilities. Over 1600 cases have been collected and entered into an Access database from 17 facilities. Not all of these records will qualify for the final study, since some cover only one year of readings, while the molecular epidemiology study requires at least two years (and preferably more) to be able to study potential effects on blood counts longitudinally.

A potential issue for the Phase 1 study is whether the records that have been abstracted are representative of the workforce. In some preliminary factory visits, it was noted that records for workers with 'normal' blood readings were stored at one location, while records for workers with 'abnormal' blood readings could be stored separately. Dr. Ni believes that this did not apply to the cases in the Yang-Pu hospital, where most of the records were collected. Nevertheless, frequency counts for 'normal' and 'abnormal' values of different blood parameters will be examined by factory and time period to ensure that this is the case.

Exposure assessment for the factories selected has been proceeding at a steady pace. So far, the EA team has visited 10 of the 17 factories, and 7 factories are awaiting a site visit from the team. The aim of these visits is to collect information beyond that which may be present in the IPHS database. This is needed to reliably link certain jobs/factory locations with appropriate retrospective data. In addition, the EA team has performed screening assessments with rapid-detecting instruments, consisting of over 250 measurements. The results of the initial assessments have been mixed: in some sites no records of benzene exposure can be found, while others have yielded good retrospective records. In other sites no current benzene exposure can be found, in others relatively low levels can be detected. So far, over 300 cases have useable exposure and blood data. We are actively working on exposure assessment and assessment of the hematologic data to assess how much useable data is present in these records.

While these efforts proceed, it seems reasonable to assume that less than half of the 1600 records will ultimately contain useable blood and exposure data. A coordinated effort is underway to identify suitable factories outside of the Shanghai area that might be used to increase the number of workers in the Phase 1 study, while also identifying sites for Phase 2A.

B. Molecular Epidemiology Phase 2

A total of 42 factories have been visited in an effort to select appropriate facilities for Phase II studies. Extensive area and personal sampling has been performed in three facilities with 984 area samples analyzed with mean airborne benzene concentrations of benzene ranging between 0.01 and 326.7 ppm. A total of 231 workers have been recruited to the Phase IIa study.

Over the past year, the ME study has encountered a number of challenges which have impacted on its progress and resulted in some mid-course adjustments. First, our success in gaining access to acceptable study sites has been very limited. Out of 42 factories visited only 5 were considered to be appropriate for initial Phase IIa activities and only 3 have been extensively analyzed. Moreover, recruitment of workers to Phase IIb, as originally designed has proven to be problematic (N = 3). In addition to the obvious ramifications of documenting high benzene exposure in the workplace along with health effects, we have encountered a variety of difficulties in obtaining the cooperation of management in many facilities. The reasons for this are many and varied. Some frequently encountered problems are culturally based: First, in state-run companies market place competition has resulted in considerable financial stress, workers are at risk being laid-off and the anxiety is high. Therefore, management typically wants to avoid anything that may trigger trouble and reactions from their workers. Second, our use of informed consents and pledge to compensate workers for any problems arising from participation in the study stands in sharp contrast to similar recent or ongoing studies that do not acquire consents or offer any forms of compensation, thereby posing an indirect threat by example. Both local occupational physicians as well as Professor Xi have informed us that it is unlikely that this issue can be resolved without major changes to our ME informed consent (IC) procedures. Accordingly, I have asked a committee comprised of senior occupational physicians from local hospitals, the Vice Director of the CDC Physical Examination Center, Dr. Ye Xibiao, Lv Ling (JCML's lead occupational physician) and Professor Fu Hua to review the problem and to make suggestions for changing the IC procedure.

These issues notwithstanding, we identified factory workers with benzene poisoning, admitted them into hospital in Shanghai, and recruited them to the DP study. This strategy was inculcated in the original approved study design and has resulted in characterization of a case series of 23 individuals with persistent dysplasia following prolonged benzene exposure and for which we have robust exposure assessments. These cases also contribute quantitative exposure assessment data to the CC/DP EA matrix (see below). To date, we have identified approximately 30 such individuals using this mechanism. In addition, we have modified the original protocol by adding peripheral blood benzene metabolite and urinary sPMA analyses to Phase IIa as opposed to limiting

metabolism studies to Phase IIb. To date, we have measured benzene metabolites in the blood of 103 exposed workers, conducted pre- and post- shift analyses of urinary sPMA in 104 workers and have integrated hematology, exposure, metabolite and urinary sPMA data for 64 workers. In addition, utilizing PCR-RFLP methods, we have conducted preliminary genotyping of CYPE2E1, NQ01-465CT and –609CT, MPO-463GA, and GSST1 polymorphisms on all 231 Phase IIa subjects.

III. EXPOSURE ASSESSMENT

A. Introduction

The past 6 months have witnessed major developments in the EA process which are outlined below. This report represents the collective effort of a large group of individuals including the Fudan SHS Exposure Assessment Team under the direction of Prof. Fu Hua, Dr. Tom Armstrong and Yimei Zhou of EMBSI, with input from Dr. Schnatter and Prof. Irons.

B. Exposure assessment strategies and goals

The EA strategy originally designed for the DP study was adapted to all SHS hospital based studies upon the recommendation of the SRP in 2001. The primary goal of the CC study is to ascertain whether benzene exposures or other variables (e.g., viruses, nutritional deficiencies or other chemicals) are associated with certain diseases (e.g., NHL). Ordinal range assessments (no, low, medium, high exposure) or where feasible, quantified ranges such as <0.4, 0.4 to < 4, 4 to 40 and > 40 mg/m3 support these study objectives. This tiered approach (Figures 2 and 3) was originally outlined in the study protocol and endorsed by IH SRP members. Quantitative benzene assessment, which is the EA strategy employed for the ME study, is targeted for a fraction of the CC/DP subjects, and is dependent on feasibility. In the ME study, factories are selected based on good EA and health effects records and a willingness to participate in extensive concurrent personal and area monitoring activities. Thus, the detailed quantitative EA analysis performed in ME supplements and reinforces EA efforts in CC/DP. The timeline for EA is depicted in Figure 4. The integration of qualitative and quantitative EA processes is targeted for the second quarter of 2006 when validation and assembly of the final CC/DP EA matrix is slated to occur. The assignment of final benzene exposure will then follow.

C. Progress

1. Data collection

During the last 6 months, Questionnaire Takers interviewed 850 subjects for an average of about 40 cases per week. The EA team conducted site monitoring at 25 factories and collected 350 personal, area and bulk samples. A total of 3375 questionnaires have been collected to date with 3308 having completed preliminary exposure assessments (EA) for substances of interest, including benzene. A total of 1885 secondary questionnaires have been administered, including 484 during the last 6-month period. Data collected was immediately reviewed and telephone interviews conducted to

clarify any remaining issues. Qualitative EA are now progressing at a rate consistent with subject accrual (See Figure 1). Semi-quantitative and quantitative exposure assessment (EA) of benzene requires more detailed data assembly, and the time line on this effort naturally lags case accrual. Nevertheless, these analyses are on schedule for completion in conjunction with detailed benzene EA which is slated for third quarter 2006, for the cases accrued to that point. Initial projections for benzene-EA were based on predictions of a caseload of 500 cases per year and an expected prevalence of 10% benzene exposed in the study subjects. The caseload is approximately 50% higher than expected and the prevalence of benzene exposed subjects is about 7%.

Exposures other than benzene were assigned in over 2600 instances. The studies by design evaluate exposures to a range of risks other than benzene, since exposures to other hazards relevant to the diseases of interest may be indicated in a subject's work and life histories.

EA team members continue to close the gap on earlier shortfalls in EA assessments and progress is now meeting the expectations of the EA Coordinating Committee (EAC). Nevertheless, unanticipated challenges have required additional staffing, and we continue to experience a shortage of qualified EA personnel. Therefore, efforts are underway to recruit additional experienced staff.

Through the first half of 2005, 93 work site inspections (CC/DP and ME) have been completed. These work site inspections included extensive current exposure monitoring:

- 4300 UltraRAE screening measurements of benzene concentrations
- 2150 area samples (20 minute breathing zone by IPHS protocol)
- 1070 personal benzene exposure samples

Additional site reviews are planned.

2. Exposure analysis for the CC/DP studies

We have experienced limited success finding exact matches for factories with exposure data corresponding to time periods relevant to subject work histories. Many factories are closed and were not previously monitored by IPHS. Some subjects are from areas of China remote from Shanghai. Review of completed assessments using exact match or limited surrogate site data showed divergent results for apparently similar work histories. Therefore, the industry sector analysis previously described in the study protocol is being emphasized for providing quantitative data to support the final benzene EA. Sector analysis was further developed in April, with a pilot test conducted in May to evaluate the capabilities of performing sector analyses as part of a comprehensive EA strategy. The study focused primarily on the shoe industry and on a segment of the rubber industry. The exercise involved:

- Sorting available IPHS data for the industries of interest
- Sorting the data into sectors within each industry

- Adding supplemental task categories (not part of the IPHS database) based on sample location descriptions
- Qualitatively evaluating the frequency-concentration distributions of the sector, by task and time period
- Calculating preliminary summary statistics for the identified sector, task and time categories

As a result of this exercise for the test industry segments, sector analysis was determined to be a useful approach for evaluating other key industrial segments found in subject work histories, and is being integrated into an overall collaborative effort with IPHS. The sector analysis evaluation yielded insights that led to further research efforts that are currently underway. One main point from the pilot study is the need for additional coding based on area descriptions given for the samples in the IPHS database. The additional codes are needed to separate the data concerning where people worked, what tasks were done and by era. The additional coding will reduce heterogeneity in the data sets thereby allowing more precise exposure assignments. This additional coding and analyses will be conducted in collaboration with IPHS.

The main stages of the CC/DP process, with responsibilities and progress dates are shown in Table 4. It is important to recognize that until the EA matrix is completed, the process is dynamic and iterative, with the methods and degree of reliance on individual information sources differing for individual sectors. Several different sources of information are being used and periodically reviewed, and previous assessments are continually being compared with ongoing ones.

For approximately 13% of the CC/DP subjects qualitatively categorized as benzene exposed, quantitative analysis has been successfully completed as part of EA in the ME study. These quantitative assessments are based on current personal exposure measurements, current area concentration measurements, detailed compilations of factory changes over time that modulate exposure, use of retrospective exposure estimating techniques, and use of past IPHS monitoring data. This has resulted in a detailed exposure matrix for these subjects. Additional publications based upon this work are in preparation.

3. Changes to the data collection process

Many of the second level questionnaires were found to be of minimal value so the set has been reduced to 23. An additional summary of key task information (e.g. task description, materials used, frequency and duration) is being provided on a "key data summary" card to better prompt the staff during questionnaire interviews. Additional question-coded data recording sheets are being considered since the primary questionnaires do not always provide sufficient writing space for detailed answers.

4. Initial screening, qualitative assessment and relative ranking

The EAT and EAC have prioritized the completion of qualitative assessments and relative ranking of the subjects for benzene and the other exposures. This provides a basis for linking the subject's work history to the project exposure assessment matrix.

Part of the review includes seeking current access to the work place for current monitoring and evaluation of changes in the operation relevant to the time the subject worked.

5. Collaboration with IPHS

Collaboration with the Shanghai IPHS has been enhanced and restructured to provide an integration of database, factory site evaluation and sector information. This involves close interaction with IPHS District staff in order to conduct a comprehensive analysis of a single district, Yang Pu, that will serve as a centerpiece for the development of tools for validating individual data sets. Work with the District staff includes verifying data in the database, and completing current monitoring for factories operating in the District that coincide with study subject work histories. This collaboration provides additional resources to further code data and to develop summaries for industrial sectors important to the subject work histories.

Reviews of the CC/DP exposure assessments have been underway for the last year, and will be expanding in number and depth as the EA matrix supporting materials and their translations reach completion. It is proposed to have members of the SRP with expertise in EA participate in a review of exposure matrix in the second half of 2006.

6. Analysis of the Chinese EA literature

Professor Liang has been asked to complete the summary and analysis of the Chinese EA literature, including categorization of articles by quality, for use on the EA process. Another important aspect of the analysis that is underway is the preparation of summary reports that outline trends impacting exposure potential for key industries. An important part of this exercise is a summary of the benzene composition time trends for key materials (e.g., glues, solvents, paints, gasoline). This is an important and significant undertaking which is needed to complete the integration of EA processes into a final exposure matrix.

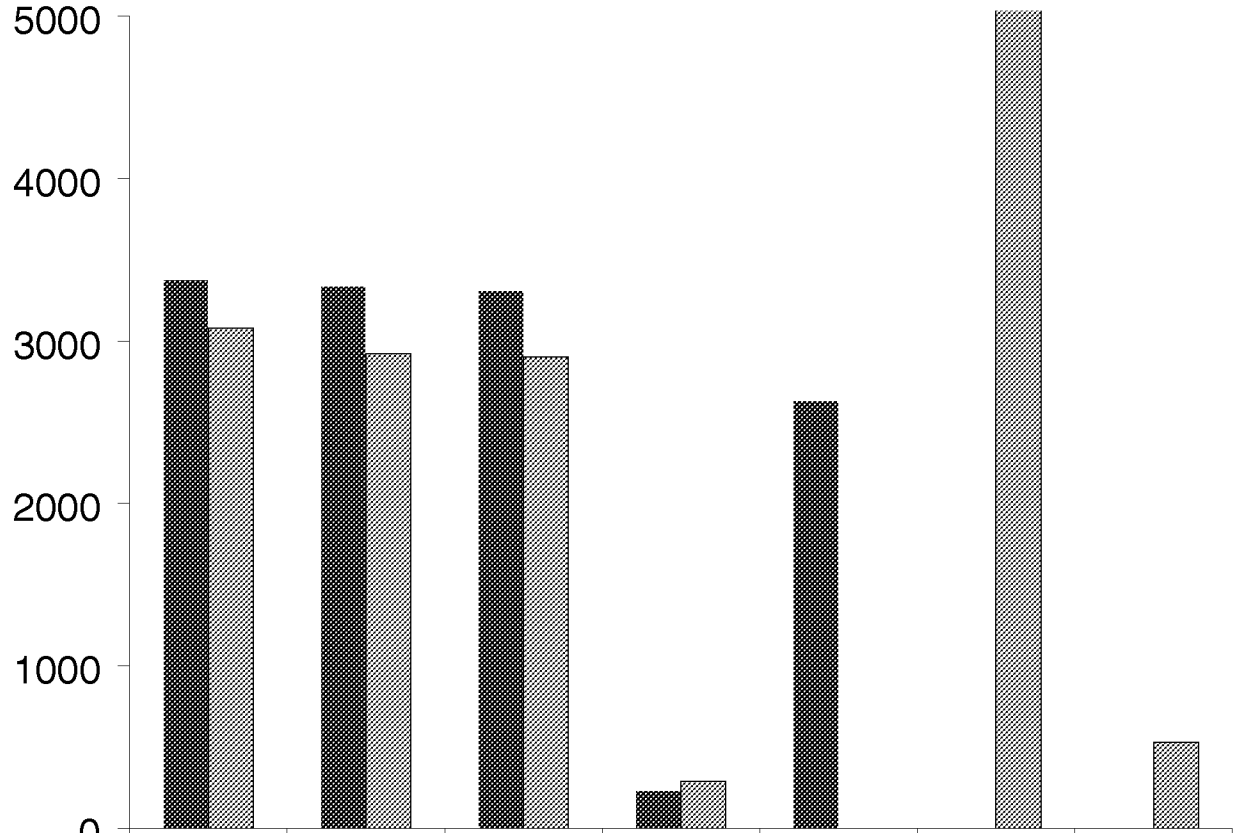
7. Management

Continued dialog and interaction among the Fudan and Western EA investigators and Consortium Committees has resulted in some streamlining of the EA committee process to improve organization and communications. The purpose of the EA Coordinating Committee (EAC) is to discuss progress and identify problems that have occurred during the past month and identify goals and approaches for the EA team (EAT) that will be followed for the next month. The EAC is under the direction of Professor Fu Hua and includes Tom Armstrong, Zhu Surong (IPHS) and Yimei Zhou (interpreter). The EAC reports directly to Dr. Irons.

The EAT, which is responsible for day-to-day EA operations, holds weekly staff meetings which are co-chaired by Ye Xibiao and Yimei Zhou. The purpose of these meetings is to manage EA operations, provide a list of weekly action items and to identify issues that need to be taken to the EAC. During the past 6 months, the team welcomed two new members who joined to fill in gaps left by a graduation and a

pregnancy. Additional staffing efforts are underway to identify an experienced individual with both IH training and bilingual language skills.

Figure 1. June 2005 CC/DP EA Status



	Case recruitment	Data Entry	Prelim EA	Benzene Exposed	Other Exp	Final Subjects	Final Benzene Exposed
06/05 Actual	3375	3335	3308	233	2631		
06/05 Planned	3080	2920	2900	290		5300	530

Figure 2. CC/DP Exposure Assessment Process Overview

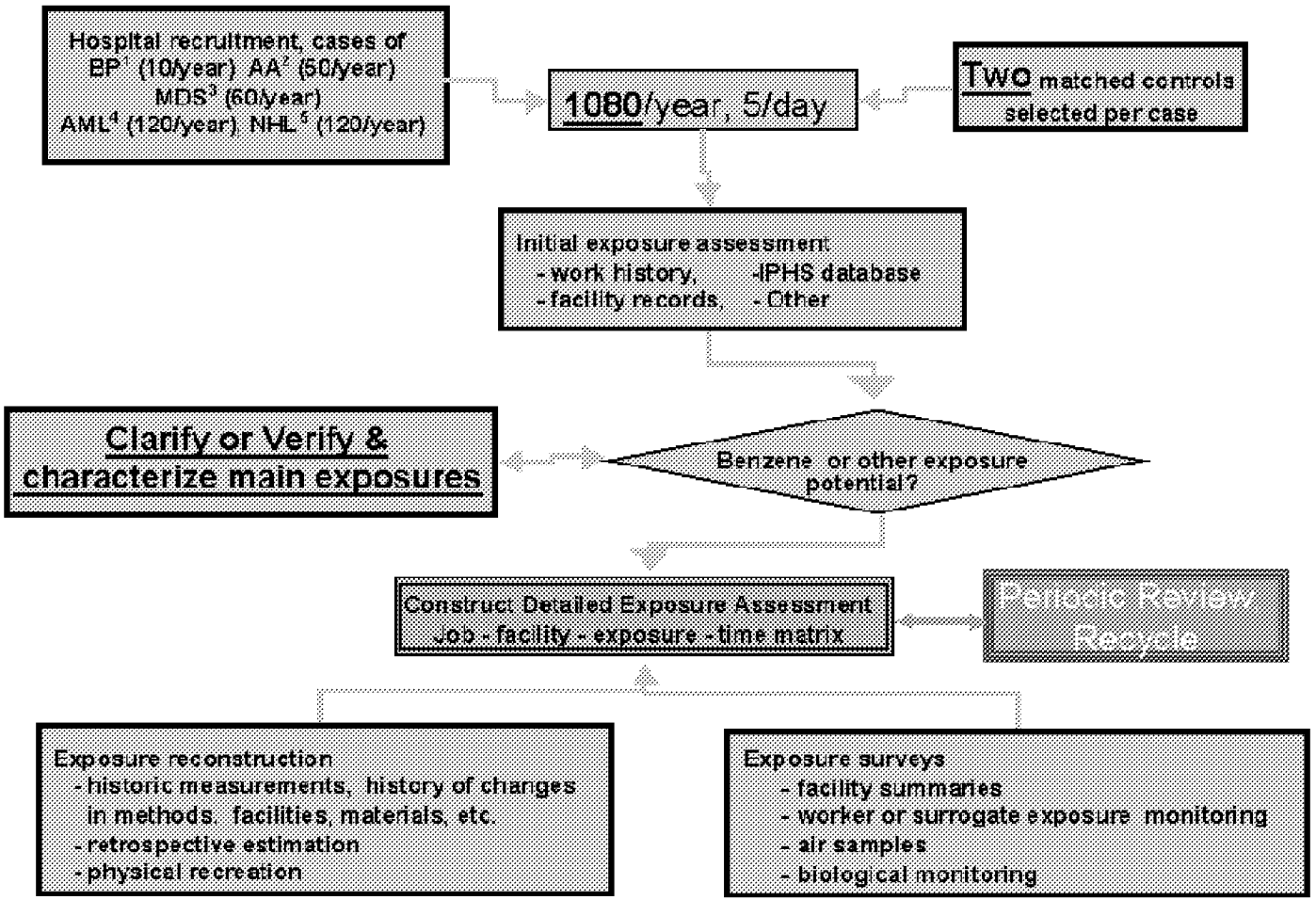


Figure 3. Case Control Studies Tiered EA Design:

Expectation of Fewer Subjects Completed at Higher Tiers

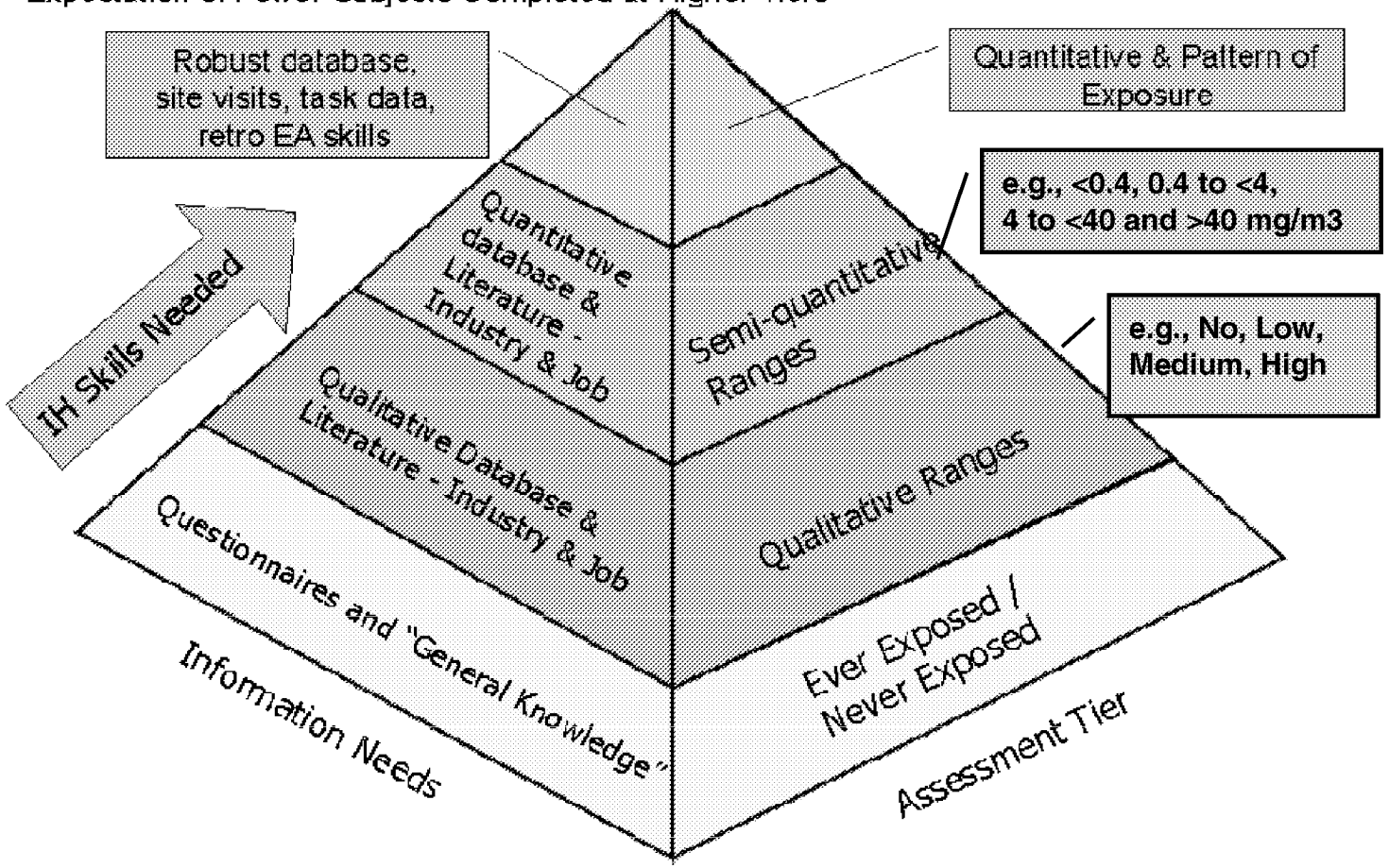


Figure 4. CC/DP EA Path Forward

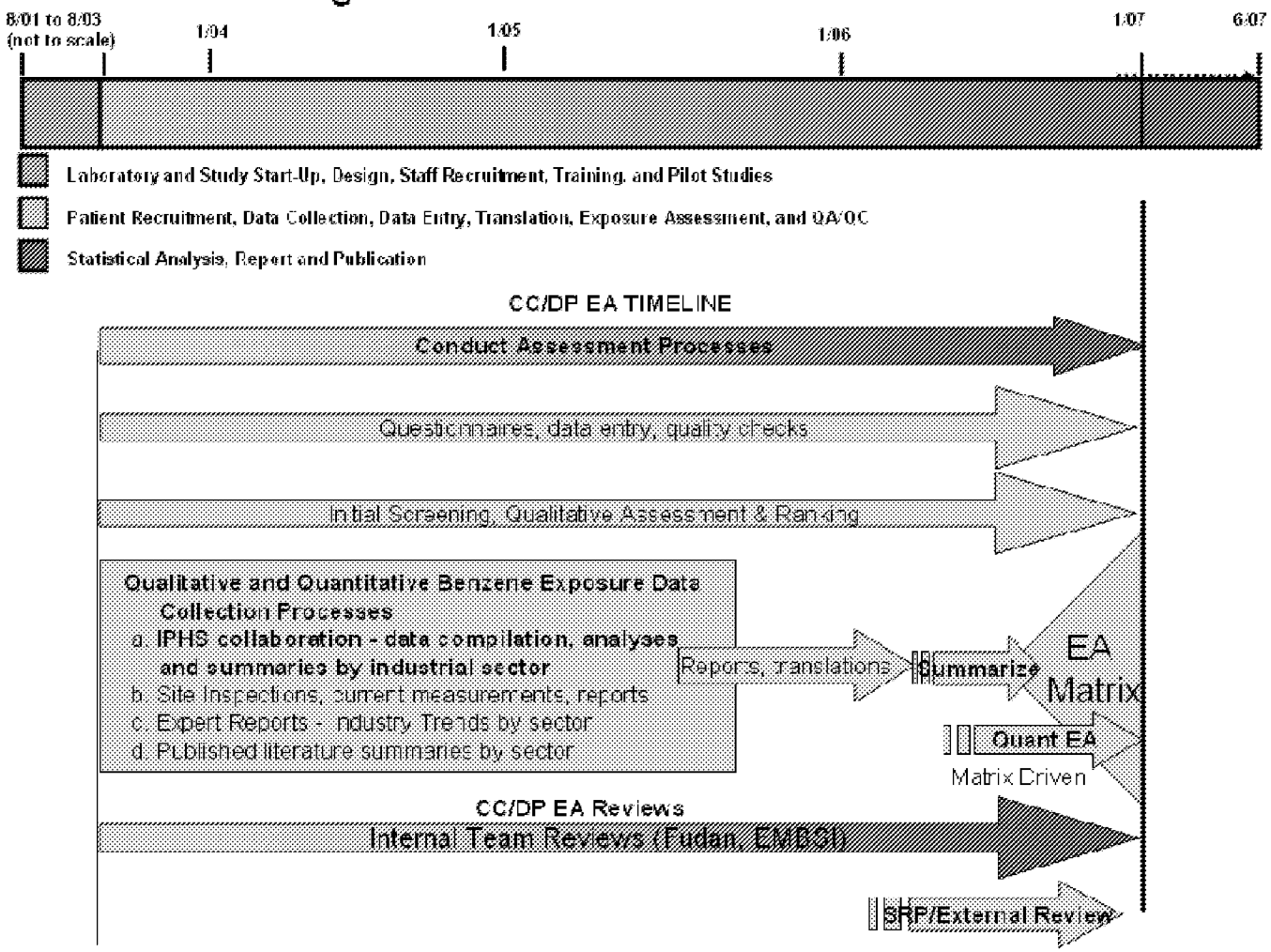


Figure 5. CC/DP Exposure Assessment for PROBABLE "BENZENE" EXPOSED Multiple Lines of Investigation and Information

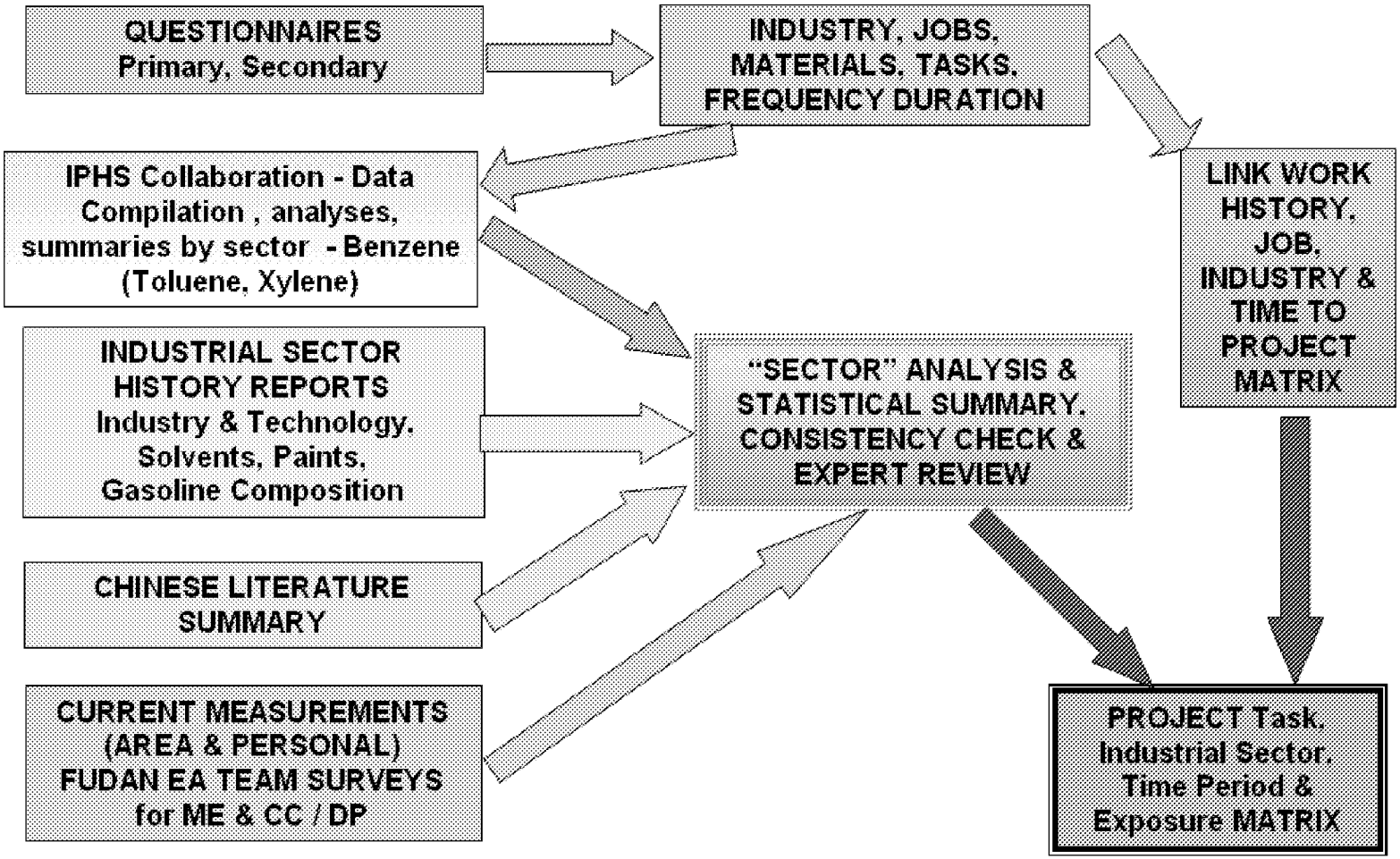


Table 4. Exposure Assessment Processes & Responsibilities for CC/DP Study

STEP	WHO	WHEN
Questionnaires – primary and secondary	Questionnaire Administration (QA)	On-going
Industry/job/materials/task/frequency/duration (key data for EA and follow-up planning)	QA/data entry/EAT	On-going
Industry codes assigned (to group work histories for consistency and follow-up planning)	EAT	On-going
Translations of key information to English	External resources	On-going
Task descriptions (essential to link to IPHS area descriptions)	EAC	On-going
Task coding (to group work histories for consistency and follow-up planning)	EAC	4Q 05'
Link work history/task/industry/time to reference data via EA matrix	EAC/EMBSI	06'
Access to IPHS data base	PIs	Continuing
Data extraction	IPHS/Fudan EAT	On-going
Industry coding (code system as for the work histories)	IPHS/Fudan EAT	Existing/verification
Key Tasks and time periods summaries	EAC	3Q 05'
Time/task coding	EAC	4Q 05'
Translation	External resources	4Q 05'
Statistical analysis of IPHS data	EMBSI	1Q 06'
Chinese literature searching/gathering (qualitative insights, quantitative information to supplement IPHS database)	Professor Liang Youxin	3Q 05'
Literature summary	Professor Liang Youxin	3Q 05'
Industry coding	Fudan EA	3Q 05'
Translation	External resources	3-4Q 05'
Exposure assessment by time/sampling objective	EAC/EMBSI	4Q 05'
Current site EA	IPHS/Fudan EA	On-going
Preliminary EA of CC/DP cases to plan follow-up actions and set investigation objectives, including site ea goals	EAC	On-going
Industry and task coding of the current data to facilitate is use including comparisons to IPHS data	EAC	4Q 05'

Translation	External resources	On-going
Expert reports – summary of industry, technology, time variations	EAC, *ad hoc* advisors (e.g, IPHS)	4Q 05'
Translation	External resources	4Q 05'
Consistency check	EMBSI	2Q 06'
Project time/industry/task exposure matrix	EMBSI/Fudan EA	3Q 06'
Final exposure assessment	EMBSI lead, Fudan support	2 – 4 Q 06'
External EA review	Fudan EA/EMBSI/External experts	4Q 06'
Final report	EMBSI/Fudan/IPHS	1Q 07'

EAT = Exposure Assessment Team. Responsible for initial screening and for field investigation work, including investigation of workplaces for current exposure sampling and historical information for the subject work history. The EAT is under the direction of the EAC.

EAC = Exposure Assessment Coordination Committee. Professor Fu Hua, Zhu Surong, Dr. Tom Armstrong, Yimei Zhou (interpreter).