

REPORT  
ON THE PILOT PROJECT  
" LEUKEMIA- LYMPHOMA "  
QUARTER VI  
( time period from 1.02.1999 to 30.04.1999 )

## TASK 1. SAMPLING

### Milestone 4.

#### ( epidemiologic group )

In deal with the providing of hematological diagnostic review in January the necessity of investigation of the availability of biological material and clinical records in several oblasts was considered by the International review panel. The fieldwork in the chosen oblasts showed the problems with the preservation of the medical material in some of them. In Sumy region it was hard to get material from original random sample because of the poor preservation. In Donetsk region most of clinical and biological material was lost because of the accident at the archive. Such reasons led us to suggestion about the examination of other oblasts. So the Ukrainian side decided to examine some oblasts (Cherkassy, Poltava, Chernigov ) where comparable number of liquidators are living and then to make the decision. Each oblast accounted about 10-11 thousands of clean-up workers.

On February 25-26, 1999 year the staff of the RCRM of AMS of Ukraine including Drs. V.Bebeshko, I.Dyagil, V.Klymenko, N.Gudzenko and O.Tsvetkova accomplished the visit to Cherkassy region.

The tasks were as follows:

1. To study the local Chernobyl register;
2. To evaluate the hematological medical care for the persons suffered following Chernobyl accident;
3. To study the availability of the clinical and biological material in the oblast;
4. To make a preliminary review of the diagnostic procedures recorded for random selected cases.

The team had the meeting with the head of local administration of the health care Dr.A. Sytnik. Dr. V.Bebeshko described briefly the tasks and the main characteristics of the joint Ukrainian-American Protocol and the perspectives of leukemia and related disorders study among liquidators.

The structure of the health care for persons suffered from Chernobyl accident in the Cherkassy oblast are presented at table 1.

### Structure of the medical care for liquidators in the Cherkassy oblast

The head of the department for the medical care of the Cherkassy oblast's State Administration  
 Symbic Anatoliy Pavlovich 45-34-58  
 Trustee - Procopencova Valentina Vasylijevna

Oblast Hospital  
 The Head - Fesun

Oblast Oncology Dispensary  
 The Head Paramonov Victor Vladimirovich - 47-01-23  
 The Depute Pustovit Yuriy Dmitrievich 47-00-05  
 Fax 47-01-23, e-mail cancer@artintel.cherkassy.ua

Center for medical statistics  
 The head - Lichogrud Nadezhda Petrovna -47-09-79  
 The Depute - Michaylichenko Tatyana Ivanovna  
 47-03-14  
 Chernobyl registry  
 Lesynskaya Oksana Ivanovna - the head  
 47-63-08  
 47-03-14 Ptashuk Taisya - operator  
 47-03-15 Ryasantseva Valentina Vasylyevna -  
 statistician

Hematology department  
 The head - Pilipenko Galina Vasylijevna - 47-00-13



The local registry in Cherkassy region is located at the local oblast hospital at the oblast's center for medical statistics. Registry head is a M.D., the staff includes one statistician and one of technical personnel. The list of the liquidators accounted 11,166. Additionally about 800 liquidators are included to the registry of Ministry of Internal Affairs. The information concerning the liquidators is in the paper and computer files. All data of the registration coupon (including identification and passport data) and the data of the coding coupon with the results of the medical examinations are available from 1987 and in computer files from 1996 to 1998.

The oblast's Cancer Registry is located at the oblast oncology dispensary (Î Î D). The staff consists of 3 persons.

Software for the Cancer Registry was created by local programmers in 1992.

The special field for the information on exposure group (liquidator, Evaquee, Contaminated area resident) is included to the individual record but not filled in carefully enough (as was told by the local staff). Data base includes all new diagnosed cases since 1992 and cases alived at the moment of CR formation. It is planed to establish the National Cancer Registry software in the Cherkassy CR.

All cases of leukemia/lymphoma registered in the CR among mail population of Cherkassy oblast were chosen to link them with Chernobyl Registry. It will help to find additional cases among liquidators and estimate both registries.

The oblast's hematological department for 60 beds is located at the OOD. The department has enough rooms, several wards for intensive care and skilled personnel. Besides in the OOD now the construction of new premises PBSC and autologous bone marrow transplantation is performed. All necessary equipment had been was bought. The staff was trained in different centers and ready for work. The head of the OOD is qualified hematologist, Dr.V.Paramonov, the former head of hematological department.

Premises for storage of medical records are located in the same building and any information could be obtained easy.

The cytological laboratory is a part of the general clinical laboratory with different kinds of the investigations. The head of clinical laboratory is qualified expert in the field of hematological cytology and cytochemistry. To evaluate the availability of the clinical and biological material the random sample from medical journals was selected according to the case requirements for the review (21 leukemia cases and related disorders and non-Hodgkin and Hodgkin lymphoma) (Table2).

Table 2.

## ORIGINAL SAMPLE ON CHERKASSY OBLAST

№	Surname	Address	Diagnosis	Year of confirmation	Med. record	Morfology	Notice
1.		Cherkasy	AL	1989		-	
2.		Cherkasy	AL	1994		+	
3.		Horodysche	AL	1994	+	+	
4.		Chyhyryn	AL	1995		-	
5.		Cherkasy	AL	1998	+	+	
6.		Chernobay	AL	1990	+	-	Slides of bone marrow not stored
7.		Kaniv	CML	1990	+	-	Patient died after 9 days stay in the hospital. He was not agree for bone marrow punction
8.		Cherkasy	CML	1987	+	+	
9.		Cherkasy	CLL	1987	+	-	
10.		Horodysche	CLL	1996	+	+	
11.		Katerynopol	OMF	1989	+	-	Diagnose was not confirmed
12.		Kaniv	OMF	1996	+	+	
13.		Cherkasy	AA	1989		-	
14.		Talnoye	MDS	1997		+	
15.		Cherkasy	MM	1988		-	
16.		Chernobay	MM	1997		+	
17.		Chernobay	MB	1997		+	
18.		Monastyrische	HD	1997		+	Hystology date is in other institution
19.		Cherkasy	NHD	1988		+	
20.		Spola	NHD	1988		-	Only 3 days stayed at the hospital
21.		Horodysche	NHD	1994		+	

The results were the following:

Table 3.

**Availability of diagnostic material for leukemia cases**

Period	Selected cases	Presence of med. Records	Presence of slides
1987-1990	4	4	0
1991-1994	2	2	2
1995-1998	4	4	3
1987-1998	10	10	5

Table 4.

**Availability of diagnostic material for cases for related disorders**

Period	Selected cases	Presence of med. Records	Presence of slides
1987-1990	2	2	0
1991-1994	-	-	-
1995-1998	2	2	2
1987-1998	4	4	2

Table 5.

**Availability of diagnostic material for lymphoma cases**

Period	Selected cases	Presence of med. Records	Presence of slides
1987-1990	3	3	1
1991-1994	1	1	1
1995-1998	3	3	3
1987-1998	7	7	5

All medical records were stored in full order during the long period of time. All the requested medical documentation was available for team.

Availability of biological material for early period (1987-1990 yy) was low for all investigated types of disorders (from 9 selected cases for this period only one sample

was found). The same results for early period we had got in other oblasts. Conditions for this period investigation have to be clarified.

For middle and late period (1991-1998) almost all samples of biological material were found and evaluated by hematologists ( from 12 selected cases for 11 slides were available) (Table 6).

Table 6.

**ORIGINAL REVIEW OF BIOLOGICAL MATERIALS ON CHERKASSY****OBLAST**

№	Surname	Volume of clinical record	Quality of slides	Coincide of diagnose	Note
1.		+	Good	CML	
2.		+	Good	MDS	
3.		+	Fair	AL	Cytochemistry absent
4.		+	Good	MM	
5.		+	Good	AL	Cytochemistry absent
6.		-	Fair	MM	
7.		+	Fair	OMF	Trepanobiopsie in other institution
8.		+	Good	AL	
9.		+	Good	CML	
10.		+	Fair	CML	
11.		+	Fair	AL	
12.		+	Fair	NHD	

The preliminary review of the slides and medical records found was conducted by the hematologists from RCRM. All original major diagnoses were confirmed by the experts. The quality of slides available was disired as good only in 50 %.

So result showed quite high level of organization of medical care in Cherkassy region and it may be background for the future study.

### MILESTONE 6.

( epidemiological group )

No clarify the feasibility of search for the persons, who were not encompassed by the routine examinations for a long time due to different causes, 50 liquidators were selected from the State Registry DB on the following basis:

- they were registered in the SR as residents of the Dnipropetrovsk oblast at any time following the accident;
- no data available in the SR DB on the death;
- No information available in the SR DB on the results of the routine examinations during the recent 3 years and more.

The search for the "lost to follow-up" and clearing up the causes for absence of the data on the routine examinations during three and more years were performed with the help of the responsible persons of the territorial polyclinics according to the latest resident's address known. These physicians in the majority of cases possess the information on the long-term absence or on absence or change of the address of the liquidators who are under surveillance since it is usually found out when they fail to appear for the routine examinations. As it was proved by the experience, a head of the oblast dispensary department for medical support of the Chernobyl victims (in the oblast having a specialized dispensary for radioactive protection of the population - a head of this institution) has to collect this information from the responsible physicians. Such specialist maintains a close contact with the responsible physicians, supervises their work, and is capable of making prompt decisions necessary under local circumstances. Involvement of the head of the dispensary department for medical support of the victims in the Dnipropetrovsk oblast clinical hospital demonstrated the advantages mentioned above.

From among 50 persons:

- 19 persons were examined in 1998; the data will be contributed to the Registry.
- 9 persons moved to the departmental subregistry of the railway transport workers. It is possible to obtain data on their health, if needed.

In the Dnipropetrovsk oblast 330 persons moved from the SR to the departmental registry of the railway transport workers:

- 2 liquidators died during 1998 (Aug.,31, 1998 and May,27,1998);
- 2 persons failed to confirm their liquidator's status and are not asked for the examinations;
- 1 liquidator is now in prison though he is registered according to the same

address;

- 1 liquidator emigrated to Israel;
- 2 liquidators moved to another oblast;

- 3 persons reside in Zhovty Vody. This town has not been reporting to the oblast registry for 2 years until 1998. Since 1998 oblast registry started again to get information on liquidators health status. One of three liquidators from Zhovty Vody failed to confirm his liquidator's status and so was not examined in 1998. 1 liquidator refuses to come for the examination. Responsible physician visited him at his home and defined his health status as good. One of three liquidators is living in Zhovty Vody although his official address is in other oblast. He does not work.

- 5 persons refuse to come for the examinations;
- 3 liquidators changed the medical institution due to different causes and have not been examined in 1998;
- 3 moved to another districts according to preliminary data;

As it is seen from the data presented above no contacts can be made with 6 persons (died, immigrated to other country, oblast, is in prison).

During the 6<sup>th</sup> quarter the following activity was initiated:

1) request to the medical facility, which provide medical care of railway transport workers, including liquidators, on the health status of those who moved from the State Chernobyl registry (9 persons from the list of "lost to follow-up");

2) request to the passport bureaus on liquidators who moved to other oblast, district according to information from the responsible physicians, who changed the medical institution and refuse to come for the examination (13 persons at all);

3) individual mail contact with liquidators who refuse to come for the examination and changed medical institution (9 persons)

The request to the railway transport workers medical facility was answered and following data were got. 8 of 9 persons were examined in 1998. The information on their health status could be got necessarily. One of 9 was not examined.

Responsible physicians did request to the passport bureaus. Answers are in the progress.

Individual mail contact was conducted with the letter, which is presented below. The letter was sent to the last known liquidator's address (to the 9 persons).

During the 6<sup>th</sup> quarter 2 answers were got. One liquidator fills himself too bad to come for medical examination. One liquidator has had long-term business trips. During the next quarter we plan to get the rest of information on «lost to follow-up» and analyze it.

Thank every-body to which this message will come for attention and consumed time! Please if the addressee is not living here any more inform us using the envelope enclosed

Dear \_\_\_\_\_!

We know that you are a participant of clean-up work after the Chernobyl accident - the biggest technogenous catastrophe in 20<sup>th</sup> century.

According to Ukrainian lows all suffered persons first of all clean-up workers have yearly medical examinations which includes examinations by specialists, laboratory tests and other special investigations.

These examinations help to find out the early stage of illnesses if they take place, to check the efficiency of previous illnesses therapy, to plan the volume of medical and prophylactic activity.

The medical department on providing of the medical care for suffered following Chernobyl accident (Oblast Hospital named by Kalinin) controls the carrying out of the regular medical examinations.

We are perturbed because of your medical data absence in our Department. It may be resulted from the fact that the envisaged necessary medical care is not given to you in the full measure.

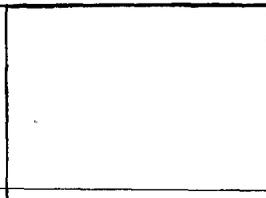
We will greatly appreciate your help in the establishment of mentioned above fact causes.

In order to minimize your time expenditure we have composed the brief list of the possible causes. Please mark one or more of them. If none of them reflects your situation please write it below.

The envelope with stems and our address is enclosed to make easy your answer.

Thank you very much for your help.

The head of the Department on providing of the medical care for suffered following Chernobyl accident (Oblast Hospital named by Kalinin)



T.I.Chekmar  
yova

**THE LIST OF POSSIBLE CAUSES**

- You were examined. The cause of your medical data absence is unknown for you
  
- You are observed in the departmental medical facility
  - for Ministry of Internal Affair
  - for Military Ministry
  - for KGB
  - for Railway Transportation workers
  - other \_\_\_\_\_
  
- You fill yourself too bad to come for medical examination
- You changed the polyclinic and were not registered there as a liquidator
  
- You have long-term business trips
  
- You more often live on the other address
  
- You have not any medical problems and decided that it is not important to come for the examination.
  
- There is no any defined cause
  
- Other cause \_\_\_\_\_

## TASK II. DOSIMETRY

### Milestone 10.

( dosimetric group )

Reconstruction of individual doses for 20 liquidators was performed by three independent experts according to the procedure developed jointly by RCRM Institute for Biophysics (IBP) and ChNPP.

During dose reconstruction using the route tests of the first days and months following the accident with no instrument dosimetry available the skilled experts help liquidators split the route into the episodes and frames, evaluate their duration and determine related dose rate for their time intervals using charts of radiation situation. In typical cases such as movement according to standard routes in the first days and months following the accident the experts use special enumeration of the episodes mostly met while estimating the route lists. This enumeration was compiled by the experts after thorough chronometry of one or another episode. Therefore, unlike the predictions that the discrepancies between the experts' estimates will be met in case of person's service in *non-uniform radiation fields*, no discrepancies are found in such cases - the estimates coincide. Hence, the error is determined by the estimation procedure and precision of the instrumental dose estimation.

The final analysis of the dose estimates comparison obtained by EPR method and by experts calculation is envisaged in the next report. As an example, we'll compare dose estimates of the liquidator who quite by chance was selected for interviewing using the International dosimetry group questionnaire and was bled for the subsequent FISH analysis. Thus, he will have four dose values set to zero by different methods. He has been a ChNPP employee since 1971; at the moment of the accident - a dosimetrist, participated in the emergency works at ChNPP from 26.04.86 till 1988, remembers details of his work well.

Table 7 presents mean estimates and low and upper boundaries of the dose intervals received by the three methods under consideration. All doses are given in sGy. As a point estimate of SEAD method a geometric mean of the dose interval boundaries was taken where membership function equals one. The same data are presented in Fig. 1 as membership functions for SEAD and analytical method and for EPR - as probability function with the corresponding mean value and standard deviation normalized in such a way so that uncertainty of the dose interval  $\pm \alpha$  may be compared with the intervals of 50% certainty determined by two other methods.

Table 7

	Low boundary	Mean	Upper boundary
EPR	4.5	7	9.5
ADR	6.3	14.9	35.1
SEAD	5.8	9.7	17.3

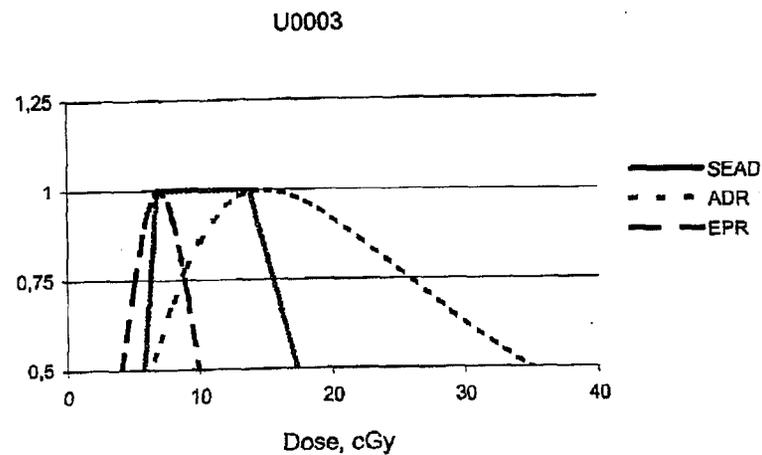


Fig 1 Juxtaposition of the dose estimates by three methods involving SEAD, ADR and EPR.

#### Milestone 12.

( dosimetric group )

On March 16-19, the first results of SEAD method testing were presented at the meeting of the International dosimetry group in Lyon. This method was applied for 62 liquidators' questionnaires involving 50 ones filled-out in Russia and 12 - in Ukraine, the latter 12 liquidators being bled for further FISH analysis. The dose estimation proper was performed by the author of SEAD method Ph.D V.P.Kryuchkov (Institute for

Biophysics, Russia) using the computer program developed by him. In the framework of the leukemia project V.P.Kryuchkov delivered a lecture in Kyiv where he presented basic principles of the method and results of the first testing. Since on the one hand, we are planning to study this method and its application further during Phase I, and on the other hand, the method has not been approved yet, its detailed description as well as the results of the subsequent testing are to be presented in the next reports or as separate appendices.

From out of 12 persons 4 liquidators had dose estimate calculated by the experts using analytical method; 7 - the dose estimated by EPR and one person had both. Comparison of these values with the values (intervals) obtained using SEAD method is presented in Fig 2.

It is known that using this method two dose intervals may be obtained: the less one in which membership function equals 1 which corresponds to 100% confidence of the expert that this dose may be involved in the given interval (the upper boundary of this interval is indicated in the Fig. by a triangle - SEAD M, and low boundary - by square SEAD m and a bigger one that corresponds to the membership function value 0.5 or 50% confidence of the expert in the dose being involved in the given interval (indicated in Fig. 2. by the error lines). The error lines for analytical estimate given in the figure correspond to the value of 0.5 for the membership function calculated by this method. As it is seen from the Fig. in 4 cases from out of 5 the results of dose reconstruction by analytical method (questionnaires 1,2,3,6) agree well with those by SEAD method. EPR estimate error is  $\pm 50$  mGy. Unfortunately, in 4 cases (questionnaires 8, 9, 10, 11) it was found out that EPR estimate can't be taken into consideration since the dose was reconstructed using the front teeth and thus, may be overestimated. In two cases from out of the rest four the EPR and SEAD dose intervals are overlapped. We hope that more substantiated conclusions as to the comparison of these methods can be done following additional testing.

### Results of cross-validation

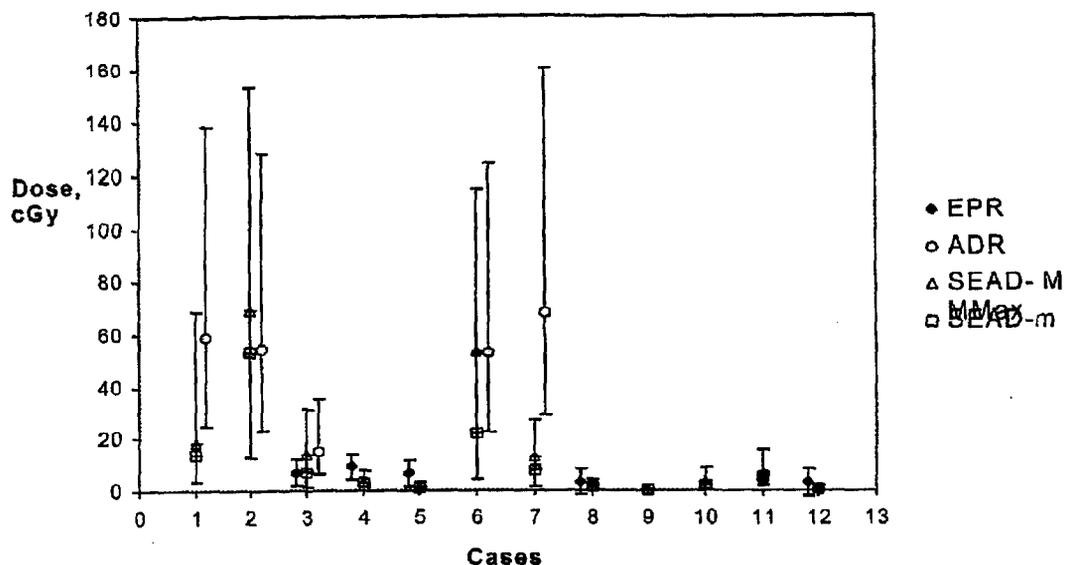


Fig. 2. Comparison of the dose estimates obtained by different methods, for 12 Ukrainian liquidators (numbers of cases are on the abscissa). Indications: EPR is dose estimate by EPR-method, ADR - by analytical method, SEAD-M and SEAD-m are upper and low boundaries of the dose interval by SEAD method.

The method will be approved at the next meeting of the International dosimetry group in June following additional testing and final modification. In particular, it is envisaged to find out how much psychological factors influence uncertainty of the final dose estimate. The matter is that any modification of the

Questionnaire (e.g. additional questions of psychological character) developed by the International dosimetry group was recognized inappropriate since it is widely used in the study case-control in Byellorus and Russia. Moreover, the physical factors are thought to play more significant role than psychological ones and information of such kind is difficult to unify.

At present the Ukrainian party is to additionally interview 38 persons among the reservists ("partisans") who donated teeth to the Bank of biosamples. To determine possible influence of an expert the questionnaires will be processed by two independent experts.

**Milestone 13.**  
( dosimetric group )

Lessons gained while accomplishing the Project's Phase I as for the teeth samples collecting and storage.

The interim results of the work for 1.5 years are summed up in the given report.

Of note, the work at the Project Phase I concerning collecting teeth samples and their storage began at the time when the dosimetry laboratory had some practical experience gained during cooperation with the stomatological institutions at the oblast polyclinic No.2 and the city students' polyclinic (Kyiv-city).

However, in the course of the Project accomplishment as a result of expanding the network of the teeth collection to four more oblast centres besides Kyiv city and oblast, there emerged a necessity to have simple, convenient and accessible means of their temporal preservation and transportation for long distances.

Thus, there was a proposal to preserve and deliver them in paper bags together with a covering note by mail.

The covering note itself - "Passport for the extracted tooth" has been significantly changed. In spite of the instructions concerning answers to the question as to the number of liquidator's registering documents in the SR, this column was not filled out correctly. Due to this, the latest variant of the passport does not contain this column however, to facilitate liquidator's search from among dozens of thousands of the persons registered it is proposed to indicate date of birth. Moreover, it is proposed to answer in more detail the question as to the term of his service in the zone. If in the previous variants of the passport there was only a question as to the year of participation in the clean-up, now he is asked about the total term, the beginning and the end of the works being exactly indicated. Such detailed information insignificant for the retrospective dosimetry on the teeth enamel may be useful for analytical dose estimation.

A significant innovation is classification of the samples collected in terms of their fitness for EPR dosimetry.

To evaluate actual amount of the material valid for dosimetry an additional procedure of visual (score) estimate of the tooth enamel condition was introduced.

The principles of the enamel condition evaluation are presented in Table 8.

Table 8.

Basic principles of the visual (score) evaluation of the tooth enamel delivered for dosimetric measurements.

No	Detailed description of the teeth enamel condition	Estimate in Scores
1.	Teeth roots, remains of teeth roots, complete absence of enamel	1
2.	Insignificant amount of enamel (10-20%), teeth under metal crowns	2
3.	Incisors, canine, entire teeth with little enamel fit for dosimetry	3
4.	Not more than 50% of enamel available (premolars, molars)	4
5.	More than 50% of enamel available	5
6.	Complete crown and root, the tooth was extracted because of paradontosis	6

Completely fit for the dosimetry estimates is the enamel evaluated not less than 4 scores : As for the incisors and canine one should be careful and use these teeth for the dosimetry only in extreme cases. It is connected with the phenomenon of additional irradiation of the front teeth enamel with hard ultraviolet which creates the same paramagnetic centres as gamma and x-ray irradiation. In case of the other teeth absence, the inner tongue surfaces of such teeth are used provided the necessary for the dosimetry enamel amount is obtained.

Introduction of the score estimate indicated that only 70% of the teeth delivered for the dosimetry measurements are fit for the retrospective dose estimation. It should be noted that the enamel quality of the teeth collected in Kyiv city and oblast is much higher: in the institutions where instructions were repeatedly carried out as to the requirements concerning enamel, nearly 100% of the samples are fit for investigations; in the institutions where all the teeth extracted are collected, only 18% of the samples may be used for the dosimetry.

Among the teeth samples provided from the oblast centres the best enamel (60% fitness) is from Poltava oblast; 54% fitness - from Dnipropetrovsk oblast, 41% fitness - from Kharkiv oblast and only 30% of fitness from Zaporizhya oblast.

General estimation of the collected materials condition necessitates enhancement of the requirements as to the enamel quality. It is envisaged to instruct additionally the specialists during seminars and dissemination of the instructions as to the requirements concerning dosimetric material.

One more problem faced with while working at the project was samples systematization aimed at developing dosimetric material coding system so that each

number given to the sample at the stage of sorting would bear complete information on it. Moreover, due to the absence in the covering notes of medical records numbers which along with complete name of the medical institutions contain information as to the donor of the biomaterial i.e. liquidator, we had to introduce our inner coding. Thus, the numbers of the samples are rather complicated however, informative. Perhaps, the numbering system introduced will be improved.

Though as it was mentioned above, the main drawback remains insufficient number of the biosamples collected.

#### Milestone 14 ( dosimetric group )

*Review of the methodical studies aimed at improving feasibilities of the EPR dosimetry technique as a result of the new resources of the modernized facilities. New version of the EPR dosimetry protocol.*

Since BRUKER Co. is going to send its service engineer on a mission to Kyiv not earlier than by the end of April in the current year a complete review of the methodical studies will be given in the next report. The same concerns a new version of the EPR dosimetry protocol. The given report only contains a brief analysis of the feasibilities and that version of the EPR dosimetry technique specified by the facilities used. Thus, only effect of gaussmeter (precision measurement of permanent magnetic field which along with a frequency meter will make it possible to calibrate spectra in terms of g-factor automatically) is not involved as well as of a new card with a processor and a feasibility to be connected with a computer network which is to make the procedure of EPR spectra processing using PC more simple.

Thus, at present the main result of methodical studies is in advantages of goniometer and highly sensitive resonator (at the stage of EPR spectra registration) as well as in advanced procedure of enamel samples preparation using low-speed saw, hydraulic press, ultrasound unit for simultaneous treatment of some dozens of samples, etc. Quantitatively, this result may be expressed as follows. Recurrence of signal measurement from the dose of 100 mGy is about 10%, the registering time of one spectra being 30 min. It means that on the one hand, the doses range essential for the epidemiologic studies is completely encompassed by EPR-dosimetry; on the other hand, the time needed to reconstruct one dose is significantly (by nearly two times) reduced. The procedure of sample preparation for EPR studies was also significantly improved. It

concerns first of all the most difficult stages in enamel extraction, i.e. tooth root extraction, tooth sawing into two parts - a cheek one and a tongue one, tooth comminution into the grains of needed fractions, etc. With the application of low-speed saw, hydraulic press, facilities for automatic sewing with the appropriate set of sieves and ultrasound bath the procedures do not need much physical efforts any more.

The EPR dosimetry protocol may be presented as follows. At the first stage a root is cut out from the tooth, the latter being sawn into the tongue and cheek halves with a low-speed saw. Then, the cheek half is comminuted with a hydraulic press to the grains of about 1-2 mm. The sample obtained is treated in NaOH solution of 3N molarity in the ultrasound bath. As it was mentioned above, nearly 40 samples can be treated at the same time.

Upon finishing the reaction indicated by the alkali color which is not changed following the regular waste solution replacement, the sample is washed for 1-2 hs. in distilled water as well as in the ultrasound bath, dried at 60 °C and comminuted to 25-850 µm fractions. If there are extra signals in EPR spectra of the sample in the vicinity of g-factor 2.0, an additional cleaning with alkali is used following comminution to 100-250 µm fraction. If the signals persist, cleaning by specific weight is used with sodium polytungstate of the necessary concentration.

EPR spectra are recorded on the modified ECS 106 Bruker spectrometer with the parameters typical for the routine enamel dosimetry. Each sample is recorded using the computer-controlled goniometer.

The procedure of estimating dosimetric signal intensity ( $g=2.0018$ ) is composed of standard stages described in the previous reports.

*Preliminary results of the interlaboratory intercalibrating of the EPR dosimetry techniques.*

Within the framework of the working plan on intercalibrating advanced method of EPR dosimetry, the doses of all ten enamel samples (from five different teeth) preliminary prepared during Quarter V were estimated. Each sample dose was estimated by six different techniques which made it possible to compare in more detail effect of different techniques on the dose estimation accuracy. Six techniques above correspond to three different configurations of EPR spectrometer involving standard rectangular, cylindrical and dielectric resonators, and to three different dosimetry techniques including that without additional irradiation, with one additional irradiation with high dose and the technique of two different powers of ultra radio-frequency field). Each

sample was additionally irradiated with five laboratory doses. Thus, 360 spectra were recorded and analyzed.

The results obtained along with the nominal doses values are presented in Table 9, the values being given in terms of air kerms.

Table 9.

Doses of the intercalibrated samples reconstructed according to the routine EPR dosimetry technique.

Sample # / dose, mGy	Config. 1		Config. 2		Config. 3		P1 and P2		W/o addit. irradi.		One addit. Irrad.	
	D, mG	$\pm\sigma$ , mGy	D, mGy	$\pm\sigma$ , mG	D, MGy	$\pm\sigma$ , mGy	D, mGy	$\pm\sigma$ , mGy	D, MGy	$\pm\sigma$ , mGy	D, mGy	$\pm\sigma$ , mGy
141a-1 / 99	97	11	95	21	108	19	126	29	91	24	90	22
141a-2 / 99	99	20	105	37	102	15	155	14	100		109	
142a-1 / 147	157	22	165	46	156	25	220	47	153		173	
142a-2 / 147	129	32	136	23	152	22	223	31	159	30	156	27
143a-1 / 327	232	26	229	19	234	18	279	38	256		253	
143a-2 / 327	199	47	249	32	210	77	257	76	239	18	227	13
144a-1 / 410	334	59	294	51	286	47	322	48	316	29	332	22
144a-2 / 410	324	42	286	63	314	89	355	136	344		377	
145a-1 / 819	603	77	618	74	632	53	713	97	612	74	611	62
145a-2 / 819	621	109	651	88	734	36	714	65	688		664	

Digits 1 and 2 in each sample number refer to the cheek and tongue parts of a tooth, respectively.. Different configurations correspond to different types of ultra r.f. resonator. Three different techniques were as follows:

P1 and P2 are techniques of two ultra r.f. powers. The values of P1 and P2 powers were 1 and 10 mWt, respectively.

W/o addit. irradi. is the technique in which universal calibrating curve is used to transfer from EPR intensity to the dose (without additional irradiation).

One addit. irradi. is a developed variant of the previous case. In this technique radiation sensitivity of each sample is evaluated following one high dose irradiation (5 Gy).

Where possible, standard deviation  $\pm \sigma$  is given for each dose value. In some cases (two last techniques) there are no errors because the necessary information is not available.

Basic configuration is configuration 1. As it can be seen from Table 9 for this configuration the dose values estimated for the tongue and cheek part of each tooth coincided within the experiment error. Therefore, arithmetic mean of the cheek and tongue parts was taken as a dose value for each tooth on the whole. These values are given in Table 10 with related errors.

Table 10.

Dose of intercalibration samples reconstructed by EPR method.

Sample #	Dose, mGy	Errors, $\pm$ mGy
141a	98	16
142a	143	27
143a	216	37
144a	329	50
145a	612	93

The respective correlation dependence with nominal dose values is shown in fig 3. It can be seen that for the two samples with minimum doses which are the most significant for the epidemiologic studies they coincide within 3%. For the samples with higher doses the correlation is worth and a deviation of EPR dose from the nominal value is within 20-34 %. In terms of the epidemiologic requirements such errors are permissible however, they are not understandable in comparison with the essentially less deviation values in the samples with low dose values. There may be two possible causes for such discrepancies. The first is associated with possible incorrect calibration of the gamma-source used for the laboratory additional sample irradiation (there should be reminded that the source used is located at the Institute for Nuclear Studies). To clear it up, an additional calibration of the gamma-source is to be carried out by irradiating alanine dosimeters. Moreover, a leading German specialist in alanine dosimetry Dr. A. Wieser will be engaged as an expert, his preliminary consent to it being received. The other possible cause of the deviations is sample irradiation with nominal doses at IAEA.

The matter is that during irradiation the effect on the nominal doses values of such significant factors as irradiation geometry, material of the phantom containing samples during irradiation, position of some samples inside the phantom was not controlled. All these factors are to be taken into account at the second stage of intercalibrating where 12 samples are to be irradiated with unknown doses at IAEA. According to the agreement with the partner laboratory of the USA (CAD, Dr.E.Haskell), the samples will be prepared and transferred to IAEA by the American party. A special covering note with the list of all requests and recommendations as to the irradiation procedure will be handed over to the person responsible for the irradiation. Then the samples will be sent to the partner laboratories for dose evaluation. The second stage of the intercalibrating will take 3-4 months for its accomplishment.

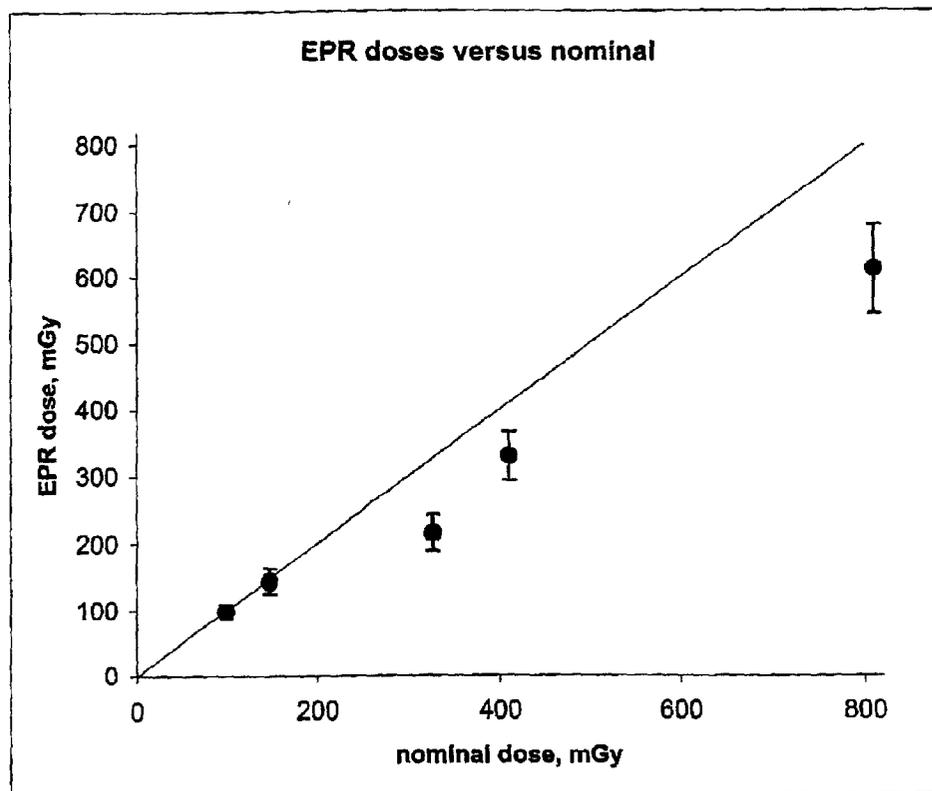


Fig 3. Correlation dependence of nominal dose data (x) and reconstructed by EPR (y) for intercalibration samples.

Milestone 15  
( dosimetric group )

During the reported period the organization work on the retrospective cytogenetic dosimetry using FISH method was in progress.

From February, 22 till March, 5 Dr. A. McFee from the Cytogenetic Lab of the Oak Ridge Associated Universities for the U.S.Department of Energy worked at the laboratory of cytogenetics at RCRM.

Dr. A. McFee delivered to Kiev a special filter (Spectrum Orange\DAPI, Vysis, USA) for luminiscent microscope and a kit for FISH technique including directly labelled by Spectrum Orange DNA probes (cocktail for chromosomes #1, 2 and 4, Vysis, USA). The number of FISH probes is enough for 150 slides. The number of slides per persons depends on the quality of slides (the number of good metaphases).

To provide the intense light needed for FISH, a 100-watt power supply, luminiscent lamp (the average life of the lamp is 200 hours) and the lamp base as well as filter were installed on the Zeiss microscope Axioscope.

Together with Dr.A.McFee the P A I N T classification for the types of chromosomal aberrations taken into consideration in the cytogenetic studies of FISH slides has been agreed (according to J.Tucker et al., 1994). A form for recording the results of FISH scoring was developed. The procedures for examining FISH slides and scoring chromosomal abnormalities were discussed.

The schedule for all FISH investigations was agreed. The microscope studies of the chromosome preparations are time consuming. According to the dosimetry studies 50 individuals are to be evaluated, or a total of 50,000 cells are to be scored. With the ordinary rate of scoring (~400 cells per working day) it is expected that it will take at least 6 months for examining the needed cells and preparing preliminary summaries of the data.

In addition to the material available two lamps for the microscope will be needed since at least 600 hours of actual scoring are expected. Some alcohol and tissue culture media in addition to the available will also be required. The tissue culture medium RPMI 1640, fetal calf serum, formamide and phosphate buffer from "Sigma" company have been ordered.

All the reagents needed for FISH method, a bank of fixed lymphocytes of liquidators and coded slides have been stored into the repaired freezer.

Milestone 16  
( dosimetric group)

Within the framework of the studies for the reported period on milestone 16 the cytogenetic examination of liquidators with different absorbed doses of gamma irradiation received following the Chernobyl accident was in progress.

The samples of venous blood from 8 persons investigated previously by EPR method and some other methods of biodosimetry in the laboratory of external radiation dosimetry (headed by Dr. Chumak) were delivered to the cytogenetic lab. The doses of radiation exposure are not indicated.

For each blood sample (~5 ml) \$10 (in hrivnas, according to the rate) were paid; the necessary documents were received.

All blood samples were incubated during 48 hours according to the standard protocol adopted in the cytogenetic lab of RCRM; the fixed sediments of peripheral lymphocytes were obtained from the part of which the metaphase chromosome slides ready for fluorescence in situ hybridization with directly labeled DNA-probes were obtained.

On the whole, the fixed cells from 40 liquidatos were obtained. All fixed sediments and coded slides are kept in the freezer at - 20° C.

Under the supervision of Dr. McFee all the solutions needed for FISH probing were prepared and properly adjusted; FISH probes were applied according to Vysis protocol for 21 slides of 18 different donors; all yielded good results. The fluorescent signal obtained was quite satisfactory and equal in brightness to that obtained in the cytogenetic labs in the USA.

750 metaphases have been scored jointly with Dr. McFee; 31 translocations have been found. Each chromosomal abnormality found by Dr. Pilinskaya was controlled by Dr. McFee, examined by Dr. Dybskiy and Dr. Cherviakova and discussed jointly to help ensure agreement in the scoring criteria to be used.

The FISH slides received from 10 liquidators have been scored. From each person nearly 1000 metaphases have been examined ( 500 cells by each scorer - Dr. Pilinskaya and Dr. Dybskiy - equally in each case) On the whole, 9,400 metaphases have been investigated. All biomarkers of radiation exposure - stable (translocations - complete and incomplete, inversions, insertions, deletions) and unstable (acentrics, dicentrics, rings) were considered in the cytogenetic analysis. The results of scoring are presented in Table 11.

Table 11

## The results of FISH analysis

№	Code	Metaphase scored	Chromosome aberrations					
			Translocations				Other	Total
			Com	Incom		Total		
t(Ab);(Ba)	t(Ab)	t(Ba)						
1	2.19.05.98	750	13	14	3	30	4	34
2	2.29.09.98	1000	5	5	2	12	5	17
3	1 22.09.98	1000	16	8	2	26	1	27
4	1 16.12.98	850	4	2	1	7	1	8
5	3 24.02.99	1000	45	8	12	65	4	69
6	1 23.02.99	1000	18	3	5	26	2	28
7	1 24.02.99	1000	5	2	0	7	0	7
8	4 15.02.99	800	17	5	2	24	2	26
9	1 27.01.98	1000	7	4	2	13	1	14
10	3 15.02.99	1000	3	2	0	5	1	6

As it is seen from Table 11 the persons examined differ essentially as to the degree of radioinduced cytogenetic effect (the frequency of chromosomal translocations, in particular) which depends on the absorbed radiation doses.

#### Milestone 17.

(dosimetric group)

In the framework of the studies the venous blood and bone marrow samples were obtained from three patients of the Hematologic Department (headed by Dr. V.I. Klimenko) – two with diagnosis chronic mieloid leukemia (CML), one – lymphoproliferative disease (LPD). The pellets of fixed cells and partly G-banded methaphase chromosome slides were prepared.

From patients with CML only bone marrow cells were incubated. Peripheral blood was not cultivated because of the initial stage of disease and thus, the number of the blasts and myelocytes was too little for receiving chromosome slides and in PHA-stimulated T-lymphocytes marker Ph (Philadelphia) chromosome was absent.

From the patient with LPD both bone marrow cells and peripheral blood lymphocytes were cultivated. The bone marrow cells were incubated during 24, 72 and 120 hours because of lack of specific mitogen for B-lymphocytes, their disorders being characteristic for the given (European) form of LPD; the optimal variant of incubation duration was selected empirically. 24 hour-incubation culture was selected for the cytogenetic analysis.

All the slides were stained according to the trypsin-Giemsa method for G-banding and analysed. The data obtained is presented in Tables 12 and 13.

Table 12

## Score of the studies

Name	Diagnosis	Metaphase scored b/m	Metaphase scored p/b
1.	CML	20	-
2.	CML	20	-
3.	LPD	10	10

Table 13

## The results of the investigation of G-banded cells

Name	Diagnosis	Karyotype, b/m	Karyotype, p/b
1	CML	46,XY,t(9;22)(q34;q11)	-
2.	CML	46,XX,t(9;22)(q34;q11)	-
3.	LPD	46,XY	46,XY

The cytogenetic analysis of the bone marrow cells in patients with CML (cases # 2 and 3) revealed the classical variant of Ph chromosome – t (9;22)(q34;q11) which permitted to confirm and verify the diagnoses of these patients. In the case # 2 the subclone with two Ph chromosomes was revealed in the bone marrow cells

In case # 3 (patient with LPD) karyotype was normal both in lymphocytes and in the bone marrow cells.

## Milestone 18.

## (dosimetric group)

During Quarter VI 102 samples were delivered to the dosimetry laboratory, 61 being from Poltava oblast, 41 - from Kyiv city (polyclinics of radiation registry) and Kyiv oblast.

## (hematologic group)

Within the framework of Task 5.2.2.2 the hematologic group was engaged in studying feasibilities of accumulating in the Bank of the tissue obtained from the persons diseased with leukemia and lymphoma.

Peripheral blood, bone marrow, lymph nodes following biopsy were collected for retention in the Bank. According to the protocol, all biological material for long-term retention was taken prior to the specific therapy and is stored at -70° C. All samples of bone marrow and peripheral blood are prepared for their transfer to the low-temperature freeze at - 193°C. There is an agreement to transfer samples in the freeze at the Kyiv station of blood transfusion.

During 18 months of the protocol accomplishment material from 27 patients was obtained, 25 of them being liquidators following the Chernobyl accident and 2 were evacuated from the zone.

The number of the biological materials samples stored for the long-term retention.

Table 14.

The number of the biological samples stored during the five quarters

Diagnosis	Number
Acute leukemia	6
Chronic lymphocytic leukemia	5
Non-Hodgkin malignant lymphoma	2
Thrombocytopenia and Leucopenia	7
Myelodysplastic syndrom	7
Total	27

### TASK III. LEUKEMIA AND LYMPHOMA

#### Milestone 21

(epidemiologic group)

To complete the data on leukemia|lymphoma cases among the liquidators registered in the Dnipropetrovsk oblast an inquiry was sent to the oblast department of the Cancer-Registry of Ukraine as to the cases of malignant tumors of lymphatic and hematopoietic tissue developed following the accident and registered in the Cancer Registry (which is situated in the Oblast Oncology Dispensary. It should be noted that to receive any information from the Cancer Registry it is necessary to have either an official inquiry from the Ministry of Health or financial support to accomplish it by the local staff members/ Another possible source to obtain data is the Cancer Registry at the National Institute for oncology and Radiology (Kiev-city) which is collecting the data from oblast level of Cancer Registry.

According to the inquiry, the data on 2058 cases of leukemia|lymphoma developed during the period 1987-1997 among the population of the Dnipropetrovsk oblast were received. The information involves family name, name, patronymic of the diseased persons, their date of birth, gender, the diagnosis defined (the code in accord with the International classification of diseases (ICD-9), year of diagnosis made. The data were received in the form of the text file with the defined length of each information field. 1072 cases of 2058 are mail cases.

Case distribution on diagnosis and year of its establishment is presented in the table #1.

**Table 15.**

**Distribution on leukemia|lymphoma cases registered in Dnipropetrovsk Cancer Registry among mail residents in 1987-1997.**

Diagnosis (ICD-9)*	Year of diagnosis establishment											
	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	At all
200	2	7	6	8	11	18	22	33	50	37	54	248
201	15	15	13	19	18	29	25	33	37	51	31	286
202	0	1	0	0	1	2	1	1	0	1	3	10
203	1	1	3	3	2	6	5	12	12	6	13	64
204	8	16	11	10	22	34	38	51	59	22	28	299
205	4	5	4	7	4	7	12	11	21	6	12	93
206	0	0	0	0	0	0	0	0	0	0	1	1
207	1	1	1	0	2	7	1	16	4	2	3	38
208	1	0	2	5	4	0	3	4	1	5	8	33
200-208	32	46	40	52	64	103	107	161	184	130	153	1072

\* 200 - lympho- and retikulosarcoma, 201-Hodgkin's disease, 202-other and uncertain types of lymphoma , 203 - multiple myeloma, 204 - lymphoid leukemia, 205 - myelogenous leukemia, 206 - monocytic leukemia, 207 - erythro- and megacariocytic leukemia, 208 - leukemia uncertain type

As it is shown above the number of cases registered is increasing substantially since 1992. It should be taken into account when we consider the Dnipropetrovsk Cancer Registry to be a source of the information.

The next step was the linkage Cancer Registry data with the Chernobyl Registry to replenish the data on cases in mail liquidators from the Dnipropetrovsk oblast under study.

It was defined by linkage that 15 records were common for both databases. The information on participation in clean-up work is recorded in 4 cases of 15. Thereby that data in the Cancer Registry are incomplete. On the other hand Cancer Registry had the records of 2 lymphoma cases and 1 multiple myeloma case which appeared in liquidators who were not registered in the State Chernobyl Registry.

Thereby the best way to complete the data on leukemia/lymphoma cases among the liquidators is the data linkage using the information from the different sources in the full volume.

3 of 15 common records didn't have the diagnosis under study recorded in the State Chernobyl Registry. Conducted fieldwork gave us the additional information on these cases. According to that 1 of 3 cases was diagnosed as polycytemia Vera in 1993. That person moved to another oblast in 1994. As for the 2<sup>nd</sup> case his medical examinations had never shown hematopoietic system pathology. The 3<sup>rd</sup> case was diagnosed as CLL. The diagnosis was performed using laboratory test and autopsy. The information of that case was not transferred to the State Chernobyl Registry in time.

The information obtained indicates the necessity to review the data from each source including Cancer Registry.

Therewith State Chernobyl Registry maintains the data about 4 leukemia cases, 1 multiple myeloma case and 2 lymphoma cases in liquidators from Dnipropetrovsk oblast which are not registered at the Cancer Registry. The term of the diagnoses establishment should be desired as one of the possible causes of that fact (6 persons were died before or during 1994, the 7<sup>th</sup> case was diagnosed in 1986 and after that was treated in Moscow). The period of Dnipropetrovsk Cancer Registry operation also should be taken into account (the data were input during 1996-1998, data base is still enlarging with retrospective cases).

The results obtained demonstrate the importance of all possible sources of information, including Cancer Registry, necessity to review all data and low self-

descriptiveness of the Dnipropetrovsk Cancer Registry for period 1987-1994. Probably Cancer Registries in other oblasts under study have more complete information for early post- accident period.

### Milestone 23

( hematologic group )

During Quarters 1-6 of the Project Pilot phase the work was in progress to reveal other hematologic diseases in the hematologic department of the clinical hospital No.4 in Dnipropetrovsk city. Particular attention was paid to such diseases as myelofibrosis, polycytemia, myelome disease, myelodisplastic syndrome involving chronic myelogenous leukemia. A detailed analysis of these diseases since 1987 to 1997 was carried out. The number of these diseases was found out to be considerably reduced as compared with 1987.

Table 16.

Disease rate of other hematologic disorders among population of the Dnipropertovsk oblast (absolute numbers)

Diagnosis	1987 y.	1997 y.
Polycytemia vera	7	8
Multiple myeloma	24	11
Aplastic-hypoplastic anemia	12	4
Osteomyelofibrosis	33	12
Myelodysplastic syndrom	5	9

A detailed analysis of the biologic material of the persons with other hematologic diseases during 1997 was carried out. The diagnostics of these diseases was found out to be at a rather high level due to the fact that FAB-classification is used at the department. At the same time, in 1987 we have hyperdiagnostics concerning aplastic anemia, myelome disease and osteomyelofibrosis. Random analysis of the patients with osteomyelofibrosis showed that in some cases we have for example, myelodysplastic syndrome. In case of aplastic anemia myelodysplastic syndrome may be diagnosed. A work is in progress to analyze material involving diagnostic and medical records to obtain clear data as to the diagnostics of other hematologic diseases in Dnipropetrovsk oblast.

**Milestone 25.****(epidemiological group)**

After the investigation of medical records in the medical facilities (hematological departments of Dnipropetrovsk, Donetsk, Kharkiv, Kyiv and RCRM ) it was established that the medical records of persons who participated the clean-up work were marked in the optional descriptive form on the first page.

The National Cancer- Registry was analyzed to determine the presence of any identifications for liquidators among cases registered. It was established that new Cancer Registry forms for each case of malignant disease contain the special field where the chernobyl status of the case was marked (liquidator, evacuated, resident of contaminated zone, child who was born from above group parents).

The completeness of these fields was investigated in the Dnipropetrovsk Cancer-Registry. The main results are presented in the report on task 21.

#### TASK IV. MOLECULAR BIOLOGY

##### Milestone 27.

( hematologic group )

During the period of the Project accomplishment 5 patients - residents of the Dnipropetrovsk oblast were examined. 1 person was diagnosed for chronic myelogenous leukemia, 2 for non-Hodgkin's malignant lymphoma and 2 - myelodysplastic syndrom.

The peripheral blood and bone marrow removed from patients were processed according to the Appendix 3.

##### Milestone 30

( hematologic and epidemiologic groups )

During Quarter VI a work was in progress to interview liquidators randomly chosen among the residents of the Dnipropetrovsk oblast registered in the SR of Ukraine.

It was envisaged to interview 40 persons and bleed 20 of them.

To achieve the necessary number of those examined, 50 persons were contacted.

First, the contacts were made with the help of the responsible physicians of the territorial polyclinics and then additionally - in written form.

The persons who hadn't appeared for the interview were sent additional letter after these contacts with the request to take part in the study and proposal to compensate their transportation expenses and working day missing.

The interview was conducted in the Dnipropetrovsk oblast hospital by the head of the dispensary department T.I. Chekmareva who had been specially trained at the seminar held in April, 1987 in Kyiv (WHO; NCI, USA; RCRM, AMS, Ukraine).

After the first letter (without proposal of compensation) and contact by responsible physician from among 50 persons requested for the interview:

- 22 were interviewed at the agreed time;
- 3 emigrated to Russia;
- 2 emigrated within the oblast area, the address being unknown;
- 1 is an alcoholic, therefore the contact with him proved difficult;
- 1 couldn't come because of disease.

Thereby we've got the response rate 22 from 46, that is about 48 %.

The 2<sup>nd</sup> letter (with proposal of compensation) was sent to the 21 liquidators (the rest of persons from selected 50 and to alcoholic). 9 persons from the 21 requested were interviewed at the agreed time.

That is the whole number of persons from the original sample who were

interviewed is 31 from 50. Taking into account no contacts can be made with 4 persons (3 emigrated to Russia, 1 couldn't come because of disease) the response rate was 31 from 46, that is about 67 %.

In order to complete the task 30 in the full volume (40 interview, 20 taking of blood sample) taking into account the response rate 17 persons were selected additionally with accord to previous definitions.

The letter with proposal of compensation were sent them. They were contacted by responsible physicians also.

From among 17 persons requested for the interview:

- 8 were interviewed at the agreed time;
- 1 addressee didn't live at that address any more. The letter was back.
- 1 is an alcoholic, therefore the contact with him proved difficult;
- 7 refused to come for the interview.

We got the response rate 8 from 16 (1 letter was returned) i.e. 50 %. That results demonstrated the necessity of recurrent contacts with liquidators and also the expected amount of the work for the future study.

It is important to accentuate following:

- 1) there was nobody who refuse to give some amount of blood after the interview;
- 2) the response rate is affected with the relation of the local personnel to the study and their responsibility
- 3) it is reasonable to manage the of interviewers training for the next stage of study with participation of Chekmaryova Tatyana who has got the experience in the process of engagement for the interview and its conducting.

Thereby 39 persons were interviewed and 19 of them were bled by the nurse of the Dnipropetrovsk oblast hospital (i.e, at the site of interviewing) after the interview.

After the interview the liquidators were requested to provide some amount of the venous blood for freezing, storage and subsequent molecular-biologic assays.

Prior to bleeding a liquidator was proposed to be acquainted with the letter of consent for blood study and sign it. All possible liquidator's reactions to the proposal for bleeding (refusal to be bled, refusal to sign a letter, etc) were to be indicated in the questionnaire (impressions on the interview). If a liquidator is ready to be bled for eventual assays, but doesn't want to sign a letter, he is not bled.

Blood was collected in the amount of 20 ml., then it was placed in the heparinized test-tube. The test-tube (vacutainer) was marked by putting a family name, name, patronymic, date of birth, code of the Dnipropetrovsk oblast (04), date and time of

bleeding. Besides, a covering note was filled out, the text being given below.

The vacutainers with the blood, cover note, a letter of consent for the assays were packed and transferred with a conductor of the so-called brigade car of the fast train Dnipropetrovsk-Kyiv. The blood was placed in an ordinary refrigerator available in the car, i.e. it was transported at the temperature of about + 5° C.

The staff members of the hematologic department at the RCRM (Kyiv) responsible for the blood receipt and processing were informed about the blood transfer over the telephone by Dr. T.I.Chekmareva who forwarded the materials from Dnipropetrovsk.

At about 8 a.m. the blood was received by the responsible persons of the hematologic department, delivered it to the Center and processed it according to the procedure described in the Protocol. The mononuclear cells extracted were frozen in the low-temperature freezer at

- 70° C. Thus, the period from the blood collection to the beginning of its processing was about 19-20 hs. During this time the blood was suitable for processing, analysis and storage but only provided that it is delivered in heparinized test-tubes at rather low temperature (in an ordinary refrigerator). The two first blood samples were placed in the vacutainers treated with K<sub>3</sub> EDTA and delivered at the ordinary temperature of a car. The attempts to process this blood were unsuccessful (mononuclears were not to be identified). Therefore, such way of blood delivery proved to be inappropriate.

### Scheme of the engagement for the interview and taking blood

