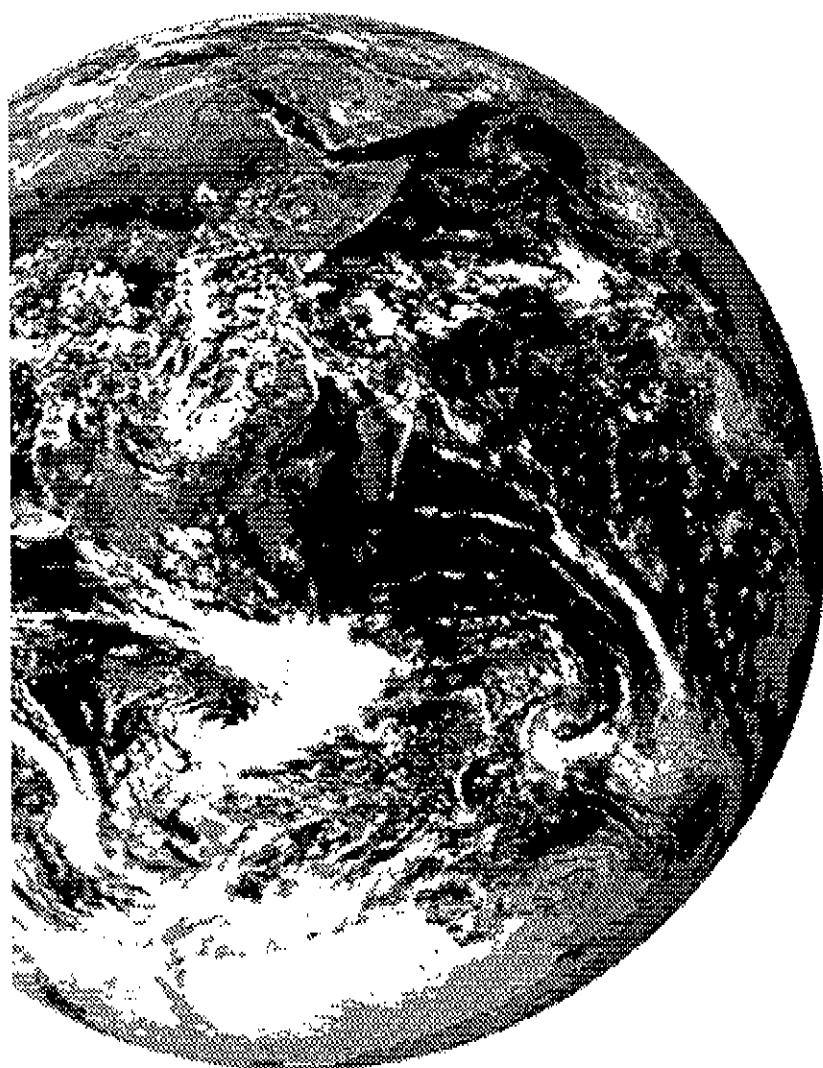




**INTERNATIONAL PROGRAMME ON THE HEALTH EFFECTS OF THE
CHERNOBYL ACCIDENT (IPHECA)**

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**"ACCIDENT RECOVERY
WORKERS" PROJECT**

**Report of the Informal
Discussion on:
OXIDATIVE STRESS IN
CHERNOBYL ACCIDENT
RECOVERY WORKERS
Paris, France,
20-21 September 1996**



**World Health Organization
Office of Global and Integrated
Environmental Health
Geneva
1997**

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1. Introduction

In May 1991, the World Health Organization (WHO) established the International Programme on the Health Effects of the Chernobyl Accident (IPHECA). The pilot phase of IPHECA was aimed at the assistance to national health authorities in early detection of thyroid diseases in children residing in contaminated territories ("Thyroid" Project), leukaemia and related diseases ("Haematology" Project), the development of epidemiological registries ("Epidemiological Registry" Project), medical examination of children exposed to radiation in utero ("Brain Damage in Utero" Project), and study of oral health in Belarus ("Oral Health" Project). The pilot phase of IPHECA came to an end in 1994.

The IPHECA Management Committee at its meeting on 17-18 March 1994 endorsed new projects within the programme on medical monitoring of Chernobyl accident-recovery workers (Accident Recovery Workers Project or "Liquidators" Project), etiology of thyroid cancer in children (International Thyroid Project), and dose reconstruction (Dose Reconstruction Project).

An International Consultation which was held in St Petersburg, 27 June to 1 July 1994, discussed main targets and objectives of the Accident Recovery Workers project. It was recommended, *inter alia*, to investigate the oxidant toxicity or clastogenic factor levels in liquidators as a radiation risk indicator. The first working group meeting, dedicated to the development of investigations of oxidative stress and antioxidant treatment in Chernobyl accident recovery workers and their families, was held in the University of Pierre and Marie Curie, from 20-21 September 1996, Paris, France.

The meeting was supported by the University of Pierre and Marie Curie, Paris, France and the Radiation Effects Research Foundation, (RERF), Hiroshima, Japan.

2. Objectives

The main objectives of the meeting were:

- a) To review studies of oxidative stress and antioxidant treatment in population exposed to radiation in Belarus, the Russian Federation and Ukraine due to the Chernobyl accident.
- b) To discuss main tasks of the subproject "Oxidative stress in Chernobyl accident recovery workers. Prevention of oxiradical-related diseases by antioxidants" within the framework of the IPHECA Accident Recovery Workers project.
- c) To discuss methodology and a protocol of the cooperative study within the subproject.

3. Meeting

Professor M. Moreau, Director of the Department of International Relations, University of Pierre and Marie Curie, opened the meeting. Dr G. Souchkevitch from the Office of Global and Integrated Environmental Health, WHO/Headquarters, Geneva, greeted the participants on

behalf of the Director-General of WHO and exposed to the audience the background and main objectives of this project, which shall be carried out within the framework of the International Programme on the Health Effects of the Chernobyl Accident (IPHECA).

Following the recommendations of the International Consultation, Dr. Emerit (University of Paris), Dr. Neriishi (RERF Hiroshima) and Dr. Souchkevitch (WHO Geneva) prepared a subproject "Oxidative stress in Chernobyl accident recovery workers. Prevention of oxyradical-related diseases by antioxidants". This subproject includes the following tasks:

- 1) Collection and analysis of the relevant data available in participating countries.
- 2) Study oxidative stress biomarkers in a group of liquidators and select the most appropriate of them for clinical trials.
- 3) Organization of pilot studies and double blind clinical trials for evaluation of the efficacy of selected antioxidants or radical scavengers and develop the optimal treatment schedule.

Dr I. Emerit and Dr K. Neriishi co-chaired the meeting. The WHO rapporteur was Dr G. Souchkevitch. A list of the participants and the agenda are attached (Appendix 1 and 2).

After the introduction and project outline provided by Dr. Souchkevitch, WHO, examples of oxidative stress reactions in A-bomb survivors were reported by Dr. Neriishi (RERF, Hiroshima). He provided data on the presence of subclinical inflammatory changes in this population, such as leucocytosis, accelerated erythrocyte sedimentation rate, increased level of acute phase proteins, and increased level of fibrinogen many years after exposure to A-bomb radiation. The reactive oxygen species generated during inflammatory reactions lead to chronic oxidative stress in A-bomb survivors. It is considered a risk factor for the development of cancer and atherosclerosis. Based on observations in A-bomb survivors, Dr. Neriishi supported the study of oxidative stress in Chernobyl recovery workers.

Representatives of six participating institutes from the former Soviet Union presented their results obtained in liquidators, related to various biomarkers of oxidative stress, and preliminary experience on antioxidant treatments. These representatives which are involved in the follow-up of liquidators and their families, expressed their interest to participate in the research project. They are as following:

- 1) **Dr Tsyb, Director of the Medical Radiological Research Centre of RAMS, Obninsk, Russian Federation.**
- 2) **Dr Nikiforov, Director of the All Russia Centre of Ecological Medicine, St. Petersburg, Russian Federation.**
- 3) **Dr Piatak, Deputy Director of the Research Centre for Radiation Medicine, AMS, Kiev, Ukraine.**
- 4) **Dr. Antipkine, Deputy Director of the Institute of Pediatrics, Obstetrics & Gynaecology, Kiev, Ukraine.**

- 5) **Dr. Shefel, Gomel Medical Institute, Gomel, Belarus.**
- 6) **Dr. Oganessian, Scientific Centre of Radiation Medicine and Burns, Yerevan, Armenia, Russian Federation**

The presentations were concluded by Dr. Emerit who reported results obtained in collaboration with the Institute of Radiation Medicine in Yerevan, Armenia, Institute of Pediatrics, Obstetrics and Gynaecology in Kiev, Ukraine, and the Centre of Ecological Medicine in St. Petersburg, the Russian Federation.

The reports of all speakers are added as Appendix 3 to this meeting report.

After the presentation of the available data from all participating institutions, the tasks of the project were the subject of a Round Table Discussion.

Task 1: There was general agreement on the presence of oxidative stress in liquidators, as well as in children irradiated as a consequence of the Chernobyl catastrophe. Lipid peroxidation was studied in most centres and found to be increased in liquidators as well as in children from the controlled territories. Conjugated dienes were found to be more sensitive indicators of lipid peroxidation than TBARS. However, as indicated by studies done at ARCEM in liquidators with pulmonary disease, TBARS can be significantly increased in macrophages from bronchoalveolar lavage fluid, while plasma levels remain in the normal range in the same individual. For comparison of subgroups of patients varying in radiation exposure, the biochemical analysis of lipid peroxidation products appeared to be less sensitive than the CF-Test, which detects lipid peroxidation products, together with other prooxidants circulating in liquidators' plasma due to their clastogenic properties.

In addition to the various methods measuring lipid peroxidation, the antioxidant defence system was studied by most of the participants. Not only the levels of antioxidant enzymes, but also the total antioxidant capacity of the plasma was measured and found to be reduced in liquidators and their children. Catalase was more affected than superoxide dismutase. However, glutathione peroxidase did not differ from control levels, or it was even increased. The level of reduced glutathione was found to be low and correlated with increased production of superoxide and hydrogen peroxide by phagocytes. Ceruloplasmin levels were regularly decreased.

The report from the Gomel Medical Institute provided results on considerable impairment of iron metabolism in liquidators exposed to radiation in doses less than 20 mGy. The important role of activated mononuclear phagocytes for disturbances of immune response was also demonstrated. The role of activated monocytes/macrophages, which produce various cytokines, including TNF alpha as one of the clastogenic components, was shown by the CF-Test.

The same biomarkers served for evaluation of the efficacy of antioxidants. Vitamin E, the classical chain breaking antioxidant and protector against lipid peroxidation, was used in several centres.

No modification of lipid peroxidation markers was noted in a study included liquidators with cardiovascular disease at the ARCEM. However, in this study, low doses of the antioxidant

and a short treatment period (only 10 days) were used. A significant decrease in lipid peroxidation products was observed in liquidators from Armenia, who were treated with vitamin E during 25 days (with a daily dose of 0.3 g). Vitamin E prescribed at an age dependent dose to children with recurrent respiratory infections (Institute of Pediatrics, Kiev) normalized the levels of hydroperoxides and increased glutathione peroxidase and ceruloplasmin levels. In addition, there was a simultaneous decrease in catalase activity after treatment. Vitamin E was also given together with vitamin C and beta carotene (Veteron from AKVA-MDT, Russia) to small groups of liquidators in Obrninsk and Kiev. Veteron contains only 6.7 mg/g of Vitamin E and Vitamin C, while beta carotene is represented at concentrations of 20 mg/g. The therapy was based on 40 mg beta-carotene per day and conducted over a period of 14-20 days. A slight decrease in MDA (16.6%, $p < 0.05$) was observed. SOD activity increased by 37%, and the antioxidant capacity of the plasma increased by 48%. Other products produced by Russian firms were tested at the MRRC in Obrninsk and considered as beneficial: Cyclocar from NIKKA-NPO "Vitamines" (beta carotene 60 mg/g, Vitamin C 17 mg/g), Chlosecar manufactured by RAMS Institute of Nutrition (a powder produced from microalgae enriched with beta-carotene 2.5 mg/g and Selenium 25 μ g/g), LECAR from AKVA-MDT (beta-carotene and Vitamin C at concentrations of 30 mg/g respectively). The RCRM in Kiev studied Veteron in comparison to Morevit, hydrolysate of oysters, known to be rich in the antioxidant microelement zinc. While both products increased catalase activity and antioxidant capacity of the plasma, only Morevit resulted in a decrease in lipid peroxidation (Morevit was given once per day, 10 mg of the dried extract, during two weeks).

Several plant extracts were used with good results. An extract of *Viscum album* (Iscador, developed by Lukas Klinik, Arlsheim Switzerland) with well-known immunomodulating as well as antioxidant and free radical scavenging properties, was given to 60 children followed at the Institute for Pediatrics in Kiev for recurrent respiratory disease. The treatment schedule was based on previous studies at the Lukas Klinik in cancer patients and used increasing doses rising from 0.001 mg to 5 mg. There was a statistically significant decrease ($p < 0.05$) in hydroperoxides, conjugated dienes and MDA, while the levels of GSH, glutathione peroxidase, catalase, Ceruloplasmin as well as the total antioxidant capacity increased. Another extract, produced at the ITOK of the Academy of Science, Armenia, from the roots of *Bryonia Alba* (called Loshtak in Armenia), contains cucurbitacin glycosides and trihydroxyoctadecadienoic acids as major active components. Experimental and clinical research undertaken in Armenia and other countries from the former Soviet Union showed that Loshtak has immunomodulatory, anti-inflammatory and stress-protective properties. In a series of 60 liquidators receiving Loshtak tablets (2-3 mg per day during 26-30 days), normalization of T-cell related immunity was observed. Results of cytogenetic analysis showed reduction of micronuclei. In 14 liquidators, the CF-Test indicated significant reduction of clastogenic activity in plasma after a one month treatment.

Two other plant extracts, whose superoxide scavenging properties were previously ascertained by pulse radiolysis or electron spin resonance studies, were tested for their anticlastogenic effects in the CF-Test. The Ginkgo biloba extract (trade name Tanakan from IPSEN-BEAUFOR, Paris, France) is a standardized extract from green leaves of Ginkgo tree. The mode of planting and harvesting and the extraction procedure are perfectly standardized. Rigorous analytical controls ascertain the amount of active components and confirm the absence of undesirable contaminants. Tanakan contains consistently 24% Ginkgo flavone glycosides and 6% Ginkgolides-bilobalides. It was given to 30 Armenian liquidators at the usual dose of 3 x 40

mg/day during 3 months. A CF-Test was performed in the beginning and during the first week after the treatment. In all CF-positive liquidators, the treatment reduced the clastogenic activity in the plasma to near control values. The benefit of the treatment persisted up to 9 months in all, while after 12 months about one third of the liquidators had become CF-positive again, indicating that the process leading to oxidative stress was not definitely suppressed. Similar results were obtained with another plant extract from Japan. The Antioxidant Biofactor AOB from A.O.A. Company, Kobe, was given to 20 Armenian liquidators at the usual dose of 6 sachets per day containing each 3 g of the lyophilized extract, which is prepared from soybean, wheat, rice, green tea, sesame, and citron according to a patented procedure. Besides minor concentrations of antioxidant oligoelements and vitamins, the extract contains various plant phenols, considered as responsible for the powerful antioxidant effects in various test systems. Except for one liquidator, who had the highest clastogenic activity before treatment, all liquidators were still negative for CF 12 months after arrest of treatment. Whether the benefit of AOB treatment benefits longer than that of Tanakan cannot be ascertained at present. Tanakan was given during 2 months and AOB during 3 months. A double blind clinical trial has now been initiated at the ARCEM, which foresees a 3 months treatment with two different doses of Tanakan. Other laboratory parameters studied during the treatment with Tanakan had indicated a reduction in leukocyte counts with a concomitant increase in T-lymphocytes (difference not significant because of the considerable individual variation). The same was true for conjugated dienes and MDA levels. No such studies were done in patients treated with AOB. Patients noted improvement in their general condition and working capacity.

All results taken together led to conclude that the oxidative stress status of liquidators can be influenced by antioxidants, as well as antioxidant vitamins, flavonoids and terpens presented in various plant extracts. However, the type of antioxidants, treatment schedule and dosage have to be standardized in order to compare obtained results.

Task 2: The participants agreed that the results available allow to make a decision concerning the oxidative stress biomarkers to be selected for the clinical trials and that it would be most appropriate to proceed immediately to task 3. Indeed, parallel measurements of oxidative stress biomarkers and antioxidant defences were performed already for a series of more than 100 liquidators at the ARCEM in St. Petersburg. These liquidators (mean age 35 years) participated in the accident recovery work in 1986-87 and developed initial or preclinical symptoms of cardiovascular, pulmonary or gastrointestinal diseases. They were compared to controls of similar age and having similar diseases, but who had not been in Chernobyl. The detailed results of these studies would be communicated to participants of this working group meeting. However, they have to be handled confidentially until acceptance of the manuscript for publication.

Three biomarkers were chosen for evaluation of efficacy of antioxidants in clinical trials of this programme:

- 1) The classical cytochrome C-assay for the study of increased superoxide production by neutrophils.
- 2) The GSH/GSSG ratio, which is also a classical test and one of the earliest markers for diminished antioxidant defence.

- 3) The CF-Test , which detects prooxidants in the plasma such as clastogenic lipid peroxidation products and cytokines.

These three assays should be regularly performed. The biochemical laboratories of the participating centres would agree on the methods for GSH and GSSG determination. Since the ARCEM has already accumulated considerable data, it was proposed to choose the methods used by this Centre.

Dr Emerit will send a detailed description of the CF-Test to all centres for preliminary application. Cytogeneticists from the different centres would come to the University of Pierre and Marie Curie, Paris with the first series of samples in order to handle these samples together with the technicians in Dr Emerit's laboratory. From each liquidator, a second sample would be studied after their return, and the results would be compared. A reprint of the cytochrome C assay was given to all participants of the meeting.

It was also agreed that each partner is free to study other biomarkers, if this is of interest to him or requested by a manufacturer, provided the antioxidant for the study. A list of biomarkers, available for the study in participating centres is given in the Annex 4. However, the three selected biomarkers should be included in any case.

Task 3: The participants agreed on a common protocol to be used for the clinical trials in order to obtain comparable results. This protocol is based on the programme prepared by Drs. Emerit and Neriishi, modified according to the suggestions of participants of the meeting.

a) **General considerations.** The major aim of this cooperative effort is the evaluation of the most appropriate antioxidant treatment for improvement of the liquidators' health. Not only the type of the antioxidant, but also its optimal dosage and the duration of the treatment have to be determined. It is suggested that the antioxidant should be tested in an open trial including not more than 30 liquidators before a more complicated and costly double blind trial would be considered. As an example, we may cite the studies done in Armenia with Tanakan. The open trial had shown that this extract of Ginkgo biloba leaves reduced the clastogenic activity in the plasma to near normal values after a 2 months treatment. The benefit persisted up to 12 months, but in about one third of the liquidators, the clastogenic activity had increased again 12 months after arrest of the treatment. Thus the treatment should be started again. On the basis of these results, the manufacturer (IPSEN-BEAUFOR) has initiated a dose-effect double blind trial at the ARCEM St. Petersburg, comparing two doses of the drug versus placebo in a total of 99 liquidators, positive for CF. In addition to the CF-Test, the cytochrome C- assay and GSH/GSSG measurements will be done before the start of the treatment and in the first week after arrest of treatment. In contrast to the open trial conducted over 2 months only, the drug will be given during 3 months in order to see whether the prolongation of the treatment will further improve the results. The liquidators will be followed again 12 months after arrest of the treatment. This schedule appeared satisfactory and was proposed for the present programme.

b) **Choice of antioxidants.** Drugs or food additives used for the present project should be well-known for good tolerance during long-term application. This means that only antioxidants introduced on the market can be used for our studies, while drugs in the experimental phase are

not acceptable. These drugs should have confirmed antioxidant properties, as evaluated by previous in vitro studies or in animal models. Since it is not known at present, whether combinations of antioxidants are preferable to application of a single drug and whether natural antioxidants are preferable to synthetic antioxidants, no recommendations are given for the choice of the antioxidant. It was agreed that a summary of the research project would be sent to manufacturers of antioxidants, together with the proposed schedule for clinical trials and the abstracts of the reports from each participant. The aim of these actions is to involve manufacturers in the study on a competitive basis.

c) **Organization of open or double blind clinical trials.** The protocols used for the trials with Tanakan and AOB have been adopted for the project :

- **Duration** of treatment 3 months
- **Dosage** to be proposed by the manufacturer
- **Collection of blood samples** for the three biological markers at start of the treatment (sample A), the first week after arrest of the treatment (B), 6 months (C) and 12 months after arrest of the treatment (D). Each liquidator should have a personal number, and attention should be paid in order not use the same number twice in the same Centre. The numbers have to be written on the tubes with a pencil ascertaining lisibility of the number even after conservation in liquid nitrogen or dry ice. The tubes are grouped in plastic bags, the flasks from 3 liquidators can be grouped in one bag for economical reasons. The numbers have to be repeated on a list, added to the samples upon transfer.
- **Open trials** designed to give preliminary data for the doses to be used in a double blind trial, the controls after 6 and 12 months may be omitted.
- **Double blind trials**, will be conducted as a randomized dose- effect trial of two (or more) different doses of the drug versus placebo, including 33 liquidators for each dose and the same number for placebo. Treatment will be allocated according to a randomization list. Randomization envelopes are kept by the manufacturer. The placebo will have the same appearance and the same packaging as the drug. Each package will be numbered. Each liquidator will have a randomization number according to the chronological order of inclusion in the study. As a function of this number in the randomization list, he will receive the relevant treatment. The packages will be stored in a place with restricted access.
- **Inclusion criteria**
 - 1) Written consent of the participant.
 - 2) Work in Chernobyl in a period between April 26, 1986 to April 26, 1987.
 - 3) Age 40-45 years (at time of examination), sex male
 - 4) Physical dosimetry indicating exposure between 200- 250 mGy
 - 5) Presence of cardiovascular, respiratory or digestive pathology in anamnesis.
 - 6) Practically healthy at time of blood sampling (outpatients only)
 - 7) Positivity of at least one of the three biological markers chosen.
- **Exclusion criteria**
 - 1) Smokers (> 15 cigarettes/ day)
 - 2) Alcoholics
 - 3) Blood pressure > 160 mm Hg, systolic
 - 4) Asthma or obstructive bronchitis
 - 5) Ulcer, hepatitis or other acute symptoms of gastrointestinal tract disease

- 6) Myocardial infarction
- 7) Any other acute disease at time of blood sampling
- 8) Intake of antioxidants immediately before inclusion visit
- 9) Intake of other drugs with possible interference

d) **Evaluation of the efficacy of the treatment.** Primary criteria for the efficacy of an antioxidant will be based on the regression or disappearance of the three biological markers of oxidative stress. In addition to these laboratory parameters, clinical improvement noted by the physician and self-evaluation by the liquidator will be included in the protocol, such as improvement of general health condition, wellbeing, working capacity etc. The use of the "Visual Analogical Scales " method is suggested.

e) **Family members of liquidators.** Children and spouses of liquidators will be included in the study and examined with the three methods: cytochrome C- assay, GSH/GSSG ratio and CF-Test. Those with abnormal results will be treated with antioxidants. The treatment schedules and respective controls of the efficacy of the treatment will be the same as for liquidators. The dosage will be determined according to age. For children, the written consent of the parents has to be obtained.

- **General Agreement:** It was suggested that cooperation of participating centres need certain requirements related to publication of results. These requirements will be included in an agreement signed by the directors of these centres. Each centre will also designate a responsible person for the study. The study will be coordinated by Drs. Emerit, Neriishi and Souchkevitch.

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AGENDA

Meeting: "Oxidative Stress in Chernobyl Accident Recovery Workers
Prevention of oxiradical-related diseases by antioxidants".

Institut biomédical des Cordeliers
Université Pierre et Marie Curie (University Paris VI), Paris, France

20 September 1996 (Friday)

- 09h00-09h15 Opening remarks: Professor Moreau, Director of International Relations
Department of University Paris VI.
- 09h15-09h45 Introduction and project outline. Dr Souchkevitch, WHO, Geneva
- 09h45-10h15 Post-irradiation oxidative stress. Examples from A-bomb survivors.
Dr Neriishi, RERF, Hiroshima
- 10h15-10h30 Coffee break

**Studies of Oxidative Stress and Antioxidant Treatments
done by the participating institutes from the CIS:**

- 10h30-11h00 Dr Nikiforov, ARCEM, St Petersburg, the Russian Federation
- 11h00-11h30 Dr Tsyb, MRRC, Obninsk, the Russian Federation
- 11h30-12h00 Dr Piatak, RCRM, Kiev, Ukraine
- 12h00-12h30 Dr Shefel, Gomel Medical Institute, Belarus
- 12h30-14h00 Lunch
- 14h00-14h30 Dr Antipkine, Institute of Pediatrics, Kiev, Ukraine
- 14h30-15h00 Dr Oganessian, RCRMB, Yerevan, Armenia
- 15h00-15h30 Dr Emerit Institut biomédical des Cordeliers, Paris, France in cooperation
with the institutes in Kiev, St Petersburg and Yerevan

General Discussions

- 15h30-19h00 - conclusions from the different reports
- methods to be used for evaluation of the oxidative stress status
- preparation of the definite programme with addenda
- publication of manuscripts
- pharmaceutical companies which could be involved in the project
- managerial and financial modalities

21 September 1996 (Saturday)

- 09h30-12h00 Continuation of discussion. Preparation of the meeting conclusion. Close
of the meeting.

ANTIOXIDANT SYSTEM IN CHILDREN AFFECTED BY THE CHERNOBYL ACCIDENT

U. Antipkine, L. Omelchenko, L. Arabskaya, I. Ossinskaya, K. Ivanov, T. Pochinok

Institute of Pediatrics, Obstetrics and Gynecology
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Two groups of children with recurrent respiratory diseases (RRD) and clinical manifestations of immunodeficiency were examined. The first group consisted of children permanently residing in radiation controlled areas. The second group consisted of children evacuated from 30-km zone, (Pripyat town). The control group included children living in clean territories.

The indices of lipid peroxidation (conjugated dienes, hydroperoxides, malon dialdehyde) and antioxidative system (reduced glutathione, glutathione peroxidase, catalase, ceruloplasmin, total antioxidant activity) were studied.

The indicators of lipid peroxidation and antioxidant activity in children with RRD depend on thyroid radiation dose and a character of clinical symptoms. We observed a sharp decrease of antioxidant activity in children living in controlled territories and activation of lipid peroxidation in comparison with children from clean regions. To correct the identified disorders in examined children, Vitamin E was used during a period of one month. Vitamin E normalized levels of hydroperoxides, increased the activity of glutathione peroxide and ceruloplasmin enzymes. At the same time, it decreased the catalase activity in children with RRD.

We observed the activation of lipid peroxidation and reducing of antioxidant system activity in children evacuated from the Pripyat. To correct the disorders we used antioxidant "Iscador" obtained from *Viscum Album*. The Iscador decreased the lipid peroxidation and increased the antioxidant activity in treated children.

POST IRRADIATION OXIDATIVE STRESS & ANTIOXIDANT TREATMENT

Ingrid Emerit MD

Institut biomédical des Cordeliers, Université Paris VI

Summary

Our laboratory has developed the CF-Test (clastogenic factor test), which detects circulating clastogenic materials in the plasma of irradiated persons. Identified components of these clastogenic factors are lipid peroxidation products, cytokines, in particular tumour necrosis factor, and unusual nucleotides, in which adenine is deaminated to hypoxanthine. They are released by cells exposed to superoxide radicals, and stimulate competent cells such as monocytes/macrophages and neutrophils for further superoxide production. This results in an oxidative stress situation and in genotoxic effects continuously induced via oxygen free radicals. CF formation is not specific for irradiated persons, but occurs in a variety of chronic inflammatory diseases, that are also accompanied by increased oxyradical production.

The CF-Test detects endogenous clastogens, produced by the body's own cells in response to oxidative stress. Aliquots of plasma ultra filtrates (filter cut-off 30000 DA) are added to blood cultures of healthy donors, and the increase in chromosomal aberrations, induced in the donor's blood culture by these ultra filtrates, is determined for a total of 100 cells.

Addition of plasma ultra filtrates from healthy persons (96 blood donors) does not modify or increases only slightly the spontaneous aberration rates of the test cultures (52% and 43% respectively for a total of 96 samples). Only 5 of the 96 ultra filtrates from healthy adults induced 6 additional aberrations/ 100 cells while higher values were not observed. In contrast to this, 41% out of 89 liquidators from Armenia and 52 % out of 200 liquidators from St. Petersburg were CF-positive, i.e. their plasma ultra filtrate induced 8 or more additional aberrations in the test cultures, set up with blood of healthy donors.

Plasma samples from 48 healthy children also did not induce chromosomal aberrations, when added to the test cultures, except for one sample (2%). Among 28 children from so-called "clean" cities in the former Soviet Union, only 8% were CF- positive, while studies of plasma samples from Ukrainian children yielded increased aberration rates, which varied in their importance as a function of their site of residence.

The studies done with the CF-Test suggest a correlation between radiation exposure and clastogenic plasma activity. There was a highly significant difference between liquidators exposed to less than 20 cGy compared to those exposed to higher doses. The data in Ukrainian children suggest correlations with the radioactive contamination of the soil.

Two pilot studies on small series of Armenian liquidators showed that the oxidative stress status can be influenced by appropriate antioxidant treatment. The first study was conducted with an extract of Ginkgo biloba leaves (trade name Tanakan, IPSEN France), the second with an

extract of various plants including soybean, wheat, rice, green tea, sesame and citron (A.O.A. Company, Japan). Both trials were conducted at the Institute for Radiation Medicine in Yerevan and are mentioned in the abstract of Oganessian et al. A CF-Test was done before the start and the first week after arrest of the 3 months treatment. Further controls were done 3,6,9 and 12 months later. They indicated that the benefit of the treatment persists for at least 9 months, in many cases even longer. However, the process leading to clastogenic activity in the plasma is not definitely inhibited by the treatment. Nevertheless the fact that antioxidant treatment can be discontinuous is of importance for long-term disease prevention on a large scale basis.

**POST-IRRADIATION OXIDATIVE STRESS
- CHRONIC SUBCLINICAL INFLAMMATION IN ATOMIC BOMB SURVIVORS**

Dr Kazuo Neriishi, M.D.

Radiation Effects Research Foundation, Hiroshima, Japan

Summary

There is accumulated evidence suggesting that persons exposed to radiation are under oxidative stress even many years after the exposure. Among the atomic bomb (A-bomb) survivors of Hiroshima and Nagasaki, subclinical inflammatory changes such as leucocytosis, accelerated erythrocyte sedimentation rate, increase acute phase proteins, and increased fibrinogen (unpublished data), have been observed even many years after exposure to A-bomb radiation. Due to the inflammatory reactions, oxygen radicals (ROS) are generated by PMN, mononuclear phagocytes, and eosinophils, leading not only to a destruction of tissues and DNA but also to a disturbance of free radical regulation of cellular processes. The oxidative stress in the form of inflammation in the A-bomb survivors is implicated in the array of conditions considered to be late effects. On this basis, indicators of post-irradiation oxidative stress among Chernobyl recovery workers should be studied.

POST-IRRADIATION OXIDATIVE STRESS AND ANTI-OXIDANT TREATMENT

A. Nikiforov, M.D., N. Zybina, G. Katashkova

All-Russia Centre of Ecological Medicine

One decade after the Chernobyl accident the actual tasks of today remain biological and health problems in the Chernobyl accident recovery workers ("Liquidators").

We analysed study data on oxidative stress markers and anti-oxidants on more than 100 Liquidators who suffered from various somatic diseases and who participated in the accident recovery work during the period 1986-1987. It should be emphasized that only those patients (35 mean age) who had initial or sometimes preclinical signs of a disease were elected for examination. "Controls" were patients of similar age who suffered from similar diseases, but who did not take part in the accident recovery work.

Studied markers of free radical production, secondary product, oxidative modification of macromolecules and antioxidant status were as follows: superoxide and hydroperoxide generation by neutrophils and mononuclear leukocytes in peripheral blood and by alveolar macrophages; primary and secondary lipid peroxidation (LP) products (conjugated dienes - CD and trienes - CT) in the same cells; the terminal lipid peroxidation product (TBRAS) in blood cells of arterial and venous plasma and alveolar macrophages; protein oxidative modification by determination of carbonyl groups; reduced glutathione of red blood cells; thiol groups of plasma proteins; antioxidant enzymes activity - superoxidismutase (SOD), catalase, glutathionperoxidase (GPA) and glutathionreductase (GR) of blood cells and alveolar macrophages.

- I. Significantly increased superoxide baseline level was detected in liquidators with pulmonary diseases as compared with controls ($p < 0,05$). The same can be observed with hydroperoxide. The bronchoalveolar lavage macrophages in liquidators showed a lower activity of SOD ($p < 0,05$) and catalase ($p < 0,01$). Lower catalase activity was also detected in blood cells of liquidators.

Obtained data demonstrated a higher degree of lipid oxidation in cell membranes of liquidators ($p < 0,05$). TBRAS contained in peripheral blood leukocytes, was higher as compared with the normal level ($p < 0,05$) but we did not find any significant statistical differences between different groups of patients.

At the same time TBRAS level in alveolar macrophages of liquidators was more than 3 times higher as compared with controls. TBRAS in plasma was less informative. In this connection, data on plasma protein oxidative modification in examined patients may be interesting for further investigations. It turned out that plasma proteins in liquidators were exposed to an oxidative damaging with a greater degree in those patients who developed bronchial obstruction. The significant decrease of reduced erythrocyte glutathione ($p < 0,01$) is detected in liquidators suffering from heart diseases characterized by superoxide and hydroperoxide hyperproduction.

Activity of antioxidant enzymes, such as GP and GR were higher in liquidators than in controls ($p < 0,05$). SOD activity was not changed.

- II. Based on obtained data, we consider that antioxidant treatment of liquidators can normalize free radical processes, correct and prevent as far as possible chromosome damages.

An antioxidant α -tocopherol was used for treatment of liquidators which suffered from heart diseases. Obtained results did not demonstrate any influence on the oxidative stress markers and antioxidant activities excluding the influence on the reduced glutathione content in erythrocytes. Glutathione level increased but did not reach a normal level. However, this treatment was conducted with a small dose and during a short period (10 days only).

**APPLICATION OF ANTIOXIDANTS FOR PREVENTION AND
TREATMENT OF IRRADIATED PERSONS**

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We studied the level of lipid peroxidation using the method of measuring of MDA in groups of liquidators and health persons. The results of investigations have shown that the level of lipid peroxidation in blood of liquidators is significantly increased in comparison with health persons. Increased levels of clastogenic factor (CF) were also identified in liquidators during the five-years period of investigation.

After treatment of liquidators with α -tocopherol the level of lipid peroxidation decreased significantly compared to background level. For CF it was shown that many antioxidants (α -tocopherol, Loshtak - extract of *Brionia alba*, Tanakan (Ipsen, France) and AOB (extract of green tea and wheat germs, produced by AOA Co., Japan) were effective as protectors from radiation injuries. The benefit of three months treatment persisted more than six months and in some patients even after one year.

**OXIDATIVE STRESS IN CHERNOBYL ACCIDENT RECOVERY WORKERS,
PREVENTION OF OXIRADICAL-RELATED DISEASES BY ANTIOXIDANTS**

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The following markers of lipid and enzyme (catalase) peroxidation and indicators of antioxidant activities were studied in liquidators: double bonds, conjugated dienes, trienes, diene ketones, malondialdehyde. The markers were determined by spectrophotometry. The increase level of dienes dissolved in heptane phase, conjugated dienes and trienes and diene ketones were identified in liquidators five or six years after the accident.

It was shown in experiments that antioxidants containing beta-carotene, vitamins E and C prevented the devastation of a thymus in mouse exposed to radiation or stress factors. Antioxidants containing β -carotene, Vitamins E and C (VETORON), and those containing β -carotene, Selenium and microalgae (CHLOSECAR) which were used for treatment of liquidators, caused the decrease by 17-24% of a malondialdehyde level in blood serum and improve health status of the patients.

The table given below provides details of antioxidants studied in MRRC.

Table **Antioxidant food additives
studied at MRRC RAMS, Obninsk**

Food additive	Concentration of antioxidants	Certificate availability	Manufacturer
VETORON	β -carotene 20 mg/g Vitamin C 6.7 mg/g Vitamin E 6.7 mg/g	+	AKVA-MDT
CYCLOCAR	β -carotene 60 mg/g Vitamin C 17 mg/g	+	NIKA-NPO "Vitamins"
CHLOSECAR	β -carotene 2.5 mg/g Biotransformed Selenium - 25 mg/g Microalgae chlorella - E-25 (powdered) 700 mg/g	+	MRRC RAMS Institute of Nutrition of RAMS
LECAR	β -carotene 30 mg/g Vitamin C 30 mg/g Natural tocopherols, lecithin, albumins	Experimental sample	AKVA-MDT MRRC RAMS

**OXIDATIVE HOMEOSTASIS IN INDIVIDUALS EXPOSED TO RADIATION
AS A RESULT OF THE CHERNOBYL NPP ACCIDENT**

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The following indices of oxidative homeostasis were investigated:

- primary (conjugated dienes, hydroperoxides, ketondienes and conjugated trienes) and final products of lipid peroxidation (malondialdehyde);
- enzyme and non-enzyme indices of an antioxidant defence system (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase): the level of reduced glutathione was measured and an "individual factor of antioxidant condition" calculated.

Examined persons included:

- 57 men, aged between 35-50, who had taken part in the clean-up of the Chernobyl NPP and had been exposed to a total radiation dose of more than 1 Gy.
- 566 men aged between 25-58, working at present time in the Chernobyl NPP
- 30 healthy blood donors.

Persons exposed to the higher radiation doses had a high level of primary lipid peroxidation products and a low level of antioxidant activity. Conjugated dienes increased by 100-115%, hydroperoxides by 75-94%, ketondienes and conjugated trienes by 31-38%. SOD activity decreased by 20-41% and catalase activity by 38-45%.

Antioxidant treatment was conducted with two products:

- 1) Veteron, containing 20 mg/g β -carotene, and 6.7 mg/g of vitamins E and C respectively, given at a dose corresponding to 40 mg β -carotene per day during four weeks.
- 2) Morevit, a hydrolysate of oysters, given as a dried extract (10 g daily during two weeks).

After Veteron, an increase of SOD activity by 37% and of the "individual factor of antioxidant condition" by 48% was noted, while the primary lipid peroxidation products remained unchanged. On the other hand, Morevit resulted in a decrease of lipid peroxidation and in an increase of catalase activity (up to 15%) and of antioxidant factor (up to 60%), while SOD activity, significantly decreased before treatment, was not influenced.

MONONUCLEAR PHAGOCYTARY SYSTEM (MPS) IN LIQUIDATORS OF THE POST-CHERNOBYL EFFECTS

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Abstract

A group of participants in the cleaning operations during 1986 to 1988 were selected for the dynamic haematological observation between 1991 to 1994. This group included 73 men aged between 24 and 40 living in Gomel and the Gomel region. Almost all of them (80%) were reservists drafted into the army with the task of overall cleaning and washing and they were billeted in the Kiev region, the Ivankov district. The rest of the group were regular rank-and-file, serving in Chernobyl. The doses accumulated during their service were under 200 mSv. In some cases doses were not recorded into their personal files. The out-patient follow-up included clinical examinations, blood tests and biochemical analyses. To check the functional activity of the MPS cells the following tests were applied: quantitative evaluation of proteins involved in the iron metabolism which are primarily produced by mononuclear phagocytes, plasma antioxidants and some immunological parameters.

Investigation of MPC cell functions demonstrated that 48% of the liquidators developed iron metabolism abnormality. It was proved by increasing level of serum ferritin ($p < 0.05$). The serum iron, TIBC and TIS levels were shown practically on normal level. Investigation of serum isoferritins revealed that 50% of the individuals with increased SF had ratio of alkaline and acidic ferritins different from the norm towards a higher acidic ferritin level. It is typical for hypersidermic conditions. The analysis of the antioxidant protection by examining of ceruloplasmin and ceruloplasmin/transferrin ratio revealed decrease of plasma antioxidant activity level among 68% of liquidators. Circulating immunocomplexes (CIC) were identified in more than 65% of the liquidators. It can be an evidence of a significant impairment of the purification function of MPS cells.

BIOMARKERS AVAILABLE IN THE PARTICIPATING INSTITUTES

	ARCEM	MRRC	USCRM	IPOG	GMI	SCRM	IBC
1. RADICAL DETECTION Chemilumescence Cytochrome c assay	+	+	+	+	+	+	+
2. LIPID PEROXIDATION Conjugated dines LDL Oxidation Antibodies to oxidized LDL Pentane exhalation	+	+	+	+	+	+	+
3. PROTEIN OXIDATION Carbonyls	+						
4. DNA OXIDATION 8-OHdG by HPLC-EC Mass spectrometry CF Test	+			+		+	+
5. ANTIOXIDANTS Vitamins Selenium Superoxide dismutase Glutathione peroxidase Glutathione reductase Glutathione GSH Ceruleplasmin Transferrin, ferritin Bilirubin Uric acid	+	+	+	+	+	+	
6. INFLAMMATION Sedimentation rate Protein electrophoresis WBC IL-6 IL-6 receptor	+	+	+	+	+	+	
7. OTHERS	+	+	+			+	

ABBREVIATIONS

ARCEM	All-Russia Centre of Ecological Medicine
CF-Test	Clastogenic Test
GHS	Glutathione (gamma-glutamylcysteinylglycine)
GMI	Gomel Medical Institute
GSSG	Oxidized glutathione
IBC	Institute biomédical des Cordeliers
IPOG	Institute for Pediatrics, Obstetrics & Gynecology, Kiev
MDA	Malondialdehyde
MRRC	Medical Radiological Research Centre, Obninsk
RAMS	Russian Academy of Medical Sciences
RCRM	Research Centre on Radiation Medicine
SCRM	Scientific Centre of Radiation Medicine, Yerevan
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
USCRM	Ukrainian Scientific Centre for Radiation Medicine