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Subject: Comments on health effects of low-level radiation for the ACRS and ACNWS meeting March 26, 1996

The enclosed copy of a recent paper by Dr. E. B. Burlakova summarizes her work during the past decade indicating that at extremely low doses, a free-radical mediated effect causes the risk of protracted low doses of radiation exposure to show a sharp rise followed by a decline in risk per rad with increasing dose-rate, and a renewed nearly linear rise at high doses and dose rates. As a result, an extrapolation from very high doses and dose-rates such as those experienced by the A-bomb survivors , either linear or linear - quadratic, leads to greatly underestimated health effects at the very lowest doses in the 1-100 mr range associated with population doses from nuclear reactors and nuclear waste sites.

This conclusion is consistent with the studies of free-radical effects on lipid cell-membranes independently found by Dr. A. Petkau at the Canadian Atomic Energy Laboratory in Pinawa, Manitoba, as discussed in the 1980 U.S. National Academy's BEIR III report on pages 464-469. The work of Burlakova and Petkau thus provides an explanation for the growing number of studies showing increased incidence of leukemia and cancer around nuclear facilities in England, France, Germany and the U.S. which have been difficult to believe when the expected incidence is based on an extrapolation from high dose and dose-rate data based on A-bomb survivor follow-up, medical radiation exposures and animal studies.

In the light of these developments, I urge the committee to review the latest data on very low dose effects for large human populations that is emerging, especially as studies of the effects of Chernobyl on distant populations become available, and to call for public hearings on radiation standards for population exposures to fission products at low doses, whose chemical concentration and nuclear decay properties differ greatly from natural background sources .

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LOW INTENSITY RADIATION: RADIOBIOLOGICAL ASPECTS

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Abstract — Biochemical, biophysical and functional properties of the genetic and membrane apparatus of the cell have been considered in mice exposed to radiation in the wide range of doses of 6 to 1800 mGy with the radiation intensity of 4.1×10^{-2} and 41×10^{-3} mGy min^{-1}. Properties considered include adsorption of DNA on nitrocellulose filters, genome restructuring, microviscosity of lipids of nuclear, mitochondrial, microsomatic, plasmatic membranes, and composition and antioxidizing activity of membrane lipids, activity and regulatory properties of the membrane and cytosolic enzymes of organs and tissues. It was shown that the dose dependence of the changes in the investigated properties is of a non-linear polymodal (bimodal) nature. The first low dose maximum was observed at doses of 10 to 50 mGy. The value of the maximum and the dose at which it was observed depend on the subject's nature, the radiation intensity and the time passed after irradiation. An essential factor is that sensitivity to other damaging effects of molecules, cells, organs and animals changes after exposure to low dose radiation. The indices of health of people exposed to low dose irradiation also change. The bimodal dose-dependence of the effect was illustrated by the leucoses death rate. The explanation is given in terms of the changes in the relation between the quantity of damage and the activity of repair systems induced by low dose irradiation.

In recent years, the effects caused by chemical agents in ultra-low doses (ULDs) and by physical factors of low intensity has been a matter of great interest[1,2].

A peculiarity of the action of ULDs of the agents is a paradoxical dose-effect dependence that is, in most cases, of a bimodal (polymodal) nature. It is significant that the regions of active concentrations or doses of physical factors are separated by a so-called 'idle' or 'dead' zone: yet, the active regions exhibit similar effects for different concentrations, 10^4 to 10^{14} times as high.

The effects of ULDs of agents have been studied for more than 10 years. For some time past, concentration has been on the investigation of the influence of low intensity radiation on the vital cellular components.

Numerous suggestions are made in the literature that damaging factors in the environment strongly affect the structure of biological objects. It was thought essential to study the influence of low intensity radiation on structural characteristics of the genome and membranes. ^{137}Cs was used as the source of radiation.

Studies confirmed the rate of alkaline elution of the

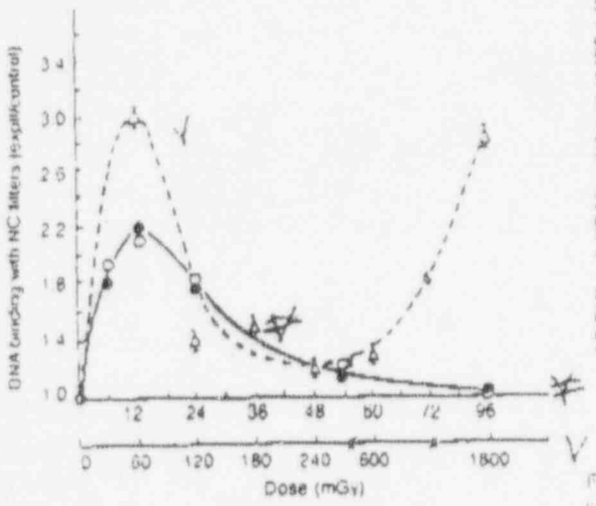

Figure 1. Per cent of binding of the exposed mice spleen DNA with nitrocellulose filters depending on the dose of irradiation (in relative units). Curves: (1) dose rate 4.1×10^{-2} mGy.min^{-1}; (2) dose rate 41×10^{-3} mGy.min^{-1}.

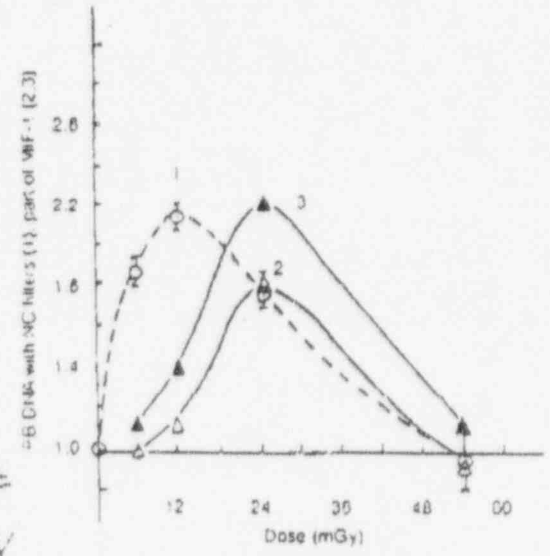

Figure 2. Per cent of binding of DNA with nitrocellulose filters: curve 1, relative units, and curves 2 and 3, the relative change of the MIF-1 intersperse reiterations share in the γ-irradiated mice spleen depending on the dose of irradiation (curve 2 was obtained by gel photometry, 3 photonegatives of the gels); dose rate 4.1×10^{-2} mGy.min^{-1}[2,3].

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DNA of lymphyocytes and liver, neutral elution and adsorption on nitrocellulose filters of the DNA of spleen, restriction of the spleen DNA by endonuclease EcoR I, and structural characteristics of nuclear, mitochondrial, synaptical, erythrocyte and leucocyte membranes extracted from the organs of exposed animals.

In addition, to determine the functional activity of cells, the activity and isoforms were studied of aldolase and lactatedehydrogenase (LDH) enzymes, the activity of acethylcholineestherase (AChE), superoxidedismutare (SOD), glutationperoxidase, the rate of formation of superoxide anion radicals, the composition and antioxidising activity of lipids of the above-mentioned membranes as well as the sensitivity of cells, membranes and DNA to the action of other damaging factors[2-6].

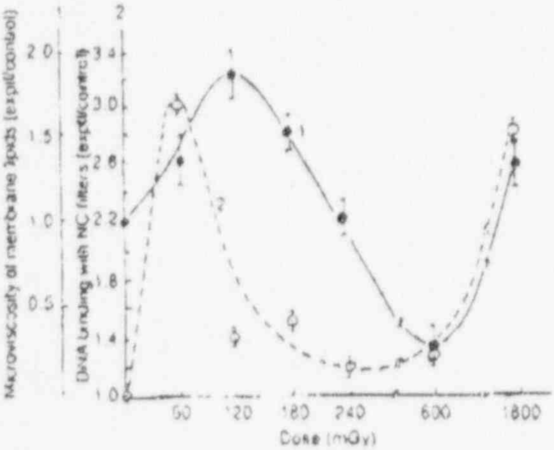

Figure 3. Curve 1, per cent binding of the spleen DNA with nitrocellulose filters (relative units) and curve 2, microviscosity of nuclear membrane lipids of the exposed mouse liver depending on the dose of radiation (intensity of radiation, 41×10^{-3} mGy min^{-1}).

Table 1. The changes in kinetic parameters of AChE, aldolase and LDH in organs of γ-irradiated mice (expt/control); dose rate 41×10^{-3} mGy min^{-1}.

Dose (mGy)	Enzyme	K_M	V_M	V_{max}/K_M
6	AChE (Synaptosomes)	1.15	0.93	0.8
	Aldolase (Synaptosomes)	2.0	1.8	0.98
	LDH	1.25	1.5	1.2
12	AChE	1.5	1.1	0.75
	Aldolase	0.5	0.75	1.25
	LDH	1.3	2	1.55
24	AChE	1.6	1.4	0.87
	Aldolase	2.5	1.2	0.5
	LDH	0.88	0.88	1.0
54	AChE	0.8	0.94	1.2
	Aldolase	0.5	0.80	1.5
	LDH	2.0	1.0	2.0

For all the parameters studied, a bimodal dependence of the effect on a dose was found, i.e. the effect increased at low doses, reached its maximum (a low dose maximum), then decreased (in some cases the sign of the effect changed to the opposite) and increased again as the dose increased. The value of the low dose maximum and the dose to reach it depended on the nature of the subjects on the intensity of radiation and the time passed since irradiation.

A general rule was that the maximum was shifted to the region of lower doses as a result of a decrease in the radiation rate. Figures 1, 2 and 3 show some of the results of the investigation.

Figure 1 shows a change in the structural properties of DNA depending on dose rate. Figure 2 shows that the change in the per cent of binding (PB) the mice spleen DNA, depending on a radiation dose (curve 1), reaches its maximum at 12 mGy (the dose rate 41×10^{-3} mGy min^{-1}). The PB values do not differ much from the control at a dose of 54 mGy. Curves 2 and 3 depict the results of the restriction analysis of the exposed mice spleen DNA; the analysis was carried out using endonuclease EcoR 1. The data showed a change in the contribution of intersperse reiterations of MIF-1 in DNA that is indicative of the genome restructuring.

Note that the dose dependence corresponds to that for the per cent of binding DNA on nitrocellulose filters.

In addition, the changes in structural properties of the genome caused by 10 to 54 mGy are comparable to those caused by doses 20 to 40 times as high.

Figure 3 shows the change in PB of the spleen DNA (radiation rate 41×10^{-3} mGy min^{-1}) and the dose dependence of microviscosity of nuclear membrane lipids. As with the structural properties of DNA (curve 1), the membrane lipid microviscosity changes dramatically with the dose of radiation; the maximum values were obtained for radiation doses of 60 to 120 mGy. The changes in the microviscosity of erythrocyte membrane lipids were the same. As in the previous cases, it was observed that the maximum was in the region of low doses. The value was comparable with the microviscosity ones obtained after irradiation by the doses of 1 to 1.2 Gy.

To evaluate the functional activity of the cells, the data on the changes in kinetic parameters of membrane and cytosol enzymes in the cells of exposed animals were used. The data are listed in Table 1. As is seen from Table 1, the changes in the enzymes' kinetic properties begin to develop at a dose of 6 mGy. Here, a prolonged retention was observed of the changed kinetic properties of enzymes caused by irradiation with

12 mGy. Also, a disturbance in the regulatory functions of enzymes was seen as a result of changes in the relation between the isozyme forms of lactatedehydrogenase and aldolase and between the activity of the enzyme and its substrate — for superoxidedismutase (Tables 2, 3). Thus, irradiation of low intensity results in a non-linear dose-dependent change in the functional activity of cells. It is important that sensitivity of separate macromolecules, cells and the organism as a whole to any other damaging factors also changed. To illustrate, an increase was observed in the sensitivity of DNA extracted from the organs of exposed mice (gamma irradiation, 60 mGy) to a repeat irradiation as compared with the control. It was found that the low dose irradiation increased a degree of erythrocyte haemolysis of exposed mice; the CNS sensitivity to neuromediators, agonists and antagonists as well as the cell response to the action of regulators, to a repeat irradiation and to introduction of radiosensitisers also change. Similar results were obtained 20 years ago by the group headed by Yu. G. Grigoryev in the experiments with dogs chronically exposed to radiation of low intensity to model the conditions of a long-term stay in space (Flight-to-Mars project).

Table 2. The change in LDH isozymes activities in mice brain cytoplasma after irradiation: dose rate 4.1 × 10^{-2} mGy.min^{-1}.

Dose (mGy)	Isozymes				
	H_4	H_3M	H_2M_2	HM_3	M_4
6	1.21 ±0.03	1.27 ±0.06	0.95 ±0.04	1.04 ±0.10	1.07 ±0.09
12	0.93 ±0.10	0.74 ±0.02	1.1 ±0.1	0.78 ±0.02	0.67 ±0.01
24	1.15 ±0.06	0.81 ±0.09	0.75 ±0.09	0.88 ±0.11	0.71 ±0.11
54	1.35 ±0.19	0.84 ±0.05	0.84 ±0.05	0.91 ±0.08	1.09 ±0.05

Table 3. The changes in the relation between the generation rate of superoxide radicals ($V_{O_2^-}$) and SOD activity in liver microsomes and liver submitochondrial particles (SMP) of mice after irradiation: dose rate 4.1 mGy.min^{-1}.

Dose (mGy)	$V_{O_2^-}/A_{SOD}$ (Microsomes)	$V_{O_2^-}/A_{SOD}$ (SMP)
0	1	1
6	1.8	1.6
12	3.4	0.8
24	1.7	1.8
54	2.0	1.2

Such a dose dependence can be explained in terms of the concept that there is a scale of the doses causing damage in biological objects and of those inducing the systems of restoration[6,7]. In fact, while the repair (adaptation) systems do not work, the effect increases with the dose; then it decreases as the repair develops, it may be eliminated or change its 'sign'; then it increases again with the dose as the damage prevails over repair. However, despite the numerous experimental findings in favour of this assumption, one cannot be quite positive about the cause of such dose-effect dependence in the region of low dose irradiation. There are different viewpoints on the nature of this dependence, e.g. a concept of existence of a particular pool of cells that are sensitive to the low intensity irradiation[8] and others[9].

Work focused on the investigation of similar biochemical and biophysical changes in the organisms of people exposed either to a chronic or to an acute low dose irradiation. Of special interest were the changes in the antioxidant status of the organism.

Numerous data obtained both at our laboratory and known from the literature permitted us to conclude that the antioxidant status of the organism is responsible for the frequency and severity of diseases, the probability of rehabilitation and sensitivity to the action of damaging factors[10].

Figure 4 shows the changes in the activity of superoxidedismutase in the blood plasma of people participating in the liquidation of the consequences of the Chernobyl catastrophe and exposed to radiation (the liquidators). Drastic changes were observed for doses less than 50 mSv. Similar dose dependences were observed while examining vitamin-antioxidants of the exposed people. In those cases the authors noted a decrease in the concentration of tocopherol and glutathione in the blood

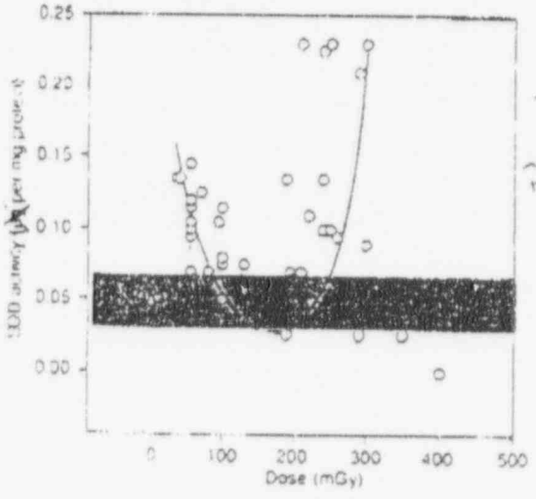

Figure 4. Superoxidedismutase activity in the blood plasma of the liquidators depending on the dose of radiation.

plasma, and a change in the A-E ratio at irradiation doses lower than 50 mSv[11].

It was essential to find out whether the changes in the health of the exposed people show similar regularities. However, the epidemiological data were not always obtained at the appropriate level to permit us to make definite qualitative conclusions.

Previously, it was noted that an increase in a number of disease cases among the liquidators shows a similar tendency to a non-monotonic, non-linear dose dependence[2]. Some biochemical and haematological parameters of the people exposed to radiation during the accidents at atomic power stations also exhibit a bimodal dose dependence[11,13]. However, it would be most interesting to study similar dependences for the long-term consequences of irradiation, i.e. malignant tumours. It is well known that ionising radiation can play the role of a promoter as well as of an initiator of malignant tumours. An increase in the radiation intensity and doses (up to certain limits) results in a decrease in the promoting and in an increase in the initiating functions of irradiation. There is a wide discussion in the literature over the influence of low doses of radiation on the occurrence of malignant radiogenic tumours and leucoses. As a rule, the scientists who deny the fact of

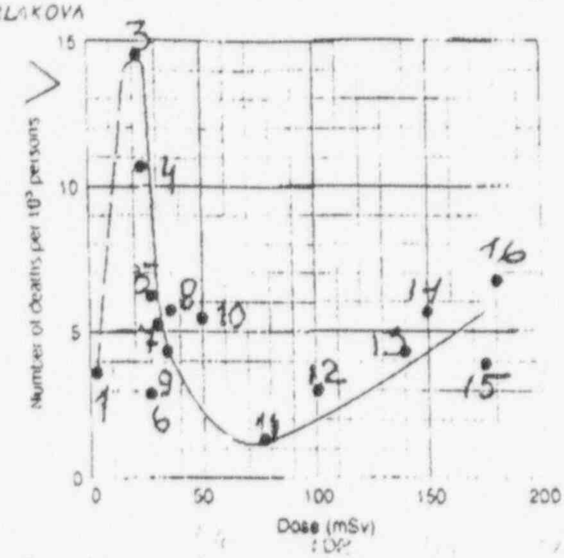

Figure 5. Leukaemia deaths per 10^5 person years depending on the dose of radiation (the numbers of the points correspond to the numbers in Table 4).

Table 4. The data on the leukaemia death rate for people after atomic bombing and nuclear accidents and for workers in the nuclear industry (the numbers in the table correspond to the numbers of the points in Figure 5).

	Irradiation locality	Dose (mSv)	Leucoses death rate per 10^5 person.years	Ref
1.	Pilgrim 1983–1988	2	3.6*	19
2.	UKAEA workers 1946–1979	20 (20–50)	4.3*	15
3.	Pilgrim 1979–1985	20	14.8*	19
4.	Oakridge National Laboratory	21	10.4	16
5.	Hanford	27	6	16
6.	American Military Agency	27.6	2.5	16
7.	Residents of Japan Group I	30	5.1	17
8.	American Nuclear Agency	33.1	5.6	16
9.	Rockyflats	35	4.0	16
10.	UKAEA workers	50	5.22	15
11.	Residents of Japan Group II	00	1.6	17
12.	UKAEA workers	100	3.0*	15
13.	Sellafield	139	4.2	16
14.	Residents of Japan Group III	150	5.7	17
15.	Residents at the Techa River, Group I	176	3.8	18
16.	Residents at the Techa River, Group II	180	6.9	18
17.	Residents at the Techa River, Group III	290	8.5	18
18.	Residents of Japan Group IV	400	8.56	17
19.	Residents at the Techa River, Group IV	610	6.5 (14.3*)	18
20.	Residents at the Techa River, Group V	780	7.9 (17.3*)	18
21.	Residents of Japan Group V	800	14.3	17
22.	Residents at the Techa River, Group VI	1640	15.3	18
23.	Residents of Japan Group VI	1800	28.6	17
24.	Residents of Japan Group VII	2600	57	17
25.	Residents of Japan Group VIII	3600	91	17

*The data are related to the control of 3.6 per 10^5 person.years

the International Agency for Cancer Research and provides the results of checking on 96,000 workers of atomic plants in Canada, Great Britain and the USA. The authors concentrated on the estimation of a relative risk of death for all kinds of cancer, excluding leu-

that it is necessary to provide this plot in an effort to concentrate the investigators on searching the regularities and mechanisms of the effects at low doses, and to emphasise that it is not permissible to pool the data obtained for low and high doses because this levels the

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verification of a high probability of occurrence of malignant tumours at low doses of irradiation put forward two arguments.

(1) It is almost impossible to reveal experimentally or epidemiologically an increase in a number of tumour carriers caused by irradiation because this increase is within the limits of the difference in the percentage of cancers in different regions and the verification requires a great bulk of experimental data.

(2) Considering the cases of cancers and leucoses caused by low dose irradiation, which exceed significantly the control values (as was shown), and extrapolating the data to the high doses in accordance with the linear dependence, one can get that the number of expected tumours exceeds the real values by orders of magnitude. This is why the conclusions about the influence of low doses are doubtful[14].

The grounds for scepticism concerning the information on an increase in the number of deaths from tumours and leucoses caused by a low dose irradiation were the results of the check when one and the same group of workers of an atomic power station yielded an increase in a number of deaths from cancer as compared with the control for doses of 20 or 50 mSv but a decrease for 100 mSv[15].

The analysis of the literature data on the leucoses death rate for different groups of exposed people working, as a rule, at atomic power stations showed the same tendency in the dose–effect dependence that was discovered in our experiments.

Recently, a fundamental paper has been published[16]. It contains the analysis of data on the cancer and leucosis death rate among the workers at atomic plants. The work had been carried out by the specialists from the International Agency for Cancer Research and provides the results of checking on 96,000 workers of atomic plants in Canada, Great Britain and the USA. The authors concentrated on the estimation of a relative risk of death for all kinds of cancer, excluding leukaemia, and for leukaemias excluding chronic lympholeucoses.

A relative risk of death from leucoses was 2.2 Sv^{-1}. This value lies between the value for the victims of the atomic bombs obtained using a linear model for the risk estimation, 3.7 Sv^{-1}, and that obtained using a linear-quadratic model, 1.9 Sv^{-1}.

In 1994, the information that had been collected for the past 35 years on medical examinations of people in Japan exposed to radiation during the atomic bombing of Nagasaki and Hiroshima were published[17]. The authors of this investigation emphasise the fact of a decrease in the leucoses death rate in the group of people exposed to a dose of 80 mSv; they regard this fact as an evidence for a hormesis caused by low dose irradiation.

In Kossenko *et al*[18], the data on the deaths from leucoses after the accident in the South Urals were presented.

An effort has been made to pool the information in the literature[15–17] on the leucoses death rate for the workers of atomic plants and exposed people and to plot a corresponding dose rate dependence curve excluding chronic leucoses. This dependence is shown in Figure 5 and Table 4. It is evident that the results of epidemiological examinations of a great number of people can be represented as a bimodal dose dependence. The dependence amalgamates the cases when irradiation resulted in a decrease in the leucoses death rate below the control. The revealed tendency shows that the rate of deaths from leucoses caused by low dose irradiation is comparable with that for the doses dozens of times higher.

Different dose–effect dependences for the regions of low and high doses permit us to suggest different mechanisms responsible for the increase in the leucoses death rate at low and high doses of irradiation.

This is only a qualitative pattern that should be subjected to statistical processing with the resulting significantly 'smoothed' effects. However it is believed that it is necessary to provide this plot in an effort to concentrate the investigators on searching the regularities and mechanisms of the effects at low doses, and to emphasise that it is not permissible to pool the data obtained for low and high doses because this levels the real effects.

Our findings and the data on experiments with exposed animals available in the literature count in favour of taking new approaches in studying the effects of low doses of radiation.

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